MEDICAL TOXICOLOGY OF DRUG ABUSE

MEDICAL TOXICOLOGY OF DRUG ABUSE

Synthesized Chemicals and Psychoactive Plants

DONALD G. BARCELOUX, MD, FAACT, FACMT, FACEP

CLINICAL PROFESSOR OF MEDICINE, DEPARTMENT OF EMERGENCY MEDICINE
DAVID GEFFEN SCHOOL OF MEDICINE
UNIVERSITY OF CALIFORNIA AT LOS ANGELES
LOS ANGELES, CALIFORNIA

SENIOR PARTNER, CEP AMERICA
EMERYVILLE, CALIFORNIA

STAFF PHYSICIAN, DEPARTMENT OF EMERGENCY MEDICINE
POMONA VALLEY HOSPITAL MEDICAL CENTER
POMONA, CALIFORNIA

ASSOCIATE EDITOR

ROBERT B. PALMER, PHD, DABAT, FAACT
TOXICOLOGY ASSOCIATES, PLLC
DENVER, COLORADO

ROCKY MOUNTAIN POISON & DRUG CENTER
UNIVERSITY OF COLORADO SCHOOL OF MEDICINE
DENVER, COLORADO

UNIVERSITY OF WYOMING COLLEGE OF HEALTH SCIENCES
LARAMIE, WYOMING

WILEY

A JOHN WILEY & SONS, INC., PUBLICATION

To my wife, Kimberly; my son, Colin; my daughter, Shannon; and my son-in-law, Michael, whose love and support sustains me through this continuing project.

CONTENTS

CONTENTS

CONTENTS

FOREWORD

It is a great pleasure for me to write this Foreword, as I have known Don Barceloux professionally for many years and we have collaborated on various projects particularly for the American Academy of Clinical Toxicology. Dr. Barceloux first established himself as a distinguished and successful author with the publication in 1988 of the first edition of *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*, which he co-authored with the late Matthew Ellenhorn.

Dr. Barceloux's reputation for producing systematic books of great quality was further enhanced in 2008 by the publication of the first volume (of four) of *Medical Toxicology*, which was entitled the *Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Plants and Venomous Animals*; he wrote 171 of the 185 chapters. At the time of publication, this book had no rival, and that continues to be the case. I use it on a daily basis in my clinical practice.

While a substantial number of textbooks on clinical toxicology have been published in the last two decades, none has focused primarily on drug abuse. Equally, many books on drug abuse have been published over the same period, but none has been written from the perspective of the clinical/medical toxicologist.

For this reason I welcome, and indeed have been waiting eagerly for, the publication of the second volume in the *Medical Toxicology* series, which uses the same helpful format as volume 1. *Medical Toxicology of Drug Abuse*: *Synthesized Chemicals and Psychoactive Plants* provides in-depth up-to-date coverage of psychoactive agents that are abused, including newer designer drugs and psychoactive plants. Detailed information is provided on the pathophysiology, toxicokinetics, clinical effects (including the features associated with abstinence syndromes and reproductive abnormalities), treatment, and prevention of drug abuse. In addition, there are sections on epidemiology, on the chemical structure and physiochemical properties of the abused substances, on impurities introduced during synthesis, on the interpretation of the results of laboratory testing, and on the characteristics and geographical distribution of psychoactive plants. All but six of the 66 chapters in the present volume were authored by Dr. Barceloux himself; the remainder have been written by other acknowledged experts. After reading the book in proof I am confident its impact and usefulness will be similar to volume 1.

I commend this excellent book to the reader.

Professor Allister Vale md frcp frcpe frcpg ffom faact fbts hon frcpsg

National Poisons Information Service (Birmingham Unit) and West Midlands Poisons Unit, City Hospital, Birmingham, UK; School of Biosciences and College of Medical and Dental Sciences, University of Birmingham
Past-President of the British Toxicology Society; Past-President of the European Association of Poisons Centres and Clinical Toxicologists; Past-Trustee of the American Academy of Clinical Toxicology

PREFACE

Medical Toxicology of Drug Abuse: *Synthesized Chemicals and Psychoactive Plants* is the second book in the Medical Toxicology Series that divides Medical Toxicology into the four following areas: Natural Substances, Drugs of Abuse, Occupational and Environmental Exposures, and Pharmaceutical Overdose. The book series is designed to provide in-depth, evidence-based coverage of the most important toxins affecting humans. This book covers a variety of older psychoactive drugs and newer designer drugs of abuse including recently popularized drug (e.g., methcathinone, mephedrone, *Salvia divinorum*, kratom). Information on a particular substance is discussed in the book commonly associated with the subject. Consequently, the most important psychoactive plants are discussed in the Drug Abuse book rather than the Natural Substances book, so the reader interested in information on drug abuse will not have to search two books. Pharmaceutical drugs (e.g., hydrocodone, morphine, oxycodone) used primarily for therapeutic purposes will be covered in the book on Pharmaceutical Overdose. Conversions for length and temperature in metric and imperial systems are provided to ease the use of this book by an international readership, whereas the metric system for mass and concentrations are retained to limit any confusion about doses in the United States. This book is designed as a convenient reference for answers to questions regarding exposure, pathophysiology, clinical effect, detection, and treatment of toxicity associated with drugs of abuse.

The format of this book follows the first book in the Medical Toxicology Series, *Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Plants, and Venomous Animals*. When the reader is familiar with the templates used in the book series, the consistency of the organization allows the reader to easily locate the appropriate information necessary for decisions regarding the sources, effect, regulation, and management of toxic exposures. The following list provides organizational details on the material under the headings for each drug:

History includes facts about the discovery, past abuse, and earlier complications of drug abuse.

Botanical Description helps the reader identify the characteristics and geographic distribution of psychoactive plants.

Identifying Characteristics includes the chemical structure, physiochemical properties, and the terminology associated with the specific drug of abuse.

Exposure discusses the epidemiology, trends, sources, production processes, impurities added during synthesis of the drug, profiling of confiscated drugs, and common methods of misuse.

Dose Effect covers clinical data on the drug doses associated with overdose and fatalities in humans. The book emphasizes dose-related effects rather than adverse or idiosyncratic reactions.

Toxicokinetics discusses the disposition of the drug in the body including the absorption, distribution, biotransformation, and elimination along with maternal and fetal kinetics, tolerance, and drug interactions.

Histopathology and Pathophysiology presents information on the mechanisms of action and toxicity, autopsies, and postmortem changes associated with drug abuse.

Clinical Response provides data on the clinical features of toxicity following the illicit use of the drug including the onset, duration, and type of clinical effects (behavioral abnormalities, mental disorders, medical complications). Additionally, this section discusses reproductive abnormalities, fatalities, and any

symptoms associated with an abstinence syndrome following cessation of use.

Diagnostic Testing presents information important to the interpretation of the clinical significance of laboratory testing. This section includes current analytic methods to identify and quantitate the drug in biologic and confiscated material, effects of storage on analytic results, biomarkers of exposure in blood, urine, and postmortem material, abnormalities detected by imaging studies and ancillary tests, and driving impairment associated with use of the drug.

Treatment includes current information on the management of toxic effects associated with drug misuse and abuse including recommendations for first responders, life-threatening problems associated with overdose, the use of antidotes, and supportive care.

Medical Toxicology of Drug Abuse: Synthesized Chemicals and Psychoactive Plants focuses on scientifically confirmed facts about specific drugs of abuse based on the medical literature and clinical experience. References are documented to validate the information and to provide sources for further inquiry. My hope is that this interdisciplinary, evidence-based approach will increase communication between traditional clinical settings and fields aligned with Medical Toxicology including those in analytic laboratories, universities, regulatory agencies, and coroner's offices . . . and thus, encourage more inquiry into the pathophysiology, clinical effects, biomarkers, treatment, and prevention of drug abuse.

DONALD G. BARCELOUX, MD

November 28, 2011

CONTRIBUTORS

Nicholas A. Buckley, MD, FRACP
Professor in Medicine, Clinical Pharmacology &
    Toxicology
University of NSW
Sydney, Australia

Timothy B. Erickson, MD, FACEP, FAACT, FACMT
Professor, Department of Emergency Medicine
Director, Division of Medical Toxicology
Director, Center for Global Health
University of Illinois at Chicago
Chicago, Illinois

Cyrus Rangan MD, FAAP, FACMT
Assistant Medical Director, California Poison Control
    System
Medical Toxicology Consultant, Children's Hospital
    Los Angeles
Director, Toxicology and Environmental Assessment
Los Angeles County Department of Public Health,
Los Angeles, California

James W. Rhee, MD, FACEP, FAAEM
Assistant Professor
Director of Medical Toxicology
Associate Program Director, Emergency Medicine
    Residency
Department of Emergency Medicine
Loma Linda University School of Medicine
Loma Linda, California

Brandon Wills, DO, MS
Fellowship Director, Medical Toxicology
Assistant Professor, Department of Emergency
    Medicine
Virginia Commonwealth University Medical Center
Associate Medical Director, Virginia Poison Center
Richmond, Virginia

REVIEW PANEL

Timothy E. Albertson, MD, MPH, PhD, FRCP, FACP, FACMT, FACEP
Professor of Medicine, Emergency Medicine,
    Anesthesiology, and Pharmacology/Toxicology
University of California, Davis, School of Medicine
Sacramento, CA

Vikhyat S. Bebarta, MD
Lieutenant Colonel, United States Air Force
Chief, Medical Toxicology
Wilford Hall Medical Center/Brooke Army Medical
    Center
San Antonio, TX

João Delgado, MD, FACEP, FACMT
Assistant Professor of Emergency Medicine
Hartford Hospital, Hartford, CT
University of Connecticut School of Medicine
Farmington, CT

Andrew Erdman, MD
Associate Head, Earl Development Safety
Genentech
South San Francisco, CA

Bruce A. Goldberger, PhD, DABFT
Professor and Director of Toxicology
Departments of Pathology & Psychiatry
University of Florida College of Medicine
Gainesville, FL

Kennon Heard, MD
Medical Toxicology Fellowship Director
Rocky Mountain Poison and Drug Center, Denver
    Health
Denver, CO
Associate Professor of Emergency Medicine
University of Colorado School of Medicine
Aurora, CO

Daniel S. Isenschmid, PhD, DABFT
NMS Labs
Willow Grove, PA

Professor A. Wayne Jones, PhD, DSc
Department of Forensic Toxicology
National Board of Forensic Medicine
Linköping, Sweden

Mark A Kostic, MD, FAAEM, FACMT
Associate Professor of Pediatrics (Emergency Medicine)
    and Emergency Medicine
Medical College of Wisconsin
Associate Medical Director, Wisconsin Poison Center
Milwaukee, WI

Mori J. Krantz, MD, FACC, FACP
Associate Professor, Medicine & Cardiology
Denver Health and the University of Colorado
Denver, CO

Ken Kulig MD, FACMT, FAACT
Toxicology Associates, Prof LLC
Denver, CO

REVIEW PANEL

ACKNOWLEDGMENTS

FIGURE 1.6. Methamphetamine pipe. (Photo courtesy of the US Drug Enforcement Agency).

FIGURE 9.2. Confiscated ecstasy tablets. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 22.2. Confiscated blotters containing lysergic acid diethylamide (LSD). (Photo courtesy of the US Drug Enforcement Agency).

Figure 31.2. Confiscated Southwest Asian brown and white heroin. The latter is also produced in Southeast Asia. (Photo courtesy of the US Drug Enforcement Agency)

Figure 31.3. Confiscated Mexican black tar heroin. (Photo courtesy of the US Drug Enforcement Agency)

Figure 31.4. Opium poppy field. (Photo courtesy of the US Drug Enforcement Agency)

Figure 31.5. Opium harvest: Collection of raw opium gum from the poppy. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 34.2. Liquid phencyclidine (PCP) and cigarette (Sherman) used to dip into the liquid PCP. (Courtesy of *Drug Bible*)
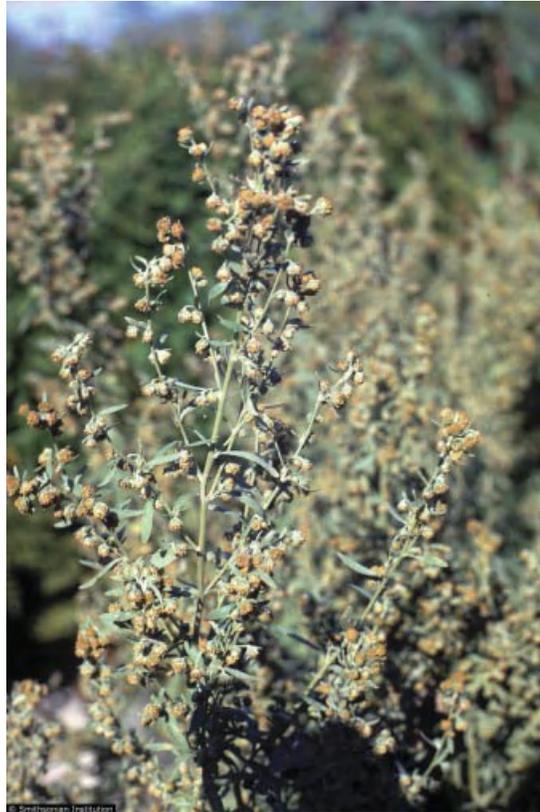
FIGURE 52.1. Bitter wormwood (*Artemisia absinthium*). (Richard A. Howard Image Collection. Courtesy of Smithsonian Institution.)

FIGURE 56.2. Confiscated cocaine hydrochloride powder. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 56.3. Crack cocaine. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 56.4. Confiscated cocaine bricks. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 58.2. Bundle of khat leaves, twigs, and stems wrapped in banana leaves. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 58.3. Dried khat leaves. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 59.1. Young kratom tree with leaves containing red stems and veins. The stems of the leaves vary from light green to red. (Photo Courtesy of Darika Sai-ngam, MA, and Sawitri Assanangkornchai, MD, PhD, Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand)

FIGURE 59.3. A man climbing his mature kratom tree to harvest the leaves for his personal use. (Photo courtesy of Darika Sai-ngam, MA, and Sawitri Assanangkornchai, MD, PhD, Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand)

FIGURE 60.1. Cultivated marijuana plant. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 60.4. Confiscated bong for smoking cannabis. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 60.5. Assorted pipes used to smoke cannabis. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 60.9. Packages of spice. (Photo courtesy of the *Drug Identification Bible*)

FIGURE 62.1. Seeds (ololiuqui) of a beach moonflower (*Ipomoea violacea* L). (Photo courtesy of Steve Hurst and the USDA-NRCS PLANTS Database).

FIGURE 62.2. Leaves and flowers of the Christmas vine (*T. corymbosa*). (Richard A. Howard Image Collection. Photo courtesy of Smithsonian Institution).

FIGURE 63.1. Flowering peyote cactus (*Lophophora williamsii*). (Richard A. Howard Image Collection. Photo courtesy of Smithsonian Institution.)

FIGURE 64.1. Wavy caps of the genus *Psilocybe*. (Photo courtesy of *Drug Identification Bible*)

FIGURE 65.2. Dried salvia leaves. (Photo courtesy of *Drug Identification Bible*)

FIGURE 65.3. Liquid extract of *Salvia divinorum*. (Photo courtesy of *Drug Identification Bible*)

FIGURE 66.1. Leaves and flowers of *Nicotiana tabacum* L. (common tobacco). (Photo courtesy of J. S. Peterson and USDA-NRCS PLANTS Database.)

FIGURE 66.7. *Duboisia hopwoodii* (F. Muell.) F. Muell. (Photography by G. Byrne, C.P. Campbell, and G Cocktern. Image used with the permission of the Western Australian Herbarium, Department of Environment and Conservation; http://florabase.dec.wa.gov.au/)

PART 1

SYNTHETIC and SEMISYNTHETIC CHEMICALS

Chapter 1

AMPHETAMINE and METHAMPHETAMINE

AMPHETAMINE

HISTORY

Amphetamine is a prototypical, noncatecholamine, sympathomimetic drug; the chemical structures of amphetamine, catecholamine-type neurohumoral transmitters (i.e., epinephrine, norepinephrine, dopamine), and the naturally occurring ephedrine are similar. Although some Chinese herbal folk remedies contained sympathomimetic drugs 5,000 years ago, Nagai did not isolate ephedrine from ma huang (*Ephedra vulgaris*) until 1887. Lazar Edeleano synthesized amphetamine in the same year.[1] Chen and Schmidt introduced ephedrine into Western medicine in the 1920s following their experience with the traditional Chinese herb, ma huang.[2]

Early US medical research on the pharmacologic effects of amphetamine began in the late 1920s during attempts to find an synthetic alternative for the use of ephedrine to treat asthma.[3,4] In the late 1920s, Alles and Prinzmetal introduced the use of racemic β-phenylisopropylamine (*d,l*-amphetamine sulfate) as a decongestant and bronchodilator.[5] Beginning in 1932, the Smith Kline & French Company marketed Benzedrine® (racemic β-phenylisopropylamine) as an inhaler for the treatment of nasal congestion and as an analeptic for the treatment of fatigue. Over the next decade, the medical applications for amphetamine were extended beyond its use as a decongestant and general stimulant to include appetite suppression, and as a treatment for narcolepsy and hyperactivity syndrome in chil-

dren.[6,7] However, in 1937, recognition of the abuse potential of amphetamine and its related compounds resulted in the restriction of the sale of amphetamine as a prescription drug in the United States.[8] Nevertheless, both the Axis and the Allies extensively used amphetamines to counter battle fatigue and to maintain alertness in their troops during World War II; amphetamines were issued in survival kits. After the war, widespread parenteral abuse of amphetamines occurred in Japan. Similar problems with amphetamine abuse occurred in Sweden during the 1950s and early 1960s.

The first major epidemic of amphetamine abuse in the United States occurred from the 1940s to the 1960s.[9] Case reports and articles from the American lay press documented the intravenous (IV) and oral abuse of amphetamine extracts from Benzedrine inhalers during the 1940s and 1950s.[10] Methods of abuse included the ingestion of folded paper strips containing amphetamine from the inhalers and the ingestion of amphetamine-moistened strips that were wrapped in cigarette paper and then dipped in coffee. Abuse of amphetamine from these papers occurred despite the addition of emetine and picric acid by the manufacturers. As a method to reduce the abuse Benzedrine® inhalers, manufacturers replaced the synthetic racemic amphetamine base (β-phenylisopropylamine) with the congener propylhexedrine. Marketing of this new product (Benzedrex®, B.F. Ascher & Co., Lenexa, KS) began in 1949. In 1959, the US Food and Drug Administration (FDA) restricted the use of these inhalers as a prescription drug because of the IV and oral abuse.

In the United States, IV amphetamine use with inhalant extracts was widespread during the 1950s and 1970s.
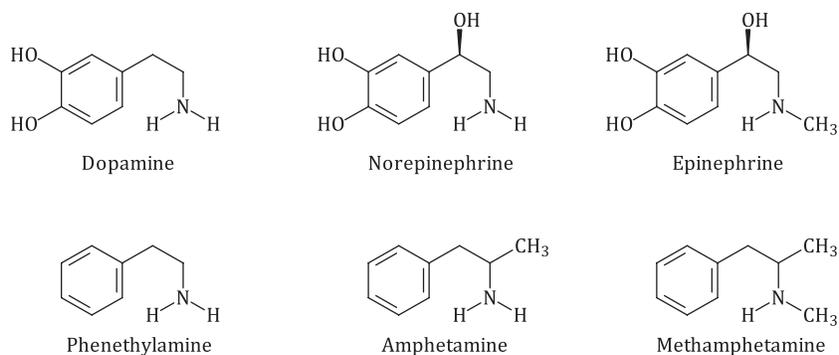
FIGURE 1.1. Chemical structures of dopamine, epinephrine, norepinephrine, phenethylamine, amphetamine, and methamphetamine.

Rampant IV drug use with methylphenidate and illicit amphetamines also occurred in the San Francisco drug culture during the 1960s. Possibly promoted by the use of amphetamine compounds commonly prescribed for the treatment of obesity and depression, the illicit use of amphetamine during this time primarily involved the diversion of drugs from pharmaceutical stocks. Initially, amphetamine and the *d*-isomer of amphetamine (dextroamphetamine) were listed as schedule III drugs; however, in 1971, these compounds were added to the list of schedule II drugs (i.e., drugs that have medical use, but significant abuse potential) in an attempt to limit the diversion of these drugs to illicit markets. Widespread IV amphetamine abuse among heroin addicts occurred in Washington, DC, as a result of the disruption of heroin supplies in the early 1970s; amphetamine control measures abruptly ended the substitution of amphetamine for heroin.[11] Until the mid-1970s, medical indications for amphetamine compounds included several common conditions (depression, fatigue, weight reduction). Subsequently, the FDA restricted the legal use of amphetamines to narcolepsy, hyperkinetic behavior in children, and short-term weight reduction. The use of amphetamine compounds for weight reduction is highly controversial; the Canadian government banned the use of amphetamine compounds for weight reduction in 1971. Case reports of amphetamine toxicity were relatively uncommon during the 1980s with use occurring primarily in deserts in the Southwestern United States.[12]

IDENTIFYING CHARACTERISTICS

Structure

Amphetamine (CAS No.300-62-9) is racemic β-phenylisopropylamine consisting of a phenyl ring substituted with an isopropylamino side chain. Amphetamine and the parent compound of sympathomimetic amines (β-phenethylamine) are structurally similar. Addition of hydroxyl substitutions on 3′-(*meta*-) and 4′-(*para*-) positions of the phenyl ring of phenethylamine produces the basic building block of the catecholamine neurotransmitters (epinephrine, norepinephrine, dopamine). Amphetamine compounds are not catecholamines because of the absence of aromatic hydroxyl moieties. Figure 1.1 compares the chemical structure of amphetamine, methamphetamine, and catecholamine neurotransmitters.

The phenylisopropylamines have a chiral center at the α-carbon, which allows enantiomers of differing biologic potencies. The dextrorotatory (*d*-) isomer of amphetamine is commercially available as dextroamphetamine (CAS:51-64-9, Dexedrine®). Alteration of the phenyl ring (e.g., chlorphentermine, fenfluramine) and the ethylamine side chain (e.g., propylhexedrine, diethylpropion, phendimetrazine, phenmetrazine) produces amphetamine derivatives with fewer side effects compared with amphetamine and methamphetamine as demonstrated in Figure 1.2.

Detailed pharmacologic investigations of phenethylamine derivatives demonstrate some basic rules for the structure–activity relationships in this class of compounds. The following 4-position on the phenethylamine nucleus can be substituted resulting in alterations of pharmacologic effect: 1) the amine nitrogen; 2) the carbon atom on the ethyl bridge, which is α to the nitrogen; 3) the carbon atom on the ethyl bridge, which is β to the nitrogen; and 4) the phenyl ring. Addition of a single aliphatic substituent to the nitrogen results in a somewhat prolonged duration of action and increased penetration of the central nervous system (CNS) relative to the nonsubstituted analogue, whereas disubstitution of the nitrogen abolishes nearly all stimulant
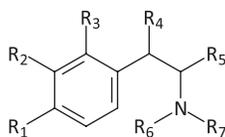
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Amphetamine	H	H	H	H	CH_3	H	H
Benzphetamine	H	H	H	H	CH_3	CH_3	CH_2CH_3
Cathinone	H	H	H	=O	CH_3	H	H
Desmethylselegiline	H	H	H	H	CH_3	H	CH_2CCH
Diethylpropion	H	H	H	=O	CH_3	CH_2CH_3	CH_2CH_3
Ephedrine	H	H	H	OH	CH_3	H	CH_3
Fenfluramine	H	CF_3	H	H	CH_3	H	CH_2CH_3
MDA	$O\text{-}CH_2\text{-}O$		H	H	CH_3	H	H
MDEA (MDE)	$O\text{-}CH_2\text{-}O$		H	H	CH_3	H	CH_2CH_3
MDMA	$O\text{-}CH_2\text{-}O$		H	H	CH_3	H	CH_3
Mescaline	OCH_3	OCH_3	OCH_3	H	H	H	H
Methamphetamine	H	H	H	H	CH_3	H	CH_3
Methcathinone	H	H	H	=O	CH_3	H	CH_3
Phenelzine	H	H	H	H	H	H	NH_2
Phentermine	H	H	H	H	$\{CH_3\}_2$	H	H
Phenylephrine	H	OH	H	OH	CH_3	H	H
Phenylpropanolamine	H	H	H	OH	CH_3	H	H
Pseudoephedrine	H	H	H	OH	CH_3	H	CH_3
Selegiline	H	H	H	H	CH_3	CH_3	CH_2CCH

Pemoline   Methylaminorex   Tranylcypromine   Phendimetrazine

Mazindol   Methylphenidate
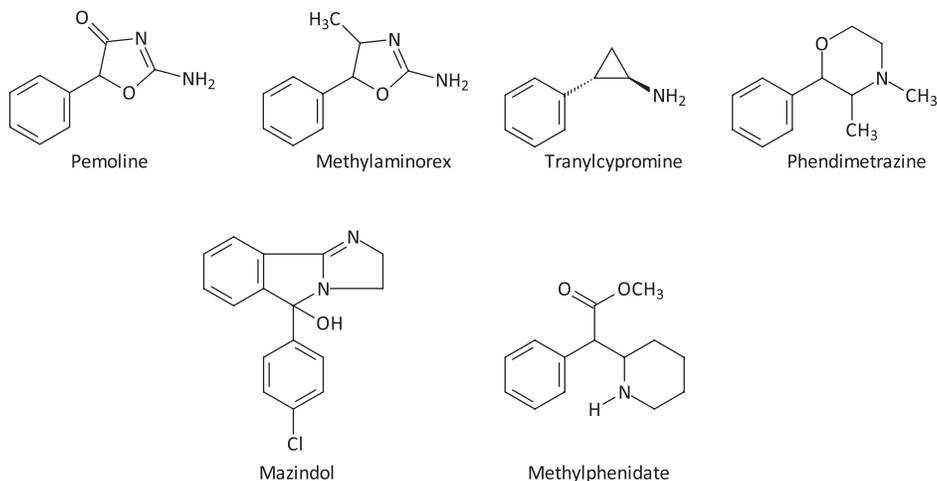
FIGURE 1.2. Amphetamine and related amphetamine structures.[99]

activity. Increasing anorectic effects result from the addition of a small aliphatic group to the α-carbon. Substitution of the β-carbon with a hydrogen bonding entity (e.g., hydroxyl moiety as in ephedrine or pseudo-ephedrine) produces strong stereochemical preferences with the (*R*) absolute configuration at this stereo-center having substantially greater adrenergic activity than the (*S*) configuration. Addition of alkoxy substitu-ents to the phenyl ring (e.g., methylenedioxymetham-phetamine [MDMA], mescaline) increases serotonergic activity and imparts hallucinogenic properties to the compound.

Physiochemical Properties

Amphetamine compounds are lipophilic, weak bases with pK_a values ranging from 8.8–10.4. The pK_a of amphetamine is 10.13. The drug base often is combined with HCl to form the hydrochloride salt, which has a melting point of 170–175°C.

5

Terminology

Although amphetamine refers specifically to racemic β-phenylisopropylamine, the term *amphetamine* frequently refers to several structurally related compounds (e.g., methamphetamine, fenfluramine, phentermine, synthetic amphetamine analogues) that share similar pharmacologic and toxicologic properties with amphetamine.[13] Amphetamine is a contraction of the older description of the prototypical compound, α-methylphenethylamine. Old trade names for amphetamine compounds include the following: Benzedrine (*d,l*-amphetamine), Biphetamine (*d,l*-amphetamine), Dexedrine (*d*-amphetamine), and Dexampex (*d*-amphetamine). Street names for amphetamine include Amp, Bennies, Black Beauties, Browns, Cranks, Fives, Goey, Hearts, Louee, Speed, Uppers, and Whiz.[14]

EXPOSURE

Epidemiology

The frequent inclusion of methamphetamine and other structurally similar amphetamine compounds (phenmetrazine, methylphenidate, diethylpropion, propylhexedrine) with racemic and *d*-amphetamine complicates the interpretation of epidemiologic data on the latter. Most studies on the misuse of prescription stimulants do not separate amphetamine from methylphenidate. Smaller studies with face-to-face interviews reported higher misuse rates, whereas larger, multisite studies reported lower rates. In a study of a convenience sample of 1,811 undergraduates at a large-public US research university, the reported lifetime rate of the illegal use of prescription stimulants (*d*-amphetamine, methylphenidate) was 34%.[15] A multisite study of 10,904 US college students reported a lifetime misuse and past year misuse of prescription stimulants (*d*-amphetamine, methylphenidate) of 6.9% and 4.1%, respectively.[16]

Sources

Approved indications for *d*-amphetamine in the United States are narcolepsy and attention deficient hyperactivity disorder (ADHD); off-label uses include the treatment of fatigue in cancer patients and the treatment of dysphoria/depression in combination with antidepressants.[17] Indications for this drug do not include the treatment of obesity, drug dependence, anxiety, or malaise.

Illicit manufacture of amphetamine remains uncommon, partly because the illicit synthesis of amphetamine is more complicated than the illicit synthesis of methamphetamine. Synthesis of amphetamine from benzylmethylketone (phenylacetone, CAS RN:103-79-7) is a reported method of illicit amphetamine production as displayed in Figure 1.3.[18] The addition of formamide or ammonium formate to benzylmethylketone produces the intermediate, *N*-formyl amphetamine. Refluxing *N*-formyl amphetamine with hydrochloric acid produces crude amphetamine that can be refined by extraction, steam distillation, or vacuum distillation. An alternate method for the clandestine synthesis of amphetamine is a 1-step reduction of phenylpropanolamine that directly yields amphetamine base. The production of amphetamine in clandestine laboratories increases the Leuckart-specific impurities (*N*-formyl amphetamine, 4-methyl-5-phenyl-pyrimidine) and yield of amphetamine compared with legally manufactured amphetamine.[19,20]

The source of some illicit racemic or *d*-amphetamine is the diversion of this drug from persons with legitimate prescriptions. Most studies on the use and misuse of prescription stimulants do not separate amphetamine from methylphenidate. In a retrospective review of published studies, the misuse and diversion of prescription stimulants for ADHD ranged from 5–35% among older adolescents and college-age populations.[21] Lifetime diversion rates of stimulant prescriptions from students with legitimate prescriptions ranged from 16–29%, when they were asked to trade, sell, or give the medication to another person.

Methods of Abuse

The effects of amphetamine appeal to individuals who interact poorly in social settings and have difficulty internalizing new experiences. Amphetamine use reduces the need for external stimuli by increasing internal arousal mechanisms. In contrast to antisocial,
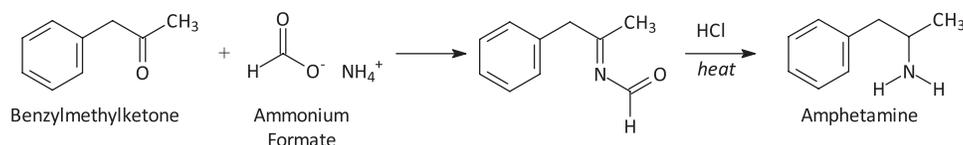
FIGURE 1.3. Synthetic preparation of amphetamine from benzylmethylketone.

schizoid personalities who tend to *abuse* amphetamines (i.e., the drug interferes with their social, economic, or medical welfare), *misuse* of amphetamines (i.e., using these drugs for illicit purposes) occurs frequently in individuals trying to enhance performance or endurance. Long-term amphetamine use causes psychologic dependence and tolerance, although physical withdrawal symptoms are typically milder following chronic amphetamine use than chronic opiate or barbiturate use.

INTERMITTENT USE

The strong CNS effects of amphetamine persist longer than most other stimulants (e.g., cocaine). Because the use of amphetamine increases physical and mental alertness, these compounds are popular among college students studying for exams, athletes, and truck drivers who require prolonged vigilance or short periods of high energy.[22] Some individuals occasionally ingest 5–20 mg of amphetamine compounds to allay fatigue, elevate mood, or prolong wakefulness. Some professional football players consume amphetamine or other stimulants to induce rage, increase endurance, improve speed, and reduce weight.[23] Amphetamine may improve the performance of tired individuals on repetitive tasks, unless jitteriness or impaired judgment adversely affects performance. The degree of improved athletic performance is relatively small, but this effect may be significant in elite sports.[24] Most sporadic users do not develop a habitual craving for amphetamine. In addition, amphetamine may increase energy expenditures, resulting in excessive fatigue. Drug-induced impairment of judgment may reduce the recognition of the hazardous consequences of fatigue and the subsequent reduction in physical performance. Amphetamine is listed as a prohibited substance by the World Anti-Doping Agency (WADA).[25]

CHRONIC ORAL ABUSE

Following the chronic daily consumption of 20–40 mg amphetamine, a reduction in amphetamine dose may cause lethargy and depression. Although some initial improvement in alertness may occur, chronic amphetamine use eventually reduces mental and physical performance without awareness by the user. Daily amphetamine doses may increase to 50–150 mg as tolerance reduces the euphoric effects of amphetamine.[26] Polydrug abuse is a common comorbidity in amphetamine abusers, in part, because of the adverse effects of chronic amphetamine abuse including insomnia and agitation.

INTRAVENOUS ABUSE

Intravenous amphetamine users usually begin with abuse of oral amphetamines; then, they progress to IV injections to experience a more intense feeling of pleasure. Other pleasurable feelings that follow the IV administration of amphetamine include a sense of extreme mental and physical power, hyperactivity, hyperexcitability, euphoria, and heightened sexual awareness. As tolerance develops, the dose and frequency of the injections increase substantially. During "runs," injection of amphetamine occurs every 2 hours throughout the day for 3–6 days until exhaustion causes the user to fall asleep (i.e., "fall out"). Sleep lasts 12–18 hours or longer with more prolonged runs. With the escalation of the amphetamine dose, frightening perceptive experiences occur including hyperacousia, hallucination, illusions, and paranoia.[27] Complications of this form of abuse include violent and bizarre behavior, slovenly dress, emaciated appearance, and major medical complications.

DOSE EFFECT

Oral doses in habitual amphetamine users often range from 50–150 mg daily. Anecdotal reports suggest that the IV use of amphetamine begins with the injection of 20- to 40-mg doses, but the dose increases substantially as tolerance develops. Experienced IV amphetamine abusers typically inject from 100–300 mg amphetamine per use; however, as tolerance increases the maximum dose during binges may exceed 1 g without the development of severe complications.[27] The presence of multiple confounding factors complicates the determination of dose-effect relationships following the use of amphetamine including underlying cardiovascular disease (e.g., coronary artery disease, angiitis), vascular abnormalities (berry aneurysms), use of other illicit drugs, smoking, reporting bias, duration of abuse, and tolerance. In a case series of 11 patients with neurologic abnormalities associated with amphetamine use, the amphetamine dose ranged from 20–200 mg.[28] However, the chronicity of amphetamine use was not well documented in this case series. In a summary of 9 case reports of myocardial infarction associated with amphetamine use, the route of abuse included chronic oral and nasal amphetamine abuse as well as IV drug use.[29] The limited data and the presence of multiple confounding factors listed above prevented the determination of dose-response relationships. The ingestion of 250 mg amphetamine following by strenuous exercise (i.e., running 1.5 miles) was associated with the development of myoglobinuria and acute renal failure.[30]

TOXICOKINETICS

Absorption

Volunteer studies indicate that peak plasma amphetamine concentrations occur within 1–2 hours following the ingestion of a pharmacologic dose of amphetamine (i.e., 10–25 mg).[31] Complete gastrointestinal (GI) absorption of therapeutic doses of standard-release amphetamine usually occurs by 4–6 hours. Absorption of amphetamine through mucosal surfaces is pH dependent. The illicit use of amphetamines before intercourse as an aphrodisiac by insertion into the vagina (i.e., "balling") suggests that absorption of amphetamine across mucosal membranes also occurs. In a volunteer study, absorption of about 50% and 80% of an amphetamine dose applied to the buccal mucosa occurred within 5 minutes at pH of 8.16 and 9.18, respectively.[32]

Sustained-release preparations are available as resin-bound rather than soluble salts. These compounds produce reduced peak blood concentrations compared with standard amphetamine preparations, but total bioavailability and time to peak concentrations are similar to standard-release preparations.[33] Although experimental studies indicate that amphetamine delays gastric emptying and decreases intestinal motility,[34] there are inadequate data to determine whether this property is clinically significant during amphetamine intoxication.

Distribution

Amphetamine distributes primarily into the kidney, lungs, and brain. The extent of plasma protein binding to amphetamine is relatively low (i.e., about 16–20%) in humans as measured by *in vitro* equilibrium dialysis.[35] Animal studies indicate that there is substantial interspecies variation in the binding of amphetamine. The protein binding of amphetamine in the plasma of mice is about 17% compared with approximately 40% in the rat.[36] The volume of distribution of amphetamine in therapeutic doses administered to humans ranges from about 3–5 L/kg. Following chronic amphetamine abuse, the volume of distribution increases slightly (up to 6 L/kg).[37] Plasma protein binding, rate of absorption, and volumes of distribution of amphetamine enantiomers are similar.[38]

Biotransformation

The biotransformation of amphetamine and methamphetamine is analogous. Figure 1.4 demonstrates the biotransformation pathways of amphetamine and methamphetamine. Metabolites of amphetamine include active compounds (e.g., *p*-hydroxyamphetamine, *o*-hydroxynorephedrine, norephedrine). The major metabolic pathway for amphetamine involves deamination (i.e., hydroxylation at the α-carbon) to phenylacetone; then, oxidation of phenylacetone to benzoic acid followed by the conjugation of benzoic acid with glucuronic acid or glycine.[39] The deamination of amphetamine to phenylacetone probably involves the CYP2C subfamily of cytochrome P450 isoenzymes.[40] Smaller amounts of amphetamine are converted to norephedrine by oxidation. β-Hydroxylation produces the active metabolite *o*-hydroxynorephedrine, which acts as a false neurotransmitter and may account for some drug effect in chronic users. The metabolism of amphetamine varies substantially between various animal species.

Elimination

Normally, the kidneys excrete about 30% of a therapeutic dose of amphetamine over 24 hours, but the actual amount of urinary excretion is highly pH dependent. In an experimental study involving 4 participants, the urinary excretion of unchanged amphetamine was about four times greater than the excretion of deaminated metabolites (hippuric and benzoic acids), when the urinary pH was acidic (pH 5.5–6.0).[41] However, the urinary excretion of deaminated metabolites and unchanged amphetamine was similar during alkaline conditions (urinary pH 7.5–8.0). In a study of 7 volunteers ingesting 10–15 mg amphetamine sulfate, unchanged amphetamine in the urine during the first 16 hours after ingestion accounted for 2.2–4.2% of the administered dose when the urine pH ranged from 7.8–8.1. However, reducing the urine pH to 4.8–5.1 resulted in urinary excretion of 48–73% of the administered amphetamine dose as unchanged amphetamine during the first 16 hours after ingestion.[42]

Consequently, the plasma elimination half-life of amphetamine is also urine pH dependent. The plasma half-life of amphetamine following a therapeutic dose is approximately 12 hours under normal urinary pH; however, experimental studies demonstrate that the plasma-elimination half-life ranges from 8–10.5 hours following urinary acidification compared with 16–31 hours following urinary alkalinization.[41] Furthermore, the *d*(+)-amphetamine enantiomer is more rapidly metabolized than the *l*(−)-enantiomer; under alkaline conditions, the mean plasma elimination half-life of the *d*(+)-amphetamine enantiomer was 12.7 hours compared with 17.0 for the *l*(−)-enantiomer.[38] Under acidic urine conditions, renal excretion of unchanged amphetamine is the major route of elimination, and
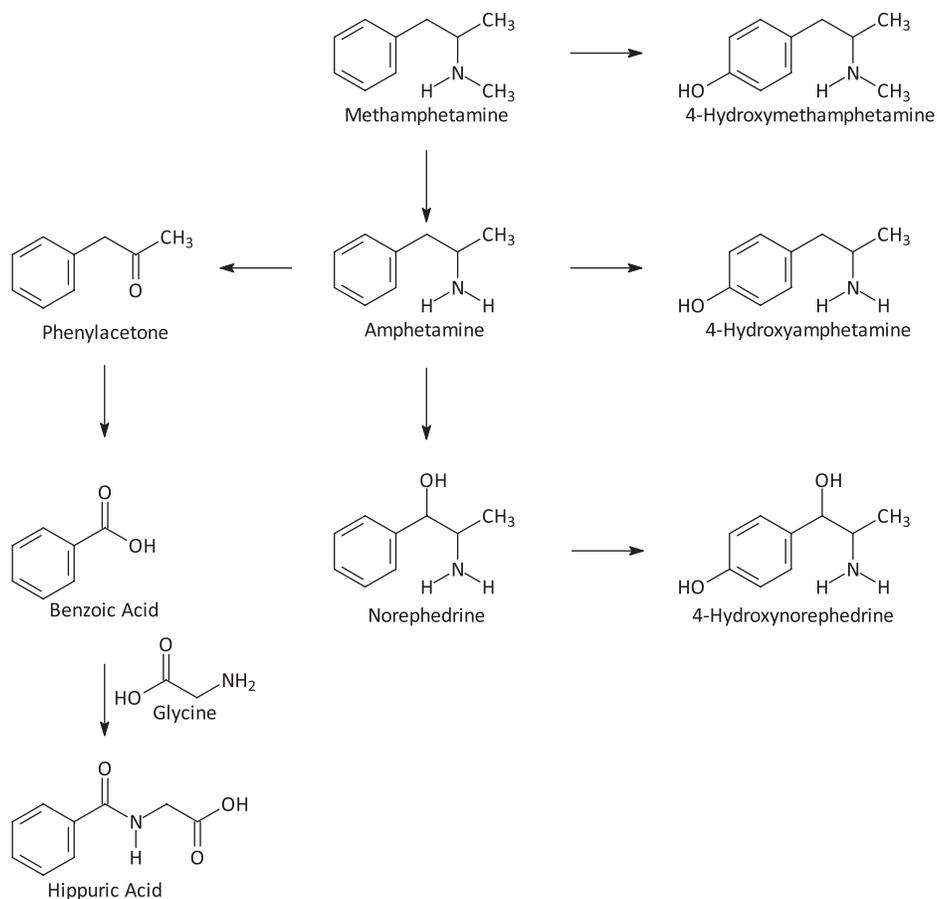
FIGURE 1.4. Pathways of amphetamine and methamphetamine biotransformation.

the plasma elimination half-life of the enantiomers is similar.

Tolerance

Acute tolerance develops to some of the subjective effects of amphetamine. In a study of healthy volunteers receiving a single 20-mg dose of *d*-amphetamine orally, the initial effects of amphetamine on mood dissipate before peak plasma amphetamine concentrations.[43] The maximum subjective ratings of "feel high" occurred between 1.5–2 hours after ingestion, whereas peak plasma amphetamine concentrations occurred about 4 hours after ingestion. In a study of 16 healthy volunteers, the administration of a second 20 mg dose of *d*-amphetamine 48 hours after the first 20 mg dose produced a slight reduction in the ratings for self-reported "feel drug," but all other mood, behavioral, and physiologic effects of *d*-amphetamine were unchanged.[44] Rapid development of tolerance to some of the side effects of amphetamine derivatives occurs during both chronic oral and IV use.

Maternal and Fetal Kinetics

Amphetamine crosses the placenta.[45] Volunteer studies indicate that *d*-amphetamine readily transfers into breast milk of lactating mothers. In a study of a lactating mother receiving 20 mg racemic amphetamine daily (5 mg every 2 h beginning at 10 AM), the milk/plasma ratio ranged from 2.8–7.5 during the days 10–42 after birth.[46] The corresponding amphetamine concentrations in milk ranged from 55–138 µg/L. In a case series of 4 mothers receiving a median daily *d*-amphetamine dose of 18 mg (range, 15–45 mg), the median milk/plasma ratio was 3.3 (range, 2.2–4.8) with the absolute infant dose of 21 µg/kg daily (range, 11–39 µg/kg/d).[47] Two of the 3 infants tested had detectable concentrations (2 µg/L, 18 µg/L) of *d*-amphetamine in their urine.

Drug Interactions

In general, the coadministration of amphetamine and monamine oxidase inhibitors is contraindicated because of the potential development of severe hypertension, hyperthermia, and altered consciousness. A case report associated the development of hyperthermia (43°C/109.4°F), agitation, seizures, opisthotonus, and coma in a 41-year-old woman following the administration of *d*-amphetamine, amobarbital, and tranylcypromine.[48] She recovered with intensive supportive measures. *d*-Amphetamine is a substrate for CYP2D6 isoenzymes; therefore, potential drug interactions may occur following the concomitant ingestion of fluoxetine, paroxetine, and, to a lesser extent, sertraline. However, there are inadequate *in vivo* human data to indicate that these potential interactions are clinically significant.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Amphetamine exhibits a wide range of profound physiologic and behavioral effects in animals as well as humans including alteration of sleep, motor activity, appetite, attention, aggression, sexual behavior, learning, classical conditioning, and operant behavior. At high doses, increased hyperadrenergic effects result from excessive norepinephrine-mediated activity. Amphetamine is structurally similar to the neurotransmitters, dopamine and norepinephrine. The physiologic effects of amphetamine result from the enzymatic inactivation, release, and uptake of catecholamine neurotransmitters (dopamine, norepinephrine), and to a lesser extent, the indoleamine, 5-hydroxytryptamine (serotonin) neurotransmitter.[49] Consequently, amphetamine is a potent indirect agonist at the various monoaminergic receptors.

The mechanism of action of amphetamine is similar to other amphetamine compounds and pharmaceutic derivatives; however, the peripheral and central α- and β-adrenergic effects of these compounds vary. Amphetamine crosses the neuronal membrane predominantly via the neuronal monamine reuptake transporters. Direct drug-monoamine competition for the reuptake transporters by amphetamine inhibits the cellular uptake of catecholamines. By inhibiting intracellular vesicular monamine transporter 2, amphetamine reduces vesicular monoamine storage while increasing extracellular monoamines (dopamine, norepinephrine, serotonin).[50] This inhibition of carrier-mediated active uptake process involving catecholamine uptake transporters mediates the cardiovascular and anorexic effects of amphetamine.

CARDIOVASCULAR SYSTEM

Therapeutic doses of amphetamine raise systolic and diastolic blood pressure and initially slow heart rate. High doses of amphetamine cause tachycardia, palpitations, and dysrhythmias. *l*-Amphetamine is a slightly stronger cardiovascular stimulant than *d*-amphetamine.

CENTRAL NERVOUS SYSTEM

BEHAVIOR.   In animal and human studies, low doses of amphetamine are general stimulants that induce alertness, euphoria, increased confidence, gregariousness, enhanced mental and physical activity, and improved self-esteem.[51] Amphetamine increases wakefulness and delays the onset and duration of rapid eye movement (REM) sleep. Withdrawal following chronic use produces the opposite pattern, at least during the first several months after cessation of use. The analgesic effects of amphetamine are minimal with no clinical significance.

Amphetamine increases the concentration of norepinephrine in the periphery and dopamine in the CNS via effects on plasmalemmal and vesicular monoamine transporters (e.g., neuronal dopamine transporter, vesicular monoamine transporter-2).[50] This compound facilitates the release of these neurotransmitters and blocks their reuptake in the presynaptic nerve terminal. The primary site of action in the CNS is the dopamine transporter. The dopamine transporter translocates *d*-amphetamine into the interior of the presynaptic terminal; subsequently, the efflux of dopamine to the synaptic cleft occurs as a result of the release of dopamine from the vesicles in the cytosol. Additionally, *d*-amphetamine inhibits the clearance of dopamine from the synapse by competitively inhibiting the reuptake of dopamine by the dopamine transporter. Cocaine is a much stronger binder of this protein in the synapse than amphetamine.[52]

Amphetamine along with other drugs of abuse (e.g., alcohol, cocaine, heroin, methamphetamine) enhance the release of the neurotransmitter dopamine in the striatum (caudate, putamen, nucleus accumbens) as a result of their effects on the dopamine transporter.[53] Animal studies suggest that brain norepinephrine is responsible for amphetamine-induced locomotor stimulation, whereas dopamine mediates stereotyped behavior similar to amphetamine psychosis in humans as well as craving and addictive behavior.[54]

Children with ADHD (old term: syndrome of minimal brain dysfunction) are characterized by hyperactivity and aggressive behavior. About 75% of these children display improvement in hyperactivity, impulsivity, distractibility, and short-attention span following the administration of amphetamine compounds or analogues. These hyperactive children excrete lower levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of CNS norepinephrine. Studies of children with and without attention deficit hyperactivity disorder (ADHD) suggest subtle differences in psychoeducational test performance in relation to urinary homovanillic acid and MHPG concentrations.[55] The administration of *d*-amphetamine increases the urinary excretion of MHPG in clinical drug responders, but not in nonresponders.[56]

ANORECTIC PROPERTIES. Although amphetamine compounds do not alter metabolic rates at normal therapeutic doses, the use of these drugs ameliorates some of the effects of hunger and fatigue depending on personality type.[57] Weight loss from therapeutic doses of most anorexiant drugs results almost entirely from reduced food consumption, but increased physical activity contributes somewhat to initial weight reduction. At usual doses, tolerance abolishes anorectic properties after several months. Patients often regain lost weight after tolerance develops or drug administration ceases. Racemic amphetamine (*d*-, *l*-amphetamine) was the first drug used for appetite suppression. However, the dextrorotatory stereoisomer (dexamphetamine) is the active anorectic constituent of the racemic mixture of *d*-, *l*-amphetamine. Consequently, the use of dexamphetamine replaced the administration of racemic amphetamine for the treatment of obesity.

Mechanism of Toxicity

An amphetamine is an indirectly acting sympathomimetic drug that increases the effects of epinephrine in the peripheral sympathetic nervous system and the effects of dopamine, norepinephrine, and serotonin in the CNS. The acute toxicity of amphetamine primarily involves an exacerbation of pharmacologic effects of this drug (i.e., hyperadrenergic state). The psychoactive effects of amphetamine also result from indirect agonist action. Amphetamine displaces catecholamines from binding sites in the storage vesicles after diffusion of these compounds into the cytoplasm. Anxiety, dysphoria, confusion, depression, nausea, vomiting, headache, sweating, apprehension, tremulousness, confusion, and fatigue limit the use of increasing doses of amphetamine despite the development of tolerance.

CARDIOVASCULAR SYSTEM

Excessive sympathetic nervous system activity and vasoconstriction produced by amphetamine causes tachycardia, hypertension, and in susceptible patients, myocardial ischemia and cardiac arrhythmias. Intravenous amphetamine abusers develop the same serious complications (e.g., opportunistic infections) as other IV drug abusers with acquired immunodeficiency syndrome (AIDS).[58]

CENTRAL NERVOUS SYSTEM

Excessive doses of amphetamine cause stereotyped movements, hyperreactivity, dystonic and dyskinetic postures, and convulsions. The administration of high doses of amphetamine to animals causes long-term depression of both dopamine and serotonin synthesis in various regions of the brain. The long-term functional and pathologic consequences of chronic depletion of dopamine and serotonin for humans are unclear.[59] *d*-Amphetamine is a positive reinforcer under controlled laboratory condition in both animals and humans.[60,61]

The etiology of the intracerebral and subarachnoid hemorrhages associated with amphetamine use is probably multifactorial. Inflammation and necrosis of small cerebral arteries (i.e., vasculitis) secondary to particulate foreign bodies or bacterial endocarditis can develop after IV drug use.[62] Subsequently, the hypertension resulting from amphetamine abuse may cause vessel rupture and hemorrhage in the weakened areas of the vessels. However, vasculitis has occurred during oral acute dextroamphetamine intoxication,[63] amphetamine withdrawal, and therapeutic use of amphetamine as an anorectic drug.[28] The presence of vasculitis after exposure by different routes suggests an immunopathologic mechanism.

Postmortem Examination

In animal studies, the IV administration of lethal doses of amphetamine produces pathologic changes resembling experimental hyperthermia.[64] Postmortem examination of dogs administered a lethal IV dose of amphetamine sulfate without anesthesia demonstrate nonspecific findings of renal, vascular, pulmonary, and hepatic congestion as well as hemorrhage in the lungs and GI tract.[65] Cardiac changes include subendocardial hemorrhage, necrosis of myocardial fibers, and hemorrhage in the cardiac valve leaflets. There are no specific postmortem findings that separate amphetamine toxicity from other causes of death. Postmortem examination of the hearts from amphetamine abusers may or may not demonstrate structural abnormalities; cerebral

embolism does not usually occur in amphetamine-related fatalities.

CLINICAL RESPONSE

Illicit Use

Acute intoxication with amphetamine, methamphetamine, or cocaine produces similar clinical effects, manifesting primarily as an accentuation of pharmacologic effects on the cardiovascular and central nervous systems. A major distinguishing feature is the prolonged effect of amphetamine compared with cocaine; many case series report the clinical effects of methamphetamine and amphetamine without distinguishing the 2 drugs. Altered mental status was the most common complaint of 127 patients presenting to the emergency department following exposure to amphetamine or methamphetamine.[66] Thin-layer chromatography indicated that 37% of these patients used either methamphetamine or amphetamine alone and 26% tested positive for both substances; analysis of urine from the remainder of the patients demonstrated other drugs of abuse. Reported CNS symptoms included agitation, anxiety, confusion, delusions, hallucination, and suicidal ideation. Convulsions occurred in 4 of 127 cases.

Following the ingestion of large doses of amphetamine, CNS symptoms begin within 30–60 minutes and persist for 4–6 hours. Large overdoses, sustained-release preparations, and alkalinization of the urine prolong the clinical features of amphetamine toxicity. Table 1.1 outlines the common clinical features of mild to severe amphetamine (or methamphetamine) intoxication. Case reports associate amphetamine abuse with a variety of organ dysfunction including rhabdomyolysis

TABLE 1.1. Categorization of Symptoms of Amphetamine Toxicity by Severity.

Severity	Signs and Symptoms
Mild	Restlessness, talkativeness, irritability, insomnia, tremor, hyperreflexia, mydriasis, flushing, diaphoresis, combativeness, dry mucous membranes, nausea, vomiting, pallor
Moderate	Hyperactivity, confusion, hypertension, tachypnea, tachycardia, premature ventricular contractions, chest discomfort, vomiting, abdominal pain, profuse diaphoresis, mild temperature elevation, impulsivity, repetitive behavior, hallucinations, panic reaction
Severe	Delirium, marked hypertension or tachycardia, hyperpyrexia (over 40°C), convulsions, focal neurologic signs, hypotension, coma, ventricular dysrhythmias

with myoglobinuria, hyperpyrexia (up to 42.8°C/109°F) with hepatorenal dysfunction and rhabdomyolysis,[67] and disseminated intravascular coagulation (DIC) with end organ failure.[68]

BEHAVIORAL ABNORMALITIES

Small doses of amphetamines produce an elevation of mood and a sense of well-being. Larger doses cause apprehension, volatility, excitement, impulsiveness, aggressiveness, and poor judgment.[26] The effects of amphetamine on aggressive behaviors are complex and the changes in behavior depend on several factors including the drug dosage, the environment, and the individual drug user.[69] Behavior abnormalities during amphetamine intoxication include belligerency, moaning, aggression, hyperactivity, anxiety, frank psychosis, and screaming.[66] Although coma may develop suddenly following IV amphetamine use, the loss of consciousness in a patient with a history of amphetamine exposure should initiate a search for other drugs or complications (cerebral hemorrhage, hyperthermia, end organ failure, DIC).

MENTAL DISORDERS

The development of acute psychosis is a common occurrence after chronic, high-dose amphetamine abuse and rarely after a single large dose of amphetamine.[70] Classically, amphetamine-induced psychoses are paranoid psychoses with delusions of persecution and hallucinations under clear consciousness.[71] In a case series of 146 IV amphetamine abusers attending an outpatient psychiatric clinic, about 35% (50 patients) reported 1 or more psychotic episodes characterized by paranoid delusions, stereotyped behavior, visual and/or auditory hallucinations, and delusions of reference.[72] Psychotic reactions may occur in amphetamine abusers with or without a prior history of a psychiatric disorder, but premorbid schizoid/schizotypal personality and early, heavy amphetamine abuse predispose users to psychosis.[73] Case reports associate hallucinations and stereotyped compulsive behavior with the therapeutic use of amphetamine in patients with compensated schizophrenic personalities.[74]

The typical presentation of amphetamine-induced psychosis involves a patient with appropriate affect, paranoid ideations, delusions of persecution, auditory illusions or hallucinations, labile mood, increased sexual drive, delusions of reference, and a sensation of being watched or followed in the presence of a clear consciousness.[75] Thought disorders are uncommon in patients with amphetamine-induced psychoses. Common behavior changes include volatility, suspi-

ciousness, hyperactivity, aggression, hostility, and anxiety. Tactile hallucinations are distinctive features of amphetamine or cocaine abuse. These alterations of thinking cause formication (delusions of parasitosis) and destructive excoriation of the skin. Feelings of suspiciousness and anxiety may become overwhelming in the psychotic patient. Curiosity and a pleasurable suspiciousness become dysphoria and fixed delusions upon which the chronic user may act violently. Stereotyped, compulsive behavior invariably is associated with amphetamine psychosis. This ritualized conduct involves fixed behavioral sequences such as cleaning, sorting, grooming, counting, pacing, and rearranging. Amphetamine-intoxicated patients usually act appropriately given their intense conviction that their paranoid and delusional state is real. Psychotic symptoms usually disappear within several days, but delusions may remain for days to months, particularly if the psychosis developed following chronic use of high doses of IV amphetamine.

MEDICAL COMPLICATIONS

NEUROLOGIC

Acute. Both ischemic and hemorrhagic strokes are well recognized, but relatively rare complications of all routes of amphetamine abuse. Frequently, patients with these complications have a structural abnormality (aneurysm, arteriovenous malformation).[76,77] In a case-control study of 414 individuals with stroke admitted to an urban hospital, 73 patients (34%) were drug abusers (cocaine, amphetamines, heroin).[78] Almost three-quarters of the strokes in this study were hemorrhagic strokes located primarily in the periphery (e.g., subcortical white matter of cerebral hemispheres). The use of amphetamine is also a strong risk factor for hemorrhagic stroke in more social and economically heterogeneous, urban populations.[79] Most of these patients were chronic drugs users; the typical presentation involved the onset of severe headache with or without nausea within a few hours of amphetamine exposure.[80] Few of these patients lost consciousness, but confusion and disorientation occurred frequently. Depending on the location of the intracranial hemorrhage, other unilateral motor or sensory deficits may develop. The admission blood pressure is usually normal, although some patients present with marked hypertension. Seizures often are not part of the initial presentation, but convulsions may complicate convalescence. Intracerebral hematomas may cause brainstem herniation and death. The average mortality of patents with amphetamine-associated intracranial hemorrhage is approximately 30%, and most survivors have mild residual deficits.

Chronic. Habitual use or massive single doses of amphetamine may produce a toxic psychosis characterized by paranoia, delusions, hallucinations, or bizarre violent behavior. The amphetamine user presents as a restless, tremulous individual who is garrulous, suspicious, and anxious. Some individuals become hostile and aggressive. Although memory, orientation, and insight are usually preserved, high or prolonged amphetamine doses may cause the individual to act upon his or her delusions, leading to suicidal or homicidal actions.

CARDIOPULMONARY. Common cardiovascular symptoms and signs during amphetamine intoxication include hypertension, chest pain, palpitations, and dyspnea. Although chest pain frequently occurs following the use of amphetamines, electrocardiographic changes other than sinus tachycardia are uncommon. A few case reports associate the development of an acute myocardial infarction with both recent use of IV amphetamine[81,82] or chronic oral amphetamine abuse.[83] Angiographic studies do not often demonstrate significant coronary artery disease in these relatively young patients. The IV administration of amphetamine has been associated with the development of myocardial injury and pulmonary edema without the presence of significant coronary artery disease. Chest pain occurs in most, but not all, patients presenting to the emergency department with myocardial injury after the use of amphetamine.[29] An acute cardiomyopathy with elevated myocardial band fraction of creatine kinase (CK-MB), mitral valve prolapse, elevated mean capillary wedge pressures, and reduced cardiac output developed 10 hours after IV use of amphetamine.[84] Three hours after the IV administration of 20–60 mg amphetamine, a 24-year-old man presented at an emergency department with pulmonary edema.[85] Coronary angiograms demonstrated patent coronary arteries and a reduced ejection fraction (32%) that normalized after 12 days. The serum creatine kinase concentration peaked at 703 IU on the fourth day.

Case studies of cardiomyopathies following the abuse of amphetamine are rare, and the contribution of drug impurities and predisposing factors to the development of these cardiomyopathies remains unclear. The chronic oral abuse of dextroamphetamine (100 mg/d intermittently for 5 years, continuously for 7 years) was associated with the development of a fatal congestive cardiomyopathy; clinical deterioration occurred during dextroamphetamine withdrawal.[86] The postmortem examination demonstrated normal coronary arteries and a focal myocarditis similar to the myocardial changes associated with pheochromocytomas. Intravenous amphetamine abusers are subject to the same complications (e.g., endocarditis, pulmonary granulomas) as heroin abusers.

Fatalities

Death from the use of amphetamine is relatively rare except following intentional ingestion of massive amounts of amphetamine or secondary to trauma during amphetamine-induced psychosis; these situations usually involve novice amphetamine users. Nontraumatic deaths related to amphetamine typically involve suicide or the intentional ingestion of amphetamine to avoid criminal prosecution. Mechanisms of nontraumatic deaths associated with the misuse or abuse of amphetamine include the following causes: cardiovascular (acute left ventricular failure, ventricular fibrillation), cerebrovascular (subarachnoid or intraparenchymal hemorrhage, cerebral edema), and hyperthermia (seizures, rhabdomyolysis, DIC, cardiovascular collapse). Predisposing factors for the development of fatal reactions to amphetamine use includes the coadministration of other stimulant or arrhythmogenic drugs, hypokalemia, or preexisting cardiovascular disease.[87] IV drug abusers are subject to the potentially fatal complications of illicit IV drug administration including septicemia, fulminant hepatitis, subacute bacterial endocarditis, cor pulmonale resulting from foreign-body granulomas, necrotizing angiitis, and AIDS.[66]

Abstinence Syndrome

A withdrawal syndrome may occur in heavy users during the first week following cessation of use. The abstinence syndrome that follows cessation of chronic amphetamine use is relatively mild compared with CNS depressants (e.g., heroin, sedative-hypnotic drugs). Chronic amphetamine and cocaine use produces a similar abstinence syndrome, but there are no common physical symptoms that characterize stimulant withdrawal.[88] Abrupt discontinuance of amphetamine does not produce seizures or life-threatening symptoms, even in those patients who habitually consume large quantities of amphetamine. The symptoms associated with abstinence syndrome following chronic amphetamine use include a dysphoric mood (depression, irritability, anxiety) and psychomotor agitation along with fatigue, insomnia, hypersomnia, poor concentration, paranoia, akathisia, and drug craving.[89] Myalgias, abdominal pain, voracious appetite, and a profound depression with suicidal tendencies may complicate the immediate postwithdrawal period; the intensity of these symptoms usually peaks 2–3 days after cessation of amphetamine use.

Reproductive Abnormalities

Available evidence suggests that the therapeutic use of *d*-amphetamine during pregnancy does not increase the risk of adverse pregnancy outcomes including teratogenicity.[90,91] The abuse of *d*-amphetamine is associated with prematurity, low birth weight, impaired neurobehavioral development, and increased maternal–fetal morbidity; however, multiple sociologic and economic variables as well as polydrug use complicate the interpretation of the effect of methamphetamine on fetal outcomes.[92]

DIAGNOSTIC TESTING

Analytic Techniques

SCREENING

The most common screening methods for amphetamine in urine involve the detection of the parent compound by immunoassay [Abbott fluorescence polarization immunoassay (FPIA; Abbott Laboratories, Abbott Park, IL); Syva EMIT® (enzyme multiplied immunoassay technique; Siemens Healthcare Diagnostics, Deerfield, IL], radioimmunoassay (RIA; Roche Diagnostic Products Corp., Indianapolis, IN), or thin-layer chromatography (TLC). Colorimetric methods, ultraviolet spectrophotometric procedures, and fluorescence methods are now superseded by immunoassays.[93] The Toxi-Lab TLC® (Varian, Inc., Palo Alto, CA) method can detect amphetamine concentrations ranging from 0.5–3.0 mg/L, but in general the use of TLC methods is less sensitive than the use of immunoassays.[94]

Immunoassays are sensitive methods for screening urine samples for the presence of amphetamine, but these techniques lack specificity. Occasionally, large ingestions of some amphetamine-like compounds may produce false-positive results including over-the-counter inhalers (*l*-isomer of methamphetamine), diet medications (phentermine), or cold preparations (phenylpropanolamine, ephedrine, pseudoephedrine) depending on the urine drug concentration and the type of immunoassay.[95] Relatively high concentrations of illicit amphetamine analogs (methylenedioxyamphetamine [MDA], methylenedioxyethylamphetamine [MDEA], methylenedioxymethamphetamine [MDMA], 3-methoxy-4,5-methylenedioxyamphetamine [MMDA]) also cross-react with some of these screening tests to produce false-positive results, but the cross-reactivity varies between different screening procedures and the concentrations of the substance in the urine sample.[96,97] Metabolism of the antiparkinson drug, selegiline produces *l*- methamphetamine and *l*-amphetamine; however, the ratio of methamphetamine/amphetamine following the biotransformation of selegiline is approximately 2.5 compared with about 10 following the metabolism of methamphetamine.[98]

TABLE 1.2. Cross-Reactivity of Amphetamine Immunoassays.[93]

Drug	RIA	FPIA (TDx®)	EMIT d.a.u.® Polyclonal	EMIT d.a.u.® Monoclonal	EMIT II®
d-Amphetamine	100	90	100	250	100
d,l-Amphetamine	50	100	100	100	67
d-Methamphetamine	2.2	57	30	100	100
d,l-Methamphetamine	—	57	—	—	53
Diethylpropion	0*	0	0*	—	0.1
Ephedrine	0	0	30	2	0.7
Fenfluramine	—	22	33	10	—
Mephentermine	0	6	75	10	10
Methylenedioxyamphetamine (MDA)	327	465	2.6	100	33
Methylenedioxymethamphetamine (MDMA)	0.6	84	2.8	33	17
Phenylethylamine	1	2	32	10	0.2
Phenmetrazine	0.1	0	30	1	17
Phentermine	1.7	6	75	333	50
Phenylpropanolamine	2	0	30	1.3	0.4
Pseudoephedrine	0	0	30	1	0.3

Abbreviations: RIA = radioimmunoassay; FPIA = fluorescence polarization immunoassay.
*Significant cross-reactivity with urinary metabolites.

Rapid screening methods with gas chromatography/ mass spectrometry separate various sympathomimetic amine compounds, but these confirmation techniques are too technician- and time-intensive for routine use in most hospital laboratories.[99] Phencyclidine, cocaine, caffeine, and barbiturates do not cross-react with reagents in the immunoassays at concentrations up to 1 mg/mL.[100] Table 1.2 lists the cross-reactivity of various immunoassays. The radioimmunoassay (Roche RIA) is relatively specific for d-amphetamine. Cross-reactivity occurs primarily with its major metabolite, p-hydroxyamphetamine, and the illicit hallucinogenic drug, p-methoxyamphetamine. Phenylpropanolamine and β-phenylethylamine produce slight cross-reactivity with radioimmunoassays, but the concentrations of these drugs in urine usually are too low to produce false-positive results except following a severe overdose of these compounds.[101] The excretion of amphetamine is highly pH dependent, and amphetamine abusers may alkalinize their urine to reduce the concentration of amphetamines present in the urine or they may acidify their urine to increase amphetamine elimination.

CONFIRMATORY

Gas chromatography, high performance liquid chromatography, and gas chromatography/mass spectrometry are the most common methods to confirm and to quantify positive results of screening tests for amphetamine.[102,103] The US National Institute on Drug Abuse (NIDA) guideline requires that positive urine samples contain amphetamine concentration exceeding 200 ng/mL. Positive screening tests for amphetamine require confirmation by more specific methods (e.g., gas chromatography/mass spectrometry, high performance liquid chromatography), which separate amphetamine from methamphetamine and other structurally similar compounds. In contrast to gas chromatography/mass spectrometry, liquid chromatography/tandem mass spectrometry does not require sample derivation or hydrolysis. The interassay, interday variability (coefficient of variation) of liquid chromatography/tandem mass spectrometry for amphetamine was approximately 10–12%.[104] Detection limits for amphetamine in serum samples using a solid-phase microextraction method combined with liquid chromatography/electrospray ionization/tandem mass spectrometry was 0.3 µg/L.[105] The use of gas chromatography/mass spectrometry does not necessarily differentiate the illicit use of methamphetamine or amphetamine from the use of prescription drugs that contain these compounds or are metabolized to amphetamine and/or methamphetamine.[106] Interpretation of drug-testing results that include the alleged use of amphetamine/methamphetamine precursor drugs requires analysis of the following factors: detection of parent drug or unique metabolite, ratio of d- and l-enantiomers of methamphetamine and amphetamine, and methamphetamine and/or amphetamine concentrations relative to the history of prescription drug use (see Confirmatory Methods under Methamphetamine).

In addition to the presence of impurities from the clandestine synthesis of amphetamine compounds, drug samples contain many adulterants. Analysis of street samples suggests wide variation in the potency of samples including the absence of amphetamine derivatives in up to 40% of purported samples. These illicit drugs contain varying amounts of phencyclidine, lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (STP), cocaine, atropine, mescaline, and strychnine, as well as additives (e.g., cornstarch, maltose, lactose, magnesium silicate, quinine, fibrous material).

STORAGE

Animal studies and examination of exhumed material indicate that amphetamine is fairly stable in the blood and bone marrow over several years of burial.[107] *In vitro* studies indicate that amphetamine is stable in urine samples stored at −20°C in 1% sodium fluoride for 2 years.[108] In a study of amphetamine concentrations in gray-top Vacutainer® (Becton, Dickinson, & Co., Franklin Lakes, NJ) tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate, the mean decrease of the amphetamine concentration at 6 months and 1 year was 31% and 77%, respectively.[109] Amphetamine was less stable than methamphetamine. *N*-ethylbenzamide (CAS RN: 614-17-5) is a thermal decomposition product of the vulcanizing agent zinc ethylphenyldithiocarbamate (CAS RN: 3037-20-2) used in rubber production. The presence of this compound in glass containers sealed with a natural rubber septum may cause false-positive results for amphetamine as measured by gas chromatography,[110] but gas chromatography/mass spectrometry accurately identifies the contaminant.

Biomarkers

BLOOD

ANTEMORTEM. Toxicologic analyses of blood and urine confirm amphetamine ingestion, but these assays are not usually available to guide clinical management; extrapolation of these amphetamine concentrations to expected clinical effects must be done cautiously.

Paranoid delusions, disorganization of thoughts, hallucinations, and poor concentration occurred in a group of 18 patients evaluated for amphetamine psychosis.[111] The peak amphetamine concentration in plasma samples drawn from 15 of these psychotic patients within 24 hours of admission ranged from 0.08–0.64 mg/L. Although these patients received ammonia chloride for urine acidification (pH 5.1–6.1), the timing of the urine acidification in relation to the plasma sample was not reported. There was no correlation between psychiatric symptoms and plasma amphetamine concentrations. The mean peak plasma amphetamine concentration in plasma samples from 8 of the 18 chronic amphetamine abusers administered 200 mg amphetamine sulfate IV followed by urinary acidification (urine pH 5.1–5.4) was approximately 0.4 mg/L (range, 0.36–0.45 mg/L) compared with 0.43 mg/L (range, 0.31–0.60 mg/L) for 10 addicts receiving 160 mg amphetamine IV (urinary pH 5.2–6.4), as measured by gas chromatography/flame ionization detection. No psychotic symptoms occurred in these patients following the administration of these 2 amphetamine doses. The ingestion of approximately 1 g amphetamine chronically by tolerant users produces whole blood concentrations in the range of 2–3 mg/L with little obvious acute signs of intoxication.[112] In a study of amphetamine abusers hospitalized for psychotic behavior, the plasma amphetamine concentration ranged from 0.161–0.530 mg/L in admission peripheral blood samples.[111]

POSTMORTEM. There are limited data on postmortem blood concentrations of amphetamine; therefore, the interpretation of the significance of a specific postmortem concentration of amphetamine requires careful analysis of the circumstances surrounding the death, the behavior of the user, the autopsy, prescription medications, anatomic site of postmortem sample collection, and the reliability of the sample integrity. In particular, the lower ranges of amphetamine concentrations must be interpreted with caution because of tolerance and the subjectivity associated with the determination of the contribution of amphetamine to the cause of death.[113] Consequently, the postmortem amphetamine concentration should not be used alone to determine the cause of death. Postmortem *d*-amphetamine blood concentrations in a case series of amphetamine-related fatalities ranged from 0.5–41 mg/L with an average of 8.6 mg/L.[114] Separation of the enantiomers by chiral derivatization may assist in the determination of the timing of ingestion (i.e., late vs. early) because of the more rapid metabolism of the (*S*)-enantiomer of amphetamine. A (*S*)-/(*R*)-enantiomer ratio of approximately 1 suggests the recent ingestion of amphetamine.[115] The use of some prescription medications (selegiline) may produce small, but detectable amounts of amphetamine and methamphetamine in postmortem blood samples.

URINE

Urine amphetamine immunoassays detect the presence of these compounds following occasional use for

approximately 1–3 days depending on several factors including the dose, duration of use, urine pH, hydration (i.e., urine creatinine, specific gravity), analytic method (sensitivity, specificity, cutoff), and individual metabolic and excretion rates.[93] Because of structural similarities, phenylpropanolamine may cross-react with some amphetamine reagents. The potential for cross-reactivity depends on a variety of factors including the assay, the drug concentration, the extraction procedure (e.g., sodium periodate), and the metabolic products of the drug. At therapeutic concentrations, the cross-reactivity of these drugs to most immunoassays are relatively low, and false-positive results are unusual.[95] Drugs that produce amphetamine as a metabolite include amphetaminil, benzphetamine, clobenzorex, dimethamphetamine, N-ethylamphetamine, fenethylline, fenproporex, and mefenorex. High concentrations of other drugs that cause potential false-positive results on amphetamine immunoassays include the following: deprenyl, famprofazone, fencamine, furfenorex, mesocarb, phenothiazines (trifluoperazine, chlorpromazine, thioridazine),[116] prenylamine, trazodone,[117] bupropion,[118] and quinolones.[119]

Abnormalities

Acute renal failure may develop secondary to acute tubular necrosis (as a result of hypotension), rhabdomyolysis, intravascular coagulation, hypovolemia, or hyperpyrexia. Hypoxemia may result from seizures, noncardiac pulmonary edema, or acute cardiac failure. Pulmonary function tests, except the carbon dioxide diffusing capacity, usually remain normal during chronic amphetamine use. Leukocytosis occurs frequently after amphetamine use; a leukemoid reaction may occur. Case reports associate microangiopathic hemolytic anemia with IV polydrug use including the abuse of amphetamine.[120] In patients with amphetamine-induced intracranial hemorrhages, computed tomography (CT) may reveal intracerebral hematomas, hemorrhage of the thalamus, cerebral edema, or transtentorial herniation. Intracranial hemorrhages are usually located in the cerebral white matter rather than abnormalities in the sites (i.e., basal ganglia, pons, cerebellum) commonly associated with chronic hypertension.

Driving

The primary benefit of low doses of amphetamines is the variable reduction of fatigue.[121] The administration of 5–15 mg of dextroamphetamine to healthy, alert (i.e., nonfatigued) adults does not produce substantial improvement of fatigue[122] or psychomotor skills.[123,124] In a study of 30 student volunteers given 15 mg d-amphet-

amine, objective measures of reaction times increased about 10% above pretreatment values.[125] However, the report did not include the actual data or any statistical analysis. Improvement occurs in some selected tasks that require rapid responses or increased alertness (vigilance, simple reaction time, motor coordination, physical endurance), particularly in restoring baseline performance by fatigued volunteers.[126,127] Enhancement of performance is most likely to occur under conditions of boredom and low intellectual demand, and the positive effects of amphetamines decrease as the complexity of the task increases.[128] Saccadic eye movements are rapid conjugate shifts of gaze that allow changes in visual fixation from 1 object (e.g., pedestrian) to another (e.g., stoplight). Smooth pursuit eye movements stabilize visual images on the retina to optimize visual acuity. Studies in volunteers indicate that 15 mg d-amphetamine orally does not alter saccadic or smooth-pursuit eye movements, whereas the IV administration of the same dose abolishes the effect of fatigue on saccadic movements and reduces saccadic reaction time.[129]

Amphetamine users frequently abuse other drugs including ethanol. The interaction of d-amphetamine and ethanol on psychomotor tasks is complex with volunteer studies demonstrating contradictory results.[130,131] The Simulator Evaluation of Drug Impairment (SEDI) task is a measure of the skills (attention, memory, recognition, decision making, reaction time) required to operate machinery with precision. Although the administration of 10 mg d-amphetamine to 12 healthy volunteers did not alter their scores on the SEDI, this dose of d-amphetamine did attenuate the decrement produced by ethanol on accuracy and reaction time performance.[132] The mean peak ethanol concentration in these volunteers was approximately 100 mg/dL.

A driving stimulation study of healthy volunteers 2 hours after receiving 0.42 mg d-amphetamine/kg body weight indicated some impairment during daytime driving, but not during nighttime driving.[133] The mean dexamphetamine blood concentration was 83 ng/mL immediately prior to testing. The behaviors primarily contributing to this impairment included "failing to stop at a red traffic light," "slow reaction times," and "incorrect signaling." However, there were a large number of the control drivers judged impaired, and there was decreased visual acuity of the left (but not the right) eye of the volunteers receiving the d-amphetamine. There is some evidence that moderate doses (up to 30 mg) of amphetamines increase self-confidence and the acceptance of greater risk. However, these effects are neither strong nor consistent among different individuals.[134] In a study of 36 healthy volunteers, the administration of 10–20 mg d-amphetamine reduced scores on several psychologic measures of impulsivity,[135] whereas

other studies suggested increased impulsivity after *d*-amphetamine administration.[136] The use of *d*-amphetamine doses up to 25 mg is difficult to detect by standardized sobriety tests of motor coordination and observation of behavior.[137] Standard field sobriety tests (horizontal gaze nystagmus, walk and turn test, one leg stand test) are not sensitive measures of the effect of dexamphetamine as measured in adult volunteers receiving 0.42 mg/kg *d,l*-dexamphetamine or placebo.[138]

Impairment of driving skills also may develop during the withdrawal phase from chronic amphetamine abuse because of the presence of exhaustion, fatigue, depression, or agitation. Neuropsychologic testing of paid volunteers did not detect rebound effects following the administration of pharmacologic doses of *d*-amphetamine.[139] Consequently, impairment may occur even at low blood amphetamine concentrations. A study of 11 amphetamine abusers suggested that their accident rate was about 3–4 times higher than the rate expected based on age, sex, and driving exposure.[140] In a retrospective study of Norwegian drivers apprehended on the suspicion of driving under the influence, medical evaluation after the arrest indicated that 73% of the drivers with blood amphetamine concentrations exceeding 0.27 mg/L (2.0 μ*M*) were severely impaired.[141] American pilots selectively used dextroamphetamine during long flights (about 17 h and 35 h) during Operation Iraqi Freedom without obvious side-effects as evaluated by retrospective interviews within 4 weeks of the 94 sorties.[142]

There are few data on the blood amphetamine concentrations associated with driving impairment. Similar to the interpretation of postmortem amphetamine concentrations in blood samples, interpretation of the significance of specific amphetamine concentrations requires careful consideration of surrounding circumstances, behavior, tolerance, and the time of the sample. In a study of 6,094 drivers suspected of driving under the influence (moving traffic violations, sobriety checkpoints, traffic accidents), the mean blood amphetamine concentration was 1.01 mg/L (median 0.80 mg/L) with a range up to 11.9 mg/L.[143] There were no other drugs detected by gas chromatography/mass spectrometry in these blood samples.

TREATMENT

The treatment of amphetamine, methamphetamine, and cocaine intoxication is similar; there are fewer clinical data on the specific treatment of amphetamine or methamphetamine intoxication than cocaine intoxication. There are no unique features of amphetamine intoxication that require treatment different than methamphetamine intoxication (see Treatment under Methamphetamine). Because amphetamine is a weak base, urinary acidification increases the excretion of unchanged drug and causes a decrease of the plasma half-life. However, the clinical efficacy of this therapeutic maneuver on clinical outcome remains doubtful, especially because cardiovascular and renal complications may develop during the use of this procedure during amphetamine intoxication. The treatment of the hallucinations and paranoia associated with amphetamine-induced psychosis typically involves the administration of dopamine agonists (e.g., haloperidol, chlorpromazine). Newer generation antipsychotic medications (e.g., olanzapine) also reduce the symptoms associated with amphetamine-induced psychosis and these medications may be better tolerated than the older antipsychotics (e.g., haloperidol).[144]

METHAMPHETAMINE

HISTORY

While attempting to synthesize ephedrine in Japan, Ogata synthesized methamphetamine in 1919.[145] Later, he sold the license for this process to Burroughs Wellcome Company, which sold methamphetamine in the United States as Methedrine® until this drug was withdrawn from the US market in 1968. During World War II, the Japanese government widely distributed amphetamine compounds in the form of over-the-counter stimulants (philopon, shabu) to their civilian workers as a method to increase worker productivity. After the war, the distribution of large legal stockpiles of methamphetamine ampules contributed to widespread parenteral abuse of methamphetamines in Japan. Between 1945 and 1955, the epidemic of methamphetamine (wake-amine) addiction involved over 2 million Japanese citizens, beginning with writers, musicians, and artists. Later, the abuse of methamphetamine extended to the Korean minorities in Japan.[146] Tight production controls of methamphetamine in Japan began with the Stimulants Control Law of 1951. A massive education program and strict penalties in Japan sharply reduced the abuse of methamphetamine by the mid-1950s. Although drug addiction in the Japanese culture was rare prior to the methamphetamine epidemic, other forms of drug abuse (heroin, methaqualone, 1,2-diphenyl-1-dimethylaminoethane [SPA]) appeared after the initial methamphetamine epidemic.

During the 1960s, one of the treatments of heroin addiction was a liquid form of methamphetamine. Although abuse of methamphetamine initially involved

the diversion of pharmaceutic products, illicit production of methamphetamine began in the early 1960s in San Francisco, a process largely controlled by motorcycle gangs in the California Bay area. Illicit production spread along the Pacific Coast of the United States during the 1960s.[147] Initially, oral methamphetamine was listed as schedule III drugs; however, in 1971, methamphetamine was added the list of schedule II drugs (i.e., drugs that have medical use but significant abuse potential) in an attempt to limit the diversion of methamphetamine to illicit markets. During the 1950s and 1960s, methamphetamine was a common prescription medication for the treatment of depression and obesity with a peak of 31 million prescriptions in the United States during 1967.[147] Parenteral methamphetamine was always listed as a schedule II drug.

During the 1980s, illicit production of a smokable form of (+)-methamphetamine hydrochloride (Ice) began in Japan and Korea. Abuse of Ice spread to Taiwan, the Philippine Islands, Hawaii, and to the mainland United States by the late 1980s. Although the abuse of Ice never reached epidemic proportions similar to freebase cocaine, illicit use and manufacture of methamphetamine spread from California to the Midwest.[147] A third epidemic of methamphetamine abuse via IV and pulmonary routes occurred in Japan during the 1990s following the importation of illicit methamphetamine from China and North Korea.[148] Since the 1980s, clandestinely manufactured methamphetamine replaced legal supplies as the primary source of methamphetamine; Mexican-based distributors and so-called superlabs in California and the southwestern United States replaced local manufacture of methamphetamine. More recently, exports from large Mexican methamphetamine laboratories replaced regional sources as the main supplier of methamphetamine in the United States.[149] Recent data from the US Substance Abuse and Mental Health Services Administration suggest that the current methamphetamine epidemic may have peaked around 2004–2005 with the use of methamphetamine reaching a plateau.

IDENTIFYING CHARACTERISTICS

Structure

Methamphetamine (CAS RN:537-46-2) is the common name for N,α-dimethylphenethylamine (desoxyephedrine, methylamphetamine, phenylisopropylmethylamine). The structures of methamphetamine ($C_{10}H_{15}N$) and amphetamine are similar, but the amino nitrogen in methamphetamine has a methyl group as demonstrated in Figure 1.1. The configuration at the chiral center of methamphetamine results in a dextrorotatory isomer [d-methamphetamine, S-(+)-methamphetamine] and

a levorotatory isomer (l-methamphetamine, R-(–)-methamphetamine] with respect to plane-polarized light.[150] The [α]D25 (specific notation) of pure d-methamphetamine is +14–20°. Metabolism of the antiparkinson drug, selegiline, produces the l-(–) enantiomer of methamphetamine. Dimethylamphetamine is a pyrolysis product of methamphetamine and an illicit stimulant sold in Japan with no medical applications.[151] Although dimethylamphetamine has abuse potential, animal models suggest that this tertiary amino compound is substantially less potent than methamphetamine.[152]

The 2 enantiomers of methamphetamine demonstrate some differences in biologic effects. In volunteer studies of methamphetamine abusers administered IV methamphetamine doses of 0.5 mg/kg, the psychodynamic effects produced by l-methamphetamine and d-methamphetamine are similar; however, the effects of the former are shorter and less desirable as reported by the participants.[153] The abuse liabilities of racemic methamphetamine and d-methamphetamine are similar. Therapeutic doses of methamphetamine produce more prominent central than peripheral effects compared with similar doses of amphetamine as a result of the increased lipophilicity and enhanced CNS penetration of methamphetamine resulting from the N-methyl substitution.

Physiochemical Properties

The addition of a methyl group to amphetamine increases lipid solubility and transport of methamphetamine across the blood–brain barrier. The molecular weight of methamphetamine (freebase) is 149.24 g/mol. Freebase methamphetamine is a lipophilic weak base with a pK_a of 9.87 that is a dark liquid at room temperature; however, methamphetamine hydrochloride (molecular weight 185.74 g/mol) is a white to translucent crystalline solid at room temperature (melting point 170–175°C/338–347°F). Unlike most methamphetamine salts, the vapor pressure of methamphetamine hydrochloride is sufficiently high to allow the efficient smoking of this salt, regardless of the size of the crystals.[154] This hydrochloride salt of methamphetamine is soluble in water, chloroform, and ethanol, but not soluble in ether; extraction of methamphetamine from biologic samples occurs easily following the use of organic solvents at alkaline pH. Both methamphetamine and methamphetamine hydrochloride easily volatilize with heat; consequently, methamphetamine may volatilize during the dry-down or evaporation phase of extraction. Unlike cocaine hydrochloride, methamphetamine hydrochloride volatilizes at 300–305°C (572–581°F) without pyrolysis. Therefore, methamphetamine hydrochloride can be smoked in the salt form, whereas the smoking of cocaine

hydrochloride requires the complicated conversion of this salt to the freebase.

Because of the presence of protonated nitrogen and a chloride nucleophile, the hydrochloride salt of methamphetamine undergoes *N*-demethylation to amphetamine more easily than the methamphetamine base decomposes to amphetamine. The heating of methamphetamine hydrochloride salt to 400°C (752°F) and to 600°C (1,112°F) converts about 5% and 10%, respectively, of the methamphetamine dose to amphetamine.[155] Experimental studies indicate that demethylation and methylation reactions are the major pyrolysis processes at temperatures below 358°C (676°F).[156] At temperatures above 315°C (599°F), amphetamine and dimethylamphetamine form from demethylation and methylation reactions, respectively. Benzyl ethyl trimethylammonium is also a pyrolysis product, and the thermal degradation of this compound produces allylbenzene, *cis*-β-methylstyrene, and *trans*-β-methylstyrene. Above 445°C (833°F), the *l*-isomers of amphetamine and methamphetamine form from the respective *d*-isomer.[156]

Smoking methamphetamine in a tobacco mixture substantially reduces the recovery of methamphetamine compared with the recovery of methamphetamine using a smoking apparatus. In a study of the pyrolysis products from smoking methamphetamine mixed with tobacco in a smoking apparatus, the amount of methamphetamine transferred to tar ranged from 6% to 17%.[154] Major pyrolysis products of a 10- or 20-mg dose of methamphetamine present in mainstream smoke included methamphetamine, amphetamine, phenylacetone, dimethylamphetamine, and *N*-formyl, *N*-acetyl-, *N*-propionyl, and *N*-cyanomethyl methamphetamine compounds. Other minor pyrolysis products include *N*-acetylmethamphetamine, phenyl ester of propanoic acid, and furfuryl methamphetamine (*d,l*-furfenorex).[157] Mainstream smoke from the smoking apparatus contained 14.5% of the initial dose of 50 μg methamphetamine as measured by gas chromatography/mass spectrometry.[158] Other major products of pyrolysis present in the mainstream smoke included phenylacetone (3.1%), *N*-cyanomethylmethamphetamine (1.9%), *trans*-β-methylstyrene (1.7%), and *N*-formyl methamphetamine (1.5%).

Terminology

The street names Speed and Crank typically refer to forms of methamphetamine hydrochloride that contain either pure *d*-methamphetamine or a racemic mixture of *d*- and *l*- methamphetamine enantiomers depending on the process used to produce the illicit methamphetamine.[159] Other names for the solid form of methamphetamine include Base, Fast, Meth, P, Point, Pure, Rabbit, Tail, Wax, and Whiz.[14] Trade names for methamphetamine compounds include Desoxyn® (Ovation Pharmaceuticals, Inc., Deerfield, IL) and the discontinued product, Methampex®. Based on the appearance of methamphetamine crystals, this form of methamphetamine hydrochloride is known as Batu (Hawaii), Crystal Meth, Crystal, Ice, Glass, and Shabu.

EXPOSURE

Epidemiology

Methamphetamine is the second most popular illicit drug (i.e., after cannabis) worldwide with annual global prevalence of about 0.4%; amphetamine is relatively more common than methamphetamine in Europe compared with Asia, Oceania, and North America.[160] Crystalline methamphetamine is one of the most prevalent illicit drugs in the United States; use began in the western United States in the early 1990s and subsequently spread across the United States. The prevalence of methamphetamine use has stabilized since 2000 with a decrease in new methamphetamine users aged 12 years and older beginning around 2005.[161] Based on weighted Internet surveys, the overall prevalence of current nonmedical methamphetamine use in 2005 was 0.27% among 18- to 49-year-olds; the estimated lifetime (i.e., at least once) was 8.9%.[162] The use of methamphetamine is also common in Taiwan and other parts of Asia. Southeast and East Asia are major global sources for methamphetamine production and trafficking, particularly Cambodia, Indonesia, Malaysia, Philippines, and the Mekong region of Vietnam.[163] In a retrospective study of autopsy cases performed in Taiwan between 1991 and 1996, methamphetamine-*related* deaths as defined by a postmortem blood methamphetamine concentration exceeding 0.1 mg/L accounted for 3.4–12.1% of the total autopsy cases.[164] Reviews of drug use in Australia indicate an increase in both importation and local manufacture of methamphetamine.[165] National household surveys of drug use in New Zealand indicate the increased use of crystal methamphetamine between 2003 and 2006 as a result of fewer respondents stopping the use of methamphetamine and more respondents reporting the increased frequency methamphetamine use.[166] The use of methamphetamine in North America varies with geographical location, type of methamphetamine, route of administration, and type of user. Furthermore, the form of methamphetamine changed from nonmedical use to the use of powder methamphetamine, and now to the use of the more potent crystal methamphetamine (Ice) with high purity.[167] The prevalence of methamphetamine use among young adults in

San Francisco has traditionally been high, particularly among homosexual males and IV drug users. In a cross-sectional study of young (median age 22 years) IV drug users in San Francisco, about 50% reported IV drug use within the last 30 days. The IV use of methamphetamine was higher in homosexual males with 60% of homosexual male, IV drug users reporting recent injection of methamphetamine compared with 47% of heterosexual male, IV drug users.[168]

Sources

Therapeutic uses of methamphetamine include the treatment of attention deficit disorder (ADD) and narcolepsy. *d*-Methamphetamine is a prescription drug (Desoxyn®, Abbott Laboratories, Abbott Park, IL) available in the United States as a schedule II drug.

ORIGIN

In contrast to the synthesis of *d*-amphetamine, the production of methamphetamine is relatively simple. Methamphetamine synthesis involves the following general steps: 1) collection and storage of chemicals, 2) isolation of precursors and catalysts from commercial products, 3) cooking (mixing, heating, filtering), 4) extraction of methamphetamine base into organic solvent, and 5) salting (precipitation and drying of water-soluble salt).

METHAMPHETAMINE POWDER. The most common methods of the illicit manufacturing of methamphetamine are phenylacetone (P2P or phenyl-2-propanone), red phosphorus/hydrogen iodide (hydroiodic or hydriodic acid) reduction (Yankee Dope, Red P, or Red, White, and Blue), and ammonia/alkali metal reduction (Nazi Dope). Methamphetamine was originally synthesized in illicit laboratories primarily by reductive amination using the Leuckart reaction, which involved the condensation of phenylacetone (phenyl-2-propanone, P2P) with methylamine in the presence of formic acid and an aluminum amalgam catalyst. This forms an intermediate imine. As the geometry of the imine is flat, the reduction of the imine to methamphetamine proceeds without stereochemical preference. Therefore, the end-product of this synthetic approach is the racemic mixture of *d,l*-methamphetamine, which frequently contains the nonreacted starting material (phenylacetone). The legal restriction placed on phenylacetone in the early 1980s and substantial differences in the pharmacologic activity of the 2 methamphetamine enantiomers resulted in a search for alternative methods of methamphetamine synthesis.

Currently, the most popular methods of illicit methamphetamine synthesis involve the reduction of *l*-ephedrine or *d*-pseudoephedrine either with red phosphorus and hydriodic acid (the "red, white, and blue" method) or with sodium or lithium metal in condensed liquid ammonia (the Birch reduction or the "Nazi" method). The latter method is more common in rural areas. Both these methods produce *d*-methamphetamine with the former process yielding 54–82% of this enantiomer.[150] The substitution of phenylpropanolamine as the precursor in either synthetic process yields amphetamine.

The advantages of the synthetic approach to illicit methamphetamine production include the following: 1) the stereochemistry of the α-carbon is fixed in the starting material, so the reduction yields a pure product containing only the more desirable *d*-methamphetamine, and 2) the reagents and precursors are inexpensive, easily purchased on the commercial and retail level. The legitimate medical and commercial uses of these methamphetamine precursors severely limit attempts to restrict access to these chemicals; *smurfing* is the process whereby criminal individuals and groups attempt to circumvent state and federal pseudoephedrine sales restriction by purchasing small quantities of pseudoephedrine at multiple retail outlets. Alternatives for drug trafficking cartels are importation of ephedrine and pseudoephedrine from Southeast Asia and South America. Figure 1.5 displays both the Leuckart reaction and the reduction of ephedrine to yield methamphetamine. Although extremely uncommon, other synthetic processes for methamphetamine include "dry reduction" using hydriodic acid/red phosphorus with small amounts of water and the reduction of phenylacetone (phenyl-2-propanone) by the mercury-aluminum amalgam reduction.[169]

CRYSTAL METHAMPHETAMINE HYDROCHLORIDE (ICE). The slow cooling of hot solutions of saturated solutions of methamphetamine hydrochloride in certain organic solvents produces large, glass-like crystals. In contrast to freebase cocaine, crystal methamphetamine is not the freebase form of methamphetamine. The smokable form of methamphetamine contains relatively pure *d*-methamphetamine in contrast to the racemic mixture of methamphetamine produced by the condensation of phenylacetone and methylamine.[170] The terms Ice, Batu (Hawaii), or Crystal refer to the relatively pure, smokable form of *d*-methamphetamine hydrochloride because this synthetic product appears as transparent, sheet-like crystals.[159] Typically, the production of Ice involves the crystallization of methamphetamine hydrochloride from a saturated solvent solution using the ephedrine- or pseudoephedrine-reduction method. This
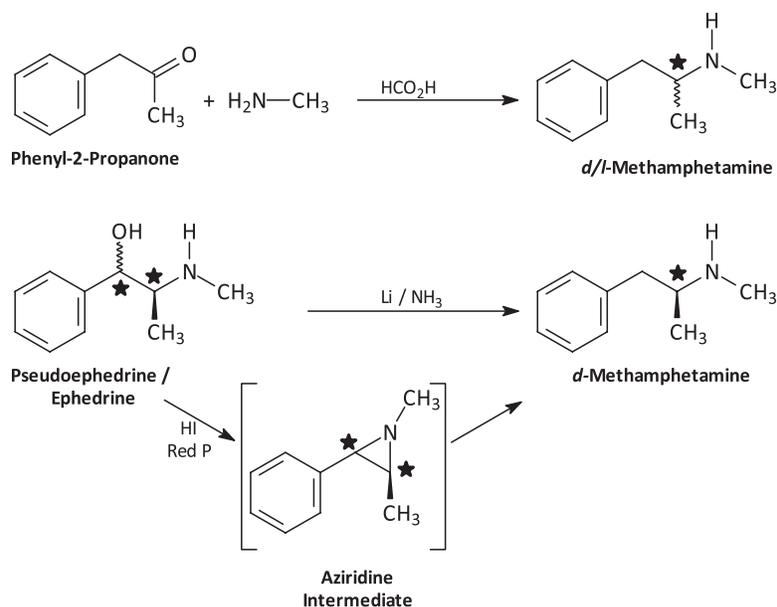
FIGURE 1.5. Synthetic routes to methamphetamine. The top scheme is the Leuckart reaction using phenyl-2-propanone (P2P) as a starting material. Reduction of the planar imine intermediate (not shown) results in a racemic mixture of *d*- and *l*-methamphetamine. The lower scheme depicts the reduction of ephedrine or pseudoephedrine (dictated by the stereochemistry of the benzylic hydroxyl) to form methamphetamine directly. Any one of a number of reductive techniques may be used for the reduction of ephedrine or pseudoephedrine to methamphetamine. However, the stereochemistry at the carbon alpha to the amine is maintained with these reductions yielding only the *d*-methamphetamine product.

process crystallizes sheets of *d*-methamphetamine hydrochloride crystals from the cooling of supersaturated, heated solutions of relatively pure *d*-methamphetamine as a result of the separation of the *d*- and *l*-isomers of ephedrine during the reduction process. Using ephedrine or pseudoephedrine as the starting material for Ice generates a more potent mixture that contains pure *d*-enantiomer rather than a racemic 50-50 mixture. The *d*-enantiomer of methamphetamine is several times more active pharmacologically than the *l*- enantiomer.[171]

METHAMPHETAMINE LABORATORIES

Retrospective, cross-sectional studies based on self-administered questionnaires suggest that symptoms experienced by law-enforcement personnel during investigations of clandestine methamphetamine laboratories include headache, sore throat, respiratory tract and mucous membrane irritation, nausea/vomiting, skin irritation, and various CNS symptoms.[172,173] Dermal burns are usually limited to persons directly involved in the production of illicit methamphetamine.[174] These injuries develop following spills, uncontrolled reactions, and/or fires during the manufacturing of methamphetamine. Phosphine gas is a by-product of the red phosphorous method (Red P) method for the clandestine

manufacturing of methamphetamine along with hydrochloric acid and iodine. In a study of a simulated red phosphorous cook, the airborne hydrogen chloride concentration during a 4-hour cooking period was 0.27 ppm; however, peak concentrations during the salting phase of the cook may substantially exceed this concentration.[175] The respirable methamphetamine concentration in this area during the cook was 720 μg/m^3. The anhydrous ammonia method generates ammonia and hydrochloric acid. Fatal concentrations of phosphine gas may form during the use of these types of manufacturing processes.[176] The increased popularity of methamphetamine and the proliferation of clandestine methamphetamine laboratories has caused an increasing incidence of burn injuries associated with laboratory accidents.[177] In a retrospective case-control study of 660 patients aged ≥16 years of age admitted to a burn center in the Midwestern United States, 10% of the 410 patients tested for drugs of abuse were positive for methamphetamine.[178] Burn injuries associated with the volatile process of methamphetamine synthesis are frequently more severe than other burn center patients including a higher incidence of third-degree burns, large burn areas, and inhalation injury.[178,179]

Children are often found during raids on homes with methamphetamine laboratories. The majority of the children display no overt clinical symptoms; however,

clinical features may range from respiratory irritation to agitation, seizures, and fatal sulfuric acid ingestion.[180] These children usually lack supervision and frequently have issues with school performance, criminal behavior, and social isolation. Protocols for the evaluation of these children include the following: 1) decontamination if external contamination is present, otherwise a bath/shower; 2) complete history, mental health, and physical examination within 48–72 hours, unless the presence of symptoms necessitates a medical evaluation earlier; 3) methamphetamine testing for legal purposes (urine drug screen with confirmation, hair samples), and placement in a safe environment.[181]

Methods of Abuse

The pattern of methamphetamine abuse is more frequent and more continuous during the daytime than cocaine abuse, which usually involves episodic use during the evening.[182] Because of the easy synthesis and available supplies of methamphetamine, users typically spend substantially less (i.e., 25%) money than cocaine users.[183] Methamphetamine is listed as a prohibited substance by WADA.[25]

Chronic Oral Abuse

"Parachuting" describes an oral method of methamphetamine abuse involving the swallowing of a methamphetamine-containing, sealed plastic bag with a hole cut on one end to dispense the amphetamine slowly during transit along the GI tract. Alternately, crushed methamphetamine tablets are rolled in a paper wrapper and swallowed. Severe, delayed (i.e., up to at least 42 h) methamphetamine toxicity can occur when the delayed release of large amounts of methamphetamine occurs during transit through the intestines.[184]

Intravenous Abuse

Intravenous methamphetamine abusers represent a subgroup of long-term, chronic drug abusers with a high prevalence of high-risk sexual and antisocial behaviors, comorbid psychiatric disorders, and multiple drug use.[185,186] A "speed freak" is a compulsive methamphetamine abuser, who uses the drug continuously for days during a "run." During this phase, the intense methamphetamine use substantially reduces food consumption, sleep, and personal hygiene. High methamphetamine doses produce extreme suspiciousness, hyperactivity, poor impulse control, or an overt paranoid psychosis that can be associated with unpredictable violent behavior.[187] Stereotyped behavior during these runs includes skin picking, bead stringing, pacing, repetitive actions,

and interminable chattering. Repeated IV injections (1–10 per day) prolong the run until exhaustion, disorganization, paranoia, severe anxiety, confusion, irritability, insomnia, or loss of drug supply terminates use, usually within several days to 1 week.

During the initial phase following cessation of methamphetamine use, exhaustion develops with the methamphetamine abuser sleeping deeply for 24–48 hours and then eating ravenously. Severe depression often occurs after the methamphetamine abuser becomes satiated. The methamphetamine abuser may resume IV drug use to relieve the depression, beginning another run.[188] To relieve anxiety, some methamphetamine abusers combine other IV drugs (e.g., heroin) with IV methamphetamine ("speedballing"). Particularly in the Pacific Northwest, methylphenidate (Ritalin®, Novartis, Basel, Switzerland) is a substitute for methamphetamine.

Occasionally, the rapid escalation of IV methamphetamine doses produces a condition called "overamped." During this situation, elevated blood pressure, temperature, and pulse, along with chest discomfort develop. Additionally, altered consciousness occurs, manifest by the inability to speak or move despite apparent awareness of the environment. Death from overdose is infrequent in tolerant individuals. In fact, habitual high-dose users commonly exhibit no apparent physical signs of dependence other than the obvious signs of economic, social, and emotional deterioration. The chronic abuser becomes unreliable, irritable, paranoid, and unstable, resulting in physical, social, and economic problems. Suicide may occur from either loss of impulse control or severe depression during the exhaustion phase. Adverse psychologic reactions from chronic methamphetamine abuse include anxiety reactions, psychosis, withdrawal reactions (e.g., exhaustion syndrome, biogenic amine depletion syndrome or [BADS]), prolonged depression, and persistent hallucinations.[189]

Smoking

The smoking of methamphetamine hydrochloride does not require conversion to the freebase in contrast to cocaine hydrochloride because of the relatively high volatility of methamphetamine hydrochloride. Typically, the methamphetamine hydrochloride crystals are heated in the base of a glass pipe and the vapors are inhaled ("chasing the white dragon"), *without* drawing the vapors through a coolant liquid that is common during the freebasing of cocaine.

Figure 1.6 displays a methamphetamine pipe. The crystallization of methamphetamine to the hydrochloride salt (Ice) is necessary because the lipid soluble, pure base form of methamphetamine evaporates easily

FIGURE 1.6. Methamphetamine pipe. (Photo courtesy of the US Drug Enforcement Agency).

at room temperatures. All crystals of methamphetamine are "smokable" regardless of the size of the crystal. In a pharmacokinetic study of chronic methamphetamine smokers, the average dose inhaled was approximately 22 mg.[190] Animal and human studies suggest that the pharmacologic effects of methamphetamine by inhalation and by IV route are similar.[191,192]

DOSE EFFECT

Illicit Use

The response to methamphetamine depends on a variety of factors including the percentage of active isomers, the setting, tolerance, and expectations. In a study of volunteers familiar with the effects of methamphetamine, the inhalation of 20–25 mg methamphetamine produced minimal subjective and cardiovascular effects, whereas a 30-mg dose of methamphetamine caused more pronounced and sustained effects.[193] The use of 40 mg methamphetamine produced extreme subjective effects characterized by feelings of omnipotence as well as decreased appetite, difficulty concentrating, insomnia, memory lapses, and intense craving for further doses. Anecdotal reports suggest that the IV use of methamphetamine begins with the injection of 20- to 40-mg doses, but as tolerance develops the dose increases substantially. The typical daily methamphetamine abuser smokes or insufflates 0.5–1 g during a 24-hour period beginning in the morning and continuing every 2–4 hours until sleep ensues. In a case series of 65 current methamphetamine abusers, the estimated mean daily dose of methamphetamine was 720 mg with a range up to 3.5 g.[194] A binge involves the use of methamphet-

amine every few hours until the user can no longer stay awake (i.e., usually 3–4 d). After the binge, the user sleeps for a prolonged period (e.g., 24–48 h) and may resume daily methamphetamine use or start on another binge. Experienced IV methamphetamine users typically inject from 100–300 mg per use with maximum doses exceeding 1 g during binges.[27] As much as 1 g may be injected every 2–3 hours by a speed freak during a binge up to a daily dose of 5–8 g for several days until exhaustion, psychosis, or loss of drug access terminates the "speed run."

Pharmaceutic Use

Medical use of methamphetamine includes the administration of daily doses up to 15 mg for obesity, up to 20–25 mg for ADD, and up to 60 mg for narcolepsy. Most methamphetamine analogues have been voluntarily removed from over-the-counter inhalants. However, the Vicks® inhaler (Procter & Gamble, Cincinnati, OH) is a nasal decongestant containing 50 mg of (*l*-methamphetamine (*l*-desoxyephedrine), menthol, camphor, methyl salicylate, and bornyl acetate. *l*-Methamphetamine has approximately 10% of the central nervous system potency of *d*-methamphetamine.[195]

Fatalities

Determination of a fatal human dose is complicated by interindividual variation including tolerance and variations in purity of illicit drugs.[196] Death from methamphetamine overdose is relatively rare compared with the prevalence of methamphetamine abuse. The minimal lethal methamphetamine dose varies with age and animal species.

ANIMAL

Experimental studies indicate that the IV LD_{50} of methamphetamine in adult, nontolerant monkeys maintained in primate chairs was approximately 15–20 mg/kg.[197] Young monkeys ($LD_{50} = 5$ mg/kg) and adult monkeys in open cages ($LD_{50} = 2$–3 mg/kg) were more vulnerable, particularly when hyperactivity and elevated body heat occur. Chronic administration of escalating doses of IV methamphetamine does not produce toxicity in some animal models. Methamphetamine is twice as toxic in animal models as amphetamine.

HUMAN

A 22-year-old man died 5 days after the ingestion of 140 mg methamphetamine hydrochloride.[198] He developed hyperthermia, hypotension, renal failure, and

hyperkalemia. Chronic methamphetamine users tolerate high doses of methamphetamine. Methamphetamine addicts can inject 1–5 g IV methamphetamine without developing severe complications as a result of the development of tolerance. A 27-year-old man survived after the injection of 75 mg methamphetamine with intensive supportive care after developing DIC, hypocalcemia, and rhabdomyolysis with myoglobinuria.[199] The estimated leakage of 20 g methamphetamine in a body packer caused his death (postmortem cardiac blood, 63.5 mg methamphetamine/L; admission blood, 8.6 mg methamphetamine/L), whereas a fellow body packer survived the estimated leakage of 18 g methamphetamine (admission blood, 7.6 mg methamphetamine/L) following intensive support for severe intoxication.[200]

TOXICOKINETICS

Absorption

ORAL

The pattern of methamphetamine absorption is similar to amphetamine, including absorption from mucosal surfaces.[32] Studies in volunteers indicate that the bioavailability of methamphetamine following ingestion is approximately 60–70%.[190] The average time between ingestion and peak plasma methamphetamine concentration was approximately 2–2.5 hours.[201] Ingestion of an 18-mg dose of methamphetamine hydrochloride by volunteers produced peak plasma concentrations of 0.035–0.038 mg/L with an average lag time from ingestion of about half an hour.[155]

INSUFFLATION

Methamphetamine easily crosses the alveolar and nasal membranes after smoking and insufflation. In a study of 11 methamphetamine abusers receiving up to 50 mg/70 kg body weight intranasally, peak cardiovascular and subjective effects occurred within 15 minutes of administration.[202] However, peak plasma methamphetamine concentrations occur about 4 hours after insufflation. Volunteer studies indicate that the bioavailability of intranasal doses of methamphetamine is near 80%.[203]

SMOKING

Absorption of methamphetamine hydrochloride (Ice) by the lungs is rapid with peak effects occurring within 15–20 minutes of the initiation of smoking.[190] Studies in volunteers indicate that the plasma methamphetamine concentration rises rapidly and then increases slowly over the next 4 hours before declining. Following the smoking of approximately 22 mg methamphetamine hydrochloride by 6 volunteers familiar with the effects of methamphetamine, the peak plasma methamphetamine concentration (0.047 ± 0.0056 mg/L) occurred at 2.5 ± 0.5 hours after initiation of smoking.[193] The peak plasma *amphetamine* (i.e., active metabolite) concentration of 0.003–0.006 mg/L in these volunteers occurred 10–24 hours after smoking began.[193] In a study of 8 experienced methamphetamine users, the mean bioavailability of the delivered doses of methamphetamine via intranasal administration and smoking were similar (i.e., 79% and 67%, respectively), depending on technique.[203] However, the absolute bioavailability of methamphetamine after smoking was substantially less (mean, 37%) than intranasal administration, primarily as a result of the amount of drug retained in the smoking apparatus.

Smoking of methamphetamine in a pipe reduces the bioavailability of the drug both by deposition of active compound in the pipe apparatus and through thermal decomposition. Following insertion of a pipe in an aluminum block heated to about 300°C, approximately 25% of the dose remained in the pipe after the completion of the smoking of a 30-mg dose of methamphetamine hydrochloride by healthy volunteers.[155] During this *in vitro* study, the recovery of intact methamphetamine base from pipes at temperatures of 400°C (752°F), 600°C (1,112°F), and 800°C (1,472°F) was approximately 98%, 88%, and 62%, respectively. The amount of amphetamine formed as a result of the complete pyrolysis methamphetamine was about 1%. The recovery of intact methamphetamine hydrochloride was slightly less than methamphetamine base with the recovery being 81%, 62%, and 38% for these temperatures, respectively.

MUCOSAL SURFACES

Case reports suggest that serious methamphetamine intoxication can develop following the concealment of methamphetamine-containing bags in the vagina. After inserting a bag of methamphetamine that leaked in her vagina, a 20-year-old woman developed seizures and apnea.[204] She recovered after intubation without obvious sequelae; however, her clinical course was complicated by aspiration pneumonia and mild rhabdomyolysis without renal dysfunction.

Distribution

Methamphetamine distributes widely to most parts of the body. The volume of distribution (V_d) of methamphetamine is approximately 3–4 L/kg, which is smaller than the V_d of phencyclidine, but higher than the V_d of cocaine. In a study of volunteers given an average

inhaled dose of 22 mg d-(+)-methamphetamine and an IV dose of 15.5 mg d-(+)-methamphetamine, the volume of distribution in the elimination phase was 3.24 ± 0.36 L/kg and 3.73 ± 0.59 L/kg, respectively.[190] Animal studies suggest that methamphetamine accumulates in the brain following distribution from the plasma. There are substantial differences in the protein binding of methamphetamine between most animal species and humans. The range of protein binding in a study of various animal species (bovine, rat, rabbit, guinea pig, horse, mouse, chicken) ranged from about 61–98%. Therefore, the pharmacokinetics of methamphetamine in animal studies must be extrapolated with caution to humans.[205]

Biotransformation

The metabolism of methamphetamine involves aromatic hydroxylation of the benzene ring at the 4-position, aliphatic hydroxylation of the β-carbon position (minor), N-demethylation to amphetamine, oxidative deamination, N-oxidation, and conjugation of nitrogen.[206,207] The 2 primary metabolites of methamphetamine biotransformation are 4-hydroxymethamphetamine and amphetamine. Amphetamine is both a minor metabolite of methamphetamine and a product of the pyrolysis of methamphetamine. Figure 1.4 demonstrates the biotransformation pathways of methamphetamine. Other minor oxidative metabolites in the urine include norephedrine, 4-hydroxynorephedrine, benzoic acid, and benzyl methyl ketoxime.[37] The metabolism of N-dimethylamphetamine also produces the active metabolite, amphetamine. Aromatic hydroxylation (4-hydroxylation) and N-demethylation of methamphetamine probably involves the cytochrome P450 isoenzyme, CYP2D6.[208] Additionally, other cytochrome P450 subfamilies (CYP2C, CYP3A) also catalyze the demethylation of methamphetamine along with cytochrome P450-independent pathways.[209] Hydroxyclobenzorex is a metabolite of illicit amphetamine that does not occur following the ingestion of therapeutic doses of amphetamines; consequently, the presence of this metabolite indicates illicit drug use.[210] The biotransformation methamphetamine varies substantially between animal species; humans metabolize a relatively smaller portion of methamphetamine and excrete a relatively larger portion of unchanged methamphetamine compared with rats and guinea pigs.[211]

Elimination

Methamphetamine is a highly basic drug with primarily renal elimination; therefore, the renal excretion of methamphetamine is dependent on urine pH, urine flow, and dose.[212] The plasma methamphetamine concentration rises rapidly after smoking and then reaches a plateau after several hours. The geometric mean plasma methamphetamine half-life in volunteers administered methamphetamine by smoking (30 mg in pipe bowl, 21.8 ± 0.3 mg estimated delivered dose) and IV injection (15.5 mg) was approximately 11 hours and 12 hours, respectively, with a range of about 8–18 hours.[190] The plasma elimination half-life of d-methamphetamine is longer than the corresponding l-enantiomer. In a study of 12 methamphetamine abusers administered IV doses of 0.5 mg/kg or 0.25 mg/kg, the plasma elimination half-life for l-methamphetamine ranged from 13.3–15.0 hours compared with 10.2–10.7 hours for d-methamphetamine.[153] The difference between the half-life of the 2 isomers was statistically significant at $P < .0001$. Metabolism of these 2 methamphetamine doses accounted for 58% and 55%, respectively, of the total clearance.

The renal excretion of amphetamine represented about 3–8% of the total methamphetamine clearance, whereas the renal excretion of unchanged methamphetamine accounted for most of the remaining clearance (i.e., about 40% of the total). Figure 1.7 displays the average urinary concentration of methamphetamine and amphetamine during the 60 hours after the administra-
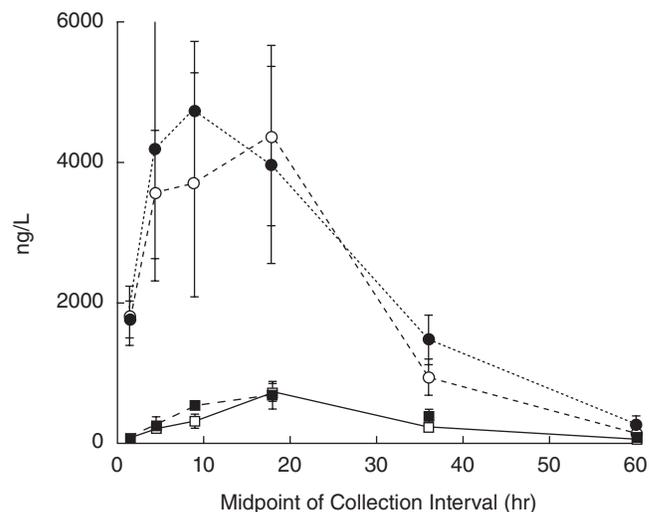
FIGURE 1.7. Urinary concentrations of amphetamine and methamphetamine after smoking 30 mg methamphetamine and the intravenous administration of 15.5 mg methamphetamine.[190] Solid circle = urine methamphetamine concentration after smoking; solid box = urine amphetamine concentration after smoking; open circle = urinary methamphetamine concentration after IV administration; open box = urinary amphetamine concentration after IV administration. (Reprinted with permission from CE Cook, AR Jeffcoat, JM Hill, DE Pugh, PK Patetta, BM Sadler, WR White, M Perez-Reyes, Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride, Drug Metabolism and Disposition, Vol. 21, p. 717, copyright 1993.)

tion of methamphetamine via smoking (30 mg in a pipe) and IV (15.5 mg) injection. Under normal conditions (urine pH 6–8), the kidney excretes about 37–54% of an absorbed methamphetamine dose as unchanged methamphetamine within the first 24 hours, 15% as *p*-hydroxymethamphetamine, and 4–7% as amphetamine. In very acidic urine (pH ≤ 5.0) the percentage of unchanged methamphetamine excreted in the urine increases up to 76%, whereas this percentage decreases in very alkaline urine (pH ≥ 8.0) to as little as 2% of the absorbed dose. In a study of 8 volunteers receiving 4 daily 10 mg (low dose) or 4 daily 20 mg (high dose) doses of *sustained-release* (*d*)-methamphetamine, the urinary terminal elimination half-life was approximately 24 ± 7 hours compared with 21 ± 7 hours for amphetamine.[213] Urinary pH was not controlled in this study and about 12% of the urine samples had a pH in excess of 8.0. Analysis of the urine data indicated that more biotransformation and less excretion of unchanged methamphetamine occurred following the high dose than the low dose. Within the first 24 hours after the *first* methamphetamine doses, the mean peak urinary excretion rate for methamphetamine was about 300 ± 200 μg/h (range, 141–600 μg/h) at about 9 ± 8 h after the last dose. In comparison, the mean peak urinary excretion rate for the low methamphetamine dose was 674 ± 571 μg/h (range, 107–1,379 μg/h) at about 10 ± 7 hours after the last dose. Consequently, the fraction of methamphetamine excreted in the urine decreases with increasing doses of methamphetamine, and the amount of urinary methamphetamine is not proportional to the absorbed dose of methamphetamine. During the course of the study from the initial administration of the 4 daily methamphetamine doses to 8 days after the last methamphetamine dose, the peak urinary methamphetamine concentrations for the low-dose and high-dose regimens were approximately 6.14 ± 2.40 μg/mL (range, 3.1–10.9) and 11.3 ± 5.1 μg/mL (range, 5.1–18.5 μg/mL), respectively. Although low amphetamine/methamphetamine ratios (<5%) suggest recent use (i.e., <3–6 h), high intra- and interindividual variability limits the use of this ratio *alone* to determine the time of methamphetamine use.

Tolerance

The progressive decline in subjective and cardiovascular effects despite the presence of high and sustained methamphetamine plasma concentrations indicates the development of tolerance.[193] This tolerance is pharmacodynamic rather than related to changes in the methamphetamine metabolism.[214] Experimental studies indicate that the administration of low doses (10 mg) of methamphetamine for 2 weeks does not alter the pharmacokinetics of methamphetamine.[215] In a 15-day residential

study, healthy volunteers received oral methamphetamine 5 or 10 mg twice daily on days 4–6 and 10–12; the volunteers received placebo on the other days.[216] Compared with baseline placebo effects, positive subjective effects of methamphetamine occurred only on day 4; however, adverse effects persisted through day 12.

Interactions

Case reports suggest that the ingestion of ethanol can potentiate the side effects of methamphetamine.[217] In volunteer studies of methamphetamine addicts, the pattern of alcohol use altered the metabolism of methamphetamine. Simultaneous ingestion of ethanol inhibited the *p*-hydroxylation and *N*-demethylation of methamphetamine.[218] However, in a double-blind, double-placebo, within-subject study of methamphetamine addicts, the administration of ethanol (1 g/kg) did not alter the subjective effects following the IV administration of 30 mg methamphetamine.[219] Case reports associate fatal serotonin syndrome with the use of methamphetamine and moclobemide.[220]

Maternal/Fetal Kinetics

Methamphetamine crosses the placenta following use by the mother during pregnancy. An infant weighing approximately 2.5 pounds died 4 hours after birth via a cesarean section for partial abruption of a low lying placenta.[221] The mother ingested methamphetamine as a diet pill during the entire pregnancy. The postmortem concentration of methamphetamine in the blood, liver, and lungs of the neonate was 0.355 μg/g, 0.246 μg/g, and 0.857 μg/g, respectively. A postmortem case series included positive methamphetamine samples from two 1-month old infants and 5 fetuses dying between the fifth and eighth month of pregnancy.[222] The postmortem fetal blood concentrations of methamphetamine and amphetamine ranged from 0.18–1.2 mg/L and from 0–0.08 mg/L, respectively. In a study of pregnant sheep, methamphetamine crossed the placenta within 30 seconds of IV administration; fetal blood pressure increased 20–37% above baseline along with a drop in fetal oxyhemoglobin saturation and arterial pH.[223] Because of the longer elimination half-life of methamphetamine in the fetal circulation, the fetal methamphetamine concentration eventually exceeded the maternal methamphetamine concentration. Pharmacokinetic studies indicate that methamphetamine diffuses into breast milk following the recreational use of methamphetamine by lactating mothers. The absolute daily infant dose of methamphetamine and amphetamine in methamphetamine equivalents in 2 infants of lactating mothers using methamphetamine was

17.5 µg/kg and 44.7 µg/kg.[224] The mothers self-administered a single IV dose of methamphetamine from their usual source (purity/dose unknown). The authors recommended withholding breast feeding 48 hours after a recreational dose of methamphetamine.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Methamphetamine stimulates the release of catecholamines from sympathetic nerve terminals, particularly dopamine in the mesocortical, mesolimbic, and nigrostriatal pathways; these structures influence emotions, motivation, reward systems, and motor output.[225] Methamphetamine enters the cytosol via passive diffusion and the membrane-bound catecholamine-uptake transporters, disrupting the pH gradient and facilitating the redistribution of monoamines in the cytosol. This process reverses the normal reuptake mechanism of the catecholamine transporters, resulting in increased movement of catecholamines into the synapses and the central and peripheral α- and β-adrenergic postsynaptic receptors.[1] Both the physiologic effects and mechanisms of action of methamphetamine and amphetamine are similar. The physiologic effects of methamphetamine result from the enzymatic inactivation, release, and uptake of catecholamine neurotransmitters (dopamine, norepinephrine), and to a lesser extent, the indoleamine, 5-hydroxytryptamine (serotonin) neurotransmitters. *In vitro* studies indicate that release of norepinephrine by methamphetamine is about twice as great as dopamine release and about 60-fold greater than serotonin release.[226] The effects of methamphetamine on the brain are complex and include dopaminergic, serotonergic, and GABAergic activity. Animal models suggest that methamphetamine stimulates the release of dopamine via several mechanisms including inhibition of monoamine oxidase and increased efflux of methamphetamine by displacement of storage vesicles. The dopamine transporter (DAT) is the primary site of action of methamphetamine as reflected by the absence of methamphetamine-induced effects in experiments with DAT knockout mice that lack the dopamine transporter.[227] Effects at monoaminergic synapses include the following: inhibition of monamine oxidase (MAO), blockade of uptake, and promotion of neurotransmitter release into the synaptic cleft. The prolonged action of methamphetamine at the synapse depletes available neurotransmitters and results in acute tolerance or tachyphylaxis. In part, this acute tolerance explains the use of escalating doses of methamphetamine during methamphetamine runs or binges.

Cardiovascular

Methamphetamine produces cardiovascular effects only in large doses, but it displays more prominent CNS effects at low doses when compared with amphetamine. A study of 2 champion cyclists demonstrated that the ingestion of 10 mg methamphetamine did not increase the capacity for aerobic exercise as measured by heart rate, minute ventilation, blood lactic acid concentration, and maximum oxygen consumption.[228] However, the administration of 10 mg methamphetamine increased their endurance of anaerobic metabolism and resulted in the ability to maintain higher levels of exercise for longer periods compared with placebo.

Central Nervous System

Experimental data indicate that at least some of the CNS effects of methamphetamine result from the alteration of pre- and postsynaptic dopamine activity in the brain. Animal data suggest an adaptive upregulation of nucleus accumbens dopamine D_1 receptor function during chronic methamphetamine administration.[229] G proteins are the intracellular messengers that link dopamine receptors to the effector enzyme, adenylyl cyclase. Dopamine receptor types are classified based on the ability to stimulate (D_1 receptors, also D_5) or inhibit (D_2 receptors, also D_3 and D_4) adenylyl cyclase through the mediation of the stimulatory or inhibitory G proteins. Although methamphetamine is usually considered more addictive than amphetamine, there are few data on the neurobiologic differences between these 2 structurally similar drugs. In a locomotor activity study of rats, methamphetamine and amphetamine similarly increased dopamine concentrations in the nucleus accumbens and the prefrontal cortex, but the dopamine concentrations in the prefrontal cortex were lower following methamphetamine than amphetamine administration.[230] The administration of amphetamine increased glutamine concentrations in the nucleus accumbens, whereas methamphetamine did not.

Mechanism of Toxicity

Cardiovascular System

Similar to amphetamine, methamphetamine causes excessive sympathetic nervous system activity and vasoconstriction, resulting in tachycardia and potentially, in susceptible patients, myocardial ischemia and cardiac arrhythmias. Dilated cardiomyopathy and congestive heart failure develops in some methamphetamine abusers, but the exact role of methamphetamine and the route of exposure require further clarification.[231] Experimental studies in animals indicate that chronic

methamphetamine administration causes myocyte atrophy, hypertrophy, cellular edema, eosinophilic degeneration, fibrosis, contraction bands, and vacuolization.[232] Ultrastructural changes include myofibrillar hypercontraction, loss of myofilaments, and mitochondrial degeneration.[233]

CENTRAL NERVOUS SYSTEM

The administration of high doses of methamphetamine to animals causes long-term depression of both dopamine and serotonin synthesis in various regions of the brain.[234,235,236] In studies of rodents, the chronic parenteral administration of methamphetamine produces marked alteration of nigrostriatal dopaminergic neurons at doses lower than those required to produce damage by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[237] A study of gerbils administered 2 subcutaneous doses of methamphetamine at 35 mg/kg demonstrated morphologic alteration of mesocortical dopamine nerve fibers and their postsynaptic structure in the frontal cortex 1 week after administration.[238] *d*-Methamphetamine is a positive reinforcer under controlled laboratory condition in both animals and humans.[239,240]

In humans, chronic abuse of high doses of methamphetamine can produce a psychosis that simulates schizophrenia. Following termination of methamphetamine administration, recurrence of the psychotic symptoms may occur after the single use of amphetamine compounds or other drugs (e.g., ethanol).[241] Although the increased release of dopamine at the synaptic vesicles and the blockade of uptake are part of the effect of methamphetamine,[242] the exact mechanism of this neurotoxic effect is unclear. Postmortem examination of chronic methamphetamine users suggests that partial desensitization of dopamine stimulation of adenylyl cyclase activity occurs in the stratum following chronic methamphetamine use (caudate, putamen, nucleus accumbens);[243] however, autopsies of brains from chronic methamphetamine users do not indicate that methamphetamine distributes preferentially to dopamine-rich areas in the brain.[244]

In a study of methamphetamine abusers, positron emission tomography (PET) scanning detected moderate reduction of dopamine transporter concentrations in the caudate and putamen averaging 27.8% and 21.1%, respectively, when compared with controls.[245] A study of former methamphetamine abusers demonstrated a reduction in the density of dopamine transporter in the nucleus accumbens and caudate/putamen, but there was no difference in the density of striatal dopamine D_2 receptors as measured by PET scanning.[246] The long-term functional and pathologic consequences of chronic depletion of dopamine and serotonin (i.e., neuroadaptation) requires further clarification to understand the effects of age, genetics, and duration of use on catecholamine reuptake transporters and presynaptic receptors.[247]

HYPERTHERMIA

Animal models indicate that intrabrain heat production is the primary cause of functional brain hyperthermia; the cerebral circulation dissipates potentially dangerous heat from brain tissue by bringing relatively cooler blood and subsequently removing warmed blood.[248] Although physiologic and behavioral activation is a transient phenomenon, brain temperature may exceed physiologic limits under severe conditions. Methamphetamine stimulates brain metabolism in a dose-dependent pattern while strongly diminishing heat dissipation as a result of peripheral vasoconstriction. The combination of neural activation in the setting of strenuous physical exercise in a hot, humid environment may cause serious elevation of brain temperatures and fatal hyperthermia.

Postmortem Examination

The most common postmortem findings include pulmonary edema, intense visceral congestion, petechial hemorrhages, relatively increased core body temperature, and increased heart weight.[249] Edema and/or congestion typically occurs in multiple organs, particularly the lung and brain.[250] Mechanisms of sudden death include ruptured berry aneurysms and aortic dissection with cardiac tamponade.[251] Hyperpyrexia with subsequent rhabdomyolysis and hepatorenal failure commonly occurs during fatal methamphetamine intoxication.[252]

HEART

Postmortem examination of hearts from methamphetamine abusers may or may not demonstrate structural abnormalities. A 31-year-old woman, who smoked methamphetamine regularly, developed diffuse systemic vasospasm, an acute myocardial infarction and fatal cardiogenic shock.[253] Postmortem examination revealed diffuse transmural myocardial ischemia with patchy interstitial fibrosis and focal acute infarction of the posterior left ventricle and lateral papillary muscle. There was no histologic evidence of an active myocarditis. Histologic examination of methamphetamine addicts, who died suddenly, reveal hemorrhage and congestion of the lungs with either no changes[254] or relatively minor cardiac abnormalities.[255,256] These cardiac abnormalities included both hypertrophy and atrophy

of myocytes, disarray of myofibrils, endocardial hemorrhage, eosinophilic changes, small round cell infiltration, and degeneration of myofibrils including myolysis and contraction band necrosis.[257] Histologic examination of cardiac tissue may reveal varying degrees of coronary arteriosclerosis, but pathologic changes of a myocardial infarction are rare.[258]

CENTRAL NERVOUS SYSTEM

Intracranial abnormalities on postmortem examination of methamphetamine abusers include intracerebral hematoma with cerebral edema and uncal herniation,[259] subarachnoid hemorrhage with or without cerebral aneurysm, and necrotizing angitis.[62] Intraventricular hemorrhage following methamphetamine use is relatively rare compared with intracerebral or subarachnoid hemorrhage.[260] The pathologic changes of the cerebral vasculitis associated with methamphetamine abuse are similar to the changes of polyarteritis nodosa, but these changes involve primarily the large arteries in contrast to the vascular changes associated with typical hypersensitivity angiitis.[261] Postmortem examination of the brains of methamphetamine users indicates that methamphetamine is distributed relatively evenly throughout the brain rather than concentrating in dopamine-rich areas.[262]

CLINICAL RESPONSE

Illicit Use

Methamphetamine, amphetamine, and cocaine produce similar clinical effects with the major distinguishing feature being the prolonged effect of methamphetamine or amphetamine compared with cocaine. Agitation and sinus tachycardia were the most common effects in a retrospective review of 47 methamphetamine exposures in children under the age of 6 years as reported to the California Poison Control System.[263] Seizures occurred in 2 cases (9%). Following the ingestion of large doses of methamphetamine, CNS symptoms begin within 30–60 minutes and persist for 4–6 hours. The onset of symptoms following the inhalation of methamphetamine is much more rapid than ingestion. Unusual complications associated with methamphetamine toxicity include decreased visual acuity secondary to retinal vasculitis[264] or hemorrhage.[265] Methamphetamine abuse is a common cause of rhabdomyolysis. In a retrospective study of 367 emergency department patients with rhabdomyolysis (serum creatine kinase ≥1000 U/L), 43% of the patients had positive urine drug screens for methamphetamine.[266] Case reports document rhabdomyolysis with myoglobinuria, hyperpyrexia, leukemoid

reaction, disseminated intravascular coagulation, and acute renal failure following IV administration of phenmetrazine and methamphetamine.[199] Headache, myalgias, lightheadedness, paresthesias, weakness, and delirium can occur along with hyperthermia.

Suggestive signs of chronic methamphetamine abuse include involuntary repetitive movements (rocking, fidgeting), multiple crusts on the skin (skin picking), extensive dental caries at base of anterior maxillary teeth, cachexia, fatigue, forgetfulness, agitation, and irritability. Case reports associate chronic amphetamine abuse with choreoathetoid movements that typically resolves with 12–24 hours.[267] Rampant dental caries often occur following the chronic abuse of methamphetamine.[268] The characteristic pattern of tooth decay involves the buccal smooth surface of the posterior teeth and the interproximal of the anterior teeth.[269] These caries often progress to complete destruction of the coronal portion of the tooth. The etiology of this pattern is unclear, but contributing factors include xerostomia, bruxism, and lack of oral hygiene.[270] Rare case reports associate methamphetamine abuse with transient visual loss, retinal vasculitis, and retinal hemorrhage.[271,272]

BEHAVIORAL ABNORMALITIES

Most methamphetamine users do not display overt signs of methamphetamine use. An acute anxiety reaction characterized by dysphoria, agitation, and muscle tremors was the most common acute psychiatric complaint of patients presenting to the Haight-Ashbury Clinic (San Francisco, CA) during the epidemic of methamphetamine use during the 1960s.[188]

MENTAL DISORDERS

The prevalence of psychotic symptoms is substantially higher in methamphetamine users than the general population, particularly in dependent methamphetamine abusers. In a cohort of 309 recruited individuals using methamphetamine at least monthly, 13% of the participants screened positive for psychosis with 23% experiencing at least 1 clinically significant symptom of suspiciousness, unusual thought content, or hallucinations in the previous year.[273] Psychotic reactions occur in methamphetamine abusers with or without a prior history of a psychiatric disorder, but premorbid schizoid/schizotypal personality and early, heavy methamphetamine abuse predispose users to methamphetamine-related psychosis.[274] Separating the effects of drug abuse and comorbid psychiatric disorder is difficult because over 50% of individuals with a history of schizophrenia have a coexisting substance

abuse disorder.[275] Furthermore, about 50% of individuals with methamphetamine dependence have comorbid psychiatric disorders other than substance abuse, particular anxiety disorders (e.g., phobias, social/generalized anxiety), mood disorders (major depression), and antisocial personalities.[276] Experimental studies indicate that a single dose of methamphetamine can cause brief (i.e., few hours) increases in psychotic features in schizophrenic patients.[277] In a experimental study of 14 psychotic patients with a history of methamphetamine abuse, the IV administration of methamphetamine in doses up to 640 mg over 1 hour produced psychotic symptoms that persisted up to 5–6 days in 2 patients.[278] The persistence of psychotic symptoms longer than a week in 1 patient was associated with recurrent methamphetamine use.

The clinical presentation of methamphetamine-induced and amphetamine-induced psychoses is similar. In a select population of 31 incarcerated, chronic IV methamphetamine abusers with psychosis, common symptoms included auditory and visual hallucinations, delusions of persecution and reference, thought broadcasting, depression, and suicidal ideations.[279] Orientation, memory, and sensorium usually remain clear, but transient, fluctuating disturbances of consciousness may occur during periods of florid psychosis after heavy methamphetamine use.[280]

Although the acute psychosis induced by methamphetamine resembles paranoid schizophrenia, several characteristics other than the clearing of psychotic symptoms with abstinence separate drug-induced psychoses from paranoid schizophrenia.[281] For example, paranoid schizophrenics typically manifest more clouding of consciousness, auditory hallucinations, thought disorders, flat affect along with less sexual stimulation and stereotyped repetitive behavior than patients with methamphetamine-induced psychoses.[282] About 29% of 31 incarcerated, chronic IV methamphetamine abusers with psychosis suffered from recurrent episodes of psychosis (flashbacks).[279] In this population of methamphetamine users, psychologic stress, cessation of antipsychotic medication, or recurrent drug use precipitated these flashbacks.[283,284] Cocaine- and methamphetamine-induced psychoses are too similar to distinguish based on presenting signs and symptoms. Other drugs associated with psychoses include bromide, alcohol, hallucinogenic drugs, L-dopa, monoamine oxidase inhibitors, and tricyclic antidepressants.

In contrast to the psychosis associated with schizophrenia, the methamphetamine-induced psychosis typically resolves shortly after cessation of drug use; occasionally, the psychotic symptoms are persistent and refractory to pharmacologic treatment despite abstinence. Most patients with methamphetamine-induced psychosis improve within hours to a week of abstinence, but the psychosis may persist over 3–6 months in some patients, particularly after prolonged methamphetamine abuse.[285,286] Spontaneous recurrence of methamphetamine-induced psychoses occasionally develop in patients with a previous history of drug-induced psychosis, particularly during periods of psychologic stress that involve fear of other people.[287,288] The acute relapse into psychosis may occur after an extended asymptomatic period during abstinence from methamphetamine use. The relapse is similar to the previous psychotic episode with typical clinical features including bizarre delusions, auditory hallucinations, delusions with jealous or persecutory content, and incoherence or loosening of association.[289] Ethanol or small doses of methamphetamine (i.e., 50 mg over 5 days) may reactivate the extreme paranoia along with negativistic and suspicious attitudes.[290] Case reports suggest that the use of stimulant drugs can exacerbate the presence of chronic fluctuating somatic and vocal tics (e.g., eye blinking, jaw jerks, hip turning, humming, panting, muscle jerks).[291] Parkinsonism is not a prominent clinical feature of methamphetamine use despite the reduction of dopamine concentrations in the caudate of chronic methamphetamine users.[292]

MEDICAL COMPLICATIONS

CENTRAL NERVOUS SYSTEM.   Cerebrovascular complications associated with methamphetamine abuse include ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage, particularly in the anterior circulation.[293] Frequently, these patients have underlying vascular pathology including atherosclerosis, stenosis, and/or aneurysms. Case reports associate the IV use of methamphetamine with the development of necrotizing angiitis and both ischemic and hemorrhagic stroke including fatal pontine hemorrhage.[294] Cerebral embolism may result from endocarditis complicating IV methamphetamine abuse. Fatal spontaneous intracranial hemorrhage and subarachnoid hemorrhages can occur without evidence of vasculitis or structural abnormalities.[295] Occasional intracerebral hemorrhages occur in the basal ganglia following methamphetamine inhalation.[28,296] Although an ischemic stroke is less common than a hemorrhagic stroke following drug abuse, ischemic strokes do occur after methamphetamine abuse by oral, IV, or pulmonary routes.[297] The cause of these strokes is multifactorial.[298] Although most of the patients with subarachnoid hemorrhages had cerebral aneurysms,[299] most of the cases of thrombosis and intracerebral hemorrhage were not associated with documented vasculitis, vascular malformations, or vasospasm.[78]

CARDIOPULMONARY SYSTEM.   A few case reports associate acute myocardial infarction with the inhalation of methamphetamine.[300] Although these patients frequently have coronary artery disease, some reports associate the inhalation of methamphetamine with the development of myocardial injury and pulmonary edema without the presence of significant coronary artery disease.[253] Epidemiologic data suggests that methamphetamine use modestly increases the risk of acute myocardial infarction. In a study of 11,011 acute myocardial infarctions identified in a quality indicators database from Texas, the adjusted odds ratio for acute myocardial infarction in methamphetamine users was 1.61 (95% CI: 1.24–2.04, $P = 0.0004$).[301] The adjusted confounders included cocaine, alcohol, and tobacco use, hypertension, diabetes mellitus, lipid disorders, obesity, congenital defects, and coagulation abnormalities. Noncardiogenic pulmonary edema following methamphetamine use is very rare. A case report associated the development of pulmonary edema following methamphetamine inhalation with normal pulmonary artery pressures.[302] No cardiac catheterization was performed. Case reports associate the development of acute aortic dissection with hypertensive crises following the use of methamphetamine. In a case series of 109 patients presenting to an urban hospital with aortic dissection, patients with methamphetamine abuse accounted for 5.5% of patients of all ages and 20% of the patients in this series under 50 years of age.[303]

Case studies of cardiomyopathies following the abuse of methamphetamine are rare; the contribution of drug impurities and predisposing factors to the development of these cardiomyopathies remains unclear. Case reports suggest that a cardiomyopathy may develop following the chronic ingestion of methamphetamine without evidence of myocardial necrosis, and the cardiomyopathy typically resolves with cessation of methamphetamine use.[304] A retrospective review of patients ≤45 years of age hospitalized for congestive heart failure suggested that a history of methamphetamine use was associated with a more severe cardiomyopathy.[305] The reduction in left ventricular ejection fraction was statistically significant at $P = 0.004$. However, there was no documentation of a dose response.

PERIPHERAL VASCULAR. Perivascular infiltration of methamphetamine can produce local necrosis, cellulitis, granulomas, and abscess formation. Intra-arterial injection causes intense vasospasm with distal cyanosis, ecchymosis, petechiae, edema, paresthesias, pain, weakness, necrosis, and decreased capillary filling. Immediate intense vasospasm is obvious after intra-arterial injections. Rarely, case reports associate ischemic colitis with methamphetamine use via exposure routes other than IV (e.g., smoking, oral).[306,307]

Fatalities

Death from methamphetamine use alone without underlying cardiac and/or vascular pathology is relatively rare except following intentional ingestion of massive amounts of methamphetamine. In one case series of postmortem examinations with detectable quantities of methamphetamine, two-thirds of fatalities involved with methamphetamine use resulted from violence, including accidents, suicide, and, less often, homicide.[254] The most common nontraumatic, nonoverdose causes of death associated with methamphetamine use include cerebrovascular hemorrhage and heart disease.[308] Rarely, hallucinations and sudden death occur during strenuous exercise in warm environments, particularly in chronic, heavy methamphetamine abusers. Persistent seizures, hyperthermia (>39–40°C/102.2–104°F), coma, severe tachycardia (>160–170 beats/minute), shock, renal failure, severe rhabdomyolysis, and focal weakness are poor prognostic signs.[309,310] Sudden death may occur during restraint of an agitated, chronic methamphetamine abuser in the same manner as the chronic use of high doses of cocaine causes excited delirium with hyperthermia, severe lactic acidosis, and sudden death during restraint occurring in both the hospital setting and police custody.[311,312]

Abstinence Syndrome

As a binge progresses, unpleasant side effects ("tweaking") replace the pleasurable effects of using methamphetamine including dysphoria, depression, fatigue, paranoia, akathisia, anxiety, irritability, confusion, insomnia, and drug craving.[313] The intensity of these symptoms usually peaks 1–3 days after cessation of methamphetamine use. The acute phase of amphetamine withdrawal initially involves increased sleeping ("nod off") and eating followed by anhedonia, irritability, poor concentration, anxiety, and craving-during the depressive phase ("crash"). Typically, these symptoms are mild and most symptoms resolve within several days to 1 week.[314] Although some symptoms may last several weeks, the persistence of severe symptoms beyond 1 week suggests an underlying disease process.[315]

Reproductive Abnormalities

Based on animal studies and limited human data, potential adverse effects of methamphetamine abuse during pregnancy include low birth weight,[316] prematurity, and malformations (cleft palate, cardiac anomalies);[317]

however, multiple sociologic and economic variables as well as polydrug use complicate the interpretation of the effect of methamphetamine on fetal outcomes.[92,318] In general, studies of mothers using illicit drugs (i.e., methamphetamine) demonstrate higher rates of prematurity and intrauterine growth retardation (reduced body weight and length, smaller head circumference) in the drug-exposed group compared with groups of mothers without a history of illicit drug use.[319,320] Case reports also associated methamphetamine use by pregnant women with fetal death and maternal complications (eclampsia, HELLP syndrome, amnionic emboli, death).[321] Follow-up studies of children from mothers using methamphetamine during pregnancy indicate a high rate of behavioral problems (aggressive behavior, problems with peers) and poor school performance (delayed development in math and language achievement), but the psychosocial effects associated with maternal drug use are difficult to separate from the effects of methamphetamine use during the prenatal period.[322] Many of these studies suffer from small sample size, inadequate control groups, poor exposure data, and confounding with other prenatal drug use.

DIAGNOSTIC TESTING

Analytic Methods

Screening

Immunoassays are sensitive methods for screening urine samples for the presence of methamphetamine, but these techniques lack specificity. Newer immunoassays (e.g., EMIT II®) are more specific than older assays (e.g., EMIT d.a.u.®), but some assays cross-react with high concentrations of phenmetrazine, phentermine, benzphetamine, illicit hallucinogenic derivatives (MDA, MDEA, MDMA), antidepressants (bupropion),[323] and some phenothiazine compounds (chlorpromazine, promethazine).[324,325] Gas chromatography is a popular method for screening methamphetamine because of the lack of native fluorescence, significant oxidative electrochemical properties at low voltage, and good ultraviolet absorption characteristics. Methamphetamine elutes early in underivatized samples; some methamphetamine may be lost during the time the detector is turned off for the elution of the solvent front.[150] High concentrations of ephedrine or pseudoephedrine may result in the formation of methamphetamine at the injector port of the gas chromatograph.[326]

Confirmatory

Illicit Use.   The NIDA guideline requires that positive urine samples contain amphetamine and methamphet-

amine concentrations exceeding 200 ng/mL and 500 ng/mL, respectively. Positive screening tests for methamphetamine require confirmation by more specific methods (e.g., gas chromatography/mass spectrometry), which provide structure-specific information. Human studies suggest that the requirement for the presence of >200 ng amphetamine/mL may result in substantial numbers of false-negative samples because of the relatively small amount of amphetamine excreted after the use of methamphetamine as a result of variations in dose and dosing frequency of methamphetamine. The pharmaceutic preparation of methamphetamine in the United States is the *d*-enantiomer; therefore, the presence of both *d*- and *l*-enantiomers suggests the use of illicit methamphetamine unless the individual ingested prescription drugs (e.g., ethylamphetamine, famprofazone, fencamine, fenproporex, prenylamine) that are metabolized to methamphetamine and contain both enantiomers.[327] Separation of these 2 enantiomers requires special chiral columns or derivatizing reagents. High performance liquid chromatography retention time data are not compound-specific like the data obtained by gas chromatography/mass spectrometry methods; therefore, more interference occurs during high performance liquid chromatography procedures compared with gas chromatography/mass spectrometry.[93] In contrast to gas chromatography/mass spectrometry, liquid chromatography/tandem mass spectrometry does not require sample derivation or hydrolysis. The interassay variability (coefficient of variation) of liquid chromatography/tandem mass spectrometry between days for methamphetamine was approximately 10–12%.[328] Detection limits for methamphetamine in serum samples using a solid phase microextraction method combined with liquid chromatography/electrospray ionization/tandem mass spectrometry was 0.04 ng/mL.[105] Enhanced polymer column extraction with gas chromatography/mass spectrometry in scan mode is a simple, reliable method to determine the presence of structurally related amphetamine compounds (e.g., MDMA, MDA, PMA) that cross-react with the methamphetamine immunoassays.[329] The lower limits of detection of these compounds ranges between 5–50 ng/mL. Excessively high temperatures in the injector can cause a confirmed positive for methamphetamine when ephedrine is present in the sample and heat-catalyzed reduction of the ephedrine to methamphetamine occurs. However, the sample should not contain the required 200 ng/mL amphetamine. Phentermine is a structural isomer of methamphetamine; consequently, the similar mass spectrum for these 2 structurally similar drugs requires careful analysis of the retention times and mass spectra for consistency. Derivatization resolves the lack of specificity of methamphetamine mass spectrum.

MEDICINAL USE.   The use of gas chromatography/mass spectrometry does not necessarily differentiate the illicit use of methamphetamine or amphetamine from the use of prescription drugs that contain these compounds or compounds that are metabolized to amphetamine and/or methamphetamine.[106] The interpretation of drug testing results involving the alleged use of amphetamine/methamphetamine precursor drugs requires analysis of the following factors: 1) detection of parent drug or unique metabolite, 2) ratio of *d*-/*l*-enantiomers of methamphetamine and amphetamine, and 3) methamphetamine and/or amphetamine concentrations relative to the history of prescription drug use. In a study of urine samples from 10 volunteers collected during the 12-hour period following the administration of 30 mg *d*-methamphetamine, about 90% of the methamphetamine-positive samples did not contain amphetamine concentrations above the 200 ng/mL standard.[330] Some biologic samples with high concentrations of methamphetamine may not contain detectable concentrations (>50 ng/mL) of *d*-amphetamine.[331] Gas chromatography/mass spectrometry with electro impact or chemical ionization separates amphetamine and methamphetamine from related amines including ephedrine, phenylpropanolamine, phentermine, and synthetic amphetamine analogues (e.g., methylenedioxymethamphetamine).[332]

Medicinal drugs that produce amphetamine or methamphetamine as metabolites include the following: amphetaminil, benzphetamine, clobenzorex, deprenyl, dimethylamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylline, fenproporex, furfenorex, mefenorex, mesocarb, prenylamine, and selegiline. Medications with unique metabolites include 4-hydroxyclobenzorex (clobenzorex), famprofazone (3-hydroxymethylpyrazolone, *p*-hydroxydesmethylfamprofazone), fenethylline (7-carboxymethyltheophylline), fenproporex (dihydroxyfenproporex, hydroxyfenproporex), furfenorex (1-phenyl-2-[*N*-methyl-*N*-γ-valerolactonylamino]-propane), mefenorex (hydroxymethoxymefenorex, hydroxymefenorex), and prenylamine (diphenylpropylamine).[327] The Vicks® inhaler contains 50 mg of *l*-methamphetamine (desoxyephedrine). Therapeutic use of these inhalers does not usually produce positive results on currently available immunoassays, but excessive use of these inhalers potentially may produce false-positive results.[333] Although the Vicks® inhaler sold in the United States contains 50 mg *l*-methamphetamine, Vicks® inhalers from other countries may contain different concentrations of enantiomers.[334] Enantiomeric separation using chiral chromatography or derivatization techniques (e.g., chiral gas chromatography/mass spectrometry or high performance liquid chromatography) differentiate the use of Vicks® inhaler from the use of racemic methamphetamine.[335] Currently, the common reduction process for methamphetamine production yields enantiomerically pure *d*-methamphetamine. Consequently, the detection of essentially pure *l*-enantiomers of methamphetamine and amphetamine indicates the use of these inhalers. Metabolism of prescription drugs that produce only the *d*- and/or *l*-enantiomer include benzphetamine (*d*-enantiomer), deprenyl (*l*-enantiomer), dimethylamphetamine (*d*-enantiomer), ethylamphetamine (*d*- and *l*-enantiomer), famprofazone (*d*- and *l*-enantiomer), fencamine (*d*- and *l*-enantiomer), fenproporex (*d*- and *l*-enantiomer), prenylamine (*d*- and *l*-enantiomer).[327]

HAIR.   Methods for the quantitation of methamphetamine in hair samples include liquid chromatography/mass spectrometry and gas chromatography/mass spectrometry (gas chromatography/mass spectrometry). Liquid chromatography/mass spectrometry is highly sensitive and reproducible, whereas capillary gas chromatography produces a sharp peak and electron ionization produces highly reproducible mass spectra. Gas chromatography/mass spectrometry coupled with micropulverized extraction, aqueous acetylation and microextraction by packed sorbent allows the determination of methamphetamine in a 1 mg hair specimen with <20% deviation from the lower limit of quantitation (0.2 ng/mg).[336]

STREET SAMPLE ANALYSIS

Impurities appear during the illicit manufacture of methamphetamine as a result of incomplete reactions and inadequate purification of intermediate and final products. Methamphetamine may contain additives (e.g., methylsulfonylmethane or dimethyl sulfone) or adulterants (e.g., other stimulants) as a result of additions to the methamphetamine after the synthetic process. There are several routes of methamphetamine synthesis, and each route produces unique organic impurities. Methods for profiling impurities in illicit methamphetamine include gas chromatography with flame ionization detector,[337] high performance liquid chromatography using column switching,[20] and gas chromatography/mass spectrometry after liquid/liquid extraction with organic solvents,[338] headspace solid phase microextraction,[339] or thermal desorption.[340] Analysis of illicit samples with these methods provides useful information regarding drug sources and trafficking routes.[341]

The presence of nonreacted phenylacetone or the presence of a racemate in an illicit drug sample of methamphetamine suggests the use of the Leuckart

method of methamphetamine synthesis. α-Benzyl-phenethylamine derivatives are the most common contaminates of the reductive amination of phenylacetone using the Leuckart procedure to produce amphetamine and methamphetamine.[342] Side reactions and incomplete conversions during the Leuckart process result in a variety of impurities and intermediate products, including benzyl methyl ketone, dibenzyl ketone, formamide, di(1-phenylisopropyl) formamide, formic acid, methylamine, N,N-dimethyl amphetamine, N-formyl amphetamine, di(1-phenylisopropyl)amine, benzylamine, and several pyrimidine and pyridine compounds.[343,344] Benzaldehyde is a common contaminant of methamphetamine produced when phenylacetone (P2P) is synthesized from the reaction of benzaldehyde with nitroethane followed by hydrogenation with iron and hydrochloric acid. However, the presence of benzaldehyde is not specific for this type of reaction.

Impurities from the clandestine synthesis of methamphetamine from ephedrine or pseudoephedrine via hydriodic acid include 1-benzyl-3-methylnaphthalene and 1,3-dimethyl-2-phenylnaphthalene.[345] An aziridine is a potentially toxic intermediate of the reduction of ephedrine, probably through the intermediate formation of iodoephedrine with the Red P/HI method. The Birch reduction of ephedrine or pseudoephedrine to form methamphetamine involves treatment of the starting material with an alkali metal (e.g., lithium or sodium in liquid ammonia). During this process, an over-reduction causes the partial reduction of the aromatic ring along with the production of small amounts of the cyclohexadienyl analogue of methamphetamine. Several of these impurities (e.g., α-benzyl-N-methyl-phenethylamine or BNMPA) are pharmacologically active, but the toxic effects of these impurities remains relatively unreported.[346,347] Rarely, lead contaminates illicit methamphetamine.[348] Although postproduction adulteration is the most likely cause of lead contamination in illicit methamphetamine, potentially lead can contaminate the methamphetamine syntheses when lead acetate and phenyl acetic acid are used to produce phenylacetone (phenyl-2-propanone).

STORAGE

Methamphetamine is relatively stable in properly stored and frozen postmortem samples. Animal studies and examination of exhumed material indicate that methamphetamine is fairly stable in the blood and bone marrow over several years.[349] *In vitro* studies indicate that methamphetamine is stable in urine samples stored at −20°C in 1% sodium fluoride for 2 years.[108] In a study of methamphetamine concentrations in samples stored at ambient temperature in gray-top Vacutainer® (Becton

Dickinson, Franklin Lakes, NJ) tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate, the mean decrease in methamphetamine concentrations at 6 months and 3 years was 9.31% and 38.1%, respectively.[109]

Biomarkers

The time course of the deposition of methamphetamine and amphetamine in oral fluids and plasma is similar, but the concentrations of these drugs are higher and the detection time shorter compared with urine. In a study of 5 volunteers receiving 10 mg or 20 mg methamphetamine daily for 4 days, the mean detection times were 0.02 and 4.8 hours, respectively, using the recommended cutoff (5 ng methamphetamine/mL, >2.5 ng amphetamine/mL).[350] The mean times to last positive specimen at this cutoff were 4.0 hours and 20.6 hours, respectively. Analysis of urine samples had higher detection times and rates compared with oral fluids.

BLOOD

THERAPEUTIC USE.   Following the ingestion of a liquid dose of 12.5 mg methamphetamine, the mean peak plasma concentration of methamphetamine was about 0.02 mg/L as measured by gas chromatography with flame-ionization detection.[201] Volunteer studies indicate that the central (arousal) and peripheral sympathomimetic effects (i.e., elevated heart rate and blood pressure) begin at plasma methamphetamine concentrations near 0.005 mg/L and 0.02 mg/L, respectively.[351] Doses of methamphetamine in clinical studies do not usually exceed 60 mg, which corresponds to a maximum blood methamphetamine concentration of approximately 0.1–0.2 mg/L.[352] The actual physical effects of methamphetamine are somewhat variable depending on the duration of drug use, psychiatric state, and the situation as well as the methamphetamine concentration. Moderate subjective effects, as defined by a mean score of 38% of the maximum "high" ever achieved, occurred in 6 experienced methamphetamine users following the smoking of 30 mg (inhaled dose of about 22 mg) methamphetamine hydrochloride.[193] The mean peak plasma concentration of methamphetamine was approximately 0.047 mg/L.

The clinical effects of methamphetamine decline more rapidly than the concentration of methamphetamine in the plasma as a result of acute tolerance to the effects of methamphetamine. Consequently, the acute subjective and cardiovascular effects of methamphetamine subside despite the presence of methamphetamine in the plasma. In a study of 8 experienced methamphetamine users, each volunteer received either a 50 mg intranasal dose of methamphetamine along with 10 mg deuterium-labeled methamphetamine
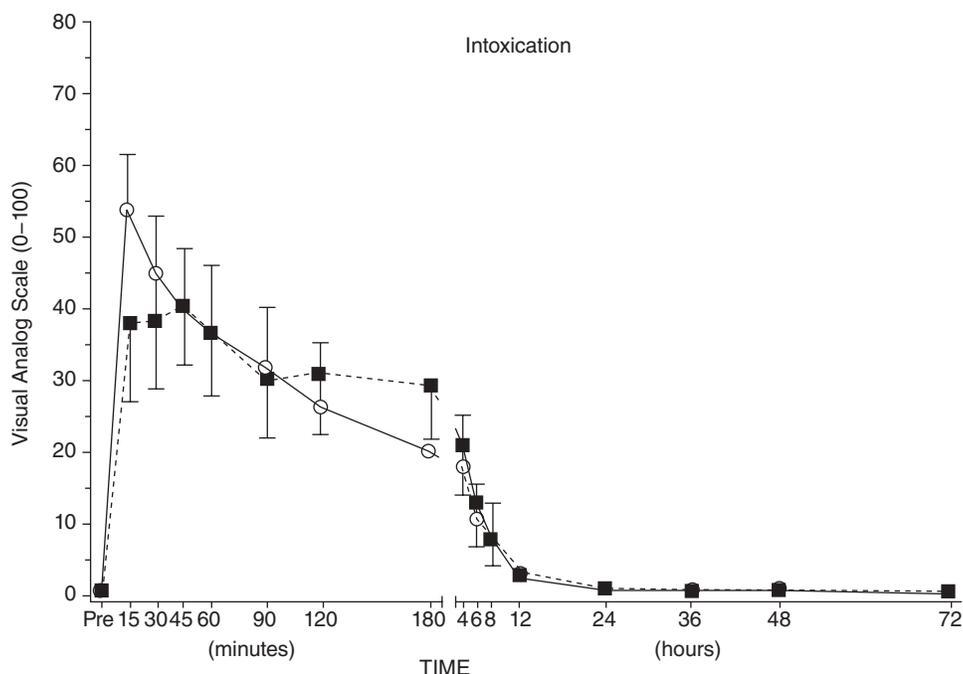
FIGURE 1.8. Subjective effects of intranasal (squares) and smoked (circles) methamphetamine based on Visual Analog Scale (0–100) over 72 hours.[203] Each participant also received 10 mg intravenous deuterium-labeled methamphetamine with the intranasal or smoked dose of methamphetamine. (Reprinted by permission from Macmillan Publishers Ltd: Clinical Pharmacology & Therapeutics, Vol. 74, DE Harris, H Boxenbaum, ET Everhart, G Sequeira, JE Mendelson et al., The bioavailability of intranasal and smoked methamphetamine, p. 484, 2003.)

intravenously or 40 mg of smoked methamphetamine along with 10 mg deuterium-labeled methamphetamine IV.[203] Subjective effects of methamphetamine were measured by a visual analog scale from 0–100 with zero being no effect and 100 being "most ever." Figure 1.8 displays the time course of the visual analog scale for intoxication following the administration of methamphetamine via smoking and intranasal administration.

Illicit Use. The clinical effects of methamphetamine depend, in part, on the presence of tolerance. In a case series of 7 chronic methamphetamine abusers exhibiting signs of violence and irrational behavior, the blood methamphetamine concentrations ranged from 0.15–0.56 mg/L as measured by gas chromatography with hydrogen-flame ionization detector.[353] Of 1,265 blood samples submitted for analysis from individuals involved in criminal activity (driving under the influence, rape, homicide, under influence of a controlled substance), about 12% (157 samples) tested positive for methamphetamine.[354] The methamphetamine concentrations ranged from 0.025–2.03 mg/L with a mean concentration of 0.308 mg/L.

Overdose. A review of animal and postmortem data suggested that mild, moderate, and serious intoxications occur at blood methamphetamine concentrations near

0.3 mg/L, 0.4–0.5 mg/L, and 3 mg/L, respectively.[355,356] Following a methamphetamine overdose, blood methamphetamine concentrations in the range of 1–2 mg/L are associated with severe methamphetamine intoxication (hyperthermia, DIC, renal failure, rhabdomyolysis) and death. A 33-year-old male was arrested for driving erratically, and his initial whole blood methamphetamine concentration was 0.23 mg/L.[357] About 3–4 hours after ingesting an unknown amount of methamphetamine, he developed confusion, agitation, and diaphoresis, followed by seizures, hyperthermia, and a marked sinus tachycardia. The serum methamphetamine concentration was 1.94 mg/L. He later developed hyperthermia and fatal DIC. Following a methamphetamine overdose, the plasma methamphetamine concentration 1 hour prior to death from hyperthermia and multiorgan failure was 6.74 μmol/dL (approximately 10 mg/L) with a blood ethanol concentration near 50 mg/dL.[358] A 41-year-old man presented to the emergency department with agitation, diaphoresis, marked sinus tachycardia (160 beats/min), hallucinations, and a temperature of 41.6°C (106.9°F).[359] The serum methamphetamine and amphetamine concentrations on admission 6 hours after the injection of methamphetamine were 0.3 mg/L and 0.04 mg/L, respectively. He recovered after a prolonged course of rhabdomyolysis and hepatorenal

failure. Rarely, survival occurs when the blood methamphetamine concentration exceeds 3–4 mg/L. A driver ingested a bag of methamphetamine following a routine traffic stop, and his serum methamphetamine concentration 1 hour later on arrival to the hospital was 9.46 mg/L.[360] He developed dysrhythmias and seizures, but he survived with supportive care. Details of his medical care were not reported. There are few data on the ratio of whole blood methamphetamine/plasma methamphetamine concentrations.

POSTMORTEM.   There are limited data on postmortem blood concentrations of methamphetamine; therefore, the interpretation of the significance of a specific postmortem concentration of methamphetamine requires careful analysis of the circumstances surrounding the death, the behavior of the individual, the autopsy, prescription medications, anatomic site of postmortem sample collection, and the reliability of the sample integrity. In particular, the lower ranges of methamphetamine concentrations must be interpreted with caution because of tolerance and the subjectivity associated with the determination of the contribution of methamphetamine to the cause of death.[164,361]

A study of 413 autopsy reports of cases with detectable methamphetamine in postmortem blood did not find a statistically significant difference ($P = 0.65$) in the mean methamphetamine concentration (2.08 mg/L) between deaths related to the medical effects of methamphetamine and the mean methamphetamine concentration (1.78 mg/L) in deaths not related to the medical effects of methamphetamine.[249] The use of some prescription medications may produce small, but detectable amounts of amphetamine and methamphetamine in postmortem blood samples. The concentration of methamphetamine and amphetamine in postmortem heart blood samples from a 72-year-old woman, who was found dead with a suicide note, was 0.28 mg/L and 0.08 mg/L, respectively.[362] Her list of prescribed medications included selegiline, desipramine, trazodone, and bromocriptine. The source of the methamphetamine and amphetamine was probably the metabolism of selegiline. Consequently, the postmortem methamphetamine concentration should not be used alone to determine the cause of death.

Concentrations.   Most deaths related to methamphetamines are associated with postmortem methamphetamine concentrations exceeding 0.5–1 mg/L. In a series of 13 autopsies in which methamphetamine was the only drug present in toxicologically significant quantities, the methamphetamine concentration in postmortem blood samples (site not reported) ranged from 0.09–18 mg/L with a median of 0.96 mg/L.[363] There is a wide range of

methamphetamine concentrations in blood samples from methamphetamine-related deaths, and the range of methamphetamine concentrations in blood samples from homicides and accidental overdoses usually overlap.[364] In a case series of 23 homicides and 9 accidental overdoses involving only methamphetamine use, the mean (± standard deviation) methamphetamine concentrations in postmortem blood from the 23 homicides and 9 accidental overdoses was 0.66 (±1) mg/L and 0.980 (±1) mg/L, respectively.[365] Amphetamine is a metabolite of methamphetamine, and case reports indicate that the amphetamine concentrations in postmortem blood from fatal cases of methamphetamine overdose are approximately 4–5% of the blood methamphetamine concentration.[366]

Redistribution.   Some postmortem redistribution of methamphetamine likely occurs because of the basic pK_a and the diffusion of methamphetamine from the sites in the pulmonary veins to the left side of the heart.[367] Additionally, failure to ligate the femoral vein during aspiration of postmortem blood may cause an increase in redistribution of methamphetamine from central to peripheral blood in cases involving the ingestion of large amounts of methamphetamine.[311] In postmortem samples from 4 methamphetamine abusers, blood samples from the left heart were 1.9–2.6 times higher than blood samples from the right heart.[368] The methamphetamine concentrations in samples from the femoral veins were closer to the concentrations in the right heart than to the left heart. In a case series of 3 fatalities, the left/right heart blood ratio was 1.0, 1.59, and 2.06 with the highest ratio occurring in autopsy blood containing the highest concentration of methamphetamine.[367] In a case series of 20 autopsies, the heart/femoral blood ratios of methamphetamine averaged 2.1 with a range of 1.2–5.0.[369] The presence of large amounts of methamphetamine in the stomach may result in the postmortem diffusion of significant amounts of methamphetamine to cardiac blood.[200] Stomach fluid and saliva may contain small amounts of amphetamine (i.e., about 0.1% of the blood methamphetamine concentration) as a result of the diffusion of amphetamine from surrounding blood vessels.[252]

URINE

The absorption of methamphetamine is rapid with the appearance of the parent compounds in the urine beginning about 20 minutes after ingestion. The concentration of methamphetamine depends on a number of variables including dose, duration of use, urine pH, urinary excretion rates, and individual pharmacokinetics. Figure 1.7 demonstrates urine methamphetamine

and amphetamine concentrations in urine samples from volunteers receiving 15.5 mg methamphetamine intravenously or 30 mg methamphetamine via a glass pipe. The mean methamphetamine concentration in urine samples from 30 outpatients presenting for treatment of methamphetamine abuse was 18.1 ± 26.2 mg/L (range, 0–101.5 mg/L).[370] In 16 urine samples from methamphetamine addicts sent to a Japanese criminal science laboratory, the median urine methamphetamine concentration was about 21 mg/L (range, 0.7–157.5 mg/L).[371] The median methamphetamine concentration in 30 forensic urine samples from Japanese users arrested for methamphetamine use was about 6.7 mg/L (range, 0.77–154.2 mg/L).[372]

Urine amphetamine immunoassays detect the presence of these compounds following occasional use for approximately 1–3 days depending on several factors including the dose, duration of use, urine pH, hydration (i.e., urine creatinine, specific gravity), analytic method (sensitivity, specificity, cutoff), and individual metabolic and excretion rates.[373] The normal urinary pH range is about 4.5–8.0 with urine creatinine concentration below 20 mg/dL and specific gravity <1.003 suggesting dilution of the urine specimen. The US DOT Regulations (49 Code of Federal Regulations Part 40) defines a substituted urine specimen as containing ≤5 mg creatinine/dL *and* a specific gravity of ≤1.001 or ≥1.020. Other criteria for an adulterated specimen include nitrite ≥500 µg/mL, urine pH ≤3 or ≥11 or the presence of substances (e.g., glutaraldehyde, chlorochromate, hydrogen peroxide, bleach, anionic surfactant, hydrochloric acid) normally absent in human urine. Studies of volunteer methamphetamine users indicate that under extreme circumstances the urine drug screen for amphetamine/methamphetamine may remain positive for approximately 1 week.[374] Current US workplace drug testing for methamphetamine requires the presence of urinary methamphetamine and urinary amphetamine concentrations above 500 ng/mL and 200 ng/mL, respectively. Because of interindividual variation and low urinary concentrations of amphetamine, the detection rate for urine specimens collected within 12 hours of ingestion and analyzed using these cutoffs is relatively low. In a volunteer study of 8 volunteers ingesting daily doses of 10 mg methamphetamine for 4 days, about 16% of the urine specimens collected within 12 hours of the dose of amphetamine were positive based on the current cutoff.[375] Final detection times after the 4 doses averaged 55.0 ± 18.7 hours with a range of 31–92 hours. Reducing the methamphetamine cutoff to 250 ng/mL extends the detection time to approximately 24 hours. In a study of 5 healthy volunteers receiving 10 mg or 20 mg methamphetamine daily for 4 days, the mean detection times for methamphetamine in urine samples based on current cutoffs were 43.6 and 66.9 hours, respectively.[350] Lowering the cutoff to 250 ng methamphetamine/mL and 100 ng amphetamine/mL increased the mean detection times to 59.5 and 79.7 hours, respectively. Although detectable concentrations of *d*-amphetamine (i.e., >50 ng/mL) are usually present in urine samples containing methamphetamine, the absence of detectable concentrations of *d*-amphetamine does not necessarily confirm the lack of methamphetamine abuse.[376]

α-Benzyl-*N*-methylphenethylamine (BNMPA) is an impurity that contaminates illicit methamphetamine produced by the Leuckart reaction using phenylacetone (phenyl-2-propanone) synthesized from phenylacetic acid. The presence of BNMPA or its major metabolite, *p*-OH-BNMPA, indicates the illicit use of methamphetamine.[377] Additionally, the presence of the (*d*)-enantiomer or a racemic mixture of methamphetamine enantiomers *suggests* the illicit use of methamphetamine in contrast to licit drugs, which contain only the (*l*)-enantiomer.[378,379] When calibrated at 0.3 mg *d*-amphetamine/L, excessive use (i.e., >twice recommended dose) of Vicks® nasal inhaler (*l*-methamphetamine) may produce false-positive results on some immunoassays, but false-positive results are unlikely during the use of a 1000 ng/mL cutoff.[380] The metabolism of the anti-parkinson drugs, selegiline and deprenyl produces *l*-amphetamine and *l*-methamphetamine, resulting in positive urine drug screens for methamphetamine. Detection of these metabolites requires chiral chromatography; routine gas chromatography/mass spectrometry does not separate the *d*- and *l*-enantiomers. Additionally, the ratio of methamphetamine/amphetamine is much lower (i.e., 2.5) following selegiline ingestion than following methamphetamine ingestion (i.e., about 10).[98] Because of structural similarities, ephedrine and pseudoephedrine may cross-react with some immunoassay screening reagents for methamphetamine.

HAIR

Sensitive methods for detecting amphetamine and methamphetamine in hair include gas chromatography/mass spectrometry[381,382] and liquid chromatography/tandem mass spectrometry liquid chromatography/tandem mass spectrometry.[383] Multiple factors complicate the interpretation of the results of hair analysis including hair growth rates, analytic techniques (washing, sample preparation), drug incorporation rates, sectional differences in drug concentrations, hair color and melanin content (increased incorporation in dark hair), life-style differences (shampooing, dyeing, bleaching), sunlight

exposure, and limited data on the correlation between chronic drug use and hair concentrations.[384] In general, the methamphetamine and amphetamine concentrations in axillary and pubic hair are somewhat higher than scalp hair. In a study of 5 methamphetamine users, the mean methamphetamine concentrations in scalp and axillary hair were 13.49 µg/g (range, 2.7–22.7 µg/g) and 18.68 µg/g (range, 2.9–53.35 µg/g), respectively.[385] There is substantial variation in the metabolite/parent drug ratio (amphetamine/methamphetamine) ratio in hair samples. The methamphetamine and amphetamine concentrations in 2,444 positive forensic samples submitted for methamphetamine abuse ranged from 0.51–193.75 µg/g and 0.13–13.39 µg/g, respectively, in head hair.[386] The amphetamine/methamphetamine ratio was 0.004–1.16. A 12-week study of 6 methamphetamine users admitted to a drug treatment program demonstrated peak methamphetamine concentrations in hair samples ranging from 1–7 cm from the root over the course of the study.[387] The incorporation of basic drugs into hair is relatively high compared with acidic and neutral drugs.[388] Consequently, the use of hair analysis to detect methamphetamine use requires further validation.

Abnormalities

RHABDOMYOLYSIS

Rhabdomyolysis may develop after the administration of large methamphetamine doses, particularly in association with hyperthermia.[389] Potential laboratory changes in patients with rhabdomyolysis include elevated serum muscle enzymes (creatine kinase, aldolase), myoglobinuria, hyperbilirubinemia, hyperuricemia, hypocalcemia, hyperkalemia, renal failure, hyperphosphatemia, and hypokalemia. Coagulopathies associated with hyperthermia and DIC may cause intramuscular hemorrhage, elevated compartment pressures, and rhabdomyolysis.

BLOOD

Leukocytosis occurs frequently after methamphetamine use. Disseminated intravascular coagulation (thrombocytopenia, hypofibrinogenemia, hypoprothrombinemia, reduced partial thromboplastin levels, elevated fibrin split products) can occur after IV or oral amphetamine use, especially in association with rhabdomyolysis and hyperthermia. Hypoglycemia is a rare complication of severe methamphetamine intoxication, probably as a result of the depletion of glycogen stores during prolonged hyperadrenergic state.[310] Elevated serum hepatic aminotransferases complicate the IV abuse of methamphetamine, but the frequency of direct drug hepatotoxicity is unclear in the absence of rhabdomyolysis and end organ failure.

CENTRAL NERVOUS SYSTEM

ACUTE. Intracranial hemorrhages in methamphetamine abusers are usually located in the cerebral white matter rather than the sites commonly associated with chronic hypertension (i.e., basal ganglia, pons, cerebellum). These intracranial hemorrhages may occur in association with aneurysms or arteriovenous malformations. Other pathologic findings associated with amphetamine use include subarachnoid hemorrhage, subdural hematoma, and drug-induced vasculitis.[390,391] Angiographic studies of involved vessels demonstrate beading of the anterior or middle cerebral arteries with partial or complete occlusion of small-caliber vessels (<1 mm).[392]

CHRONIC. Neuropsychologic testing suggests that high-dose methamphetamine/amphetamine abuse reduces scores on the Wechsler Memory Scale-Revised tests on attention/concentration, delayed recall indices, and verbal memory compared with controls who do not use illicit drugs.[393] There were no statistical differences in neuropsychologic scores between controls ($n = 9$) and low-dependence methamphetamine/amphetamine users as defined by the severity of dependence scale (i.e., intensity of use rather than cumulative dose). Total lifetime street-use of methamphetamine/amphetamine was not significantly different between the high-dose ($n = 11$) and low-dose methamphetamine/amphetamine ($n = 15$) groups. About 68% of the drug users used methamphetamine/amphetamines intravenously; the study did not separate amphetamine users from methamphetamine users. A study of cognitive function in chronic methamphetamine users demonstrated statistically significant differences in test scores on visual discrimination learning and shifting as well as smaller differences in visuospatial memory, when compared with age- and IQ-matched controls.[394] In a meta-analysis of 18 studies on neuropsychologic testing in chronic methamphetamine abusers, moderate to large deficits occurred in domains of learning ($d = -0.66$), executive functions ($d = -0.63$), and memory ($d = -0.59$).[395] These results suggest detrimental effects on the limbic and frontostriatal circuits. The most prominent effects on memory occurred in episodic memory, which is a multifactorial ability (e.g., encoding, consolidation, retrieval) dependent on multiple regions in the brain (e.g., frontal, temporal). Slightly smaller effects were observed in attention/working memory, language, and

visuoconstruction. The relationship between these deficits and characteristics of methamphetamine use (e.g., route, duration of use, abstinence) and confounders (e.g., premorbid psychologic conditions, comorbid diseases) are not well defined.

Although there are limited data, human studies suggests that chronic methamphetamine abuse produces permanent alteration of brain chemistry and function. PET scans detected a reduction in dopamine transporters in the caudate nucleus and putamen of 6 abstinent methamphetamine users.[396] However, none of these former methamphetamine abusers had clinical signs of parkinsonism. The reported mean period of abstinence for these former methamphetamine users was 3 years. A brain proton magnetic resonance spectroscopy (MRS) study of 26 abstinent methamphetamine abusers demonstrated an approximate 5% reduction in the concentration of the neuronal marker, *N*-acetylaspartate, in the basal ganglia and in frontal white matter.[397] The median time from the imaging study to the last reported use of methamphetamine was about 4 months.

Driving

There are limited data on the causal relationship between methamphetamine use and trauma, including traffic accidents. In general, the increasing prevalence of methamphetamine in postmortem samples from drivers in fatal accidents reflects the increasing abuse of this drug. A retrospective study of patients admitted to a California trauma center indicated that the percentage of urine samples positive for amphetamines (presumably mostly methamphetamine based on prevalence of use) and for cocaine was 13.2% versus 6.2%, respectively.[398] Studies of postmortem blood from fatal traffic accident victims demonstrated that the rate of methamphetamine use ranged from 1.8%–8.0%.[352,399,400] In a study of 17 drivers involved in fatal traffic accidents, the postmortem methamphetamine concentration ranged from 0.05–2.6 mg/L with a median of 0.35 mg/L.[363] Accident investigation of these fatal accidents indicated that 16 of the 17 drivers were responsible for the fatal accident. Drifting out of the lane of travel and reckless (high speed) driving were the most prominent causal factors. In a study of postmortem blood samples from 370 fatally injured drivers, detectable concentrations of methamphetamine occurred in 18 (4.9%). The methamphetamine concentrations ranged from <0.01–1.08 mg/L with mean and median concentrations of 0.73 mg/L and 0.26 mg/L, respectively.[401] Culpability for the accidents was not reported.

A study of 26 positive blood samples from drivers apprehended for driving under the influence of methamphetamine demonstrated a mean methamphet-amine concentration of 0.55 mg/L with a range up to 1.88 mg/L.[352] Two additional drivers developed irrational, agitated, violent behavior after ingesting methamphetamine during their apprehension. The methamphetamine concentrations were 2.58 mg/L and 9.46 mg/L. The latter driver developed seizures. Driving abnormalities associated with methamphetamine intoxication includes tailgating, rapid lane changes without signaling, and speeding.[402]

The effect of methamphetamine on driving is complex and dose-related. In general at low doses, methamphetamine is a stimulant, but psychomotor skills, reasoning, and cognition deteriorate as the dose and duration of use increases.[150] Furthermore, CNS depression during the withdrawal phase and psychotic symptoms from chronic abuse of high doses of methamphetamine may impair driving skills. The intensity of effects at a specific methamphetamine concentration depends in part on the amount of tolerance to amphetamines. Studies of volunteers given methamphetamine indicate that the effects of methamphetamine on the psychomotor skills required to operate machinery effectively are similar to the effects of *d*-amphetamine. The administration of 15–20 mg methamphetamine to healthy volunteers slightly improves performance on some simple tasks (reaction times, vigilance, attention), particularly in fatigued subjects. However, the positive effects of methamphetamine on psychomotor skills is neither consistent or without adverse effects. In study of 70 college-age volunteers receiving IV methamphetamine (0.2 mg/kg or 0.3 mg/kg) or placebo, methamphetamine alone produced subjective arousal and a small improvement in recall of recently learned words; however, the number of incorrect responses increased substantially.[403] In a study of 36 male college students receiving 15 mg methamphetamine/150 pounds intravenously, there was slightly improved speed on some repetitive motor tasks when compared with baseline.[404] However, there were no differences between methamphetamine and placebo on several tests of reaction time. Standard field sobriety tests (horizontal gaze nystagmus, walk and turn test, one leg stand test) are not sensitive measures of the effect of methamphetamine as measured in adult volunteers receiving 0.42 mg/kg *d,l*-methamphetamine or placebo.[138]

TREATMENT

Stabilization

The treatment of amphetamine, methamphetamine, and cocaine intoxications are similar; however, there are fewer clinical data on the specific treatment of methamphetamine intoxication compared with cocaine. The

major life-threatening complications of acute methamphetamine intoxication include hyperthermia, hypertension, seizures, cardiovascular instability, and trauma. Coma, shock, acute renal failure, severe hyperthermia (i.e., temperature >41°C/105.8°F), and seizures are poor prognostic indicators.[405] Severely intoxicated patients require IV access, cardiac monitoring, pulse oximetry, an electrocardiogram (ECG), and supplemental oxygen. Respiratory depression does not usually occur during mild to moderate methamphetamine intoxication, but pulmonary edema (cardiogenic shock, acute respiratory distress syndrome) can develop during severe methamphetamine poisoning. Most patients tolerate sinus tachycardia without pharmacologic intervention. Both hypertension and tachycardia often respond to IV benzodiazepines (adult: lorazepam 2 mg or diazepam 5 mg bolus titrated to effect). Core body temperatures should be measured in any agitated patient. Patients with suspected myocardial ischemia should be managed with nitrates, morphine, benzodiazepines, aspirin, and benzodiazepines. Hypotension may respond to fluid challenges, but often a vasopressor is needed. Shock is a poor prognostic sign that indicates the need for monitoring of cardiac output to determine the most efficacious combination of fluid and vasopressors.

HYPERTENSION

Elevated blood pressure during methamphetamine intoxication does not usually require treatment unless the hypertension contributes to myocardial ischemia. Benzodiazepines can be administered for agitation. Treatment for severe hypertension includes IV sodium nitroprusside (0.5–8 µg/kg/min) or phentolamine (2.5–5 mg initial IV bolus). Phenothiazine compounds reduce amphetamine-induced hypertension in animal models, but there are inadequate clinical data to determine the efficacy of these drugs during methamphetamine toxicity. Theoretical concerns about potential reduction in the seizure threshold have limited the administration of phenothiazine compounds in clinical practice. Beta blockers should be avoided because the blockade of β₂-mediated vasodilation can cause vasoconstriction and paradoxical worsening of the hypertension associated with the methamphetamine.

SEIZURES

Seizures usually respond to benzodiazepines (lorazepam, diazepam). Therapeutic options for the treatment of status epilepticus include phenobarbital and anesthetic agents (e.g., propofol). Hyperthermia, acidosis, hypoxemia, and rhabdomyolysis may complicate the clinical course during status epilepticus; these patients should be evaluated for the presence of these complications.

AGITATION

Diazepam (5–10 mg IV in adults or 0.1–0.3 mg/kg in children) or lorazepam (1–2 mg IV in adults) are the safest drugs for agitation; IV midazolam (initial starting dose, 0.03 mg/kg/h) titrated to effect is a short-acting alternative to IV lorazepam or diazepam, particularly in critical patients. Lorazepam is preferred to diazepam when IV access is unavailable.[66] Benzodiazepines should be titrated to the desired clinical effect. Intravenous droperidol (2.5–5 mg) is an alternative to diazepam or lorazepam for the treatment of acutely agitated methamphetamine-intoxicated patients. In a prospective clinical study of adult patients, the administration of IV droperidol (2.5–5 mg) produced more rapid and profound sedation compared with IV lorazepam (2–4 mg) in a group of 146 patients with methamphetamine intoxication.[406] More repeat doses of lorazepam were administered during the first hour of treatment than repeat doses of droperidol. A study of 18 pediatric patients with methamphetamine intoxication suggested that IV haloperidol is a safe adjunctive therapy with benzodiazepines for the treatment of acute methamphetamine intoxication in children.[407] The starting dose of haloperidol was 0.025–0.05 mg/kg, repeated every 15–30 minutes as necessary for the treatment of delirium. Hypotension, respiratory depression, and dystonic reactions are potential complications of butyrophenone use. A calm environment and familiar faces may also help alleviate agitation.

HYPERTHERMIA

Severe temperature elevation (i.e., >40–42°C/104–107.6°F) is a poor prognostic sign, and hyperthermia frequently occurs with evidence of end-organ failure (hypotension, hyperkalemia, metabolic acidosis, rhabdomyolysis, elevated serum creatinine kinase, coagulopathy).[309] Elevated core body temperature should be treated aggressively, especially when temperatures exceed 39–40°C (102.2–104°F). Cooling measures include removal of clothing, cool rooms, cool mist spray to the skin, liberal use of fans, ice baths, and sedation (benzodiazepines). Ice-bath immersion produces the most rapid response, but technical difficulties frequently limit the use of this modality. The use of hypothermic blankets or the application of ice packs often does not produce sufficient cooling for these patients. Neuromuscular paralysis, sedation (midazolam, continuous propofol infusion), and mechanical ventilation may be necessary to reduce the muscle rigidity and

hyperactivity associated with refractory hyperthermia.[408] Vital signs should be followed closely (i.e., every 15–20 minutes) and cooling measures continued until core temperatures are below 38.5–39°C (101.3–102°F). Less severe temperature elevations (<38–40°C/100.4–104°F) can be treated by placement in a cool room, removal of clothes, minimization of physical activity (sedation), tepid sponging, or evaporative methods (cool mist spray, fans).

Gut Decontamination

There are few data on the effect of decontamination procedures on the clinical outcome of methamphetamine intoxication. Most patients requiring treatment for ingesting methamphetamine are "body stuffers," who attempted to conceal evidence by swallowing variable quantities of methamphetamine. Animal studies indicate that the administration of activated charcoal within 1 minute after the oral administration of a lethal dose of methamphetamine reduces early mortality in mice, but mortality at 48 hours was similar between the treated and untreated groups of mice.[409] All animals demonstrated signs (piloerection, tremor, agitation) of methamphetamine toxicity within 9 minutes of oral administration of the methamphetamine. The administration of activated charcoal, sedation as needed, and supportive care are treatment options for body stuffers. More aggressive decontamination (polyethylene glycol electrolyte lavage solution) is necessary for body packers; however, the ingestion of methamphetamine by body packers is unusual compared with cocaine because of the ease of local clandestine manufacture of methamphetamine.

Elimination Enhancement

Currently, the use of forced diuresis with urinary acidification is not recommended. There are inadequate clinical data on the efficacy of hemodialysis, peritoneal dialysis, and hemoperfusion during amphetamine intoxication to recommend the use of these procedures. The large volume of distribution of methamphetamine suggests that these measures will not significantly increase the elimination rate of methamphetamine. Animal studies do not support the use of multiple-dose activated charcoal as a means of enhancing methamphetamine elimination.[410]

Antidotes

There are no specific antidotes for methamphetamine intoxication.

Supplemental Care

Most methamphetamine body stuffers with serious methamphetamine intoxication present to the emergency department with a pulse >120 bpm and/or body temperature ≥38.0°C (100.4°C).[411] Delayed symptoms may occur following the ingestion of well-wrapped baggies or rolled plastic baggies that simulate sustained release packaging ("parachuting methamphetamine"). Monitoring of these patients may be necessary up to 24 hours after ingestion.

ANCILLARY TESTS

Depending on clinical judgment and severity of poisoning, laboratory examination includes complete blood count, serum electrolytes, calcium, phosphorus, uric acid, creatine kinase, hepatic transaminases, creatinine, glucose, blood urea nitrogen (BUN), coagulation profile (platelet count, fibrin split products, fibrinogen, prothrombin time, partial thromboplastin time), urinalysis including myoglobin, arterial blood gases, chest x-ray, ECG, cardiac monitoring, and appropriate diagnostic neurologic testing (i.e., CT of the brain with or without angiography, lumbar puncture). The use of the urine dipstick is a convenient method to detect the presence of hemoglobin or myoglobin at concentrations above 5–10 mg/L.[412] After the cessation of the myoglobin formation, hepatic metabolism and renal excretion clear plasma myoglobin within 1–6 hours. The presence of myoglobinuria necessitates generous fluid replacement, but alkalinization of the urine is not routinely recommended because the excretion of amphetamine compounds decreases substantially in alkaline urine and there are inadequate data to support the efficacy of alkalinization in this setting.

METHAMPHETAMINE PSYCHOSIS

Fulminant psychosis in chronic methamphetamine abusers requires hospitalization to provide a protective environment, particularly when suicidal ideations are present. There are limited clinical data on the treatment of methamphetamine psychosis.[413] Empirical treatment includes the use of chlorpromazine at dosages of 50 mg orally or intramuscularly every 4 hours. Depending on adverse effects (sedation, orthostatic hypotension) and clinical response, the total daily dose varies up to 200–800 mg divided in 4–6 doses. The administration of oral or intramuscular doses (2–5 mg) of haloperidol every 6 hours is an alternative to chlorpromazine. Newer antipsychotic medications include olanzapine; limited clinical data suggest that olanzapine is as effective as

haloperidol for methamphetamine psychosis with lower risk of adverse effects.[414] Long-term management of the habitual methamphetamine user is difficult because of frequent relapses. Treatment of these patients involves group therapy and restructured lifestyles. Gradual withdrawal with methamphetamine is unnecessary. Heavy abusers become irritable and depressed during this period; antidepressants and suicide precautions are often required. In addition to behavioral group therapy, experimental pharmaceutic treatment of underlying methamphetamine abuse include the use of bupropion or risperidone.[415,416] Other experimental pharmacologic agents for the treatment of methamphetamine abuse include baclofen, mirtazapine, and topiramate.[417] The concomitant use of antipsychotic drugs does not necessarily prevent the exacerbation of psychotic symptoms in patients, who continue to use methamphetamine.

COMPLICATIONS

Severe methamphetamine intoxication may be complicated by a variety of problems, including acute renal failure, rhabdomyolysis, acute compartment syndrome, subarachnoid hemorrhage, intracerebral hemorrhage, cerebral edema with transtentorial herniation, DIC, and acute respiratory distress syndrome/acute lung injury. Laboratory examinations and repeat physical examinations are necessary to diagnose these conditions early. Management is primarily supportive, but surgery may be required for intracranial lesions or compartment syndrome.

Distal ischemia is usually an isolated problem resulting from inadvertent intra-arterial administration of methamphetamine compounds; there are few clinical data on outcomes to guide treatment of the distal ischemia. Therapeutic options for the treatment of ischemia include intra-arterial tolazoline (12–25 mg/limb), IV nitroprusside infusions (0.5–8 µg/kg/min), dextran 40 (80 mL/h), local nerve blocks, and axillary nerve block. Other therapeutic measures include the use of heparin, thrombolytics, and papaverine. Monitoring of intra-arterial pressure during these infusions is mandatory. Supportive care for compartment syndrome includes analgesics, elevation of extremities, and elimination of methamphetamine exposure. Fasciotomies are usually unnecessary unless there is clear evidence of high compartment pressures and associated distal ischemia.

References

1. Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. Prog Neurobiol 2005;75:406–433.
2. Chen KK, Schmidt DF. Ephedrine and related substances. Med (Baltimore) 1930:9:1–117.
3. Edeleano L. Uber einige derivate der phenylmethacryl-saure und der phenylisobuttersaure. Berl Dtsch Chem Ges 1887;20:616–622.
4. Baldessarini RJ. 1. Pharmacology of the amphetamines. Pediatrics 1972;49:694–701.
5. Alles GA, Prinzmetal M. The comparative physiological actions of *d,l*-β-phenylisopropylamines. II. Bronchial effect. J Pharmacol Exp Ther 1933;48:161–174.
6. Prinzmetal M, Bloomberg W. The use of benzedrine for the treatment of narcolepsy. JAMA 1935;105:2051–2054.
7. Bradley C. The behavior of children receiving Benzedrine. Am J Psychiatry 1937;94:577–585.
8. Editorial. Benzedrine sulfate "pep pills." JAMA 1937;108:1973–1974.
9. Rasmussen N. America's first amphetamine epidemic 1929–1971: a quantitative and qualitative retrospective with implications for the present. Am J Public Health 2008;98:974–985.
10. Monroe R, Drell H. Oral use of stimulants obtained from inhalers. JAMA 1947;135:909–915.
11. Greene MH, DuPont RL. Amphetamines in the District of Columbia. I. Identification and resolution of an abuse epidemic. JAMA 1973;226:1437–1440.
12. Karch SB. Karch's pathology of drug abuse. 3rd ed. Boca Raton, FL: CRC Press LLC; 2002.
13. Ellenhorn MJ, Barceloux DG. Medical toxicology. Diagnosis and treatment of human poisoning. New York: Elsevier; 1988.
14. Green SL, Kerr F, Braitberg G. Review article. amphetamines and related drugs of abuse. Emerg Med Australasia 2008;20:391–402.
15. DeSantis AD, Webb EM, Noar SM. Illicit use of prescription ADHD medications on a college campus: a multi-methodological approach. J Am Coll Health 2008;57:315–323.
16. McCabe SE, Knight JR, Teter CJ, Wechsler H. Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. Addiction 2005;100:96–106.
17. Janowsky DS. Depression and dysphoria effects on the interpersonal perception of negative and positive moods and caring relationships: effects of antidepressants, amphetamine, and methylphenidate. Curr Psychiatry Rep 2003;5:451–459.
18. Blachut D, Czarnocki Z, Wojtasiewicz K. α-Phenylethylamine in illegally produced amphetamine. Forensic Sci Int 2001;123:182–190.
19. Huizer H, Theeuwen AB, Verweij AM, Sinnema A, van der Toorn JM. Impurities in illicit amphetamine. J Forensic Sci Soc 1981;21:225–232.
20. Lambrechts M, Tonnesen F, Rasmussen KE. Profiling of impurities in illicit amphetamine samples by

high-performance liquid chromatography using column switching. J Chromatogr 1986;369:365–377.

21. Wilens TE, Adler LA, Adams J, Sgambati S, Rostrosen J, Sawtelle R, et al. Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. J Am Acad Child Adolesc Psychiatry 2008;47:21–31.

22. Davey J, Richards N, Freeman J. Fatigue and beyond: patterns of and motivations for illicit drug use among long-haul truck drivers. Traffic Inj Prev 2007;8:253–259.

23. Mandell AJ. The Sunday syndrome: a unique pattern of amphetamine abuse indigenous to American professional football. Clin Toxicol 1979;15:225–232.

24. Laties VG, Weiss B. The amphetamine margin in sports. Fed Proc 1981;40:2689–2692.

25. Docherty JR. Pharmacology of stimulants prohibited by the World Anti-Doping Agency (WADA). Br J Pharmacol 2008;154:606–622.

26. Council on Scientific Affairs. Clinical aspects of amphetamine abuse. JAMA 1978;240:2317–2319.

27. Kramer JC, Fischman VS, Littlefield DC. Amphetamine abuse pattern and effects of high doses taken intravenously. JAMA 1967;201:305–309.

28. Harrington H, Heller HA, Dawson D, Caplan L, Rumbaugh C. Intracerebral hemorrhage and oral amphetamines. Arch Neurol 1983;40:503–507.

29. Waksman J, Taylor RN Jr, Bodor GX, Daly FF, Jolliff HA, Dart RC. Acute myocardial infarction associated with amphetamine use. Mayo Clin Proc 2001;76:323–326.

30. Cartledge JJ, Chow WM, Hamilton Stewart PA. Acute renal failure after amphetamine presenting with loin pain. Br J Urol 1998;81:160–161.

31. Rowland M. Amphetamine blood and urine levels in man. J Pharm Sci 1969;4:508–509.

32. Beckett AH, Boyes RN, Triggs EJ. Kinetics of buccal absorption of amphetamines. J Pharm Pharmacol 1968:20;92–97.

33. Hinsvark ON, Truant AP, Jenden DJ, Steinborn JA. The oral bioavailability and pharmacokinetics of soluble and resin bound forms of amphetamine and phentermine in man. J Pharmacokinet Biopharm 1973;1:319–328.

34. Northup DW, Van Liere EJ. Effect of the isomers of amphetamine and desoxyephedrine on gastric emptying in man. J Pharmacol Exp Ther 1953;109:358–360.

35. Franksson G, Anggard E. The plasma protein binding of amphetamine, catecholamines and related compounds. Acta Pharmacol Toxicol 1970;28:209–214.

36. Baggot JD, Davis LE, Neff CA. Extent of plasma protein binding of amphetamine in different species. Biochem Pharmacol 1972;21:1813–1816.

37. Busto U, Bendayan R, Sellers EM. Clinical pharmacokinetics of non-opiate abused drugs. Clin Pharmacokinet 1989;16:1–26.

38. Wan SH, Matin SB, Azarnoff DL. Kinetics, salivary excretion of amphetamine isomers and effect of urinary pH. Clin Pharmacol Ther 1978;23:585–590.

39. Dring LG, Smith RL, Williams RT. The fate of amphetamine in man and other animals. J Pharm Pharmacol 1966;18:402–405.

40. Shiiyama S, Soejima-Ohkuma T, Honda S, Kumagai Y, Cho AK, Yamada H, et al. Major role of the CYP2C isozymes in deamination of amphetamine and benzphetamine: evidence for the quinidine-specific inhibition of the reactions catalyzed by rabbit enzyme. Xenobiotica 1997;27:379–387.

41. Davis JM, Kopin IJ, Lemberger L, Axelrod J. Effects of urinary pH on amphetamine metabolism. Ann NY Acad Sci 1971;179:493–501.

42. Beckett AH, Rowland M. Urinary excretion kinetics of amphetamine in man. J Pharm Pharmacol 1965;17:628–639.

43. Brauer LH, Ambre J, de Wit H. Acute tolerance to subjective but not cardiovascular effects of *d*-amphetamine in normal, healthy men. J Clin Psychopharmacol 1996;16:72–76.

44. Wachtel SR, de Wit H. Subjective and behavioral effects of repeated *d*-amphetamine in humans. Behav Pharmacol 1999;10:271–281.

45. Bost RO, Kemp P, Hniclica V. Tissue distribution of methamphetamine and amphetamine in premature infants. J Anal Toxicol 1989;13:300–302.

46. Steiner E, Villén T, Hallberg M, Rane A. Amphetamine secretion in breast milk. Eur J Clin Pharmacol 1984;27:123–124.

47. Ilett KF, Hacket P, Kristensen JH, Kohan r. Transfer of dexamphetamine into breast milk during treatment for attention deficit hyperactivity disorder. Br J Clin Pharmacol 2006;63:371–375.

48. Kriskó I, Lewis E, Johnson JE 3rd. Severe hyperpyrexia due to tranylcypromine-amphetamine toxicity. Ann Intern Med 1969;70:559–564.

49. Seiden LS, Sabol KE, Ricaurte GA. Amphetamine: effects on catecholamine systems and behavior. Ann Rev Pharmacol Toxicol 1993;32:639–677.

50. Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR. New insights into mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 2007;47:681–698.

51. Ellinwood EH Jr, Kilbey MM. Fundamental mechanisms underlying altered behavior following chronic administration of psychomotor stimulants. Biol Psychiatry 1980;15:749–757.

52. Schenk JO. The functioning neuronal transporter for dopamine: kinetic mechanisms and effects of amphetamines, cocaine and methylphenidate. Prog Drug Res 2002;59:111–131.

53. Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, et al. SPECT imaging of striatal dopamine release after amphetamine challenge. J Nucl Med 1995;36:1182–1190.

54. Taylor KM, Synder SH. Differential effects of *d*- and *l*-amphetamine on behavior and on catecholamine deposi-

tion in dopamine and norepinephrine containing neurons of rat brain. Brain Res 1971;28:295–309.

55. Shekim WO, Sinclair E, Glaser R, Horwitz E, Javaid J, Bylund DB. Norepinephrine and dopamine metabolites and educational variables in boys with attention deficit disorder and hyperactivity. J Child Neurol 1987;2:50–56.

56. Shekim WO, Javaid J, Davis JM, Bylund DB. Urinary MHPG and HVA excretion in boys with attention deficit disorder and hyperactivity treated with *d*-amphetamine. Biol Psychiatry 1983;18:707–714.

57. Meyer RE, DiMascio A, Stifler L. Personality differences in the response to stimulant drugs administered during a sleep-deprived state. J Nerv Mental Dis 1970;150:91–100.

58. Micozzi MS, Wetli CV. Intravenous amphetamine abuse, primary cerebral mucormycosis and acquired immunodeficiency. J Forensic Sci 1985;30:504–510.

59. McCann UD, Ricaurte GA. Amphetamine neurotoxicity: accomplishments and remaining challenges. Neurosci Biobehavior Rev 2004;27:821–826.

60. Hart CL, Ward AS, Haney M, Foltin RW, Fischman MW. Methamphetamine self-administration by humans. Psychopharmacology 2001;157:75–81.

61. Munzar P, Baumann MH, Shoaib M, Goldberg SR. Effects of dopamine and serotonin-releasing agents on methamphetamine discrimination and self-administration in rats. Psychopharmacology 1999;141:287–296.

62. Citron BP, Halpern M, McCarron M, Lundbery GD, McCormick R, Pingus IJ, et al. Necrotizing angiitis associated with drug abuse. N Engl J Med 1970;283:1003–1011.

63. Matick H, Anderson D, Brumlik J. Cerebral vasculitis associated with oral amphetamine overdose. Arch Neurol 1983;40:253–254.

64. Clausing P, Bowyer JF. Time course of brain temperature and caudate/putamen microdialysate levels of amphetamine and dopamine in rats after multiple doses of *d*-amphetamine. Ann N Y Acad Sci 1999;890:495–504.

65. Zalis EG, Lundberg GD, Knutson RA. The pathophysiology of acute amphetamine poisoning with pathological correlation. J Pharmacol Exp Ther 1967;158:115–127.

66. Derlet RW, Rice P, Horowitz Z, Lord RV. Amphetamine toxicity: experience with 127 cases. J Emerg Med 1989;7:157–161.

67. Jones AL, Jarvie DR, McDermid G, Proudfoot AT. Hepatocellular damage following amphetamine intoxication. Clin Toxicol 1994;32:435–444.

68. Ginsberg MD, Hertzman M, Schmidt-Nowara WW. Amphetamine intoxication with coagulopathy, hyperthermia and reversible renal failure. A syndrome resembling heatstroke. Ann Intern Med 1970;75:81–85.

69. Murray JB. Psychophysiological aspects of amphetamine-methamphetamine abuse. J Psychol 1998;132:227–237.

70. Siomopoulos V. Thought disorder in amphetamine psychosis: a case report. Psychosomatics 1976;17:42–44.

71. Yanagita T, Ellinwood EH Jr. Psychotoxic manifestations in amphetamine abuse. Psychopharmacol Bull 1986;22:751–756.

72. Geerglings PJ. Social and psychiatric factors in amphetamine users. Psychiatria Neurologia Neurochirurgia 1972;75:219–224.

73 Ellinwood H Jr. Amphetamine psychosis: a multidimensional process. Semin Psychiatry 1969;1:208–226.

74. West WA. Interaction of low dose amphetamine use with schizophrenia in outpatients. Three case reports. Am J Psychiatry 1974;131:321–323.

75. Hall RC, Popkin MK, Beresford TP, Klassen Hall A. Amphetamine psychosis: clinical presentations and differential diagnosis. Psychiatric Med 1988.6:73–79.

76. Buxton N, McConachie NS. Amphetamine abuse and intracranial haemorrhage. J R Soc Med 2000;93:472–477.

77. Chaudhuri C, Salahudeen AK. Massive intracerebral hemorrhage in an amphetamine addict. Am J Med Sci 1999;317:350–352.

78. Kaku DA, Lowenstein DH. Emergence of recreational drug abuse as a major risk factor for stroke in young adults. Ann Intern Med 1990;113:821–827.

79. Pettitti DB, Sidney S, Quesenberry C, Bernstein A. Stroke and cocaine or amphetamine use. Epidemiology 1998;9:596–600.

80. Kase CS. Intracerebral hemorrhage: non-hypertensive causes. Stroke 1986;17:590–595.

81. Packe GE, Garton MJ, Jennings K. Acute myocardial infarction caused by intravenous amphetamine abuse. Br Heart J 1990;64:23–24.

82. Ragland AS, Ismail Y, Arsura EL. Myocardial infarction after amphetamine use. Am Heart J 1993;125:247–249.

83. Bashour TT. Acute myocardial infarction resulting from amphetamine abuse: a spasm-thrombus interplay? Am Heart J 1994;128:1237–1239.

84. Call TD, Hartneck J, Dickinson WA, Hartman CW, Bartel AG. Acute cardiomyopathy secondary to intravenous amphetamine use. Ann Intern Med 1982;97:559–560.

85. O'Neill ME, Arnolda LF, Coles DM, Nikolic G. Acute amphetamine cardiomyopathy in a drug addict. Clin Cardiol 1983;6:189–191.

86. Smith JH, Roche AHG, Jagusch MF, Herdson PB. Cardiomyopathy associated with amphetamine administration. Am Heart J 1976;91:792–797.

87. Jelliffe RW, Hill D, Tatter D, Lewis E Jr. Death from weight control pills: A case report with objective post mortem confirmation. JAMA 1969;208:1843–1847.

88. Lago JA, Kosten TR. Stimulant withdrawal. Addiction 1994;89:1477–1481.

89. Hodding GC, Jann M, Ackerman IP. Drug withdrawal syndromes. A literature review. West J Med 1980;133:383–391.

90. Humphreys C, Garcia-Bournissen F, Ito S, Koren G. Exposure to attention deficit hyperactivity disorder

medications during pregnancy. Can Fam Physician 2007;53:1153–1155.

91. Milkovich L, van der Berg BJ. Effects of antenatal exposure to anorectic drugs. Am J Obstet Gynecol 1977;129: 637–642.

92. Golub M, Costa L, Crofton K, Frank D, Fried P, Gladen B, et al. NTP-CERHR Expert Panel Report on the reproductive and developmental toxicity of amphetamine and methamphetamine. Birth Defects Res B Dev Reprod Toxicol 2005;74:471–584.

93. Braithwaite RA, Jarvie DR, Minty PSB, Simpson D, Widdop B. Screening for drugs of abuse. I: Opiates, amphetamines and cocaine. Ann Clin Biochem 1995; 32:123–153.

94. Wilson JF, Williams J, Walker G, Toseland PA, Smith BL, Richens A, et al. Performance of techniques used to detect drugs of abuse in urine: study based on external quality assessment. Clin Chem 1991;37:442–447.

95. D'Nicuola J, Jones R, Levine B, Smith ML. Evaluation of six commercial amphetamine and methamphetamine immunoassays for cross-reactivity to phenylpropanolamine and ephedrine in urine. J Anal Toxicol 1992;16: 211–213.

96. Przekop MA, Manno JE, Kunsman GW, Cockerham KR, Manno BR. Evaluation of the Abbott ADx amphetamine/methamphetamine II abused drug assay: comparison to TDx, EMIT, and GC/MS methods. J Anal Toxicol 1991; 15:323–326.

97. Ruangyuttikarn W, Moody D. Comparison of three commercial amphetamine immunoassays for detection of methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, and methylenedioxyethylamphetamine. J Anal Toxicol 1988;12:229–233.

98. Fujita Y, Takahashi K, Takei M, Niitsu H, Aoki Y, Onodera M, et al. Detection of levorotatory methamphetamine and levorotatory amphetamine in urine after ingestion of an overdose of selegiline. Yakugaku Zasshi 2008;128: 1507–1512.

99. Valentine JL, Middleton R. GC-MS identification of sympathomimetic amine drugs in urine: rapid methodology applicable for emergency clinical toxicology. J Anal Toxicol 2000;24:211–222.

100. Budd RD. Amphetamine EMIT—structure versus reactivity. Clin Toxicol 1981;18:91–110.

101. Budd RD. Amphetamine radioimmunoassay—structure versus reactivity. Clin Toxicol 1981;18:299–316.

102. Thurman EM, Pedersen MJ, Stout RL, Martin T. Distinguishing sympathomimetic amines from amphetamine and methamphetamine in urine by gas chromatography/mass spectrometry. J Anal Toxicol 1992;16:19–27.

103. Aziz K. Drugs-of-abuse testing. Screening and confirmation. Clin Lab Med 1990;10:493–502.

104. Andersson M, Gustavsson E, Stephanson N, Beck O. Direct injection LC–MS/MS method for identification and quantification of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymehtamphetamine in urine drug testing. J Chromatogr B 2008:861:22–28.

105. Chou C-C, Lee M-R. Solid phase microextraction with liquid chromatography-electrospray ionization-tandem mass spectrometry for analysis of amphetamine and methamphetamine in serum. Analytica Chim Acta 2005;538:49–56.

106. Kraemer T, Maurer HH. Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine. J Chromatogr B 1998;713:163–187.

107. Nagata T, Kimura K, Hara K, Kudo K. Methamphetamine and amphetamine concentrations in postmortem rabbit tissues. Forensic Sci Int 1990;48:39–47.

108. Moody DE, Monti KM, Spanbauer AC. Long-term stability of abused drugs and antiabuse chemotherapeutical agents stored at –20 degrees C. J Anal Toxicol 1999;23:535–540.

109. Giorgi SN, Meeker JE. A 5-year stability study of common illicit drugs in blood. J Anal Toxicol 1995;19:392–398.

110. Christophersen AS, Bugge A, Dahlin E, Morland J, Wethe G. Interference with analysis of amphetamine in blood by n-ethylbenzenamine from rubber septums. J Anal Toxicol 1988;12:147–149.

111. Anggård E, Gunne LM, Jönsson LE, Niklasson F. Pharmacokinetic and clinical studies on amphetamine dependent subjects. Eur J Clin Pharmacol 1970;3:3–11.

112. Baselt RC, Cravey RH. A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. J Anal Toxicol 1977;1:81–103.

113. Verschraagen M, Maes A, Ruiter B, Bosman IJ, Smink BE, Lusthof KJ. Post-mortem cases involving amphetamine-based drugs in the Netherlands comparison with driving under the influence cases. Forensic Sci Int 2007;170:163–170.

114. Baselt RC. Disposition of toxic drugs and chemicals in man, 8th ed. Foster City, CA: Biomedical Publications, 2008.

115 Meyer E, van Bocxlaer JF, Dirinck IM, Lambert WE, Thienpont L, de Leenheer AP. Tissue distribution of amphetamine isomers in a fatal overdose. J Anal Toxicol 1997;21:236–239.

116. Moore FM, Jarvie DR, Simpson D. Comparison of polyclonal and monoclonal assays for routine screening of urines for amphetamines. Ann Clin Biochem 1996;33: 78–81.

117. Roberge RJ, Luellen JR, Reed S. False-positive amphetamine screen following a trazodone overdose. Clin Toxicol 2001;39:181–182.

118. Weintraub D, Linder MW. Amphetamine positive toxicology secondary to bupropion. Depression Anxiety 2000;12:53–54.

119. Vandevenne M, Vandenbussche H, Verstraete A. Detection time of drugs of abuse in urine. Acta Clinica Belgica 2000;55–6:323–333.

120. Schofferman J, Billesdon J, Hall R. Microangiopathic hemolytic anemia: Another complication of drug abuse. JAMA 1974;230:721.

121. Hamilton MJ, Smith PR, Peck AW. Effects of bupropion, nomifensine and dexamphetamine on performance, subjective feelings, autonomic variables and electroencephalogram in healthy volunteers. Br J Clin Pharmacol 1983; 15:367–374.

122. Spiegel R. Effects of amphetamines on performance and on polygraphic sleep parameters in man. Adv Biosci 1978;21:189–201.

123. Evans MA, Martz R, Lemberger L, Rodda BE, Forney RB. Effects of dextroamphetamine on psychomotor skills. Clin Pharmacol Ther 1976;19:777–781.

124. Berchou R, Block RI. Use of computerized psychomotor testing in determining CNS effects of drugs. Percept Mot Skills 1983;57:691–700.

125. Frankenhaeuser M, Post B. Objective and subjective performance as influenced by drug-induced variations in activation level. Scand J Psychol 1966;7:168–178.

126. Cochran JC, Thorne DR, Penetar DM, Newhouse PA. Parsing attentional components during a simple reaction time task using sleep deprivation and amphetamine intervention. Percept Mot Skills 1992;75:675–689.

127. Newhouse PA, Belenky G, Thomas M, Thorne D, Sing HC, Fertig J. The effects of *d*-amphetamine on arousal, cognition, and mood after prolonged total sleep deprivation. Neuropsychopharmacology 1989;2:153–164.

128. Weiss B, Laties VG. Enhancement of human performance by caffeine and the amphetamines. Pharmacol Rev 1962; 14:1–36.

129. Tedeschi G, Bittencourt PR, Smith AT, Richens A. Effect of amphetamine on saccadic and smooth pursuit eye movements. Psychopharmacology 1983;79:190–192.

130. Hughes FW, Forney RB. Dextro-amphetamine, ethanol and dextro-amphetamine-ethanol combination on performance of human subjects stressed with auditory feedback (DAF). Psychopharmacologia 1964;6:234–238.

131. Wilson L, Taylor JD, Nash CW, Cameron DF. The combined effects of ethanol and amphetamine sulfate on performance of human subjects. Can Med Assoc J 1966; 94:478–484.

132. Perez-Reyes M, White WR, McDonald SA, Hicks RE. Interaction between ethanol and dextroamphetamine. Effects on psychomotor performance. Alcohol Clin Exp Res 1992;16:75–81.

133. Silber BY, Papafotiou K, Croft RJ, Ogden E, Swann P, Stough C. The effects of dexamphetamine on simulated driving performance. Psychopharmacology (Berl) 2005; 179:536–543.

134. Hurst PM. Amphetamines and driving behavior. Accid Anal Prev 1976;8:9–13.

135. de Wit H, Enggasser JL, Richards JB. Acute administration of *d*-amphetamine decreases impulsivity in healthy volunteers. Neuropsychopharmacology 2002;27:813–825.

136. Hurst PM, Weidner MF, Radlow R. The effects of amphetamines upon judgments and decisions. Psychopharmacologia 1967;11:397–404.

137. Shinar D, Schechtman E. Drug identification performance on the basis of observable signs and symptoms. Accid Anal Prev 2005;37:843–851.

138. Silber BY, Papafotiou K, Croft RJ, Stough CK. An evaluation of the sensitivity of the standardized field sobriety tests to detect the presence of amphetamine. Psychopharmacology 2005;182:153–159.

139. Hurst PM, Chubb NC, Bagley SK, Ross S. Rebound from *d*-amphetamine. Psychol Rep 1971;29:1023–1033.

140. Smart RG, Schmidt W, Bateman K. Psychoactive drugs and traffic accidents. J Safety Res 1969;1:67–73.

141. Bjorneboe A, Bjorneboe GE, Gjerde H, Bugge A, Drevon CA, Morland J. A retrospective study of drugged driving in Norway. Forensic Sci Int 1987;33:243–251.

142. Kenagy DN, Bird CT, Webber CM, Fischer JR. Dextroamphetamine use during B-2 combat missions. Aviat Space Environ Med 2004;75:381–386.

143. Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of central stimulant amines: age and gender differences in concentrations of amphetamine, methamphetamine, and ecstasy in blood. J Stud Alcohol Drugs 2008;69:202–208.

144. Shoptaw SJ, Kao U, Ling WW. Treatment for amphetamine psychosis. Cochrane Database Syst Rev 2008;(4):CD003026.

145. Ogata A. Methamphetamine. J Pharm Soc Jpn 1919;451:751.

146. Brill H, Hirose T. The rise and fall of a methamphetamine epidemic: Japan 1945–1955. Semin Psychiatry 1969;1: 179–194.

147. Anglin MD, Burke C, Perrochet B, Stamper E, Dawud-Noursi S. History of the methamphetamine problem. J Psychoactive Drugs 2000;32:137–141.

148. Ujike H, Sato M. Clinical features of sensitization to methamphetamine observed in patients with methamphetamine dependence and psychosis. Ann NY Acad Sci 2004;1025:279–287.

149. Dye LR. Recipe for disaster: Mexican methamphetamine. J Med Toxicol 2006;2:81–82.

150. Logan BK. Methamphetamine – effects on human performance and behavior. Forensic Sci Rev 2002;14: 134–151.

151. Katagi M, Tatsuno M, Miki A, Nishikawa M, Tsuchihashi H. Discrimination of dimethylamphetamine and methamphetamine use: simultaneous determination of dimethylamphetamine-*N*-oxide and other metabolites in urine by high-performance liquid chromatography-electrospray ionization mass spectrometry. J Anal Toxicol 2000; 24:354–358.

152. Witkin JM, Ricaurte GA, Katz JL. Behavioral effects of *N*-methylamphetamine and *N,N*-dimethylamphetamine in rats and squirrel monkeys. J Pharmacol Exp Ther 1990;253:466–474.

153. Mendelson J, Uemura N, Harris D, Nath RP, Fernandez E, Jacob P 3rd, et al. Human pharmacology of the meth- amphetamine stereoisomers. Clin Pharmacol Ther 2006; 80:403–420.

154. Sekine H, Nakahara Y. Abuse of smoking methamphet- amine mixed with tobacco: 1. Inhalation efficiency and pyrolysis products of methamphetamine. J Forensic Sci 1987;32:1271–1280.

155. Cook CE. Pyrolytic characteristics, pharmacokinetics, and bioavailability of smoked heroin, cocaine, phencycli- dine, and methamphetamine. NIDA Res Monogr 1991; 115:6–23.

156. Sato M, Hida M, Nagase H. Analysis of pyrolysis prod- ucts of methamphetamine. J Anal Toxicol 2004;28: 638–643.

157. Lee M-R, Jeng J, Hsiang W-S, Hwang B-H. Determination of pyrolysis products of smoked methamphetamine mixed with tobacco by tandem mass spectrometry. J Anal Toxicol 1999;23:41–45.

158. Sekine H, Nakahara Y. Abuse of smoking methamphet- amine mixed with tobacco: II. The formation of mecha- nism of pyrolysis products. J Forensic Sci 1990;35: 580–590.

159. Cho AK. Ice: a new dosage form of an old drug. Science 1990;249:631–634.

160. United Nations Office on Drugs and Crime. 2007 World Drug Report. Vienna, Austria: United Nations Office on Drugs and Crime, 2007.

161. Office of Applied Studies. Results from the 2005 National Survey on Drug Use and Health: national findings. Rockville, MD: Substance Abuse and Mental Health Services Administration. DHS publication SMA 06-4194 NSDH Series H-30, 2006.

162. Durell TM, Kroutil LA, Crits-Christoph P, Barchha N, van Brunt DL. Prevalence of nonmedical methamphet- amine use in the United States. Subst Abuse Treat Prev Policy 2008;3:19

163. McKetin R, Kozel N, Douglas J, Ali R, Vicknasingam B, Lund J, Li J–H. The rise of methamphetamine in Southeast and East Asia. Drug Alcohol Rev 2008;27: 220–228.

164. Shaw K-P. Human methamphetamine-related fatalities in Taiwan during 1991–1996. J Forensic Sci 1999;44: 27–31.

165. Degenhardt L, Roxburgh A, Black E, Bruno R, Campbell G, Kinner S, Fetherston J. The epidemiology of metham- phetamine use and harm in Australia. Drug Alcohol Rev 2008;27:243–252.

166. Wilkins C, Sweetsur P. Trends in population drug use in New Zealand: findings from national household survey- ing of drug use in 1998, 2001, 2003, and 2006. NZMJ 2008; 121:61–71.

167. Maxwell JC, Rutkowski BA. The prevalence of metham- phetamine and amphetamine abuse in North America: a review of the indicators, 1992–2007. Drug Alcohol Rev 2008;27:229–235.

168. Inglez-Dias A, Hahn JA, Lum PJ, Evans J, Davidson P, Page-Shafer K. Trends in methamphetamine use in young injection drug use in San Francisco from 1998 to 2004: the UFO Study. Drug Alcohol Rev 2008;27:286–291.

169. Lurie IS, Bailey CG, Anex DS, Bethea MJ, McKibben TD, Casale JF. Profiling of impurities in illicit methamphet- amine by high-performance liquid chromatography and capillary electrochromatography. J Chromatogr A 2000;870:53–68.

170. Frank RS. The clandestine drug laboratory situation in the United States. J Forensic Sci 1983;28:18–31.

171. Derlet RW, Heischober B. Methamphetamine stimulant of the 1990s? West J Med 1990;153:625–628.

172. Thrasher DL, von Derau K, Burgess JL. Health effects from reported exposure to methamphetamine labs: a poison center-based study. J Med Toxicol 2009;5: 200–204.

173. Witter RZ, Martyny JW, Mueller K, Gottschall B, Newman LS. Symptoms experienced by law enforcement personnel during methamphetamine lab investigations. J Occup Environ Hyg 2007;4:895–902.

174. Burgess JL, Barnhart S, Checkoway H. Investigating clandestine drug laboratories: adverse medical effects in law enforcement personnel. Am J Ind Med 1996;30: 488–494.

175. Van Dyke M, Erb N, Arbuckle S, Martyny J. A 24-hour study to investigate persistent chemical exposures associ- ated with clandestine methamphetamine laboratories. J Occup Eviron Hyg 2009;6:82–89.

176. Willers-Russo LJ. Three fatalities involving phosphine gas, produced as a result of methamphetamine manufac- turing. J Forensic Sci 1999;44:647–652.

177. Santos AP, Wilson AK, Hornung CA, Polk HC Jr, Rodriguez JL, Franklin GA. Methamphetamine labora- tory explosions: a new and emerging burn injury. J Burn Care Rehabil 2005;26:228–232.

178. Burke BA, Lewis RW 2nd, Latenser BA, Chung JY, Willoughby C, Kealey GP, Wibbenmeyer LA. Methamphetamine-related burns in the cornbelt. J Burn Care Res 2008;29:574–579.

179. Spann MD, McGwin G Jr, Kerby JD, George RL, Dunn S, Rue LW 3rd, Cross JM. Characteristics of burn patients injured in methamphetamine laboratory explosions. J Burn Care Res 2006;27:496–501.

180. Burge M, Hunsaker JC III, Dais GJ. Death of a toddler due to ingestion of sulfuric acid at a clandestine home methamphetamine laboratory. Forensic Sci Med Pathol 2009;5:298–301.

181. Mecham N, Melini J. Unintentional victims. Development of a protocol for the care of children exposed to chemi- cals at methamphetamine laboratories. Pediatr Emerg Care 2002;18:327–332.

182. Simon SL, Richardson K, Dacey J, Glynn S, Domier CP, Rawson RA, Ling W. A comparison of patterns of meth- amphetamine and cocaine use. J Addict Dis 2002;21: 35–44.

183. Rawson RA, Huber A, Brethen PB, Obert JL, Gulati V, Shoptaw S, Ling W. Methamphetamine and cocaine users: differences in characteristics and treatment retention. J Psychoactive Drugs 2000;32:233–238.

184. Hendrickson RG, Horowitz Z, Orton RL, Notenboom H. "Parachuting" meth: a novel delivery method for methamphetamine and delayed-onset toxicity from "body stuffing." Clin Toxicol 2006;44:379–382.

185. Farabee D, Prendergast M, Cartier J. Methamphetamine use and HIV risk among substance-abusing offenders in California. J Psychoactive Drugs 2002;34:295–300.

186. Wu LT, Pilowsky DJ, Wechsberg WM, Schlenger WE. Injection drug use among stimulant users in a national sample. Am J Drug Alcohol Abuse 2004;30:61–83.

187. Ellinwood EH Jr. Assault and homicide associated with amphetamine abuse. Am J Psychiatry 1971;127:1170–1175.

188. Smith DE. Physical vs psychological dependence and tolerance in high dose methamphetamine abuse. Clin Toxicol 1969;2:99–103.

189. Smith DE, Fisher CM. An analysis of 310 cases of acute high dose methamphetamine toxicity in Haight Ashbury. Clin Toxicol 1970;3:117–124.

190. Cook CE, Jeffcoat AR, Hill JM, Pugh DE, Patetta PK, Sadler BM, et al. Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. Drug Metab Dispos 1993;21:717–723.

191. Perez-Reyes M, White R, McDonald S, Hill J, Jeffcoat R, Cook CE. Pharmacologic effects of methamphetamine vapor inhalation (smoking) in man. NIDA Res Monogr 1991;105:575–577.

192. Meng Y, Dukat M, Bridgen DT, Martin BR, Lichtman AH. Pharmacological effects of methamphetamine and other stimulants via inhalation exposure. Drug Alcohol Depend 1999;53;111–120.

193. Perez-Reyes M, White WR, McDonald SA, Hill JM, Jeffcoat AR, Cook CE. Clinical effects of methamphetamine vapor inhalation. Life Sci 1991;49:953–959.

194. Simon SL, Domier C, Carnell J, Brethen P, Rawson R, Ling W. Cognitive impairment in individuals currently using methamphetamine. Am J Addict 2000;9:222–231.

195. Gal J. Amphetamines in nasal inhalers. J Toxicol Clin Toxicol 1982;19:577–578.

196. Albertson TE, Derlet RW, Van Hoozen BE. Methamphetamine and the expanding complications of amphetamines. West J Med 1999;170:214–219.

197. Schuster CR, Fischman MW. Amphetamine toxicity: behavioral and neuropathological indexes. Fed Proc 1975;34:1845–1851.

198. Zalis EG, Parmley LF Jr. Fatal amphetamine poisoning. Arch Intern Med 1963;112:822–826.

199. Kendrick WC, Hull AR, Knochel JP. Rhabdomyolysis and shock after intravenous amphetamine administration. Ann Intern Med 1977;86:381–387.

200. Takekawa K, Ohmori T, Kido A, Oya M. Methamphetamine body packer. Acute poisoning death due to massive leaking of methamphetamine. J Forensic Sci 2007;52:1219–1222.

201. Driscoll RC, Barr FS, Gragg BJ, Moore GW. Determination of therapeutic blood levels of methamphetamine and pentobarbital by GC. J Pharm Sci 1971;60:1492–1495.

202. Hart CL, Gunderson EW, Perez A, Kirkpatrick MG, Thurmond A, Comer SD, Foltin RW. Acute physiological and behavioral effects of intranasal methamphetamine in humans. Neuropsychopharmacology 2008;33:1847–1855.

203. Harris DS, Boxenbaum H, Everhart ET, Sequeira G, Mendelson JE, Jones RT. The bioavailability of intranasal and smoked methamphetamine. Clin Pharmacol Ther 2003;74:475–486.

204. Kashani J, Ruha A-M. Methamphetamine toxicity secondary to intravaginal body stuffing. J Toxicol Clin Toxicol 2004;42:987–989.

205. Yanagisawa Y, Nakazato K, Nagai T. Binding of methamphetamine to serum albumin in various species *in vitro*. Pharmacol Res 1997;35:99–102.

206. Musshoff F. Illegal or legitimate use? Precursor compounds to amphetamine and methamphetamine. Drug Metab Rev 2000;32:15–44.

207. Kraemer T, Maurer H. Toxicokinetics of amphetamines: metabolism and toxicokinetics data of designer drugs, amphetamine, methamphetamine, and their *N*-alkyl derivatives. Ther Drug Monit 2002;24:277–289.

208. Law MY, Slawson MH, Moody DE. Selective involvement of cytochrome P450 2D subfamily in *in vivo* 4-hydroxylation of amphetamine in rat. Drug Metab Dispos 2000;28:348–353.

209. Dostalek M, Jurica J, Pistovcakova J, Hanesova M, Tomandl J, Linhart I, Sulcova A. Effect of methamphetamine on cytochrome P450 activity. Xenobiotica 2007;37:1355–1366.

210. Maurer HH, Kraemer T, Ledvinka O, Schmitt CJ, Weber AA. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in toxicological analysis. Studies on the detection of clobenzorex and its metabolites within a systematic toxicological analysis procedure by GC-MS and by immunoassay and studies on the detection of alpha- and beta-amanitin in urine by atmospheric pressure ionization electrospray LC-MS. J Chromatogr B Biomed Sci Appl 1997;689:81–89.

211. Caldwell J, Dring LG, Williams RT. Metabolism of [14C] methamphetamine in man, the guinea pig and the rat. Biochem J 1972;129:11–22.

212. Beckett AH, Rowland M. Urinary excretion kinetics of methylamphetamine in man. J Pharm Pharmacol 1965;17(suppl):109S–114S.

213. Kim I, Oyler JM, Moolchan ET, Cone EJ, Huestis MA. Urinary pharmacokinetics of methamphetamine and its metabolite, amphetamine, following controlled oral administration to humans. Ther Drug Monit 2004;26:664–672.

214. Cook CE, Jeffcoat AR, Sadler BM, Hill JM, Voyksner RD, Pugch DE, et al. Pharmacokinetics of oral methamphetamine and effects of repeated daily dosing in humans. Drug Metab Dispos 1992;20:856–862.

215. Perez-Reyes M, White WR, McDonald SA, Hicks RE, Jeffcoat AR, Hill JM, Cook CE. Clinical effects of daily methamphetamine administration. Clin Neuropharmacol 1991;14:352–358.

216. Comer SD, Hart CL, Ward AS, Haney M, Foltin RW, Fischman MW. Effects of repeated oral methamphetamine administration in humans. Psychopharmacology 2001;155:397–404.

217. Harajiri S, Koima H, Arikawa K, Miura C, Inanaga K. Synergism between methamphetamine and alcohol in a case of methamphetamine psychosis. Kurme Med J 1986;33:163–165.

218. Shimosato K, Oda H, Ohmae M, Tomita M, Doi Y. Biphasic effects of alcohol drinking on methamphetamine metabolism in man. Alcohol Alcoholism 1988;23:351–357.

219. Mendelson J, Jones RT, Upton R, Jacob P III. Methamphetamine and ethanol interactions in humans. Clin Pharmacol Ther 1995;57:559–568.

220. Pilgrim JL, Gerostamolos D, Drummer OH, Bollmann M. Involvement of amphetamines in sudden and unexpected death. J Forensic Sci 2009;54:478–485.

221. Garriott JC, Spruill FG. Detection of methamphetamine in a newborn infant. J Forensic Sci 1973;18:434–436.

222. Stewart JL, Meeker JE. Fetal and infant deaths associated with maternal methamphetamine abuse. J Anal Toxicol 1997;21:515–517.

223. Burchfield DJ, Lucas VW, Abrams RM, Miller RL, DeVane CL. Disposition and pharmacodynamics of methamphetamine in pregnant sheep. JAMA 1991;265:1968–1973.

224. Bartu A, Dusci LJ, Ilett KF. Transfer of methylamphetamine and amphetamine into breast milk following recreational use of methylamphetamine. Br J Clin Pharmacol 2009;67:455–459.

225. Schep LJ, Slaughter RJ, Beasley DM. The clinical toxicology of metamfetamine. Clin Toxicol 2010;48:675–694.

226. Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 2001;39:32–41.

227. Fumagalli F, Gainetdinov RR, Valenzano KJ, Caron MG. Role of dopamine transporter in methamphetamine-induced neurotoxicity: evidence from mice lacking the transporter. J Neurosci 1998;18:4861–4869.

228. Wyndham CH, Rogers GG, Benade AJ, Strydom NB. Physiological effects of the amphetamines during exercise. South Afr Med J 1971;45:247–252.

229. Worsley JN, Moszczynska A, Falardeau P, Kalasinsky KS, Schmunk G, Guttman M, et al. Dopamine D_1 receptor protein is elevated in nucleus accumbens of human, chronic methamphetamine users. Mol Psychiatry 2000;5:664–672.

230. Shoblock JR, Sullivan EB, Maisonneuve IM, Glick SD. Neurochemical and behavioral differences between *d*-methamphetamine and *d*-amphetamine in rats. Psychopharmacology (Berl) 2003;165:359–369.

231. Wijetunga M, Seto T, Lindsay J, Schatz I. Crystal methamphetamine-associated cardiomyopathy: tip of the iceberg? J Toxicol Clin Toxicol 2003;41:981–986.

232. He SY, Matoba R, Fujitani N, Sodesaki K, Onishi S. Cardiac muscle lesions associated with chronic administration of methamphetamine in rats. Am J Forensic Med Pathol 1996;17:155–162.

233. Maeno Y, Iwasa M, Inoue H, Koyama H, Matoba R, Nagao M. Direct effects of methamphetamine on hypertrophy and microtubules in cultured adult rat ventricular myocytes. Forensic Sci Int 2000;113:239–243.

234. Yamanaka Y, Takano R, Egashira T. Methamphetamine-induced behavioral alterations following repeated administration of methamphetamine. Japan J Pharmacol 1986;41:147–154.

235. Woolverton WL, Ricaurte GA, Forno LS, Seiden LS. Long-term effects of chronic methamphetamine administration in rhesus monkeys. Brain Res 1989;486:73–78.

236. Schmidt CJ, Ritter JK, Sonsalla PK, Hanson GR, Gib JW. Role of dopamine in the neurotoxic effects of methamphetamine. J Pharmacol Exp Ther 1985;233:539–544.

237. Sonsalla PK, Heikkila RE. Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine in several strains of mice. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:345–354.

238. Wahnschaffe U, Esslen J. Structural evidence for the neurotoxicity of methylamphetamine in the frontal cortex of gerbils (*Meriones unguiculatus*): a light and electron microscopical study. Brain Res 1985;337:299–310.

239. Hart CL, Ward AS, Haney M, Foltin RW, Fischman MW. Methamphetamine self-administration by humans. Psychopharmacology 2001;157:75–81.

240. Munzar P, Baumann MH, Shoaib M, Goldberg SR. Effects of dopamine and serotonin-releasing agents on methamphetamine discrimination and self-administration in rats. Psychopharmacology 1999;141:287–296.

241. Ando K, Hironaka N, Yanagita T. Psychotic manifestations in amphetamine abuse – experimental study on the mechanism of psychotic recurrence. Psychopharmacol Bull 1986;22:763–767.

242. Seiden LS. Methamphetamine: toxicity to dopaminergic neurons. NIDA Res Monogr 1985;62:100–116.

243. Tong J, Ross BM, Schmunk GA, Pereti FJ, Kalasinsky KS, Furukawa Y, et al. Decreased striatal dopamine D_1 receptor-stimulated adenylyl cyclase activity in human methamphetamine users. Am J Psychiatry 2003;160:896–903.

244. Kalasinsky KS, Bosy TZ, Schmunk GA, Reiber G, Anthony RM, Furukawa Y, et al. Regional distribution

of methamphetamine in autopsied brain of chronic human methamphetamine users. Forensic Sci Int 2001; 116:163–169.

245. Volkow ND, Chang L, Wang G-J, Leonido-Yee M, Franceschi D, Sedler MJ, et al. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am J Psychiatry 2001;158: 377–382.

246. Iyo M, Sekine Y, Mori N. Neuromechanism of developing methamphetamine psychosis: a neuroimaging study. Ann NY Acad Sci 2004;1025:288–295.

247. Seger D. Cocaine, metamfetamine, and MDMA abuse: the role and clinical importance of neuroadaptation. Clin Toxicol 2010;48:695–708.

248. Kiyatkin EA. Brain hyperthermia during physiological and pathological conditions: causes, mechanisms, and functional implications. Curr Neurovasc Res 2004;1: 77–90.

249. Karch SB, Stephens BG, Ho C-H. Methamphetamine-related deaths in San Francisco: demographic, pathologic, and toxicologic profiles. J Forensic Sci 1999;44:349–368.

250. Inoue H, Ikeda N, Kudo K, Ishida T, Terada M, Matoba R. Methamphetamine-related sudden death with a concentration which was of a "toxic level". Legal Med 2006;8:150–155.

251. Davis GG, Swalwell CI. Acute aortic dissections and ruptured berry aneurysms associated with methamphetamine abuse. J Forensic Sci 1994;39:1481–1485.

252. Kojima T, Une I, Yashiki M, Noda J, Sakai K, Yamamoto K. A fatal methamphetamine poisoning associated with hyperpyrexia. Forensic Sci Int 1984;24:87–93.

253. Hong R, Matsuyama E, Nur K. Cardiomyopathy associated with the smoking of crystal methamphetamine. JAMA 1991;265:1152–1154.

254. Kalant H, Kalant OJ. Death in amphetamine users: Causes and rates. CMA J 1975;112:299–304.

255. van Hoof F, Heyndricks A, Timperman J. Report of a human fatality due to amphetamine. Arch Toxicol 1974;32:307–312.

256. Orrenius S, Machly AC. Lethal amphetamine intoxication. A report of three cases. J Legal Med 1970;67: 184–189.

257. Matoba R, Shikata I, Fujitani N. Cardiac lesions in methamphetamine abusers. Acta Med Leg Soc (Liege) 1985;36: 51–55.

258. Matoba R, Onishi S, Shikata I. Cardiac lesions in cases of sudden death in methamphetamine abusers. Heart Vessels 1985;1:298–300.

259. Hall CD, Blanton DE, Scatliff JH, Morris CE. Speed kills: fatality from the self-administration of methamphetamine intravenously. South Med J 1973;66:650–652.

260. Moriya F, Hashimoto Y. A case of fatal hemorrhage in the cerebral ventricles following intravenous use of methamphetamine. Forensic Sci Int 2002;129:104–109.

261. Shibata S, Mori K, Sekine I, Suyama H. Subarachnoid and intracerebral hemorrhage associated with necrotiz-ing angiitis due to methamphetamine abuse an autopsy case. Neurol Med Chir (Tokyo) 1991;31:49–52.

262. Kalasinshy KS, Boxy TZ, Schmunk GA, Reiber G, Anthony RM, Furukawa Y, et al. Regional distribution of methamphetamine in autopsied brain of chronic human methamphetamine users. Forensic Sci Int 2001; 116:163–169.

263. Matteucci MJ, Auten JD, Crowley B, Combs D, Clark RF. Methamphetamine exposures in young children. Pediatr Emerg Care 2007;23:638–640.

264. Shaw HE, Lawson JG, Stulting RD. Amaurosis fugax and retinal vasculitis associated with methamphetamine inhalation. J Clin Neuroopthalmol 1985;5:169–176.

265. Wallace RT, Brown GC, Benson W, Sivalingham A. Sudden retinal manifestations of intranasal cocaine and methamphetamine abuse. Am J Ophthalmol 1992;114: 1658–160.

266. Richards JR, Johnson EB, Stark RW, Derlet RW. Methamphetamine abuse and rhabdomyolysis in the ED: a 5-year study. Am J Emerg Med 1999;17:681–685.

267. Downes MA, Whyte IM. Amphetamine-induced movement disorder. Emerg Med Australasia 2005;17: 277–280.

268. Shaner JW. Caries associated with methamphetamine abuse. J Mich Dent Assoc 2002;84:42–47.

269. Shaner JW, Kimmes N, Saini T, Edwards P. "Meth mouth": rampant caries in methamphetamine abusers. Aids Patient Care STDS 2006;20:146–150.

270. Goodchild JH, Donaldson M. Methamphetamine abuse and dentistry. A review of the literature and presentation of a clinical case. Quintessence Int 2007;38:583–590.

271. Poulsen EJ, Mannis MJ, Chang SD. Keratitis in methamphetamine abusers. Cornea 1996;15:477–482.

272. Wallace RT, Brown GC, Benson W, Sivalingham A. Sudden retinal manifestations of intranasal cocaine and methamphetamine abuse. Am J Ophthalmol 1992;114: 158–160.

273. McKetin R, McLaren J, Lubman DI, Hides L. The prevalence of psychotic symptoms among methamphetamine users. Addiction 2006;101:1473–1478.

274. Chen C-K, Lin S-K, Sham PC, Ball D, Loh E-W, Hsiao CC, et al. Pre-morbid characteristics and co-morbidity of methamphetamine users with and without psychosis. Psychol Med 2003;33:1407–1414.

275. Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. JAMA 1990;264:2511–2518.

276. Glasner-Edwards S, Mooney LJ, Marinelli-Casey P, Hillhouse M, Ang A, Rawson RA and the Methamphetamine Treatment Project Corporate Authors. Psychopathology in methamphetamine-dependent adults 3 years after treatment. Drug Alcohol Rev 2010;29:12–20.

277. Curran C, Byrappa N, McBride A. Stimulant psychosis: systematic review. Br J Psychiatr 2004;185:196–204.

278. Bell DS. The experimental reproduction of amphetamine psychosis. Arch Gen Psychiatry 1973;29:35–40.

279. Akiyama K. Longitudinal clinical course following pharmacological treatment of methamphetamine psychosis which persists after long-term abstinence. Ann NY Acad Sci 2006;1074:125–134.

280. Nakatani Y, Hara T. Disturbance of consciousness due to methamphetamine abuse a study of 2 patients. Psychopathology 1998;31:131–137.

281. Yeh H-S, Lee Y-C, Sun H-J, Wan S-R. Six months follow-up of patients with methamphetamine psychosis. Chin Med J (Taipei) 2001;64:388–394.

282. Tomiyama G. Chronic schizophrenia-like states in methamphetamine psychosis. Jap J Psychiatry Neurol 1990;44:531–539.

283. Dore G, Sweeting M. Drug-induced psychosis associated with crystalline methamphetamine. Australas Psychiatry 2006;14:86–89.

284. Yui K, Ikemoto S, Ishiguro T, Goto K. Studies of amphetamine or methamphetamine psychosis in Japan. Relation of methamphetamine psychosis to schizophrenia. Ann NY Acad Sci 2000;914:1–12.

285. Szuster RR. Methamphetamine in psychiatric emergencies. Hawaii Med J 1990;49:389–391.

286. Iwanami A, Sugiyama A, Kuroki N, Toda S, Kato N, Nakatani Y, et al. Patients with methamphetamine psychosis admitted to a psychiatric hospital in Japan. A preliminary report. Acta Psychiatr Scand 1994;89:428–432.

287. Yui K, Goto K, Ikemoto S, Ishiguro T. Stress induced spontaneous recurrence of methamphetamine psychosis: the relation between stressful experiences and sensitivity to stress. Drug Alcohol Depend 2000;58:67–75.

288. Yui K, Ishiguro T, Goto K, Ikemoto S. Precipitating factors in spontaneous recurrence of methamphetamine psychosis. Psychopharmacology 1997;134:303–308.

289. Sato M. A lasting vulnerability to psychosis in patients with previous methamphetamine psychosis. Ann NY Acad Sci 1992;654:160–170.

290. Sato M, Chen C-C, Akiyama K, Otsuki S. Acute exacerbation of paranoid psychotic state after long-term abstinence in patients with previous methamphetamine psychosis. Biol Psychiatry 1983;18:429–440.

291. Golden GS. The effect of central nervous system stimulants on Tourette syndrome. Ann Neurol 1977;2:69–70.

292. Moszczynska A, Fitzmaurice P, Ang L, Kalasinsky KS, Schmunk GA, Peretti FJ, et al. Why is parkinsonism not a feature of human methamphetamine users? Brain 2004;127:363–370.

293. Ho EL, Josephson SA, Lee HS, Smith WS. Cerebrovascular complications of methamphetamine abuse. Neurocrit Care 2009;10:295–305.

294. Miyashita T, Hayashi T, Ishida Y, Tsuneyama K, Kimura A, Kondo T. A fatal case of pontine hemorrhage related to methamphetamine abuse. J Forensic Leg Med 2007; 14:444–447.

295. McGee SM, McGee DN, McGee MB. Spontaneous intracerebral hemorrhage related to methamphetamine abuse. Autopsy findings and clinical correlation. Am J Forensic Med Pathol 2004;25:334–337.

296. Yen DJ, Wang SJ, Ju TH, Chen CC, Liao KK, Fuh JL, Hu HH. Stroke associated with methamphetamine inhalation. Eur Neurol 1994;34:16–22.

297. Rothrock JF, Rubenstein R, Lyden PD. Ischemic stroke associated with methamphetamine inhalation. Neurology 1988;38:589–592.

298. Perez JA Jr, Arsura EL, Strategos S. Methamphetamine-related stroke: four cases. J Emerg Med 1999; 17:469–471.

299. Davis GG, Swalwell CI. The incidence of acute cocaine or methamphetamine intoxication in deaths due to ruptured cerebral (berry) aneurysms. J Forensic Sci 1996; 41:626–628.

300. Furst SR, Fallon SP, Reznik GN, Shah PK. Myocardial infarction after inhalation of methamphetamine. N Engl J Med 1990;323:1147–1148.

301. Westover AN, Nakonezny PA, Haley RW. Acute myocardial infarction in young adults who abuse amphetamines. Drug Alcohol Depend 2008;96:49–56.

302. Nestor TA, Tamamoto WI, Kam TH, Schultz T. Crystal methamphetamine-induced acute pulmonary edema: a case report. Hawaii Med J 1989;48:457–459.

303. Wako E, LeDoux D, Mitsumori L, Aldea GS. The emerging epidemic of methamphetamine-induced aortic dissections. J Card Surg 2007;22:390–393.

304. Jacobs LJ. Reversible dilated cardiomyopathy induced by methamphetamine. Clin Cardiol 1989;12:725–727.

305. Ito H, Yeo K-K, Wijetunga M, Seto TB, Tay K, Schatz IJ. A comparison of echocardiographic findings in young adults with cardiomyopathy: with and without a history of methamphetamine abuse. Clin Cardiol 2009;32:e18–e22.

306. Holubar SD, Hassinger JP, Dozois EJ, Masuoka HC. Methamphetamine colitis a rare case of ischemic colitis in a young patient. Arch Surg 2009;144:780–782.

307. Herr RD, Caravati EM. Acute transient ischemic colitis after oral methamphetamine ingestion. Am J Emerg Med 1991;9:406–409.

308. Pilgrim JL, Gerostamoulos D, Drummer OH, Bollmann M. Involvement of amphetamines in sudden and unexpected death. J Forensic Sci 2009;54:478–485.

309. Chan P, Chen JH, Lee MH, Deng JF. Fatal and nonfatal methamphetamine intoxication in the intensive care unit. Clin Toxicol 1994;32:147–155.

310. Lan K-C, Lin Y-f, Yu F-C, Lin C-S, Chu P. Clinical manifestations of prognostic features of acute methamphetamine intoxication. J Formosa Med Assoc 1998;97: 528–533.

311. Kiely E, Lee CJ, Marinetti L. A fatality from an oral ingestion of methamphetamine. J Anal Toxicol 2009;33:557–560.

312. Stratton SJ, Rogers C, Green K. Sudden death in individuals in hobble restraints during paramedic transport. Ann Emerg Med 1995;25:710–712.

313. Zweben JE, Cohen JB, Christian D, Galloway GP, Salinardi M, Parent D, Iguchi M. Methamphetamine Treatment Project. Psychiatric symptoms in methamphetamine users. Am J Addict 2004;13:181–190.

314. Newton TF, Kalechstein AD, Duran S, Vansluis N, Ling W. Methamphetamine abstinence syndrome: preliminary findings. Am J Addict 2004;13:248–255.

315. McGregor C, Srisurapanont M, Jittiwutikarn J, Laobhripatr S, Wongtan T, White JM. The nature, time course and severity of methamphetamine withdrawal. Addiction 2005;100:1320–1329.

316. Nguyen D, Smith LM, Lagasse LL, Derauf C, Grant P, Shah R, et al.. Intrauterine growth of infants exposed to prenatal methamphetamine: results from the infant development, environment, and lifestyle study. J Pediatr 2010;157:337–339.

317. Plessinger MA. Prenatal exposure to amphetamines. Risks and adverse outcomes in pregnancy. Obstet Gynecol Clin North Am 1998;25:119–138.

318. Good MM, Solt I, Acuna JG, Rotmensch S, Kim MJ. Methamphetamine use during pregnancy: maternal and neonatal implications. Obstet Gynecol 2010;116: 330–334.

319. Little BB, Snell LM, Gilstrap LC III. Methamphetamine abuse during pregnancy: outcome and fetal effects. Obstet Gynecol 1988;72:541–544.

320. Oro AS, Dixon SD. Perinatal cocaine and methamphetamine exposure: maternal and neonatal correlates. J Pediatr 1987;111:571–578.

321. Catanzarite VA, Stein DA. "Crystal" and pregnancy methamphetamine-associated maternal deaths. West J Med 1995;162:454–457.

322. Eriksson M, Jonsson B, Steneroth G, Zetterstrom R. Amphetamine abuse during pregnancy: environmental factors and outcome after 14–15 years. Scand J Public Health 2000;28:154–157.

323. Vidal C, Skripuletz T. Bupropion interference with immunoassays for amphetamines and LSD. Ther Drug Monit 2007;29:373–375.

324. Poklis A, Hall KV, Eddleton RA, Fitzgerald RL, Saady JJ, Bogema SC. EMIT-d.a.u. monoclonal amphetamine/ methamphetamine assay. I. Stereoselectivity and clinical evaluation. Forensic Sci Int 1993;59:49–62.

325. Dasgupta A, Saldona S, Kinnaman G, Smith M, Johansen K. Analytical performance evaluation of EMIT II monoclonal amphetamine/methamphetamine assay: more specificity than EMIT d.a.u. monoclonal amphetamine/ methamphetamine assay. Clin Chem 1993;39:104–108.

326. Hornbeck CL, Carrig JE, Czarny RJ. Detection of a GC/ MS artifact peak as methamphetamine. J Anal Toxicol 1993;17:257–263.

327. Cody JT. Precursor medications as a source of methamphetamine and/or amphetamine positive drug testing results. J Occup Environ Med 2002;44:435–450.

328. Andersson M, Gustavsson E, Stephanson N, Beck O. Direct injection LC–MS/MS method for identification and quantification of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymehtamphetamine in urine drug testing. J Chromatogr B 2008:861:22–28.

329. Kudo K, Ishida T, Hara K, Kashimura S, Tsuji A, Ikeda N. Simultaneous determination of 13 amphetamine related drugs in human whole blood using an enhanced polymer column and gas chromatography-mass spectrometry. J Chromatogr B 2007;855:115–120.

330. Valentine JL, Kearns GL, Sparks C, Letzig LG, Valentine CR, Shappell SA, et al. GC-MS determination of amphetamine and methamphetamine in human urine for 12 hours following oral administration of dextro-methamphetamine: lack of evidence supporting the established forensic guidelines for methamphetamine confirmation. J Anal Toxicol 1995;19:581–590.

331. Long C, Crifasi J. Methamphetamine identification in four forensic cases. J Forensic Sci 1996;41:713–714.

332. Dallakian P, Budzikiewicz H, Brzezinka H. Detection and quantitation of amphetamine and methamphetamine: electron impact and chemical ionization with ammonia – comparative investigation on Shimadzu QP 5000 GC-MS system. J Anal Toxicol 1996;20:255–261.

333. Cody JT, Schwarzhoff R. Interpretation of methamphetamine and amphetamine enantiomer data. J Anal Toxicol 1993;17:321–326.

334. Hornbeck CL, Czarny RJ. Retrospective analysis of some *l*-methamphetamine/*l*-amphetamine urine data. J Anal Toxicol 1993;17:23–25.

335. Jirovsky D, Lemr K, Sevcik J, Smysl B, Stransky Z. Methamphetamine – properties and analytical methods of enantiomer determination. Forensic Sci Int 1998;96; 61–70.

336. Miyaguchi H, Iwata YT, Kanamori T, Tsujikawa K, Kuwayama K, Inoue H. Rapid identification and quantification of methamphetamine and amphetamine in hair by gas chromatography/mass spectrometry coupled with micropulverized extraction, aqueous acetylation and microextraction by packed sorbent. J Chromatogr A 2009;1216:4063–4070.

337. Tanaka K, Ohmori T, Inoue T. Analysis of impurities in illicit methamphetamine. Forensic Sci Int 1992;56: 157–162.

338. Lambrechts M, Rasmussen KE. Leuckart-specific impurities in amphetamine and methamphetamine seized in Norway. Bull Narc 1984;36:47–57.

339. Kuwayama K, Tsujikawa K, Miyaguchi H, Kanamori T, Iwata Y, Inoue H, et al. Identification of impurities and the statistical classification of methamphetamine using headspace solid phase microextraction and gas chromatography-mass spectrometry. Forensic Sci Int 2006;160:44–52.

340. Kuwayama K, Inoue H, Kanamori T, Tsujikawa K, Miyaguchi H, Iwata Y, et al. Contribution of thermal desorption and liquid-liquid extraction for identification

and profiling of impurities in methamphetamine by gas chromatography-mass spectrometry. Forensic Sci Int 2007;171:9–15.

341. Dujourdy L, Dufey V, Besacier F, Miano N, Marquis R, Lock E, et al. Drug intelligence based on organic impurities in illicit MA samples. Forensic Sci Int 2008;177: 153–161.

342. Noggle FT Jr, Clark CR, Davenport TW, Coker ST. Synthesis, identification, and acute toxicity of α-benzylphenethylamine and α-benzyl-N-methylphenethylamine. Contaminants in clandestine preparation of amphetamine and methamphetamine. J Assoc Off Anal Chem 1985;68:1213–1222.

343. Dayrit FM, Dumlao MC. Impurity profiling of methamphetamine hydrochloride drugs seized in the Philippines. Forensic Sci Int 2004;144:29–36.

344. Lurie IS, Bailey CG, Anex DS, Bethea MJ, McKibben TD, Casale JF. Profiling of impurities in illicit methamphetamine by high-performance liquid chromatography and capillary electrochromatography. J Chromatogr A 2000; 870:53–68.

345. Cantrell TS, John B, Johnson L, Allen AC. A study of impurities found in methamphetamine synthesized from ephedrine. Forensic Sci Int 1988;39:39–53.

346. Moore KA, Lichtman AH, Poklis A, Borzelleca JF. α-Benzyl-N-methylphenethylamine (BNMPA), an impurity of illicit methamphetamine synthesis: pharmacological evaluation and interaction with methamphetamine. Drug Alcohol Depend 1995;39:83–89.

347. Bal TS, Gutteridge DR, Johnson B. Adverse effects of the use of unusual phenethylamine compounds sold as illicit amphetamine. Med Sci Law 1989;29:186–188.

348. Allcott JV III, Barnhart RA, Mooney LA. Acute lead poisoning in two users of illicit methamphetamine. JAMA 1987;258:510–511.

349. Kojima T, Okamoto I, Miyazaki T, Chikasue F, Yashiki M, Nakamura K. Detection of methamphetamine and amphetamine in a skeletonized body buried for 5 years. Forensic Sci Int 1986;31:93–102.

350. Huestis MA, Cone EJ. Methamphetamine disposition in oral fluid, plasma, and urine. Ann NY Acad Sci 2007; 1098:104–121.

351. Morselli PL, Placidi GF, Maggini C, Gomeni R, Guazelli M, De Lisio G, et al. An integrated approach for the evaluation of psychotropic drug in man. I. Studies on amphetamine. Relationship between drug levels and psychophysiological measurements. Psychopharmacologia 1976;46:211–217.

352. Logan BK. Methamphetamine and driving impairment. J Forensic Sci 1996;41:457–464.

353. Lebish P, Finkle BS, Brackett JW Jr. Determination of amphetamine, methamphetamine, and related amines in blood and urine by gas chromatography with hydrogen-flame detector. Clin Chem 1970;16:195–200.

354. Rasmussen S, Cole R, Spiehler V. Methamphetamine in antemortem blood and urine by radioimmunoassay and GC/MS. J Anal Toxicol 1989;13:263–267.

355. Nishida M, Namera A, Yashiki M, Kojima T. On-column derivatization for determination of amphetamine and methamphetamine in human blood by gas chromatography-mass spectrometry. Forensic Sci Int 2002;125:156–162.

356. Hara K, Nagata T, Kimura K. Forensic toxicologic analysis of methamphetamine and amphetamine in body materials by gas chromatography/mass spectrometry. Z Rechtsmed 1986;96:93–104.

357. Molina NM, Jejurikar SG. Toxicological findings in a fatal ingestion of methamphetamine. J Anal Toxicol 1999;23:67–68.

358. Katsumata S, Sato K, Kashiwade H, Yamanami S, Zhou H, Yonemura I, et al. Sudden death due presumably to internal use of methamphetamine. Forensic Sci Int 1993;62:209–215.

359. Kamijo Y, Soma K, Nishida M, Namera A, Ohwada T. Acute liver failure following intravenous methamphetamine. Vet Hum Toxicol 2002;44:216–217.

360. Logan BK, Weiss EL, Harruff RC. Case report: distribution of methamphetamine in a massive fatal ingestion. J Forensic Sci 1996;41:322–323.

361. Fukunaga T, Mizoi Y, Adachi J, Tatsuno Y, Fujiwara S, Ueno Y. Methamphetamine concentrations in blood, urine, and organs of fatal cases after abuse. Jpn J Legal Med 1987;41:328–334.

362. Meeker JE, Reynolds PC. Postmortem tissue methamphetamine concentrations following selegiline administration. J Anal Toxicol 1990;14:330–331.

363. Logan BK, Fligner CL, Haddix T. Cause and manner of death in fatalities involving methamphetamine. J Forensic Sci 1998;43:28–34.

364. Zhu B-L, Oritani S, Shimotouge K, Ishida K, Quan L, Fujita MQ, et al. Methamphetamine-related fatalities in forensic autopsy during 5 years in the southern half of Osaka city and surrounding areas. Forensic Sci Int 2000;113:443–447.

365. Bailey DN, Shaw RF. Cocaine- and methamphetamine-related deaths in San Diego County (1987): homicides and accidental overdoses. J Forensic Sci 1987;34: 407–422.

366. Kojima T, Une I, Yashiki M. CI-mass fragmentographic analysis of methamphetamine and amphetamine in human autopsy tissues after acute methamphetamine poisoning. Forensic Sci Int 1983;21:253–258.

367. Moriya F, Hashimoto Y. Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages postmortem. J Forensic Sci 1999;44:10–16.

368. Miyazaki T, Kojima T, Yashiki M, Wakamoto H, Iwasaki Y, Taniguchi T. Site dependence of methamphetamine concentrations in blood samples collected from cadavers of people who had been methamphetamine abusers. Am J Forensic Med Pathol 1993;14:121–124.

369. Barnhart FE, Fogacci JR, Reed DW. Methamphetamine—a study of postmortem redistribution. J Anal Toxicol 1999; 23:69–70.

370. Batki SL, Moon J, Delucchi K, Bradley M, Hersh D, Smolar S, et al. Methamphetamine quantitative urine concentrations during a controlled trial of fluoxetine treatment. Preliminary analysis. Ann NY Acad Sci 2000; 909:260–262.

371. Shimosato K, Tomita M, Ijiri I. Urinary excretion of *p*-hydroxylated methamphetamine metabolites in man. I. A method for determination by high-performance liquid chromatography-electrochemistry. Arch Toxicol 1986;59: 135–140.

372. Nagai T, Matsushima K, Nagai T, Yanagisawa Y, Fujita A, Kurosu A, Tokudome S. Interpretation and enantiomer analysis of methamphetamine abusers' urine and illegally brewed methamphetamine crystals. J Anal Toxicol 2000;24:140–145.

373. Dasgupta A, Saldana S, Kinnaman G, Smith M, Johansen K. Analytical performance evaluation of EMIT® II monoclonal amphetamine/methamphetamine assay: more specificity than EMIT® d.a.u.™ monoclonal amphetamine/methamphetamine assay. Clin Chem 1993; 39:104–108.

374. Smith-Kielland A, Skuterud B, Morland J. Urinary excretion of amphetamine after termination of drug abuse. J Anal Toxicol 1997;21:325–329.

375. Oyler JM, Cone EJ, Joseph RE Jr, Moolchan ET, Huestis MA. Duration of detectable methamphetamine and amphetamine excretion in urine after controlled oral administration of methamphetamine to humans. Clin Chem 2002;47:1703–1714.

376. Long C, Crisfasi J. Methamphetamine identification in four forensic cases. J Forensic Sci 1996;41:713–714.

377. Moore KA, Ismaiel A, Poklis A. α-Benzyl-*N*-methylphenethylamine (BNM:A), an impurity of illicit methamphetamine synthesis: III. Detection of BNMPA and metabolites in urine of methamphetamine users. J Anal Toxicol 1996;20:89–92.

378. Cooke BJ. Chirality of methamphetamine and amphetamine from workplace urine samples. J Anal Toxicol 1994;18:49–51.

379. Goldberger BA, Cone EJ. Confirmatory tests for drugs in the workplace by gas chromatography-mass spectrometry. J Chromatogr A 1994;674:73–86.

380. Poklis A, Moore KA. Stereoselectivity of the TDxADx/FLx amphetamine/methamphetamine II amphetamine/methamphetamine immunoassay – response of urine specimens following nasal inhaler use. Clin Toxicol 1995;33:35–41.

381. Nakahara Y, Takahashi K, Shimamine M, Takeda Y. Hair analysis for drug abuse: I. Determination of methamphetamine and amphetamine in hair by stable isotope dilution gas chromatography/mass spectrometry method. J Forensic Sci 1991;36:70–78.

382. Nakahara Y, Takahashi K, Takeda Y, Konuma K, Fukui S, Tokui T. Hair analysis for drug abuse, part II. Hair analysis for monitoring of methamphetamine abuse by isotope dilution gas chromatography/mass spectrometry. Forensic Sci Int 1990;46:243–254.

383. Hegstad S, Khiabani HZ, Kristoffersen L, Kunøe N, Lobmaier PP, Christophersen AS. Drug screening of hair by liquid chromatography-tandem mass spectrometry. J Anal Toxicol 2008;32:364–372.

384. Saito T, Yamamoto I, Kusakae T, Huang X-L, Yukawa N, Takeichi S. Determination of chronic methamphetamine abuse by hair analysis. Forensic Sci Int 2000;112:65–71.

385. Han E, Yang W, Lee J, Park Y, Kim E, Lim M, Chung H. Correlation of methamphetamine results and concentrations between head, axillary, and pubic hair. Forensic Sci Int 2005;147:21–24.

386. Han E, Park Y, Yang W, Lee J, Lee S, Kim E, Lim M, Chung H. The study of metabolite-to-parent drug ratios of methamphetamine and methylenedioxymethamphetamine in hair. Forensic Sci Int 2006;161:124–129.

387. Lin D-L, Yin R-M, Liu H-C, Wang C-Y, Liu RH. Deposition characteristics of methamphetamine and amphetamine in fingernail clippings and hair sections. J Anal Toxicol 2004;28:411–417.

388. Nakahara Y, Kikura R. Hair analysis for drugs of abuse SIII. Effect of structural factors on incorporation of drugs into hair: the incorporation rates of amphetamine analogs. Arch Toxicol 1996;70:841–849.

389. Kao C-H, Liao S-Q, Wang S-J, Yeh S-H. Tc-99m PYP imaging in amphetamine intoxication associated with nontraumatic rhabdomyolysis. Clin Nucl Med 1992; 17:101–102.

390. Conci F, D'Angelo V, Tampieri D, Vecchi G. Intracerebral hemorrhage and angiographic beading following amphetamine abuse. Ital J Neurol Sci 1988;9:77–81.

391. Yu YJ, Cooper DR, Wellenstein DE, Block B. Cerebral angiitis and intracerebral hemorrhage associated with methamphetamine abuse. J Neurosurg 1983;58:109–111.

392. Rumbaugh CL, Bergeron RT, Fang HCH, McCormick R. Cerebral angiographic changes in the drug abuse patient. Radiology 1971;101:335–344.

393. McKetin R, Mattick RP. Attention and memory illicit amphetamine users: comparison with non-drug-using controls. Drug Alcohol Depend 1998;50:181–184.

394. Orstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, Robbins TW. Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. Neuropsychopharmacology 2000;23:113–126.

395. Scott JC, Woods SP, Matt GE, Meyer RA, Heaton RK, Atkinson JH, Grant I. Neurocognitive effects of methamphetamine: a critical review and meta-analysis. Neuropsychol Rev 2007;17:275–297.

396. McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11C]WIN-35,428. J Neurosci 1998;28: 8417–8422.

397. Ernst T, Chang L, Leonido-Yee M, Speck O. Evidence for long-term neurotoxicity associated with methamphetamine abuse A 1H MRS study. Neurology 2000;54:1344–1349.

398. Schermer CR, Wisner DH. Methamphetamine use in trauma patients: a population-based study. J Am Coll Surg 1999;189:442–449.

399. Crouch DJ, Birky MM, Gust SW, Rollins DE, Walsh JM, Moulden JV, et al. The prevalence of drugs and alcohol in fatally injured truck drivers. J Forensic Sci 1993;38:1342–1353.

400. Logan BK, Schwilke EW. Drug and alcohol use in fatally injured drivers in Washington State. J Forensic Sci 1996;41:505–510.

401. Schwilke EW, dos Santos MI, Logan BK. Changing patterns of drug and alcohol use in fatally injured drivers in Washington state. J Forensic Sci 2006;51:1191–1198.

402. Lemos NP. Methamphetamine and driving. Sci Just 2009;49:247–249.

403. Mewaldt SP, Ghoneim MM. The effects and interactions of scopolamine, physostigmine and methamphetamine on human memory. Pharmacol Biochem Behav 1979;10:205–210.

404. Talland GA, Quarton GC. Methamphetamine and pentobarbital effects on human motor performance. Psychopharmacologia 1965;8:241–250.

405. Lan KC, Lin YF, Yu FC, Lin CS, Chu P. Clinical manifestations and prognostic features of acute methamphetamine intoxication. J Formosa Med Assoc. 1998;97:528–533.

406. Richards JR, Derlet RW, Duncan DR. Methamphetamine toxicity: treatment with a benzodiazepine versus a butyrophenone. Eur J Emerg Med 1997;4:130–135.

407. Ruha A-M, Yarema MC. Pharmacologic treatment of acute pediatric methamphetamine toxicity. Pediatr Emerg Care 2006;22:782–785.

408. Callaway CW, Clark RF. Hyperthermia in psychostimulant overdose. Ann Emerg Med 1994;24:68–76.

409. McKinney PE, Tomaszewski C, Phillips S, Brent J, Kulig K. Methamphetamine toxicity prevented by activated charcoal in a mouse model. Ann Emerg Med 1994;24:220–223.

410. Hutchaleelaha A, Mayersohn M. Influence of activated charcoal on the disposition kinetics of methamphetamine enantiomers in the rat following intravenous dosing. J Pharm Sci 1996;85:541–545.

411. West PL, McKeown NJ, Hendrickson RG. Methamphetamine body stuffers: an observational case series. Ann Emerg Med 2010;55:190–197.

412. Curry SC, Chang D, Connor D. Drug and toxin-induced rhabdomyolysis. Ann Emerg Med 1989;18:1068–1084.

413. Srisurapanont M, Kittiratanapaiboon P, Jarusuraisin N. Treatment for amphetamine psychosis. Cochrane Database Syst Rev 2001;(4):CD003026.

414. Shoptaw SJ, Kao U, Ling W. Treatment for amphetamine psychosis. Cochrane Database Syst Rev 2009;(1):CD003026.

415. Meredith CW, Jaffe C, Yanasak E, Cherrier M, Saxon AJ. An open-label pilot study of risperidone in the treatment of methamphetamine dependence. J Psychoactive Drugs 2007;39:167–172.

416. Elkashef AM, Rawson RA, Anderson AL, Li S-H, Holmes T, Smith EV, et al. Bupropion for the treatment of methamphetamine dependence. Neuropsychopharmacology 2008;33:1162–1170.

417. Rose ME, Grant JE. Pharmacotherapy for methamphetamine dependence: a review of the pathophysiology of methamphetamine addiction and the theoretical basis and efficacy of pharmacotherapeutic interventions. Ann Clin Psychiatr 2008;20:145–155.

Chapter 2

METHYLPHENIDATE

HISTORY

Methylphenidate was first synthesized in 1944 by Ciba-Geigy Pharmaceutical Company as an analeptic agent for the treatment of barbiturate overdose. Initial indications for the use of methylphenidate were lethargy, chronic fatigue, depression (with or without psychosis), senile behavior, and narcolepsy. Now methylphenidate is primarily prescribed in the United States for the treatment of attention deficit disorder (ADD) and attention deficit hyperactivity disorder (ADHD) in children and adolescents.[1] Narcolepsy-associated somnolence is also treated with methylphenidate. In 1960, a case report documented the daily ingestion of up to 125 tablets of methylphenidate in a 37-year-old woman, who required hospitalization for her addiction.[2] Sweden withdrew methylphenidate from their list of approved drugs in 1968. During the 1970s, the intravenous (IV) abuse of methylphenidate occurred, particularly on the West Coast of the United States.[3] Intravenous drug abusers used methylphenidate as a stimulant in combination with opiates (e.g., pentazocine); however, daily use of methylphenidate was low (i.e., <1–2%) compared with most other drugs of abuse.[4]

IDENTIFYING CHARACTERISTICS

Physiochemical Properties

Methylphenidate is a white, odorless, crystalline powder. Table 2.1 lists some physiochemical properties of methylphenidate.

Methylphenidate (CAS RN: 113-45-1) is a piperidine-substituted phenylisopropylamine that is structurally related to amphetamine. Figure 2.1 displays the chemical structure of methylphenidate. The molecular formula of methylphenidate is $C_{14}H_{19}NO_2$. Methylphenidate exists as 4 isomers as a result of the presence of 2 chiral centers; however, the pharmaceutical formulation of methylphenidate (Ritalin®, Novartis Pharmaceutical Corp., East Hanover, NJ) contain a racemic mixture of *threo*-(*S*,*S*)-(−)- and *threo*-(*R*,*R*)-(+)-isomers. The pharmacologic and toxic actions of methylphenidate result primarily from the *threo*-(+)-enantiomer, which selectively competes for the dopamine uptake binding site on the dopamine transporter in the basal ganglia.[5] The binding of the *threo*-(−)-enantiomer to the dopamine transporter is diffuse and nonspecific.[6]

Terminology

The IV use of crushed pentazocine (Talwin®, Sanofi-Aventis US, Bridgewater, NJ) and methylphenidate (Ritalin®) is called "Ts and blues," "poor man's heroin," or "Ts and Rs."[7] The name, "Ts and blues" also refers to the IV use of the combination of pentazocine (Talwin®) and the antihistamine tripelennamine (Pyribenzamine®, Novartis Pharmaceutical Corp., East Hanover, NJ). Street names for methylphenidate include Skippy, R-ball, and Vitamin R.

Form

Because of the technical difficulty associated with stereospecific synthesis, drug diversion is the primary source of illicit methylphenidate rather than clandestine synthesis. Consequently, illicit methylphenidate is usually pharmaceutic grade and impurities are usually not clinical significant following ingestion. The IV use of crushed Ritalin® tablets results in the injection of talc (magnesium silicate) particles.

TABLE 2.1. Physical Properties of Methylphenidate.

Physical Property	Value
Molecular Mass	
Methylphenidate	233.31 g/mol
Methylphenidate HCl	269.77 g/mol
pKa	8.77
log P (Octanol-Water)	0.2
Water Solubility	2.01E+05 mg/L (25°C)
Vapor Pressure (Free Base)	5.89E-05 mm Hg (25°C)
Henry's Law Constant	4.38E-09 atm-m^3/Mole (25°C)
Atmospheric OH Rate Constant	1.11E-10 cm^3/molecule-second (25°C)
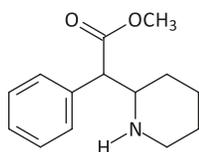
FIGURE 2.1. Chemical structure of methylphenidate.

EXPOSURE

Epidemiology

The abuse of methylphenidate is significantly less common than other major stimulant drugs of abuse (e.g., methamphetamine, cocaine). However, a small percentage of adolescents and young adults use methylphenidate for nonmedical purposes. In a survey of US high school seniors (Monitoring the Future Study), the nonmedical use of Ritalin® among high school seniors increased over the last 2 decades with the approximate percentage ranging from about 2–3% in 2000.[8] In a study of substance-abusing adolescents with comorbid psychiatric conditions, methylphenidate misuse was 4 times higher than methylphenidate abuse with about 5% of the study population abusing methylphenidate on a regular basis.[9] The primary method of methylphenidate abuse was the insufflation of crushed Ritalin® tablets. Misuse of methylphenidate occurs on college campuses reportedly as a means to improving study skills.[10]

Most studies on the misuse of prescription stimulants do not separate amphetamine from methylphenidate. The rate of misuse is substantially higher than the rate of daily abuse, and methylphenidate abuse is probably much less common than most other substances of abuse. In a study of 450 adolescents referred for treatment in a Canadian substance abuse program, 23% reported the nonmedical use of methylphenidate or *d*-amphetamine and 6% abused one or both of these substances.[9] Smaller studies with face-to-face interviews reported higher misuse rates, whereas larger, multisite studies reported lower rates. In a study of a convenience sample of 1,811 undergraduates at a large-public US research university, the reported lifetime rate of the illegal use of prescription stimulants (*d*-amphetamine, methylphenidate) was 34%.[11] A multisite study of 10,904 US college students reported a lifetime misuse and past-year misuse of prescription stimulants (*d*-amphetamine, methylphenidate) of 6.9% and 4.1%, respectively.[12]

Sources

The US Controlled Substances Act lists methylphenidate (Ritalin®) as a schedule II substance (i.e., a medically prescribed drug with the highest abuse potential and dependence profile). Methylphenidate is a well-established treatment for ADHD in immediate-release tablets of 5 mg, 10 mg, and 20 mg. Off-label uses of methylphenidate include the adjunctive treatment of depression and the treatment of poststroke cognitive impairment. Extended-release tablets of methylphenidate in 18 mg-, 36 mg-, and 54 mg-formulations are available as Concerta® (ALZA Corporation, McNeil Consumer & Specialty Pharmaceuticals, Fort Washington, PA). These tablets are coated with immediate-release methylphenidate surrounding an osmotic pump resulting in continuous methylphenidate release over a 10-hour period. Although these preparations contain both *d*- and *l-threo*-methylphenidate, only the *d*-enantiomer has significant pharmacologic activity. Most reports in the medical literature regarding methylphenidate involve adults injecting pharmaceutic-grade methylphenidate or the diversion of pharmaceutic products by adolescents or young adults.[13]

Methods of Abuse

Volunteer studies indicate that oral methylphenidate has some abuse potential; however, high doses (supratherapeutic) are necessary to produce stimulant-like subjective effects. The use of up to 48 mg methylphenidate did not increase the desire to take the drug again in a volunteer study.[14] This study suggested that the use of methylphenidate was associated with significant dysphoric effects that would reduce the abuse potential of the drug. A small percentage of adolescents and young adults treated for ADD will use methylphenidate for nonmedical purposes.[15] Methylphenidate is a drug of abuse on college campuses where the insufflation or ingestion of this drug is used to increase concentration, improve alertness, or for recreational purposes (i.e., getting high, experimenting).[16] In a retrospective review

of published studies, the misuse and diversion of prescription stimulants for ADHD ranged from 5–35% among older adolescents and college-age populations.[13] Lifetime diversion rates of stimulant prescriptions from students with legitimate prescription was 16–29%, when asked to trade, sell, or give the medication to another person. Case series indicate that the abuse pattern and toxicity of IV methylphenidate and cocaine/amphetamine sulfate abuse are similar, characterized by cyclical binging and crashing.[17]

DOSE EFFECT

Medical uses for methylphenidate include the treatment of narcolepsy in adults and ADD in children. The usual daily oral dose of immediate-release methylphenidate for ADD is 10–60 mg. In healthy individuals, the administration of methylphenidate increases activity, arousal, sociability, and mood; however, in volunteer studies, there is substantial variation in the response of healthy volunteers despite receiving the same IV dose of 20 mg methylphenidate.[18] Clinical effects in this study ranged from euphoria to dysphoria, anxiety, and malaise. In a retrospective study of 113 exposures to methylphenidate reported to poison control centers, adverse effects in pediatric patients were not associated with methylphenidate ingestions of ≤1 mg/kg.[19] Side effects of methylphenidate are dose-related with the incidence of these effects substantially increasing at doses above 2 mg/kg.[20] In animal studies, the administration of methylphenidate in doses of 10 times the maximum therapeutic dose increases systolic blood pressure, but there are no electrocardiographic or conduction abnormalities.[21] Common adverse effects include headache, anxiety, irritability, abdominal pain, anorexia, palpitations, and tachycardia. Case reports indicate that abuse of prescription methylphenidate by adolescents occurs at daily doses of 200 mg via insufflation[22] and ingestion.[23] Habitual IV methylphenidate abusers can tolerate substantially higher daily doses.

TOXICOKINETICS

Absorption

Methylphenidate is available in immediate-release and long-acting formulations. The gastrointestinal tract rapidly absorbs pharmaceutic doses of immediate-release methylphenidate with peak plasma concentrations (C_{max}) occurring within 1–3 hours of ingestion.[24] The absorption of long-acting preparations is bimodal with the second peak occurring about 3–4 hours after the peak associated with immediate-release preparations. Substantial presystemic elimination of *d-threo-*

methylphenidate occurs following the ingestion of therapeutic doses, resulting in relatively low absolute bioavailability (i.e., 0.11–0.53).[25] As a result of the wide variation in the bioavailability between individuals, the plasma methylphenidate concentrations vary substantially between individuals receiving the same dose of methylphenidate.[1] In a study of healthy adults, the mean maximal concentration (C_{max}) of methylphenidate in plasma following the ingestion of 0.15 mg/kg and 0.3 mg/kg was 3.5 ± 0.4 ng/mL and 7.8 ± 0.8 ng/mL, respectively.[26]

The time to reach peak concentrations in the brain is similar for both cocaine and methylphenidate. In a positron emission tomography (PET) study of healthy volunteers receiving 0.5 mg methylphenidate/kg intravenously, the time between administration and peak uptake in the brain for cocaine and methylphenidate was 2–8 minutes and 4–10 minutes, respectively.[5]

The pharmacokinetics of the *threo*-(*R*,*R*)-(+)-isomer and the racemic mixture are similar with the exception of first-pass metabolism. The extensive presystemic clearance and the relatively rapid clearance of the less active *threo*-(*S*,*S*)-(–)-isomer results in very low plasma concentrations compared with the active (+)-enantiomer following ingestion of pharmaceutic doses of methylphenidate. In a randomized, single-dose, cross-over study of 21 healthy volunteers receiving 40 mg racemic methylphenidate orally, the peak concentration of the *threo*-(*S*,*S*)-(–)-isomer was approximately 18- to 22-fold lower than active *threo*-(*R*,*R*)-(+)-isomer.[27] The IV administration of the racemic mixture bypasses the presystemic clearance of the (–)-enantiomer; consequently, the plasma concentrations of the 2 enantiomers are similar after IV administration. Methylphenidate is a basic compound that accumulates in the acidic environment of the stomach as a result of ion trapping, even following IV administration.

Distribution

Methylphenidate has a large volume of distribution ranging from about 10–50 L/kg in children receiving chronic daily oral doses of methylphenidate up to 1.3 mg/kg.[28] In a study of 8 boys with ADHD, the mean volume of distribution was 39.28 ± 7.67 L/kg with a range of 27.19–47.93 L/kg.[29] In a study of 8 children, the mean protein-bound methylphenidate was 15.2% ± 5.2%.[28] The binding of methylphenidate to plasma proteins is relatively low (i.e., 10–33%).[30] The relatively low protein binding and high lipid solubility of methylphenidate results in the large volume of distribution and high tissue binding, particularly in the brain where methylphenidate concentrations typically exceed plasma methylphenidate concentrations.

59
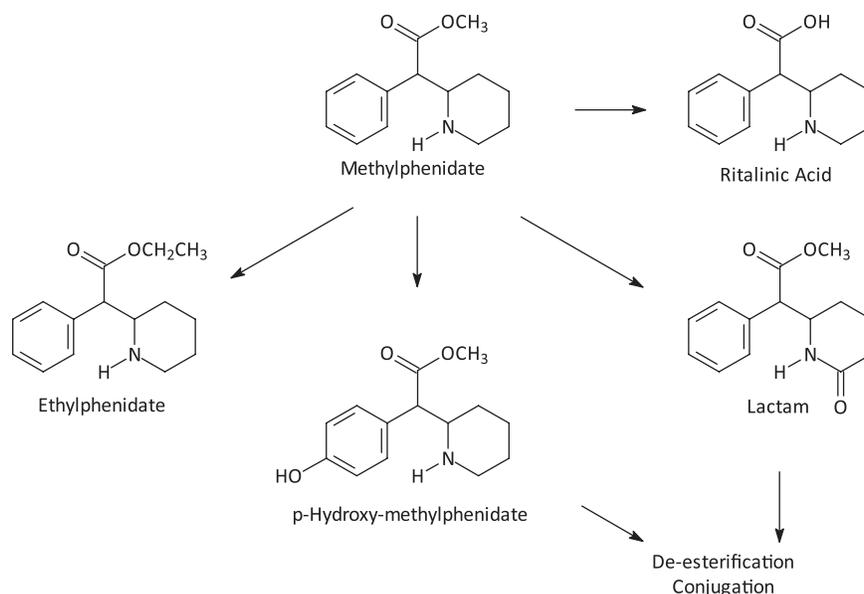
FIGURE 2.2. Biotransformation of methylphenidate.

Biotransformation

The main metabolic pathway in humans is the rapid de-esterification of methylphenidate to an inactive metabolite, ritalinic acid (CAS RN: 19395-41-6, α-phenyl-2-piperidineacetic acid) via hydrolytic esterase found throughout the body. During the biotransformation of methylphenidate, the hydrolysis of the methyl ester linkage with the subsequent formation of ritalinic acid accounts for over 80% of the absorbed methylphenidate dose.[31] Figure 2.2 demonstrates the biotransformation of methylphenidate. The *p*-hydroxylation of methylphenidate to *p*-hydroxymethylphenidate and the microsomal oxidation to 6-oxomethylphenidate (lactam) and 5-hydroxy-6-oxomethylphenidate are minor pathways in humans. Ritalinic acid appears in the urine unchanged or as 6-oxoritalinic acid. Ethylphenidate is a metabolite formed in the presence of ethanol via a carboxylesterase-dependent transesterification, similar to the formation of cocaethylene;[32] however, volunteer studies indicate that the formation of ethylphenidate is limited in the presence of moderate ethanol doses.[33] The metabolism of methylphenidate is stereoselective with a gradual increase in the *threo*-(+)/(−) ratio beginning about 1.5 hours after ingestion.[30] There is no evidence of interconversion between enantiomers of methylphenidate.

Elimination

The kidney eliminates less than 1% of an oral dose of 20 mg methylphenidate dose unchanged, although acid-ification will somewhat enhance excretion; fecal elimination is minimal.[34] The plasma elimination of methylphenidate is relatively rapid with a mean elimination half-life of about 3 hours (range, 2–8 h) after the ingestion of pharmaceutic doses of methylphenidate. In a study of 8 boys with ADHD, the mean plasma elimination half-life was 3.33 ± 0.65 hours with a range of 2.24–4.31 hours.[29] In healthy volunteer studies, the clearance of the inactive *threo*-(*S*,*S*)-(−)-isomer is approximately 2½ times greater than the active *threo*-(*R*,*R*)-(+)-isomer. The plasma half-life of ritalinic acid is approximately 3–5 hours with the mean plasma elimination half-life being slightly longer following IV administration (4.8 ± 0.5 h) than ingestion on an empty stomach (3.4 ± 0.4 hours).[25] Renal excretion of ritalinic acid accounts for approximately 60–80% of the methylphenidate dose.[31] In PET studies of the brains of healthy volunteers after the IV administration of methylphenidate, the clearance of methylphenidate from the striatum was significantly longer (90 minutes) than the clearance of cocaine (20 minutes).[5] Unchanged methylphenidate accounts for about 1% of the methylphenidate dose.

Maternal and Fetal Kinetics

There are limited data on the distribution of methylphenidate during pregnancy or lactation as a result of the use of this drug primarily in children and adolescents. Limited data suggests that substantial amounts of methylphenidate are not excreted in breast milk. Evaluation of breast milk from a 31-year-old woman receiving

methylphenidate doses of 5 mg in the morning and 10 mg at noon indicated that the infant dose was approximately 0.16% of the maternal dose.[35] Breastfeeding in the morning prior to the first dose of methylphenidate probably does not transfer methylphenidate to the infant because of the short elimination half-life of methylphenidate. In a study of a 26-year-old lactating mother on 40 mg methylphenidate twice daily, the milk/plasma ratio of methylphenidate was 2.7 with a relative infant dose of 0.2% of the weight-adjusted maternal dose.[36]

Tolerance

Children treated for ADD do not usually experience the mood elevation or euphoria commonly present in adult IV methylphenidate abusers, even at the beginning of therapy. The development of acute tolerance during the slow onset of action following the ingestion of methylphenidate mitigates the reinforcing effects and abuse potential of methylphenidate.[37] The injection of large doses of methylphenidate produces a rapid increase in synaptic dopamine concentrations that is not modulated by acute tolerance, and the short interval between administration and perceived effects enhances the addictive potential of the drug.

Drug Interactions

In pharmacokinetic studies, the administration of ethanol 30 minutes before or after racemic methylphenidate increases the peak plasma concentration and the area under the concentration-time curve (AUC) by approximately 40% and 25%, respectively.[38] The *threo*-(*S*,*S*)-(−)-isomer and high doses of methylphenidate inhibit the action of monoamine oxidase. As a result, the concomitant use of methylphenidate and monoamine oxidase inhibitors potentially can cause a hypertensive emergency.[39] In general, stimulants may exacerbate symptoms of schizophrenia. Even in the absence of psychotic disorders, the administration of methylphenidate may antagonize the central dopaminergic effects of haloperidol or similar antipsychotic medications.[40] A case report associated the coadministration of disulfiram and methylphenidate with the onset of psychotic episode that resolved within 1 day.[41]

There are few data on drug interactions associated with chronic methylphenidate use. Potential interactions include the inhibition of the metabolism of the coumarin, ethyl biscoumacetate, anticonvulsants (diphenylhydantoin, primidone, phenobarbital), and tricyclic antidepressants.[42] However, there are inadequate *in vivo* human data to indicate that these potential interactions are clinically significant. Rare case reports suggest the possibility that the administration of methylphenidate with a selective serotonin reuptake inhibitor (SSRI) may increase the risk of serotonin syndrome.[43] In a study of cocaine-dependent volunteers, the coadministration of methylphenidate and cocaine reduced some of the desired effects of cocaine (e.g., "good effects," "desire for cocaine"); however, the administration of methylphenidate did not alter the pharmacokinetics of cocaine.[44]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Methylphenidate is a noncatecholamine sympathomimetic drug with both direct and indirect adrenergic agonist activity. Modification of the side chain of the basic-phenylamine moiety into a heterocyclic structure (e.g., methylphenidate) attenuates the anorectic and cardiovascular effects of amphetamine, but this structural change maintains the central nervous system (CNS) stimulant properties. The dopamine transporter (DAT) recycles extracellular dopamine from perisynaptic regions to the interior of the neuron. Methylphenidate binds to presynaptic dopamine transporters, resulting in the reversible inhibition of dopamine reuptake and increased synaptic dopamine concentrations.[45] The affinity of cocaine and methylphenidate for dopamine transporters in rat striatum is similar (i.e., 640 nmol/L and 390 nmol/L, respectively).[46] The result is prolonged postsynaptic and presynaptic dopamine receptor interactions. Depending on ionic conditions, the dopamine transporter functions as a mediator of inward-directed dopamine transport (i.e., reuptake) and/or outward directed dopamine transport (i.e., release). Methylphenidate impedes the clearance of dopamine from the synapse by competing with dopamine for the uptake-binding site. In contrast to amphetamine, methylphenidate is probably not a substrate for entry into the cytoplasm by the dopamine transporter; therefore, little presynaptic dopamine release occurs following the administration of methylphenidate.[47] Of the 2 enantiomers (*d*- and *l-threo*-methylphenidate) of methylphenidate, only the *d-threo*-methylphenidate binds significantly to the dopamine transporters.

The distribution of methylphenidate and cocaine in the brain is similar. Although both cocaine and methylphenidate occupy similar sites in the striatum and compete directly for these sites, the clearance of these 2 drugs from these sites differs significantly.[48] The clearance of cocaine from the dopamine transporter is much more rapid than methylphenidate, and the abuse potential for methylphenidate is substantially less

than cocaine. Therapeutic oral doses produce peak methylphenidate concentrations in the brain within 60–90 minutes, and these concentrations result in the inhibition of >50% of the dopamine transporters.[5] To a lesser extent, methylphenidate also binds to norepinephrine transporters, resulting in increased synaptic concentrations of norepinephrine. The serotonin transporter probably is not affected by methylphenidate.[1] Some increase in cortical acetylcholine may occur as a result of the indirect stimulation of cortical D_1 receptors.[49] In rodent studies, *d*-methylphenidate is a relatively weak substrate for the polymorphic efflux transporter P-glycoprotein in the brain.[50]

Mechanism of Toxicity

The IV administration or insufflation of methylphenidate produces rapid increases in brain methylphenidate concentrations with associated euphoria. However, the ingestion of therapeutic doses of methylphenidate results in slower uptake of methylphenidate in the brain with significantly reduced euphoric effects compared with the IV and nasal routes of methylphenidate administration. The ability of most drugs of abuse to raise dopamine concentrations in the nucleus accumbens correlates to the self-administration (i.e., reinforcing properties) of the drug. Psychostimulant drugs (e.g., methylphenidate) raise extracellular dopamine concentrations by inhibiting the dopamine transporter.[51] Oral therapeutic doses (0.25–1 mg/kg) of methylphenidate block about 50–75% of the dopamine transporters, but the abuse potential of oral methylphenidate is substantially less than IV methylphenidate.[52] Based on PET studies of the human brain, IV administration produces peak brain methylphenidate concentrations 8–15 minutes after use compared with 60–90 minutes following the oral administration of methylphenidate.[5] Although IV methylphenidate produces rapid increases of extracellular dopamine in the brain, the slow elimination of methylphenidate from the brain reduces the reinforcing effects that lead to repeated use, especially compared with the rapid elimination and high abuse potential of cocaine.[53] This slower egress of methylphenidate from the brain may decrease drug craving compared with cocaine. The highest density of methylphenidate uptake occurs in the striatum with lesser amounts in the prefrontal cortex and limbic regions.[1] The behavioral and cardiovascular effects of methylphenidate may involve more than 1 neurotransmitter.[51]

Postmortem Examination

The IV injection of dissolved tablets produces tiny particles of talc that migrate to the pulmonary vessels and pulmonary interstitium, particularly in intravascular or perivascular locations. A foreign body reaction develops manifest by birefringent talc crystals under polarized light. These particles produce granulomas that can cause widespread pulmonary vascular obstruction, pulmonary hypertension, biventricular enlargement, and cor pulmonale.[54] The α-adrenergic properties of methylphenidate may enhance the effects of the pulmonary granulomas on the intima and muscular layers of the pulmonary vessels, resulting in pulmonary hypertension and right ventricular hypertrophy.[55] A case series of postmortem examination of 7 young, IV Ritalin® abusers associated IV administration of dissolved Ritalin® tablets with the development of a symmetric, panlobular emphysema, particularly in the lower lobes.[56] Panlobular emphysema is an uncommon complication of IV abuse of other drugs. Microscopic examination of the lungs demonstrated variable amounts of talc granulomas, inflammatory infiltrates, and occlusive vascular lesions. There was minimal interstitial fibrosis. Talc (magnesium silicate) is a filler and lubricant in the Ritalin® tablet. These pathologic findings are similar to the histologic features of emphysema associated with α_1-antitrypsin deficiency rather than the micronodularity, pleural abnormalities, and progressive massive fibrosis associated with other types of IV drug abuse.[57] Furthermore, obstructive lung disease is an uncommon complication of more common forms of IV drug abuse (e.g., heroin).[58] Pleural abnormalities and progressive massive fibrosis do not usually occur following chronic IV methylphenidate use. The postmortem examination of heart from a 19-year-old demonstrated microscopic foci of necrosis in myocardial fibers surrounded by degenerating polymorphonuclear leukocytic and histocytic cells, similar to the cardiac lesions associated with catecholamine cardiomyopathy; however, contraction band necrosis was absent.[59] He had a cardiac arrest shortly after insufflating methylphenidate and died 16 hours later after sustaining severe hypoxic brain damage and global myocardial ischemia. Traditional anatomic findings of IV drug abuse were absent as was a history of IV drug use.

CLINICAL RESPONSE

The toxic effects of methylphenidate are an extension of pharmacologic effects that include agitation, hallucination, psychosis, seizures, lethargy, tachydysrhythmia, hypertension, weight loss, and hyperthermia. Similar to other stimulant drugs (e.g., cocaine, methamphetamine, pemoline), the use of methylphenidate may exacerbate the tics associated with Tourette syndrome.[60] Rare case reports associate orofacial and extremity dyskinesias with the ingestion of methylphenidate.[61] These dyskinetic movements begin soon after the ingestion of methylphenidate and resolve the same day.

Medical Use

Adverse effects associated with the therapeutic use of methylphenidate include headache, irritability, insomnia, anorexia, abdominal pain, weight loss, irritability, jitteriness, motor tics, anxiety, palpitations, tachycardia, and visual hallucinations.[62] Dose-related increases in blood pressure and heart rate may occur depending on tolerance.[63] In clinical studies, the therapeutic use of methylphenidate is associated with small, but clinically insignificant increases in the mean heart rate and blood pressure without changes in the electrocardiogram; minor elevations in the mean systolic blood pressure (<5 mm Hg) also may persist at least 6 months.[64] Rare case reports and reports from the US Food and Drug Administration Adverse Event Reporting System document sudden death in adolescents on therapeutic doses of methylphenidate; however, the number of sudden deaths in the stimulant-treated children was similar to the general pediatric population.[65,66] In a matched case-control study of 564 cases of sudden death occurring in children aged 7–19 years from 1985–1996, there was a statistically significant incidence of methylphenidate use in the cases of sudden death when compared with a control group of deaths from motor vehicle accidents matched within 3 years of age.[67] There was no analytic confirmation of methylphenidate use; therefore, potential reporting bias limits conclusions regarding causation.

Illicit Use

Following IV injection of crushed methamphetamine tablets, adverse effects include agitation, anxiety, sense of doom, hypoesthesias, chest pain, hypertension, dyspnea, tachycardia, mydriasis, elevated body temperature, and rhabdomyolysis. In addition to the typical complications of IV drug use (e.g., endocarditis, HIV), case reports indicate that long-term, intravenous Ritalin® abusers can develop severe panlobar emphysema, particularly in the lower lobes.[68] These drug abusers develop profound obstructive lung disease characterized by severe hypoxia, decreased diffusing capacities, and radiologic evidence of hyperinflation. These severe pulmonary deficits develop at a relatively young age (4th and 5th decade) similar to α_1-antitripsinase deficiency. However, case series of these patients have not detected this enzyme defect.[56] Multiorgan failure developed in a 32-year-old Ritalin® abuser, who increased her IV dose from 30 mg to 110 mg over 5 months.[69] Two hours after the last dose of 110 mg Ritalin® (methylphenidate) dissolved in tap water, she developed nausea, vomiting, increasing dyspnea, lethargy, and paresthesias. Over the next 24 hours these symptoms progressed to marked tachypnea, metabolic acidosis, hypoxia, hypotension, and multiorgan failure including disseminated intravascular coagulation (DIC). She survived with intensive cardiorespiratory support.

An acute cardiomyopathy (global hypokinesis with left ventricular ejection fraction of 30–35%) and diffuse ST elevations in inferolateral leads developed in a 17-year-old adolescent after the ingestion of 1 methylphenidate tablet at a party.[70] No cardiac coronary artery catheterization was performed. He also smoked marijuana and drank alcohol at this party as confirmed on a urine drug screen that detected no other drugs. A case report associated the chronic ingestion of 60 mg methylphenidate daily for 6 months with the development of a cerebral lacunar infarction manifested by left-sided weakness without loss of consciousness.[71] A computed tomography (CT) scan of the brain demonstrated a lacunar infarction involving the head of the right caudate nucleus and adjacent anterior limb of the internal capsule; the cerebral angiography was normal.

Overdose

The symptoms of methylphenidate intoxication are similar to the clinical features associated with an overdose of other sympathomimetic drugs. Symptoms reported to poison control centers following excessive exposure to methylphenidate include agitation/anxiety, mydriasis, abdominal pains, vomiting, palpitations, tachycardia, and lethargy.[72,73] Severe symptoms are unusual in these case series; typically, the level of conscious and vital signs are normal. Methylphenidate overdose can produce a variety of CNS and cardiovascular effects. Neurologic effects associated with methylphenidate intoxication in addition to the symptoms listed above include restlessness, tremors, muscle twitching, hyperreflexia, lightheadedness, euphoria, hallucination, delusions, paranoia, psychosis, and seizures.[17,20] Cardiac effects include tachycardia, dysrhythmias, hypertension, and chest pain. A 28-year-old woman developed chest pain, ST and T wave depression in her inferolateral leads, and global ventricular hypokinesia (ejection fraction, 30%) after an overdose of methylphenidate.[74] Although the troponin I was elevated, the serum creatine kinase and coronary angiogram were normal. She recovered without sequelae. Syncope may occur, primarily immediately after injection. Fever, diaphoresis, tachypnea, mydriasis, vomiting, and abdominal pain also complicate methylphenidate overdose. Case reports associate the insufflation of crushed methylphenidate tablets with sudden loss of consciousness and cardiopulmonary arrest.[59] The differential diagnosis of methylphenidate intoxication includes toxicity from the use of cocaine, methamphetamine,

3,4-methylenedioxymethamphetamine (MDMA), mescaline, psilocybin, lysergic acid diethylamide (LSD), phencyclidine, anticholinergic agents, pseudoephedrine, ephedrine, phenylpropanolamine, and caffeine.

Abstinence Syndrome

There are few clinical data on the symptoms associated with withdrawal from chronic abuse of methylphenidate. Withdrawal symptoms are probably similar to the abstinence syndrome associated with amphetamine compounds including apathy, lethargy, depression, and paranoia.[22]

Reproductive Abnormalities

There is insufficient data on the developmental toxicity of chronic methylphenidate use in humans. Although some evidence suggests that the maternal abuse of methylphenidate during pregnancy reduces growth and increases prematurity, the lack of adequately controlled studies limits conclusions regarding the causal relationship between methylphenidate and retarded growth. Therapeutic doses of methylphenidate do not increase the incidence of tics or movement disorders in children or increase the incidence of malformations in surveillance studies.[75] A case series of IV pentazocine and methylphenidate-abusing mothers suggested that the IV abuse of these drugs does not produce unique teratogenic abnormalities, but the neonates of these mothers have a relatively high rate of prematurity and growth retardation similar to neonates of other drug-abusing mothers.[76] Adverse pregnancy outcomes in this series included low intelligence, neonatal withdrawal, and malformations; however, the lack of a control group and multiple confounders (e.g., polydrug abuse, smoking, maternal health) limit conclusions regarding the etiology of these abnormalities.

DIAGNOSTIC TESTING

Analytic Methods

Techniques used to detect methylphenidate include enzyme immunoassay (ELISA), gas chromatography, and gas chromatography/mass spectrometric detection of the *O*-trimethylsilyl, *N*-trifluoroacetyl derivatives.[77] ELISA testing results in a relatively high number (i.e., 10%) of false-positive assays. Liquid chromatography/ tandem mass spectrometry is a more rapid, specific method with a limit of quantitation for methylphenidate and ritalinic acid of 23 μg/L (100 nM) and 117 μg/L (500 nM), respectively.[78] Analysis of urine by chiral derivatization gas chromatographic/mass spectrometry with detection by electron impact ionization/selected ion monitoring allows the determination of methylphenidate isomers.[79] Methods for the quantitation of methylphenidate in hair include liquid chromatography/ positive electrospray ionization/ mass spectrometry in selected ion monitoring acquisition mode[80] and gas chromatography/mass spectrometry with *N*-methyl-bisheptafluorobutyric amide derivatization and analysis of the perfluorobutyryl derivatives in SIM.[81] Using the former method, the methylphenidate concentrations ranged from 0.15–4.17 ng/mg in hair, with decreasing drug concentration in distal hair segments. The limit of detection was 0.02 ng/mg in hair. The methylphenidate concentrations following analysis by the latter method ranged from 0.073–1.1 ng/mg in hair, but the methylphenidate concentration did not correlate closely to the methylphenidate dose based on dividing samples from 17 children into low- (10–15 mg daily), medium- (20 mg daily), and high-dose (40–54 mg daily) groups.

Biomarkers

Blood

There is substantial individual variation in plasma methylphenidate concentrations in individuals given the same dose. In a study of 4 children receiving oral methylphenidate doses of 10–15 mg, the plasma methylphenidate and ritalinic acid concentrations ranged from 4–25 μg/L and 80–250 μg/L, respectively, 3–6 hours after ingestion.[28] A fatality occurred about 1 hour after the IV injection of an estimated 40 mg methylphenidate. The methylphenidate postmortem blood level was 2,800 μg/L and the stomach contents (140 mL) contained 1.6 mg methylphenidate.[82] The presence of small amounts of methylphenidate in the stomach does not imply the ingestion of methylphenidate because this drug is basic and accumulates in the acidic environment of the stomach. There are few data on the blood/plasma ratio of methylphenidate. In a study of a single patient, the whole blood/plasma ratio of methylphenidate was about 0.7–0.8.[34]

Urine

Routine urine immunoassay drug screens are relatively insensitive to the presence of methylphenidate. Liquid chromatography/electrospray ionization/tandem mass spectrometry is a rapid, sensitive, and highly specific method for the detection of methylphenidate and the metabolite, ritalinic acid in urine samples.[78]

Abnormalities

Emphysema can complicate the IV abuse of methylphenidate. Plain radiographs demonstrate a symmetrical distribution, primarily involving the lung bases, while CT scans demonstrate low attenuation in the basilar areas.[57] Right ventricular hypertrophy may develop as a result of pulmonary hypertension. Electrocardiographic abnormalities (e.g., QT_c prolongation, QRS widening, dysrhythmias) are unusual in case series of methylphenidate overdoses reported to poison centers.[72] Pulmonary function tests typically reveal a variety of defects as a result of polydrug use including obstructive defects, restrictive patterns, and decreased diffusing capacity as well as hypoxia.[17]

Driving

The blood sample from a person arrested for erratic driving contained approximately 500 µg methylphenidate/L as measured by gas chromatography with flame ionization detection.[83] This individual performed poorly on balance tests, and he reported that he ingested an indeterminate number of methylphenidate tablets 12 hours prior to his apprehension. Individual variability and tolerance to methylphenidate complicates the correlation of plasma methylphenidate concentrations to effects. An increased frequency of vehicular crashes, traffic citations, driving performance deficits, and driving-related cognitive impairments occur in adolescents and adults with ADHD. Methylphenidate treatment of individuals with ADHD improves driving performance. In a study of high-risk ADHD patients, methylphenidate treatment resulted in improvements in information processing (e.g., visuomotor coordination under high-stress conditions), visual orientation, and sustained visual attention compared with baseline and an untreated ADHD control group.[84] In a virtual driving simulator study of 53 adults with ADHD, two 20-mg doses of methylphenidate reduced impulsiveness, variability of steering in the standard driving course, and driving speed during the obstacle course.[85]

TREATMENT

There are few data on the treatment of serious methylphenidate intoxication; however, the treatment of serious methylphenidate intoxication is similar to the treatment of methamphetamine and amphetamine toxicity. (See Amphetamine/Methamphetamine Treatment section). Most agitated patients intoxicated with methylphenidate respond to reassurance, a quiet environment, and IV benzodiazepines (lorazepam, diazepam).

Haloperidol is an option for the treatment of agitation, delirium, or hallucination associated with methylphenidate intoxication, when optimal benzodiazepine therapy does not control these abnormalities. Similar to methamphetamine, the major life-threatening complications of acute methylphenidate intoxication include hyperthermia, hypertension, seizures, cardiovascular instability, and trauma. Coma, shock, acute renal failure, severe hyperthermia (i.e., temperature >41°C/105.8°F), and seizures are poor prognostic indicators. Severely intoxicated patients require IV access, cardiac monitoring, pulse oximetry, 12-lead ECG, and supplemental oxygen. Respiratory depression does not usually occur during mild to moderate methylphenidate intoxication, but pulmonary edema (cardiogenic or due to acute lung injury) can develop during severe methylphenidate poisoning. Underlying pulmonary hypertension occurs in habitual IV methylphenidate users that may intensify during acute methylphenidate intoxication.

Most patients tolerate sinus tachycardia without pharmacologic intervention. Both hypertension and tachycardia often respond to IV benzodiazepines (adults: lorazepam 2 mg or diazepam 5 mg IV bolus titrated to effect). Core temperatures should be measured in any agitated patient. Patients with suspected myocardial ischemia as a result of underlying cardiac disease should be managed with nitrates, morphine, benzodiazepines, and aspirin. Hypotension may respond to fluid challenges, but a vasopressor is often needed. Shock is a poor prognostic sign that indicates the need for monitoring of cardiac output to determine the most efficacious combination of fluid and vasopressors. There are no specific antidotes for methylphenidate intoxication, and there are no clinical data to support the use of methods to enhance the elimination of methylphenidate.

References

1. Leonard BE, McCartan D, White J, King DJ. Methylphenidate: a review of its neuropharmacological, neuropsychological and adverse clinical effects. Hum Psychopharmacol 2004;19:151–180.
2. Rioux B. Is Ritalin an addiction-producing drug? Dis Nerv Syst 1960;21:346–349
3. Spensley J, Rockwell DA. Psychosis during methylphenidate abuse. N Engl J Med 1972;286:880–881.
4. Haglund RM, Howerton LL. Ritalin: consequences of abuse in a clinical population. Int J Addict 1982;17:349–356.
5. Volkow ND, Fowler JS, Wang G, Ding Y, Gatley SJ. Mechanism of action of methylphenidate: insights from

PET imaging studies. J Atten Disord 2002;6(Suppl 1):S31–S43.

6. Markowitz JS, Patrick KS. Differential pharmacokinetics and pharmacodynamics of methylphenidate enantiomers: does chirality matter? J Clin Psychopharmacol 2008;28 (Suppl 2):S54–S61.

7. Carter HS, Watson WA. IV pentazocine/methylphenidate abuse – the clinical toxicity of another Ts and blues combination. Clin Toxicol 1994;32:541–547.

8. Johnston L, O'Malley P, Bachman J. Monitoring the Future National Survey results on drug use, 1975-2000. Bethesda, MD, National Institute on Drug Abuse, NIH Publication No. 01-4924, 2001.

9. Williams RJ, Goodale LA, Shay-Fiddler MA, Golster SP, Chang SY. Methylphenidate and dextroamphetamine abuse in substance-abusing adolescents. Am J Addict 2004;13:381–389.

10 Weyandt LL, Janusis G, Wilson KG, Verdi G, Paquin G, Lopes J, et al. Nonmedical prescription stimulant use among a sample of college students. J Atten Disord 2009; 13:284–296.

11. DeSantis AD, Webb EM, Noar SM. Illicit use of prescription ADHD medications on a college campus: a multi-methodological approach. J Am Coll Health 2008;57: 315–323.

12. McCabe SE, Knight JR, Teter CJ, Wechsler H. Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. Addiction 2005;100:96–106.

13. Wilens TE, Adler LA, Adams J, Sgambati S, Rostrosen J, Sawtelle R, et al. Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. J Am Acad Child Adolesc Psychiatry 2008;47:21–31.

14. Stoops WW, Glaser PE, Fillmore MT, Rush CR. Reinforcing, subject-rated, performance and physiological effects of methylphenidate and d-amphetamine in stimulant abusing humans. J Psychopharmacol 2004;18: 534–543.

15. Kollins SH, MacDonald EK, Rush CR. Assessing the abuse potential of methylphenidate in nonhuman and human subjects a review. Pharmacol Biochem Behavior 2001;68:611–627.

16. Babcock Q, Byrne T. Student perceptions of methylphenidate abuse at a public liberal arts college. J Am Coll Health 2000;49:143–145.

17. Parran TV Jr, Jasinski DR. Intravenous methylphenidate abuse. Prototype for prescription drug abuse. Arch Intern Med 1991;151:781–783.

18. Joyce PR, Donald RA, Nicholls MG, Livesey JH, Abbott RM. Endocrine and behavioral responses to methylphenidate in normal subjects. Biol Psychiatry 1986;21: 1015–1023.

19. Foley R, Mrvos R, Krenzelok EP. A profile of methylphenidate exposures. J Toxicol Clin Toxicol 2000;38:625–630.

20. Klein-Schwartz W. Abuse and toxicity of methylphenidate. Curr Opin Pediatrics 2002;14:219–223.

21. Wakamatsu A, Nomura S, Tate Y, Shimizu S, Harada Y. Effects of methylphenidate hydrochloride on the cardio-vascular system *in vivo* and *in vitro*: a safety pharmacology study. J Pharmacol Toxicol Methods 2009;59:128–134.

22. Jaffe SL. Intranasal abuse of prescribed methylphenidate by an alcohol and drug abusing adolescent with ADHD. J Am Acad Child Adolesc Psychiatry 1991;30:773–775.

23. Goyer PF, Davis GC, Rapoport JL. Abuse of prescribed stimulant medication by a 13-year-old hyperactive boy. J Am Acad Child Psychiatry 1979;18:170–175.

24. Aoyama T, Sasaki T, Kotaki H, Sawada Y, Sudoh Y, Honda Y, Iga T. Pharmacokinetics and pharmacodynamics of (+)-*threo*-methylphenidate enantiomer in patients with hypersomnia. Clin Pharmacol Ther 1994;55:270–276.

25. Chan YP, Swanson JM, Soldin SS, Thiessen JJ, Macleod SM, Logan W. Methylphenidate hydrochloride given with or before breakfast: II. Effects on plasma concentration of methylphenidate and ritalinic acid. Pediatrics 1983; 72:56–59.

26. Wargin W, Patrick K, Kilts C, Gualtieri CT, Ellington K, Mueller RA, et al. Pharmacokinetics of methylphenidate in man, rat and monkey. J Pharmacol Exp Ther 1983;226: 382–386.

27. Wong YN, King SP, Laughton WB, McCormick GC, Grebow PE. Single-dose pharmacokinetics of modafinil and methylphenidate given alone or in combination in healthy male volunteers. J Clin Pharmacol 1998;38: 276–282.

28. Hungund BL, Perel JM, Hurwic MJ, Sverd J, Winsberg BG. Pharmacokinetics of methylphenidate in hyperkinetic children. Br J Clin Pharmacol 1979;8:571–576.

29. Greenhill LL, Perel JM, Rudolph G, Feldman B, Curran S, Puig-Antich J, Gardner R. Correlations between motor persistence and plasma levels in methylphenidate-treated boys with ADHD. Int J Neuropsychopharmacol 2001;4: 207–215.

30. Kimko HC, Cross JT, Abernethy DR. Pharmacokinetics and clinical effectiveness of methylphenidate. Clin Pharmacokinet 1999;37:457–470.

31. Faraj BA, Israili ZH, Perel JM, Jenkins ML, Holtzman SG, Cucinell SA, Dayton PG. Metabolism and disposition of methylphenidate-14C: studies in man and animals. J Pharmacol Exp Ther 1974;191:535–547.

32. Markowitz JS, DeVane CL, Boulton DW, Nahas Z, Risch SC, Diamond F, Patrick KS. Ethylphenidate formation in human subjects after the administration of a single dose of methylphenidate and ethanol. Drug Metab Dispos 2000;28:620–624.

33. Koehm M, Kauert GF, Toennes SW. Influence of ethanol on the pharmacokinetics of methylphenidate's metabolites ritalinic acid and ethylphenidate. Arzneimittelforschung 2010;60:238–244.

34. Redalieu E, Bartlett MF, Waldes LM, Darrow WR, Egger H, Wagner WE. A study of methylphenidate in man with respect to its major metabolite. Drug Metab Dispos 1982;10:708–709.

35. Spigset O, Brede WR, Zahlsen K. Excretion of methylphenidate in breast milk. Am J Psychiatry 2007;164:348.

36. Hackett LP, Kristensen JH, Hale TW, Franzcp RP, Ilett KF. Methylphenidate and breast-feeding. Ann Pharmacother 2006;40:1890–1891.

37. Swanson JM, Volkow ND. Serum and brain concentration of methylphenidate: implications for use and abuse. Neurosci Biobehav Rev 2003;27;615–621.

38. Patrick KS, Straughn AB, Minhinnett RR, Yeatts SD, Herrin AE, DeVane CL, et al. Influence of ethanol and gender on methylphenidate pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 2007;81:346–353.

39. Sherman M, Hauser GC, Glover BH. Toxic reactions to tranylcypromine. Am J Psychiatry 1964;120:1019–1021.

40. Wald D, Ebstein RP, Belmaker RH. Haloperidol and lithium blocking of the mood response to intravenous methylphenidate. Psychopharmacology (Berl) 1978;57: 83–87.

41. Caci H, Bayle F. A case of disulfiram-methylphenidate interaction: implications for treatment. Am J Psychiatry 2007;164:1759.

42. Markowitz JS, Morrison SD, DeVane CL. Drug interactions with psychostimulants. Int Clin Psychopharmacol 1999;14:1–18.

43. Ishii M, Tatsuzawa Y, Yoshino A, Nomura S. Serotonin syndrome induced by augmentation of SSRI with methylphenidate. Psychiatry Clin Neurosci 2008;62:246.

44. Winhusen T, Somoza E, Singal BM, Harrer J, Apparaju S, Mezinskis J, et al. Methylphenidate and cocaine: a placebo-controlled drug interaction study. Pharmacol Biochem Behav 2006;85:29–38.

45. Froimowitz M, Patrick KS, Cody V. Conformational analysis of methylphenidate and its structural relationship to other dopamine reuptake blockers such as CFT. Pharm Res 1995;12:1430–1434.

46. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 1987;237:1219–1223.

47. Hitri A, Hurd YL, Wyatt RJ, Deutsch SI. Molecular, functional and biochemical characteristics of the dopamine transporter: regional differences and clinical relevance. Clin Neuropharmacol 1994;17:1–22.

48. Volkow ND, Ding Y-S, Fowler JS, Wang G-J, Logan J, Gatley JS, et al. Is methylphenidate like cocaine: studies on their pharmacokinetics and distribution in the human brain. Arch Gen Psychiatry 1995;52:456–463.

49. Acquas E, Fibiger HC. Chronic lithium attenuates dopamine D1-receptor mediated increases in acetylcholine release in rat frontal cortex. Psychopharmacology (Berl) 1996;125:162–167.

50. Zhu HJ, Wang JS, DeVane CL, Williard RL, Donovan JL, Middaugh LD, et al. The role of the polymorphic efflux transporter P-glycoprotein on the brain accumulation of *d*-methylphenidate and *d*-amphetamine. Drug Metab Dispos 2006;34:1116–1121.

51. Volkow ND, Wang G-J, Gatley SJ, Fowler JS, Ding Y-S, Logan J, et al. Temporal relationships between the pharmacokinetics of methylphenidate in the human brain and its behavioral and cardiovascular effects. Psychopharmacology 1996;123:26–33.

52. Volkow ND, Wang G-J, Fowler JS, Logan J, Gerasimov M, Maynard L, et al. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 2001;21:RC121.

53. Huss M, Lehmkuhl U. Methylphenidate and substance abuse: a review of pharmacology, animal, and clinical studies. J Atten Disord 2002;6(suppl 1):S65–S71.

54. Lewman LV. Fatal pulmonary hypertension from intravenous injection of methylphenidate (Ritalin) tablets. Hum Pathol 1972;3:67–70.

55. Lundquest DE, Young WK, Edland JF. Maternal death associated with intravenous methylphenidate (Ritalin®) and pentazocine (Talwin®) abuse. J Forensic Sci 1987;32:798–801.

56. Schmidt RA, Glenny RW, Godwin JD, Hampson NB, Cantino ME, Reichenbach DD. Panlobular emphysema in young intravenous Ritalin® abusers. Am Rev Respir Dis 1991;143:649–656.

57. Stern EJ, Frank MS, Schmutz JF, Glenny RW, Schmidt RA, Godwin JD. Panlobular pulmonary emphysema caused by IV injection of methylphenidate (Ritalin): findings on chest radiographs and CT scans. Am J Roentgenol 1994; 162:555–560.

58. Overland ES, Nolan AJ, Hopewell PC. Alteration of pulmonary function in intravenous drug abusers. Prevalence, severity, and characterization of gas exchange abnormalities. Am J Med 1980;68:231–237.

59. Massello W III, Carpenter DA. A fatality due to the intranasal abuse of methylphenidate (Ritalin®). J Forensic Sci 1999;44:220–221.

60. Pollack MA, Cohen NL, Friedhoff AJ. Gilles de la Tourette's syndrome. Arch Neurol 1977;34:630–632.

61. Balazs J, Besnyo M, Gadoros J. Methylphenidate-induced orofacial and extremity dyskinesia. J Child Adolesc Psychopharmacol 2007;17:378–381.

62. Halevy A, Shuper A. Methylphenidate induction of complex visual hallucinations. J Child Neurol 2009;24: 1005–1007.

63. Kelly KL, Rapport MD, DuPaul GJ. Attention deficit disorder and methylphenidate: a multi-step analysis of dose-response effects on children's cardiovascular functioning. Int Clin Psychopharmacol 1988;3:167–181.

64. Hammerness P, Wilens T, Mick E, Spencer T, Doyle R, McCreary M, et al. Cardiovascular effects of longer-term, high-dose OROS methylphenidate in adolescents with attention deficit hyperactivity disorder. J Pediatr 2009; 155:84–89.

65. Daly MW, Custer G, McLeay PD. Cardiac arrest with pulseless electrical activity associated with methylphenidate in an adolescent with a normal baseline echocardiogram. Pharmacotherapy 2008;28:1408–1412.

66. Knight M. Stimulant-drug therapy for attention-deficit disorder (with or without hyper activity) and sudden cardiac death. Pediatrics 2007;119:154–155.

67. Gould MS, Walsh BT, Munfakh JL, Kleinman M, Duan N, Olfson M, et al. Sudden death and use of stimulant medications in youths. Am J Psychiatry 2009;166:992–1001.

68. Sherman CB, Hudson LD, Pierson DJ. Severe precocious emphysema in intravenous methylphenidate (Ritalin) abusers. Chest 1987;92:1085–1087.

69. Stecyk O, Loludice TA, Demeters S, Jacobs J. Multiple organ failure resulting from intravenous abuse of methylphenidate hydrochloride. Ann Emerg Med 1985;14:597–599.

70. Dadfarmay S, Dixon J. A case of acute cardiomyopathy and pericarditis associated with methylphenidate. Cardiovasc Toxicol 2009;9:49–52.

71. Sadeghian H. Lacunar stroke associated with methylphenidate abuse. Can J Neurol Sci 2004;31:109–111.

72. Hill SL, El-Khayat RH, Sandilands EA, Thomas SH. Electrocardiographic effects of methylphenidate overdose. Clin Toxicol 2010;48:342–346.

73. White SR, Yadao CM. Characterization of methylphenidate exposures reported to a regional poison control center. Arch Pediatr Adolesc Med 2000;154:1199–1203.

74. Wong OF, Tsui KL, Fung HT. Acute coronary syndrome secondary to methylphenidate overdose. Hong Kong J Emerg Med 2010;16:66–70.

75. Golub M, Costa L, Crofton K, Frank D, Fried P, Gladen B, et al. NTP-CERHR expert panel report on the reproductive and developmental toxicity of methylphenidate. Birth Defects Res 2005 (Part B);74:300–381.

76. Debooy VD, Seshia MM, Tenenbein M, Casiro OG. Intravenous pentazocine and methylphenidate abuse during pregnancy maternal lifestyle and infant outcome. Am J Dis Child 1993;147:1062–1065.

77. Solans A, Carnicero M, De La Torre R, Segura J. Simultaneous detection of methylphenidate and its main metabolite, ritalinic acid, in doping control. J Chromatogr B Biomed Appl 1994;658:380–384.

78. Eichhorst J, Etter M, Lepage J, Lehotay DC. Urinary screening for methylphenidate (Ritalin) abuse: a comparison of liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry, and immunoassay methods. Clin Biochem 2004;37:175–183.

79. LeVasseur NL, Zhu HJ, Markowitz JS, DeVane CL, Patrick KS. Enantiospecific gas chromatographic-mass spectrometric analysis of urinary methylphenidate: implications for phenotyping. J Chromatogr B Analyt Technol Biomed Life Sci 2008;862:140–149.

80. Marchei E, Muñoz JA, García-Algar O, Pellegrini M, Vall O, Zuccaro P, Pichini S. Development and validation of a liquid chromatography-mass spectrometry assay for hair analysis of methylphenidate. Forensic Sci Int 2008;176:42–46.

81. Sticht G, Sevecke K, Käferstein H, Döpfner M, Rothschild MA. Detection of methylphenidate in the hair of children treated with Ritalin. J Anal Toxicol 2007;31:588–591.

82. Levine B, Caplan YH, Kauffman G. Fatality resulting from methylphenidate overdose. J Anal Toxicol 1986;10:209–210.

83. Schubert B. Detection and identification of methylphenidate in human urine and blood samples. Acta Chem Scand 1970;24:433–438.

84. Sobanski E, Sabljic D, Alm B, Skopp G, Kettler N, Mattern R, Strohbeck-Kühner P. Driving-related risks and impact of methylphenidate treatment on driving in adults with attention-deficit/hyperactivity disorder (ADHD). J Neural Transm 2008;115:347–356.

85. Barkley RA, Murphy KR, O'Connell T, Connor DF. Effects of two doses of methylphenidate on simulator driving performance in adults with attention deficit hyperactivity disorder. J Safety Res 2005;36:121–131.

Chapter 3

PROLINTANE

HISTORY

Prolintane hydrochloride is a central nervous system (CNS) stimulant that has been marketed in Europe since the 1960s as an antidepressant (i.e., antifatigue), analeptic, and vasopressor (i.e., orthostatic hypotension) agent. The first reports of prolintane abuse appeared in Europe during the early 2000s when prolintane was identified in tablets distributed at a rave party (i.e., all-night dancing with electronically synthesized music).[1] Later reports documented the recreational use of prolintane in the United States.[2]

IDENTIFYING CHARACTERISTICS

Prolintane (CAS RN: 493-92-5, $C_{15}H_{23}N$) is a synthetic sympathomimetic amine with pharmacologic properties similar to d-amphetamine. The molecular weight (MW) of prolintane is 217.35 g/mol, whereas the MW of the hydrochloride salt (CAS RN: 1211-28-5) is 253.8 g/mol. Prolintane hydrochloride is a water-soluble compound that is a white powder with a melting point of approximately 133ºC. Figure 3.1 demonstrates the chemical structure of prolintane [(R,S)-1-(α-propylphenethyl)-pyrrolidine].

EXPOSURE

Therapeutic uses of prolintane in Africa, Europe, and Australia include the treatment of narcolepsy, attention deficit hyperactivity disorder (ADHD), fatigue, and orthostatic hypotension. Prolintane is formulated with multivitamins including ascorbic acid and sold as an orange tablet (e.g., "kitovit") or a tonic. Proprietary preparations include Villescon® (Boehringer Ingelheim, Berkshire, UK), Promotil® (Boehringer Ingelheim, Paris, France), and Catovit® (Boehringer Ingelheim, New South Wales, Australia). This drug is not approved for pharmaceutic use in the United States. Prolintane is a popular rave drug in Europe, but the documented use of prolintane in this setting in the United States is rare.[1] Additionally, prolintane has been implicated as a doping agent in athletics (e.g., cycling);[3] this compound is now banned by the US National Collegiate Athletic Association and the World Anti-Doping Agency (WADA).[4]

DOSE EFFECT

Typical adult therapeutic doses of prolintane are 10–40 mg daily. In therapeutic trials, 20 mg prolintane is a mild stimulant equivalent to approximately 100 mg caffeine.[5] The use of therapeutic doses of prolintane is associated with a subjective feeling of improved concentration and decreased fatigue, but there are minimal changes in objective measures of fatigue and mental activity.[6] The stimulant properties of prolintane are substantially less than d-amphetamine. In a study of fatigued volunteers, the administration of 20 mg or 40 mg prolintane produced similar, but less intense effects than 20 mg d-amphetamine.[7] These clinical effects included stimulation, euphoria, anorexia, and mild elevation of systolic blood pressure. In experimental studies of healthy volunteers, prolintane has little cardiovascular activity following the administration of a single dose of 20 mg.[8] This dose slightly improves some mental tasks (e.g., sign
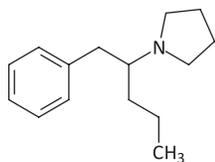
FIGURE 3.1. Chemical structure of prolintane.

recordings), but not others (e.g., digit span). The clinical significance of these results on memory, learning, and concentration is unclear. In volunteer studies, the administration of prolintane (20–40 mg) increases wakefulness and improves performance on some perceptual and arithmetic tasks in fatigued individuals.[7,9]

TOXICOKINETICS

The first-pass effect of prolintane is large. Prolintane undergoes extensive oxidation and *N*-dealkylation in the body with the formation of at least 18 metabolites. The metabolism of prolintane primarily involves biotransformation. The major metabolic pathways are aromatic hydroxylation (*para*- and *meta*-positions), lactam formation, γ-amino acid formation, heterocyclic hydroxylation (3- and 4-positions), and propyl hydroxylation at ω-, (ω-1)-positions.[10] Oxidation of the α-carbon of the pyrrolidine ring forms the major metabolite, oxoprolintane. In a study of 5 healthy volunteers receiving 0.15 mg prolintane/kg intravenously, the mean plasma elimination half-life was approximately 4.5 hours.[11] The urine pH of these volunteers was within the normal range. The kidney excretes minimal amounts (i.e., <1–2%) of prolintane unchanged.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Prolintane is a CNS stimulant with many pharmacologic properties similar to *d*-amphetamine. However, rodent studies indicate that prolintane enhances brain dopamine turnover produced by dopamine receptor antagonists similar to methylphenidate and cocaine, whereas amphetamine and methamphetamine do not cause similar effects.[12]

CLINICAL RESPONSE

Prolintane is a sympathomimetic amine that has psychostimulant properties similar to *d*-amphetamine and phentermine. Adverse effects of therapeutic doses include nausea, vomiting, anxiety, irritability, insomnia, lightheadedness, headache, euphoria, palpitations, diaphoresis, confusion, agitation, disorientation, and acute

psychosis.[13] Clinical effects associated with the abuse of prolintane include lightheadedness, insomnia, irritability, and nervousness. Excessive use of prolintane may also cause hypertension, tachycardia, hyperreflexia, mydriasis, hyperthermia, agitation, confusion, disorientation, hallucinations, and psychosis.

DIAGNOSTIC TESTING

Analytic methods for the detection of prolintane include thin-layer chromatography,[14] gas chromatography with nitrogen-selective detection,[11] high performance liquid chromatography with ultraviolet detection (252 nm, 258 nm, 264 nm),[2] gas chromatography/mass spectrometry,[15] and capillary zone electrophoresis with β-cyclodextrin-modified micellar electrokinetic chromatography.[16] The limit of detection (LOD) and lower limit of quantitation (LLOQ) for the latter method were 300 ± 100 ng/mL and $1,000 \pm 100$ ng/mL, respectively. Prolintane is 1 of 14 tertiary amine stimulants detected by screening and confirmation with gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry.[17] The LOD in urine samples using this method is 10 ng/mL; this method meets WADA's requirements for sensitivity. Following the intravenous (IV) injection of 0.15 mg prolintane/kg to 5 healthy volunteers, the mean peak plasma prolintane concentration was approximately 60 ng/mL.[11] Prolintane use is not usually detectable by routine urine immunoassay drug screens.

TREATMENT

Treatment is supportive, similar to the treatment of *d*-amphetamine intoxication.

References

1. Gaulier JM, Canal M, Pradeille JL, Marquet P, Lachâtre G. [New drugs at "rave parties": ketamine and prolintane]. Acta Clin Belg 2002;(Suppl):41–46. [French]
2. Kyle PB, Daley WP. Domestic abuse of the European rave drug prolintane. J Anal Toxicol 2007;31:415–418.
3. Delbeke FT. Doping in cyclism: results of unannounced controls in Flanders (1987–1994). Int J Sports Med 1996; 17:434–438.
4. World Anti-Doping Agency List Committee. The world doping code. The 2008 prohibited list international standard. Montreal, Canada: World Anti-Doping Agency; 2007:8.
5. McGuinness BW. A therapeutic trial of prolintane. Practitioner 1965;195:363–365.
6. Newbold GF. Prolintane in debility and fatigue report of a trial among college students in Cardiff. Practitioner 1974;213:868–870.

7. Hollister LE, Gillespie HK. A new stimulant, prolintane hydrochloride, compared with dextroamphetamine in fatigued volunteers. J Clin Pharmacol 1970;10:103–109.

8. Kuituenen T, Karkkainen S, Ylitalo P. Comparison of the acute physical and mental effects of ephedrine, fenfluramine, phentermine and prolintane. Meth Find Exp Clin Pharmacol 1984;6:265–270.

9. Nicholson AN, Stone BM, Jones MM. Wakefulness and reduced rapid eye movement sleep: studies with prolintane and pemoline. Br J Clin Pharmacol 1980;10:465–472.

10. Rucker G, Neugebauer M, Zhong D. Study on the metabolism of racemic prolintane and its optically pure enantiomers. Xenobiotica 1992;22:143–152.

11. Schmid J. Assay of prolintane in plasma by capillary gas chromatography with nitrogen-selective detection. J Chromatogr 1981;222:129–134.

12. Fuller RW, Snoddy HD. Effects of prolintane on 3,4-dihydroxyphenylacetic acid concentration in rat brain after spiperone treatment. Pharmacol Biochem Behav 1979;10:561–563.

13. Martinez-Mir I, Catalan C, Palop V. Prolintane: a "masked" amphetamine. Ann Pharmacother 1997;31:256.

14. Musumarra G, Scarlata G, Cirma G, Romano G, Palazzo S, Clementi S, Giulietti G. Qualitative organic analysis. I. Identification of drugs by principal components analysis of standardized thin-layer chromatographic data in four eluent systems. J Chromatogr 1985;350:151–168.

15. Lillsunde P, Korte T. Comprehensive drug screening in urine using solid-phase extraction and combined TLC and GC/MS identification. J Anal Toxicol 1991;15:71–81.

16. Espartero AG, Perez JA, Zapardiel A, Bermejo E, Hernandez L. Direct determination of prolintane and its metabolite oxoprolintane in human urine by capillary zone electrophoresis and β-cyclodextrin-modified micellar electrokinetic chromatography. J Chromatogr A 1997;778: 355–361.

17. Lu J, Wang S, Dong YU, Wang X, Yang S, Zhang J, et al. Simultaneous analysis of fourteen tertiary amine stimulants in human urine for doping control purposes by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. Anal Chim Acta 2010;657:45–52.

Chapter 4

PROPYLHEXEDRINE

HISTORY

The Smith, Kline, and French Company introduced Benzedrine® inhalers during the early 1930s as racemic amphetamine. One Benzedrine® inhaler contained the equivalent of 250 mg of racemic amphetamine. These inhalers remained available as over-the-counter products until 1949, when Smith Kline & French voluntarily removed amphetamine inhalers from the market in response to reports of abuse and sudden death.[1,2] The Benzedrine® inhaler was replaced by the Benzedrex® inhaler (B.F. Ascher & Co., Lenexa, KS), which contained 250 mg of propylhexedrine (i.e., no amphetamine) in a combination with 12.5 mg methanol and various aromatics hydrocarbons. Some airlines (e.g., Pan American) distributed Benzedrex® inhalers in the 1950s as decongestants to reduce discomfort resulting from changes in cabin pressures. The usual method of Benzedrine® and Benzedrex® abuse involved the chewing and swallowing of the amphetamine-containing cotton pledget, preferably with chewing gum to reduce the irritant properties of the base. Occasionally, the strips were soaked in beverages (i.e., coffee, alcohol) or swallowed whole. One study of an incarcerated population in the 1940s revealed that 25% of the inmate population used the inhalant material for recreational purposes.[3] By the mid-1970s, the medical literature contained reports of both oral and intravenous (IV) abuse of the propylhexedrine inhalers. The abuse of propylhexedrine was sporadic throughout North America with cases reported from Dallas, Toronto, and San Francisco during the 1980s;[4] however, there are little current data on propylhexedrine abuse because most national US databases on drug abuse do not include propylhexedrine.

IDENTIFYING CHARACTERISTICS

Propylhexedrine (CAS RN: 101-40-6, MW = 155.3 g/mol) is an α-adrenergic agonist with a molecular formula of $C_{21}H_{10}N$. This slightly water-soluble compound is a clear, colorless liquid that vaporizes slowly at room temperature. The hydrochloride salt of propylhexedrine (MW = 191.7 g/mol) is a crystalline solid with a melting point of approximately 127°C/260.6°F. Table 4.1 displays some physical properties of propylhexedrine.

This drug is an alicyclic aliphatic sympathomimetic amine structurally similar to methamphetamine. Figure 4.1 displays the structural similarity between propylhexedrine and other common aromatic sympathomimetic compounds including methamphetamine and the neurotransmitter, epinephrine.

EXPOSURE

The primary medicinal use of propylhexedrine is the symptomatic relief of nasal congestion without rebound. Propylhexedrine is a potent α-adrenergic sympathomimetic agent, which is a "stimulant of last resort" for abusers when more desirable stimulants are unavailable. However, the abuse potential of propylhexedrine is low compared with methamphetamine, phenmetrazine, or methylphenidate.[5] Most drug users prefer cocaine and methamphetamine to propylhexedrine, and propylhexedrine abuse is typically brief as the users rapidly lose interest in the drug.[3] The street names for

TABLE 4.1. Physical Properties of Propylhexedrine.

Physical Property	Value
Boiling Point	205°C/401°F
log P (Octanol-Water)	3.500
Atmospheric OH Rate Constant	9.87E-11 cm³/molecule-second (25°C)
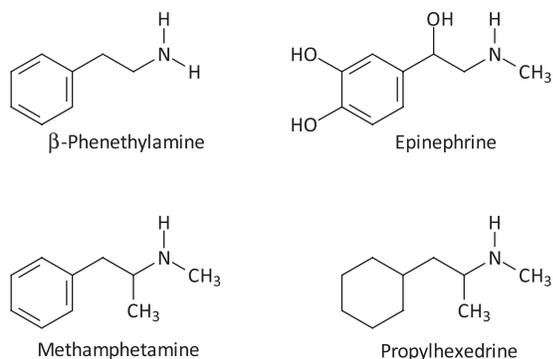
FIGURE 4.1. Structural similarities of methamphetamine, β-phenylethylamine, epinephrine, and propylhexedrine.

the propylhexedrine extract include Bathtub Crystal, Crystal, and Bathtub Crank. Abuse of propylhexedrine nasal inhalers involves habitual inhalation, ingestion or chewing of the propylhexedrine-containing cotton plug, or injection of an acidic solution prepared from the dismantled inhaler. Nasal abuse of propylhexedrine is uncommon.

Habitual use of these inhalers involves the daily use of the contents of 2 to 11 inhalers. Following the disassembly of the inhaler, the cotton pledget is soaked in muriatic (hydrochloric) acid to remove the propylhexedrine.[4] Heating of this extract produces a brown residue that subsequently is redissolved in water and injected. Depending on the purity of the process, the resultant solution contains a variety of impurities including polymeric material, fine fibers, methanol, and lavender oil. Some patients with previous amphetamine experience consider the euphoria induced by the IV injection of Benzedrex® extracts, 15 mg of methamphetamine, and 75 mg of phenmetrazine similar.[6] Benzedrex® abuse is commonly associated with polydrug abuse and homosexual activities.[7] Euphoria apparently does not result from inhalation.

DOSE EFFECT

The ingestion of a hot water extract of a cotton pledget containing 250 mg propylhexedrine produced anxiety, agitation, tremors, nausea, and diaphoresis that resolved without sequelae 4 hours after ingestion.[8] Ingestion of an estimated 375 mg propylhexedrine by a 3-year-old caused tremor, muscle spasticity, tachycardia, and insomnia that resolved without sequelae.[9]

TOXICOKINETICS

Propylhexedrine penetrates the blood–brain barrier rapidly. In rodent studies, this drug has a large volume of distribution ($V_d = 19.3$ L/kg) after IV administration.[10] Biotransformation of propylhexedrine involves N-demethylation, N-oxidation, and 4″-hydroxylation with urinary metabolites including norpropylhexedrine, cyclohexylacetoxime, and 4-hydroxypropylhexedrine.[11] The approximate elimination half-life of propylhexedrine after IV administration to rodents is about 2.5 hours.[8]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Propylhexedrine is a potent local vasoconstrictor with substantially less (i.e., <10%) CNS stimulating effect compared with dextroamphetamine sulfate. The acute toxicity of propylhexedrine is an exacerbation of pharmacologic effects of this drug. The psychoactive effects of these compounds result from indirect agonist actions that cause the displacement of catecholamines from binding sites in the storage vesicles.

At least in part, the central effects of propylhexedrine result from alterations in the neurotransmitters, dopamine and norepinephrine, because of the close structural resemblance of amphetamine compounds to these neurotransmitters. Increasing the synaptic concentration of these neurotransmitters either by direct release from storage vesicles or by inhibition of reuptake is the most likely mechanism of toxicity.

Postmortem Examination

Autopsies of individuals with a history of IV propylhexedrine abuse usually demonstrate foreign body granulomas in the pulmonary parenchyma containing birefringent crystalline material along with variable amounts of pulmonary hypertension and right ventricular hypertrophy.[12] Histologic examination reveals diffuse interstitial alveolar fibrosis, pulmonary edema with focal intra-alveolar hemorrhage, intimal and medial proliferation with luminal obstruction, medial hypertrophy of the muscular arteries, and focal myocardial fibrosis (usually without clinically significant coronary artery stenosis).[13]

CLINICAL RESPONSE

Illicit Use

Propylhexedrine is a potent local vasoconstrictor with mild to moderate CNS stimulant effects. Although propylhexedrine seems structurally similar to methamphetamine, the substitution of a cyclohexyl ring for the phenyl moiety substantially reduces the desirable CNS effects of propylhexedrine. Mild intoxication causes restlessness, irritability, anxiety, mydriasis, diaphoresis, nausea, and tachycardia. More severe effects associated with serious propylhexedrine intoxication include hypertension, tachypnea, cardiac dysrhythmias, hyperpyrexia, seizures, psychosis, and coma.

MENTAL DISORDERS

A case report associated the development of an acute paranoid psychosis with the daily chewing of 10–12 propylhexedrine inhalers by 2 amphetamine abusers.[14] The psychosis in both of these patients resolved after the cessation of drug use. Initially, the propylhexedrine abuser can experience headache, nausea, and double vision followed by euphoria that lasts several hours.[4] Case reports associate deterioration of chronic paranoid schizophrenia with the chewing of propylhexedrine decongestant inhalants as manifest by the onset of acute delusional mood and paranoid delusions.[15] Other factors (sleep deprivation, predrug personality, idiosyncratic reactions) contribute to the development of the toxic psychosis. Homicidal and suicidal ideations may occur during drug-induced psychosis.[5]

MEDICAL COMPLICATIONS

Peripheral vascular complications of IV propylhexedrine abuse include local ischemia with necrosis of the digits requiring amputation,[16] cellulitis, sterile abscess, and a fatal abscess in the neck after an attempted central venous injection of an aqueous extract of propylhexedrine-containing inhalers.[17] Incidental injection of the propylhexedrine extract into a peripheral artery caused local vasoconstriction, inflammation, infection, and necrosis manifest by intense pain, marked edema, blanching of the extremity, and loss of distal arterial pulses.[18] A case report of a 22-year-old man associated the development of pulmonary edema, hypotension, anterior myocardial infarction on electrocardiogram (ECG), accelerated junctional rhythm, and pericardial effusion with the ingestion of 250 mg of propylhexedrine obtained from a Benzedrex® inhaler several hours prior to the onset of symptoms.[19] The patient recovered after intensive cardiorespiratory support. There was no cardiac angiography or echocardiography. Pulmonary edema is a common finding among habitual IV propylhexedrine abusers presenting to emergency departments with dyspnea and cardiovascular instability; cor pulmonale can develop in these patients.[11] Case reports associated the IV use of propylhexedrine extracts with the development of brainstem dysfunction including transient diplopia, right-internuclear ophthalmoplegia, and focal paralysis of the tongue.[7]

Fatalities

Several case reports associated cardiac arrest with the chronic IV administration of propylhexedrine, particularly during extreme exertion (e.g., resisting arrest, fleeing from an assailant or a crime scene)[20] or working outside.[21] These patients are frequently severely acidotic, hypotensive, and unresponsive to the standard techniques of advanced cardiac life support. Right ventricular hypertrophy and pulmonary hypertension is often present. A 29-year-old man was transported to a hospital with abdominal pain, nausea, vomiting, and chest pain.[11] Shortly after arrival, he developed a seizure, severe metabolic acidosis, hypotension, and cardiopulmonary arrest. He had no evidence of ischemia on the ECG, and the chest x-ray demonstrated right ventricular cardiomegaly and pulmonary edema.

DIAGNOSTIC TESTING

Analytic Methods

Analysis of biologic samples for propylhexedrine is similar to the methods used to detect methamphetamine and amphetamine; typically these methods involve gas chromatographic separation with detection by flame ionization (FID), mass spectrometry (MS), or nitrogen–phosphorus detection (NPD). Challenges associated with inadequate chromatographic resolution or co-elution of propylhexedrine with methamphetamine can be resolved by the use of selective ion monitoring (SIM) mode in the mass spectrometer. Preanalytical derivatization with heptafluorobutyric anhydride or 4-carbethoxyhexafluorobutyryl chloride following chromatographic separation and MS detection in SIM mode also effectively distinguishes methamphetamine from propylhexedrine. Mass spectral fragmentation of methamphetamine includes the tropylium ion (m/z 91) from phenyl ring expansion and benzyl carbon incorporation; the lack of a phenyl ring in propylhexedrine eliminates this ion from its mass spectrum. Additionally, the mass spectrum of propylhexedrine includes a prominent occurrence at m/z 182, which is

absent in the mass spectrum of methamphetamine.[22] High performance liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry does not require preanalytical derivatization necessary for gas chromatographic analysis. This method allows the separation of sympathomimetic amines including propylhexedrine with limits of detection in the range of 0.001–0.005 mg/L.[23]

Biomarkers

The blood propylhexedrine concentration in blood samples drawn 30 minutes after the insufflation of 15 puffs from a Benzedrex® inhaler by a healthy volunteer was 0.1 mg/L. The typical dose of Benzedrex® inhaler is 2 puffs in each nostril. A 30-year-old man was found dead in his residence with multiple cotton pledgets from disassembled Benzedrex® inhalers.[24] The postmortem blood propylhexedrine concentration was 30 mg/L. Typically, the postmortem blood samples of IV abusers found dead after propylhexedrine injection ranges from approximately 1.0–2.5 mg/L. The postmortem propylhexedrine concentration in blood samples from 2 IV propylhexedrine abusers found dead was 0.3 mg/dL and 2.0 mg/L compared with 1.6 mg/L in postmortem blood from an IV propylhexedrine abuser who died 3 hours after admission to the emergency department with pulseless electrical activity.[10] The propylhexedrine concentrations in postmortem blood samples from patients dying from causes other than propylhexedrine intoxication can overlap with the concentrations associated with fatal IV propylhexedrine intoxication. The propylhexedrine concentrations in postmortem blood from a 23-year-old hanging victim, a 25-year-old with fatal head wounds, and a 27-year-old man with fatal gunshot wounds to the chest were 2.5 mg/L, 1.1 mg/L, and 0.8 mg/L, respectively.[11]

Abnormalities

With the exception of the carbon monoxide diffusing capacity, pulmonary function tests are often normal in IV propylhexedrine abusers.[5] Pulmonary fibrosis, right ventricular hypertrophy, and pulmonary hypertension are frequent findings in chronic IV propylhexedrine abusers.

TREATMENT

Stabilization

The treatment of propylhexedrine intoxication is similar to the treatment of methamphetamine and amphetamine toxicity. Most agitated patients intoxicated with propylhexedrine respond to reassurance, a quiet environment, and IV benzodiazepines (lorazepam, diazepam). Similar to methamphetamine, the major life-threatening complications of acute propylhexedrine intoxication include hyperthermia, hypertension, seizures, cardiovascular instability, and trauma. Coma, shock, acute renal failure, severe hyperthermia (i.e., temperature >41ºC/105.8ºF), and seizures are poor prognostic indicators. Severely intoxicated patients require IV access, cardiac monitoring, pulse oximetry, an ECG, and supplemental oxygen. Respiratory depression does not usually occur during mild to moderate propylhexedrine intoxication, but pulmonary edema (i.e., cardiogenic, acute lung injury) can develop during severe propylhexedrine poisoning. Underlying pulmonary hypertension can occur in habitual IV propylhexedrine users.

Most patients tolerate sinus tachycardia without pharmacologic intervention unless the tachycardia results from right-sided heart failure. Both hypertension and tachycardia often respond to IV benzodiazepines (Adult: lorazepam 2 mg or diazepam 5 mg IV bolus titrated to effect). Core temperatures should be measured in any agitated patient. Patients with suspected myocardial ischemia should be managed according to the usual guidelines for myocardial ischemia including aspirin, antiplatelet agents, thrombolytics, primary percutaneous coronary intervention, and blood pressure control as needed. Hypotension may respond to fluid challenges, but often a vasopressor is needed. Shock is a poor prognostic sign that indicates the need for monitoring of cardiac output to determine the most efficacious combination of fluid and vasopressors. There are no specific antidotes for propylhexedrine intoxication or efficacious methods to enhance the elimination of propylhexedrine.

Supportive Care

Treatment for local tissue damage resulting from the IV administration of extracts containing propylhexedrine includes antibiotics, vasodilators, debridement, and local wound care. The presentation of serious deep infections in the setting of distal ischemia may be subtle; therefore, suspected areas of infection should be imaged with ultrasound or magnetic resonance imaging (MRI) and early drainage. Distal ischemia is usually an isolated problem resulting from inadvertent intra-arterial administration of propylhexedrine; there are few clinical data on outcomes to guide treatment of the distal ischemia. Therapeutic options for the treatment of ischemia include intra-arterial tolazoline (12–25 mg/limb), IV nitroprusside infusions (0.5–8 μg/kg/min), Dextran 40 (80 mL/h), local nerve blocks, and axillary nerve block.

If a compartment syndrome is suspected, compartment pressures should be measured to help guide the need for fasciotomy. Criteria for fasciotomy include <30 mm Hg difference between compartment and diastolic blood pressures or obvious clinical symptoms of compartment syndrome (severe pain unrelieved by splinting, severe pain with passive stretching unrelated to injury).[25] Supportive care for compartment syndrome includes analgesics, elevation of extremities, and consideration of the use of anticoagulant or antiplatelet drugs.[26] Although the use of prostanoids (e.g., iloprost) is an option for the treatment of critical distal ischemia, there is no conclusive evidence of the long-term effectiveness and safety of different prostanoids including iloprost.[27]

References

1 Smith LC. Collapse with death following the use of amphetamine sulfate. JAMA 1939;113:1022–1023.

2. Peterson BH, Somerville DM. Excessive use of "Benzedrine" by a psychopath. Med J Aust 1949;2: 948–949.

3. Monroe RR, Drell HJ. Oral use of stimulants obtained from inhalers. JAMA 1947;135:909–915.

4. Smith DE, Wesson DR, Sees KL, Morgan JP. An epidemiological and clinical analysis of propylhexedrine abuse in the United States. J Psychoactive Drugs 1988;20: 441–442.

5. Wesson DR. Propylhexedrine. Drug Alcohol Depend 1986;17:273–278.

6. Anderson RJ, Reed WG, Hillis LD, Morgan CD, Garriott JC. History, epidemiology, and medical complications of nasal inhaler abuse. J Toxicol Clin Toxicol 1982;19: 95–107.

7. Fornazzari L, Carlen PL, Kapur BM. Intravenous abuse of propylhexedrine (Benzedrex®) and the risk of brainstem dysfunction in young adults. Can J Neurol Sci 1986;13: 337–339.

8. Liggett SB. Propylhexedrine intoxication: clinical presentation and pharmacology. South Med J 1982;75:250–251.

9. Polster H. [On poisoning with the appetite depressant propylhexedrine, "obesin" in a 3-year-old child]. Arch Toxicol 1965;20:271–273. [German]

10. Iven H, Feldbusch E. Pharmacokinetics of phenobarbital and propylhexedrine after administration of barbexaclone in the mouse. Naunyn Schmiedebergs Arch Pharmacol 1983;324:153–159.

11. Midha KK, Beckett AH, Saunders A. Identification of the major metabolites of propylhexedrine *in vivo* (in man) and *in vitro* (in guinea pig and rabbit). Xenobiotica 1974;4: 627–635.

12. Di Maio VJM, Garriott JC. Intravenous abuse of propylhexedrine. J Forensic Sci 1977;22:152–158.

13. Anderson RJ, Garza HR, Garriott JC, DiMaio V. Intravenous propylhexedrine (Benzedrex®) abuse and sudden death. Am J Med 1979;67:15–20.

14. Pallis DJ, Barraclough BM, Tsiantis J. Psychosis and nasal decongestants. Practitioner 1972;209:676–678.

15. Johnson J, Johnson DA, Robins AJ. Propylhexedrine chewing and psychosis. Br J Med 1972;3:529–530.

16. Mancusi-Ungaro HR Jr, Decker WJ, Forshan VR, Blackwell SJ, Lewis SR. Tissue injuries associated with parenteral propylhexedrine abuse. Clin Toxicol 1983–84; 21:359–372.

17. Perez J, Burton BT, McGirr JG. Airway compromise and delayed death following attempted central vein injection of propylhexedrine. J Emerg Med 1994;12:795–797.

18 Covey DC, Nossaman BD, Albright JA. Ischemic injury of the hand from intra-arterial propylhexedrine injection. J Hand Surg 1988;13A:58–61.

19. Marsden P, Sheldon J. Acute poisoning by propylhexedrine. Br Med J 1972;1:730.

20. DiMaio VJ, Garriott JC. Intravenous abuse of propylhexedrine. J Forensic Sci 1988;22:152–158.

21. Sturner WA, Spruill FG, Garriott JC. Two propylhexedrine-associated fatalities: Benzedrine revisited. J Forensic Sci 1974;19:572–574.

22. Thurman EM, Pedersen MJ, Stout RL, Martin T. Distinguishing sympathomimetic amines from amphetamine and methamphetamine in urine by gas chromatography/mass spectrometry. J Anal Toxicol 1992; 16:19–27.

23. Bogusz MJ, Kruger K-D, Maier R-D. Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 2000;24:77–84.

24. Riddick L, Reisch R. Oral overdose of propylhexedrine. J Forensic Sci 1981;26:834–839.

25. Frink M, Hildebrand F, Krettek C, Brand J, Hankemeier S. Compartment syndrome of the lower leg and foot. Clin Orthop Relat Res 2010;468:940–950.

26. Partanen TA, Vikatmaa P, Tukiainen E, Lepanalo M, Vuola J. Outcome after injections of crushed tablets in intravenous drug abusers in the Helsinki University Central Hospital. Eur J Vasc Endovasc Surg 2009;37:704–711.

27. Ruffolo AJ, Romano M, Ciapponi A. Prostanoids for critical limb ischaemia. Cochrane Database Syst Rev 2010;(1):CD006544.

Chapter 5

FLUNITRAZEPAM

HISTORY

In 1975, Hoffmann-La Roche (Roche) Pharmaceuticals (Basel, Switzerland) introduced flunitrazepam (Rohypnol®) into European markets as a more potent hypnotic drug than diazepam. Case reports of flunitrazepam abuse appeared in the United States in the early 1990s despite the lack of approval of this drug for medical use. The first US confiscation of illicit flunitrazepam occurred in 1989; in 1996, the importation of flunitrazepam across the US border was banned.[1] In 1995, the World Health Organization (WHO) recommended the change of flunitrazepam from a schedule IV classification (small, but significant risk of abuse) to a schedule III (a substantial risk to public health) of the Convention on Psychotropic Substances based on the widespread abuse of flunitrazepam by opiate abusers. In an attempt to reduce the abuse of flunitrazepam in the late 1990s, Roche Pharmaceuticals reduced the number of Mexican distributors, discontinued the sale of the 2-mg tablet, halted direct sale of flunitrazepam to pharmacies, and added blue dye to the tablets.

IDENTIFYING CHARACTERISTICS

Flunitrazepam (2H-1,4-benzodiazepin-2-one,1,3-dihydro-5-(2-fluorophenyl)-1-methyl-7-nitro-) has a molecular formula of $C_{16}H_{12}FN_3O_3$. Figure 5.1 displays the chemical structure of flunitrazepam (CAS RN: 1622-62-4), which is similar to the chemical structure of diazepam. The pKa of flunitrazepam is 1.82; at 37°C, the water solubility decreases as the pH increases resulting in a decrease in solubility as the drug moves from the acidic environment of the stomach to the more alkaline environs of the small intestines. At body temperature, the water solubility of this compound is 850 µg/mL (pH 1), whereas the water solubility decreases to 6 µg/mL at pH 7.4. Flunitrazepam is tasteless, colorless, and soluble in ethanol. However, reformulation of flunitrazepam tablets in the late 1990s resulted in the slow dissolution of the tablets and a cloudy, blue-tinged solution. Flunitrazepam is highly lipid soluble, with rapid penetration of the blood–brain barrier and fast onset of central nervous system (CNS) action compared with most other benzodiazepines. Table 5.1 lists some physiochemical properties of flunitrazepam.

Street names for Rohypnol® include Roofies, Rophies, Ropies, Ruffiew, Ribs, Ropes, Ropers, Roches, Rochas, Rochas Dos (2-mg tablet), Rophs, R-25, R05-4200, Roach-2s, Rubies, Forget Pills, Mexican Valium, Circles, Darkene, La Roche, Mind Erasers, Papas, Peanuts, Trip and Fall, Remember All, Wolfies, and the Date Rape Drug. A variety of pharmaceutic companies market flunitrazepam in some countries throughout the world as tablets and solutions under different trade names including Darkene® (Bayer Schering Pharma, Berlin, Germany), Ilman® (Demo S.A., Athens, Greece), Insom® (Aspen Pharmacare, Johannesburg, South Africa), Hipnosedon® (Roche), Hypnodorm® (Alphapharm, New South Wales, Australia; Teva, Petach Tikva, Israel), Nilium® (HELP S.A. Pharmaceutical Co., Athens, Greece), Roipnol® (Roche), Valsera® (Polifarma, Rome, Italy), and Vulbegal® (Coup Pharmaceutical Products S.A., Athens, Greece).[2] The tablets are crushed by flunitrazepam addicts for insufflation or injection. Flunitrazepam is a pharmaceutic preparation that is
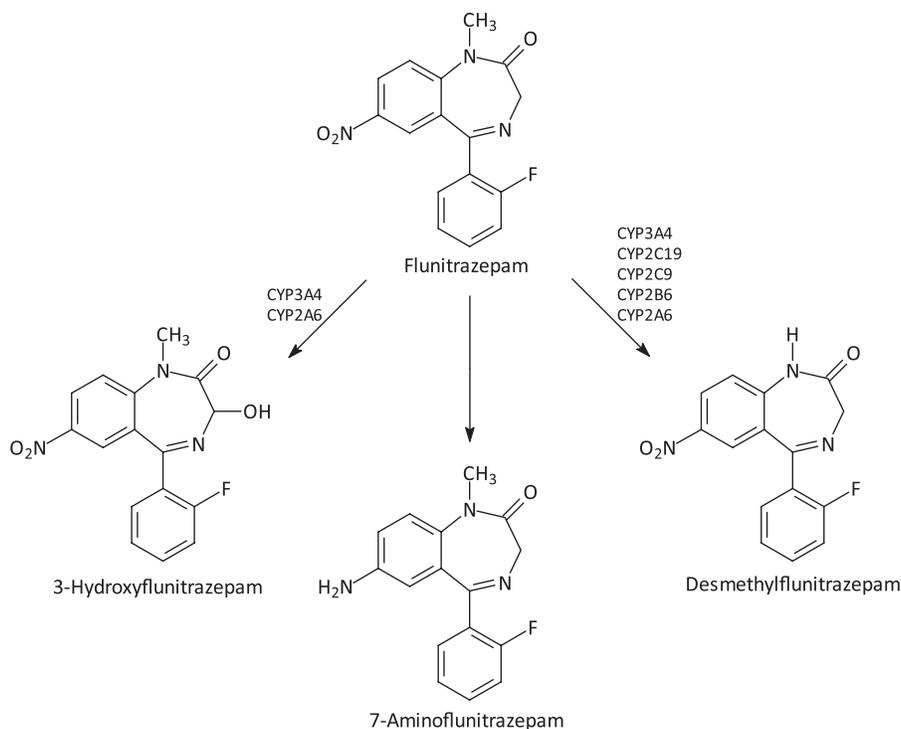
FIGURE 5.1. Biotransformation of flunitrazepam.

TABLE 5.1. Some Physiochemical Properties of Flunitrazepam.

Physical Property	Value
Melting Point	166–167°C (~331–333°F)
log P (Octanol-Water)	2.06
Water Solubility	72.8 mg/L (25°C)
Vapor Pressure	2.54E-09 mm Hg (25°C)
Henry's Law Constant	2.26E-11 atm-m³/mole (25°C)
Atmospheric OH Rate Constant	6.05E-12 cm³/molecule-second (25°C)

relatively pure. However, surreptitious substitution of other benzodiazepines (e.g., lorazepam, diazepam, clonazepam, bromazepam) for flunitrazepam may occur.

EXPOSURE

Epidemiology

Although flunitrazepam has never been approved for medical use in the United States, this compound is a frequently prescribed hypnotic drug in many countries, particularly in Scandinavia. The US National

Institute on Drug Abuse lists flunitrazepam as a "club drug" along with methamphetamine, 3,4-methylenedioxymethamphetamine (ecstasy, MDMA), LSD (*d*-lysergic acid diethylamide), ketamine, and GHB (γ-hydroxybutyrate). The use of ketamine and flunitrazepam among American youth is the least prevalent drug among these club drugs with a lifetime use of 0.4% for US youth aged 16–23 years based on the annual National Survey on Drug Use and Health.[3] With the exception of use of this drug during sexual assault, the abuse of flunitrazepam occurs primarily among multidrug users of club drugs, particularly women.

Sources

Flunitrazepam is a sedative-hypnotic drug available in many countries in Europe, Asia, Australia, and South America. This drug has not been approved for therapeutic use in the United States or Canada. Medical uses outside the United States include the treatment of insomnia, preanesthetic anxiety, and agitation. Flunitrazepam is marketed as 0.5-mg and 1-mg tablets as well as an injectable form. The United States banned the importation of flunitrazepam in 1996, but Mexico and some Latin American countries remain sources for smuggling flunitrazepam into the United States. An epi-

demic of anticholinergic poisoning (i.e., mydriasis, visual hallucinations, plucking behavior, agitation, coma) occurred when scopolamine was sold as illicit Rohypnol®.[4]

Methods of Abuse

Flunitrazepam produces a sense of relaxation and disinhibition similar to ethanol. Certain individuals use flunitrazepam to enhance the effects of ethanol or marijuana. The primary route of flunitrazepam abuse is ingestion, particularly in combination with the abuse of other drugs by polydrug abusers.[5,6] The abuse of flunitrazepam alone is uncommon. Other routes of flunitrazepam abuse include the injection or insufflation of crushed flunitrazepam tablets.[7,8] Flunitrazepam is a common drug of abuse among benzodiazepine-using heroin addicts.[9] Volunteer studies in methadone-maintained patients suggest that high doses of flunitrazepam (4 mg) produce euphoria in these patients.[10] Similar to other benzodiazepines, medicinal use of flunitrazepam frequently involves the chronic administration of this drug. In this setting, there is no definite evidence that the abuse of flunitrazepam is greater than other potent benzodiazepines.[11]

Flunitrazepam is one of numerous drugs used to chemically subdue victims in a sexual assault or robbery.[12,13] The victim's suggestibility and passivity after flunitrazepam ingestion provide the opportunity for assault with little or no recollection by the victim. Anecdotal reports suggest that the victim may develop drowsiness after the ingestion of a drink laced with the drug, and then loss of memory; subsequently, the victim remembers awakening in a place different than the original location.[14] Despite widespread media attention, flunitrazepam is an uncommon agent of suspected rapists based on the analysis of urine specimens from cases reported to rape treatment centers.[15,16] Ethanol remains the most common drug detected in samples collected from cases of suspected drug-facilitated sexual assault. In a prospective case series of 78 inner-city emergency department patients alleging the ingestion of a spiked drink, unexplained sedative drugs were detected in only 2 patients.[17] The mean time between alleged exposure and biologic sampling was 5.9 hours (range, 1–12 h). Sixty percent of the participants in the study had serum ethanol concentrations exceeding 150 mg/dL. Analysis of 2,003 urine samples of suspected chemical assault victims referred from a US rape treatment center demonstrated that alcohol and marijuana metabolites were present in 63% and 30% of these samples, respectively.[14] Flunitrazepam was detected in <0.5% of these urine samples, usually in combination with other drugs.

DOSE EFFECT

Illicit Use

Interviews with flunitrazepam abusers suggest that flunitrazepam use is occasional in most of the polydrug users, but some chronic abusers ingest up to 7–15 tablets at one time, usually with alcohol.[7] Drug-facilitated sexual assault case reports indicate that the ingestion of flunitrazepam doses as low as 1 mg can cause antero-grade amnesia, drowsiness, and confusion within the immediate period after consumption of an adulterated drink.[18] Despite these effects, case reports suggest that some automated tasks (e.g., driving) can be accomplished during the period of amnesia with some impairment of psychomotor skills.

Medical Use

The usual therapeutic dose of flunitrazepam for the treatment of insomnia is 1–2 mg at bedtime. Similar to other benzodiazepines, volunteer studies indicate that flunitrazepam doses between 1–4 mg produce dose-dependent effects on psychomotor performance (e.g., reaction time, balance, hand-eye coordination), vigilance, attention, and short-term memory, particularly explicit memory (recall/recognition of recent events and personal interactions).[19,20] Flunitrazepam induces memory loss during the time of peak effect of the drug. This inability to recall information occurs at the same doses used to promote sleep. Higher doses produce longer periods of memory loss, but the limited data to date do not indicate that the amnesic action of flunitrazepam is significantly greater than other potent benzodiazepines.[21]

Toxicity

Based on French poison center data, the ingestion of 0.25–0.29 mg flunitrazepam/kg was a potentially toxic dose of flunitrazepam that required medical supervision.[22] A review of the toxicity associated with the ingestion of excessive doses of flunitrazepam suggested that the ingestion of single doses of 10–30 mg by adults produces coma, whereas the ingestion of 40–50 mg causes respiratory depression.[21] The ingestion of 40 mg flunitrazepam by a 21-month-old boy was associated with coma, respiratory depression, hypotonia, bradycardia, and hypotension.[23] Tolerance to the effects of flunitrazepam is not well defined. The suicidal ingestion of 100 mg flunitrazepam by a 24-year-old woman was associated with sedation and hypoventilation, requiring the continuous infusion of the antidote, flumazenil.[24]

TOXICOKINETICS

Absorption

The oral bioavailability of flunitrazepam is approximately 80–90%.[25] Volunteer studies indicate that the peak plasma concentration of flunitrazepam occurs about 2 hours after ingestion of 2 mg flunitrazepam with initial peak flunitrazepam concentrations ranging from about 10–15 ng/mL.[26,27]

Distribution

Following the intravenous (IV) administration of flunitrazepam to patients prior to anesthesia, the average volume of distribution at steady state is about 2.5–3.5 L/kg.[28,29] Plasma protein binding is approximately 80%.[30] Distribution kinetics rather than elimination kinetics are the primary determinate of the duration of action of flunitrazepam with an open 3-compartment model best describing the disappearance of flunitrazepam from the blood.

Biotransformation

Flunitrazepam is structurally similar to diazepam, and the oxidative pathways of these 2 benzodiazepines are similar. The biotransformation of flunitrazepam produces many metabolites including the major metabolites *N*-desmethylflunitrazepam, 3-hydroxyflunitrazepam, and 7-aminoflunitrazepam. Existing data suggests that metabolites account for minor, if any effects of flunitrazepam. Receptor binding studies suggest that the binding of desmethylflunitrazepam is similar to flunitrazepam, whereas the binding of 7-aminoflunitrazepam is an order of magnitude less than flunitrazepam.[31] There are inadequate human studies to determine if any of these metabolites are present in sufficient concentrations to produce clinical or toxic effects; the main hypnotic effects of flunitrazepam probably result from the parent compound. *In vitro* studies suggest that CYP3A4 is the major CYP isoform involved with the oxidation of flunitrazepam to *N*-desmethyl-flunitrazepam and 3-hydroxy-flunitrazepam as displayed in Figure 5.1.[32] Although *in vitro* studies suggest the involvement of CYP2C19 in the oxidation of flunitrazepam,[33] *in vivo* studies indicate that the role of CYP2C19 in the formation of *N*-desmethylflunitrazepam and 3-hydroxyflunitrazepam is minor.[34]

Elimination

Flunitrazepam is almost completely metabolized with only small amounts of this drug appearing in the urine unchanged. The plasma elimination half-life of flunitrazepam is about 24–48 hours. Following IV administration of a mean flunitrazepam dose of 21 μg/kg (range, 14–33 μg/kg) to 12 patients undergoing anesthesia, the mean serum elimination half-life of flunitrazepam was about 25 hours (range, 15–66 h).[28] In a study of 8 patients ingesting 2 mg flunitrazepam daily for 28 days, the terminal plasma elimination half-life was 20–36 hours.[26] Another study of healthy adults receiving single and multiple doses of flunitrazepam, the terminal elimination half-life averaged about 13 hours and 19 hours, respectively.[29] The plasma elimination half-life of flunitrazepam substantially exceeds the duration of action as a result of the rapid distribution of flunitrazepam into the tissues.

Maternal and Fetal Kinetics

Flunitrazepam crosses the placenta. The fetus is considered part of the deep (3rd) compartment. In a study of pregnant women near term receiving intramuscular flunitrazepam, there was no statistically significant difference between maternal and fetal venous plasma concentrations of flunitrazepam.[30] Although flunitrazepam appears in the milk of breast-feeding mothers ingesting flunitrazepam, clinical effects in the breast-feed infant are not expected unless high doses of flunitrazepam are ingested.[35]

Tolerance

Although tolerance to benzodiazepines develops during chronic administration, there are few data on tolerance in flunitrazepam users.

Drug Interactions

Flunitrazepam potentially enhances the effect of other sedative-hypnotic drugs, ethanol, and buprenorphine. In volunteer studies, the combination of a morning dose of ethanol (0.5 g/kg body weight) and prior-evening dose of flunitrazepam (2 mg) significantly impaired standing steadiness, tracking, and reaction times when compared with flunitrazepam and ethanol alone.[36]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Commercially available benzodiazepines produce therapeutic effects by binding to benzodiazepine receptors within the γ-aminobutyric acid$_A$ (GABA$_A$)-complex. However, these drugs differ in their effects from phar-

macologic (i.e., duration and onset of action) and pharmacodynamic actions. Similar to other benzodiazepines, flunitrazepam is an indirect γ-aminobutyric-acid (GABA) agonist that enhances the binding of GABA to postsynaptic neuronal chloride channels. This action opens the chloride channel of post-synaptic neurons, resulting in an influx of chloride and hyperpolarization of the cell along with a reduction in cell excitability. The enhancement of inhibitory neurotransmission in the brain and spinal cord causes sedation and reduced anxiety. Benzodiazepines potentiate GABA-mediated inhibition in a more selective manner than barbiturates and ethanol by increasing the affinity of GABA for its receptor. The potency of a specific benzodiazepine compound depends on the drug concentrations at the receptor site as well as the degree of affinity for the corresponding receptor. Flunitrazepam is highly lipophilic, and this drug is one of the most potent benzodiazepine compounds. The affinity of flunitrazepam for benzodiazepine receptors is somewhat greater than some (e.g., alprazolam, diazepam, and oxazepam), but not all (e.g., triazolam, midazolam) commercial benzodiazepines.[37] The rapid onset of action and the high potency of this drug increase the abuse liability of flunitrazepam compared with other, less potent benzodiazepines.

Mechanism of Toxicity

The pharmacodynamic interaction of flunitrazepam with the GABA$_A$-benzodiazepine receptor complex throughout the central nervous system causes ataxia, sedation, drowsiness, sleep, reduced anxiety, anticonvulsant effects, and impairment of memory. The disturbance of memory is dose-dependent. This impairment of memory probably involves the process of the consolidation of information into memory storage rather than input or retrieval of information. The types of memory storage include short-term memory storage (primary memory), middle course memory storage (secondary memory), and long-term memory storage (tertiary memory). Short-term storage includes the information necessary for thought processes and understanding. The consolidation of short-term memory into long-term memory occurs both as explicit memory ("conscious") and implicit ("unconscious") memory. The former is semantic (facts, general information), episodic (events personally experienced) and autobiographic (e.g., recall of names) memory, whereas implicit memory is the unconscious memory for automated tasks (i.e., actions of the normal daily routine, such as driving a car). As with most hypnotic-sedative drugs, the loss of memory associated with flunitrazepam is primarily anterograde rather than retrograde amnesia (i.e., loss of early memories). Most cases of anterograde amnesia involve the inability to remember their normal daily routine (e.g., driving, talking to people, work activities). In contrast to the loss of memory associated with ethanol, individuals with anterograde amnesia following the use of flunitrazepam can perform complex tasks (e.g., driving a car). Volunteer studies suggest that 2-mg doses of flunitrazepam impair memory and prolong rapid eye movement (REM) latency without changing the percentage of REM sleep.[38] However, not all volunteers developed memory impairment at this dose. With intermediate doses (e.g., 2 mg) of flunitrazepam, impairment of explicit memory can occur without significant changes in psychomotor sedation (e.g., reduced simple choice reaction times), vigilance, or attention.[39] The anterograde amnesia probably results, at least in part, from impairment of memory consolidation secondary to REM sleep suppression. In very high doses, flunitrazepam can cause respiratory depression, usually before clinically significant reduction in blood pressure.

Postmortem Examination

There are no specific pathologic features associated with postmortem examination of flunitrazepam-induced fatalities.[40] Postmortem examination of individuals, who died after ingesting flunitrazepam, demonstrates typical findings associated with respiratory depression and anoxia (i.e., pulmonary and/or cerebral edema).

CLINICAL RESPONSE

The pharmacologic effects of flunitrazepam include sedation, reduced anxiety, muscle relaxation, and increased seizure threshold. The onset of action following ingestion is about 15–20 minutes and the duration of action of is approximately 4–8 hours. Adverse effects associated with the abuse of flunitrazepam include lethargy, lightheadedness, gastrointestinal distress, psychomotor retardation, poor coordination, slurred speech, confusion, tremors, nightmares, and low blood pressure.[41] Benzodiazepines (e.g., lorazepam, diazepam, flunitrazepam) produce dose-dependent amnesia. Anterograde amnesia is associated with the administration of flunitrazepam, particularly with doses exceeding 4 mg; loss of memory begins 5–60 minutes after ingestion of flunitrazepam, depending on the dose.[42] Retrograde amnesia (i.e., recalling objects prior to the administration of the drug) is usually preserved. Flunitrazepam is mostly a sedating drug. However, some individuals experience idiosyncratic, paradoxical reactions towards benzodiazepines including flunitrazepam. These unexpected reactions include talkativeness, agitation, confusion, loss of impulse control, aggression,

and violent behavior.[43,44] Individuals with increased risk of paradoxical reactions include elderly, children, alcoholics, and psychiatric patients with personality or psychotic disorders.

Illicit Use

In some criminals, the use of large doses of flunitrazepam is associated with feelings of power, enhanced self-esteem, omnipotence, loss of fear and episodic memory, and impulsive violence, particularly when ingested with alcohol.[45] However, the role of flunitrazepam in producing the impulsive behavior remains unclear. Rare case reports associate the abuse of flunitrazepam with a variety of inappropriate behavior including loss of impulse control, irritability, aggression, verbal hostility, physical assaults, and suicidal actions.[46] However, the causal role of flunitrazepam in these behaviors is difficult to determine, and there is no definite evidence that flunitrazepam is more often associated with these behaviors than other benzodiazepines.[47] A case report associated the IV administration of dissolved flunitrazepam tablets into the femoral artery with the development of distal peripheral ischemia that resolved following treatment with anticoagulation and intraarterial urokinase and prostaglandins.[48] Flunitrazepam is occasionally administered during drug-facilitated sexual assaults and robberies as a means to induce amnesia and disinhibition.[49]

Overdose

The clinical effects of an overdose of flunitrazepam are similar to the toxicity of other benzodiazepines. In a study of children ingesting flunitrazepam, common clinical features of a flunitrazepam overdose were related to alterations of consciousness, particularly drowsiness, agitation, and ataxia.[22] Less common effects of flunitrazepam overdose include bradycardia, hypotension, coma, and hypotonia.

Abstinence Syndrome

Abrupt termination of the chronic use of flunitrazepam causes withdrawal reactions including restlessness, agitation, irritability, headache, myalgias, paresthesias, and confusion. This abstinence syndrome is similar to other benzodiazepines. In a study of 28 addicts using up to 8–10 mg flunitrazepam daily, withdrawal symptoms (e.g., anxiety, fear, insomnia, confusion, shivering, tachycardia, involuntary movement, paresthesias, perceptual changes) began about 36 hours after cessation of flunitrazepam use.[50] Withdrawal seizures can occur up to about 1 week after the cessation of flunitrazepam use,

particularly in alcoholics. Other withdrawal effects include derealization (i.e., perception that external world is strange and dreamlike), depersonalization, delirium, and hallucinations. Rebound insomnia (i.e., a temporary worsening of sleep after discontinuation of hypnotic drugs) is a sensitive indicator of physiological dependence on benzodiazepine drugs (i.e., flunitrazepam).[21] In a study of 6 insomniacs receiving 2 mg flunitrazepam nightly for 4 weeks, sleep latency and total wake time were significantly increased above baseline for the first 3 nights after discontinuation of the drug.[51] These parameters returned to baseline within 2 weeks. The capacity of flunitrazepam to produce physiologic dependence or withdrawal symptoms after administration of an antagonist or discontinuation of treatment is similar to other potent benzodiazepines.[21]

Reproductive Abnormalities

There is no clear association between the use of flunitrazepam by pregnant women and the development of reproductive abnormalities, including delayed neurobehavioral development.[52]

DIAGNOSTIC TESTING

Analytic Methods

Screening

Benzodiazepine immunoassays (e.g., enzyme immunoassays, fluorescence polarization immunoassays, radioimmunoassays) are relatively specific, but the sensitivity of older immunoassay screening tests for the small flunitrazepam concentrations typically present in urine samples after either therapeutic use or abuse is relatively low. Furthermore, most benzodiazepine immunoassay reagents react poorly with flunitrazepam metabolites (e.g., 7-aminonitrazepam).[53] More recently developed immunoassays (e.g., Cozart ELISA, Cozart Bioscience, Oxfordshire, UK; Immunalysis ELISA, Immunalysis Corporation, Pomona, CA) are more sensitive (i.e., 30 ng/mL) for 7-aminoflunitrazepam than older immunoassays.[54] Significant cross-reactivity with this assay occurs only with diazepam.[55] Radioreceptor assays lower the limit of detection to pharmacologically active concentrations of flunitrazepam, but these techniques do not identify specific compounds.[56] Although gas chromatography with electron capture detection has high sensitivity, this technique lacks specificity and has poor sensitivity for flunitrazepam metabolites (e.g., 7-aminoflunitrazepam, 3-hydroxy-flunitrazepam). Rapid drug detector kits (e.g., Drink Detective®, SureScreen Diagnostics, London) are commercially

available for the detection of club drugs including fluni-trazepam, but the these products lack both sensitivity and specificity, particularly in drinks containing tonic water and fruit juices.[57] Other methods for the detection of flunitrazepam and other drugs of abuse in food and drink include lateral-flow immunoassay with confirmation by high-performance liquid chromatography coupled with quadrupole ion trap/time-of-flight tandem mass spectrometry.[58]

CONFIRMATORY

Methods to quantitate flunitrazepam and associated metabolites in biologic samples include gas chromatography, high performance liquid chromatography with ultraviolet detection (250 nm),[34] gas chromatography/mass spectrometry,[59] high performance liquid chromatography/tandem mass spectrometry,[60,61] ion trap gas chromatography/tandem mass spectrometry,[62] liquid chromatography/atmospheric pressure photoionization/mass spectrometry,[63] and micellar electrokinetic chromatography.[64] Gas chromatography/mass spectrometry with electron impact or negative chemical ionization detection are sensitive and specific confirmatory methods for the quantitation of flunitrazepam, but these methods do not detect polar metabolites without derivatization. In contrast to gas chromatography/electron impact/mass spectrometry and gas chromatography/negative chemical ionization/mass spectrometry, liquid chromatography/mass spectrometry methods using atmospheric pressure chemical ionization or electrospray ionization are sensitive, specific analytic methods that do not require derivatization. The lower limit of quantitation (LLOQ) for flunitrazepam using high performance liquid chromatography with ultraviolet detection is about 0.5 ng/mL, whereas the LLOQ for flunitrazepam and 7-aminoflunitrazepam using gas chromatography/mass spectrometry is 0.1 ng/mL and 0.01 ng/mL, respectively. The limit of detection (LOD) for high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry is 0.2 ng/mL for flunitrazepam and 7-aminonitrazepam and 0.1 ng/mL for *N*-desmethylflunitrazepam and 3-hydroxyflunitrazepam.[65] Using liquid chromatography/electrospray ionization/mass spectrometry with solid-phase extraction, the LOD in biologic samples for these 4 compounds was 0.025 ng/mL, 0.25 ng/mL, 0.04 ng/mL, and 0.2 ng/mL, respectively.[66]

STORAGE

Flunitrazepam is relatively stable in sterile blood, but rapid conversion of this compound to 7-aminoflunitrazepam occurs in the presence of bacteria and elevated temperature. An experimental study of non-fluoridated postmortem blood contaminated with bacteria indicated that the conversion of flunitrazepam to 7-aminoflunitrazepam is almost complete within 8 hours at room temperature.[67] In a study of nonfluoridated blood samples spiked with 0.25 mg/L flunitrazepam, the flunitrazepam concentration over 3 months of storage decreased by 90%, 70%, and 15%, when stored at room temperature, 4°C, and –20°C, respectively.[68] Flunitrazepam is relative stable when stored in sodium fluoride at –20°C, but some loss of the 7-aminoflunitrazepam occurs. Although 7-aminoflunitrazepam is more resistant than flunitrazepam to bacteria and elevated temperature, degradation of this metabolite occurs during prolonged storage. After storage at –20°C (–4°F) for 2 months and 24 months with preservatives (1% sodium fluoride/potassium oxalate), the original concentration of 7-aminoflunitrazepam decreased 29% and 68%, respectively.[67] Consequently, analysis of these samples preferably should occur within 1 week.

Biomarkers

Using sensitive methods (e.g., gas chromatography/negative chemical ionization/mass spectrometry), volunteer studies indicate that 7-aminofluntrazepam is detectable in hair for at least 28 days after the ingestion of 2 mg flunitrazepam.[69] However, timing of the use of flunitrazepam is difficult to determine using hair analysis. Flunitrazepam is less basic than 7-aminoflunitrazepam; therefore, the flunitrazepam concentrations in hair are lower and more difficult to detect than 7-aminonitrazepam. Using tandem mass spectrometry, hair analysis complements rather than replaces the analysis of blood and urine for flunitrazepam and associated metabolites.[70] Flunitrazepam diffuses poorly into vitreous humor, and the postmortem vitreous concentrations of flunitrazepam are about one-third of the peripheral blood.[71]

BLOOD

The whole blood/plasma ratio of flunitrazepam is about 0.75 with a range of 0.65–0.80.[29]

THERAPEUTIC/ILLICIT USE. The elimination of flunitrazepam from the blood is rapid, and detection of flunitrazepam in blood samples requires sensitive analytic methods. Peak flunitrazepam concentrations occur about 2–4 hours after oral administration with peak concentrations reaching 10–15 ng/mL after single 2-mg IV doses and 15–20 ng/mL after 1 month of daily oral dosing of 2 mg flunitrazepam, respectively.[26,72] Flunitrazepam was detectable in whole blood samples,

but not in plasma samples up to 4 hours after the oral administration of 2 mg flunitrazepam, as measured by gas chromatography/mass spectrometry (LOD, 5 ng/mL).[73] Flunitrazepam concentrations in blood do not correlate to agitated or aggressive behavior. In a Norwegian study of drivers apprehended for suspected driving under the influence and suspects of violent crimes, agitation or aggressive behavior was not more common in the group with the highest blood flunitrazepam concentration, when compared with individuals with therapeutic concentrations of flunitrazepam.[80] Although the onset and duration of anterograde amnesia associated with the administration of flunitrazepam is dose-dependent, impairment of explicit memory (recall/recognition of recent events and personal interactions) can occur following ingestion of therapeutic concentrations of flunitrazepam.[39]

OVERDOSE.   In a study of 588 blood samples collected from patients presenting to a French emergency department, 7 serum samples were positive only for flunitrazepam, as measured by gas chromatography/mass spectrometry (LOD, ~4 ng/mL).[74] The mean serum flunitrazepam concentration was 284 ± 342 ng/mL with a range of 19–1,011 ng/mL. The clinical conditions of these patients were not described in this paper.

POSTMORTEM.   A study of antemortem and postmortem samples from 4 individuals suggests that postmortem diffusion of flunitrazepam and 7-aminoflunitrazepam into peripheral tissues (e.g., femoral blood) is probably too small to confound the interpretation of postmortem flunitrazepam concentrations; the study was too small to provide statistical analysis.[71] However, as a result of the postmortem conversion of flunitrazepam to 7-aminoflunitrazepam, postmortem blood concentrations of flunitrazepam alone may not accurately reflect antemortem flunitrazepam concentrations.[40] In such cases, the individuals typically have high concentrations (0.4–0.8 mg/L) of 7-aminoflunitrazepam because the total blood concentrations of nitrobenzodiazepine compounds are relatively stable during the initial postmortem interval.

URINE

The duration of positive urine samples after the use of flunitrazepam depends on the sensitivity of the analytic method, preparation techniques (e.g., enzymatic hydrolysis), route of exposure, interindividual variation, and the flunitrazepam dose. The major metabolite, 7-aminoflunitrazepam, was detectable in urine samples from volunteers for 3 days after the ingestion of 0.5 mg fluni-

trazepam, as measured by gas chromatography/mass spectrometry (LOD, 2 ng/mL).[75] Using liquid chromatography/tandem mass spectrometry with a cutoff of 0.5 ng/mL, 7-aminoflunitrazepam was detectable in hydrolyzed urine for 5 days in all 16 volunteers receiving 0.5 mg flunitrazepam, whereas the window of detection was extended to 10 days in 2 volunteers.[76] The ratio of 7-aminodesmethylflunitrazepam/7-aminoflunitrazepam increased with time independent of dose in this study; a ratio of 0.1 suggested ingestion within 4 hours compared with >72 hours for ratios exceeding 0.6. In a study of 10 healthy volunteers receiving 2 mg flunitrazepam, 7-aminoflunitrazepam was detectable for 14 days, 21 days, and 28 days in 6, 1, and 3 volunteers, respectively, as measured by gas chromatography/negative chemical ionization/mass spectrometry.[77] The LLOQ for 7-aminoflunitrazepam in hydrolyzed urine was 10 pg/mL (0.010 ng/mL). The flunitrazepam concentrations in these urine specimens were 2 orders of magnitude below the 7-aminoflunitrazepam concentrations. In the study using liquid chromatography/tandem mass spectrometry, 7 of the 16 volunteers receiving a single dose of 2 mg flunitrazepam had detectable 7-aminoflunitrazem concentrations in hydrolyzed urine for 10 days. The window of detection for 7-aminoflunitrazepam in urine is substantially shorter (48–96 h), when using liquid chromatography with diode array detection, gas chromatography/mass spectrometry, or liquid chromatography/mass spectrometry rather than gas chromatography/negative chemical ionization/mass spectrometry.[78] Following chronic abuse of flunitrazepam, high concentrations of 3-hydroxyflunitrazepam also occur in hydrolyzed urine samples from these individuals. Typically, the concentration of total 7-aminoflunitrazepam in urine samples is several fold higher than the concentration of free 7-aminoflunitrazepam.[79]

Driving

Volunteer studies indicate that the ingestion of flunitrazepam at night can produce some residual effects on psychomotor skills involved with driving during the following morning and perhaps some effect on driving skills in the following afternoon. In a study of 20 male volunteers, the ingestion of 2 mg flunitrazepam at night caused some detrimental effects on standing steadiness, tracking, choice reaction, and flicker recognition.[36] A study of 29 healthy volunteers suggested that an IV flunitrazepam dose of 0.03 mg/kg causes some impairment of reaction times and coordination 4 hours after administration.[72] The ingestion of ethanol at doses of 0.5 g/kg in the morning produced synergistic effects on psychomotor skills. Examination of individuals under

the influence of flunitrazepam demonstrate typical effects of a central nervous system depressant including poor coordination, slurred speech, and horizontal gaze nystagmus.[53] Flunitrazepam was the only drug detected in blood samples from 415 Norwegian drivers suspected of driving under the influence and 7 suspects of violent crime.[80] Only a relatively small subset (i.e., 6%) displayed restlessness or agitated behavior, and the number of individuals with this type of behavior was not more prevalent in the individuals with high flunitrazepam concentrations. Volunteer studies suggest that acute tolerance develops to some psychomotor skill (e.g., simple and choice reaction times) following the administration of medium to large doses (i.e., 1–2 mg) of flunitrazepam.[81]

TREATMENT

The treatment of flunitrazepam toxicity is usually supportive. Most patients ingesting excessive amounts of flunitrazepam develop only minor symptoms of drowsiness, ataxia, confusion, slurred speech, and anterograde amnesia. These effects usually resolve with observation for the development of toxicity from the coingestion of other drugs or the presence of accidental trauma. The primary life-threatening effects of a large overdose of flunitrazepam are increased risk of trauma, coma, respiratory depression, and aspiration. These patients should have IV access established along with monitoring of oxygenation (i.e., pulse oximetry, arterial blood gases) as needed. Gut decontamination (i.e., activated charcoal) is usually not necessary because of the mild toxicity associated with flunitrazepam unless indicated by the coingestion of a more toxic drug. Flumazenil is a specific antidote for benzodiazepine toxicity, but the use of this antidote is limited by the infrequent occurrence of serious toxicity during benzodiazepine toxicity and the potential development of withdrawal in benzodiazepine-dependent patients. Additionally, flumazenil increases the risk of seizures following the ingestion of proconvulsant drugs, particularly during tricyclic antidepressant toxicity.[82] The use of flumazenil during flunitrazepam toxicity requires an assessment of the potential benefits of reversing life-threatening cardiorespiratory depression related to flunitrazepam and the risk of precipitating adverse reactions (withdrawal, ventricular dysrhythmias, seizures). The onset of action of IV flumazenil is 1–2 minutes with peak effects 5–10 minutes after injection. The initial adult dose is 0.2 mg IV followed by 0.3 mg and 0.5 mg at 1- to 2-minute intervals up to 3 mg titrated to desired clinical effects. If the administration of a total of 3 mg produces a partial response, then additional doses up to 2 mg may be administered intravenously. Pure benzodiazepine over-

doses typically require about 0.5–1 mg. Failure to respond to a total of 5 mg flumazenil indicates that the altered consciousness probably is not related to benzodiazepine toxicity. The pediatric dose of flumazenil is not well established, but IV doses of 0.01–0.02 mg/kg (maximum dose, 0.2 mg) have been administered successfully without complications.[83] The plasma half-life of flunitrazepam far exceeds the half-life of flumazenil; therefore, patients responding to flumazenil must be observed for 24 hours to prevent recurrence of adverse effects.[23] Methods to enhance elimination (e.g., hemodialysis, hemofiltration, hemoperfusion) are not effective for highly lipophilic and widely distributed drugs, such as flunitrazepam.

References

1. Simmons MM, Cupp MJ. Use and abuse of flunitrazepam. Ann Pharmacother 1998;32:117–119.
2. Mandrioli R, Mercolini L, Raggi MA. Benzodiazepine metabolism: an analytical perspective. Curr Drug Metab 2008;9:827–844.
3. Wu L-T, Schlenger WE, Galvin DM. Concurrent use of methamphetamine, MDMA, LSD, ketamine, GHB, and flunitrazepam among American youths. Drug Alcohol Depend 2006-84:102–113.
4. Vallersnes OM, Lund C, Duns AK, Netland H, Rasmussen IA. Epidemic of poisoning caused by scopolamine disguised as Rohypnol tablets. Clin Toxicol (Phila) 2009;47: 889–893.
5. Druid H, Holmgren P, Ahler J. Flunitrazepam: an evaluation of use, abuse, and toxicity. Forensic Sci Int 2001;122: 136–141.
6. Calhoun SR, Wesson DR, Galloway GP, Smith DE. Abuse of flunitrazepam (Rohypnol) and other benzodiazepines in Austin and South Texas. J Psychoactive Drugs 1996;28:183–189.
7 Bond A, Seijas D, Dawling S, Lader M. Systemic absorption and abuse liability of snorted flunitrazepam. Addiction 1994;89:821–830.
8. Pratikto TH, Strubel G, Biro F, Kroger K. Intra-arterial injection of dissolved flunitrazepam tablets. Vasa 2004;33:52–54.
9. San L, Tato J, Torrens M, Castillo C, Farre M, Cami J. Flunitrazepam consumption among heroin addicts admitted for in-patient detoxification. Drug Alcohol Depend 1993;32:281–286.
10. Farre M, Teran MT, Roset PN, Mas M, Torrens M, Cami J. Abuse liability of flunitrazepam among methadone-maintained patients. Psychopharmacology (Berl) 1998;140:486–495.
11. Geiselmann B, Linden M. Prescription and intake patterns in long-term and ultra-long-term benzodiazepine treatment in primary care practice. Pharmacopsychiatry 1991; 24:55–61.

12. Bismuth C, Dally S, Borron SW. Chemical submission: GHB, benzodiazepines, and other knock out drops. Clin Toxicol 1997;35:595–598.

13. Marc B, Baudry F, Vaquero P, Zerrouki L, Hassnaoui S, Douceron H. Sexual assault under benzodiazepine submission in a Paris suburb. Arch Gynecol Obstet 2000; 263:193–197.

14. Slaughter L. Involvement of drugs in sexual assault. J Reprod Med 2000;45:425–430.

15. Juhascik MP, Negrusz A, Faugno D, Ledray L, Greene P, Lindner A, et al. An estimate of the proportion of drug-facilitation of sexual assault in four U.S. localities. J Forensic Sci 2007;52:1396–1400.

16. ElSohly MA, Salamone SJ. Prevalence of drugs used in cases of alleged sexual assault. J Anal Toxicol 1999;23: 141–146.

17. Greene SL, Shiew CM, Streete P, Mustchin SJ, Hugget D, Earl B, Dargan PI. What's being used to spike your drink? Alleged spiked drink cases in inner city London. Postgrad Med J 2007;83:754–758.

18. Ohshima T. A case of drug-facilitated sexual assault by the use of flunitrazepam. J Clin Forensic Med 2006;13:44–45.

19. Dowd SM, Strong MJ, Janicak PG, Negrusz A. The behavioral and cognitive effects of two benzodiazepines associated with drug-facilitated sexual assault. J Forensic Sci 2002;47:1101–1107.

20. Mintzer MZ, Griffiths RR. Flunitrazepam and triazolam: a comparison of behavioral effects and abuse liability. Drug Alcohol Depend 1998;53:49–66.

21. Woods JH, Winger G. Abuse liability of flunitrazepam. J Clin Psychopharmacol 1997;3(Suppl 2):1S–57S.

22. Pulce C, Mollon P, Frantz P, Descotes J. Acute poisonings with ethyl loflazepate, flunitrazepam, prazepam and triazolam in children. Vet Hum Toxicol 1992;34:141–143.

23. Roald OK, Dahl V. Flunitrazepam intoxication in a child successfully treated with the benzodiazepine antagonist flumazenil. Crit Care Med 1989;17:1355–1356.

24. Brammer G, Gibly R, Walter FG, Bey T, Torres R, Kohler S. Continuous intravenous flumazenil infusion for benzodiazepine poisoning. Vet Hum Toxicol 2000;42:280–281.

25. Cano JP, Soliva M, Hartmann D, Ziegler WH, Amrein R. Bioavailability from various galenic formulations of flunitrazepam. Arzneimittelforschung 1977;27:2383–2388.

26. Grahnen A, Wennerlund P, Dahlstrom B, Eckernas SA. Inter-and intraindividual variability in the concentration-effect (sedation) relationship of flunitrazepam. Br J Clin Pharmacol 1991;31:89–92.

27. Wickstrom E, Amrein R, Haefelfinger P, Hartmann D. Pharmacokinetic and clinical observations on prolonged administration of flunitrazepam. Eur J Clin Pharmacol 1980;17:189–196.

28. Kangas L, Kanto J, Pakkanen A. A pharmacokinetic and pharmacodynamic study of flunitrazepam. Int J Clin Pharmacol Ther Toxicol 1982;20:585–588.

29. Boxenbaum HG, Posmanter HN, Macasieb T, Geitner KA, Weinfeld RE, Moore JD, et al. Pharmacokinetics of fluni-

30. Kanto J, Erkkola R, Kangas L, Pitkanen Y. Placental transfer of flunitrazepam following intramuscular administration during labour. Br J Clin Pharmacol 1987;23:491–494.

31. Haefely W. The preclinical pharmacology of flumazenil. Eur J Anaesthesiol 1988;2(Suppl):25–36.

32. Hesse LM, Venkatakrishnan K, von Moltke LL, Shader RI, Greenblatt DJ. CYP3A4 is the major CYP isoform mediating the in vitro hydroxylation and demethylation of flunitrazepam. Drug Metab Dispos 2001;29:133–140.

33. Kilicarslan T, Haining RL, Rettie AE, Busto U, Tyndale RF, Sellers EM. Flunitrazepam metabolism by cytochrome P450S 2C19 and 3A4. Drug Metab Dispos 2001;29: 460–465.

34. Gafni I, Bsto UE, Tyndale RF, Kaplan HL, Sellers EM. The role of cytochrome P450 2C19 activity in flunitrazepam metabolism in vivo. J Clin Psychopharmacol 2003;23: 169–175.

35. Kanto JH. Use of benzodiazepines during pregnancy, labour and lactation, with particular reference to pharmacokinetic considerations. Drugs 1982;23:354–380.

36. Seppala T, Nuotto E, Dreyfus JF. Drug-alcohol interactions on psychomotor skills: zopiclone and flunitrazepam. Pharmacology 1983;27(Suppl 2):127–135.

37. Braestrup C, Squires RF. Pharmacological characterization of benzodiazepine receptors in the brain. Eur J Pharmacol 1978;48:263–270.

38. Misaki K, Nakagawa H, Koshino Y, Kishi H, Ota T, Okuda K, et al. Effect flunitrazepam on sleep and memory. Psychiatr Clin Neurosci 1998;52:327–332.

39. Bareggi SR, Ferini-Strambi L, Pirola R, Smirne S. Impairment of memory and plasma flunitrazepam levels. Psychopharmacology 1998;140:157–163.

40. Drummer OH, Syrjanen ML, Cordner SM. Deaths involving the benzodiazepine flunitrazepam. Am J Forensic Med Pathol 1993;14:238–243.

41. Smith KM, Larive LL, Romanelli F. Club drugs: methylenedioxymethamphetamine, flunitrazepam, ketamine hydrochloride, and gamma-hydroxybutyrate. Am J Health Syst Pharm 2002;59:1067–1076.

42. McKay AC, Dundee JW. Effect of oral benzodiazepines on memory. Br J Anaesth 1980;52:1247–1257.

43. Hall RC, Zisook S. Paradoxical reactions to benzodiazepines. Br J Clin Pharmacol 1981;11(Suppl 1):99S–104S.

44. Mancuso CE, Tanzi MG, Gabay M. Paradoxical reactions to benzodiazepines: literature review and treatment options. Pharmacotherapy 2004;24:1177–1185.

45. Daderman AM, Lidberg L. Flunitrazepam (Rohypnol) abuse in combination with alcohol causes premeditated, grievous violence in male juvenile offenders. J Am Acad Psychiatry Law 1999;27:83–99.

46. Teo SH, Chee KT, Tan CT. Psychiatric complications of Rohypnol abuse. Singapore Med J 1979;20:270–273.

trazepam following single- and multiple-dose oral administration to healthy human subjects. J Pharmacokinet Biopharm 1978;6:283–293.

47. Dietch JT, Jennings RK. Aggressive dyscontrol in patients treated with benzodiazepines. J Clin Psychiatry 1988;49: 184–188.

48. Leifert JA, Bossaller L, Uhl M. Acute ischaemia of the leg following accidental intra-arterial injection of dissolved flunitrazepam tablets. Vasa 2008;37:374–378.

49. Barnett JM, Broad RM. Flunitrazepam used in a case of poisoning. J Clin Forensic Med 2003;10:89–91.

50. Vescovi PP, Gerra G, Ippolito L, Caccavai R, Maestri D, Passeri M. Nicotinic acid effectiveness in the treatment of benzodiazepine withdrawal. Curr Ther Res Clin Exp 1987;41:1017–1021.

51. Scharf MB, Bixler EO, Kales A, Soldatos CR. Long-term sleep laboratory evaluation of flunitrazepam. Pharmacology 1979;19:173–181.

52. McElhatton PR. The effects of benzodiazepine use during pregnancy and lactation. Reprod Toxicol 1994;8:461–475.

53. Raymon LP, Steel BW, Walls HC. Benzodiazepines in Miami-Dade County, Florida driving under the influence (DUI) cases (1995–1998) with emphasis on Rohypnol®: GC-MS confirmation, patterns of use, psychomotor impairment, and results of Florida legislation. J Anal Toxicol 1999;23:490–499.

54. Wang PH, Liu C, Tsay WI, Li JH, Liu RH, Wu TG, et al. Improved screen and confirmation test of 7-aminoflunitrazepam in urine specimens for monitoring flunitrazepam (Rohypnol) exposure. J Anal Toxicol 2002;26:411–418.

55. Lin D-L, Yin R-M, Chen C-H, Chen Y-L, Liu RH. Performance characteristics of 7-aminoflunitrazepam specific enzyme-linked immunosorbent assays. J Anal Toxicol 2005;29:718–722.

56. Bruhwyler J, Hassoun A. Potential use of a radioreceptor assay for the determination of benzodiazepine compounds in serum. J Anal Toxicol 1993;17:403–407.

57. Beynon CM, Sumnall HR, McVeigh J, Cole JC, Bellis MA. The ability of two commercially available quick test kits to detect drug-facilitated sexual assault drugs in beverages. Addiction 2006;101:1413–1420.

58. Zuckschwerdt JB, Nixon CE, Ciner FL, Croley TR. Liquid chromatography/quadrupole ion trap/time-of-flight determination of the efficacy of drug test kits for rapid screening of food. J Food Protect 2008;71:1007–1014.

59. Elian AA. Detection of low levels of flunitrazepam and its metabolites in blood and bloodstains. Forensic Sci Int 1999;101:107–111.

60. Gunn J, Kriger S, Terrell AR. Simultaneous determination and quantification of 12 benzodiazepines in serum or whole blood using UPLC/MS/MS. Methods Mol Biol 2010; 603:107–119.

61. Dussy FE, Hamberg C, Briellmann TA. Quantification of benzodiazepines in whole blood and serum. Int J Legal Med 2006;120:323–330.

62. Terada M, Masui S, Hayashi T, Watanabe R, Inoue H, Iino M, et al. Simultaneous determination of flunitrazepam and 7-aminoflunitrazepam in human serum by ion trap gas chromatography-tandem mass spectrometry. Legal Med 2003;5(Suppl 1):S96–S100.

63. Marchi I, Schappler J, Veuthey JL, Rudaz S. Development and validation of a liquid chromatography-atmospheric pressure photoionization-mass spectrometry method for the quantification of alprazolam, flunitrazepam, and their main metabolites in hemolyzed blood. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:2275–2283.

64. Huang C-W, Jen H-P, Wang R-D, Hsieh Y-Z. Sweeping technique combined with micellar electrokinetic chromatography for the simultaneous determination of flunitrazepam and its major metabolites. J Chromatogr A 2006;1110: 240–244.

65. Bogusz MJ, Maier RD, Kruger KD, Fruchtnicht W. Determination of flunitrazepam and its metabolites in blood by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Chromatogr B Biomed Sci Appl 1998;713: 361–369.

66. Jourdil N, Bessard J, Vincent F, Eysseric H, Bessard G. Automated solid-phase extraction and liquid chromatography-electrospray ionization-mass spectrometry for the determination of flunitrazepam and its metabolites in human urine and plasma samples. J Chromatogr B 2003;788:207–219.

67. Robertson MD, Drummer OH. Stability of nitrobenzodiazepines in postmortem blood. J Forensic Sci 1998;43: 5–8.

68. El Mahjoub A, Staub C. Stability of benzodiazepines in whole blood samples stored at varying temperatures. J Pharm Biomed Anal 2000;23:1057–1063.

69. Negrusz A, Moore CM, Hinkel KB, Stockham TL, Verma M, Strong MJ, et al. Deposition of 7-aminoflunitrazepam and flunitrazepam in hair after a single dose of Rohypnol®. J Forensic Sci 2001;46:1143–1151.

70. Kintz P, Villain M, Ludes B. Testing for the undetectable in drug-facilitated sexual assault using hair analyzed by tandem mass spectrometry as evidence. Ther Drug Monit 2004;26:211–214.

71. Robertson MD, Drummer OH. Postmortem distribution and redistribution of nitrobenzodiazepines in man. J Forensic Sci 1998;43:9–13.

72. Korttila K, Linnoila M. Amnesic action of and skills related to driving after intravenous flunitrazepam. Acta Anaesth Scand 1976;20:160–168.

73. ElSohly MA, Feng S, Salamone SJ, Brenneisen R. GC-MS determination of flunitrazepam and its major metabolite in whole blood and plasma. J Anal Toxicol 1999;23: 486–489.

74. Divanon F, Debruyne D, Moulin M, Leroyer R. Benzodiazepines: toxic serum concentrations in positive enzyme immunoassay responses. J Anal Toxicol 1998;22: 559–566.

75. Feely J, Kavanagh PV, McNamara SM. The detection and quantitation of 7-amihoflunitrazepam, the major urinary metabolite of flunitrazepam, by gas chromatography/mass spectrometry. Isr J Med Sci 1999;168:189–192.

76. Forsman M, Nystrom I, Roman M, Berglund L, Ahlner J, Kronstrand R. Urinary detection times and excretion patters of flunitrazepam and its metabolites after a single oral dose. J Anal Toxicol 2009;33:491–501.

77. Negrusz A, Moore CM, Stockham TL, Poiser KR, Kern JL, Palaparthy R, et al. Elimination of 7-aminofluitrazepam and flunitrazepam in urine after a single dose of Rohypnol®. J Forensic Sci 2000;45:1031–1040.

78. Verstraete AG. Detection times of drugs of abuse in blood, urine, and oral fluid. Ther Drug Monit 2004;26:200–205.

79. Snyder H, Schwenzer KS, Pearlman R, McNally AJ, Tsilimidos M, Salamone SJ, et al. Serum and urine concentrations of flunitrazepam and metabolites, after a single oral dose, by immunoassay and GC-MS. J Anal Toxicol 2001;25:699–704.

80. Bramness JG, Skurtveit S, Morland J. Flunitrazepam: psychomotor impairment, agitation and paradoxical reactions. Forensic Sci Int 2006;159:83–91.

81. Ingum J, Bjorklund R, Volden R, Morland J. Development of acute tolerance after oral doses of diazepam and flunitrazepam. Psychopharmacology 1994;113:304–310.

82. Haverkos GP, DiSalvo RP, Imhoff TE. Fatal seizures after flumazenil administration in a patient with mixed overdose. Ann Pharmacother 1994;28:1347–1349.

83. Weinbroum AA, Flaishon R, Sorkine P, Szold O, Rudick V. A risk-benefit assessment of flumazenil in the management of benzodiazepine overdose. Drug Saf 1997;17: 181–196.

Chapter 6

GAMMA HYDROXYBUTYRATE and RELATED DRUGS

γ-HYDROXYBUTYRATE (GHB)

HISTORY

Although Saytzeff isolated GHB in 1874,[1] the first synthesis of gamma hydroxybutyrate (GHB) occurred during the early 1960s, when Henry Laborit searched for an active, synthetic analogue of γ-aminobutyric acid (GABA) that readily crossed the blood–brain barrier and persisted longer than GABA.[2] Preliminary studies demonstrated the similar sedative effects of GHB and γ-butyrolactone; however, animal studies in the late 1960s revealed that the biologic and behavioral effects of γ-butyrolactone resulted from the rapid conversion of this compound to GHB by an active lactonase.[3] Early uses of GHB included anesthesia and sleep-induction. In 1962, Blumenfeld et al reported the first use of GHB for surgical anesthesia in humans.[4] Although GHB was an effective anesthetic with minimal cardiorespiratory depression, recognition of undesirable postoperative sequelae (nausea, vomiting, myoclonus, Cheyne-Stokes respirations), lack of muscle relaxation, and poor analgesia limited the use of GHB as an anesthetic.[5] In 1964, Helrich et al induced sleep in healthy volunteers following the intravenous (IV) administration of GHB at doses up to 9 g (100 mg/kg).[6] Before 1990, GHB was sold as a dietary supplement in the United States that was a replacement for L-tryptophan. In the United States, reports of enhanced growth hormone release and potential anabolic effects resulted in the misuse of GHB by body builders during the early 1990s[7]; however, the lack of scientific support for the anabolic effects of GHB resulted in the subsequent reduction of GHB use for anabolic purposes.[8] In the 1990s, the discovery of the euphoric, sedative, and short-term growth hormone-releasing properties of GHB in the club drug and body-building scenes resulted in the popularization of GHB as a substance of abuse.[9] However, case reports associated serious toxic effects (coma, seizures) with the recreational use of GHB.[10,11] The US Food and Drug Administration (FDA) issued a recall for GHB- and GBL-containing nutritional supplements in early 1999. The Hillory J. Farias and Samantha Reid Date-Rape Drug Prohibition Act of 2000 reclassified GHB as a schedule I controlled substance as a result of reports of physical dependence and withdrawal following chronic GHB and the use of GHB for sedation and anterograde amnesia during drug-facilitated sexual assaults.[12] GHB became a controlled substance in most European Union member states in 2001. Amendments to the US Controlled Substances Act allowed the clinical development of sodium oxybate (i.e., oral formulation of GHB) to proceed via a unique, bifurcated schedule. Subsequently, the FDA approved the use of sodium oxybate for the treatment of cataplexy in 2002 as a schedule II drug. To promote the safe use of this drug and to alleviate concerns over possible diversion and abuse following product approval, a proprietary restricted drug distribution system (Xyrem® Success Program) was established. Components of the program include a centralized distribution and dispensing system with tracking of prescription shipments, a physician and

patient registry, and compulsory educational materials for patients and physicians along with an initial post-marketing surveillance program.[13] In 2005, the FDA extended the therapeutic use of sodium oxybate (GHB) to include the treatment of excessive daytime sleepiness in patients with narcolepsy. The treatment of cataplexy in adult patients with narcolepsy was approved in the European Union in 2005.

IDENTIFYING CHARACTERISTICS

GHB (CAS RN: 591-81-1, $C_4H_8O_3$, MW: 104.10 g/mol) is an endogenous short-chain fatty acid that is an active metabolite of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA).[14] Sodium oxybate (CAS RN: 502-85-2, MW: 127.09 g/mol) is the sodium salt of GHB that is the current oral form used to administer GHB therapeutically. One level teaspoon (i.e., 5 mL) of pure GHB weighs 4.4 g.[11] Common street names for GHB include Cherry Meth, Easy Lay, Everclear, G, George, Gina, Grievous Bodily Harm (GBH), Georgia Homeboy, Great Hormones at Bedtime, Goops, Growth Hormone Booster, Liquid Ecstasy, Liquid X, Organic or Nature's Quaalude, Oxy-Sleep, Poor Man's Heroin, Salty Water, Scoop, Soap, Vitamin G, Wolfies, and Zonked. GHB appears on the illicit market as a clear, odorless, oily liquid, a crystalline powder, or a gel (sodium salt). The liquid form has a slightly salty taste; occasionally, food dyes or cinnamon are added to improve palatability. The colorless, odorless, highly water soluble properties of the liquid allows the surreptitious use of GHB in the drinks of drug-facilitated sexual assault victims.

EXPOSURE

Epidemiology

Illicit GHB use in the United States declined dramatically following the scheduling of GHB as a schedule I controlled substance; GHB exposures reported to the California Poison Control System decreased 76% from 1999 ($n = 426$) to 2003 ($n = 101$).[15] Based on Drug Abuse Warning Network (DAWN) data, approximately 0.3–0.4% of drug-related emergency department (ED) visits in the United States are associated with GHB or GHB analogues.[16] Data from the 2004 National Drug Strategy Household Survey indicated that the prevalence of GHB use was relatively low in Australia.[17] About 0.5% of Australians aged 14 years or older reported the use of GHB at least once in their lifetime and 0.1% reported recent use. The prevalence of GHB use was highest among 20- to 29-year-olds. Although

detection issues complicate the interpretation of studies of GHB use during drug-facilitated sexual assault, existing studies suggest that GHB use in this setting is uncommon (<3%), similar to the use of flunitrazepam. In a systematic review of 11 studies on the involvement of GHB in reported sexual assaults, the range of detectable GHB concentrations in reported sexual assaults was 0.2–4.4%.[18] However, the short window of GHB detection limits conclusions regarding the prevalence of GHB use during drug-facilitated sexual assault.[19,20] Past and present medicinal uses of GHB include weight loss, sedation, the treatment of narcolepsy and alcohol or opiate withdrawal, and the induction of rapid eye movement sleep and general anesthesia. The use of GHB as an anesthetic has been discarded because of unpredictable anesthetic effects and adverse reactions.

Sources

The illicit manufacture of GHB involves the heating of a GBL mixture alkalinized with lye. After the addition of acetone, the mixture is dried. The ester hydrolysis of γ-butyrolactone produces clear solutions containing an amount of GHB equivalent to about 70% of the GBL weight. In a series of 418 samples seized at homosexual-oriented clubs in Australia, 225 samples were liquid with 85 (37.8%) containing GHB and 140 (62.2%) containing GBL.[21] None of the samples contained 1,4-butanediol (1,4-BD). In the United States, GHB is available as sodium oxybate (Xyrem®, Jazz Pharmaceuticals, Palo Alto, CA) for the treatment of cataplexy in patients with narcolepsy. GHB is available in Europe (Alcover®, CT Laboratorio Farmaceutico, SRL, San Remo, Italy) for use as an anesthetic or for the treatment of alcohol or opiate withdrawal.[22] Although randomized clinical trials indicate that GHB is superior to placebo for the treatment of alcohol withdrawal, the use of GHB for this indication is not clearly better than benzodiazepines or chlormethiazole in maintaining abstinence.[23]

Methods of Abuse

By the late 1990s and early 2000s, GHB was a popular club drug that was associated with drug-facilitated sexual assault.[24] The use of GHB at party settings appears more common among men than women, and the use of GHB frequently occurs in polydrug users (ethanol, ecstasy, methamphetamine, cocaine, ketamine).[25,26] Although GHB has a reputation as a club drug, an online survey of 189 individuals using GHB at least once in their lifetime suggested that private homes are a more common venue for GHB use than nightclubs.[27] Reports from GHB users describe the effects of

GHB as similar to sedative-hypnotic or alcohol intoxication, and chronic use may cause tolerance and dependence.[28] Desirable effects associated with the GHB use recreationally include euphoria, relaxation, loss of inhibitions, increased sociability, better mood, and enhanced sexual awareness. Although increased sexual desire, arousal, and activity are often related to GHB use, the use of GHB is also associated with decreased sexual performance and impaired memory of the sexual experience.[29] Visual or auditory hallucinations and involuntary limb movements are not typically associated with GHB use. Preclinical studies of GHB suggest that the abuse potential of GHB is relatively low; however, GHB abuse is a reported complication of the treatment of alcohol dependence/withdrawal with GHB.[30] In a double-blind study of 14 volunteers with histories of drug abuse, measures of likelihood of abuse (e.g., ratings of liking, reinforcing effects) indicate that the abuse likelihood of GHB is lower than pentobarbital and greater than triazolam.[31] Memory impairment was less with GHB compared with pentobarbital or triazolam, and GHB produced significantly greater adverse effects (e.g., nausea). A study of 12 healthy recreational users of GHB demonstrated that the ratings for euphoria and pleasurable effects following oral administration of 40–60 mg GHB/kg were slightly higher than 0.7 mg ethanol/kg or 1.25 mg flunitrazepam.[32]

DOSE EFFECT

For the treatment of narcolepsy, the standard dose of sodium oxybate is 4.5–9.0 g at bedtime. Single oral doses of 10 mg GHB/kg produce short-term amnesia and hypotonia, whereas the ingestion of 20–30 mg/kg causes drowsiness. In clinical trials, therapeutic doses of GHB for the induction of sleep typically range from 15–30 mg/kg with doses up to 30–50 mg/kg producing sleep that is reversible by external stimulation compared with 50–150 mg/kg for the treatment of alcoholics in withdrawal.[33,34] Euphoria may occur at GHB doses used to induce sleep. GHB doses above 60 mg/kg are associated with obtundation and coma.[35] Intravenous GHB doses of 60–70 mg/kg induce sleep within 5–15 minutes and coma lasting about 1–2 hours followed by rapid arousal.[36] Adverse effects at these doses include nausea, vomiting, hypotonia, bradycardia, respiratory depression, and Cheyne-Stokes respiration.[37]

The typical street dose of GHB is 2–6 g (25–75 mg/kg or approximately ½–1¼ teaspoon of pure GHB), but experienced users may ingest up to 30 g.[11] Withdrawal symptoms usually occur in high-dose, chronic GHB users, whereas the administration of 50 mg GHB/kg daily for 6 months to alcoholic patients was not associated with withdrawal following abrupt cessation of GHB therapy at the end of the study.[38] In a case series of 48 narcolepsy patients treated with 2.25–3 g GHB daily for up to 9 years, evidence of withdrawal did not occur.[39] The exact dose of GHB associated with withdrawal symptoms is difficult to estimate because the origin of the GHB often is a powder obtained over the Internet and the patients are frequently polydrug abusers. The escalating use of GHB up to an estimated 40 g daily was associated with withdrawal symptoms including anxiety, tremors, diaphoresis, hallucination, paranoia, nystagmus, impaired memory, hypertension, and tachycardia based on case reports.[40] Prevention of withdrawal symptoms may require frequent administration in the day and night. Typically, individuals ingest GHB doses every 2–4 hours during the night to prevent insomnia or anxiety.

TOXICOKINETICS

Absorption

Volunteer studies indicate that the absorption of GHB is rapid and capacity-limited with peak plasma GHB concentrations occurring <1 hour after ingestion.[41] Peak GHB plasma concentrations occur within about 20–45 minutes after the ingestion of 25–50 mg GHB/kg body weight by healthy volunteers.[42] In a study of alcoholic patients receiving 25 mg GHB/kg twice daily for 7 days, the mean maximum plasma GHB concentration was 55 ± 19 mg/L with a range from 32–85 mg/L.[43] The median time to peak plasma GHB concentration was 30 minutes (range, 20–45 min). Following the oral administration of single GHB (sodium oxybate) doses of 60 mg/kg and 72 mg/kg, the mean maximum plasma GHB concentrations were 113.5 ± 20.1 mg/L (range, 90.3–134.7 mg/L) and 130.1 ± 10.7 mg/L (range, 122.5–137.6 mg/L), respectively.[44] Figure 6.1 displays the time-concentration curves for GHB after the oral administration of a single dose of 12.5 mg/kg, 25 mg/kg, or 50 mg/kg to 8 healthy male volunteers. The absorption of sodium oxybate (Xyrem®, Orphan Medical, Minnetonka, MN) in solution is also rapid with peak GHB concentrations occurring within 30–60 minutes after administration.[45]

Distribution

GHB is poorly protein bound, and the apparent volume of distribution is relatively small (0.4–0.6 L/kg). In a study of 8 healthy volunteers receiving a single GHB dose of 25 mg/kg, the mean volume of distribution was 52.7 ± 15.0 L (range, 19.1–155 L).[42]
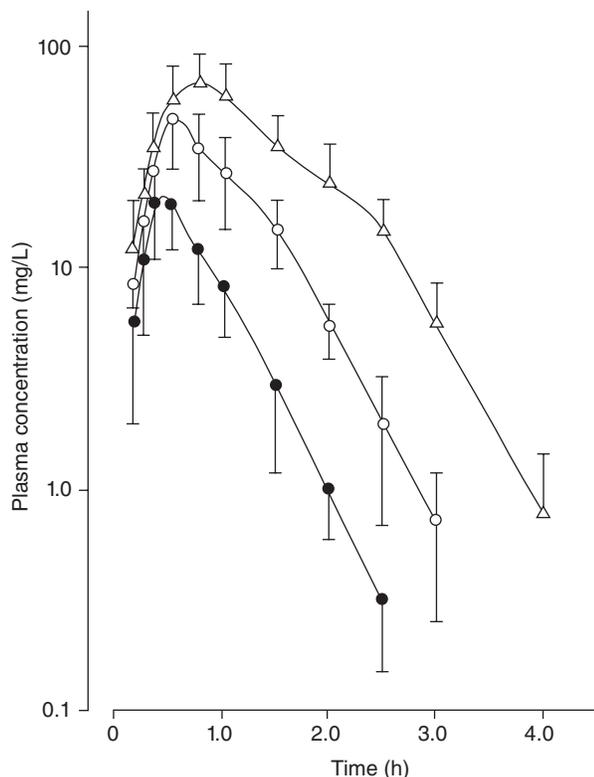
FIGURE 6.1. Plasma concentrations of γ-hydroxybutyrate (GHB) following oral administration of 12.5 mg/kg (closed circle), 25 mg/kg (open circle), and 50 mg/kg (open triangle). (Reprinted with kind permission from Springer Science+Business Media: European Journal of Clinical Pharmacology, Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers, Vol. 45, 1993, p. 354, P. Palatini, Fig. 1A.)

Biotransformation

The metabolism of GHB primarily involves conversion to succinic semialdehyde and then succinate followed by the conversion of succinate to carbon dioxide and water via the tricarboxylic acid cycle (Krebs cycle). Several minor pathways of GHB metabolism also exist, including the reduction of GHB to succinic semialdehyde by the mitochondrial enzyme, GHB-oxoacid-transhydrogenase (D-2-hydroxyglutarate transhydrogenase), and β-oxidation as displayed in Figure 6.2.

Elimination

Elimination of GHB results primarily from biotransformation rather than renal excretion. The kidneys excrete <1–2% of a therapeutic GHB dose (25 mg/kg twice daily for 7 days) unchanged.[43] The elimination of GHB is rapid, but nonlinear as a result of capacity-limited kinetics following the administration of GHB doses

above 50 mg/kg. Following typical therapeutic or recreational doses of GHB, the plasma elimination half-life is approximately 20–30 minutes. In a study of alcoholic patients receiving 25 mg GHB/kg twice daily for 7 days, the median plasma elimination half-life was 27 ± 5 minutes with a range of 20–45 minutes.[43] In a study of 6 narcolepsy patients, the plasma elimination half-life of GHB was 53 ± 19 minutes following administration of two 3 g-doses at a 4-hour interval.[46] Plasma GHB concentrations were nondetectable within approximately 4–6 hours after ingestion as measured by gas chromatography with mass selective detection (lower limit of quantitation [LLOQ], ~7 mg/L). The elimination of GHB following the administration of therapeutic doses of sodium oxybate (Xyrem®) is also rapid with an average plasma elimination half-life of about 40 minutes.[45] Case reports of GHB overdoses suggest that zero-order or saturation (Michaelis-Menten) kinetics best describe the elimination of GHB following the ingestion of very large doses of GHB.[47]

Drug Interactions

The illicit use of GHB frequently occurs with the ingestion of other drugs, particularly ethanol; GHB and ethanol share similar sedative effects. In a double-blind, placebo-controlled, crossover study, 16 healthy adults received GHB (50 mg sodium oxybate/kg) and/or ethanol (0.6 g/kg, estimated blood ethanol concentration 50 mg/dL). The combination of GHB and ethanol produced more significant adverse effects (vomiting, decreased blood pressure, reduced oxygen saturation) compared with placebo or each drug alone.[48] However, there were minimal pharmacokinetic interactions (i.e., no statistically significant reduction in elimination half-life or maximal plasma GHB concentrations).

Case reports suggest that concomitant use of human immunodeficiency virus 1 (HIV-1) protease inhibitors with GHB may exacerbate the respiratory depression and bradycardia associated with GHB intoxication.[49] Valproate, phenobarbital, barbital, and chlorpromazine inhibit NADPH-dependent aldehyde reductase, which catalyze the formation of GHB from succinic semialdehyde;[50] however, the clinical relevance of this inhibition in regard to GHB concentrations and clinical effects is unclear.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

GHB is a natural constituent of the brain that is both a precursor and metabolic product of GABA. GHB
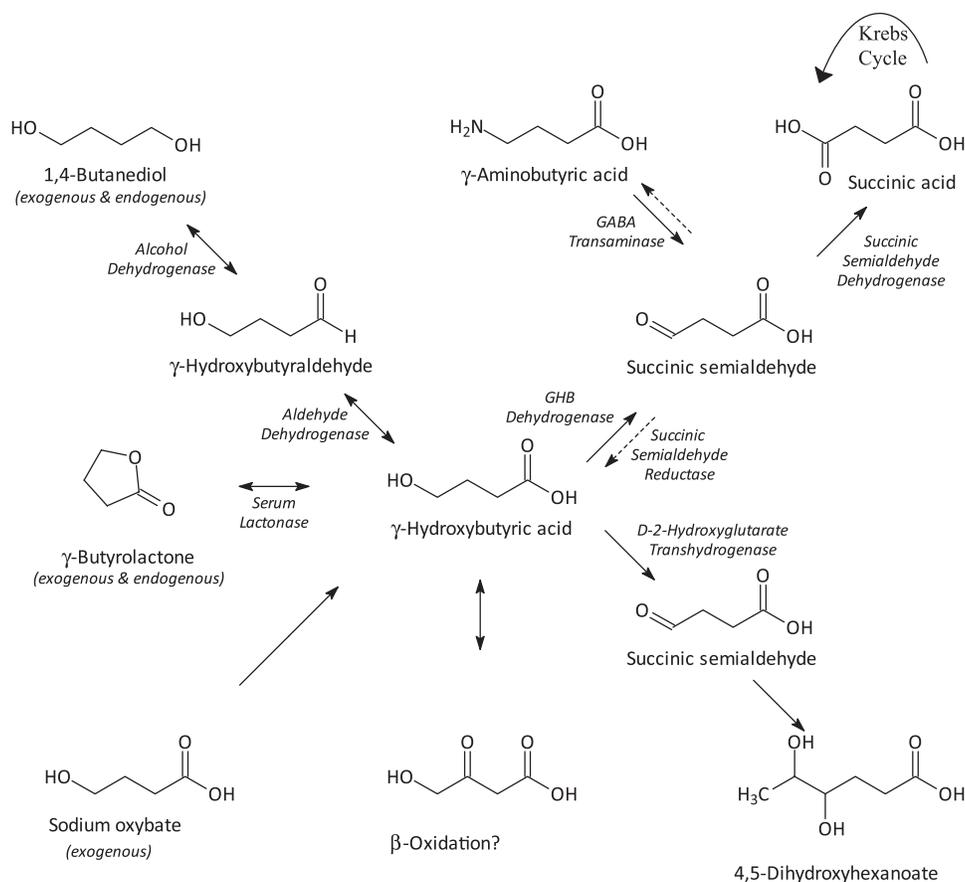
FIGURE 6.2. Synthesis and metabolism of γ-hydroxybutyrate (GHB) with major pathways in the solid arrows and minor pathways in dashed arrows. GABA = γ-aminobutyric acid.[55]

occurs in micromolar quantities (1–4 μM) in all parts of mammalian brains and some peripheral organs (heart, kidney, liver, muscle, brown fat), but the highest GHB concentrations appear in the cytosolic and synaptosomal fractions of the striatum, particularly in the developing brain.[51] GABA is the major precursor of GHB, and the GHB concentrations in the brain are approximately 0.1% of the GABA concentrations.[52] Synthesis of GHB occurs throughout the central nervous system (CNS), where GHB is released into the synaptic cleft from storage vesicles by potassium-dependent depolarization; then, GHB uptake into the nerve terminal occurs. GHB synthesis primarily involves the conversion of GABA to succinic semialdehyde via mitochondrial GABA transaminase and the subsequent reduction of succinic semialdehyde to GHB via cytosolic succinic semialdehyde reductase.

Although GHB is biologically active and ubiquitous in the CNS, the exact mechanism of action is unclear. Experimental evidence suggests that GHB acts as a presynaptic, G-protein coupled neurotransmitter and/or a neuromodulator of the GABA_B receptor, particularly in the mesocorticolimbic dopamine pathways. Most physiologic and pharmacologic effects of exogenous GHB probably result from modulation of GABA_B receptors directly as a partial agonist and indirectly through GHB-derived GABA[53]; however, drug discrimination studies suggest that some subtypes of the GABA_A and GABA_B receptors are also involved as a result of the differences in effect between the GABA_B receptor agonist, baclofen and GHB.[54] GABA_B receptors mediate slow inhibitory postsynaptic potentials by activation of calcium channels and G-protein-coupled, inwardly rectifying potassium channels via signaling through the adenylate cyclase system. GHB binding sites occur throughout the brain including the hippocampus, dentate gyrus, olfactory system, nucleus accumbens, septum, caudate putamen, substantia nigra, ventral tegmental area, pons, and the cortex with lower concentrations of binding sites in the amygdala and the thalamus.[55] Under *endogenous* conditions, GHB can increase or decrease neuronal activity by inhibiting the release of neurotransmitters located near the endogenous source of GHB. Volunteer studies indicate that the

administration of 2.5–3.5 g GHB at bedtime doubles growth hormone secretion, primarily during the first 2 hours after sleep onset.[56] The stimulation of growth hormone secretion correlated to an increase in the amount of sleep stage IV (slow wave, nonrapid eye movement sleep). However, the exact mechanism of growth hormone release remains unclear as other neurotransmitter pathways (serotonergic, cholinergic) may affect the increased growth hormone release associated with GHB administration. Additionally, the physiologic importance of growth hormone release remains unclear because there is no direct evidence that short-term elevation in growth hormone increases muscle mass and GHB use does not increase the muscle mass of alcoholic patients.[8]

Mechanism of Toxicity

GHB is a CNS depressant with complex neuropharmacologic and neurophysiologic effects including alteration of dopaminergic transmission in the basal ganglia.[57] The exogenous administration of GHB produces sedation, and at sufficiently high doses, anesthesia. Following exogenous administration, GHB produces behavioral effects by selective agonist activity at the $GABA_B$ receptors. These metabotropic receptors cause slow inhibitory neurotransmission by hyperpolarization of the postsynaptic membrane via a biochemical cascade rather than alteration of electrical activity. Similar to GABA (the primary inhibitory neurotransmitter in the CNS), GHB binding sites are G-protein coupled receptors. The binding of GHB for the $GABA_B$ receptors is weak ($K_i = 80$–120 μM); therefore, supraphysiologic GHB concentrations from endogenous administration are necessary for clinically significant binding of GHB to $GABA_B$ receptors.[58] The binding of GHB to postsynaptic $GABA_B$ receptors increases potassium conductance via inwardly rectifying potassium (GIRK or Kir3) currents. $GABA_B$ stimulation causes multiple pre- and postsynaptic effects including postsynaptic silencing, modulation of intracellular calcium dynamics, and inhibition of neurotransmitter release. The active uptake of GHB from the synaptic cleft terminates GHB activity. Experimental *in vitro* and *in vivo* animal studies indicate that exogenous GHB administration affects a variety of neurotransmitter systems in the brain including dopamine release and synthesis, serotonin turnover, increased dynorphin and enkephalin concentrations, noradrenergic transmission, and inhibition of glutamate release.[55] GHB is not an agonist at the μ-, δ-, or κ-opioid receptors.[59] Experimental data suggests that GHB abuse and withdrawal may result from the inhibition of GABAergic neurons by stimulation of $GABA_B$ receptors and inhibition of presynaptic GABA release in

mesocorticolimbic dopaminergic pathways by GHB receptor activation with subsequent increased dopaminergic activity in these pathways. However, delineation of the exact role of endogenous GHB as a neuromodulator and neurotransmitter requires further studies.[53]

Postmortem Examination

Autopsies of fatalities associated with GHB are nonspecific (e.g., pulmonary edema, visceral congestion). Common postmortem findings include cerebral edema, pulmonary congestion and hemorrhage, usually in association with ethanol and/or other drugs and occasionally with evidence of aspiration.[60]

CLINICAL RESPONSE

Illicit Use

Adverse effects associated with the illicit use of GHB are highly variable; symptoms include euphoria, sedation, memory loss, nausea, vomiting, and lightheadedness. As the dose escalates, agitation, confusion, hallucination, loss of peripheral vision, myoclonus, bradycardia, mild hypothermia, respiratory insufficiency, hypotension, and coma occur, particularly following the concomitant administration of other drugs. Although case reports associate GHB use with seizures,[11] volunteer studies do not document epileptiform changes on the electroencephalogram and random clonic movements of the face and extremities occur during induction of anesthesia with GHB.[61] Additionally, GHB use commonly occurs with the use of other drugs of abuse associated with seizure activity. The onset of action following oral GHB administration is rapid (15–30 min), while the duration of action of GHB is relatively short. Based on questionnaires, the acute effects of a single recreational dose of GHB persists about 2–4 hours (range, <2–6 h).[62] The patient frequently demonstrates anterograde (i.e., after onset of alteration of consciousness), but not retrograde amnesia (i.e., prior to administration).

Overdose

Overdoses of GHB are common complications of GHB use as determined by questionnaires. In a cross-sectional survey of 76 Australian GHB users, 53% of the respondents reported at least 1 overdose as defined by the loss of consciousness with inability to awaken following external stimulation.[63] The percentage of at least 1 overdose in individuals using GHB over 15 times was 75%. The profile of a patient presenting to an emergency department with GHB intoxication is a young male, polydrug user (alcohol, illicit drugs) with altered con-

sciousness. The classical clinical features of GHB intoxication include vomiting, myoclonus, somnolence, obtundation, stupor, coma, bradycardia, respiratory depression, and hypothermia. Paradoxical excitation and aggressive behavior may occur. In a case series of 104 emergency department patients with GHB intoxication, all patients had impaired consciousness and 17% of the cases had a Glasgow Coma Score (GCS) of 3.[64] Other clinical features of GHB intoxication reported in these patients included the following: vomiting, 23%; sinus bradycardia, 20%; agitation, 5%; myoclonus, 3%, and seizures, 3%. Pupil size is variable, and blood pressure is typically normal despite the presence of respiratory insufficiency. Despite severe respiratory depression, violent agitation may occur during attempts to intubate the patient or to insert a urinary catheter. Hypotension is uncommon. In a case series of 170 patients presenting to an emergency department with GHB intoxication, hypotension was present in 6 patients (4%).[65]

The duration of the coma is relatively short with rapid awakening. Even deeply comatose patients awaken within 4–6 hours after ingestion.[66] Recovery typically is spontaneous, frequently abrupt, and often associated with an emergence phenomenon (myoclonic jerks, transient confusion, combativeness).[67] In a case series of 5 unconscious patients (GCS = 3) presenting to an ED, the GCS remained unchanged for a median time of 60 minutes (range, 20–110 min).[68] Awakening from coma (GCS 3→15) was rapid (median, 30 min; range, 20–60 min).

Death following the ingestion of GHB alone is uncommon, and GHB-associated fatalities usually occur in the setting of polydrug use outside the hospital including abuse of sodium oxybate.[69,70] Patients with GHB intoxication typically survive if they arrive at the emergency department without anoxic brain damage.[71] Potential causes of GHB-associated deaths include aspiration, respiratory depression, and traumatic injury.

Abstinence Syndrome

Withdrawal symptoms may occur following the use of prolonged, high-doses of GHB within 1–6 hours following the cessation of GHB use. Typically, these withdrawal symptoms develop in individuals using GHB every 1–3 hours; whereas the daily use of GHB during the treatment of narcolepsy is not usually associated with withdrawal.[72] The minimum daily GHB dose causing withdrawal is difficult to predict because of recall bias and the variable concentrations of GHB in illicit materials. The clinical features of GHB withdrawal are similar to ethanol and sedative-hypnotic (benzodiazepine, barbiturate) withdrawal, but the onset of symptoms (anxiety, delirium) occurs much sooner (i.e.,

<6–12 h) after cessation of use.[73] The symptoms associated with GHB withdrawal are highly variable, depending primarily on the daily dose and frequency of use.[74] The presentation of the GHB abstinence syndrome includes nausea, vomiting, anxiety, tachycardia, insomnia, tremor, agitation, diaphoresis, and hallucinations; rarely withdrawal symptoms progress to delirium, primarily in severely dependent patients. In a review of 38 published cases of GHB withdrawal, a majority of patients had tremor, tachycardia, anxiety, hallucinations, and delirium. Seizures do not usually occur during GHB withdrawal.[73] The abstinence syndrome progresses over the first 2–3 days with mild autonomic instability (diaphoresis, hypertension, tachycardia, tremor), anxiety, and hallucinations. Confusion, disorientation, agitation, or combative behavior may require restraint and sedation. Symptoms of withdrawal typically resolve within 3–12 days, but may persist up to 15 days.[75] Case reports suggest that insomnia, anxiety, dysphoria, and poor memory may persist for several months after withdrawal.[76]

DIAGNOSTIC TESTING

Analytic Methods

SALIVA

GHB is detectable in saliva as determined by gas chromatography/mass spectrometry in selective ion monitoring mode after silyl derivatization.[77] The LLOQ was 0.5 mg/L with coefficients of variation ranging from 2.1–12.5%. However, determination of GHB concentrations in saliva probably does not extend the window of detection when compared with blood or urine.[78]

SCREENING

Rapid colorimetric methods are available to determine the presence of GHB and GBL in urine samples using the ferric hydroxamate test.[79] The limit of detection (LOD) with this method is 0.1 mg/mL in a 1-mL urine sample; this test does not distinguish between GHB and GBL. Positive samples turn purple. More sophisticated qualitative methods for the detection of GHB and GBL include gas chromatography/flame ionization detection.[83] This method can detect both compounds by the analysis of samples with and without acid hydrolysis to convert GHB to GBL. The LOD is 0.5 mg/L. Other screening methods that lack sensitivity at low, endogenous GHB concentrations include ^1H nuclear magnetic resonance spectroscopy, micellar electrokinetic chromatography, capillary zone electrophoresis with indirect ultraviolet absorption, and high performance liquid

chromatography with ultraviolet or ultraviolet-visible spectrophotometry.[80] Commercial immunoassays for the detection of GHB are generally unavailable.

CONFIRMATORY

High performance liquid chromatography with ultraviolet (254 nm) and micellar electrokinetic chromatography techniques are generally less sensitive than gas chromatography/mass spectrometry.[81,82] Gas chromatography/mass spectrometry methods include both the conversion of GHB to GBL by acid hydrolysis (lactonization) and the direct analysis of GHB after liquid-liquid extraction. The latter method typically involves extraction with an organic solvent and derivatization to counter the thermal degradation and improve the chromatographic peak shapes of GHB with heptafluorobutyric anhydride or bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS). Direct methods for the quantitation of GHB include gas chromatography/flame ionization detection,[83] gas chromatography/mass spectrometry with silyl-derivatization,[84] gas chromatography/electron impact/mass spectrometry,[85] gas chromatography/mass spectrometry with selected-ion monitoring,[86,87] and gas chromatography/positive ion chemical ionization/mass spectrometry.[88] Gas chromatography/mass spectrometry is the preferred method for quantitation of GHB. The LLOQ for the above 3 gas chromatography/mass spectrometric methods was 1 mg/L, 1 mg/L, and 2.5 mg/L, respectively. The LLOQ for gas chromatography/tandem mass spectrometry[89] and liquid chromatography/tandem mass spectrometry[90] is similar (i.e., 2.5 mg/L and 1 mg/L, respectively).

STREET SAMPLE ANALYSIS

Many illicit samples of GHB may contain GBL; analysis of illicit samples for GHB precursors by gas chromatography/mass spectrometry or high performance liquid chromatography/ultraviolet-visible spectrophotometry (e.g., GBL, 1,4-butanediol, maleic anhydride) provides information regarding the origin of the samples.[91] Determination of δ^{13}C-values with gas chromatography/combustion-isotope ratio/mass spectrometry is an alternative method for the identification of drug origin and the separation of endogenous production of GHB from external sources.[92]

STORAGE

In vitro production of GHB may occur during storage at ambient temperatures in unpreserved samples or in the presence of certain collection tube additives, par-

ticularly in postmortem blood samples stored >60 days. In a convenience sample of 26 postmortem blood samples stored at 4°C (39.2°F), the mean GHB concentration in containers with sodium fluoride was 19 mg/L compared with a mean GHB concentration of 32 mg/L in containers without preservatives.[93] Analysis of these samples occurred within 2 months of the autopsies. At room temperature, the GHB concentration was approximately 3-fold higher in unpreserved containers than in containers with sodium fluoride. Artificial production of GHB can occur in blood stored in yellow-top anticoagulant-citrate buffer tubes (trisodium citrate, citric acid, dextrose), whereas blood stored in purple-top tubes (edetate disodium acetate, EDTA) usually remains GHB-negative.[94] In general, postmortem blood preserved with sodium fluoride (10 mg/mL) can be stored at either 25°C (room temperature, 77°F) or 4°C (refrigerator, 39.2°F) with little change in the GHB concentration.[80] There was no significant increase in GHB concentrations (i.e., <3 mg/L) in pooled antemortem human serum aliquots stored for 8 months either with or without sodium fluoride preservative when stored at 4°C (39.2°F) or –20°C (–4°F).[95]

GHB is more stable in urine samples than in blood samples, particularly in sodium fluoride-preserved urine samples stored at –20°C (–4°F). The increase in GHB concentrations in urine samples stored over 1 year typically is below 5–7 mg/L, even in preserved (i.e., 1% sodium fluoride) specimens stored at room temperature.[96] In urine samples stored without preservatives at refrigerated temperatures, the *in vitro* production of GHB does not usually exceed the suggested 10 mg/L cutoff. In a study of 100 urine samples stored for about 1 year at room temperature with sodium fluoride, the maximum GHB concentration was 7 mg/L.[97] In a study of endogenous GHB concentrations in pooled urine samples from 2 participants stored at room (~25°C/77°F), refrigerator (5°C/41°F), and freezer (–10°C/14°F) temperatures over 6 months, the greatest increase (~400%) in endogenous GHB concentrations occurred at room temperature, whereas smaller increases occurred in refrigerated samples (~140–200%) and frozen samples (~100%).[98] The GHB concentrations in these samples were below the GHB concentrations (i.e., <1 mg/L) typically associated with GHB use.

Biomarkers

There are few data on the GHB concentrations in vitreous humor. In a case series of postmortem examinations with detectable concentrations of GHB, 3 cases had the following measurable GHB vitreous humor/femoral blood concentrations in mg/L (ratio): 78/97 (0.80), 250/180 (1.39), and 280/210 (1.33).[99] The GHB concen-

tration was measured after conversion of GHB to gamma-butyrolactone and analysis of the latter compound by gas chromatography/flame ionization detection (LOD, 30 mg/L). Although there are few data on the distribution of GHB between whole blood and serum, the lack of protein binding and high water solubility of GHB suggest that the whole blood/serum ratio is similar to ethanol.

BLOOD

THERAPEUTIC/ILLICIT USE.   GHB is a trace, natural constituent of body fluids along with the 2 isomers, α-hydroxybutyric acid and β-hydroxybutyric acid. Although there is no consensus on the optimal antemortem GHB concentration that distinguishes between endogenous production and exogenous administration, several studies suggest that a plasma GHB cutoff of 4–5 mg/L reliably identifies the ingestion of GHB antemortem.[100] In a study of 240 antemortem specimens from individuals without known GHB exposure, the mean GHB concentration was 0.74 mg/L (range, 0.34–5.75 mg/L). The mean endogenous GHB concentration in 50 serum samples from residents of a detoxification program was 1.14 mg/L (range, 0.62–3.24 mg/L; median, 0.97 mg/L).[101] There is substantial intraindividual variation in endogenous GHB concentrations over time of day and during the week.

The presence of other drugs in a majority of GHB intoxication complicates the correlation of plasma GHB concentrations to clinical effects. In a study of the use of GHB as an IV anesthetic, serum GHB concentrations correlated to clinical effects.[102] Occasional eye opening and spontaneous movement occurred at GHB concentrations ≤150 mg/L, whereas serum GHB concentrations exceeding 260 mg/L produced profound coma. Blood and urine samples from patients with rare inborn error of metabolism (i.e., 4-hydroxybutyric aciduria) may contain GHB concentrations exceeding 30 mg/L and 200 mg/L, respectively.[103]

OVERDOSE.   The level of consciousness during GHB-induced coma does not correlate well to plasma GHB concentrations, in part because of the frequent concomitant ingestion of other drugs including ethanol, ecstasy, marijuana, methamphetamine, cocaine, ketamine, and antidepressants. Plasma GHB concentration in patients with GHB overdose typically exceed 100 mg/L. The median plasma GHB concentration in blood from a convenience sample of 54 patients presenting to an emergency department with GHB intoxication was 103 mg/L (mean, 137 mg/L) with a range of 29–490 mg/L.[104] About two-thirds of these patients were unconscious or unresponsive on admission to the ED;

there were no deaths in this series. Approximately two-thirds of the blood samples contained other drugs (e.g., ethanol, methylenedioxymethamphetamine). In a series of 15 unconscious patients presenting to an emergency department with GHB intoxication (GCS ≤8), the median plasma GHB concentration was 212 mg/L (range, 112–430 mg/L).[68] The single patient with only GHB detected in his blood was an 18-year-old man with an initial GCS score of 6. His plasma GHB concentration on presentation was 186 µg/mL, and he awoke within 1 hour of presentation.

In a series of 16 patients with severe GHB intoxication (GCS ≤8), the serum GHB concentration ranged from 45–295 mg/L (median, 180 mg/L), whereas patients with a GCS of 3 had serum GHB concentrations ranging from 72–300 mg/L (median, 193 mg/L).[105] The severe coma in these patients persisted 1–2 hours. Awakening from coma occurs relatively rapidly with relatively small changes in the plasma GHB concentration. The mean plasma GHB concentration (i.e., measured as total GBL) in a case series of 27 patients presenting to EDs in the UK with altered consciousness secondary to GHB use was 245 mg/L (range, 86–551 mg/L).[106] There were no reported sequelae. A 23-year-old woman ingested ethanol and GHB along with smoking marijuana shortly before becoming unconscious.[107] She presented to the emergency department comatose (GCS = 6) with normal vital signs except mild bradycardia (56 bpm) and respiratory depression (8 breaths/min). In admission blood, her serum ethanol and GHB concentrations were 134 mg/dL and 125 mg/L, respectively. She awoke within 1 hour and left the emergency department against medical advice.

POSTMORTEM
GHB Production Animal and postmortem human studies indicate that postmortem production of GHB increases as the postmortem interval increases, depending on the specific analytic technique, type of biologic sample, and storage methods. Potential sources for the postmortem production of GHB include the enzymatic conversion of succinic acid, γ-aminobutyric acid (GABA) and/or putrescine to GHB. Endogenous GHB concentrations in vitreous and in blood samples from different parts of the body are similar in postmortem examinations performed within 48 hours of death. In a study of 25 well-preserved autopsy cases (postmortem interval, 6–48 h), the highest endogenous GHB concentrations occurred in the femoral blood (4.6 ± 3.4 mg/L), whereas the lowest concentration appeared in the urine (0.6 ± 1.2 mg/L) as measured by headspace gas chromatography/flame ionization detection after conversion of GHB to GBL.[108] Some differences in postmortem GHB concentrations may result from the use

of different analytic methods. In 20 postmortem blood specimens (postmortem interval not reported) from cases without known GHB exposure, the blood GHB concentrations ranged from 3.2–168 mg/L as measured by gas chromatography/flame ionization detection and gas chromatography/mass spectrometry after conversion of GHB to GBL (i.e., total GBL).[109]

The exact pathway of postmortem GHB formation remains unclear because of the failure to detect endogenous precursors, 1,4-butanediol or succinate semialdehyde (GABA transamination) as potential sources of GHB formation.[110] Although endogenous GHB concentrations do not correlate to the concentrations of common putrefactive makers (e.g., tryptamine, phenyl-2-ethylamine), experimental studies suggest that *Pseudomonas aeruginosa* species can produce GHB in unpreserved blood specimens.[111] In well-preserved (e.g., sodium fluoride) postmortem blood and urine samples, the presence of GHB concentrations exceeding 30–50 mg/L in blood samples or 20 mg/L in urine samples suggests the presence of exogenous GHB. A study of 38 postmortem blood and 15 urine samples preserved with sodium fluoride from autopsy cases unrelated to GHB ingestion demonstrated mean GHB concentrations of 12.3 mg/L (range, 2–29 mg/L) and 4.8 mg/L (range, 0–10 mg/L), respectively.[112] However, determination of the presence of exogenous GHB in postmortem samples exceeding these cutoffs requires careful analysis of the storage methods (e.g., preservatives), analytic techniques, multiple biologic specimens (e.g., blood, urine, vitreous) and clinical history, particularly when examining postmortem cardiac blood. In 71 autopsy cases (postmortem interval, 12–72 hours) without known GHB exposure, 14 postmortem unpreserved heart blood samples contained GHB concentrations exceeding 50 mg/L (range, 50.8–409 mg/L).[113] The femoral blood and vitreous humor samples in all these cases were below the 50 mg/L cutoff. The heart/femoral blood ratio for 5 cases in this series of presumed endogenous GHB production was 1.24, 1.28, 1.85, 5.65, and 24.3.

GHB Concentrations   Interpretation of the significance of postmortem GHB concentrations requires analysis of sample storage, postmortem interval, and the correlation of the medical/case history to the postmortem GHB concentration. There are limited data on the postmortem redistribution of GHB with heart/femoral blood ratios typically ranging from about 0.5–2. A 35-year-old man was found dead after ingesting wine and GHB at a party. The GHB concentrations in heart and femoral blood samples were 276 mg/L and 461 mg/L, respectively (heart/femoral blood ratio, 0.56)[114] compared with 3,385 mg/L and 2,936 mg/L (heart/femoral blood ratio, 1.15) in a 43-year-old man pronounced dead soon after being found unconscious by relatives.[115] The screen for drugs of abuse and ethanol was negative. The vitreous humor contained 48 mg GHB/L. Heart and femoral blood samples from a 22-year-old woman dying after the ingestion of a single dose of GHB contained GHB concentrations of 648 mg/L and 330 mg/L, respectively (heart blood/femoral blood ratio, 1.96).[116] The autopsy demonstrated no other cause of death and the comprehensive drug screen was negative with the exception of GHB.

In a convenience sample of 78 deaths from throughout the United States attributed to GHB intoxication without co-intoxicants, the median postmortem blood concentration (site not specified) was 347 mg/L with a range of 18–4,400 mg/L.[117] All of the cases with a medical history had a cardiorespiratory arrest prior to the arrival of the paramedics. The median GHB concentration in femoral blood samples from 18 deaths attributed to GHB intoxication was 235 mg/L (range, 55–2,200 mg/L).[118] Although other drugs were present in postmortem blood samples from the vast majority of the samples, almost half of these cases had minor or no clinically significant amounts of other drugs. In a case series of 8 autopsy cases attributed to GHB intoxication (along with other drugs), the mean postmortem/perimortem GHB concentration (method not reported) in blood samples (source not specified) was 231 mg/L (range, 77–370 mg/L). Similar to antemortem samples, the clinical significance of postmortem GHB concentration requires careful analysis of the analytic techniques, storage methods, postmortem interval, and clinical history including resuscitation measures and survival times. The GHB heart/femoral blood ratio in postmortem blood samples from a 38-year-old man found dead at his home was 1.13 (1,052 mg/L/932 mg/L).[119]

URINE

Urine drug of abuse screens do not routinely detect GHB, and rapid immunoassays for the detection of GHB overdose are usually not available in the hospital setting. GHB occurs in the urine as well as other body fluids as a result of endogenous production. Proposed cutoff concentrations of GHB in urine to distinguish endogenous production from exogenous administration range from about 5–10 mg/L with a majority of urine specimens containing GHB concentrations below 1–2 mg/L.[120] In a convenience sample of 119 urine specimens from GHB-free study participants, the maximal urinary GHB concentration was 3 mg/L,[100] whereas the maximum GHB concentration in urine samples from 55 participants without known exposure to GHB was 3.5 mg/L (median, 1.3 mg/L).[121] This study demonstrated substantial intra-individual variation in the GHB concentration in urine that is independent of

TABLE 6.1. Range of Endogenous Concentrations of γ-hydroxybutyrate (GHB) and Associated Isomers in the Urine of Healthy (n = 30) and Diabetic (*n* = 20) Volunteers.[122]

Substance	Healthy (mg/L)	Diabetic (mg/L)
GHB	0.16–2.14	0.17–3.03
α-Hydroxybutyric acid	0.10–2.68	0.14–124
β-Hydroxybutyric acid	8.51–34.7	4.94–4,520

urinary creatinine concentration and standard diets. Some foods (e.g., ripe guava fruit) contain relatively high concentrations of GHB; however, there is no clear evidence that these GHB concentrations produce clinical effects. The urinary GHB concentrations of healthy volunteers and diabetics are similar; however, diabetic patients typically have higher concentrations of GHB isomers, particular during diabetic ketoacidosis.[122] Table 6.1 compares the range of endogenous GHB and GHB isomers in the urine of 30 healthy and 20 diabetic volunteers. The highest concentration of GHB isomers occurred in a diabetic with ketoacidosis. Analysis of 50 urine samples donated by healthy women demonstrated a maximum urinary GHB of 1.46 mg/L as measured by gas chromatography/mass spectrometry with silyl derivation (LOD, 0.1 mg/L).[123] Pregnant women have a modestly higher urine GHB concentration when compared with nonpregnant women. The mean GHB concentration in urine samples from 66 pregnant women was 0.36 mg/L compared with 0.24 mg/L in 69 nonpregnant women as measured by liquid chromatography/tandem mass spectrometry.[124] The mean urine gamma-butyrolactone (GBL) concentration was 4.3-fold higher in pregnant women.

The GHB concentration in urine samples from patients with GHB overdoses usually exceed 1,000 mg/L. The mean GHB concentration (i.e., measured as total GBL) in urine samples from a case series of 27 patients presenting to EDs in the United Kingdom with altered consciousness secondary to GHB use was 1,732 mg/L (range, 5–5,581 mg/L).[106] There were no reported sequelae. The window of detection for GHB following typical recreational doses is approximately 12 hours with peak GHB urine concentrations usually occurring within 3 hours of ingestion as measured by gas chromatography/mass spectrometry. Following the administration of a single GHB (Xyrem®) dose of 50 mg/kg to 16 healthy adults, the percentage of urine samples *below* the 10-mg/L cutoff during specific time intervals after administration were as follows: 3–6 hours, 12.5%; 6–12 hours, 81.3%; 12–24 hours, 100%.[125]

Following large GHB ingestions, the urine contains high GHB concentrations. A 28-year-old man was found comatose after ingesting a liquid confirmed to be GHB.[126] He was intubated on arrival at the emergency department; after transfer to the intensive care unit, he was rapidly extubated. The GHB concentrations in his urine sample from admission and 6-hours postadmission were 3,020 mg/L and 2,324 mg/L, respectively, as measured by gas chromatography/mass spectrometry in selective ion monitoring.

Abnormalities

Evaluation of serum chemistry during GHB intoxication is usually normal including normal serum potassium concentrations. Case reports associate electrocardiographic abnormalities with GHB intoxication including U waves, right bundle branch block, 1st degree A-V block, and ventricular premature beats;[127] however, other ingested drugs may contribute to the appearance of these abnormalities.

Driving

Alteration of consciousness and diminution of motor skills (e.g., depressed consciousness, impaired balance, nystagmus) seriously impairs driving skills of individuals under the influence of GHB.[128,129] Typical behaviors of GHB-intoxicated drivers include erratic driving (weaving, swerving, ignoring road signs), abnormal behavior (falling asleep at a red light, stopping in the middle of the road), ataxia, confusion, disorientation, agitation, slurred speech, and jerking movements along with poor performance on field sobriety tests.[130,131] The rapid elimination of GHB complicates the correlation of GHB blood concentrations to driving impairment. Additionally, individual responses to specific blood GHB concentrations are highly variable. In a series of 6 individuals with similar impairment after arrest for driving under the influence (DUI), the whole blood GHB concentrations ranged from 16–350 mg/L.[132] The latter value was substantially higher than the postmortem blood GHB concentration (174 mg/L, with 20 mg ethanol/dL) from a 36-year-old man found unconscious and failing to respond to cardiopulmonary resuscitation in the emergency department. In these samples, the presence of substantially higher (i.e., 10-fold) GHB concentrations in urine samples suggests an exogenous GHB source. In a case series of 185 individuals arrested for suspected driving under the influence of drugs or ethanol and testing positive only for GHB, the mean GHB concentration in venous blood samples was 92 mg/L (median, 86 mg/L) with an upper limit of 270 mg/L as measured by gas chromatography/flame ionization detection (LLOQ, 8 mg/L).[133] The typical range of time between arrest and blood sampling was

approximately 30–90 minutes (i.e., ~1–3 plasma elimination half-lives). Abnormalities reported by police officers included sedation, unsteady gait, slurred speech, irrational behavior, agitation, jerking, mydriasis, and spitting.[134] The reported blood GHB concentrations (i.e., GHB plus GBL) were similar to a smaller cases series of 8 individuals with only GHB in their toxicology screens after arrest for suspected DUI.[135] Venous blood samples were drawn up to 2 hours after arrest; the blood GHB concentration ranged from 26–155 mg/L, as measured by gas chromatography/mass spectrometry.

TREATMENT

Stabilization

The management of GHB intoxication typically involves stabilization of the airway, support of respirations, and prevention of aspiration. Because of the alteration of consciousness typically associated with GHB intoxication, these patients require monitoring of oxygenation with pulse oximetry and if needed, arterial blood gases. Supportive care includes supplemental oxygenation, suctioning and positioning for spontaneous vomiting, cardiac monitoring (primarily for bradycardia) and IV access. The mouth should be inspected for the presence of mucosal ulcerations suggestive of alkali burns from the sodium hydroxide used to synthesize GHB.[136] If present, the patient should be evaluated for the possibility of serious pulmonary complications from aspiration of alkali material. Intubation is usually unnecessary unless hypoventilation, hypoxemia, or the absence of a gag reflex occurs. When indicated, rapid-sequence intubation usually requires only paralysis with succinylcholine because of the sedation and amnesia associated with GHB intoxication.[137] The hypothermia associated with GHB use is typically mild and responds well to passive warming techniques.

Gut Decontamination

Measures for gastrointestinal (GI) decontamination (e.g., lavage, activated charcoal) are not routinely recommended for GHB intoxication alone because of the rapid absorption of GHB and potential for aspiration secondary to altered consciousness. The decision to use GI decontamination depends on the clinical severity of other ingested substances.

Elimination Enhancement

Measures to enhance the elimination of GHB are usually unnecessary because of the rapid endogenous elimination of GHB.

Antidotes

There are no specific antidotes for GHB intoxication, and naloxone should be administered only for the concomitant presence of opiate intoxication.[138] Experimental studies and case reports indicate that flumazenil does not reverse the clinical effects of GHB.[139] Although case reports and observational studies suggest that low doses of physostigmine may attenuate the coma associated with severe GHB intoxication,[140,141] there is insufficient scientific evidence to support the use of physostigmine in the treatment of γ-hydroxybutyrate toxicity.[142] Consequently, the routine use of physostigmine is not recommended, particularly in the setting of the frequent presence of other drugs of abuse.[143]

Supplemental Care

The recovery from GHB intoxication is rapid as a result of the short plasma elimination half-life of GHB. Typically, patients can be discharged within 6 hours after ingestion following an uneventful recovery. The persistence of symptoms over 6 hours after ingestion suggests the presence of other drugs or complications (trauma, hypoxia, drug overdose). The treatment of withdrawal symptoms typically involves the use of benzodiazepines (starting dose, 10–20 mg diazepam or 2–4 mg lorazepam), frequently in high doses (i.e., daily starting dose, 80–150 mg diazepam) tapered over 7 days.[40] Baclofen is a $GABA_B$ agonist that is a potentially useful for the treatment of benzodiazepine-resistant GHB withdrawal; however, there are inadequate clinical data to determine the efficacy of this drug.

The Clinical Institute Withdrawal Assessment of Alcohol Scale may aid in the management of withdrawal reactions; however, there is a lack of validation of this scale in the treatment of GHB withdrawal. Antipsychotic medications are not usually administered for the treatment of GHB withdrawal, in part because of concern about lowering the seizure threshold. Quetiapine is a potential alternative; however, there are few clinical data to assess the clinical efficacy of this antipsychotic for severe GHB withdrawal. For patients with delirium secondary to GHB withdrawal that is unresponsive to high diazepam doses (i.e., 200 mg/day), the administration of pentobarbital (or chloral hydrate) in the intensive care setting is a therapeutic option.[73] Therapy with pentobarbital begins with small doses (1–2 mg/kg IV) every 30–60 minutes titrated to response. Typically, sensorium and tachycardia of diazepam-resistant patients with severe GHB withdrawal respond to pentobarbital within 2–6 hours.[144] Case reports suggest that patients with severe withdrawal symptoms should be monitored for hyperthermia and rhabdomyolysis.[145]

GHB ANALOGUES

HISTORY

GHB analogues include 1,4-butanediol (1,4-BD), γ-butyrolactone (GBL), and γ-valerolactone (GVL). Restrictions of the sale and use of GHB during the 1990s resulted in the use of GHB analogues (e.g., 1,4-BD, GBL) as precursors and surrogates of GHB.[146] GBL was sold over-the-counter and through the Internet either alone or with instructions and components for the home synthesis of GHB. After a FDA warning regarding the health risks of γ-butyrolactone, γ-butyrolactone supplements were voluntarily recalled in 1999.[147] Subsequently, 1,4-BD products were marketed as replacements for γ-butyrolactone.

IDENTIFYING CHARACTERISTICS

There are several popular GHB analogues as displayed in Figure 6.3. These analogues are distinct chemicals with differing toxicity and pharmacokinetic profiles. GBL (CAS RN: 96-48-0, $C_4H_6O_2$) has a molecular weight of 86.10 g/mol and density of 1.13 g/mL, whereas 1,4-BD (CAS RN: 110-63-4, $C_4H_{10}O_2$) has a molecular weight of 90.10 g/mol and density of 1.02 g/mL. The latter is a lactone-ring analogue of GHB; 1,4-BD is an aliphatic alcohol. γ-Hydroxyvalerate (GHV) is the 5-carbon analogue of GHB. Both GBL and 1,4-BD are water- and alcohol-soluble, colorless compounds. Common names for GBL include Blue Nitro, Blue Nitro Vitality, Fire Water, Gamma-G, GH Revitalize, Remforce, Renewtrient, Revivarant, and Revivarant-G; whereas street synonyms for 1,4-BD include Blueraine, Dream On, Fubar, Pine Needle Oil, Rejuv@Nite, Somatopro, and Thunder.[146]

EXPOSURE

GBL is a list I chemical under the Chemical Diversion and Trafficking Act of 1988 that requires specific record keeping and reporting to the Drug Enforcement Administration for the import, manufacture, distribution, and export of this chemical; 1,4-BD is not included in this list of controlled chemicals. In contrast to the lack of industrial uses for GHB, GBL and 1,4-BD are common chemicals for a variety of industrial or research purposes including the synthesis of polybutylene terephthalate resins, polyurethanes, and tetrahydrofuran. GBL is a constituent of some superglue removers, paint strippers, nonacetone nail-polish remover pads, stain removers, and solvents; unadulterated wine may contain small amounts of GBL. 1,4-BD is a precursor for the industrial synthesis of GBL. The industrial grades of GHB analogues may contain impurities. GBL is frequently substituted for GHB. In a study of 158 emergency department presentations in London during 2006, self-reported use of GHB and GBL was about 95% and 5%, respectively.[148] However, analysis of 225 liquid samples seized from club attendees in the same catchment area during this period demonstrated that only 85 (37.8%) contained GHB, whereas 140 (62.2%) contained GBL. None of the samples contained detectable quantities of 1,4-butanediol.

1,4-butanediol is a natural trace constituent of the human body similar to GHB, whereas GBL is not a normal constituent of the body. The synthesis of GHB by saponification of the lactone in GBL via hydroxide anions (sodium hydroxide) is a relatively simple process; whereas the complexity of the conversion of 1,4-BD to GHB limits the use of this method of producing GHB. Processes for the synthesis of GBL include the hydrogenation of maleic anhydride and the dehydrogenation of 1,4-butanediol with acetylene and formaldehyde (classical Reppe process).
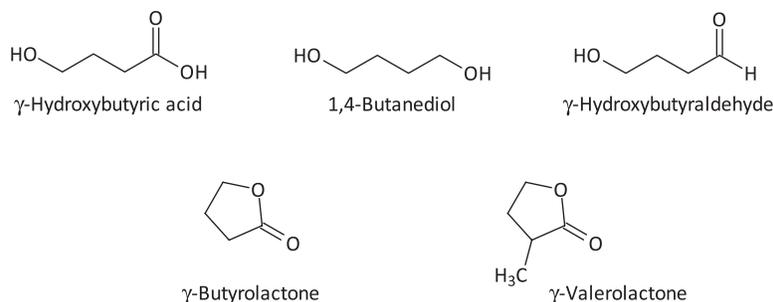
FIGURE 6.3. Chemical structures of γ-hydroxybutyric acid and various analogues.

DOSE EFFECT

GBL and 1,4-BD are more potent in animal studies than GHB based on the D_{50} (dose at which the response decreases by 50%). In a study of rats, the D_{50} for GBL, 1,4-BD, and GHB were 116 mg/kg, 151 mg/kg, and 297 mg/kg, respectively.[149] A case series of adverse events from 1,4-BD use associated the ingestion of approximately 1 g 1,4-butanediol with diaphoresis, confusion, agitation, ataxia, and myoclonus without any clinically significant alteration in vital signs, whereas the ingestion of about 6–8 g 1,4-BD was associated with loss of consciousness, urinary incontinence, vomiting, and mild bradycardia.[150] In this report, the estimated ingestions of 1,4-BD by a man and woman found dead in 2 separate incidents were 5.4 g and 20 g based on history from companions.

TOXICOKINETICS

Kinetics

Animal studies indicate that GBL is a prodrug that is rapidly and almost completely absorbed from the GI tract following the oral administration of low therapeutic doses.[151] Higher doses produce relatively lower bioavailability because of capacity-limited transport. Percutaneous absorption of GBL is limited (<10%) based on rodent studies.[152] Animal studies indicate that the conversion of GBL and 1,4-BD to GHB occurs *in vivo*.[153] Limited human data confirms the rapid formation of GHB following the administration of GBL or 1,4-BD. In a doubled-blind, placebo-controlled, crossover study of 8 healthy volunteers receiving 25 mg 1,4-BD/kg, the mean time to maximum plasma concentration (T_{max}) of 1,4-BD was 24 ± 12 min compared with the mean T_{max} for GHB of 39.4 ± 11.2 min after 1,4-BD ingestion.[154] The latter T_{max} is similar to the T_{max} (30–40 min) following the administration of GHB. The mean plasma elimination half-life of 1,4-BD was 39.3 ± 11.0 min.

Drug Interactions

Alcohol dehydrogenase catalyzes the formation of γ-hydroxybutyraldehyde and aldehyde dehydrogenase catalyzes the formation of γ-hydroxybutyrate from γ-hydroxybutyraldehyde. Animal studies indicate that the interaction of ethanol and 1,4-BD is complex, resulting from the inherent properties of this diol (pharmacodynamic interaction) and the ethanol-induced inhibition of the conversion of 1,4-BD to GHB (pharmacokinetic interaction).[155] 1,4-BD may potentiate some of the behavioral effects of ethanol as well as delay and attenuate the formation of GHB.[156]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Similar to GHB, GBL is a natural constituent of the brain that easily crosses the blood–brain barrier.[157] The relatively higher potencies of GBL and 1,4-BD probably do not result from enhanced penetration of the blood–brain barrier by these compounds. Experimental studies indicate that GBL is inactive following intracerebroventricular administration when compared with GHB; thus, the metabolic activation of GBL to GHB probably occurs in the periphery rather than in the brain. In animal studies, GBL has negligible affinity for the GHB receptor. Postmortem examination of fatalities associated with 1,4-BD are usually nonspecific (e.g., pulmonary edema).[150]

CLINICAL RESPONSE

The clinical features of GBL and 1,4-BD intoxication include CNS depression and fluctuating levels of consciousness that are indistinguishable from GHB intoxication.[158,159] Consequently, there are no pathognomonic signs or symptoms to separate poisoning by GHB analogues from GHB intoxication. Like GHB, the ingestion of GBL may cause coma and life-threatening respiratory depression.[160] Myoclonus occurs commonly during GHB and GHB analogue poisoning; the presence of myoclonic jerks may be confused with seizure activity. Although animal studies suggest that GBL and GHB produce electroencephalographic and behavioral changes resembling generalized absence seizures,[161] generalized seizures during intoxication with GBL or 1,4-BD more likely results from hypoxia, hypoglycemia, or the concomitant ingestion of other drugs of abuse (cocaine, methylenedioxymethamphetamine). The common effects associated with GHB analogue induced intoxication include nausea, vomiting, diaphoresis, bradycardia, incontinence, and short duration (i.e., 4–6 h) of coma along with variable pupillary responses.[162] GHB and GHB analogues are not strong cardiac depressants, and hypotension is not usually present even in comatose patients unless accompanied by bradycardia. Rarely, case reports associate fatalities with the ingestion of only 1,4-BD.[163] Potentially, caustic burns of the upper GI tract may occur if the sodium hydroxide used to convert GHL to GHB was incompletely neutralized. Limited animal data suggest that GBL is not carcinogenic or teratogenic.[164]

The clinical features of withdrawal from the chronic use of GHB analogues and GHB are similar including tremors, insomnia, confusion, anxiety, agitation, delirium, hallucinations, and autonomic instability (hypertension, tachycardia, diaphoresis).[165] Case reports

indicate that the abuse of GHB analogues continue 2 months to 4 years prior to the development of withdrawal.[166] Limited human data suggest that abstinence symptoms begin within a few hours of cessation of 1,4-BD use and up to 72 hours after chronic GBL use.[167] These symptoms (e.g., insomnia, anxiety, depression) persist for 3–15 days and in some patients up to weeks after withdrawal.[168] Case reports of seizures during GHB withdrawal are rare and may represent other etiologies.[165]

DIAGNOSTIC TESTING

Analytic Methods

Detection of the use of GHB analogues typically involves the detection of GHB because of the *in vivo* conversion of GBL and 1,4 BD to GHB. Most urine immunoassays for drugs of abuse do not detect GHB or GHB analogues. Chromatographic methods are necessary for the detection and quantitation of GHB and GHB analogues (See section on Analytic Methods for GHB above). Methods to separate GHB and GHB analogues include high performance liquid chromatography high performance liquid chromatography with photodiode array or ultraviolet (254 nm) detection and gas chromatography/mass spectrometry.[169,170]

Biomarkers

Following the oral administration of 25 mg 1,4-BD/kg to 8 healthy volunteers, the mean maximum plasma GHB concentration was 45.6 ± 19.7 mg/L with substantial interindividual variation in the rate of metabolism, particularly in Asian participants.[154]

In postmortem blood (source not reported) from 2 adults found dead after the ingestion of 1,4-BD and ethanol, the 1,4-BD concentrations were 845 mg/L and 1,756 mg/L compared with GHB concentrations of 432 mg/L and 837 mg/L, respectively.[150] A 25-year-old man was found in bed in cardiac arrest (asystole) with a history of ingesting GBL the previous evening.[171] He was pronounced dead in emergency department after unsuccessful cardiopulmonary resuscitation. The postmortem examination demonstrated pulmonary aspiration of gastric contents and pulmonary edema. The total GBL concentration (GBL plus GHB) in postmortem femoral blood was 282 mg/L. There are few data on the postmortem distribution of GHB analogues. In a case report of a 21-year-old man found dead after the ingestion of 1,4-BD, the 1,4-BD concentration in heart and peripheral postmortem blood was similar (78 mg/L and 70 mg/dL, respectively).[169]

Case reports associate nonspecific electrocardiographic changes with GHB and GHB analogue intoxication including the presence of U waves, junctional bradycardia, widened QRS, and right bundle branch block.[159,172]

TREATMENT

The management of intoxication by GHB analogues is supportive, similar to the management of GHB intoxication. The main life-threatening features of intoxication are respiratory depression and aspiration. In rare cases, intubation and mechanical ventilation may be necessary, but most cases of poisoning with clinically significant altered consciousness require only continuous monitoring of blood pressure, cardiac rhythm, and pulse oximetry along with IV access and rapid evaluation of blood glucose. Similar to GHB intoxication, these patients may respond violently to intubation; therefore, rapid sequence intubation with paralytic agents is usually necessary to protect the airway and ventilate the select group of patients with severe respiratory compromise. Gastrointestinal decontamination measures are unnecessary in patients with intoxication by GHB analogues only. There are no effective antidotes to GHB analogue intoxication. The administration of 4-methylpyrazole (fomepizole) potentially blocks the conversion of 1,4-BD to GHB; however, there are inadequate clinical data to determine if the use of this antidote improves clinical outcome. The persistence of coma 4–6 hours after presentation indicates the presence of other causes of coma beside GHB or GHB analogues. GHB abuse frequently occurs with the abuse of other drugs (ethanol, cocaine, methamphetamine, methylenedioxymethamphetamine). The treatment of the abstinence syndrome associated with chronic 1,4-BD or GBL abuse is similar to GHB withdrawal with initial treatment with benzodiazepines and pentobarbital in refractory cases.[173]

References

1. Saytzeff A. Naturally occurring metabolite of GABA thought to function as a neurotransmitter or neuromodulator. The highest concentration in humans is found in fetal cerebellum and adult hypothalamus. Justus Liebigs Annalen der Chemie 1874;171:258. [German]

2. Laborit H. Sodium 4-hydroxybutyrate. Int J Neuropharmacol 1964;3:433–451.

3. Roth RH, Levy R, Giarman NJ. Dependence of rat serum lactonase upon calcium. Biochem Pharmacol 1967;16:596–598.

4. Blumenfeld M, Suntay RG, Harmel MH. Sodium gamma-hydroxybutyric acid: a new anaesthetic adjuvant. Anesth Analg 1962;41:721–726.

5. Appleton PJ, Burn JM. A neuroinhibitory substance: gamma hydroxybutyric acid preliminary report of first clinical trial in Britain. Anesth Analg 1968;47:164–170.

6. Helrich M, Mcaslan TC, Skolnik S, Bessman SP. Correlation of blood levels of 4-hydroxybutyrate with state of consciousness. Anesthesiology 1964;25:771–775.

7. Dyer JE, Kreutzer R, Quattrone A, Kizer KW, Geller RJ, Smith JD, et al. Multistate outbreak of poisonings associated with illicit use of gamma hydroxybutyrate. MMWR Morb Mortal Wkly Rep 1990;39:861–863.

8. Addolorato G, Ca[risto E, Gessa GL, Caputo F, Stefanini GF, Gasbarrini G. Long-term administration of GHB does not affect muscular mass in alcoholics. Life Sci 1999;65:PL191–PL196.

9. Chin M-Y, Kreutzer RA, Dyer JE. Acute poisoning from γ-hydroxybutyrate in California. West J Med 1992;156: 380–384.

10. Centers for Disease Control and Prevention (CDC). Gamma hydroxy butyrate use—New York and Texas, 1995–1996. MMWR Morb Mortal Wkly Rep 1997;46: 281–283.

11. Dyer JE. Gamma-hydroxybutyrate: a health-food product producing coma and seizurelike activity. Am J Emerg Med 1991;9:321–324.

12. Drug Enforcement Agency, Department of Justice. Schedules of controlled substances: addition of gamma hydroxybutyric acid to schedule I. Fed Regist 2000;65: 13235–13238.

13. Fuller DE, Hornfeldt CS, Kelloway JS, Stahl PJ, Anderson TF. The Xyrem risk management program. Drug Saf 2004;27:293–306.

14. Bessman SP, Fishbein WN. Gamma-hydroxybutyrate, a normal brain metabolite. Nature 1963;200:1207–1208.

15. Anderson IB, Kim SY, Dyer JE, Burkhardt CB, Iknoian JC, Walsh MJ, Blanc PD. Trends in gamma-hydroxybutyrate (GHB) and related drug intoxication: 1999 to 2003. Ann Emerg Med 2006;47:177–183.

16. Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Drug Abuse. Warning Network, 2005: National Estimates of Drug-Related Emergency Department Visits. DAWN Series D-29, DHHS Publication No. (SMA) 07-4256, Rockville, MD, 2007.

17. Degenhardt L, Dunn M. The epidemiology of GHB and ketamine use in an Australian household survey. Int J Drug Policy 2008;19:311–316.

18. Németh Z, Kun B, Demetrovics Z. The involvement of gamma-hydroxybutyrate in reported sexual assaults: a systematic review. J Psychopharmacol 2010;24:1281–1287.

19. Djezzar S, Questel F, Burin E, Dally S, the French Network of Centers for Evaluation and Information on Pharmacodependence. Chemical submission: results of 4-year French inquiry. Int J Legal Med 2009;123:213–219.

20. Slaughter L. Involvement of drugs in sexual assault. J Reprod Med 2000;45:425–430.

21. Wood DM, Warren-Gash C, Ashraf T, Greene SL, Shather Z, Trivedy C, et al. Medical and legal confusion surrounding gamma-hydroxybutyrate (GHB) and its precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4 BD). Q J Med 2008;101:23–29.

22. Gallimberti L, Schifano F, Forza G, Miconi L, Ferrara SD. Clinical efficacy of gamma-hydroxybutyric acid in treatment of opiate withdrawal. Eur Arch Psychiatry Clin Neurosci 1994;244:113–114.

23. Leone MA, Vigna-Taglianti F, Avanzi G, Brambilla R, Faggiano F. Gamma-hydroxybutyrate (GHB) for treatment of alcohol withdrawal and prevention of relapses. Cochrane Database Syst Rev 2010;2:CD006266.

24. Varela M, Nogué S, Orós M, Miró O. Gamma hydroxy-butyrate use for sexual assault. Emerg Med J 2004;21: 255–256.

25. Miró O, Nogué S, Espinosa G, To-Figueras J, Sánchez M. Trends in illicit drug emergencies: the emerging role of gamma-hydroxybutyrate. J Toxicol Clin Toxicol 2002; 40:129–135.

26. Van Sassenbroeck DK, Calle PA, Rousseau FM, Verstraete AG, Belpaire FM, Monsieurs KG, et al. Medical problems related to recreational drug use at nocturnal dance parties. Eur J Emerg Med 2003;10:302–308.

27. Sumnall HR, Woolfall K, Edwards S, Cole JC, Beynon CM. Use, function, and subjective experiences of gamma-hydroxybutyrate (GHB). Drug Alcohol Depend 2008;92: 286–290.

28. Miotto K, Darakjian J, Basch J, Murray S, Zogg J, Rawson R. Gamma-hydroxybutyric acid: patterns of use, effects and withdrawal. Am J Addict 2001;10:232–241.

29. Barker JC, Harris SL, Dyer JE. Experiences of gamma hydroxybutyrate (GHB) ingestion: a focus group study. J Psychoactive Drugs 2007;39:115–129.

30. Addolorato G, Caputo F, Capristo E, Stefanini GF, Gasbarrini G. Gamma-hydroxybutyric acid efficacy, potential abuse, and dependence in the treatment of alcohol addiction. Alcohol 2000;20:217–222.

31. Carter LP, Richards BD, Mintzer MZ, Griffiths RR. Relative abuse liability of GHB in humans: a comparison of psychomotor, subjective, and cognitive effects of supratherapeutic doses of triazolam, pentobarbital, and GHB. Neuropsychopharmacology 2006;31:2537–2551.

32. Abanades S, Farre M, Barral D, Torrens M, Closas N, Langohr K, et al. Relative abuse liability of γ-hydroxybutyric acid, flunitrazepam, and ethanol in club drug users. J Clin Psychopharmacol 2007;27:625–638.

33. Nimmerrichter AA, Walter H, Gutierrez-Lobos KE, Lesch OM. Double-blind controlled trial of gamma-hydroxybutyrate and clomethiazole in the treatment of alcohol withdrawal. Alcohol Alcohol 2002;37:67–73.

34. Moncini M, Masini E, Gambassi F, Mannaioni PF. Gamma-hydroxybutyric acid and alcohol-related syndromes. Alcohol 2000;20:285–291.

35. Mamelak M, Escriu JM, Stokan O. The effects of gamma-hydroxybutyrate on sleep. Biol Psychiatry 1977;12: 273–288.

36. Vickers MD. Gamma hydroxybutyric acid. Clinical pharmacology and current status. Proc R Soc Med 1968;61: 821–824.

37. Appleton PJ, Burn JM. A neuroinhibitory substance: gamma hydroxybutyric acid. Preliminary report of first clinical trial in Britain. Anesth Analg 1968;47:164–170.

38. Addolorato G, Castelli E, Stefanini GF, Casella G, Caputo F, Marsigli L, et al. An open multicentric study evaluating 4-hydroxybutyric acid sodium salt in the medium-term treatment of 179 alcohol dependent subjects. GHB Study Group. Alcohol Alcohol 1996;31:341–345.

39. Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with gamma-hydroxybutyrate. A review of clinical and sleep laboratory findings. Sleep 1986;9:285–289.

40. Craig K, Gomez HF, McManus JL, Bania TC. Severe gamma-hydroxybutyrate withdrawal: a case report and literature review. J Emerg Med 2000;18:65–70.

41. Palatini P, Tedeschi L, Frison G, Padrini R, Zordan R, Orlando R, et al. Dose-dependent absorption and elimination of gamma-hydroxybutyric acid in healthy volunteers. Eur J Clin Pharmacol 1993;45:353–356.

42. Brenneisen R, Elsohly MA, Murphy TP, Passarelli J, Russmann S, Salamone SJ, Watson DE. Pharmacokinetics and excretion of gamma-hydroxybutyrate (GHB) in healthy subjects. J Anal Toxicol 2004;28:625–630.

43. Ferrara SD, Zotti S, Tedeschi L, Frison G, Castagna F, Gallimberti L, et al. Pharmacokinetics of gamma-hydroxybutyric acid in alcohol dependent patients after single and repeated oral doses. Br J Clin Pharmacol 1992; 34:231–235.

44. Abanades S, Farre M, Segura M, Pichini S, Barral D, Pacifici R, et al. γ-Hydroxybutyrate (GHB) in humans pharmacodynamics and pharmacokinetics. Ann NY Acad Sci 2006;1074:559–576.

45. Borgen LA, Okerholm RA, Lai A, Scharf MB. The pharmacokinetics of sodium oxybate oral solution following acute and chronic administration to narcoleptic patients. J Clin Pharmacol 2004;44:253–257.

46. Scharf MB, Lai AA, Branigan B, Stover R, Berkowitz DB. Pharmacokinetics of gamma-hydroxybutyrate (GHB) in narcoleptic patients. Sleep 1998;21:507–514.

47. Jones AW, Eklund A, Kronstrand R. Concentration-time profiles of gamma-hydroxybutyrate in blood after recreational doses are best described by zero-order rather than first-order kinetics. J Anal Toxicol 2009;33:332–335.

48. Thai D, Dyer JE, Benowitz NL, Haller CA. Gamma-hydroxybutyrate and ethanol effects and interactions in humans. J Clin Psychopharmacol 2006;26:524–529.

49. Harrington RD, Woodward JA, Hooton TM, Horn JR. Life-threatening interactions between HIV-1 protease inhibitors and the illicit drugs MDMA and gamma-hydroxybutyrate. Arch Intern Med 1999;159:2221–2224.

50. Cash CD, Maitre M, Mandel P. Purification from human brain and some properties of two NADPH-linked aldehyde reductases which reduce succinic semialdehyde to 4-hydroxybutyrate. J Neurochem 1979;33:1169–1175.

51. Doherty JD, Hattox SE, Snead OC, Roth RH. Identification of endogenous gamma-hydroxybutyrate in human and bovine brain and its regional distribution in human, guinea pig and rhesus monkey brain. J Pharmacol Exp Ther 1978;207:130–139.

52. Maitre M. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. Prog Neurobiol 1997;51:337–361.

53. Snead OC III, Gibson KM. γ-Hydroxybutyric acid. N Engl J Med 2005;352:2721–2732.

54. Carter LP, Koek W, France CP. Behavioral analyses of GHB: receptor mechanisms. Pharmacol Ther 2009; 121:100–114.

55. Pardi D, Black J. γ-Hydroxybutyrate/sodium oxybate. CNS Drugs 2006;20:993–1018.

56. Van Cauter E, Plat L, Scharf MB, Leproult R, Cespedes S, L'Hermite-Balériaux M, Copinschi G. Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young men. J Clin Invest 1997;100:745–753.

57. Tunnicliff G. Sites of action of gamma-hydroxybutyrate (GHB)—a neuroactive drug with abuse potential. Clin Toxicol 1997;35:581–590.

58. Mathivet P, Bernasconi R, De Barry J, Marescaux C, Bittiger H. Binding characteristics of gamma-hydroxybutyric acid as a weak but selective GABA$_B$ receptor agonist. Eur J Pharmacol 1997;321:67–75.

59. Vayer P, Mandel P, Maitre M. Gamma-hydroxybutyrate, a possible neurotransmitter. Life Sci 1987;41:1547–1557.

60. Jones C. Suspicious death related to gamma-hydroxybutyrate (GHB) toxicity. J Clin Forensic Med 2001;8:74–76.

61. Entholzner E, Mielke L, Pichlmeier R. EEG changes during sedation with gamma-hydroxybutyric. Anesthetist 1995;44:345–350.

62. Kim SY, Barker JC, Anderson IB, Dyer JE, Earnest G, Blanc PD. Systematic assessment of gamma hydroxybutyrate (GHB) effects during and after acute intoxication. Am J Addict 2008;17:312–318.

63. Degenhardt L, Darke S, Dillon P. The prevalence and correlates of gamma-hydroxybutyrate (GHB) overdose among Australian users. Addiction 2003;98:199–204.

64. Miro O, Nogue S, Espinosa G, To-Figueras J, Sanchez M. Trends in illicit drug emergencies: the emerging role of gamma-hydroxybutyrate. Clin Toxicol 2002;40:129–135.

65. Munir VL, Hutton JE, Harney JP, Buykx P, Weiland TJ, Dent AW. Gamma-hydroxybutyrate: a 30 month emergency department review. Emerg Med Australasia 2008; 20:521–530.

66. Viera AJ, Yates SW. Toxic ingestion of gamma-hydroxybutyric acid. South Med J 1999;92:404–405.

67. Chin RL, Sporer KA, Culison B, Dyer JE, Wu TD. Clinical course of γ-hydroxybutyrate overdose. Ann Emerg Med 1998;31:716–722.

68. Van Sassenbroeck DK, De Neve N, De Paepe P, Belpaire RM, Verstraete AG, Calle PA, Buylaert WA. Abrupt awakening phenomenon associated with gamma-hydroxybutyrate use: a case series. Clin Toxicol 2007;45:533–538.

69. Zovsec DL, Smith SW, Hall BF. Three deaths associated with use of Xyrem®. Sleep Med 2009;10:490–493.

70. Caldicott DG, Chow FY, Burns BJ, Felgate PD, Byard RW. Fatalities associated with the use of γ-hydroxybutyrate and its analogues in Australasia. Med J Aust 2004;181:310–313.

71. Knudsen K, Greter J, Verdicchio M. High mortality rates among GHB abusers in Western Sweden. Clin Toxicol 2008;46:187–192.

72. Dyer JE, Roth B, Hyma BA. Gamma-hydroxybutyrate withdrawal syndrome. Ann Emerg Med 2001;37:147–153.

73. McDonough M, Kennedy N, Glasper A, Bearn J. Clinical features and management of gamma-hydroxybutyrate (GHB) withdrawal: a review. Drug Alcohol Depend 2004;75:3–9.

74. Glasper A, McDonough M, Bearn J. Within-patient variability in clinical presentation of gamma-hydroxybutyrate withdrawal: a case report. Eur Addict Res 2005;11:152–154.

75. Galloway GP, Frederick SL, Staggers FE Jr, Gonzales M, Stalcup SA, Smith DE. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction 1997;92:89–96.

76. McDaniel CH, Miotto KA. Gamma hydroxybutyrate (GHB) and gamma butyrolactone (GBL) withdrawal: five case studies. J Psychoactive Drugs 2001;33:143–149.

77. De Paoli G, Bell S. A rapid GC-MS determination of gamma-hydroxybutyrate in saliva. J Anal Toxicol 2008;32:298–302.

78. Kintz P, Goulle J-P, Cirimele V, Ludes B. Window of detection of γ-hydroxybutyrate in blood and saliva. Clin Chem 2001;47:2033–2034.

79. Alston WC 2nd, Ng K. Rapid colorimetric screening test for gamma-hydroxybutyric acid (liquid X) in human urine. Forensic Sci Int 2002;126:114–117.

80. Morris-Kukoski CL. γ-Hydroxybutyrate bridging the clinical-analytical gap. Toxicol Rev 2004;23:33–43.

81. Mercer JW, Oldfield LS, Hoffman K, Shakleya DM, Bell SC. Comparative analysis of gamma-hydroxybutyrate and gamma-hydroxyvalerate using GC/MS and HPLC. J Forensic Sci 2007;52:383–388.

82. Dahlén J, Vriesman T. Simultaneous analysis of gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol by micellar electrokinetic chromatography. Forensic Sci Int 2002;125:113–119.

83. LeBeau MA, Montgomery MA, Miller ML, Burmeister SG. Analysis of biofluids for gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by headspace GC-FID and GC-MS. J Anal Toxicol 2000;24:421–428.

84. McCusker RR, Paget-Wilkes H, Chronister CW, Goldberger BA. Analysis of gamma-hydroxybutyrate (GHB) in urine by gas chromatography-mass spectrometry. J Anal Toxicol 1999;23:301–305.

85. Couper FJ, Logan BK. Determination of gamma-hydroxybutyrate (GHB) in biological specimens by gas chromatography-mass spectrometry. J Anal Toxicol 2000;24:1–7.

86. Ferrara SD, Tedeschi L, Frison G, Castagna F, Gallimberti L, Giorgetti R, et al. Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry. J Pharm Biomed Anal 1993;11:483–487.

87. Elian AA. GC-MS determination of gamma-hydroxybutyric acid (GHB) in blood. Forensic Sci Int 2001;122:43–47.

88. Chen M, Andrenyak DM, Moody DE, Foltz RL. Stability of plasma gamma-hydroxybutyrate determined by gas chromatography-positive ion chemical ionization-mass spectrometry. J Anal Toxicol 2003;27:445–448.

89. Paul R, Tsanaclis L, Kingston R, Berry A, Guwy A. GC-MS-MS determination of gamma-hydroxybutyrate in blood and urine. J Anal Toxicol 2006;30:375–379.

90. Wood M, Laloup M, Samyn N, Morris MR, de Bruijn EA, Maes RA, et al. Simultaneous analysis of gamma-hydroxybutyric acid and its precursors in urine using liquid chromatography-tandem mass spectrometry. J Chromatogr A 2004;1056:83–90.

91. Mesmer MZ, Satzger RD. Determination of gamma-hydroxybutyrate (GHB) and gamma-butyrolactone (GBL) by HPLC/UV-VIS spectrophotometry and HPLC/thermospray mass spectrometry. J Forensic Sci 1998;43:489–492.

92. Saudan C, Augsburger M, Kintz P, Saugy M, Mangin P. Detection of exogenous GHB in blood by gas chromatography-combustion-isotope ratio mass spectrometry: implications in postmortem toxicology. J Anal Toxicol 2005;29:777–781.

93. Stephens BG, Coleman DE, Baselt RC. In vitro stability of endogenous gamma-hydroxybutyrate in postmortem blood. J Forensic Sci 1999;44:231.

94. LeBeau MA, Montgomery MA, Jufer RA, Miller ML. Elevated GHB in citrate-buffered blood. J Anal Toxicol 2000;24:383–384.

95. Beránková K, Mutnanská K, Balíková M. Gamma-hydroxybutyric acid stability and formation in blood and urine. Forensic Sci Int 2006;161:158–162.

96. Kerrigan S. In vitro production of gamma-hydroxybutyrate in antemortem urine samples. J Anal Toxicol 2002;26:571–574.

97. LeBeau MA, Montgomery MA, Morris-Kukoski C, Schaff JE, Deakin A. Further evidence of in vitro production of gamma-hydroxybutyrate (GHB) in urine samples. Forensic Sci Int 2007 169:152–156.

98. LeBeau MA, Miller ML, Levine B. Effect of storage temperature on endogenous GHB levels in urine. Forensic Sci Int 2001;119:161–167.

99. Kugelberg FC, Holmgren A, Eklund A, Jones AW. Forensic toxicology findings in deaths involving gamma-hydroxybutyrate. Int J Legal Med 2010;124:1–6.

100. Elliott SP. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. Forensic Sci Int 2003;133:9–16.

101. Andresen H, Sprys N, Schmoldt A, Mueller A, Iwersen-Bergmann S. Gamma-hydroxybutyrate in urine and serum: additional data supporting current cut-off recommendations. Forensic Sci Int 2010;200:93–99.

102. Helrich M, McAslan TC, Skolnik S, Bessman SP. Correlation of blood levels of 4-hydroxybutyrate with state of consciousness. Anesthesiology 1964;25:771–775.

103. Divry P, Baltassat P, Rolland MO, Cotte J, Hermier M, Duran M, Wadman SK. A new patient with 4-hydroxybutyric aciduria, a possible defect of 4-aminobutyrate metabolism. Clin Chim Acta 1983;129:303–309.

104. Couper FJ, Thatcher JE, Logan BK. Suspected GHB overdoses in the emergency department. J Anal Toxicol 2004;28:481–484.

105. Sporer KA, Chin RL, Dyer JE, Lamb R. γ-Hydroxybutyrate serum levels and clinical syndrome after severe overdose. Ann Emerg Med 2003;42:3–8.

106. Elliott SP. Nonfatal instances of intoxication with γ-hydroxybutyrate in the United Kingdom. Ther Drug Monit 2004;26:432–440.

107. Louagie HK, Verstraete AG, De Soete CJ, Baetens DG, Calle PA. A sudden awakening from a near coma after combined intake of gamma-hydroxybutyric acid (GHB) and ethanol. Clin Toxicol 1997;35:591–594.

108. Moriya F, Hashimoto Y. Site-dependent production of γ-hydroxybutyric acid in the early postmortem period. Forensic Sci Int 2005;148:139–142.

109. Fieler EL, Coleman DE, Baselt RC. γ-Hydroxybutyrate concentrations in pre-and postmortem blood and urine. Clin Chem 1998;44:692.

110. Sakurada K, Kobayashi M, Iwase H, Yoshino M, Mukoyama H, Takatori T, Yoshida K-I. Production of γ-hydroxybutyric acid in postmortem liver increases with time after death. Toxicol Lett 2002;129:207–217.

111. Elliott S, Lowe P, Symonds A. The possible influence of micro-organism and putrefaction in the production of GHB in post-mortem biological fluid. Forensic Sci Int 2004;139:183–190.

112. Elliott SP. Further evidence for the presence of GHB in postmortem biological fluid: implications for the interpretation of findings. J Anal Toxicol 2004;28:20–26.

113. Kintz P, Villain M, Cirimele V, Ludes B. GHB in postmortem toxicology discrimination between endogenous production from exposure using multiple specimens. Forensic Sci Int 2004;143:177–181.

114. Mazarr-Proo S. Distribution of GHB in tissues and fluids following a fatal overdose. J Anal Toxicol 2005;29:398–400.

115. Kintz P, Villain M, Pélissier AL, Cirimele V, Leonetti G. Unusually high concentrations in a fatal GHB case. J Anal Toxicol 2005;29:582–585.

116. Kalasinsky KS, Dixon MM, Schmunk GA, Kish SJ. Blood, brain, and hair GHB concentrations following fatal ingestion. J Forensic Sci 2001;46:728–730.

117. Zvosec DL, Smith SW, Porrata T, Strobl AQ, Dyer JE. Case series of 226 γ-hydroxybutyrate-associated deaths: lethal toxicity and trauma. Am J Emerg Med 2011; 29:319–332.

118. Knudsen K, Jonsson U, Abrahamsson J. Twenty-three deaths with γ-hydroxybutyrate overdose in western Sweden between 2000 and 2007. Acta Anaesthesiol Scand 2010;54:987–992.

119. Lenz D, Rothschild MA, Kroner L. Intoxications due to ingestion of γ-butyrolactone: organ distribution γ-hydroxybutyric acid and γ-butyrolactone. Ther Drug Monit 2008;30:755–761.

120. Elian AA. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in antemortem urine and blood. Forensic Sci Int 2002;128:120–122.

121. Yeatman DT, Reid K. A study of urinary endogenous gamma-hydroxybutyrate (GHB) levels. J Anal Toxicol 2003;27:40–42.

122. Shima N, Miki A, Kamata T, Katagi M, Tsuchihashi H. Urinary endogenous concentrations of GHB and its isomers in healthy humans and diabetics. Forensic Sci Int 2005;149:171–179.

123. Crookes CE, Faulds MC, Forrest AR, Galloway JH. A reference range for endogenous gamma-hydroxybutyrate in urine by gas chromatography-mass spectrometry. J Anal Toxicol 2004;28:644–649.

124. Raknes G, Aronsen L, Fuskevåg OM. Urinary concentrations of gamma-hydroxybutyric Acid and related compounds in pregnancy. J Anal Toxicol 2010;34:394–399.

125. Haller C, Thai D, Jacob P III, Dyer JE. GHB urine concentrations after single-dose administration in humans. J Anal Toxicol 2006;30:360–364.

126. Bodson Q, Denooz R, Serpe P Charlier C. Gamma-hydroxybutyric acid (GHB measurement by GC-MS in blood, urine and gastric contents, following an acute intoxication in Belgium. Acta Clin Belg 2008;63:200–208.

127. Li J, Stokes SA, Woeckener A. A tale of novel intoxication: seven cases of γ-hydroxybutyric acid overdose. Ann Emerg Med 1998;313:723–728.

128. Al-Samarraie MS, Karinen R, Mørland J, Stokke Opdal M. Blood GHB concentrations and results of medical examinations in 25 car drivers in Norway. Eur J Clin Pharmacol 2010;66:987–998.

129. Barker JC, Karsoho H. Hazardous use of gamma hydroxybutyrate: driving under the influence. Subst Use Misuse 2008;43:1507–1520.

130. Bosman IJ, Lusthof KJ. Forensic cases involving the use of GHB in The Netherlands. Forensic Sci Int 2003;133: 17–21.

131. Stephens BG, Baselt RC. Driving under the influence of GHB? J Anal Toxicol 1994;18:357–358.

132. Pan YM, Gill GN, Tilson CS, Wall WH, McCurdy HH. Improved procedure for the analysis of

gamma-hydroxybutyrate and ethylene glycol in whole blood. J Anal Toxicol 2001;25:328–332.

133. Jones AW, Holmgren A, Kugelberg FC. Gamma-hydroxybutyrate concentrations in the blood of impaired drivers, users of illicit drugs, and medical examiner cases. J Anal Toxicol 2007;31:566–572.

134. Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of gamma-hydroxybutyrate (GHB). Forensic Sci Med Pathol 2008;4:205–211.

135. Couper FJ, Logan BK. GHB and driving impairment. J Forensic Sci 2001 46:919–923.

136. Dyer JE, Reed JH. Alkali burns from illicit manufacture of GHB. J Toxicol Clin Toxicol 1997;35:553. (abstr)

137. Li J, Stokes SA, Woeckener A. A tale of novel intoxication: a review of the effects of γ-hydroxybutyric acid with recommendations for management. Ann Emerg Med 1998;31:729–736.

138. Thomas G, Bonner S, Gascoigne A. Coma induced by abuse of gamma-hydroxybutyrate (GBH or liquid ecstasy): a case report. BMJ 1997;314(7073):35–36.

139. Gerra G, Caccavari R, Fontanesi B, Marcato A, Fertonani Affini G, Maestri D, et al. Flumazenil effects on growth hormone response to gamma-hydroxybutyric acid. Int Clin Psychopharmacol 1994;9:211–215.

140. Holmes CM, Henderson RS. The elimination of pollution by a non inhalational technique. Anaesth Intensive Care 1978;6:120–124.

141. Caldicott DG, Kuhn M. Gamma-hydroxybutyrate overdose and physostigmine: teaching new tricks to an old drug? Ann Emerg Med 2001;37:99–102.

142. Traub SJ, Nelson LS, Hoffman RS. Physostigmine as a treatment for gamma-hydroxybutyrate toxicity: a review. J Toxicol Clin Toxicol 2002;40:781–787.

143. Zvosec DL, Smith SW, Litonjua R, Westfal RE. Physostigmine for gamma-hydroxybutyrate coma: inefficacy, adverse events, and review. Clin Toxicol (Phila) 2007;45:261–265.

144. Sivilotti ML, Burns MJ, Aaron CK, Greenberg MJ. Pentobarbital for severe gamma-butyrolactone withdrawal. Ann Emerg Med 2001;38:660–665.

145. Bowles TM, Sommi RW, Amiri M. Successful management of prolonged γ-hydroxybutyrate and alcohol withdrawal. Pharmacotherapy 2001;21:254–257.

146. Palmer RB. γ-Butyrolactone and 1,4-butanediol abused analogues of γ-hydroxybutyrate. Toxicol Rev 2004; 23:21–31.

147. Centers for Disease Control and Prevention (CDC). Adverse events associated with ingestion of gamma-butyrolactone—Minnesota, New Mexico, and Texas, 1998–1999. MMWR Morb Mortal Wkly Rep 1999; 43:137–140.

148. Wood DM, Warren-Gash C, Ashraf T, Greene SL, Shather Z, Trivedy C, et al. Medical and legal confusion surrounding gamma-hydroxybutyrate (GHB) and its precursors gamma-butyrolactone (GBL) and 1,4-butanediol (1,4-BD). Q J Med 2008;101:23–29.

149. Carter LP, Koek W, France CP. Lack of effects of GHB precursors GBL and 1,4-BD following i.c.v. administration in rats. Eur J Neurosci 2006;24: 2595–2600.

150. Zvosec KL, Smith SW, McCutcheon JR, Spillane J, Hall BJ, Peacock EA. Adverse events, including death, associated with the use of 1,4-butanediol. N Engl J Med 2001; 34:87–94.

151. Arena C, Fung H-L. Absorption of sodium γ-hydroxybutyrate and its prodrug γ-butyrolactone: relationship between *in vitro* transport and *in vivo* absorption. J Pharm Sci 1980;69:356–358.

152. Fung H-L, Lettieri JT, Bochner R. Percutaneous butyrolactone absorption in rats. J Pharm Sci 1979;68: 1198–1200.

153. Lettieri J, Fung HL. Improved pharmacological activity via pro-drug modification: comparative pharmacokinetics of sodium gamma-hydroxybutyrate and gamma-butyrolactone. Res Commun Chem Pathol Pharmacol 1978;22:107–118.

154. Thai D, Dyer JE, Jacob P, Haller CA. Clinical pharmacology of 1,4-butanediol and gamma-hydroxybutyrate after oral 1,4-butanediol administration to healthy volunteers. Clin Pharmacol Ther 2007;31:178–184.

155. Poldrugo F, Snead OC 3rd. 1,4 Butanediol, gamma-hydroxybutyric acid and ethanol: relationships and interactions. Neuropharmacology 1984;23:109–113.

156. Poldrugo F, Barker S, Basa M, Mallardi F, Snead OC. Ethanol potentiates the toxic effects of 1,4-butanediol. Alcohol Clin Exp Res 1985;9:493–497.

157. Snead OC 3rd, Furner R, Liu CC. *In vivo* conversion of gamma-aminobutyric acid and 1,4-butanediol to gamma-hydroxybutyric acid in rat brain. Studies using stable isotopes. Biochem Pharmacol 1989;38:4375–4380.

158. Ingels M, Rangan C, Bellezzo J, Clark RF. Coma and respiratory depression following the ingestion of GHB and its precursors: three cases. J Emerg Med 2000;19: 47–50.

159. Rambourg-Schepens MO, Buffet M, Durak C, Mathieu-Nolf M. Gamma butyrolactone poisoning and its similarities to gamma hydroxybutyric acid: two case reports. Vet Hum Toxicol 1997;39:234–235.

160. Dupont P, Thorton J. Near-fatal gamma-butyrolactone intoxication—first report in the UK. Hum Exp Toxicol 2001;20:19–22.

161. Snead OC III. The γ-hydroxybutyrate model of absence seizures: correlation of regional brain levels of γ-hydroxybutyric acid and γ-butyrolactone with spike wave discharges. Neuropharmacology 1991;30:161–167.

162. Higgins TF Jr, Borron SW. Coma and respiratory arrest after exposure to butyrolactone. J Emerg Med 1996;14: 435–437.

163. Theron L, Jansen K, Skinner A. New Zealand's first fatality linked to use of 1,4-butanediol (1,4-B, Fantasy): no evidence of coingestion or comorbidity. N Z Med J 2003; 116(1184):U650.

164. Kronevi T, Holmberg B, Arvidsson S. Teratogenicity test of γ-butyrolactone in the Sprague-Dawley rat. Pharmacol Toxicol 1988;62:57–58.

165. Catalano MC, Glass JM, Catalano G, Burrows SL, Lynn WA, Weitzner BS. Gamma butyrolactone (GBL) withdrawal syndromes. Psychosomatics 2001;42:83–88.

166. Herold AH, Sneed KB. Treatment of a young adult taking gamma-butyrolactone (GBL) in a primary care clinic. J Am Board Fam Pract 2002;15:161–163.

167. Wojtowicz JM, Yarema MC, Wax PM. Withdrawal from gamma-hydroxybutyrate, 1,4-butanediol and gamma-butyrolactone: a case report and systematic review. CJEM 2008:10:69–74.

168. Bell J, Collins R. Gamma-butyrolactone (GBL) dependence and withdrawal. Addiction 2011; 106:442–447.

169. Duer WC, Byers KL, Martin JV. Application of a convenient extraction procedure to analyze gamma-hydroxybutyric acid in fatalities involving gamma-hydroxybutyric acid, gamma-butyrolactone, and 1,4-butanediol. J Anal Toxicol 2001;25:576–582.

170. Mercer JW, Oldfield LS, Hoffman KN, Shakleya DM, Bell SC. Comparative analysis of gamma-hydroxybutyrate and gamma-hydroxyvalerate using GC/MS and HPLC. J Forensic Sci 2007;52:383–388.

171. Dargan PL, Button J, Davies S, Ramsey J, George S, Holt DW, Wood DM. The first reported UK fatality related to gamma-butyrolactone (GBL) ingestion. J R Soc Med 2009;102:545–547.

172. Tancredi DN, Shannon MW. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 30-2003. A 21-year-old man with sudden alteration of mental status. N Engl J Med 2003; 349:1267–1275.

173. Schneir AB, Ly BT, Clark RF. A case of withdrawal from the GHB precursors gamma-butyrolactone and 1,4-butanediol. J Emerg Med 2001;21:31–33.

Chapter 7

KETAMINE

HISTORY

Calvin Stevens synthesized ketamine hydrochloride at the Park-Davis Laboratories in 1962. Early clinical studies suggested that ketamine was an effective anesthetic agent with less toxicity, little respiratory depression at anesthetic doses, and fewer emergence reactions than phencyclidine.[1] During the late 1960s, ketamine was marketed as the dissociative anesthetic, Ketalar® (JHP Pharmaceuticals, Parsippany, NJ); this compound was used to treat soldiers in the Vietnam War. The abuse potential of ketamine was recognized in the early 1970s,[2] but reports of ketamine abuse in human and veterinary medicine did not appear until the early 1980s in Australia and in the early 1990s in the United States.[3,4]

IDENTIFYING CHARACTERISTICS

Ketamine is the arylcycloalkylamine compound, 2(2-chlorophenyl)-2-(methylamino)-cyclohexanone, which is structurally similar to phencyclidine and cyclohexamine (N-ethyl-1-phenylcyclohexylamine). Figure 7.1 displays the chemical structure of ketamine (CAS RN: 6740-88-1, MW 237.75 g/mol). Ketamine is a chiral molecule that is available in commercial preparations as a racemic mixture of equal amounts of R(−)-ketamine and S(+) ketamine enantiomers. The latter enantiomer is about 2–4 times a more potent analgesic than R(−)-enantiomer, but the R(−)-enantiomer may contribute to the emergence reactions associated with ketamine anesthesia.[5] In mice, the hypnotic effect of the S-(+)-ketamine isomer is approximately 1.5 times greater than the R-(−)-ketamine isomer.[6] Ketamine is a highly lipid soluble, weakly basic amino compound that usually occurs in pharmaceutic preparations as the hydrochloride salt. Table 7.1 lists some physical properties of ketamine.

Trade names for ketamine include Ketalar®, Ketaject® (Phoenix Pharmaceutical, Burlingame, CA), Ketaset®, and Vetalar®. Synonyms for ketamine on the street include K, Kit-Kat, Kay, Jet, Vitamin K, Special K, Super Acid, Super K, Super C, Special LA Coke, and 1980 Acid. In the United States, ketamine is a Drug Enforcement Agency (DEA) schedule III substance (drugs with currently accepted medical indications and abuse potential for low to moderate physical dependence or high psychologic dependence). The usual form of ketamine is a pharmaceutic liquid, but evaporation of this liquid crystalizes ketamine. The crystal residue is ground into a powder, similar in appearance to cocaine or heroin. Like phencyclidine, ketamine is smoked as a cigarette after the addition of ketamine to tobacco or marijuana. Because of the difficulty manufacturing ketamine clandestinely, illicit ketamine is usually a pharmaceutic preparation diverted from legal stocks, particularly from veterinarian clinics. Although ketamine is occasionally an adulterant of heroin, illicit stocks of ketamine are usually relatively pure.

EXPOSURE

Epidemiology

The use of ketamine in the general population is relatively low with the highest prevalence of use in individuals (medical personnel, veterinarian professionals, club drug users) with direct access to this compound.[7,8] The
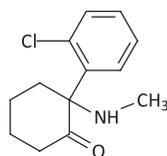
FIGURE 7.1. Chemical structure of ketamine.

TABLE 7.1. Some Physical Properties of Ketamine.

Physical Property	Value
Melting Point	92.5°C (198.5°F)
pKa	7.5
Molecular Weight	238 g/mol
log P (Octanol-Water)	3.120
Atmospheric OH Rate Constant	7.71E–11 cm^3/molecule-second (25°C/77°F)

abuse of ketamine usually occurs in the setting of poly-drug use. Although the typical pattern of ketamine use involves insufflation of ketamine as one of several club drugs, a subset of ketamine abusers use ketamine intravenously.[9] These high-risk populations include gay men, marginalized youth, and intravenous (IV) polydrug users.[10] Cross-sectional surveys of ketamine users suggest that the negative adverse effects (e.g., poor coordination, difficulty speaking, increased body temperature, blurred vision) associated with ketamine use reduce some of the desire to continue chronic use of ketamine.[11] Experimental medicinal uses of ketamine include the treatment of anorexia, chronic pain, and palliative care.[12]

Sources

The typical source of illicit ketamine is diversion of legal human or veterinarian supplies of ketamine and, less often, illegal importation. Although the structure of ketamine appears relatively simple, the synthesis of this compound requires several complex steps. This process involves the use of numerous solvents, reagents, and precursors (e.g., methylamine, *o*-chlorobenzonitrile, cyclopentyl bromide) as well as heating in a steel pressure vessel (bomb) for several days. Because of this complex synthetic process and the difficulty obtaining these precursors, most illicit ketamine is diverted from pharmaceutic stocks rather than synthesized in clandestine laboratories.

Methods of Abuse

The most common method of ketamine abuse is insufflation and, less commonly, IV administration by poly-drug users.[13] Use of ketamine typically occurs in a carefully preplanned setting that is comfortable, familiar, and secure. Frequently, this setting includes the use of other club drugs (e.g., ecstasy, methamphetamine) in a social setting (e.g., nightclubs, dance parties, rave parties).[14] The use of ketamine by novice users in public or unfamiliar settings is associated with an increased risk of behavioral complications; however, the effect of the environment diminishes as the dose of ketamine increases and psychedelic effects predominate over stimulant effects.[15] Ketamine abusers describe the effects of ketamine use as "falling into a K-hole" where physical immobilization and social detachment persists up to about 1 hour.[16] This experience typically involves spiritual journeys, disengagement with time and space, illusions, and fantasy interactions with famous or fictitious persons. Additionally, some ketamine abusers report near-death experiences manifest by altered perception of time and a strong sense of detaching from their physical body along with a sense of peace and/or joy.[17] Typically, these experiences occur during the first few uses of ketamine. Because of the short elimination half-life of ketamine, abusers usually administer sequential doses of this drug to maintain the psychedelic effects.[18] These ketamine abusers administer ketamine in cyclical binges similar to cocaine or methamphetamine abusers.[19]

DOSE EFFECT

Illicit Use

Recreational doses are highly variable (50–200 mg), in part, because of the rapid development of tolerance to ketamine.[20] Typical illicit doses of ketamine are 25–50 mg intramuscularly, 30–75 mg insufflation, and 75–300 mg orally. The intense, dissociative experience persists approximately 1 hour. Case reports document the daily use of up to 4–7 g ketamine.[21] Insufflating 30–50 mg ketamine (i.e., a small line) produces a dreamy state, whereas the intramuscular administration of 60–125 mg results in a more intense dissociative state ("K-hole").

Medical Use

Ketamine is a rapidly acting, relatively safe anesthetic agent that is a common agent for conscious sedation in children. The bronchodilating properties are useful for the sedation of asthmatic patients requiring intubation. The typical anesthetic dose of ketamine for conscious sedation is 1–2 mg/kg intravenously or 4–5 mg/kg intramuscularly. Experimental uses of ketamine include the use of low-dose ketamine for postoperative pain relief,

for regional or local anaesthesia, and for opioid-sparing effects.[22] The emergence reaction after ketamine administration is not dose-dependent.

Toxicity

In a case series of 9 children receiving intramuscular (IM) or IV ketamine doses up to 50 mg/kg, the primary effect was transient respiratory depression in 4 children and prolonged sedation up to 24 hours in all patients.[23] In another case report, the inadvertent IM administration of 10 times the normal ketamine dose (450 mg) to a 3-year-old child was associated with prolonged sedation and 4 episodes of desaturation relieved by supplemental oxygen and repositioning of the airway.[24] All children recovered without sequelae.

TOXICOKINETICS

Absorption

The bioavailability of oral ketamine is low. In a study of 3 volunteers receiving ketamine 50 mg orally, the average bioavailability was approximately 18% ± 5%, whereas the mean maximum ketamine concentration was about 42 ± 13 ng/mL at 33 ± 13 minutes after administration.[25] For the 25-mg nasal dose, the bioavailability of ketamine was approximately 44% ± 11%; the mean maximum ketamine concentration was about 29 ± 17 ng/mL at 23 ± 10 minutes after administration. In a study of 32 children undergoing halothane administration and receiving 9 mg ketamine/kg intranasally, the mean bioavailability of ketamine was approximately 50%.[26] The mean peak concentration of ketamine in plasma samples was 2,104 ± 965 ng/mL at a mean time of 42 ± 19 minutes after intranasal administration. The peak norketamine concentration occurred about 2 hours after the nasal administration of ketamine.

Distribution

In a study of 12 intensive care patients receiving ketamine, the mean volume of distribution was 16 ± 8.6 L/kg, based on analysis of arterial plasma samples following administration of analgesic doses (2 mg/kg) of IV ketamine.[27] Ketamine rapidly distributes into the brain and highly perfused tissues after IV administration with a distribution half-life of approximately 7–11 minutes. *In vitro* studies of human serum using ultrafiltration indicate that the protein binding of ketamine and norketamine at 30°C (86°F) were approximately 69% and 60%, respectively.[28]

Biotransformation

The major pathway of ketamine biotransformation involves *N*-demethylation to the active metabolite, norketamine, followed by hydroxylation to the much less-active metabolite, dehydronorketamine. Ring hydroxylation without prior *N*-demethylation is a minor metabolic pathway. The principal cytochrome P450 isoform responsible for the *N*-demethylation of ketamine to norketamine is CYP3A4 with relatively minor contributions from CYP2B6 and CYP2C9 isoforms.[29,30] There are few studies on the potency of the active metabolites of ketamine. Based on animal studies, the anesthetic potency of ketamine is about 3–5 times greater than norketamine.[31]

Elimination

The plasma half-life of ketamine is about 2–4 hours.[32] In a study of 31 patients receiving 2 mg ketamine/kg intravenously, the mean plasma elimination half-life was 79 ± 8 minutes,[33] whereas the mean elimination half-life of ketamine in 12 hemodynamically stable, intensive care patients was 4.9 ± 1.6 hours.[27] Although the pharmacokinetics of the *R*-(−)- and the *S*-(+)-enantiomers of ketamine are somewhat similar, the presence of the *R*-(−)-enantiomer inhibits the clearance of the *S*-(+)-enantiomer. In a study of 10 healthy young men receiving an infusion of ketamine, the mean clearance of the *S*-(+)-enantiomer of ketamine from arterial blood was 26.3 ± 3.5 mL/kg/min in the pure form compared with 18.5 ± 0.7 mL/kg/min in the racemic mixture containing 50% of each enantiomer.[34] Elimination of ketamine occurs primarily in the urine as a result of the renal excretion of the conjugated products of glucuronic acid and norketamine or hydroxylated metabolites (e.g., 6-hydroxy-norketamine) of norketamine. Renal excretion of unchanged ketamine and norketamine accounts for <4% of the absorbed dose of ketamine, whereas fecal excretion of ketamine accounts for <5% of the absorbed dose.[35]

Maternal and Fetal Kinetics

Ketamine easily crosses the placenta, and ketamine concentrations in the mother and fetus are similar.[36]

Tolerance

Case reports suggest that both acute and chronic tolerance develops rapidly after the use of ketamine.[21,37,38] Anecdotal reports suggest that chronic use dulls the psychedelic experience associated with ketamine use as

well as memories of ketamine effects.[19] Based on rodent studies, the chronic administration of ketamine induces hepatic cytochrome P450 isoenzymes.[39] Physical dependence does not usually develop following chronic ketamine abuse.[40] However, anecdotal reports suggest that nonspecific symptoms may develop with a few days after cessation of ketamine use including restless, irritability, hypervigilance, and difficulty with concentration.[19]

Drug Interactions

Because biotransformation of ketamine primarily involves the CYP3A4 isoform, drugs that alter this isoform would be expected to affect the metabolism of ketamine. Benzodiazepines (e.g., midazolam, lorazepam) attenuate the altered perception and thought processes associated with the use of ketamine. Although these drugs reduce the ketamine-associated emotional distress, the cognitive or behavioral effects of ketamine are not reduced. Acute administration of diazepam increases the half-life of ketamine. Lamotrigine significantly decreases ketamine-induced perceptual abnormalities, but lamotrigine enhances the mood elevating effects of ketamine. Haloperidol causes little change in the psychosis, perceptual changes, or euphoria associated with ketamine use. Animal studies suggest that the concomitant administration of ketamine (20 mg/kg) and caffeine (20 mg/kg) enhances locomotor activity and reduces coordination, whereas ketamine (200 mg/kg) and caffeine (20–100 mg/kg) produces a dose-dependent increase in seizures and death.[41]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Ketamine is a dissociative anaesthetic with analgesic and amnestic properties that causes impairment of working and episodic memory during intoxication and the immediate period after the administration of ketamine.[42] Similar to phencyclidine (PCP), ketamine is a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor for glutamate, but the mechanism of action of ketamine is more complex than PCP because of the interaction of ketamine with many others receptors (opioid, noradrenaline, serotonin, muscarinic cholinergic). These effects also include decreased catecholamine reuptake and the stereoselective inhibition of the dopamine transporter with the S(+)-enantiomer being approximately 8 times more potent than the R(−)-enantiomer (K_i = 46.9 μM and 390 μM, respectively).[43]

The affinity of PCP for the NMDA receptor is approximately 10-fold greater than ketamine. The administration of subanesthetic doses of ketamine to schizophrenic individuals produces short-term activation of psychotic symptoms, which are similar to their usual psychotic episodes.[44,45] Following anesthetic doses (>2 mg/kg), ketamine produces a dose-related increase in heart rate and pressure without altering stroke index.[33] Ketamine does not cause significant respiratory depression except following very large doses or rapid IV administration.

Mechanism of Toxicity

The psychedelic effect of ketamine results, at least in part, from the disruption of the normal thalamocortical filtering of external and internal information to the cortex. Animal studies suggest that subanesthetic doses of ketamine increase dopamine D_2 receptor binding in the hippocampus and decrease glutamate receptor binding in the frontal cortex, while dopamine D_1 receptor binding remains unchanged.[46] Ketamine impairs or antagonizes N-methyl-D-aspartate glutamate receptor neurotransmission and increases glutamate release. This effect causes aberrant perceptual processing of auditory and visual association and reduced integration of aberrant perception in visceromotor function and the sense of self.[47] Additionally, the density of dopamine transporters increase in the striatum, while the density of 5-HT transporters increase in the striatum, the hippocampus, and the frontal cortex. However, in a study of chronic ketamine abusers imaged by positron emission tomography, dorsolateral prefrontal cortex D_1 receptor availability was significantly upregulated when compared with healthy controls.[48] The main dopaminergic receptors in the human cortex are D_1, and sustained reduction in prefrontal dopamine produces an upregulation of prefrontal dopamine D_1 receptors. The cognitive performance of these study participants was normal; therefore, the clinical significance of this study remains unclear. Stimulation of dopamine release is a common effect of most drugs of abuse. NMDA receptors are densely localized in areas of the cerebral cortex and the hippocampus, which are important areas for executive function and memory. Human memory probably involves NMDA receptor-dependent long-term potentiation as well as other processes associated with neuronal learning. Preliminary clinical studies suggest some impairment of episodic memory (recognition of objects and source of information) in chronic ketamine abusers,[49] but the extent and type of these memory impairments remain undefined. Typically, executive function and attention are not affected at subanesthetic doses of ketamine.

Postmortem Examination

In the absence of trauma, postmortem examinations of patients dying of ketamine intoxication are typically unremarkable with nonspecific positive findings primarily involving pulmonary congestion.[50] Postmortem changes associated with death from respiratory depression include cerebral edema, pulmonary edema, interstitial and intra-alveolar hemorrhage in the lungs, and hyperemia of the airways. The postmortem examination of a 34-year-old woman, who died after several poisoning attempts with ketamine, demonstrated widespread fibrosis of cardiac muscle fibers around small arteries along with chronic hypoxic changes in the brain.[51] These latter changes included hemorrhage in the internal capsule and cerebral edema. The causal relationship between ketamine and these changes is unclear.

CLINICAL RESPONSE

Ketamine is a relatively safe anesthetic agent with few serious side effects as a result of the lack of cardiorespiratory depression in therapeutic doses. This drug produces a cataleptic state characterized by open eyes, staring gaze, slow nystagmus, increased salivation, and intact corneal and light reflexes. The major side effect is development of psychotic symptoms during emergence from ketamine anesthesia, particularly in adults and schizophrenic patients.[52] Rarely, laryngospasm occurs following dissociative sedation that may require intubation.[53] The postanesthesia emergence reaction involves alterations in mood and body image, dissociative (extracorporeal) state, vivid illusions, floating sensation, and frank delirium. These vivid dreams and illusions usually disappear shortly after awakening. The incidence of this reaction ranges from about 5–30%, depending on risk factors (age >16 years, female participants, IV ketamine doses >2 mg/kg, IV administration exceeding 40 mg/min).[54] In studies of healthy young adults, subanesthetic doses of ketamine produce dose-related psychedelic effects similar to the IV administration of *N,N*-dimethyltryptamine, manifest by alterations of mood, perception, body awareness, self-control, and mentation.[55] These effects are distinct from schizophrenia, and true hallucinations do not occur. In this study, the visual analog scales for anxiety, suspicious, and meaning were low compared with rating scales for reality, time, surroundings, thought, sound, and being high.

Illicit Use

Based on self-reported cases of ketamine use to a US regional poison center, the symptoms of ketamine intoxication are relatively brief with one-half of cases being asymptomatic on evaluation in the emergency department (ED).[56] The most common complaints were anxiety, chest pain, and palpitations with tachycardia being the most common physical finding. In contrast to patients intoxicated with phencyclidine, rotary nystagmus was rare (i.e., 3 of 20 patients). The most common symptoms in a retrospective study of 233 ketamine abusers presenting to an emergency department in Hong Kong were as follows: impaired consciousness, 45%; abdominal pain, 21%; lower urinary tract symptoms, 12%, and dizziness, 12%.[57] The frequency of abnormal physical findings were as follows: high blood pressure, 40%; tachycardia, 39%; abdominal tenderness, 18%; and white powder in nostril, 17%. Occasional case reports associate cystitis (dysuria, frequency, hematuria, proteinuria, urgency) with chronic ketamine abuse.[58,59]

Desired effects associated with ketamine intoxication include euphoria, depersonalization, derealization, universal empathy, sudden insights into the nature of existence or oneself, and perceptual distortions (sounds, shapes, colorful visions, floating feeling, absence of time, body image). The "K-hole" refers to the development of tunnel vision during intoxication followed by the feeling of rising above the body like a near-death experience. Although some individuals experience recurrent psychoses following repeated binges on ketamine, there is insufficient evidence to support the development of sensitization to the behavioral effects of ketamine.[60]

The principal risk of acute ketamine intoxication is physical trauma resulting from a state of confusion including falls, burns, drowning, or motor vehicle accidents. Adverse effects associated with case series of chronic ketamine users include perforated nasal septum (insufflation), abdominal pain, anorexia, weight loss, ataxia, slurred speech, dizziness, anxiety, hyperexcitability, insomnia, confusion, and blurred vision.[61] Case reports associate ketamine abuse with urinary retention, hydronephrosis, and renal dysfunction.[62]

Overdose

There are relatively few cases of ketamine overdose reported in the medical literature, primarily involving inadvertent administration of excessive ketamine doses to children undergoing sedation or anesthesia. In these patients, the main complications are transient respiratory depression and prolonged sedation up to 24 hours.[23] Fatalities associated with ketamine are usually related to either behavioral-induced trauma or multiple-drug overdose.[63] In a series of 15 nonhospital-associated deaths with detectable postmortem concentrations of ketamine, 12 involved multiple drugs and 2 involved trauma.[64] The other death resulted from natural causes (sarcoidosis).

Abstinence Syndrome

Case reports of abstinence following chronic ketamine abuse associated the development of intense drug craving, anxiety, fatigue, diaphoresis, and tremulousness within 8 hours after cessation of use.[21] Within 24–48 hours after ketamine use ceases, lethargy, depression, and anorexia occur. After a period of deep sleep, the individual feels better with some residual weakness.

Reproductive Abnormalities

Ketamine is an anesthetic agent used during induction for cesarean sections as a method to reduce postoperative pain.[65] Clinical studies suggest that ketamine increases uterine contractions.[66] However, there are few data on the teratogenic effect of ketamine use during the first and second trimester of pregnancy.

DIAGNOSTIC TESTING

Analytic Methods

Techniques to detect ketamine and norketamine in biologic samples include liquid chromatography/ TurboIonSpray®/tandem mass spectrometry,[67] gas chromatography/mass spectrometry,[68,69] liquid chromatography/mass spectrometry in selected ion mode,[70] and high performance liquid chromatography with ultraviolet detection (220 nm).[71] The lower limit of quantitation (LLOQ) for ketamine and norketamine is in the range of 5 ng/mL, as analyzed by isocratic high performance liquid chromatography[72] or gas chromatography/mass spectrometry.[73] The limit of detection (LOD) and LLOQ for ketamine in urine samples analyzed by gas chromatography/mass spectrometry with selected-ion monitoring were 0.5–1.0 ng/mL and 1.5–3.0 ng/mL, respectively, with interassay variation <10%.[68,74] The detection limits for ketamine and norketamine were 0.03 ng/mL and 0.05 ng/mL, respectively, as analyzed by ultra performance liquid chromatography/tandem mass spectrometry.[75] Ketamine and norketamine in plasma samples are stable when transported at 4°C (39.2°F) within 2 days and stored at –20°C (–4°F) for 10 weeks.[76] The plasma concentrations of dehydronorketamine can change initially during refrigeration as a result of the rapid permeation of this compound into erythrocytes.

Biomarkers

BLOOD

THERAPEUTIC USE.   Drowsiness and perceptual distortions occur with plasma ketamine concentration from 50–200 ng/mL, whereas analgesia begins at plasma ketamine concentrations >100–160 ng/mL.[32,55] The maximum plasma concentrations of ketamine after the administration of 50 mg orally or 25 mg intranasally range from 30–50 ng/mL at 20–30 minutes after administration.[25] Experimental studies of healthy volunteers indicate that subanesthetic doses (i.e., 200 ng/mL plasma) of ketamine causes general central nervous system depression, perceptual distortions, delusions of reference, and flattening of affect.[77] However, true hallucinations do not occur, and the overall clinical features of ketamine intoxication do not mimic schizophrenia. In a study of healthy, young men, plasma ketamine concentrations of 200 ng/mL were associated with lateral gaze nystagmus and alteration of visual analog scale for euphoria, feelings of unreality, perceptual alterations (shapes, sounds), and difficulty controlling thoughts.[55] There were no significant changes in blood pressure, heart rate, or oxygen saturation when compared with baseline. Plasma ketamine concentrations were undetectable by high performance liquid chromatography within 4–6 hours after intranasal and IV administration.[25] In a study of 5 volunteers receiving subanesthetic doses of IV ketamine, plasma ketamine concentrations exceeding 70 ng/mL were associated with memory impairment and perceptual distortions, as measured by a simple memory test and questionnaire.[78] Analgesic concentrations of ketamine in plasma range from about 100–200 ng/mL, whereas plasma concentrations in patients awakening from anesthesia typically range from approximately 600–1,100 ng/mL. During anesthesia, plasma ketamine concentrations reach 2,000–3,000 ng/mL without significant cardiovascular depression. Behavioral abnormalities do not correlate well to blood ketamine concentrations.

POSTMORTEM.   Fatalities associated with only ketamine use are rare. A 26-year-old man was found dead with a tourniquet on his arm and syringes of ketamine near his body.[50] The death scene suggested that he probably died before the distribution phase of ketamine ended. The ketamine concentrations in postmortem heart and femoral blood were 6.9 mg/L and 1.8 mg/L, respectively (heart/femoral blood ratio, 3.8). There were no other drugs detectable in postmortem blood. A 31-year-old women was found dead in her bathroom with a syringe and bottle of ketamine near her.[79] The postmortem blood sample (source not reported) contained 7 mg ketamine/L, and no other drugs were detected. The postmortem ketamine concentration in a blood sample (site not reported) from an 18-year-old man found dead after the homicidal administration of at least 3 doses of IM ketamine was 27.6 mg/L.[80] High ketamine concentrations (15.2 mg/L) were also

present in postmortem bile samples. A 34-year-old woman was found dead in her apartment after drinking a cup of tea surreptitiously prepared with 300 mg ketamine. Postmortem blood contained 3.8 mg ketamine/L. In a case series on postmortem drug redistribution that included ketamine, the postmortem heart/femoral blood ratios for ketamine in 2 cases were 0.8 and 2.3.[81]

URINE

Ketamine is not a routine drug in urine immunoassay screens for drugs of abuse. Some cross-reactivity occurs between ketamine and phencyclidine on immunoassays. The detection window for ketamine and norketamine in urine is dependent both on the analytic method and individual toxicokinetics. In a study of hospitalized children undergoing ketamine anesthesia, ketamine and norketamine were detectable up to 2 days and 14 days, respectively, as analyzed by gas chromatography/negative chemical ionization/mass spectrometry.[82] Using liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry, the windows of detection for norketamine in urine were 6 days and 11 days, respectively. The LOD for ketamine with these 2 analytic techniques were 20 ng/mL (gas chromatography/negative chemical ionization/mass spectrometry) and 2 ng/mL (liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry), respectively. Over the 16-day observation period, urine samples from 1 child in this cohort receiving ketamine anesthesia did not contain detectable concentrations of ketamine or norketamine by either method. In a case series of 33 urine samples submitted to a reference laboratory from the US Department of Defense criminal investigative agencies for ketamine use, the ketamine and norketamine concentrations ranged from 6–7,744 ng/mL and 7–7,986 ng/mL, respectively, as measured by gas chromatography/mass spectrometry.[68] In a study of 6 healthy volunteers ingesting 50 mg ketamine, urine samples contained detectable amounts of dehydronorketamine up to 10 days as measured by ultra performance liquid chromatography/tandem mass spectrometry.[75]

Abnormalities

Laboratory examination of blood from patients with ketamine intoxication is typically unremarkable. A case report associated the insufflation of ketamine with the development of the Brugada syndrome (coved downsloping 4 mm ST elevation with negative T wave in V_1 and V_2), rhabdomyolysis, renal dysfunction, confusion, and respiratory failure.[83] Additionally, the left ventricular

ejection fraction was 40% together with hypokinesia of the right ventricle.

Driving

There are few data on the effect of ketamine on driving performance. However, the administration of ketamine would be expected to substantially impair driving skills based on the cognitive impairments, perceptual distortions, and thought disorders produced in volunteer studies after the administration of ketamine.[84] Potential impairments include increased distractibility, blurred vision, visual illusions, spatial distortions, increased reaction time, and impaired memory (immediate and delayed recall, working memory).[85]

TREATMENT

Treatment is supportive with careful attention to the evaluation and treatment of any cardiorespiratory dysfunction. Symptoms are typically brief with most patients discharged from the emergency department within 5 hours of presentation.[56] The failure of symptoms to ameliorate after 2 hours of observations suggests another drug or disease process, particularly if seizures, hemodynamic changes, or hyperthermia are present. The most serious adverse effects of ketamine intoxication are respiratory depression, agitation, and rhabdomyolysis. Patients should be placed in a quiet environment with minimal external stimulation. There are no specific antidotes. The treatment of emergence reactions primarily involves the use of benzodiazepines (e.g., adults: lorazepam 1–2 mg, IV, titrated to effect). Preliminary clinical studies suggest that haloperidol (5 mg IM, repeated in 30 minutes if needed) is an alternative to benzodiazepines.[86] However, there are inadequate data to assess the safety or efficacy of haloperidol in this setting.

References

1. Domino EF, Chodoff P, Corssen G. Pharmacologic effects of CI-581, a new dissociative anesthetic, in man. Clin Pharmacol Ther 1965;6:279–291.
2. Collier BB. Ketamine and the conscious mind. Anaesthesia 1972;27:120–134.
3. Shomer RR. Misuse of ketamine. J Am Vet Med Assoc 1992;200:256–257.
4. Ahmed SN, Petchkovsky L. Abuse of ketamine. Br J Psychiatry 1980;137:303.
5. White PF, Schuttler J, Shafer A, Stanski DR, Horai Y, Trevor AJ. Comparative pharmacology of the ketamine

isomers. Studies in volunteers. Br J Anaesth 1985; 57:197–203.

6. Ryder S, Way WL, Trevor AJ. Comparative pharmacology of the optical isomers of ketamine in mice. Eur J Pharmacol 1978;49:15–23.

7. Moore NN, Bostwick JM. Ketamine dependence in anesthesia providers. Psychosomatics 1999;40:356–359.

8. Copeland J, Dillon P. The health and psycho-social consequences of ketamine use. Int J Drug Policy 2005;16: 122–131.

9. Lankenau SE, Clatts MC. Drug injection practices among high-risk youths: the first shot of ketamine. J Urban Health 2004;81:232–248.

10. Lampinen TM, McGhee D, Martin I. Increased risk of "club" drug use among gay and bisexual high school students in British Columbia. J Adolesc Health 2006;38:458–461.

11. Dillon P, Copeland J, Jansen K. Patterns of use and harms associated with non-medical ketamine use. Drug Alcohol Depend 2003;69:23–28.

12. Mills IH, Park GR, Manara AR, Merriman RJ. Treatment of compulsive behavior in eating disorders with intermittent ketamine infusions. QJM 1998;91:493–503.

13. Lankenau SE, Clatts MC. Patterns of polydrug use among ketamine injectors in New York City. Subst Use Misuse 2005;40:1381–1397.

14. Lua AC, Lin HR, Tseng YT, Hu AR, Yeh PC. Profiles of urine samples from participants at rave party in Taiwan: prevalence of ketamine and MDMA abuse. Forensic Sci Int 2003;136:47–51.

15. Wolff K, Winstock AR. Ketamine from medicine to misuse. CNS Drugs 2006;20:199–218.

16. Maxwell JC. Party drugs: properties, prevalence, patterns, and problems. Subst Use Misuse 2005;40:1203–1240.

17. Corazza O, Schifano F. Near-death states reported in a sample of 50 misusers. Subst Use Misuse 2010;45: 916–924.

18. Hurt PH, Ritchie EC. A case of ketamine dependence. Am J Psychiatry 1994;151:779.

19. Jansen KL, Darracot-Cankovic R. The nonmedical use of ketamine, part two: a review of problem use and dependence. J Psychoactive Drugs 2001;33:151–158.

20. Dalgarno PJ, Shewan D. Illicit use of ketamine in Scotland. J Psychoactive Drugs 1996;28:191–199.

21. Critchlow DG. A case of ketamine dependence with discontinuation symptoms. Addiction 2006;101:1212–1213.

22. Annetta MG, Iemma D, Garisto C, Tafani C, Proietti R. Ketamine: new indications for an old drug. Curr Drug Targets 2005;6:789–794.

23. Green SM, Clark R, Hostetler MA, Cohen M, Carlson D, Rothrock SG. Inadvertent ketamine overdose in children: clinical manifestations and outcome. Ann Emerg Med 1999;34:492–497.

24. Capape S, Mora E, Mintegui S, Gardcia S, Santiago M, Benito J. Prolonged sedation and airway complications after administration of an inadvertent ketamine overdose in emergency department. Eur J Emerg Med 2008; 15:92–94.

25. Yanagihara Y, Ohtani M, Kariya S, Uchino K, Hiraishi T, Ashizawa N, et al. Plasma concentration profiles of ketamine and norketamine after administration of various ketamine preparations to healthy Japanese volunteers. Biopharm Drug Dispos 2003;24:37–43.

26. Malinovsky JM, Servin F, Cozian A, Lepage JY, Pinaud M. Ketamine and norketamine plasma concentrations after IV, nasal and rectal administration in children. Br J Anaesth 1996;77:203–207.

27. Hijazi Y, Bodonian C, Bolon M, Salord F, Boulieu R. Pharmacokinetics and hemodynamics of ketamine in intensive care patients with brain or spinal cord injury. Br J Anaesth 2003;90:155–160.

28. Hijazi Y, Boulieu R. Protein binding of ketamine and its active metabolites to human serum. Eur J Clin Pharmacol 2002;58:37–40.

29. Hijazi Y, Boulieu R. Contribution of CYP3A4, CYP2B6, and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2002; 30:853–858.

30. Yanagihara Y, Kariya S, Ohtani M, Uchino K, Aoyama T, Yamamura Y, Iga T. Involvement of CYP2B6 in n-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2001;29:887–890.

31. White PF, Johnston RR, Pudwill CR. Interaction of ketamine and halothane in rats. Anesthesiology 1975;42:179–186.

32. Clements JA, Nimmo WS, Grant IS. Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. J Pharm Sci 1982;71:539–542.

33. Idvall J, Ahlgren I, Aronsen KR, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. Br J Anaesth 1979;51:1167–1173.

34. Ihmsen H, Geisslinger G, Schuttler J. Stereoselective pharmacokinetics of ketamine: R(-)-ketamine inhibits the elimination of S(+)-ketamine. Clin Pharmacol Ther 2001; 70:431–438.

35. Wieber J, Gugler R, Hengstmann JH, Dengler HJ. Pharmacokinetics of ketamine in man. Anaesthesist 1975; 24:260–263.

36. Ellingson A, Haram K, Sagen N, Solheim E. Transplacental passage of ketamine after intravenous administration. Acta Anaesthesiol Scand 1977;21:41–44.

37. Kamaya H, Krishna PR. Ketamine addiction. Anesthesiology 1987;67:861–862.

38. Pal HR, Berry N, Kumar R, Ray R. Ketamine dependence. Anaesth Intensive Care 2002;30:382–384.

39. Marietta MP, Vore ME, Way WL, Trevor AJ. Characterization of ketamine induction of hepatic microsomal drug metabolism. Biochem Pharmacol 1977;26:2451–2453.

40. Lim DK. Ketamine associated psychedelic effects and dependence. Singapore Med J 2003;44:31–34.

41. Hsu H-R, Mei Y-Y, Wu C-Y, Chiu P-H, Chen H-H. Behavioral and toxic interaction profile of ketamine in combination with caffeine. Basic Clin Pharmacol Toxicol 2009;104:379–383.

42. Curran HV, Morgan C. Cognitive, dissociative and psychotogenic effects of ketamine in recreational users on the night of drug use and 3 days later. Addiction 2000;95:575–590.

43. Nishimura M, Sato K. Ketamine stereoselectively inhibits rat dopamine transporter. Neurosci Lett 1999;274:131–134.

44. Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, Breier A. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology 1997;17:141–150.

45. Lahti AC, Holcomb HH, Medoff DR, Tamminga CA. Ketamine activates psychosis and alters limbic blood flow in schizophrenia. Neuroreport 1995;6:869–872.

46. Becker A, Peters B, Schroeder H, Mann T, Huether G, Grecksch G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:687–700.

47. Deakin JF, Lees J, McKie S, Hallak JE, Williams SR, Dursun SM. Glutamate and the neural basis of the subjective effects of ketamine. Arch Gen Psychiatry 2008;65:154–164.

48. Narendran R, Frankle WG, Keefe R, Gil R, Martinez D, Slifstein M, et al. Altered prefrontal dopaminergic function in chronic recreational ketamine users. Am J Psychiatry 2005;162:2352–2359.

49. Morgan CJ, Riccelli M, Maitland CH, Curran HV. Long-term effects of ketamine: evidence for a persisting impairment of source memory in recreational users. Drug Alcohol Depend 2004;75:301–308.

50. Lalonde BR, Wallage HR. Postmortem blood ketamine distribution in two fatalities. J Anal Toxicol 2004;28:71–74.

51. Tao LY, Chen XP, Qin ZH. A fatal chronic ketamine poisoning. J Forensic Sci 2005;50:173–176.

52. White PF, Ham J, Way WL, Trevor AJ. Pharmacology of ketamine isomers in surgical patients. Anesthesiology 1980;52:231–239.

53. Cohen VG, Krauss B. Recurrent episodes of intractable laryngospasm during dissociative sedation with intramuscular ketamine. Pediatr Emerg Care 2006;22:247–249.

54. White PF, Way WL, Trevor AJ. Ketamine—its pharmacology and therapeutic uses. Anesthesiology 1982;56:119–136.

55. Bowdle TA, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesthesiology 1998;88:82–88.

56. Weiner AL, Vieira L, McKay CA Jr, Bayer MJ. Ketamine abusers presenting to the emergency department: a case series. J Emerg Med 2000;18:447–451.

57. Ng SH, Tse ML, Ng HW, Lau F. Emergency department presentation of ketamine abusers in Hong Kong: a review of 233 cases. Hong Kong Med J 2010;16:6–11.

58. Chen L-Y, Chen K-P, Huang M-C. Cystitis associated with chronic ketamine abuse. Psychiatr Clin Neurosci 2009;63:591.

59. Chu PS, Kwok SC, Lam KM, Chu TY, Chan SW, Man CW, et al. 'Street ketamine'-associated bladder dysfunction: a report of ten cases. Hong Kong Med J 2007;13:311–313.

60. Cho HS, D'Souza DC, Gueorguieva R, Perry EB, Madonick S, Karper LP, et al. Absence of behavioral sensitization in healthy human subjects following repeated exposure to ketamine. Psychopharmacology (Berl) 2005;179:136–143.

61. Hansen G, Jensen SB, Chandresh L, Hilden T. The psychotropic effect of ketamine. J Psychoactive Drugs 1988;20:419–425.

62. Chu PS, Kwok SC, Lam KM, Chu TY, Chan SW, Man CW, et al. "Street ketamine"-associated bladder dysfunction: a report of ten cases. Hong Kong Med J 2007;13:311–313.

63. Breitmeier D, Passie T, Mansouri F, Albrecht K, Kleemann WJ. Autoerotic accident associated with self-applied ketamine. Int J Legal Med 2002;116:113–116.

64. Gill JR, Stajic M. Ketamine in non-hospital and hospital deaths in New York City. J Forensic Sci 2000;45:655–658.

65. Kee WD, Khaw KS, Ma ML, Mainland PA, Gin T. Postoperative analgesic requirement after cesarean section: a comparison of anesthetic induction with ketamine or thiopental. Anesth Analg 1997;85:1294–1298.

66. Oats JN, Vasey DP, Waldron BA. Effects of ketamine on the pregnant uterus. Br J Anaesth 1979;51:1163–1166.

67. Wang KC, Shih TS, Cheng SG. Use of SPE and LC/TIS/MS/MS for rapid detection and quantitation of ketamine and its metabolite, norketamine, in urine. Forensic Sci Int 2005;147:81–88.

68. Rohrig TP, Gamble M, Cox K. Identification and quantitation of ketamine in biological matrices using gas chromatography-mass spectrometry (GC-MS). Methods Mol Biol 2010;603:317–326.

69. Moore KA, Sklerov J, Levine B, Jacobs AJ. Urine concentrations of ketamine and norketamine following illegal consumption. J Anal Toxicol 2001;25:583–588.

70. Rodriguez Rosas ME, Patel S, Wainer IW. Determination of the enantiomers of ketamine and norketamine in human plasma by enantioselective liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003;794:99–108.

71. Svensson JO, Gustafsson LL. Determination of ketamine and norketamine enantiomers in plasma by solid-phase extraction and high-performance liquid chromatography. J Chromatogr B Biomed Appl 1996;678:373–376.

72. Bolze S, Boulieu R. HPLC determination of ketamine, norketamine, and dehydronorketamine in plasma with a high-purity reversed-phase sorbent. Clin Chem 1998;44:560–564.

73. Olmos-Carmona ML, Hernandez-Carrasquilla M. Gas chromatographic-mass spectrometric analysis of veteri-

nary tranquilizers in urine: evaluation of method performance. J Chromatogr B Biomed Sci Appl 1999;734: 113–120.

74. Huang MK, Liu C, Li JH, Huang SD. Quantitative detection of ketamine, norketamine, and dehydronorketamine in urine using chemical derivatization followed by gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005;820:165–173.

75. Parkin MC, Turfus SC, Smith NW, Halket JM, Braithwaite RA, Elliott SP, et al. Detection of ketamine and its metabolites in urine by ultra high pressure liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008;876:137–142.

76. Hijazi Y, Bolon M, Boulier R. Stability of ketamine and its metabolites norketamine and dehydronorketamine in human biological samples. Clin Chem 2001;47:1713–1715.

77. Pomarol-Clotet E, Honey GD, Murray GK, Corlett PR, Absalom AR, Lee M, et al. Psychological effects of ketamine in healthy volunteers phenomenological study. Br J Psychiatry 2006;189:173–179.

78. Hartvig P, Valtysson J, Lindner K-J, Kristensen J, Karlsten R, Gustafsson LL, et al. Central nervous system effects of subdissociative doses of (S)-ketamine are related to plasma and brain concentrations measured with positron emission tomography in health volunteers. Clin Pharmacol Ther 1995;58:165–173.

79. Peyton SH, Couch AT, Bost RO. Tissue distribution of ketamine: two case reports. J Anal Toxicol 1988;12: 268–269.

80. Licata M, Pierini G, Popoli G. A fatal ketamine poisoning. J Forensic Sci 1994;39:1314–1320.

81. Dalpe-Scott M, Degouffe M, Garbutt D, Drost M. A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. Can Soc Forensic Sci 1995;28:113–121.

82. Adamowicz P, Kala M. Urinary excretion rates of ketamine and norketamine following therapeutic ketamine administration: method and detection window considerations. J Anal Toxicol 2005;29:376–382.

83. Rollin A, Maury P, Guilbeau-Frugier C, Brugada J. Transient ST elevation after ketamine intoxication: a new cause of acquired Brugada ECG pattern. J Cardiovasc Electrophysiol 2011;22:91–94.

84. Adler CM, Goldberg TE, Malhotra AK, Pickar D, Breier A. Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. Biol Psychiatry 1998;43:811–816.

85. Hetem LA, Danion JM, Diemunsch P, Brandt C. Effect of a subanesthetic dose of ketamine on memory and conscious awareness in healthy volunteers. Psychopharmacology (Berl) 2000;152:283–288.

86. Giannini AJ, Underwood NA, Condon M. Acute ketamine intoxication treated by haloperidol: a preliminary study. Am J Ther 2000;7:389–391.

Chapter 8

METHCATHINONE, MEPHEDRONE, and METHYLONE

METHCATHIONE

HISTORY

Methcathinone was first synthesized in Germany and France in the late 1920s as an intermediate in the synthesis of ephedrine. The optical isomers [$S(-)$- and $R(+)$-methcathinone] were discovered in the following decade. Parke-Davis Pharmaceuticals obtained the patent for $S(-)$-methcathinone in 1957 as an analeptic; however, reports of adverse effects (trembling, seizures, incoordination, spasticity, labored respirations) in animal studies prevented the marketing of this drug.[1] Methcathinone was a popular drug of abuse in the former Soviet Union during the 1970s and 1980s with street names of Ephedrone, Jeff, Cosmos, and Jee Cocktail.[2] During the early 1990s, the first reports associating atypical parkinsonism with intravenous (IV) administration of ephedrone (methcathinone) synthesized by the oxidation of ephedrine or pseudoephedrine with potassium permanganate appeared in Russia. Subsequently, similar case reports associated IV methcathinone abuse with persistent movement disorders in Estonia, Georgia, Latvia, Ukraine, and an Azerbaijani resident in Canada.[3,4] In 1992, methcathinone was added to the list of controlled substances in the United States,[5] followed by cathinone in 1993.

IDENTIFYING CHARACTERISTICS

Methcathinone (CAS RN: 5650-44-2, monomethylpropion) is structurally similar to methamphetamine, and this β-keto-α-methylphenethylamine compound is a more potent stimulant than cathinone based on animal studies.[6] Figure 8.1 displays the structural similarities between the optical isomers of methcathinone, methamphetamine, cathinone, and amphetamine. Methcathinone contains an asymmetric carbon; thus, methcathinone is potentially available as a racemic mixture or as enantiomers [$S(-)$, $R(+)$]. In rodent behavioral studies (locomotor stimulation, drug discrimination), the $S(-)$-methcathinone enantiomer is about 5 times more potent than the $R(+)$-enantiomer.[7] The World Health Organization includes methcathinone in schedule I (i.e., most controlled substance category as a result of high abuse potential) of the United Nations Convention on Psychotropic Substances. Although some analogs of methcathinone (e.g., N-monoethylcathinone, N-mono-n-propylcathinone) produce stimulant effects similar to analogs of methamphetamine in animal studies, the potency of structural analogs of methcathinone are not necessarily predicted by structure–activity relationships of similar methamphetamine structural analogs.[8] Methcathinone has a distinctive pleasant smell (i.e., likened to pistachio ice cream), bitter taste, and dark color that differentiate this drug from amphetamine and cocaine.[1] In addition, methcathinone causes irritation of the mucous membranes in contrast to the anesthetic properties of cocaine.

EXPOSURE

Clandestine laboratories synthesize methcathinone easily by the oxidation of l-ephedrine with household chemicals (sulfuric acid, paint thinner, acetone). Consequently, most illicit methcathinone contains the
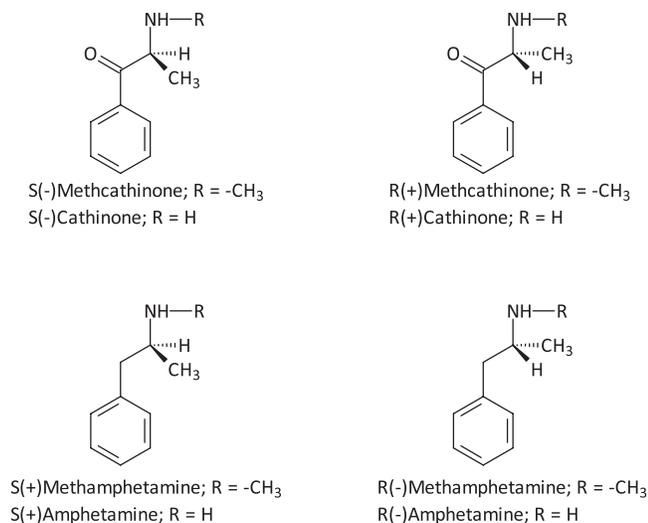
FIGURE 8.1. Structural and stereochemical relationships between the optical isomers of methcathinone, methamphetamine, cathinone, and amphetamine.

$S(−)$-enantiomer with small amounts of the $R(+)$-enantiomer as a result of spontaneous racemization of the $S(−)$-enantiomer during extraction procedures. Abuse of methcathinone involves the use of this drug via the IV, pulmonary, or nasal routes. During a typical episode of methcathinone use, the drug user inhales the drug for 24–36 hours until the drug supply is depleted or the drug user is physically exhausted. Desirable effects reported by methcathinone during acute intoxication include intense physical stimulation, sexual arousal, euphoria, and tolerance for alcohol.[1] During binges, methcathinone users report the rapid development of strong psychologic craving and tolerance for this drug.[2]

DOSE EFFECT

The intranasal use (80–250 mg) of methcathinone produces euphoria within 10 minutes along with visual illusions and hallucination followed by a 5- to 8-hour period of feeling invincibility and increased libido.[2] Undesirable side effects associated with these doses include headache, abdominal pain, diaphoresis, and agitation. In a case series of 13 male opiate addicts developing movement disorders after chronic IV ephedrone (methcathinone) abuse, the reported daily use of ephedrone was 8–20 mL 1–6 times daily (mean reported daily use, 42 ± 28 mL).[9]

TOXICOKINETICS

Following the ingestion of recreational doses (e.g., 30 mg) of methcathinone, unchanged methcathinone,

(+) threo-ephedrine (d-pseudoephedrine), (−)-threo-ephedrine (l-pseudoephedrine), and phenylpropanolamine appear in the urine along with small amounts of phenylpropanolamine.[10] Based on 2 trials in a single volunteer, about one-third of this dose appeared in the urine as unchanged methcathinone and about one-half as ephedrine (about 60% l-pseudoephedrine, 40% d-pseudoephedrine); phenylpropanolamine was a minor urinary metabolite (<5%). Reports from methcathinone users indicate that cessation of methcathinone use is associated with prolonged sleep, irritability, dysphoria, hyperphagia, and depression similar to the cessation of acute, high-dose amphetamine use.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Rodent studies indicate that methcathinone is a potent CNS stimulant with effects on the dopaminergic and serotonergic receptors.[11] These effects are species and enantiomer dependent. In mice, both $R(+)$- and $S(−)$-enantiomers produce toxic effects on dopamine neurons based on reduction in dopamine, 3,4-dihydroxyphenylacetic acid (dopamine metabolite), and dopamine uptake in rat brain; the $R(+)$-enantiomer is more potent than the $S(−)$-enantiomer in these studies.[12] Neither enantiomer produces serotonin toxicity at intraperitoneal doses ranging from 40 mg/kg every 2 hours for 4 doses to 120 mg/kg every 2 hours for 2 doses, then 2 doses at 4-hour intervals. Both enantiomers produce dopamine depletion in the stratum of rats with the $S(−)$-enantiomer slightly greater than the $R(+)$-enantiomer at doses of 25 mg/kg and 50 mg/kg twice daily for 4 days. Only the $S(−)$-enantiomer produces serotonin depletion in the hippocampus and neocortex of rats. Following the administration of methcathinone, decreased tyrosine hydroxylase and tryptophan hydroxylase occur along with reductions in dopamine and serotonin in the frontal cortex, neostriatum, and hippocampus. Additionally, methcathinone is a substrate for the dopamine and serotonin transports, resulting in the uptake of methcathinone by dopamine and serotonin neurons.[13] The similarity of the movement disorder associated with IV methcathinone abuse and chronic manganism suggest a role for manganese-induced neuropathy as the cause of this movement disorder in ephedrone addicts. The IV administration of ephedrone produced by oxidation of ephedrine or pseudoephedrine with potassium permanganate releases large amounts of manganese into the blood following IV administration. A case series of 3 IV drug users reported the development of similar extrapyramidal disorders and postural instability following the chronic IV use of a solution of potassium permanganate, ephedrine, and acetylsalicylic acid.[14] In

the 2 addicts recently using this IV solution, their blood manganese concentrations were very high (2,100 ng/mL, 3,176 ng/mL), whereas the addict that did not use the solution for 6 months had a normal blood manganese concentration (2.4 ng/mL; normal <19 ng/mL). The solution these drug abusers injected did not contain methcathinone, suggesting that the motor disorder does not result from methcathinone.

CLINICAL RESPONSE

Acute intoxication with methcathinone produces clinical features similar to stimulant effects of amphetamine abuse. Reported desirable effects from the IV abuse of methcathinone include "rush," "feeling happy," and "sense of flying" within 15 minutes after injection followed by several hours of jocularity and increased alertness. Reported adverse effects of methcathinone use include nausea, abdominal pain, facial erythema, epistaxis, anxiety, irritability, difficulty concentrating, agitation, paranoia, tachycardia, insomnia, and muscle cramps. Complications of chronic methcathinone use include paranoid psychosis with auditory hallucinations, weight loss, dehydration, tremor, agitation, personality changes, antisocial behavior, and depression. Case reports associate the development of paranoia with auditory hallucinations, confusion slurred speech, tremor, disorientation, and intermittent anxiety following sustained, IV use of methcathinone.[2] These symptoms usually resolve over 24–36 hours. Occasionally, transient hypotension and bradycardia may also occur, but these abnormalities usually respond rapidly to supportive care.

Case reports associate the IV abuse of methcathinone synthesized by the oxidation of ephedrine or pseudoephedrine by potassium permanganate with levodopa-resistant atypical parkinsonism, profound hypophonia, and gait disturbances.[15] In a case series of 13 male opiate addicts developing movement disorders after chronic IV ephedrone (methcathinone) abuse, the mean time between the initiation of ephedrone abuse and the first neurologic symptoms was 8.5 ± 3.2 months.[9] Clinical abnormalities include bradykinesias, dystonia, emotional lability, dysarthria, risus sardonicus, involuntary laughter, and retropulsion with falls backwards.[9] These patients did not typically demonstrate olfactory abnormalities or declines in cognitive ability with the exception of mild dysfunction of executive skills and mild depression. These patients had several clinical features distinguishing them from patients with Parkinson disease including particular difficulty walking backward, a symmetric motor disorder that resulted in walking on the first metatarsal-phalangeal joints ("cock walk"), profoundly soft speech, and lack of a resting tremor.

They were unresponsive to levodopa, and the neurologic abnormalities remained after cessation of methcathinone abuse. Progression of neurologic symptoms after cessation of ephedrone use occurs in up to one-third of these patients. Some patients improve slowly, but complete reversal of neurologic symptoms does not usually occur.

DIAGNOSTIC TESTING

Analytic Methods

Analytic methods for the quantitation of methcathinone and other amphetamine analogs in biologic samples include gas chromatography,[16] high performance liquid chromatography with ultraviolet (UV) detection,[17] gas chromatography/mass spectrometry (GC/MS),[18] and liquid chromatography/electrospray ionization/tandem mass spectometry.[19] The lower limit of quantitation (LLOQ) of methcathinone using the latter method is 10 ng/mL with intermediate precision (<15%). The use of high performance liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry allows the quantitation of methcathinone and other underivatized amphetamines in hair samples with a limit of detection (LOD) in the range of 0.20 ng/mg.[20] Commonly available immunoassays do not sufficiently screen methcathinone from other amphetamine analogs. *In situ* derivatization of urine samples by extractive acylation with penta-fluoropropionic anhydride followed by rapid chromatography on a microbore capillary column and MS in selected-ion mode allows the rapid confirmation of various amphetamine analogs including methcathinone.[21] Methcathinone is not a metabolite of phenylpropanolamine or any other over-the-counter medications. Although methcathinone is stable for 3 days in refrigerated samples (2–4°C/35.6°–39.2°F) and in frozen samples (-18°C/-0.4°F) for 2 months, urine samples stored at 2–4°C (35.6°–39.2°F) for 3 months lost about 79% of the methcathinone.[18]

Abnormalities

The magnetic resonance images (MRIs) of patients with ephedrine-induced movement disorders demonstrate hyperintensity T1-weighted signals from the globus pallidus, substantia nigra, dentate nucleus, and the pontine tegmentum.[22] The whole blood manganese concentration in samples from these patients are typically elevated about 3–4 times above the upper reference range; whole blood manganese concentrations remain elevated months after cessation of IV methcathinone produced by potassium permanganate oxidation. Both the MRI

and the blood manganese concentration may be normal in the presence of an obvious movement disorder (bradykinesia, postural instability, slowed facial expressions, dysarthria, generalized dystonia).[23] Blood manganese concentrations do not correlate well to clinical symptoms.

TREATMENT

The treatment of methcathinone intoxication is supportive, similar to amphetamine intoxication. The movement disorders associated with IV ephedrone (methcathinone) abuse and potassium permanganate oxidation does not usually respond to the drugs (L-dopa, amantadine, bromocriptine, trihexyphenidyl) typically used to treat Parkinson disease. There are inadequate clinical data to determine if chelation of elevated manganese concentrations improves the chronic movement disorder associated with IV ephedrone abuse. Although the use of calcium disodium EDTA is associated with partial improvement in some patients, other patients did not improve and patients did not typically recover completely.[22]

MEPHEDRONE (4-METHYLMETH-CATHINONE)

Mephedrone (2-aminoethyl-1-tolyl-propan-1-one, 4-methylmethcathinone) is a β-keto amphetamine that is the synthetic 4-methyl aromatic analogue of methcathinone. Figure 8.2 compares the chemical structure, structural formula, and molecular weight of mephedrone and methcathinone. Common names include 4-MMC, Bubbles, Meph, Meow Meow, Miaow, TopCat, MMCAT, and Crab. Limited analytic data on mephedrone samples purchased from the Internet suggest that these sample contain high concentrations of 4-methylmethcathinone

in a racemic mixture.[24] Mephedrone is an uncontrolled cathinone derivative in many countries with sympathomimetic properties (tachycardia, mydriasis, blurred vision, agitation) similar to methcathinone. This compound is a substitute for ecstasy (MDMA) in the club scene. Mephedrone is an illegal drug in Denmark, Finland, Israel, Norway, Sweden, and the United Kingdom. Potential postprohibition mephedrone-like products include the 2-aminopentanophenone compounds.[25] These monoamine oxidase inhibitors are marketed as Energy 1 (NRG-1) or naphyrone (naphthylpyrovalerone, O-2482), although these products may contain illicit mephedrone or other cathinones/adulterants. In a study of 24 products purchased online from 18 UK-based websites within 6 weeks after the ban on mephedrone and other derivatives (3,4-methylenedioxypyrovalerone, butylone), 70% of the NRG-1 and NRG-2 products contained a mixture of banned cathinones.[26] Mephedrone is usually sold as a white crystalline or off-white–yellow powder (hydrochloride salt), frequently from street vendors and less often on the Internet as "bath salts."[27]

The common methods of administration are ingestion, insufflation, and rarely by injection with heroin. Biotransformation of mephedrone involves hydroxylation at the 4-methyl group followed by oxidation to the corresponding 4-carboxy metabolite and demethylation of the β-keto group to the corresponding alcohol.[28] The clinical features of mephedrone overdose reflect sympathomimetic toxicity similar to cocaine and methamphetamine intoxication including elevated blood pressure, tachycardia, palpitations, mydriasis, hyperactivity, and agitation as well as anxiety, paresthesias, confusion, vomiting, chest pain, and headache.[29] Based on data on 131 telephone enquiries to the UK National Poisons Information Service concerning mephedrone alone or in combination with alcohol, common clinical features reported were as follows: agitation or aggression ($n = 32$, 24%, 95% CI: 18–33%), tachycardia ($n = 29$, 22%, 95% CI: 16–30%), confusion or psychosis ($n = 18$, 14%, 95% CI: 9–21%), chest pain ($n = 17$, 13%, 95% CI: 8–20%), nausea ($n = 15$, 11%, 95% CI: 7–18%), palpitations ($n = 14$, 11%, 95% CI: 6–18%), peripheral
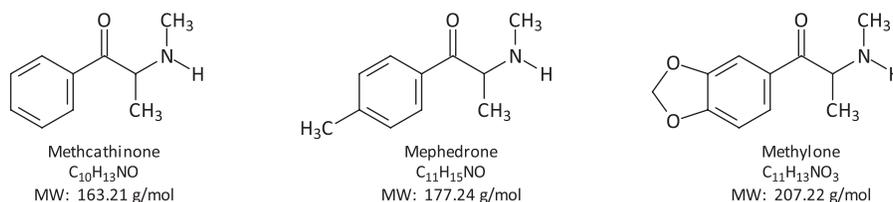
FIGURE 8.2. Comparison of chemical structures, structural formulas, and molecular weights of methcathinone, mephedrone (4-methylmethcathinone), and methylone.

vasoconstriction ($n = 10$, 8%, 95% CI: 4–14%), headache ($n = 7$, 5%, 95% CI: 2–11%), and seizures ($n = 4$, 3%, 95% CI: 1–8%).[30] A 22-year-old man ingested 200 mg mephedrone purchased from an Internet supplier; when he did not develop the desired effects, he injected 3,800 mg intramuscularly into his thighs.[31] Shortly thereafter, he developed chest pressure, diaphoresis, blurred tunnel vision, and a feeling of being unwell. At the emergency department (ED), he was agitated, anxious, and hypertensive (blood pressure = 177/111) with mydriasis. His symptoms resolved after 4 hours of observation and 1 mg oral lorazepam. Other complications associated with the use of mephedrone include hypo-osmotic hyponatremia with encephalopathy manifest by altered consciousness, nausea, vomiting, and elevated intracranial pressure.[32] Elevation of serum creatine kinase and rarely, hyponatremia may occur. Methods of detection include GC/MS in selective ion monitoring mode using methamphetamine-d_{14} as an internal standard.[33] The LOD and LLOQ with this method are 0.010 mg/L and 0.025 mg/L, respectively, with between-day accuracy ranging from 0.2–12.4%.

METHYLONE (3,4-METHYLENEDIOXY-METHCATHINONE)

Shulgin et al investigated the psychoactive properties of methylone in the 1970s; he and Jacob patented the drug as an antidepressant and antiparkinson agent in the middle 1990s. Methylone appeared as a designer drug of abuse in Europe and Japan in the middle 2000s.[34] Methylone (2-methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one, 3,4-methylenedioxymethcathinone) is the β-ketone analogue of 3,4-methylenedioxymethamphetamine (MDMA) as displayed in Figure 8.2. Major metabolic pathways for methylone include 1) *N*-demethylation of the side-chain to methylenedioxycathinone, and 2) demethylenation and *O*-methylation of the hydroxyl group on the benzene ring to form 4-hydroxy-3-methoxymethcathinone and 3-hydroxy-4-methoxymethcathinone. In rodent studies, the kidney excretes most of the metabolites as conjugates with small amounts (i.e., ~3%) appearing in the urine as unchanged methylone.[35] The major urinary metabolite of methylone was 4-hydroxy-3-methoxymethcathinone.

In vitro studies indicate that methylone increases the concentration of monoamine neurotransmitters (serotonin, norepinephrine) in the synaptic cleft by inhibition of the plasma monoamine reuptake transporters with relatively little effect on dopamine concentrations.[36] In contrast to MDMA, the effect of methylone on the vesicular monamine transporter is weak. The clinical effects of methylone are similar to MDMA, although the reported euphoric and stimulatory effects of methylone are milder than MDMA. There are few data on the effects of methylone in humans. Expected adverse effects include mydriasis, diaphoresis, nausea, vomiting, agitation, confusion, dysphoria, tachycardia, and hypertension based on structural similarity to MDMA. Methods for the quantitation of methylone include GC/MS, liquid chromatography/electrospray ionization/mass spectrometry, and gas chromatography/electron impact mass spectrometry.[35,37] Commercial immunoassays for drugs of abuse may be insensitive to the presence of methylone in the urine.[38] The use of GC/MS in electron impact mode allows the quantitation of methylone, mephedrone, and butylone in biologic samples.[28]

References

1. Calkins RF, Aktan GB, Hussain KL. Methcathinone: the next illicit stimulant epidemic? J Psychoactive Drugs 1995;27:277–285.

2. Emerson TS, Cisek JE. Methcathinone: a Russian designer amphetamine infiltrates the rural Midwest. Ann Emerg Med 1993;22;1897–1903.

3. de Bie RM, Gladstone RM, Strafella AP, Ko J-H, Lang AE. Manganese-induced parkinsonism associated with methcathinone (ephedrone) abuse. Arch Neurol 2007; 64:886–889.

4. Sikk K, Taba P, Haldre S, Bergquist J, Nyholm D, Zjablov G, et al. Irreversible motor impairment in young addicts—ephedrone, manganism or both? Acta Neurol Scand 2007; 115:385–389.

5. Bonner RC. Schedules of controlled substances temporary placement of methcathinone into schedule I. Fed Regist 1992;57:18824–18825.

6. Glenon RA, Yousef M, Naiman N, Kalix P. Methcathinone: a new potent amphetamine-like agent. Pharmacol Biochem Behav 1987;26:547–551.

7. Glennon RA, Young R, Martin BR, Dal Cason TA. Methcathinone ("cat"): an enantiomeric potency comparison. Pharmacol Biochem Behav 1995;50:601–606.

8. Dal Cason TA, Young R, Glennon RA. Cathinone: an investigation of several *N*-alkyl and methylenedioxy-substituted analogs. Pharmacol Biochem Behav 1997;58:1109–1116.

9. Selikhova M, Fedoryshyn L, Matviyenko Y, Komnatska I, Kyrylchuk M, Krolicki L, et al. Parkinsonism and dystonia caused by the illicit use of ephedrone—a longitudinal study. Mov Disord 2008;23:2224–2231.

10. Markantonis SL, Kyroudis A, Beckett AH. The stereose-lective metabolism of dimethylpropion and monomethyl-propion. Biochem Pharmacol 1986;35:529–532.

11. Young R, Glennon RA. Discriminative stimulus effects of S(−)-methcathinone (CAT): a potent stimulant drug of abuse. Psychopharmacology 1998;140:250–256.

12. Sparago M, Wlos J, Yuan J, Hatzidimitriou G, Tolliver J, Dal Cason TA. Neurotoxic and pharmacologic studies on enantiomers of the N-methylated analog of cathinone (methcathinone): a new drug of abuse. J Pharmacol Exp Ther 1996;279:1043–1052.

13. Cozzi NV, Foley KF. Methcathinone is a substrate for the serotonin uptake transporter. Pharmacol Toxicol 2003;93:219–225.

14. Varlibas F, Delipoyraz I, Yuksel G, Filiz G, Tireli H, Gecim NO. Neurotoxicity following chronic intravenous use of "Russian cocktail." Clin Toxicol 2009;47:157–160.

15. Stepens A, Logina I, Liguts V, Aldins P, Iksteina I, Platkajis A, et al. A parkinsonian syndrome in methcathinone users and the role of manganese. N Engl J Med 2008;358:1009–1017.

16. Soriano C, Muñoz-Guerra J, Carreras D, Rodríguez C, Rodríguez AF, Cortés R. Automated analysis of drugs in urine. J Chromatogr B Biomed Appl 1996;687:183–187.

17. Belhadj-Tahar H, Sadeg N. Methcathinone: a new postin-dustrial drug. Forensic Sci Int 2005;153:99–101.

18. Paul BD, Cole KA. Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. J Anal Toxicol 2001;25:525–530.

19. Beyer J, Peters FT, Kraemer T, Maurer HH. Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. J Mass Spectrom 2007;42:150–160.

20. Stanaszek R, Piekoszewski W. Simultaneous determina-tion of eight underivatized amphetamines in hair by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). J Anal Toxicol 2004;28:77–85.

21. Marais AA, Laurens JB. Rapid GC-MS confirmation of amphetamines in urine by extractive acylation. Forensic Sci Int 2009;183:78–86.

22. Sanotsky Y, Lesyk R, Fedoryshyn L, Komnatska I., Matviyenko Y, Fahn S. Manganic encephalopathy due to "ephedrine" abuse. Mov Disord 2007;22:1337–1343.

23. Varlibas F, Delipoyraz I, Yuksel G, Filiz G, Tireli H, Gecim NO. Neurotoxicity following chronic intravenous use of "Russian cocktail." Clin Toxicol 2009;47:157–160.

24. Gibbons S, Zloh M. An analysis of the "legal high" mephedrone. Bioorg Med Chem Lett 2010;20:4135–4139.

25. Meltzer PC, Butler D, Deschamps JR, Madras BK. 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (Pyrovalerone) analogues: a promising class of mono-amine uptake inhibitors. J Med Chem 2006;49:1420–1432.

26. Brandt SD, Sumnall HR, Measham F, Cole J. Analyses of second-generation "legal highs" in the UK: initial findings. Drug Test Anal 2010;2:377–382.

27. Dargan PI, Albert S, Wood DM. Mephedrone use and associated adverse effects in school and college/university students before the UK legislation change. QJM 2010;103:875–879.

28. Meyer MR, Wilhelm J, Peters FT, Maurer HH. Beta-keto amphetamines: studies on the metabolism of the designer drug mephedrone and toxicological detection of mephedrone, butylone, and methylone in urine using gas chromatography-mass spectrometry. Anal Bioanal Chem 2010;397:1225–1233.

29. Regan L, Mitchelson M, Macdonald C. Mephedrone toxic-ity in a Scottish emergency department. Emerg Med J 2011;28:1055–1058.

30. James D, Adams RD, Spears R, Cooper G, Lupton DJ, Thompson JP, Thomas SH. Clinical characteristics of mephedrone toxicity reported to the UK National Poisons Information Service. Emerg Med J 2011;28:686–689.

31. Wood DM, Davies S, Puchnarewica M, Button J, Archer R, Ovaska H, et al. Recreation use of mephedrone (4-methylmethcathinone, 4-MMC) with associated sympa-thomimetic toxicity. J Med Toxicol 2010;6;327–330.

32. Sammler EM, Foley PL, Lauder GD, Wilson SJ, Goudie AR, O'Riordan JI. A harmless high? Lancet 2010;376:742.

33. Dickson AJ, Vorce SP, Levine B, Past MR. Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin. J Anal Toxicol 2010;34:162–168.

34. Bossong MG, van Dijk JP, Niesink RJ. Methylone and mCPP, two new drugs of abuse? Addict Biol 2005;10:321–323.

35. Kamata HT, Shima N, Zaitsu K, Kamata T, Miki A, Nishikawa M, et al. Metabolism of the recently encoun-tered designer drug, methylone, in humans and rats. Xenobiotica 2006;36:709–723.

36. Nagai F, Nonaka R, Satoh Hisashi Kamimura K. The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 2007;559:132–137.

37. Kikura-Hanajiri R, Kawamura M, Saisho K, Kodama Y, Goda Y. The disposition into hair of new designer drugs; methylone, MBDB and methcathinone. J Chromatogr B 2007;855:121–126.

38. Shimizu E, Watanabe H, Kojima T, Hagiwara H, Fujisaki M, Miyatake R, et al. combined intoxication with methy-lone and 5-MeO-MIPT. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:288–291.

Chapter 9

METHYLENEDIOXYMETHAMPHETAMINE (ECSTASY, MDMA)

Nicholas A. Buckley, MD

HISTORY

Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is a central nervous system (CNS) stimulant that is one of the most commonly abused drugs in the United States, Canada, United Kingdom, Western Europe, and Australia.[1] The structurally similar compound, methamphetamine is a common precursor of MDMA. Merck synthesized MDMA in 1912, and in 1914 Merck received a patent for MDMA as an appetite suppressant. However, Merck never marketed MDMA. The US military conducted the first documented animal toxicity studies on MDMA in the 1950s. This research was part of a program that investigated the effect of psychedelic drugs on animals and humans, presumably as substances that might increase the effectiveness of interrogations. These studies also included the administration of 3,4-methylenedioxyamphetamine (MDA), but not MDMA to human participants. The frequently referenced chemist, Alexander Shulgin became a strong proponent of the use of MDMA in psychotherapy after rediscovering MDMA in the 1970s.[2] In addition to the use of MDMA as an adjunctive treatment during psychotherapy, suggested therapeutic uses of MDMA included the treatment of Parkinson disease, posttraumatic stress disorder, depression, autism, and substance abuse.[1,2] In the mid-1970s to mid-1980s, some psychoanalysts administered MDMA ("Adam") as adjunctive therapy based on the belief that MDMA inhibits the fear response to a perceived emotional threat, which would allow the patient to place the emotional sequelae of past experiences into a more realistic perspective in current relationships.[3]

The use of MDMA occurs primarily in well-developed Western countries, particularly Western Europe, Australia, Canada, and the United States.[4] The first widespread use of MDMA began in the "rave" club scene including dance clubs and at rave parties. The abuse of MDMA increased despite the introduction of legal restrictions in the 1970s and 1980s that proscribed the use and manufacture of MDMA. Most countries with regulatory drug systems subsequently declared MDMA an illegal substance. The use of MDMA became illegal in the United Kingdom in 1977. In 1985, the US Drug Enforcement Agency (DEA) restricted the use of MDMA because of the growing abuse problem, particularly prevalent in Texas.[5] The initial restriction was temporary, based on the structural and pharmacologic similarities of MDMA to banned hallucinogenic amphetamines (e.g., MDA). Some authors proposed that MDMA represented a novel class of drugs, the entactogens (i.e., "touching within") that were not hallucinogenic.[6] However, the DEA did not accept this new classification. Subsequent animal studies on the pharmacologic and toxicologic effects of MDMA on serotonergic and dopaminergic neurons supported the similarity between this substance and the banned substance MDA.[7] In 1988, the DEA permanently restricted the use of MDMA by classifying MDMA as a schedule I controlled substance. The

pharmacologic classification of MDMA remains controversial with many authors referring to MDMA as an entactogen, while others classify MDMA as an amphetamine designer drug or a hallucinogenic amphetamine.

Illicit production of MDMA began in the mid-1980s shortly after the implementation of legal restrictions in the United States. In the late 1990s, a large increase in MDMA use occurred in Western countries beginning in the United Kingdom and other Western European countries as part of the increased popularity of dance party and rave club scenes.[8] Illicit MDMA originated in the Netherlands, Belgium, and Germany. More recently, large increases in MDMA use appeared in the United States and Australia. The rates of MDMA confiscations, MDMA-related hospital visits, reported rates of previous and current use all increased 2- to 20-fold during the 1990s in the United States.[6]

Although the therapeutic use of MDMA remains controversial, some clinical research on the effects of MDMA continues. The US Food and Drug Administration (FDA) approved studies of MDMA in Parkinson disease in the 1990s, and in 2002 the FDA approved human studies of MDMA as an adjunctive treatment for psychotherapy in posttraumatic stress disorder.[9] This clinical trial began in 2004, and a similar trial commenced in Spain. These trials generated some controversy as institutional review board (IRB) approval was initially withheld for over a year because of animal data suggesting that a Parkinson-like syndrome develops in primates receiving recreational doses of MDMA.[10] However, the animal study of primates was subsequently withdrawn because of concern that methamphetamine was inadvertently substituted for MDMA during these experiments.[9]

IDENTIFYING CHARACTERISTICS

MDMA (CAS RN: 42542-10-9) is racemic 3-4 methylenedioxymethamphetamine (1-benzo[1,3]dioxol-5-yl-N-methyl-propan-2-amine). The molecular weight of MDMA is 193.242 g/mol, and the structural formula is $C_{11}H_{15}NO_2$. MDMA is structurally similar to MDA and methamphetamine. Figure 9.1 displays the chemical structure of MDMA and structurally related amphetamine compounds. Methamphetamine-based compounds (e.g., MDMA, MDA) contain substituents on the aromatic ring and act primarily on serotonin receptors, whereas amphetamine-based compounds (e.g., ephedrine, phenylpropanolamine, phentermine) do not contain substituents on the aromatic ring and act primarily on dopamine receptors at therapeutic doses.[11] The $S(+)$- and $R(-)$-enantiomers have different pharmacokinetics and pharmacologic effects;[12,13] however, illicit and therapeutic use of MDMA usually involves the racemic (1:1) mixture of the enantiomers. Most animal toxicology studies on MDMA also administer racemic MDMA. The $S(+)$ form of MDMA is a more potent inhibitor of serotonin reuptake than the $R(-)$-enantiomer.[14,15] The subjective effects of MDMA correlate better to the pharmacokinetic profile of the $S(+)$-enantiomer.[16] The response to MDMA enantiomers may vary between animal species. For example, the hyperthermic response to the $R(-)$-enantiomer differs in non-human primates and rodents.[17]

MDMA is a white, crystalline powder that is a weak base with an estimated pK_a value between 9–10. The boiling point of the free base of MDMA is too high to volatilize sufficient amounts of MDMA to cause pharmacologic effects. The salts of MDMA are also not volatile, but the water solubility of these compounds
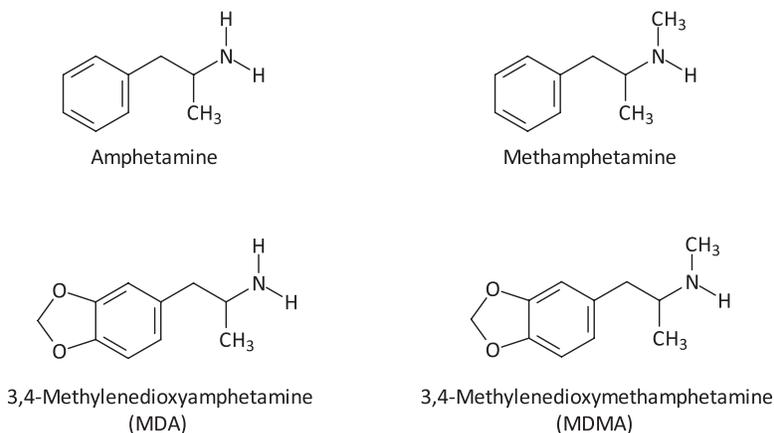
FIGURE 9.1. Structure of 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), amphetamine, and methamphetamine.

FIGURE 9.2. Confiscated ecstasy tablets. (Photo courtesy of the US Drug Enforcement Agency)

allows administration via insufflation, ingestion, or intravenous (IV) injection.[18] MDMA hydrochloride has a melting point of 148–153ºC (298–307°F). This salt is soluble in water, chloroform, and alcohol, but not soluble in ether. Besides ecstasy, common street names for MDMA include XTC, E, and Adam. Figure 9.2 displays samples of confiscated MDMA tablets.

EXPOSURE

Epidemiology

Historically, MDMA has been an experimental adjunct to psychotherapy in the United States for a wide variety of disorders including depression and substance abuse. Most countries list MDMA as a substance with no approved use (e.g., Drug Enforcement Agency schedule I of the Controlled Substances Act in United States). MDMA use occurs predominantly in dance clubs and party venues. The use of MDMA became popular as a means to enhance sociability, energy, endurance, and sexual feelings.

Sources

Like amphetamine and MDA, MDMA is a synthetic drug that does not occur naturally. Alexander Shulgin publicized a process for synthesizing MDMA in his book *PIHKAL* (*Phenylethylamines I Have Known and Loved*).[19] The initial precursor of this process was MDA (3,4-methylenedioxyamphetamine), and the process utilized formic acid; however, newer methods for the synthesis of MDMA use nonrestricted precursor agents.

The clandestine manufacture of MDMA now involves a large number of variations of the original synthetic process including safrole bromination, variations of the Leuckart method, and reductive amination with various reducing agents (Al/Hg, $NaBH_4$, $NaBH_3CN$).[20] 3,4-Methylenedioxyphenyl-2-propanone (MDP-2-P) is the starting material in a Leuckart reaction and reductive amination. MDP-2-P is commonly prepared by 2 different synthesis methods: (1) oxidation of isosafrole in an acid medium, and (2) reduction of 1-(3,4-methylenedioxyphenyl)-2-nitropropene.[21] The latter compound is prepared by condensation of piperonal and nitroethane. The end-product of all methods is a racemic mixture of $S(+)$- and $R(-)$-MDMA.

Methods of Abuse

MDMA is nearly always consumed as tablets of "ecstasy." Rarely, MDMA abuse involves other routes of exposure including nasal, pulmonary, rectal, and IV administration.[29] These tablets are typically branded with many colors and logos; there are thousands of different brands of tablets. Ecstasy tablets contain widely varying amounts of MDMA with the usual dose ranging from approximately 30–150 mg; occasionally, other phenethylamine drugs (e.g., MDA, MDEA, PMA, MBDB) are marketed as ecstasy. Analysis of 101 confiscated ecstasy tablets from nightclubs and late night venues in London and Swansea in 2006 demonstrated a mean MDMA content/tablet of 58.7 ± 22.9 mg with a range of 20–131 mg.[22] The vast majority of the tablets (i.e., 96%) contained <100 mg MDMA; there was a bimodal distribution of MDMA content at 20–40 mg and 60–80 mg. Regular users typically ingest 2–3 tablets of ecstasy, whereas the most experienced users may take 10–25 tablets in a single session.[23] Although escalation of the MDMA dose helps maintain the positive drug experience in tolerant users, bingeing (i.e., taking multiple tablets at 1 time or repeated doses during an evening) is relatively uncommon among MDMA users.[24] Frequently, adverse effects increase during dose escalation.

Desired effects of the use of MDMA include enhancement of mood, increased energy, feeling of closeness to others, empathy, increased sociability, mild perceptual disturbances (colors, sounds), sense of well-being, and emotional sensitivity. Structured interviews of recreational MDMA users suggest that the use of MDMA is associated with the perception of greater satisfaction with sex, but sexual performance is often impaired.[25] Early studies suggested that the use of MDMA was self-limited and primarily limited to ingestion.[26] The typical pattern of MDMA abuse is occasional social use with only a small proportion of users ingesting MDMA

more frequently than weekly.[27] Within a subset of intravenous polydrug users, the use of IV MDMA is much higher. However, structured interviews of these polydrug users indicate that the majority of these user switch back from IV MDMA use to oral or intranasal routes of exposure.[28]

Sporadic MDMA users do not usually develop dependence on MDMA; psychological dependence is rare.[29] Although non-human primates self-administer MDMA, the decreasing use of MDMA by these animals suggests a reduction in the reinforcing properties of the drug following continued use.[30] The frequent use of MDMA is usually associated with polydrug use, and most of these MDMA users prefer other drugs. Rarely, case reports associate the abuse of MDMA with self-treatment for post-traumatic stress syndrome.[29] A cohort of daily users of only MDMA has not been identified; therefore, research on the chronic effects of MDMA use in humans is invariably confounded.[31]

DOSE EFFECT

The potency of MDMA and MDA are similar (i.e., effective oral dose 100–160 mg). There are differences in the potency of MDMA stereoisomers with the $S(+)$ isomer of MDMA (effective dose 80–120 mg) being more potent than the $R(-)$-isomer (effective dose 300 mg).[32] Below doses of 1 mg MDMA/kg, volunteer studies indicate a lack of cardiovascular effects; above the 1 mg/kg-dose, the desired psychological effects are prominent along with adverse effects.[33] In a study of 13 MDMA-naïve healthy volunteers, a typical recreational dose (1.7 mg/kg) enhanced mood and caused a sense of well-being along with increased emotional sensitivity.[34] These subjective effects occurred in addition to moderate elevation of blood pressure. In this study, 1 participant developed transient severe hypertension (240/145 for 20 min) that resolved without complications or treatment. In a double-blind crossover study of 8 volunteers familiar with MDMA, the administration of a placebo and 0.5 mg/kg of MDMA produced no discernible subjective effects in a controlled laboratory setting. A 1.5 mg/kg oral dose of MDMA produces significant subjective effects and increased heart rate with the greatest effect 1–2 hours after dosing. In a study of 8 healthy volunteers, the ingestion of 1.5 mg MDMA/kg increased the mean peak values of the heart rate by 28 bpm, the systolic blood pressure by 25 mm Hg, and cardiac output by 2 L/min when compared with baseline.[35] The cardiovascular effects of this MDMA dose were similar to the effects of dobutamine at 20–40 μg/kg/min, but MDMA had no inotropic effects. This dose of MDMA also significantly increases plasma cortisol, prolactin, and dehydroepiandrosterone (DHEA) levels.[36] The administration of MDMA doses up to 1.9 mg/kg to volunteers does not significantly increase body temperature >0.4°C above baseline.[31]

Illicit Use

At recreational doses, MDMA produces dose-dependent subjective responses that are different from classical stimulants or hallucinogens. Most casual users of MDMA typically ingest 1–2 tablets (i.e., about 60–300 mg racemic MDMA) in a social setting (rave, concert, party, disco).[37,38] Tolerance to some of the effects of MDMA develops during chronic use, and more experienced MDMA users typically ingest larger doses than novice users.[23] In a survey of >1,000 ravers/clubbers, 54% reported the use of ≥5 tablets per session, whereas 2% reported the use of ≥20 tablets per session. The variables that potentially affect the response to MDMA include the dose, the presence of impurities or adulterants, individual metabolic rates, ambient temperature, fluid intake, strenuous exercise, cardiovascular fitness, and hyperthermia.[32] The maximum tolerated dose in healthy individuals has not been determined.

Human Toxicity

Available data suggests that serious toxicity from the ingestion of MDMA is idiosyncratic rather than dose-related, reflecting individual variation in the clinical response to MDMA. After the ingestion of 40 tablets of ecstasy by a 19-year-old man, the only adverse effects were a heart rate of 95 bpm, mydriasis, confusion, mild sedation, and retrograde amnesia.[39] A 30-year-old man developed coma and seizures after the ingestion of 50 tablets of ecstasy.[40] His peak recorded core temperature was 38.7°C (101.7°F); he survived with supportive care. The ingestion of 1 tablet of ecstasy by a 13-month-old child was associated with hypertension, tachyarrhythmias, and seizures, but there was no significant increase in body temperature.[41] The child survived with intensive supportive care.

The minimum fatal human dose is difficult to estimate because of the wide variability of drug dose per tablet, concomitant ingestion of other drugs, and the idiopathic response of individuals to MDMA. Compared with the prevalence of MDMA abuse, fatalities associated with MDMA are extremely rare. Additionally, most of these fatalities involve the concomitant ingestion of other substances. However, deaths from MDMA ingestion alone occasionally occur in young healthy adults following the ingestion of recreational doses of MDMA.[42]

Animal Toxicity

MDMA and amphetamine have similar toxicity in animal models. The minimal lethal MDMA dose varies with age, route of exposure, environmental conditions, and animal species. Data from animal studies demonstrated the following LD_{50}: mice, 97 mg/kg intraperitoneal; rats, 49 mg/kg intraperitoneal; guinea pig, 98 mg/kg intraperitoneal; dog, 14 mg/kg IV; and monkey, 22 mg/kg IV.[43] The LD_{50s} of MDMA following ingestion are about 3-fold higher than intraperitoneal administration. However, high ambient temperatures and strenuous activity greatly increase the lethal toxicity of MDMA as seen with other stimulants (e.g., methamphetamine).[44] The IV LD_{50} of MDMA in adult, nontolerant monkeys maintained in primate chairs was approximately 15–20 mg/kg. Young monkeys ($LD_{50} = 5$ mg/kg) and adult monkeys in open cages ($LD_{50} = 2$–3 mg/kg) were more vulnerable, particularly when hyperactivity and elevated body heat occur.[45]

TOXICOKINETICS

The pharmacokinetics of MDMA are nonlinear, resulting in a disproportionate increase in the maximum MDMA concentration as the dose increases.[46] Although there are limited human data, existing pharmacokinetic data suggest that the bioavailability is many times higher, the half-life longer and total and hepatic clearance shorter with increasing doses of MDMA. The irreversible inhibition of CYP2D6 by MDMA and MDA contributes to nonlinear pharmacokinetics of MDMA by increasing bioavailability and reducing clearance of MDMA following repeat dosing.[47,48] Consequently, crossover pharmacokinetic studies in volunteers and recreational MDMA users must be interpreted cautiously because of the potential inhibition of hepatic clearance by repeat dosing. The pharmacokinetics of MDMA are also stereoselective. The bioavailability (f), estimated volumes of distribution (V_d), and clearance (Cl) of the 2 MDMA enantiomers are significantly different. V_d/f was 1.5 times higher for the more potent S(+)-enantiomer; total oral clearance (Cl/f) was 2.5 times higher for the S(+)-enantiomer (131 vs. 55 L/h) compared with the R(−)-enantiomer.[16]

Absorption

Volunteer studies indicate that peak plasma MDMA concentrations occur within 2–3 hours after the ingestion of 100 mg tablets.[46] As a weak base, absorption of MDMA probably occurs predominantly in the small intestine, and the amount of MDMA absorption depends on gastric emptying and pH. There are few data on the bioavailability of MDMA following ingestion because of the lack

of pharmacokinetic data on the IV administration of MDMA. However, pharmacokinetic studies suggest a substantial first-pass metabolism because the time to peak metabolite concentrations occurs prior to the peak MDMA concentration.[16] In a study of 8 healthy volunteers ingesting 100 mg MDMA, the mean maximum MDMA concentration was 222 ± 26 ng/mL at 1–3 hours after ingestion.[46] Table 9.1 lists pharmacokinetic data on peak concentrations and time-to-peak concentrations of MDMA and some of the MDMA metabolites following the ingestion of 100 mg MDMA by 8 healthy volunteers. There are few pharmacokinetic data on MDMA following routes of exposure other than ingestion.

In a study of 8 healthy volunteers receiving low dose (1 mg/kg) and high dose (1.6 mg/kg) MDMA, the mean peak plasma MDMA concentrations were 161.4 ± 11.5 ng/mL and 305.7 ± 16.9 mg/mL, respectively.[49] Peak subjective effects occurred 1–2 hours after ingestion with no secondary peak effects. Figure 9.3 displays the plasma MDMA concentration during the first 47 hours after ingestion of a low dose (1 mg/kg) and higher dose (1.6 mg/kg) of MDMA.

Distribution

MDMA distributes widely throughout the body including the liver, kidney, lungs, and brain. MDMA binds with weak affinity to albumin. Although no human data are available on the percentage of binding of MDMA to proteins, extrapolation of animal and human data from related amphetamine compounds suggests that the binding of plasma MDMA to proteins in humans is low (approximately 20%).[46] The volume of distribution of MDMA in therapeutic doses is about 6 L/kg.[16] The distribution of MDMA is stereoselective. In a postmortem study of the distribution of MDMA enantiomers, the concentration of the R(−)-enantiomer was slightly

TABLE 9.1. Mean (± Standard Deviation) Pharmacokinetic Parameters for 3,4-methylenedioxymethamphetamine (MDMA) and MDMA Metabolites Based on the Administration of 100 mg MDMA to 8 Volunteers).[46]

Compound	C_{max} (ng/mL)	t_{max} (Hours)	$t_{1/2}$ (Hours)
MDMA	222.5 ± 26.1	2.3 ± 1.1	9.0 ± 2.3
MDA	13.1 ± 4.5	6.7 ± 2.6	24.9 ± 14.5
DHMA	154.5 ± 76.6	1.2 ± 0.3	13.4 ± 8.1
HMMA	236.7 ± 87.1	2.3 ± 0.9	11.2 ± 2.9
HMA	7.5 ± 4.0	8.2 ± 1.7	37.4 ± 17.9

Abbreviations: C_{max} = peak plasma concentration; t_{max} = time to peak plasma concentration; $t_{1/2}$ = plasma elimination half-life; DHMA = 4-dihydroxymethamphetamine; HMMA = 4-hydroxy-3-methoxymethamphetamine; MDA = 3,4-methylenedioxyamphetamine; HMA = 4-hydroxy-3-methoxyamphetamine.
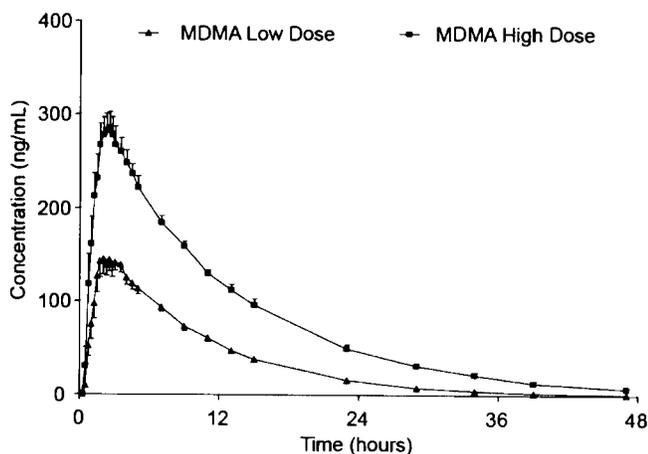
FIGURE 9.3. Mean 3,4-methylenedioxymethamphetamine (MDMA) plasma concentration ($n = 8$) in a double-blind, randomized, controlled clinical trial following the ingestion of low dose (1mg/kg) and high dose (1.6 mg/kg) MDMA. (Reprinted with permission from EA Kolbrich, RS Goodwin, DA Gorelick, RJ Hayes, EA Stein, MA Huestis, Physiological and subjective responses to controlled oral 3,4-methylenedioxy-methamphetamine administration, Journal of Clinical Psychopharmacology, Vol. 28, Issue 4, p. 438, copyright 2008.)

greater than the $S(+)$-enantiomer in the blood, while the concentration of the $R(-)$-enantiomer was substantially greater than the $S(+)$-enantiomer in the bile, vitreous humor, and urine.[50] Most of the tissues contained higher concentrations of $R(-)$-enantiomer than the $S(+)$-enantiomer with the exception of the liver, where the concentrations of the 2 enantiomers were similar.

Biotransformation

The metabolism of MDMA involves 2 main pathways as demonstrated in Figure 9.4.[51] The major pathway is *O*-demethylenation of MDMA to 3,4-dihydroxy-methamphetamine (DHMA) and the subsequent *O*-methylation by catechol-*O*-methyltransferase (COMT) to 4-hydroxy-3-methoxymethamphetamine (HMMA) and/or conjugation with glucuronide or sulfate. The minor pathway is *N*-dealkylation to 3,4-methylene-dioxyamphetamine (MDA). This pathway subsequently follows a similar metabolic pathway as MDMA with *O*-demethylenation to 3,4-dihydroxyamphetamine (HHA) and *O*-methylation to 4-hydroxy-3-methoxy-amphetamine (HMA). A small proportion of these
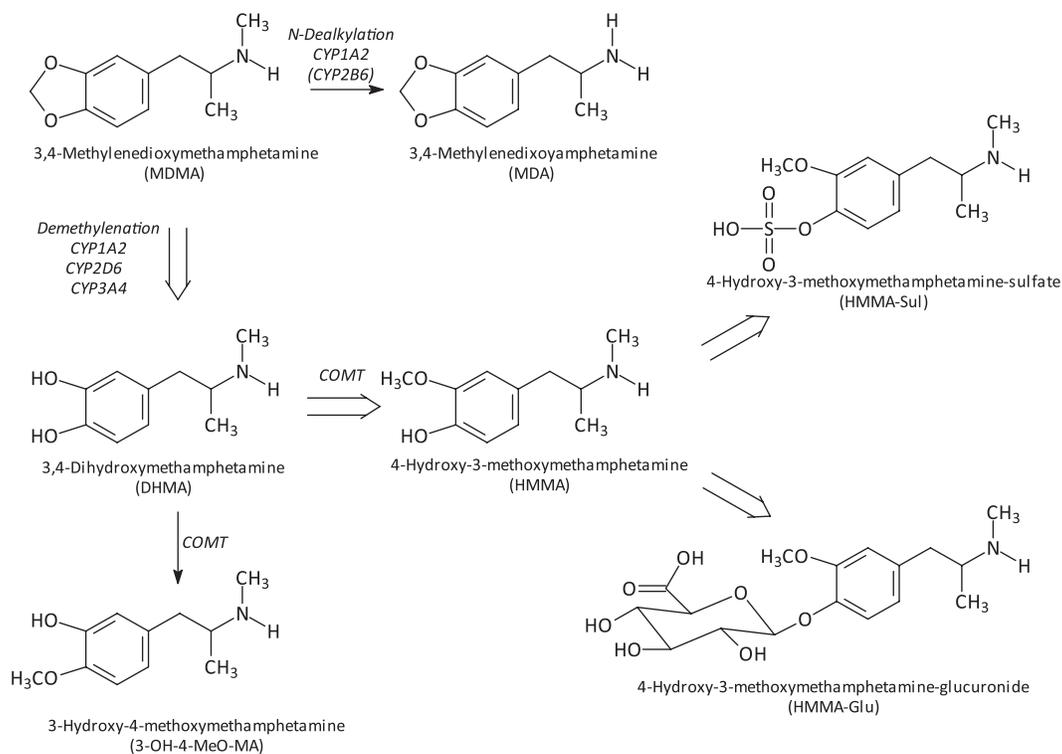
FIGURE 9.4. Biotransformation of 3,4-methylenedioxymethamphetamine (MDMA) with major (thicker line) and minor (thinner line) pathways. DHMA = 3,4-dihydroxymethamphetamine; COMT = catechol-*O*-methyltransferase; HMMA = 4-hydroxy-3-methoxymethamphetamine; MDA = 3,4-methylenedioxyamphetamine; HHA = 3,4-dihydroxyamphetamine; HMA = 4-hydroxy-3-methoxyamphetamine.

metabolites undergo further *N*-dealkylation, deamination, and oxidation to benzoic acid derivatives followed by conjugation with glycine. All the psychologically inactive metabolites (HMMA, DHMA, HHA, HMA, benzoic acid derivatives) are subsequently conjugated with glucuronide, sulfate, or glycine.[52]

The main CYPP450 isoenzymes involved with the demethylenation pathway are CYP2D6, CYP1A2, and CYP2B6 as demonstrated in Figure 9.4.[53] *In vitro* studies indicate that this pathway demonstrates biphasic kinetics with high- and low-affinity components. CYP2D6 is the primary CYP450 isoenzyme involved with the low-affinity component, whereas CYP1A2 and to a lesser extent CYP2B6 and CYP3A4 regulate the high-affinity component.[46] The minor pathway of *N*-dealkylation to MDA occurs primarily via CYP1A2 and CYP2B6 isoenzymes. This pathway has a maximal capacity 7- to 40-fold lower than the major demethylenation pathway *in vitro*, and the contribution of this pathway to the overall biotransformation of MDMA is relatively small compared with the demethylenation pathway. Some minor metabolites of MDMA are more neurotoxic *in vitro* than MDMA or MDA including *N*-methyl-α-methyldopamine (DHMA), α-methyldopamine (HHA), and their conjugates (glutathione, *N*-acetylcysteine).[54] However, the clinical significance of these minor metabolites is equivocal because of the low concentration of these metabolites. Although MDA is a metabolite of MDMA, the concentration of MDA is probably too low to cause pharmacologic or toxic effects.[46]

Approximately 5–10% of Caucasians are "poor metabolizers" who lack significant CYP2D6 activity. However, these individuals are not significantly more susceptible to adverse effects from the ingestion of MDMA because this pathway has low capacity and the auto-inhibition of CYP2D6 by repeat dosing of MDMA rapidly converts "extensive metabolizer" phenotype to the "poor metabolizer" phenotype.[55] In a study of poor and extensive metabolizers of CYP2D6, the poor metabolizer had relatively higher concentrations of MDMA after the first dose of 100 mg racemic MDMA, but all participants had similar MDMA concentrations following the second dose of MDMA 24 hours later.[57] CYP2D6 has a high affinity (i.e., low K_m), but this isoenzyme also has a low capacity for MDMA. Other enzymes with higher capacity (e.g., CYP1A2, CYP3A4) metabolize most of the MDMA in humans.[56] Consequently, the metabolism of MDMA at high MDMA concentrations by patients with low CYP2D6 activity (i.e., poor metabolizer) is similar to the more common phenotypes.[57] Additionally, studies of fatalities associated with the ingestion of MDMA do not support an association between variations in CYP2D6 genotype and fatal outcome.[58]

The extrapolation of pharmacokinetic data from animals to humans is limited by the substantial variation in metabolism between various animal species. Although the metabolic pathways via CYP450 isoenzymes are similar between animal species, the amount of MDMA metabolized by various pathways differs between animal species. In rats, the main metabolite is MDA compared with DHMA in humans.[59] Animal studies suggest that some potential MDMA metabolites (e.g., 2,4,5-trihydroxymethamphetamine) may be neurotoxic, but the relevance of these metabolites to human toxicity is unclear.[60]

Elimination

The kidneys excrete from 10–20% of a typical dose of MDMA unchanged in urine, depending on the dose and urine pH.[46] Although there are limited human data, the amount of urinary excretion probably decreases when the urine pH is alkaline. However, changes in urine pH probably do not significantly affect the elimination half-life of MDMA because the relatively clearance of unchanged MDMA in the urine is small. The clearance of MDA formed from the biotransformation of MDMA also varies with urine pH. The average plasma elimination half-life of racemic MDMA is about 7–8 hours following ingestion of recreational doses of MDMA (i.e., 100 mg).[18] The elimination half-lives of the 2 enantiomers of MDMA are variable and dose-related, but the ratio of the *R*(−)-isomer to the *S*(+)-isomer of MDMA always exceeds 1 because of the relatively faster elimination of the *S*-(+)-isomer. Following the ingestion of 100 mg racemic MDMA by healthy recreational drug users, the mean plasma elimination half-lives of *S*(+)-MDMA and *R*(−)-MDMA enantiomers were 4.8 hours and 14.8 hours, respectively.[13] In a study of 8 healthy volunteers ingesting 40 mg racemic MDMA, the plasma elimination half-lives of *S*(+)-MDMA and *R*(−) MDMA enantiomers were 3.6 hours and 5.8 hours, respectively.[16] During the first 29 hours following the ingestion of MDMA by an 8-month-old child sufficient to cause seizures, tachycardia, hypertension, elevated body temperature (38.9°C/102.0°F), and rhabdomyolysis, the estimated serum elimination half-life of MDMA was about 6 hours.[61] The mean Cl/f values of the *S*(+) enantiomer was 131 L/h (range, 51–276 L/h), whereas the mean value for the *R*(−)-enantiomer was 55 L/h (range, 19–109 L/h). In another study of recreational MDMA users, values for the clearance of racemic MDMA ranged from 26–800 L/h with the highest value occurring in only 1 individual ingesting a dose of 50 mg.[46] Renal clearance of MDMA typically ranges from 5–20 L/h. Hepatic clearance is far more variable with values ranging from 15–150 L/h.[16] The main metabolites excreted in the urine are conjugates of HMMA, and

these metabolites account for over 50% of MDMA metabolites in the urine. Unchanged MDMA accounts for the majority of the rest of the measured excretion with an increasing proportion of unchanged MDMA appearing in the urine as the dose of MDMA increases. MDA and HMA conjugates accounted for less than 5% of total MDMA elimination.[46]

Tolerance

Current data indicate that chronic pharmacodynamic tolerance develops following the regular use of MDMA based on the escalation of dose ingested by more experienced ecstasy users. Anecdotally, human volunteers report less desired effects following repeated MDMA doses, and regular MDMA users usually ingest higher doses than naïve MDMA users. In a multicenter Internet study of 109 novice ecstasy users (<10 lifetime occasions), 136 moderate ecstasy users (10–99 lifetime occasions), and 37 heavy ecstasy users (>100 lifetime occasions), the number of novice and heavy users reportedly ingesting more than 10 tablets was 0% and 35%, respectively.[62] Tolerance to some of the acute effects of MDMA occurs rapidly (i.e., within a day); the development of this tolerance is not related to changes in MDMA metabolism as the administration of repeated doses of MDMA is associated with reduced clearance (i.e., higher concentrations).[55]

Interactions

There are many theoretical pharmacokinetic and pharmacodynamic interactions (e.g., inhibition of CYP2D6, CYP1A2, CYP3A4, and CYP2B6) in addition to acidification of the urine (e.g., vitamin C). However, the clinical relevance of these potential interactions is questionable because the biotransformation of MDMA involves several CYP450 isoenzymes and the renal clearance of unchanged MDMA is low. *In vivo* studies of healthy volunteers indicate that inhibitors of CYP2D6 (e.g., paroxetine) increase the plasma MDMA concentration 20–30% compared with control volunteers, but pharmacodynamic effects of pretreatment with paroxetine attenuates the physiologic and psychologic effects of MDMA despite these pharmacologic changes.[63] Other pharmacodynamic interactions include attenuation of the clinical effects of MDMA by serotonin reuptake inhibiting drugs (SSRIs, tricyclic antidepressants), which competitively inhibit the binding of MDMA to the serotonin transporter.[64] The risk of the serotonin syndrome after ingestion of MDMA increases following the concomitant use of stimulants (cocaine, methamphetamine), inhibitors of serotonin metabolism, and monoamine oxidase inhibitors.[65] Case reports of

extremely potent inhibitors of multiple CYP450 isoenzymes (CYP2D6, CYP2B6, CYP3A4, CYP1A2, CYP2C9) suggest that some of these potent inhibitors (e.g., ritonavir) enhance the clinical effects of MDMA[66] and these effects may be potentially fatal.[67] Case reports associated the ingestion of MDMA and moclobemide with the development of fatal serotonin syndrome.[68] A 50-year-old man developed severe hypertension, diaphoresis, agitated delirium, and hypertonicity lasting 5–6 hours after the ingestion of MDMA and the nonselective monoamine oxidase inhibitor, phenelzine.[69] In a cross-over study of 9 healthy volunteers, the concomitant administration of 0.8 g ethanol/kg increased the mean plasma MDMA concentration approximately 13% and the plasma ethanol concentration decreased 9–15%, when compared with the administration of each drug alone.[70] The MDMA–alcohol combination induced longer lasting euphoria and well-being than MDMA or alcohol alone, but MDMA did not reverse the impairment of ethanol on psychomotor skills. Animal studies suggest that the concomitant administration of caffeine with MDMA may potentiate the hyperthermic and cardiovascular response to MDMA,[71,72] but there are few human data to determine the clinical significance of this potential interaction. The coadministration of 100 mg MDMA and 16 mg tetrahydrocannabinol did not result in any statistically significant alternation in plasma MDMA concentrations.[73]

Maternal and Fetal Kinetics

In studies of rats, MDMA and MDA easily cross the placenta with peak amniotic fluid and fetal brain concentrations occurring within 2 hours. The pattern of MDMA and MDA concentrations in the amniotic fluid mirrors the change in maternal plasma concentrations, but the peak concentrations in amniotic fluid were approximately half that of maternal plasma.[74] There are inadequate data in humans to evaluate the pharmacokinetics of MDMA during pregnancy.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

The primary physiologic effects of MDMA result from alterations of the release, uptake, and metabolism of neurotransmitters (serotonin, dopamine, norepinephrine) that cause an acute increase in the intrasynaptic concentration of these neurotransmitters followed by a period of depletion.[51] These compounds are involved in the control of mood, sleep, appetite, reward, thermoregulation, and the autonomic nervous system.[75]

133

In general, the response to MDMA is stronger in women than men including higher ratings for positive mood and altered perception (time, space) as assessed by standardized psychometric rating scales in double-blind, placebo controlled clinical trials with MDMA doses ranging from 70–150 mg.[76] Additionally, the increased sensitivity to MDMA in women is associated with more side effects (difficulty concentrating, thirst, impaired balance, decreased appetite, jaw clenching). The overall psychologic effects of MDMA largely result from the carrier-mediated, presynaptic release of serotonin, whereas the more stimulant-like euphoric effects of MDMA probably result, at least in part, from dopamine D_2 receptor stimulation.[77] In animal studies, the administration of selective serotonin reuptake inhibitors blocks the MDMA-induced release of serotonin and dopamine along with reducing the behavioral effects of MDMA.[78] MDMA has a high affinity for the presynaptic serotonin transporter ($EC_{50} = 8\,\mu M$), and reversal of the serotonin uptake transporter is the main mechanism for the increase in the presynaptic release of serotonin.

Binding to the presynaptic serotonin transporter inhibits reuptake and increases movement of serotonin into the synapse. This action initially causes increased concentrations of serotonin and major serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), but subsequently 5HIAA concentrations decline. The administration of MDMA (20 mg/kg subcutaneously, twice daily for 4 days) to rats caused marked reductions (30–60%) in the concentration of 5HIAA in cerebral cortex, hippocampus, striatum, hypothalamus, and midbrain at 2 weeks after the cessation of MDMA administration.[79] In addition, MDMA caused substantial reductions (50–75%) in the density of [3H]-paroxetine-labeled serotonin uptake sites in these regions of the brain. Long-term, the administration of MDMA causes a significant loss of serotonin and 5-HIAA in most regions of the forebrain. A similar pattern in extracellular serotonin concentrations occurs in animal studies. The administration of MDMA also causes a marked, irreversible inhibition of the rate-limiting enzyme tryptophan hydroxylase in the synthesis of serotonin. The onset of this inhibition begins several hours after peak CNS concentrations occur, and this effect persists many days to weeks until the synthesis of new enzymes. Nonneurotoxic doses of MDMA cause a major loss of tryptophan hydroxylase activity for up to 2 weeks, whereas a neurotoxic dose decreases tryptophan hydroxylase activity for up to 110 days following the administration of MDMA.[80]

In addition to interacting with the serotonin transporter site, MDMA has moderate affinity for serotonin receptors. Serotonergic 5-HT$_2$ receptor stimulation probably accounts for the mild hallucinogen-like perceptual effects associated with MDMA.[81] The affinity of MDMA for the norepinephrine and dopamine transporters is at least 10-fold lower than the serotonin transporter. The effect of MDMA on noradrenaline and dopamine release and the subsequent depletion of these compounds are less potent than the effects of MDMA on serotonin. MDMA binds to many receptors other than the serotonin transporter in 1–100 µM range including 5-HT$_1$ and 5-HT$_2$, H$_1$-histaminic, M$_1$- and M$_2$-muscarinic, and α_1-, α_2- and β-adrenergic receptors.[82] Activation of norepinephrine receptors produces sympathomimetic effects including elevation of blood pressure, whereas the stimulation of dopamine D_2 receptors probably accounts for at least part of the euphoria associated with MDMA.[81] In the rat brain, MDMA has the highest affinity for the serotonin transporter ($K_i = 0.61\,\mu M$) and lesser affinity for the α_2-adrenergic ($K_i = 3.6\,\mu M$), 5-HT$_2$ ($K_i = 5.1\,\mu M$), H$_1$-histaminic ($K_i = 5.7\,\mu M$), and M$_1$-muscarinic ($K_i = 5.8\,\mu M$) receptors.[81] Both enantiomers of MDMA competitively inhibit serotonin catabolism in the brain by monoamine oxidase A (MAOA) and B (MAOB). Based on *in vitro* studies of rat brain homogenates, the inhibition of MAOA and MAOB by MDMA enantiomers is similar. In these studies, the mean IC_{50} values of *S*(+)-MDMA for these 2 enzymes were $44 \pm 6.06\,\mu M$ and $370 \pm 4.68\,\mu M$, respectively, compared with $56 \pm 8.24\,\mu M$ and $378 \pm 6,29\,\mu M$, respectively, for *R*(−)-MDMA.[83] The differences in the mean IC_{50} values between the 2 enantiomers were not statistically significant.

Mechanism of Toxicity

MDMA acts primarily as an indirect serotonergic, dopaminergic, and sympathomimetic drug; therefore, MDMA causes a very wide range of physiologic and behavioral effects in animals and humans. These primarily affect CNS functions (e.g., thermoregulation, thirst, appetite, sleep, motor activity, attention, aggression, sexual and operant behavior) and to a lesser extent, the cardiovascular system. Secondary effects occur on other neurotransmitters and hormones including increased release of acetylcholine in the CNS as well as systemic increases in corticosterone, prolactin, renin, aldosterone, oxytocin, and vasopressin.[51]

Immunohistochemical studies indicate that the administration of MDMA to experimental animals causes the apparent loss of serotonin nerve terminals. Biochemical studies demonstrate loss of [3H]-paroxetine binding to the presynaptic serotonin transporter and a decrease in tryptophan hydroxylase activity. The most common manifestations of acute toxicity from MDMA are predictable from the pharmacologic properties of MDMA. Behavioral effects (e.g., increased activity, impaired hypothalamic homeostatic functions) result in

mild hyperthermia, fatigue, and dehydration. The magnitude of these effects is increased by high ambient temperatures and fluid depletion. The psychologic effects of MDMA can cause acute anxiety, paranoia, and hyperventilation in susceptible individuals.

CENTRAL NERVOUS SYSTEM

ANIMAL.   In animal studies, low doses of MDMA cause increased alertness and physical activity, increased social interaction, and reduced aggression. Larger doses cause stereotyped movements, hyperreactivity, dystonic and dyskinetic postures, piloerection, and salivation.[84] These results are consistent with subjective reports from human studies, where low doses of MDMA result in increased alertness, euphoria, increased confidence, gregariousness, enhanced mental and physical activity, and improved self-esteem.[85] MDMA causes selective and persistent neurotoxic effects on central serotonergic neurons in laboratory animals. In these studies, the administration of MDMA produces persistent reductions in regional brain concentrations of serotonin, the rate-limiting enzyme (tryptophan hydroxylase) of serotonin synthesis, serotonin transporter, and vesicular monoamine transporters up to 8 weeks after administration of MDMA.[86,87] The affinity of MDMA is much less for the dopamine transporter than the norepinephrine transporter.[88] At single MDMA doses (10–20 mg/kg) typically associated with serotonin depletion in rats, increased markers of neurotoxic damage (i.e., cell death, reactive gliosis, silver staining) are not reliably present.[89] Immunohistochemical techniques document a loss of serotonin axons and axon terminals in MDMA-treated animals with higher doses of MDMA (20 mg/kg, subcutaneously, twice daily for 4 days).[90] However, extrapolation of animal data to effects found in humans is complicated by the following factors: 1) animal models of MDMA-induced neurotoxicity do not mimic typical human patterns of ecstasy use, 2) lack of correlation of functional deficits to brain imaging, 3) confounding variables in existing epidemiologic data (polydrug use, drug purity, reporting bias, premorbid personality abnormalities), and 4) the possible role of preexisting deficits in serotonergic systems, impulse control, and cognition that potentially predispose individuals to the abuse of drugs (i.e., MDMA).[85,91] Animal studies indicate that the $S(+)$-isomer of MDMA is more neurotoxic than the $R(-)$-isomer.[92]

HUMAN.   Clinical studies on abstinent MDMA users suggest the possibility of neurotoxic effects of MDMA on brain serotonin neurons, but the findings are inconclusive. In current MDMA users, positron emission tomography (PET) scanning demonstrates alterations of the serotonin transporters in some serotonin-rich areas of the brain (mesencephalon, putamen, caudate, thalamus). The reduction in serotonin density is dose- and time-dependent; the changes correlate to reported use and slowly resolve during long-term abstinence.[86,93] Although these effects are at least partially reversible in some recreational MDMA, studies to date have not determined the full reversibility of these abnormalities. In a study of current MDMA users, former MDMA users, and controls (drug-naïve controls, other psychoactive drugs than MDMA) using the serotonin transporter ligand [11]C-(+)-McN5652, PET scanning detected a statistically significant reduction in serotonin availability in the mesencephalon and thalamus in the current users when compared with controls.[94] These changes persisted at least several weeks, but there was no statistically significant reduction in serotonin availability in former MDMA users, when compared with controls. Subsequent re-analysis of this data using Voxel-based comparisons between the groups with statistical parametric mapping confirmed the reduced serotonin binding potential in the striatum and the thalamus, but not in the neocortical areas of the brain of current MDMA users.[95] Depletion of serotonin is not necessarily synonymous with anatomic damage to neurons.

THERMOREGULATION

In animal studies, MDMA impairs thermoregulation, and the administration of MDMA activates mechanisms that conserve and generate heat. The animal data suggest that MDMA-induced stimulation of serotonergic pathways at relatively warm ambient temperatures causes an elevation in metabolic rate and peripheral vasoconstriction. In male, Long-Evans rats, the administration of 30 mg MDMA/kg doubled the metabolic rate at room temperatures of 20°C (68°F), when compared to the saline placebo group.[96] However, there was no difference in metabolic rate between these 2 groups at a room temperature of 10°C (50°F). This dose of MDMA caused a 3.2°C increase in core temperature at an ambient temperature of 30°C (86°F) compared with no change at 20°C (68°F) and a 2.0°C decrease at a room temperature of 10°C (50°F). Thus, the increased metabolic rate and peripheral vasoconstriction may produce life-threatening elevations in core temperature at high ambient temperatures.

The effect of MDMA on body temperature is complex because this drug affects the release and receptor activation of all 3 major monoamine neurotransmitters (i.e., serotonin, dopamine, norepinephrine).[97] In animal studies, low environmental temperatures reduce the inhibition of serotonin synthesis and the neurotoxicity associated with the administration of MDMA, whereas high environmental temperatures enhance the

hyperthermia and neurotoxicity associated with MDMA.[98,99] Although high ambient temperatures enhance the effect of MDMA on core temperatures, the most important hyperthermic effects probably are centrally mediated. Both serotonin and dopamine are involved in the central control of thermoregulation; however, these effects are primarily mediated by dopamine rather than serotonin based on animal studies. In these studies, the hyperthermic response is blocked by D_1 receptor antagonists, but not by serotonin 5-HT$_2$ antagonists or fluoxetine. Rodent studies suggest that alpha$_1$- and beta$_3$-adrenoreceptors modulate the hyperthermic response to MDMA in some animals;[100] however, alpha$_1$-adrenoreceptor antagonists attenuate the MDMA-induced hyperthermic response to a substantially greater degree than beta$_3$-adrenoreceptor antagonists.[101] This response is relatively small and the clinical significance is unclear.

HYPONATREMIA

The administration of MDMA to healthy volunteers produces a slight increase in body temperature. Following the ingestion of 1.5 mg MDMA/kg by 14 healthy volunteers, the mean peripheral body temperature increased 0.3ºC; the concomitant ingestion of a 5-HT$_{2A}$ antagonist (ketanserin) abolished this increase.[102] Consequently, other factors probably contribute to the development of severe hyperthermia including an idiopathic response, strenuous physical activity, inadequate fluid replacement, and high ambient temperatures. MDMA-induced hyponatremia and cerebral edema is a rare cause of death that is not associated with hyperthermia. Hyponatremia may result from the excessive consumption of water while exercising vigorously during MDMA intoxication. Additionally, the ingestion of MDMA is associated with the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). Animal studies indicate that in addition to MDMA, metabolites (e.g., 4-hydroxy-3-methoxymethamphetamine, HMMA) of MDMA contribute to the increased secretion of antidiuretic hormone, arginine vasopressin.[103,104] Case reports of MDMA-related hyponatremia suggest that the cause of low serum sodium concentrations is multifactorial (i.e., MDMA-induced arginine vasopressin secretion, drug-induce thirst, ingestion of hypotonic fluids).[105]

HEPATOTOXICITY

The variable patterns of hepatotoxicity following MDMA ingestions suggest different mechanisms of toxicity. Severe hepatic damage may occur during MDMA intoxication as a result of severe hyperthermia.[106] However, case reports suggest that hepatic failure in chronic MDMA users may develop in the absence of documented hyperthermia.[107] In some of these cases, the presence of acute, recurrent hepatitis in chronic MDMA users is suggestive of features of autoimmune hepatitis including eosinophilic infiltration of the liver.[108] Typically, the severity of the liver damage does not correlate with the frequency or amount of MDMA consumption; thus, the reaction may be idiopathic or the result of a contaminant.

Postmortem Examination

In animal studies, the administration of lethal doses of MDMA produces pathologic changes resembling experimental hyperthermia. The results are similar in most fatal MDMA intoxications, although there is some variability due to contributory mechanisms of death (cerebral edema, multiorgan failure). In a case series of 5 fatalities related to MDMA, the most common postmortem abnormalities occurred in the liver and ranged from focal hepatocyte necrosis to centrilobular necrosis.[109] Postmortem abnormalities in most of these cases were consistent with hyperthermia. Centrilobular necrosis of the liver is particularly common in MDMA deaths associated with hyperthermia. Sinusoidal congestion and dilation, portal and sinusoidal inflammation, microvesicular fatty changes, and cholestasis may be present in these fatal cases. Myocardial abnormalities predominantly involve contraction band necrosis and patchy foci of necrosis with mixed inflammatory infiltrates, similar to catecholamine-induced myocardial damage. In the brain, perivascular hemorrhage and hypoxic changes are consistent with generalized organ dysfunction, and congestion of the internal organs is usually present. Gross cerebral edema, focal hemorrhage in the brain, uncal herniation, and pituitary necrosis occur in cases associated with fatal hyponatremia. Postmortem examination of individuals who died after MDMA use found a relatively high incidence of cardiovascular disease, similar to cocaine and methamphetamine abusers. A retrospective review of 82 autopsy cases with MDMA listed as a cause of death demonstrated the following pathology: mild atherosclerosis, 18%; moderate atherosclerosis, 9%; severe atherosclerosis, 14%; cardiomegaly, 18%; and ventricular hypertrophy, 7%.[110] Cerebrovascular malformations occurred in 4% of the cases. The median age of these cases was 26 years (range, 17–58 years).

CLINICAL RESPONSE

Illicit Use

The adverse effects of MDMA usually result from an accentuation of the pharmacologic properties (i.e., sym-

pathomimetic stimulation) of MDMA on the cardiovascular and central nervous systems. Serious effects following MDMA use are relatively rare.[37,110] However, the concomitant use of other drugs of abuse (e.g., ethanol, cocaine, amphetamines) and adulteration of ecstasy tablets with other amphetamine designer drugs (e.g., PMA) commonly complicate the clinical presentation of MDMA users and increases the incidence of serious effects (e.g., delusions, delirium, acute psychosis, suicidal ideation, seizures, hyperthermia, hypotension).[111,112] In a case series of emergency department (ED) visits resulting from MDMA use, the concomitant use of cocaine use increased the incidence of panic attacks compared with the use of MDMA alone.[37] Similarly, the concomitant use of opiates or γ-hydroxybutyrate was more commonly associated with deep coma than the use of MDMA alone. Adverse effects associated with the recreational use of MDMA include bruxism,[113] trismus, anorexia, dry mouth, thirst, diaphoresis, blurred vision, impaired gait and balance, restless legs, difficulty sleeping, mydriasis, excitement, tachycardia, palpitations, lethargy, poor concentration, incontinence, paresthesias, and other psychiatric symptoms.[114] With successive doses, the adverse effects become more common and the desired effects less intense. Larger doses may cause an acute panic attack characterized by dysphoria, agitation, and hyperventilation.[115,116] Dysphoria, nausea, vomiting, headache, sweating, apprehension, confusion, and tremulousness complicate the use of MDMA as the dose increases. Altered mental status is the most common abnormality in patients presenting to the emergency department following MDMA use, particularly among naive users.[37] MDMA abuse frequently is associated with minor movement disorders such as tics (e.g., eye blinking, jaw jerks, hip turning, humming, panting, muscle jerks) based on case reports. Table 9.2 compares the frequency of reported clinical abnormalities by patients presenting to 3 EDs with self-reported MDMA intoxication.

Behavioral Abnormalities

The recreational use of MDMA is associated with moderate derealization (i.e., loss of sense that people or objects in your surroundings are real), but not typically with hallucinations. Moderate doses of MDMA generally produce effects described in positive terms by the recipients including a sense of closeness to others, elevation of mood, talkativeness, and less commonly, altered visual perceptions. In a survey of 20 psychiatrists self-administering MDMA, structured interviews revealed the following subjective effects: altered time perception, 90%; increased communication, 85%; decreased defensiveness, 80%; reduced fear, 65%; reduced sense of

TABLE 9.2. Comparison of Reported Clinical Features of 3,4-methylenedioxymethamphetamine (MDMA) Intoxication from Emergency Department Studies in Zurich,[37] London,[111] and Barcelona.[116]

Study Location	Zurich	London	Barcelona
Study Size (n)	52	48	135
Physical Signs (%)			
Tachycardia (>100/min)	54	67	25
Bradycardia (<60/min)	17	NR	2
Hypertension (SBP >160 mm Hg)	15	12	7
Hypotension (SBP <80 mm Hg)	4	NR	1
Hyperthermia (>38°C)	4	19	1
Hypothermia (<35°C)	11	NR	NR
Tachypnea (>20 breaths/min)	13	21	14
Loss of Consciousness	36	23	9
GCS = 3	21	NR	NR
GCS < 8	33	NR	NR
GCS < 12	38	NR	9
Mydriasis	42	37	NR
Agitation	29	21	27
Confusion/Delirium	13	4	10
Tremor	11	2	11
Myoclonus	10	NR	10
Seizures	6	4	7
Symptoms (%)			
Palpitations	19	21	18
Dizziness/Weakness/Unwell	15	31	6
Anxiety/Panic	13	23	53
Nausea/Vomiting	10	23	1
Dyspnea	8	8	NR
Chest Pain	8	8	1
Thirst	2	6	NR
Headache	2	12	NR
Complications (%)			
Severe Rhabdomyolysis	6	0	1
Cardiopulmonary Arrest	6	0	1
Disseminated Intravascular Coagulation	4	0	1
Liver Failure	2	0	1
Renal Failure	2	0	0
Death	2	0	1
Laboratory Abnormalities (%)			
Leukocytosis (>10,000/mm³)	46	NR	NR
Creatine Kinase (>300 U/L)	33	NR	16
Elevated Serum Creatinine (>105 mmol/L)	15	NR	NR
Hyponatremia (<135 meq/L)	0	NR	NR

Abbreviations: SBP = systolic blood pressure; GCS = Glasgow Coma Scale; NR = not reported.

137

alienation from others, 60%; altered visual perception, 50%; and reduced aggression.[117] The subjective effects of MDMA typically begin within 30–60 minutes after ingestion and resolve within 4–6 hours.

MENTAL DISORDERS

Determining the chronic effects of MDMA use is complicated by the difficulty identifying a cohort of users of MDMA only. Self-rating questionnaires of heavy recreational ecstasy users (30–1,000 occasions) reveal higher scores for somatization, paranoid ideation, psychosis, anxiety, hostility, phobias, impulsiveness, altered appetite, and difficulty sleeping when compared with non-MDMA users.[118] Symptoms associated with chronic, heavy use of MDMA based on responses to questionnaires include depression, anxiety, volatility, tremors/twitches, and weight loss.[119] Although anxiety is a frequent minor side-effect of MDMA use, occasionally an acute panic attack occurs. Typically, this reaction resolves quickly with supportive care and benzodiazepines, but symptoms (e.g., agoraphobia) may persist for several months.[120] In contrast to amphetamines and methamphetamines, psychosis is an uncommon manifestation of MDMA abuse based on clinical features of patients presenting to EDs with MDMA intoxication.

MEDICAL COMPLICATIONS

Alteration of consciousness following MDMA use is usually not a direct effect of MDMA, and these patients usually have other causes of altered mental status including hyponatremia, hyperkalemia, metabolic acidosis, hyperthermia, seizures, or the concomitant ingestion of CNS depressants.[121] Rare case reports associate MDMA use with subarachnoid hemorrhage,[122] intracerebral hematoma,[123] and cerebral venous thrombosis,[124] but underlying structural abnormalities (e.g. aneurysm, arteriovenous malformation) may account for some of these events.[125,126]

The physiologic response to recreational doses of MDMA includes elevated heart rate, increased blood pressure, and palpitations.[35] Cardiovascular complications associated with MDMA intoxication include hypertension, chest pain, palpitations, dyspnea, and atrial fibrillation.[37,127] Case reports temporally associate the use of MDMA with the development of an acute myocardial infarction both with[128] and without[129] coronary thrombosis. Spontaneous pneumothorax, pneumomediastinum with cervical emphysema, difficulty swallowing, precordial crepitations, dyspnea, and thoracic pain may occur following the ingestion of MDMA as a result of Valsalva-type maneuvers (sustained respiratory muscle activity with a closed glottis) or pro-

tracted vomiting.[130,131] Rarely, case reports associate the ingestion of MDMA with isolated noncardiac pulmonary edema, but this complication usually occurs with multiorgan failure.[132]

MDMA is associated with the serotonin syndrome (confusion, agitation, diaphoresis, hypertonicity, hyperreflexia, shivering, tremor, myoclonus, diarrhea, and autonomic instability). The serotonin syndrome is similar to acute hyperthermia and multiorgan failure associated with MDMA toxicity, but significant physical exertion is not required for the development of hyperthermia in the setting of the serotonin syndrome. Mild cases of serotonin syndrome resolve spontaneously, but severe cases require intensive care.[133]

MDMA is structurally and pharmacologically similar to fenfluramine; the phentermine–fenfluramine combination was associated with valvular heart disease following chronic use as anorexic agents. Like fenfluramine, MDMA causes proliferation of cardiac valvular interstitial cells *in vitro* by activation of the 5-HT_{2B} receptor.[134] A small study of MDMA abusers suggested a possible association between chronic MDMA use and valvular heart disease. In a study of 29 current and past MDMA users, 8 MDMA users (28%) with no other known risk factors had echocardiographic evidence of valvular lesions compared with none for the gender- and age-matched controls.[135] The lesions included valvular stranding and regurgitation of the mitral, tricuspid, or aortic valves as defined by US Food and Drug Administration criteria for appetite suppressant-induced valvular heart disease. The valvular lesions detected in this study were mild to moderate compared with phentermine–fenfluramine associated valvular disease, but the size of the study was small.

Overdose

Table 9.3 outlines the common clinical features of mild to severe MDMA intoxication. In a study of ecstasy-related admissions to Israeli EDs, the most common clinical features of MDMA toxicity were agitation, restlessness, disorientation, shaking, elevated blood pressure, headache, and loss of consciousness.[136] The most common serious complications of acute MDMA toxicity are coma with respiratory insufficiency and severe hyperthermia (>41ºC/105.8ºF) with associated hypertonicity, hyperreflexia, altered consciousness, multiorgan failure, hepatocellular damage, rhabdomyolysis, acute renal failure with myoglobinuria, severe electrolyte disturbance (hyponatremia, hypernatremia, hyperkalemia), and disseminated intravascular coagulation (DIC).[137,138] Patients with severe hyperthermia may collapse at the scene, arriving at the emergency department in cardiopulmonary arrest.[121] The severity and duration

TABLE 9.3. Symptoms by Severity of 3,4-Methylenedioxymethamphetamine (MDMA) Intoxication.

Severity	Signs and Symptoms
Mild	Anxiety, agitation, irritability, insomnia, dizziness, tremor, hyperreflexia, mydriasis, flushing, diaphoresis, thirst, nausea, vomiting, pallor
Moderate	Confusion, delirium, hypertension, tachypnea, tachycardia, premature ventricular contractions, chest discomfort, vomiting, abdominal pain, profuse diaphoresis, mild temperature elevation, hallucinations, panic reaction, elevated creatine kinase
Severe	Hyperpyrexia (>40°C), seizures, coma, focal neurologic signs, shock, disseminated intravascular coagulation, hepatic failure, pulmonary edema, serious electrolyte imbalance ($K^+ > 6.5$ meq/L, $Na^+ < 125$ meq/L), rhabdomyolysis, serious ventricular dysrhythmias

of hyperpyrexia are prognostic indicators of the risk of death. Few patients survive peak core temperatures exceeding 42°C (107.6°F).[139] However, a patient survived MDMA-induced hyperthermia reaching a peak core temperature of 42.9°C (109.2°F). Complications included seizures, rhabdomyolysis, metabolic acidosis, and respiratory failure.[140] Headache, myalgias, light-headedness, paresthesias, weakness, and delirium can be an early symptom of hyperthermia.[121]

Although seizures occur occasionally following ingestion of ecstasy tablets, the development of seizures often indicates ingestion of tablets adulterated with *para*-methoxyamphetamine (PMA).[169] Severe liver disease may occur in MDMA users without hyperthermia.[141] Although these individuals develop jaundice, hypoglycemia, prolonged prothrombin times, and markedly elevated serum hepatic aminotransferase concentrations, hepatic encephalopathy does not usually occur.[142] Rare case reports describe orthotopic liver transplantation for severe hepatic encephalopathy associated with the use of ecstasy.[143]

Fatalities

Death after the use of MDMA alone is very rare, and many deaths from ecstasy follow ingestion of tablets subsequently found to contain substances other than MDMA.[110] The clinical features of lethal MDMA toxicity are similar to the serotonin syndrome. Most MDMA-induced fatalities are associated with hyperthermia along with multiorgan failure, rhabdomyolysis, and DIC. Other causes of death include dilutional hyponatremia

with cerebral edema and uncal herniation, cerebrovascular events, and hepatic failure without hyperthermia.[42] Potential predisposing factors for the development of fatal reactions to MDMA use include use of monoamine oxidase (MAO) inhibitors, ingestion of other stimulants, preexisting cardiovascular disease, and strenuous activity in high ambient temperatures. However, fatalities from the ingestion of MDMA may occur in individuals who are not participating in physical activities.[144]

Abstinence Syndrome

Experimental studies indicate that the administration of multiple daily doses of MDMA does not induce classical manifestations of physical dependence.[145] In contrast to ethanol, sedatives, and opioid withdrawal, there are no physical symptoms that characterize MDMA withdrawal. Symptoms that follow cessation of MDMA use are mild, and these effects may result from mild residual toxicity rather than an abstinence syndrome. Up to about one-third of recreational users of MDMA report difficulty concentrating, myalgias, fatigue, depressive symptoms, and confusion the day following MDMA use.[85] When compared with ethanol control groups, increased depressive symptoms commonly occur during the several days following MDMA use; however, confounding variables (e.g., preexisting psychiatric conditions) complicate the interpretation of these studies.[146] In a 9-day study of regular MDMA users, these participants had increased scores on negative mood parameters (depression, irritability, anxiety, rumination) and cognition (impairment of concentration and memory) 24 hours after using MDMA, when compared with controls (abstinent ecstasy users).[147] The negative cognitive effects resolved after 2 days, and the negative effects on mood persisted 3–4 days. Sleeping was disrupted for 2 days.

Reproductive Abnormalities

There are few data on the reproductive effects of chronic MDMA abuse. A prospective follow-up study of 136 babies exposed to ecstasy *in utero* suggested that maternal MDMA exposure may be associated with a significantly increased number of congenital defects (15.4%, 95% CI: 8.2–25.4), particularly the incidence of cardiovascular (26/1000 livebirths, 95% CI: 3–90) and musculoskeletal (38/1000, 95% CI: 8–109) anomalies.[148] However, these studies are confounded by smoking, heavy alcohol intake, and polydrug use combined with a higher than expected rate of unplanned pregnancies that increase the risk of fetal exposure to potentially harmful substances.[149] Available data to date do not

show strong support for a causal relationship between chronic MDMA use and birth defects; however, data on the use of MDMA during pregnancy are limited.[150]

DIAGNOSTIC TESTING

Analytic Methods

SCREENING

Most urine drug screens do not contain MDMA-specific immunoassays (e.g., DRI Ecstasy, Microgenics Corp., Fremont, CA);[151] therefore, detection of this compound depends on the cutoffs and relative cross-reactivities with commercial reagents for amphetamine and methamphetamine that are not specifically designed to detect MDMA. In general, immunoassays for amphetamines are based on antibodies derived from amphetamine or methamphetamine conjugates that are modified at an amine group. Any amine-containing compound structurally similar (e.g., MDMA) to amphetamine will react with the assay. MDMA cross-reacts at 135% with the ELISA (enzyme-linked immunosorbent assay) methamphetamine assay (Immunalysis, Pomona, CA), but the cross-reactivity of MDMA with the enzyme multiplied immunoassay technique, EMIT II (Dade-Behring/Siemens Healthcare Diagnostics, Deerfield, IL) is only 3%.[152] As a result of the variable degree of cross-reactivity between assays, more specific methods (e.g., high performance liquid chromatography [HPLC], gas chromatography/mass spectrometry [GC/MS]) are required to identify and quantify MDMA.

CONFIRMATORY

Analytic methods for the quantitation of MDMA include GC with nitrogen-selective detection,[153] HPLC with fluorescence detection,[154] capillary electrophoresis,[155] liquid chromatography/electrospray ionization/tandem mass spectrometry,[156] and GC/MS.[157] The limit of detection (LOD) for the latter method is 50 ng/mL.[158] In urine samples, liquid chromatography/electrospray ionization/mass spectrometry[159] and GC/MS[160] can simultaneously quantify MDMA and common metabolites. The lower limit of quantification (LLOQ) for the latter method is about 25 ng/mL. Liquid chromatography/mass spectrometry is an alternative to GC/MS as a confirmation technique that simplifies sample preparation and eliminates derivatization procedures as well as the hydrolysis of urine samples. The LOD using liquid chromatography/electrospray ionization/tandem mass spectrometry for methamphetamine and MDMA in urine samples is 8 ng/mL with a coefficient of variation (CV) between 5–16%.[161] The LLOQ is 28 ng/mL and

26 ng/mL, respectively. The LLOQ for these compounds in oral fluid by GC/MS with electron impact ionization ranges between 5–25 ng/mL with a CV below 8.3%.[162] The HPLC retention data is not as precise as the data obtained by GC/MS methods; therefore, more interference occurs during HPLC procedures compared with GC/MS. Gas chromatography/mass spectrometry with electron impact or chemical ionization separates MDMA from amphetamine, methamphetamine, and related amines including ephedrine, phenylpropanolamine, and phentermine. Gas chromatography/negative ion chemical ionization/mass spectrometry and HPLC with peroxyoxalate chemiluminescence detection are sensitive and accurate methods for the detection of MDMA in human hair.[163,164] Methods to quantify the concentration of R-(−)- and S-(+)-isomers of MDMA and MDA include negative-ion chemical ionization GC/MS[165] and gas chromatography/electron ionization/mass spectrometry with selective ion monitoring.[166]

STREET SAMPLE ANALYSIS

The purity of MDMA tablets and the adulterants added to these tablets varies by year and by country. The purity of MDMA tablets was relatively high before the mid to late 1990s, when the composition of ecstasy tablets varied widely, even among visually similar tablets with the same brand.[167] In some studies of the purity of MDMA tablets, less than one-half of such tablets contained unadulterated MDMA and many tablets contained other compounds.[112] These tablets may contain pharmaceutic preparations with little psychoactive properties including acetaminophen (paracetamol), caffeine, carisoprodol, codeine, dextromethorphan, diazepam, diethylpropion, diphenhydramine, fentanyl, lidocaine, procaine, ephedrine, pseudoephedrine, and zolpidem. However, many tablets contain psychoactive adulterants including MDA, MDEA, methamphetamine, PMA, N-benzylpiperazine (BZP), 1-(3-chlorophenyl)piperazine (mCPP), 2,5-dimethoxy-4-bromophenethylamine (2C-B), 2,5-dimethoxy-4-bromoamphetamine (DOB), cocaine, and ketamine. In a study of the pharmaceutic content of tablets sold as ecstasy across the United States, 39% of the tablets contained only MDMA, 46% contained substances other than MDMA, and 15% were mixtures of MDMA and adulterants.[168] Methamphetamine, MDA, MDEA, caffeine and pseudoephedrine are common adulterant of MDMA tablets in the United States.[112] Rarely, serious toxins (e.g., strychnine) are adulterants of MDMA.[112] The substitution of PMA for MDMA in ecstasy tablets caused a number of deaths in Europe,[169] North America,[170] and Australia.[171] The starting materials (e.g., anise) for synthesizing PMA are substantially less

expensive and more readily available than common starting materials (safrole, isosafrole) for MDMA.[172] In the Netherlands, monitoring of the illicit market between 1993 and 2008 demonstrated a peak MDMA content in confiscated ecstasy of 86% between 2004–2006.[173] After that period, the purity of MDMA tablets decreased with the increasing adulteration of these tablets with mCPP, BZP, and caffeine.

Impurities include by-products of contaminants produced from unintended side reactions as well as by-products of reactions from the starting materials. A 31-year-old man died following a massive MDMA overdose; his postmortem blood contained small amounts of 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine (MDDM or MDDA).[174] The authors of this case report suggested that MDDM was probably a synthetic by-product or contaminant in the MDMA tablets.

Drug profiling involves the extraction of physical and/or chemical profiles from drug samples to collect information on the source and similarity of confiscated batches of drugs. Analysis of these impurities after extraction under alkaline conditions and GC/MS usually identifies the probable method of synthesis and precursor agents.[21] The synthesis of MDMA produces highly variable constituents in the final mixture as a result of differences in manufacturing parameters. Consequently, determination of the presence of organic impurities [e.g., 3,4-methylenedioxyphenyl-2-propanone, 3,4-methylenedioxyphenyl-2-propanol, 3-(3,4-methylenedioxyphenyl)-3-buten-2-one, *N*-formyl-*N*-methyl-3,4-methylenedioxyamphetamine] provides information on the source of the MDMA tablets.[175] Analysis of the physical characteristics (e.g., diameter, thickness, weight, score) of the tablets resulting from compression of the mixed powder during the tablet-pressing process also provides drug intelligence on the source of the MDMA tablets.[176]

STORAGE

MDMA is relatively stable during most storage conditions (-20°C/-4°F, 4°C/39.2°F, 20°C/68°F). In a controlled study of MDMA stored at -20°C (-4°F), 4°C (39.2°F), and 20°C (68°F) in urine and whole blood samples, there was no significant loss of MDMA for 21 weeks and 5 weeks, respectively.[177] Sample degradation limited analysis of MDMA after these times.

Biomarkers

BLOOD

Animal studies indicate that MDMA concentrates in erythrocytes with a mean erythrocyte/plasma ratio of 1.48 ± 0.04, similar to MDA.[178]

PHARMACOLOGY STUDIES.   Pharmacokinetic studies in volunteers indicate that the kinetics of MDMA is non-linear.[179] MDMA doses of 50 mg and 150 mg produce mean peak plasma concentrations of 0.05 mg/L, and 0.46 mg/L, respectively, about 1–2 hours after ingestion.[180] The mean peak plasma MDMA concentration in 6 healthy volunteers about 2 hours after the ingestion of 100 mg MDMA was approximately 0.2 mg/L.[181] These studies indicate that the central (arousal) and peripheral sympathomimetic effects (i.e., elevated heart rate and blood pressure) follow the time-profile of plasma MDMA concentrations, but not necessarily the quantitative plasma concentration of MDMA. At the end of a dance party, the mean plasma MDMA concentration in 27 regular MDMA users (i.e., 1–12 d/mo) was 0.31 ± 0.21 mg/L with a range up to 0.84 mg/L.[182] Individuals in this group reported the following subjective effects at the time of the blood sampling: no effect, 22%; mild effects, 52%; and strong effects, 26%. The reported drug use did not correlate well to the plasma MDMA concentration. Although there was a small progressive increase in temperature during and after MDMA use, MDMA concentrations and temperature were not statistically correlated.

OVERDOSE.   The MDMA concentrations in blood samples from asymptomatic users and users with serious side effects are similar.[42,183] Consequently, the plasma MDMA concentration alone is not a reliable predictor of acute MDMA-induced medical complications. A 17-month-old toddler developed generalized tonic-clonic seizures, elevated temperature (38.5°C/101.3°F), tachycardia, and hypertension after ingesting a tablet of MDMA.[184] The serum MDMA concentration on admission to the emergency department was 0.30 mg/L. The child survived without sequelae following intensive care. Three hours after admission for seizures, elevated temperature (38°C/100.4°F), hypertension, and tachycardia, the serum MDMA concentration in blood samples from a 14-month-old baby was 0.591 mg/L.[185] The child fully recovered with intensive supportive care. A 27-year-old man presented to an emergency department 6 hours after ingesting ecstasy.[128] He was agitated, diaphoretic, and complaining of chest pain with electrocardiographic evidence of an inferior myocardial infarction. His plasma MDMA concentration at admission was 1.1 mg/L with no detectable MDA. A 20-year-old man developed seizures, marked trismus, and a temperature of 38.6°C (101.5°F) soon after ingestion 18 ecstasy tablets.[186] The plasma MDMA concentration was 4.05 mg/L. Thirteen hours following the ingestion of 40 tablets of ecstasy by a 19-year-old man, the serum concentration of MDMA was 4.3 mg/L.[39] Clinical features of toxicity were limited to elevated heart rate,

mydriasis, confusion, lethargy, and retrograde amnesia. The serum MDMA concentration in a 32-year-old woman 3 hours after the ingestion of MDMA was 6.5 mg/L.[187] She was agitated, combative diaphoretic, hypoxic, and hyperthermic (41.6°C/106.9°F). Ten hours after ingestion, she was hemodynamically stable and responsive, but agitated while intubated. She was extubated 25 hours after ingestion, and she survived with intensive supportive care. A 30-year-old man developed seizures, coma, respiratory failure, and modest elevation of his core temperature (38.7°C/101.7°F). His serum S(+)- and R(−)-MDMA concentrations 4 hours after ingestion were 42 mg/L and 44 mg/L as determined by GC/MS. He survived with supportive care.

A 20-year-old presented to the emergency department comatose with hyperthermia (43°C/109.4°F), hypotension, and hyperkalemia (7.7 meq/L).[121] His serum MDMA concentration at admission was 2.4 mg/L; he died 1 hour later. In the same case series, a 22-year-old man presented to an emergency department with coma, hyperkalemia (6.8 meq/L), hypotension, and elevated temperature (38.5°C/101.3°F). His serum MDMA concentration at admission was 0.93 mg/L; he died 58 hours after admission.

Postmortem. Postmortem MDMA concentrations must be interpreted along with other antemortem and postmortem evidence because of the overlap of postmortem MDMA concentrations of fatal acute MDMA intoxication and fatalities associated with trauma during MDMA use. There are limited data on postmortem blood concentrations of MDMA. Deaths occasionally occur in young healthy adults following the ingestion of typical recreational doses of MDMA. In these cases, the postmortem MDMA concentrations overlap with MDMA concentrations from blood samples taken from recreational MDMA users during or after rave parties.[31] Additionally, the use of MDMA frequently occurs with polydrug use. In a case series of 22 deaths with positive postmortem MDMA concentrations, the cases were divided into the following 3 groups with associated mean MDMA concentrations: 1) co-intoxications with cocaine and/or opiates, 0.58 mg/L; 2) intoxications without cocaine or opiates, 1.04 mg/L; and 3) trauma-related deaths, 0.97 mg/L.[188] MDA is a metabolite of MDMA; MDA concentrations up to approximately 10% to 15% of the blood MDMA concentration may be present following the ingestion of MDMA only.[189] In the above case series, the mean postmortem blood MDMA and MDA concentrations were 0.86 mg/L and 0.16 mg/L, respectively. In a case series of 15 deaths attributed to accidental MDMA intoxication, the postmortem blood (site not specified) contained MDMA

concentrations from 0.48–10 mg/L and the range of the MDA concentrations was <0.05–0.15 mg/L.[190]

The MDMA concentrations in femoral blood samples from 9 individuals with fatal MDMA intoxication ranged from 1.2–22 mg/L; the mean and median concentrations were 8.4 mg/L and 4.4 mg/L, respectively.[191] None of these postmortem blood samples contained significant concentrations of other drugs. A 22-year-old man was agitated, acting bizarrely, and mumbling incoherently at a student club.[192] He was initially unconscious with stable vital signs at the ED; he had a sudden cardiopulmonary arrest and could not be resuscitated. At postmortem examination 24 hours after death, his femoral blood contained the following drugs: MDMA, 1.42 mg/L and MDA, 0.17 mg/L. A 19-year-old woman died about 3 hours after ingesting MDMA despite 30 minutes of cardiopulmonary resuscitation.[144] The postmortem femoral blood contained MDMA and MDA concentrations of 3.8 mg/L and trace, respectively. A 19-year-old man died of hyperthermia and multiorgan failure 7 hours after arriving comatose at the ED.[193] The concentrations of MDMA and MDA in autopsy blood (site not reported) were 1.09 mg/L and 0.08 mg/L, respectively, as measured by GC/MS.

MDMA concentrations in blood samples from autopsies may demonstrate postmortem redistribution, particularly in the presence of high MDMA postmortem concentrations in the liver, stomach, and lungs.[194] Case reports suggest heart/femoral blood ratio of MDMA ranging between 1.4–3.9.[194,195] The postmortem/serum perimortem MDMA ratio in blood samples from 2 fatalities from MDMA intoxication were 1.9 for postmortem brachial blood and 1.1 for postmortem femoral blood.[196] Perimortem blood was collected within 1 hour before death.

Urine

The kidney excretes MDMA in the urine beginning shortly after ingestion. Urine amphetamine immunoassays may detect the presence of MDMA following use for approximately 1–2 days, depending on several factors including the dose, duration of use, urine pH, hydration (i.e., urine concentration), analytic method, cutoffs, and individual metabolic and excretion rates. The sensitivity and specificity of individual immunoassays varies. The cross-reactivity of MDMA with reagents in some urine drug screens [e.g., Abuscreen® OnLine HS AMP/MDMA (Abbott Laboratories, Abbott Park, IL), CEDIA®-Amp/MDMA (Microgenics Corp., Fremont, CA), Abbott TDx AMP/MAM: II (Abbott Laboratories, Abbott Park, IL)] is high (>90%), compared with other immunoassays.[197] In a study using a

MDMA cutoff of 0.5 mg/L urine, the false-positive percentage for TDx®-Amp, COBAS®-Amp (Roche, Nutley, NJ), OnLine-Amp/MDMA (Roche Diagnostics Co., Indianapolis, IN), and CEDIA®-Amp/MDMA ranged between 11–21%, whereas the false-negative percentage ranged between 10–17%.[198] The EMIT®-Amp assay (Dade Behring Inc., Deerfield, IL) has high cross-reactivity toward MDMA, but this assay is unlike to detect MDMA at concentrations below 0.5 mg/L concentration. The cutoff concentrations of racemic MDA and MDMA for the EMIT-d.a.u. monoclonal amphetamine/methamphetamine immunoassay is approximately 3 mg/L.[199] The mean urine MDMA concentration in 43 urine samples collected from participants 1–8 hours after a rave party was 19.2 mg/L with a range of 0.11–173 mg/L.[197]

OTHER SAMPLING SITES

The vitreous humor is an alternate sampling site for postmortem MDMA concentrations, particularly following prolonged toxicity. Animal studies indicate that equilibration of the vascular compartment and vitreous humor occurs within 1 hour of the IV administration of MDMA, and MDMA remains relatively stable in the vitreous humor despite high ambient temperatures.[200] An 18-year-old man was found dead after a party the preceding evening.[201] The postmortem MDMA concentrations in samples from the right ventricular blood and the vitreous humor were 0.42 mg/L and 0.36 mg/L, respectively. The vitreous humor from a man found dead with high concentrations of MDMA (33.17 mg/L) in his stomach contained 1.63 mg MDMA/L, whereas the femoral blood contained 1.13 mg/L.[202] The concentrations of MDMA in urine and bile from this case were 0.79 mg/L and 25.42 mg/L, respectively. MDMA concentrations in hair suggest exposure to MDMA, but quantitative MDMA concentrations in hair (i.e., >1 ng/mg) are not reliable measures of the cumulative use of MDMA.[203] The distribution of enantiomers in hair is similar to other tissues with most of the hair specimens demonstrating a predominance of the R(−)-enantiomer.[204] However, the ratio of MDA/MDMA in hair samples from the hair is highly variable. In a study of hair from the heads of 53 chronic ecstasy users, this ratio varied from 1–110% with a mean of 12%.[205]

Abnormalities

BIOCHEMICAL

Leukocytosis occurs frequently after MDMA use.[37] Dilutional hyponatremia is a potential complication of MDMA use, particularly after the ingestion of large amounts of water. Severe hyperkalemia usually occurs in association with hyperthermia, metabolic acidosis, rhabdomyolysis, and renal failure, but hyperkalemia may develop in the absence of renal dysfunction.[206] Disseminated intravascular coagulation (thrombocytopenia, hypofibrinogenemia, hypoprothrombinemia, reduced partial thromboplastin levels, elevated fibrin split products) can occur after MDMA use, usually in association with multiple organ failure, rhabdomyolysis, and hyperthermia. Hypoglycemia is a rare complication of severe MDMA intoxication. Laboratory evidence of hepatic failure (elevated serum hepatic aminotransferases, alkaline phosphatase, bilirubin, prolonged prothrombin time, and activated partial thromboplastin time) may occur in the absence of hyperthermia.[106] Rhabdomyolysis may develop following MDMA use. Laboratory changes include elevated serum muscle enzymes (creatine kinase, aldolase), myoglobinuria, hyperbilirubinemia, hyperuricemia, hypocalcemia, hyperkalemia, renal failure, hyperphosphatemia, and hypokalemia.[121,139] Acute renal failure may develop secondary to acute tubular necrosis (as a result of hypotension), rhabdomyolysis, intravascular coagulation, hypovolemia, or hyperpyrexia. Hypoxemia may result from respiratory paralysis secondary to seizures, noncardiac pulmonary edema, or coma.

NEUROPSYCHOLOGIC

The heavy users have a high incidence of polydrug use (cocaine, lysergic acid diethylamide, amphetamine), and interpretation of the significance of the neuropsychologic scores is complicated by polydrug use as well as potential premorbid personality disorders. Although MDMA causes selective neurotoxic damage of central serotonergic neurones in laboratory animals,[207] the existence of neurotoxic syndrome following chronic MDMA abuse remains unproven as a result of issues related to retrospective data, methodologic flaws, and confounding (polydrug use, reporting bias, drug purity, premorbid personality disorders).[208] Despite substantial methodologic problems, the majority of the evidence suggests that residual alterations of serotonergic transmission occur in chronic MDMA users.[209] These changes at least partially resolve after long-term abstinence. The most consistent findings in heavy MDMA abuse are subtle, small–medium cognitive impairments,[210] particularly in short-term and long-term memory (e.g., verbal more than visual memory) and in the speed of information processing.[211,212] Volunteer studies suggest that verbal memory tasks with high cognitive complexity (e.g., California Verbal Learning Test, Verbal Paired

Associates) are more affected than low cognitive complexity tasks when comparing ecstasy users and non-users.[213] However, the lack of a clear dose-response relationship and similarity of changes in polydrug controls raise questions about the causal nature of this association.[214,215] Other deficits include increased impulsivity, poor problem solving, and deficits in executive skills. Studies of chronic ecstasy users suggest that the most vulnerable cognitive function is associative memory performance rather than working memory or attention, primarily in users who also abuse amphetamines.[216] More basic cognitive tasks are usually preserved including simple and choice reaction times, number vigilance, Stroop task (selective attention), and trail making (visuospatial ability, simple executive function).[217]

Driving

There are few data on the association of MDMA with vehicular accidents and related fatalities. Most studies to date indicate that the presence of MDMA is uncommon in impaired drivers or accident-related fatalities.[115] MDMA is rarely (<1%) detected in drivers associated with fatal accidents, but fatal injuries may result from reckless behavior during MDMA intoxication, particularly in association with ethanol.[218] The relationship between MDMA and driving is complex because of a number of factors including rapid tolerance, idiosyncratic responses, cognitive deficits following acute intoxication, and fatigue associated with strenuous activity during MDMA intoxication. In a case series of 6 drivers arrested for driving while impaired, blood samples contained only MDMA and the range of MDMA concentrations was <0.05–0.58 mg/L as measured by GC/MS.[183] The demeanor of these 6 individuals did not correlate to MDMA concentrations as the person with the highest concentration fell asleep in the police car during his arrest. Most of the individuals displayed muscle twitching, poor balance and coordination, mydriasis, and diaphoresis. In a Norwegian study of 9,013 drivers apprehended during 1998 and 1999 for suspicion of driving under the influence of alcohol or drugs, blood samples from 177 drivers (1.96%) contained detectable amounts of MDMA.[219] The median concentration of MDMA was 0.155 mg/L with a range from 0.019–1.14 mg/L. Many of these drivers were multidrug users, but the study did not separate the drivers into a group of MDMA-only users. In a Swiss study of 440 living drivers suspected of driving under the influence of drugs, blood samples from approximately 6% of the drivers contained MDMA.[220] The median MDMA concentration for this group was 0.218 mg/L with a range from 0.01–2.48 mg/L. In a subsequent Swiss study of 4,794 drivers apprehended for suspected driving under

the influence of drugs,[223] (4.7%) tested positive for MDMA in whole blood samples with a median MDMA concentration of 0.206 mg/L (Mean, 0.279 mg/L; range, 0.011–2.60 mg/L).[221] In a study of 493 drivers suspected of driving under the influence (moving traffic violations, sobriety checkpoints, traffic accidents), the mean blood MDMA concentration was 0.23 mg/L (median, 0.10 mg/L) with a range up to 3.5 mg/L.[222] The blood samples from some of these drivers contained other drugs as measured by GC/MS; the impairment of specific drivers in this series was not reported.

MDMA moderates the impairment in some, but not most driving skills associated with the ingestion of low to moderate doses of ethanol. In experimental studies on light-to-moderate MDMA users, the administration of a single dose of MDMA improved psychomotor performance on a compensatory tracking task and in a weaving and an on-the-road driving task, whereas estimation of time to contact in a dual attention task deteriorated.[223] In volunteer studies, the administration of MDMA (100 mg) and ethanol (0.8 g/kg) produced longer lasting euphoria and well-being than MDMA or ethanol alone.[70] MDMA reversed the sedative effects of ethanol, but the concomitant administration of MDMA did not alter the feeling of drunkenness. Eighteen healthy volunteers participated in a double-blind, placebo-controlled, cross-over study involving the administration of MDMA doses of 0, 75, and 100 mg with (mean blood alcohol content [BAC] = 0.042) and without alcohol.[224] Alcohol alone impaired critical tracking performance (critical tracking task, object movement estimation task) and actual driving performance parameters (i.e., standard deviation of lateral position [SDLP], brake reaction time, and coherence). MDMA alone reduced SDLP and standard deviation of speed. MDMA significantly moderated alcohol-induced impairment of road tracking performance, but MDMA did not modify the ethanol-induced impairments of car-following and laboratory task performance. In studies of simulated driving performance, recreational doses of MDMA did not dramatically alter lateral or longitudinal vehicle control, but the use of multiple drugs with MDMA significantly impaired driving performance.[225] The use of MDMA alone was associated with a decreased sense of risk taking. In a study of 12 healthy volunteers receiving 75 mg MDMA, this dose enhanced speed of manual movements and capacity to steer or track fast moving objects, including a divided attention task, when compared with placebo.[226] Additionally, there was no effect on visual search or planning. However, MDMA impaired the ability to perceive and predict motion, which is a measure of the ability of drivers to judge if another car would collide with their car.

TREATMENT

Stabilization

The major life-threatening complications of acute MDMA toxicity include hyperthermia, electrolyte abnormalities, cerebral edema, hypertension, seizures, ischemic complications, arrhythmias, hepatitis, and trauma. Severe hyperthermia (i.e., temperature >42°C/107.6°F) causes multiorgan failure and DIC, which are very poor prognostic signs. Patients with serious complications require IV access and close monitoring of temperature and electrolytes. Continuous cardiac monitoring is required if the initial electrocardiogram (ECG) or subsequent ECG is abnormal. Supplemental oxygen should be given to hypoxic patients and those with ischemic complications. Respiratory complications are not usually a major feature of toxicity. However, respiratory depression with impaired consciousness may occur, particularly following serious CNS complications (e.g., stroke, cerebral edema, hyponatremia).

Sinus tachycardia is common and this rhythm does not usually require direct pharmacologic intervention. Both hypertension and tachycardia often respond to benzodiazepines (e.g., adults: lorazepam 2 mg or diazepam 5 mg by IV bolus, repeated as required). Benzodiazepines are also the most appropriate first-line treatment for agitation not responding to simple reassurance. Pharmacologic agents are preferable to physical restraints in agitated patients, if possible. Core temperatures should also be measured frequently in any agitated patient, as the temperature may rise rapidly in response to physical activity including resistance to restraints.

Patients with suspected myocardial ischemia should be managed with nitrates, morphine, benzodiazepines, and aspirin. Hypotension may respond to fluid challenges, but often a vasopressor is needed. Beta blockers may lead to increased vasoconstriction and paradoxical worsening of hypertension (by blocking beta$_2$-mediated vasodilatation); in general, these drugs should be avoided.

SEIZURES

Seizures usually respond to benzodiazepines (lorazepam, diazepam). Therapeutic options for the treatment of recurrent seizures include phenobarbital and anesthetic agents (e.g., propofol). Sodium channel blocking drugs (e.g., phenytoin) are unlikely to terminate the seizures associated with MDMA toxicity. Hyperthermia, acidosis, hypoxemia, hyponatremia, and rhabdomyolysis may complicate the clinical course during status epi-

lepticus, and the patient should be evaluated for the presence of these complications. Mild to moderate hyponatremia usually responds to fluid restriction, but the use of hypertonic saline may be necessary with severe hyponatremia (<125 meq/L) associated with seizures. Central pontine myelinosis (osmotic demyelination syndrome) is usually associated with rapid restoration of serum sodium concentrations in patients with chronic hyponatremia; consequently, limitations on sodium replacement probably do not apply to patients with complications from *severe* hyponatremia secondary to acute MDMA intoxication.

AGITATION

Diazepam (Adults: 5 mg IV, children: 0.1–0.3 mg/kg) or lorazepam (1–2 mg IV in adults) are the safest drugs to use for agitation. Although hypotension, seizures, respiratory depression, and dystonic reactions are potential complications of antipsychotic drug use during MDMA intoxication, experimental studies on healthy volunteers receiving 1.5 mg MDMA/kg orally and 1.4 mg haloperidol intravenously did not detect any clinically significant adverse reactions.[227] In this study, the pretreatment of participants with haloperidol did not alter the physiologic responses (blood pressure, heart rate, body temperature) to MDMA compared with MDMA alone. The antipsychotic drugs (e.g., droperidol) should probably be second-line agents. Attention to environmental factors (soft lighting, minimal noise, familiar faces) may also help alleviate agitation.

HYPERTHERMIA

Severe hyperthermia (i.e., >42°C/107.6°F) is a very poor prognostic sign, particularly in association with multiorgan failure (hepatic failure, DIC, hypotension, rhabdomyolysis, hyperkalemia, metabolic acidosis); death may occur within a few days. Temperatures exceeding 39–40°C (102.2–104°F) should therefore be treated aggressively, while the core temperature is continuously monitored. Cooling should always include appropriate use of sedation (benzodiazepines). Other simple measures include removal of clothing, evaporative cooling with water applied with sponging or sprays and fans. Cooling blankets and ice packs may be used, but these measures are probably not superior to evaporative cooling. For refractory hyperthermia, neuromuscular paralysis, sedation, and mechanical ventilation may be necessary to reduce hypertonicity and hyperactivity. Immersion in an ice bath can also be used in refractory cases, but this approach often presents logistical problems. All vital signs should be monitored frequently and cooling measures continued until core temperatures are

below 38.5°C (101.3°F). If the temperature has exceeded 41°C (105.8°F) at any time, laboratory testing is required to detect multiorgan failure. If the hyperthermic patient does not respond rapidly to cooling measures, the IV administration of dantrolene is a therapeutic option. Case reports associate improvement in hyperthermia (42.9°C/109.2°F and 40.2°C/104.4°F), hyperkalemia, and rhabdomyolysis with the early administration of dantrolene (60 mg bolus q10 min ×3 and 1 mg/kg bolus ×3 over 1 hour).[140,228] However, the adjunctive role of dantrolene in the treatment of hyperthermia remains controversial.[139,229]

Gut Decontamination

There are no data to support the use of decontamination procedures on the clinical outcome of MDMA intoxication. Patients with adverse effects following recreational use are unlikely to have significant unabsorbed MDMA; decontamination is not usually warranted. Gut decontamination might be warranted in the case of symptomatic "body packers," and administration of activated charcoal and polyethylene glycol electrolyte lavage solution would be standard treatment. However, smuggling MDMA via body packers has not been reported to date.

Elimination Enhancement

MDMA is a weak base and acidification of urine increases the clearance of unchanged MDMA. However, it is unlikely that alteration of the urine pH will favorably improve the outcome of MDMA toxicity. Adverse effects usually occur early after ingestion and the half-life is relatively short (i.e., about 7–8 h). Most individuals already have acidic urine and systemic acidosis might increase cardiovascular adverse effects. There are no clinical data to confirm the efficacy of urinary acidification. There are no clinical data to support the use of hemodialysis, peritoneal dialysis, multiple-dose activated charcoal, or hemoperfusion. The large volume of distribution (6 L/kg) and the high endogenous clearance of MDMA suggest that these measures will not cause a clinically relevant increase in MDMA clearance.

Antidotes

There is no specific antidote for MDMA intoxication. Benzodiazepines (and occasionally other GABA agonist sedative drugs, such as barbiturates and propofol) provide adequate pharmacologic treatment for most manifestations of MDMA toxicity. Beta blockers are considered relatively contraindicated as they may increase hypertension and increase ischemia as a result of unopposed alpha agonist adrenergic effects. Serotonin antagonists, such as cyproheptadine 4–8 mg orally 3 times daily as needed or chlorpromazine 25–50 mg intramuscularly as needed in volume-replete patients, could be considered where the clinical syndrome resembles severe serotonin toxicity (i.e., severe hyperthermia, marked hyperreflexia with clonus).[133]

Supplemental Care

ANCILLARY TESTS

In severe MDMA toxicity, laboratory examination should include complete blood count, glucose, serum electrolytes, creatinine, blood ureas nitrogen (BUN), calcium, phosphorus, uric acid, creatine kinase, hepatic transaminases, a full coagulation profile (fibrin split products, fibrinogen, prothrombin time, partial thromboplastin time), and urine dipstick analysis (i.e., primarily to look for a positive response to the test for blood that might indicate myoglobinuria). An ECG should be done routinely and neuroimaging should be done in those with impaired consciousness, severe headache, or neurologic signs.

COMPLICATIONS

Severe MDMA toxicity may be complicated by a variety of problems, including multiorgan failure, hyperkalemia, hyponatremia, hepatitis, acute renal failure, rhabdomyolysis, cerebrovascular accident, myocardial infarction, arrhythmias, DIC, and acute respiratory distress syndrome. These conditions should be diagnosed early; however, management of these conditions should follow usual management procedures with appropriate supportive care and monitoring. There are no specific management considerations that relate to MDMA, except for the relative contraindication against using beta-blockers in the treatment of dysrhythmias, myocardial infarction, and hypertension. Hemodialysis may be required to correct electrolyte abnormalities in the presence of renal dysfunction. Patients with isolated fulminant acute liver failure after MDMA use may require liver transplantation.[106] Myoglobinuria is a frequent complication of serious MDMA toxicity. Although urinary alkalinization is frequently recommended for myoglobinuria, alkalinization may decrease MDMA clearance. Consequently, unless there is marked myoglobinuria, generous fluid replacement with high-volume urine flow is preferable to attempts to alter urine pH. Patients with mild MDMA intoxication may be discharged after 4 hours of observation if vital signs including temperature remain stable and the mental status returns to normal baseline.

References

1. Pentney AR. An exploration of the history and controversies surrounding MDMA and MDA. J Psychoactive Drugs 2001;33:213–221.

2. Shulgin AT. History of MDMA. In: Peroutka S (Ed). Ecstasy: the clinical, pharmacological and neurotoxicological effects of the drug MDMA. Norwell, MA: Kluwer Academic Publishers, 1990.

3. Greer GR, Tolbert R. A method of conducting therapeutic sessions with MDMA. J Psychoactive Drugs 1998;30: 371–379.

4. Landry MJ. MDMA: a review of epidemiologic data. J Psychoactive Drugs 2002;34:163–169.

5. Lawn JC. Schedules of controlled substances; temporary placement of 3,4-methylenedioxymethamphetamine (MDMA) into schedule I. Fed Regist 1985; 50:23118–23120.

6. Nichols DE. Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J Psychoactive Drugs 1986;18:305–313.

7. Stone DM, Stahl DC, Hanson GR, Gibb JW. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur J Pharmacol 1986;128: 41–48.

8. Pentney AR. An exploration of the history and controversies surrounding MDMA and MDA. J Psychoactive Drugs 2001;33:213–221.

9. Check E. Psychedelic drugs: the ups and downs of ecstasy. Nature 2004;429:126–128.

10. Ricaurte GA, Yuan J, Hatzidimitriou G, Cord BJ, McCann UD. Severe dopaminergic neurotoxicity in primates after a common recreational dose regimen of MDMA ("ecstasy"). Science 2002;297:2260–2263.

11. Cho AK, Melega WP. Patterns of methamphetamine abuse and their consequences. J Addict Dis 2002;21: 21–34.

12. Hiramatsu M, Nabeshima T, Kameyama T, Maeda Y, Cho AK. The effect of optical isomers of 3,4-methylenedioxymethamphetamine (MDMA) on stereotyped behavior in rats. Pharmacol Biochem Behav 1989; 33:343–347.

13. Pizarro N, Farre M, Pujadas M, Peiro AM, Roset PN, Joglar J et al. Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). Drug Metab Dispos 2004;32:1001–1007.

14. Johnson MP, Hoffman AJ, Nichols DE. Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused rat brain slices. Eur J Pharmacol 1986;132: 269–276.

15. Steele TD, Nichols DE, Yim GK. Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [3H]monoamines into synaptosomes from different regions of rat brain. Biochem Pharmacol 1987;36: 2297–2303.

16. Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA, Hutt AJ. Stereospecific analysis and enantiomeric disposition of 3, 4-methylenedioxymethamphetamine (Ecstasy) in humans. Clin Chem 1999;45:1058–1069.

17. Taffe MA, Lay CC, Von Huben SN, Davis SA, Crean RD, Katner SN. Hyperthermia induced by 3,4-methylenedioxymethamphetamine in unrestrained rhesus monkeys. Drug Alcohol Depend 2006;82:276–281.

18. Kalant H. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs. CMAJ 2001;165:917–928.

19. Shulgin AT, Shulgin A. PIHKAL. A chemical love story. Berkeley CA: Transform Press; 1991.

20. Swist M, Wilamowski J, Parczewski A. Basic and neutral route specific impurities in MDMA prepared by different synthesis methods. Comparison of impurity profiles. Forensic Sci Int 2005;155:100–111.

21. Swist M, Wilamowski J, Parczewski A. Determination of synthesis method of ecstasy based on the basic impurities. Forensic Sci Int 2005;152:175–184.

22. Wood DM, Stribley V, Dargan PI, Davies S, Holt DW, Ramsey J. Variability in the 3,4-methylenedioxymethamphetamine content of "ecstasy" tablets in the UK. Emerg Med J 2011;28:764–765.

23. Parrott AC. Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. J Psychopharmacology 2005;19:71–83.

24. Hammersley R, Ditton J, Smith I, Short E. Patterns of ecstasy use by drug users. Br J Criminol 1999;39: 625–647.

25. Zemishlany Z, Aizenberg D, Weizman A. Subjective effects of MDMA ("ecstasy") on human sexual function. Eur Psychiatry 2001;16:127–130.

26. Solowij N, Hall W, Lee N. Recreational MDMA use in Sydney: a profile of "ecstasy" users and their experiences with the drug. Br J Addict 1992;87:1161–1172.

27. von Sydow K, Lieb R, Pfister H, Hofler M, Wittchen H-U. Use, abuse and dependence of ecstasy and related drugs in adolescents and young adults – a transient phenomenon? Results from a longitudinal community study. Drug Alcohol Depend 2002;66:174–159.

28. Topp L, Hando J, Dillon P, Roche A, Solowij N. Ecstasy use in Australia: patterns of use and associated harm. Drug Alcohol Depend 1999;55:105–115.

29. Jansen KL. Ecstasy (MDMA) dependence. Drug Alcohol Depend 1999;53:121–124.

30. Piper BJ, Vu HL, Safain MG, Oliver AJ, Meyer JS. Repeated adolescent 3,4-methylenedioxymethamphetamine (MDMA) exposure in rats attenuates the effects of a subsequent challenge with MDMA or a 5-hydroxytryptamine(1A)

receptor agonist. J Pharmacol Exp Ther 2006;317: 838–849.

31. Cole JC, Sumnall HR. Altered states: the clinical effects of ecstasy. Pharmacol Ther 2003;98:35–58.

32. Schifano F. A bitter pill. Overview of ecstasy (MDMA, MDA) related fatalities. Psychopharmacology 2004;173: 242–248.

33. Dumont GJ, Verkes RJ. A review of acute effects of 3,4-methylenedioxymethamphetamine in healthy volunteers. J Psychopharmacol 2006;20:176–187.

34. Vollenweider FX, Gamma A, Liedchti M, Huber T. Psychological and cardiovascular effects and short-term sequelae of MDMA ("ecstasy") in MDMA-naïve healthy volunteers. Neuropsychopharmacology 1998;19:241–251.

35. Lester SJ, Baggott M, Welm S, Schiller NB, Jones RT, Foster E, Mendelson J. Cardiovascular effects of 3,4-methylenedioxymethamphetamine a double-blind, placebo-controlled trial. Ann Intern Med 2000;133:969–973.

36. Harris DS, Baggott M, Mendelson JH, Mendelson JE, Jones RT. Subjective and hormonal effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology (Berl) 2002;162:396–405.

37. Liechti ME, Kunz I, Kupferschmidt H. Acute medical problems due to Ecstasy use. Case-series of emergency department visits. Swiss Med Wkly 2005;135:652–657.

38. Weir E. Raves: a review of the culture, the drugs and the prevention of harm. CMAJ 2000;162:1843–1848.

39. Regenthal R, Kruger M, Rudolph K, Trauer H, Preiss R. Survival after massive "ecstasy" (MDMA) ingestion. Intensive Care Med 1999;25:640–641.

40. Ramcharan S, Meenhortst PL, Otten JM, Koks CH, de Boer D, Maes RA, Beijnen JH. Survival after massive ecstasy overdose. Clin Toxicol 1998;36:727–731.

41. Bedford Russell AR, Schwartz RH, Dawling S. Accidental ingestion of "ecstasy" (3,4-methylenedioxy-methylamphetamine). Arch Dis Child 1992;67: 1114–1115.

42. Henry JA, Jeffreys KJ, Dawling S. Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). Lancet 1992;340:384–387.

43. Hardman HF, Haavik CO, Seevers MH. Relationship of the structure of mescaline and seven analogs to toxicity and behavior in five species of laboratory animals. Toxicol Appl Pharmacol 1973;25:299–309.

44. Malpass A, White JM, Irvine RJ, Somogyi AA, Bochner F. Acute toxicity of 3,4-methylenedioxymethamphetamine (MDMA) in Sprague-Dawley and Dark Agouti rats. Pharmacol Biochem Behav 1999;64:29–34.

45. Davis WM, Hatoum HT, Waters IW. Toxicity of MDA (3,4-methylenedioxyamphetamine) considered for relevance to hazards of MDMA (ecstasy) abuse. Alcohol Drug Res 1987;7:123–134.

46. de la Torre, Farre M, Roset PN, Pizarro N, Abanades S, Segura M et al. Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. Ther Drug Monit 2004;26:137–144.

47. Heydari A, Yeo KR, Lennard MS, Ellis SW, Tucker GT, Rostami-Hodjegan A. Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. Drug Metab Dispos 2004;32:1213–1217.

48. Wu D, Otton SV, Inaba T, Kalow W, Sellers EM. Interactions of amphetamine analogs with human liver CYP2D6. Biochem Pharmacol 1997;53:1605–1612.

49. Kolbrich EA, Goodwin RS, Gorelick DA, Hayes RJ, Stein EA, Huestis MA. Physiological and subjective responses to controlled oral 3,4-methylenedioxymethamphetamine administration. J Clin Psychopharmacol 2008;28:432–440.

50. Moore KA, Mozayani A, Fierro MF, Poklis A. Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning. Forensic Sci Int 1996;83:111–119.

51. Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev 2003;55:463–508.

52. Kraemer T, Maurer HH. Toxicokinetics of amphetamines: metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine, and their N-alkyl derivatives. Ther Drug Monit 2002;24:277–289.

53. Kreth K, Kovar K, Schwab M, Zanger UM. Identification of the human cytochromes P450 involved in the oxidative metabolism of "Ecstasy"-related designer drugs. Biochem Pharmacol 2000;59:1563–1571.

54. Capela JP, Macedo C, Branco PS, Ferreira LM, Lobo AM, Fernandes E et al. Neurotoxicity mechanisms of thioether ecstasy metabolites. Neuroscience 2007;146: 1743–1757.

55. Farre M, de la Torre, Mathuna BO, Roset PN, Peiro AM, Torrens M et al. Repeated doses administration of MDMA in humans: pharmacological effects and pharmacokinetics. Psychopharmacology (Berl) 2004;173: 364–375.

56. Segura M, Farre M, Pichini S, Peiro AM, Roset PN, Ramirez A et al. Contribution of cytochrome P450 2D6 to 3,4-methylenedioxymethamphetamine disposition in humans: use of paroxetine as a metabolic inhibitor probe. Clin Pharmacokinet 2005;44:649–660.

57. de la Torre R, Farre M, Mathuna BO, Roset PN, Pizarro N, Segura M et al. MDMA (ecstasy) pharmacokinetics in a CYP2D6 poor metabolizer and in nine CYP2D6 extensive metabolizers. Eur J Clin Pharmacol 2005;61: 551–554.

58. Gilhooly TC, Daly AK. CYP2D6 deficiency, a factor in ecstasy related deaths? Br J Clin Pharmacol 2002;54: 69–70.

59. de la Torre R, Farre M. Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. Trends Pharmacol Sci 2004;25:505–508.

60. Johnson M, Elayan I, Hanson GR, Foltz RL, Gibb JW, Lim HK. Effects of 3,4-dihydroxymethamphetamine and 2,4,5-trihydroxymethamphetamine, two metabolites of 3,4-methylenedioxymethamphetamine, on central sero-

tonergic and dopaminergic systems. J Pharmacol Exp Ther 1992;261:447–453.

61. Eifinger F, Roth B, Kroner L, Rothschild MA. Severe ecstasy poisoning in an 8-month-old infant. Eur J Pediatr 2008;167:1067–1070.

62. Scholey AB, Parrott AC, Buchanan T, Heffernan TM, Ling J, Rodgers J. Increased intensity of ecstasy and poly-drug usage in the more experienced recreational ecstasy/MDMA users: a WWW study. Addict Behav 2004;29: 743–752.

63. Farré M, Abanades S, Roset PN, Peiró AM, Torrens M, O'Mathúna B, Segura M, de la Torre R. Pharmacological interaction between 3,4-methylenedioxymethamphetamine (ecstasy) and paroxetine: pharmacological effects and pharmacokinetics. J Pharmacol Exp Ther 2007;323:954–962.

64. Liechti ME, Vollenweider FX. The serotonin uptake inhibitor citalopram reduces acute cardiovascular and vegetative effects of 3,4-methylenedioxymethamphetamine ("ecstasy") in healthy volunteers. J Psychopharmacol 2000;14:269–274.

65. Silins E, Copeland J, Dillon P. Qualitative review of serotonin syndrome, ecstasy (MDMA) and the use of other serotonergic substances: hierarchy of risk. Aust N Z J Psychiatry 2007;41:649–655.

66. Harrington RD, Woodward JA, Hooton TM, Horn JR. Life-threatening interactions between HIV-1 protease inhibitors and the illicit drugs MDMA and gamma-hydroxybutyrate. Arch Intern Med 1999;159:2221–2224.

67. Henry JA, Hill IR. Fatal interaction between ritonavir and MDMA. Lancet 1998;352(9142):1751–1752.

68. Vuori E, Henry JA, Ojanperä I, Nieminen R, Savolainen T, Wahlsten P, Jäntti M. Death following ingestion of MDMA (ecstasy) and moclobemide. Addiction 2003;98: 365–368.

69. Smilkstein MJ, Smolinske SC, Rumack BH. A case of MAO inhibitor/MDMA interaction: agony after ecstasy. J Toxicol Clin Toxicol 1987;25:149–159.

70. Hernández-López C, Farré M, Roset PN, Menoyo E, Pizarro N, Ortuño J, et al. 3,4-Methylenedioxymethamphetamine (ecstasy) and alcohol interactions in humans: psychomotor performance, subjective effects, and pharmacokinetics. J Pharmacol Exp Ther 2002;300: 236–244.

71. McNamara R, Maginn M, Harkin A. Caffeine induces a profound and persistent tachycardia in response to MDMA ("ecstasy") administration. Eur J Pharmacol 2007;555:194–198.

72. McNamara R, Kerans A, O'neill B, Harkin A. Caffeine promotes hyperthermia and serotonergic loss following co-administration of the substituted amphetamines, MDMA ("ecstasy") and MDA ("love"). Neuropharmacology 2006;50:69–80.

73. Dumont GJ, Kramers C, Sweep FC, Touw DJ, van Hasselt JG, de Kam M, et al. Cannabis coadministration potentiates the effects of "ecstasy" on heart rate and temperature in humans. Nature 2009;86:160–166.

74. Campbell NG, Koprich JB, Kanaan NM, Lipton JW. MDMA administration to pregnant Sprague-Dawley rats results in its passage to the fetal compartment. Neurotoxicol Teratol 2006;28:459–465.

75 Morton J. Ecstasy: pharmacology and neurotoxicity. Curr Opin Pharmacol 2005;5:79–86.

76. Liechti ME, Gamma A, Vollenweider FX. Gender differences in the subjective effects of MDMA. Psychopharmacology (Berl) 2001;154:161–168.

77. Liechti ME, Vollenweider FX. Which neuroreceptors mediate the subjective effects of MDMA in humans? A summary of mechanistic studies. Hum Psychopharmacol 2001;16:589–598.

78. Callaway CW, Wing LL, Geyer MA. Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J Pharmacol Exp Ther 1990;254:456–464.

79. Battaglia G, Yeh SY, O'Hearn E, Molliver ME, Kuhar MJ, De Souza EB. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites. J Pharmacol Exp Ther 1987;242: 911–916.

80. Stone DM, Merchant KM, Hanson GR, Gibb JW. Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 1987;26:1677–1683.

81. Vollenweider FX, Liechti ME, Gamma A, Greer G, Geyer M. Acute psychological and neurophysiological effects of MDMA in humans. J Psychoactive Drugs 2002; 34:171–184.

82. Battaglia G, Brooks BP, Kulsakdinun C, De Souza EB. Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. Eur J Pharmacol 1988;149:159–163.

83. Leonardi ET, Azmitia EC. MDMA (ecstasy) inhibition of MAO type A and type B: comparisons with fenfluramine and fluoxetine (Prozac). Neuropsychopharmacology 1994;10:231–238.

84. Frith CH, Chang LW, Lattin DL, Walls RC, Hamm J, Doblin R. Toxicity of methylenedioxymethamphetamine (MDMA) in the dog and the rat. Fundam Appl Toxicol 1987;9:110–119.

85. Peroutka SJ, Newman H, Harris H. Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. Neuropsychopharmacology 1988;1:273–277.

86. Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS. Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 1987;241:338–345.

87. Schmidt CJ, Taylor VL. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem Pharmacol 1987;36:4095-4102.

88. Karlsen SN, Spigset O, Slordal L. The dark side of ecstasy: neuropsychiatric symptoms after exposure to 3,4-methylenedioxymethamphetamine. Basic Clin Pharmacol Toxicol 2007;102:15–24.

89. Baumann MH, Wang X, Rothman RB. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology 2007;189:407–424.

90. O'Hearn E, Battaglia G, De Souza EB, Kuhar MJ, Molliver ME. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 1988;8:2788–2803.

91. Lyvers M. Recreational ecstasy use and the neurotoxic potential of MDMA: current status of the controversy and methodological issues. Drug Alcohol Rev 2006;25:269–276.

92. Fitzgerald RL, Blanke RV, Rosecrans JA, Glennon RA. Stereochemistry of the metabolism of MDMA to MDA. Life Sci 1989;45:295–301.

93. McCann UD, Szabo Z, Seckin E, Rosenblatt P, Mathews WB, Ravert HT, et al. Quantitative PET studies of the serotonin transporter in MDMA users and controls using [11C]McN5652 and [11C]DASB. Neuropsychopharmacology 2005;30:1741–1750.

94. Buchert R, Thomasius R, Nebeling B, Petersen K, Obrocki J, Jenicke L, et al. Long-term effects of "ecstasy" use on serotonin transporters of the brain investigated by PET. J Nucl Med 2003;44:375–384.

95. Buchert R, Thiele F, Thomasius R, Wilke F, Petersen K, Brenner W, et al. Ecstasy-induced reduction of the availability of the brain serotonin transporter as revealed by [11C](+)McN5652-PET and the multi-linear reference tissue model: loss of transporters or artifact of tracer kinetic modelling? J Psychopharmacol 2007;21:628–634.

96. Gordon CJ, Watkinson WP, O'Callaghan JP, Miller DB. Effects of 3,4-methylenedioxymethamphetamine on autonomic thermoregulatory responses of the rat. Pharmacol Biochem Behav 1991;38:339–344.

97. Docherty JR, Green AR. The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives. Br J Pharmacol 2010;160:1029–1044.

98. Sanchez V, O'shea E, Saadat KS, Elliott JM, Colado MI, Green AR. Effect of repeated ("binge") dosing of MDMA to rats housed at normal and high temperature on neurotoxic damage to cerebral 5-HT and dopamine neurones. J Psychopharmacol 2004;18:412–416.

99. O'Shea E, Orio L, Escobedo I, Sanchez V, Camarero J, Green AR, Colado MI. MDMA-induced neurotoxicity: long-term effects on 5-HT biosynthesis and the influence of ambient temperature. Br J Pharmacol 2006;148:778–785.

100. Sprague JE, Moze P, Caden D, Rusyniak DE, Holmes C, Goldstein DS, Mills EM. Carvedilol reverses hyperthermia and attenuates rhabdomyolysis induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) in an animal model. Crit Care Med 2005;33:1311–1316.

101. Bexis S, Docherty JR. Role of alpha 1- and beta 3-adrenoceptors in the modulation by SR59230A of the effects of MDMA on body temperature in the mouse. Br J Pharmacol 2009;158:259–266.

102. Liechti ME, Saur MR, Gamma A, Hell D, Vollenweider FX. Psychological and physiological effects of MDMA ("ecstasy") after pretreatment with the 5-HT(2) antagonist ketanserin in healthy humans. Neuropsychopharmacology 2000;23:396–404.

103. Forsling ML, Fallon JK, Shah D, Tilbrook GS, Cowan DA, Kicman AT, Hutt AJ. The effect of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its metabolites on neurohypophysial hormone release from the isolated rat hypothalamus. Br J Pharmacol 2002;135:649–656.

104. Fallon JK, Shah D, Kicman AT, Hutt AJ, Henry JA, Cowan DA, Forsling M. Action of MDMA (ecstasy) and its metabolites on arginine vasopressin release. Ann N Y Acad Sci 2002;965:399–409.

105. Campbell GA, Rosner MH. The agony of ecstasy: MDMA (3,4-methylenedioxymethamphetamine) and the kidney. Clin J Am Soc Nephrol 2008;3:1852–1860.

106. Ellis AJ, Wendon JA, Portmann B, Williams R. Acute liver damage and ecstasy ingestion. Gut 1996;38:454–458.

107. Jones AL, Simpson KJ. Review article: mechanisms and management of hepatotoxicity in ecstasy (MDMA) and amphetamine intoxications. Aliment Pharmacol Ther 1999;13:129–133.

108. Fidler H, Dhillon A, Gertner D, Burroughs A. Chronic ecstasy (3,4-methylenedioxymetamphetamine) abuse: a recurrent and unpredictable cause of severe acute hepatitis. J Hepatol 1996;25:563–566.

109. Milroy CM, Clark JC, Forrest AR. Pathology of deaths associated with "ecstasy" and "eve" misuse. J Clin Pathol 1996;49:149–153.

110. Kaye S, Darke S, Duflou J. Methylenedioxymethamphetamine (MDMA)-related fatalities in Australia: demographics, circumstances, toxicology and major organ pathology. Drug Alcohol Depend 2009;104:254–261.

111. Williams H, Dratcu L, Taylor R, Roberts M, Oyefeso A. "Saturday night fever": ecstasy related problems in a London accident and emergency department. J Accid Emerg Med 1998;15:322–326.

112. Parrott AC. Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. Psychopharmacology (Berl) 2004;173:234–241.

113. Dinis-Oliveira RJ, Caldas I, Carvalho F, Magalhães T. Bruxism after 3,4-methylenedioxymethamphetamine (ecstasy) abuse. Clin Toxicol 2010;48:863–864.

114. Cregg MT, Tracey JA. Ecstasy abuse in Ireland. Ir Med J 1993;86:118–120.

115. Mørland J. Toxicity of drug abuse—amphetamine designer drugs (ecstasy): mental effects and consequences of single dose use. Toxicol Lett 2000; 112–113:147–152.

116. Sanjurjo E, Nogué S, Miró O, Munné P. [Analysis of patients attended in an emergency department due to ecstasy consumption]. Med Clin (Barc) 2004;123:90–92. [Spanish]

117. Liester MB, Grob CS, Bravo GL, Walsh RN. Phenomenology and sequelae of 3,4-methylenedioxymethamphetamine use. J Nerv Ment Dis 1992; 180:345–352

118. Parrott AC, Sisk E, Turner JJ. Psychobiological problems in heavy "ecstasy" (MDMA) polydrug users. Drug Alcohol Depend 2000;60:105–110.

119. Parrott AC, Buchanan T, Scholey AB, Heffernan T, Ling J, Rodgers J. Ecstasy/MDMA attributed problems reported by novice, moderate and heavy recreational users. Hum Psychopharmacol 2002;17:309–312.

120. Pallanti S, Mazzi D. MDMA (Ecstasy) precipitation of panic disorder. Biol Psychiatry 1992;32:91–95.

121. Greene SL, Dargan PI, O'Connor N, Jones AL, Kerins M. Multiple toxicity from 3,4-methylenedioxymethamphetamine ("ecstasy"). Am J Emerg Med 2003; 21:121–124.

122. Gledhill JA, Moore DF, Bell D, Henry JA. Subarachnoid haemorrhage associated with MDMA abuse. J Neurol Neurosurg Psychiatry 1993;56:1036–1037.

123. Harries DP, De Silva R. "Ecstasy" and intracerebral haemorrhage. Scott Med J 1992;37:150–152.

124. Rothwell PM, Grant R. Cerebral venous sinus thrombosis induced by "ecstasy". J Neurol Neurosurg Psychiatry 1993;56:1035.

125. Manchanda S, Connolly MJ. Cerebral infarction in association with ecstasy abuse. Postgrad Med J 1993;69: 874–875.

126. Muntan CD, Tuckler V. Cerebrovascular accident following MDMA ingestion. J Med Toxicol 2006;2:16–18.

127. Madhok A, Boxer R, Chowdhury D. Atrial fibrillation in an adolescent – the agony of ecstasy. Pediatr Emerg Care 2003;19:348–349.

128. Lai T-L, Hwang J-J, Fang C-C, Chen W-J. Methylene 3,4dioxymethamphetamine-induced acute myocardial infarction. Ann Emerg Med 2003;42:759–762.

129. Qasim A, Townend J, Davies MK. Ecstasy induced acute myocardial infarction. Heart 2001;85:e10.

130. Mazur S, Hitchcock T. Spontaneous pneumomediastinum, pneumothorax and ecstasy abuse. Emerg Med (Fremantle) 2001;13:121–123.

131. Mortelmans LJ, Bogaerts PJ, Hellemans S, Volders W, Van Rossom P. Spontaneous pneumomediastinum and myocarditis following Ecstasy use: a case report. Eur J Emerg Med 2005;12:36–38.

132. Chang SH, Lai TI, Chen WJ, Fang CC. MDMA-induced acute pulmonary edema in a patient without other organ dysfunction. Am J Emerg Med 2006;24:734–736.

133. Isbister GK, Buckley NA, Whyte IM. Serotonin toxicity: a practical approach to diagnosis and treatment. Med J Aust 2007;187:361–365.

134. Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glennon RA, Blough B, et al. 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells *in vitro*. Mol Pharmacol 2003;63: 1223–1229.

135. Droogmans S, Cosyns B, D'haenen H, Creeten E, Weytjens C, Franken PR, et al. Possible association between 3,4-methylenedioxymethamphetamine abuse and valvular heart disease. Am J Cardiol 2007;100: 1442–1445.

136. Halpern P, Moskovich J, Avrahami B, Bentur Y, Soffer D, Peleg K. Morbidity associated with MDMA (ecstasy) abuse: a survey of emergency department admissions. Hum Exp Toxicol 2011;30:259–266.

137. Walubo A, Seger D. Fatal multi-organ failure after suicidal overdose with MDMA, "ecstasy": case report and review of the literature. Hum Exp Toxicol 1999;18: 119–125.

138. Ben-Abraham R, Szold O, Rudick V, Weinbroum AA. "Ecstasy" intoxication: life-threatening manifestations and resuscitative measures in the intensive care setting. Eur J Emerg Med 2003;10:309–313.

139. Hall AP, Henry JA. Acute toxic effects of "ecstasy" (MDMA) and related compounds: overview of pathophysiology and clinical management. Br J Anaesth 2006; 96:678–685.

140. Mallick A, Bodenham AR. MDMA induced hyperthermia: a survivor with an initial body temperature of 42.9 degrees C. J Accid Emerg Med 1997;14:336–338.

141. Guneysel O, Onur E, Akoglu H, Denizbasi A. Ecstasy-induced recurrent toxic hepatitis in a young adult. Curr Ther Res Clin Exp 2008;69:260–265.

142. Andreu V, Mas A, Bruguera M, Salmeron JM, Moreno V, Nogue S, Rodes J. Ecstasy: a common cause of severe acute hepatotoxicity. J Hepatol 1998;29:394–397.

143. Brauer RB, Heidecke CD, Nathrath W, Beckurts KT, Vorwald P, Zilker TR, et al. Liver transplantation for the treatment of fulminant hepatic failure induced by the ingestion of ecstasy. Transpl Int 1997;10:229–233.

144. Libiseller K, Pavlic M, Grubwieser P, Rabl W. Ecstasy—deadly risk even outside rave parties. Forensic Sci Int 2005;153:227–230.

145. Robledo P, Balerio G, Berrendero F, Maldonado R. Study of the behavioural responses related to the potential addictive properties of MDMA in mice. Naunyn Schmiedebergs Arch Pharmacol 2004;369:338–349.

146. Curran HV, Travill RA. Mood and cognitive effects of +/-3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"): week-end "high" followed by mid-week low. Addiction 1997;92:821–831.

147. Huxster JK, Pirona A, Morgan MJ. The sub-acute effects of recreational ecstasy (MDMA) use: a controlled study in humans. J Psychopharmacol 2006;20:281–290.

148. McElhatton PR, Bateman DN, Evans C, Pughe KR, Thomas SH. Congenital anomalies after prenatal ecstasy exposure. Lancet 1999;354:1441–1442.

149. Ho E, Karimi-Tabesh L, Koren G. Characteristics of pregnant women who use ecstasy (3, 4-methylenedioxymethamphetamine). Neurotoxicol Teratol 2001; 23:561–567.

150. van Tonningen-van Driel MM, Garbis-Berkvens JM, Reuvers-Lodewijks WE. [Pregnancy outcome after ecstasy use; 43 cases followed by the Teratology Information Service of the National Institute for Public Health and Environment (RIVM)]. Ned Tijdschr Geneeskd 1999;143:27–31. [Dutch]

151. Stout PR, Klette KL, Wiegand R. Comparison and evaluation of DRI methamphetamine, DRI ecstasy, Abuscreen ONLINE amphetamine, and a modified Abuscreen ONLINE amphetamine screening immunoassays for the detection of amphetamine (AMP), methamphetamine (MTH), 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA) in human urine. J Anal Toxicol 2003;27:265–269.

152. Marin SJ, Keith L, Merrell M, McMillin GA. Comparison of drugs of abuse detection in meconium by EMIT® II and ELISA. J Anal Toxicol 2009;33:148–154.

153. Ortuño J, Pizarro N, Farré M, Mas M, Segura J, Camí J, et al. Quantification of 3,4-methylenedioxymethamphetamine and its metabolites in plasma and urine by gas chromatography with nitrogen-phosphorus detection. J Chromatogr B Biomed Sci Appl 1999;723: 221–232.

154. Clauwaert KM, Van Bocxlaer JF, De Letter EA, Van Calenbergh S, Lambert WE, De Leenheer AP. Determination of the designer drugs 3, 4-methylenedioxymethamphetamine, 3,4-methylenedioxyethylamphetamine, and 3,4-methylenedioxyamphetamine with HPLC and fluorescence detection in whole blood, serum, vitreous humor, and urine. Clin Chem 2000;46: 1968–1977.

155. Pizarro N, Ortuño J, Farré M, Hernández-López C, Pujadas M, Llebaria A, et al. Determination of MDMA and its metabolites in blood and urine by gas chromatography-mass spectrometry and analysis of enantiomers by capillary electrophoresis. J Anal Toxicol 2002;26:157–165.

156. Shima N, Katagi M, Mamata H, Zaitsu K, Kamata T, Miki A, et al. Conjugates of p-hydroxymethamphetamine and 4-hydroxy-3-methoxymethamphetamine in blood obtained from methamphetamine and 3,4-methylenedioxymethamphetamine users: analysis by LC-MS-MS. Forensic Toxicol 2008;26:58–65.

157. Butler D, Guilbault GG. Analytical techniques for ecstasy. Anal Lett 2004;37:2003–2030.

158. Gan BK, Baugh D, Liu RH, Walia AS. Simultaneous analysis of amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA) in urine samples by solid-phase extraction, derivatization,

and gas chromatography/mass spectrometry. J Forensic Sci 1991;36:1331–1341.

159. Shima N, Kamata H, Katagi M, Tsuchihashi H, Sakuma T, Nemoto N. Direct determination of glucuronide and sulfate of 4-hydroxy-3-methoxymethamphetamine, the main metabolite of MDMA, in human urine. J Chromatogr B 2007;857:123–129.

160. Pirnay SO, Abraham TT, Huestis MA. Sensitive gas chromatography-mass spectrometry method for simultaneous measurement of MDEA, MDMA, and metabolites HMA, MDA, and HMMA in human urine. Clin Chem 2006;52:1728–1734.

161. Andersson M, Gustavsson E, Stephanson N, Beck O. Direct injection LC-MS/MS method for identification and quantification of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in urine drug testing. J Chromatogr B 2008;861:22–28.

162. Scheidweiler KB, Huestis MA. A validated gas chromatographic-electron impact ionization mass spectrometric method for methylenedioxymethamphetamine (MDMA), methamphetamine and metabolites in oral fluid. J Chromatogr B 2006;835:90–99.

163. Nakamura S, Wada M, Crabtree BL, Reeves PM, Montgomery JH, Byrd HJ, et al. A sensitive semi-micro column HPLC method with peroxyoxalate chemiluminescence detection and column switching for determination of MDMA-related compounds in hair. Anal Bioanal Chem 2007;387:1983–1990.

164. Martins LF, Yegles M, Chung H, Wennig R. Sensitive, rapid and validated gas chromatography/negative ion chemical ionization-mass spectrometry assay including derivatisation with a novel chiral agent for the enantioselective quantification of amphetamine-type stimulants in hair. J Chromatogr B Analyt Technol Biomed Life Sci 2006;842:98–105.

165. Peters FT, Samyn N, Lamers CT, Riedel WJ, Kraemer T, de Boeck G, Maurer HH. Drug testing in blood: validated negative-ion chemical ionization gas chromatographic-mass spectrometric assay for enantioselective measurement of the designer drugs MDEA, MDMA, and MDA and its application to samples from a controlled study with MDMA. Clin Chem 2005;51:1811-1822.

166. Rasmussen LB, Olsen KH, Johansen SS. Chiral separation and quantification of R/S-amphetamine, R/S-methamphetamine, R/S-MDA, R/S-MDMA, and R/S-MDEA in whole blood by GC-EI-MS. J Chromatogr B 2006;842:136–141.

167. Sherlock K, Wolff K, Hay AW, Conner M. Analysis of illicit ecstasy tablets: implications for clinical management in the accident and emergency department. J Accid Emerg Med 1999;16:194–197.

168. Tanner-Smith EE. Pharmacological content of tablets sold as "ecstasy": results from an online testing service. Drug Alcohol Depend 2006;83:247–254.

169. Johansen SS, Hansen AC, Muller IB, Lundemose JB, Franzmann MB. Three fatal cases of PMA and PMMA poisoning in Denmark. J Anal Toxicol 2003;27:253–256.

170. Martin TL. Three cases of fatal paramethoxyamphetamine overdose. J Anal Toxicol 2001;25:649–651.

171. Ling LH, Marchant C, Buckley NA, Prior M, Irvine RJ. Poisoning with the recreational drug paramethoxyamphetamine ("death"). Med J Aust 2001;174:453–455.

172. Waumans D, Bruneel N, Tytgat J. Anise oil as paramethoxyamphetamine (PMA) precursor. Forensic Sci Int 2003;133:159–170.

173. Vogels N, Brunt TM, Rigter S, van Dijk P, Vervaeke H, Niesink RJ. Content of ecstasy in the Netherlands: 1993–2008. Addiction 2009;104:2057–2066.

174. De Letter EA, Lambert WE, Bouche M-P, Cordonnier JA, Van Bocxlaer JF, Piette MH. Postmortem distribution of 3,4-methylenedioxy-,N,N-dimethyl-amphetamine (MDDM or MDDA) in a fatal MDMA overdose. Int J Legal Med 2007;121:303–307.

175. Weyermann C, Marquis R, Delaporte C, Esseiva P, Lock E, Aalberg L, et al. Drug intelligence based on MDMA tablets data I. Organic impurities profiling. Forensic Sci Int 2008;177:11–16.

176. Marquis R, Weyermann C, Delaporte C, Esseiva P, Aalberg L, Besacier R, et al. Drug intelligence based on MDMA tablets data 2. Physical characteristics profiling. Forensic Sci Int 2008;178:34–39.

177. Clauwaert KM, Van Bocxlaer JF, De Leenheer AP. Stability study of the designer drugs "MDA, MDMA and MDEA" in water, serum, whole blood, and urine under various storage temperatures. Forensic Sci Int 2001;124:36–42.

178. Garrett ER, Seyda K, Marroum P. High performance liquid chromatographic assays of the illicit designer drug "ecstasy," a modified amphetamine, with applications to stability, partitioning and plasma protein binding. Acta Pharm Nord 1991;3:9–13.

179. de la Torre R, Farre M, Ortuno J, Mas M, Brenneisen R, Roset PN, et al. Non-linear pharmacokinetics of MDMA ("ecstasy") in humans. Br J Clin Pharmacol 2000;49:104–109.

180. de la Torre R, Farré M, Navarro M, Pacifici R, Zuccaro P, Pichini S. Clinical pharmacokinetics of amfetamine and related substances: monitoring in conventional and non-conventional matrices. Clin Pharmacokinet 2004;43:157–185.

181. Pacifici R, Zuccaro P, Hernandez López C, Pichini S, Di Carlo S, Farré M, et al. Acute effects of 3,4-methylenedioxymethamphetamine alone and in combination with ethanol on the immune system in humans. J Pharmacol Exp Ther 2001;296:207–215.

182. Irvine RJ, Keane M, Felgate P, McCann UD, Callaghan PD, White JM. Plasma drug concentrations and physiological measures in "dance party" participants. Neuropsychopharmacology 2006;31:424–430.

183. Logan BK, Couper FJ. 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) and driving impairment. J Forensic Sci 2001;46:1426–1433.

184. Duffy MR, Swart M. Severe ecstasy poisoning in a toddler. Anaesthesia 2006;61:498–501.

185. Melian AM, Burillo-Putze G, Campo CG, Padron AG, Ramos CO. Accidental ecstasy poisoning in a toddler. Pediatr Emerg Care 2004;20:534-535.

186. Roberts L, Wright H. Survival following intentional massive overdose of "ecstasy". J Accid Emerg Med 1993;11:53–54.

187. Brown C, Osterloh J. Multiple severe complications from recreational ingestion of MDMA ("ecstasy"). JAMA 1987;258:780–781.

188. Gill JR, Hayes JA, deSouza IS, Marker E, Stajic M. Ecstasy (MDMA) deaths in New York City: a case series and review of the literature. J Forensic Sci 2002;47:121–126.

189. Liu RH, Liu H-C, Lin D-L. Distribution of methylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA) in postmortem and antemortem specimens. J Anal Toxicol 2006;30:545–550.

190. Lin D-L, Liu H-C, Liu RH. Methylenedioxymethamphetamine-related deaths in Taiwan: 2001–2008. J Anal Toxicol 2009;33:366–371.

191. Verschraagen M, Maes A, Ruiter B, Bosman IJ, Smink BE, Lusthof KJ. Post-mortem cases involving amphetamine-based drugs in the Netherlands. Comparison with driving under the influence cases. Forensic Sci Int 2007;170:163–170.

192. Klys M, Rojek S, Wozniak K, Rzepecka-Wozniak E. Fatality due to the use of a designer drug MDMA (ecstasy). Leg Med 2007;9:185–191.

193. Garcia-Repetto R, Moreno E, Soriano T, Jurado C, Gimenez MP, Menendez M. Tissue concentrations of MDMA and its metabolite MDA in three fatal cases of overdose. Forensic Sci Int 2003;135:110–114.

194. De Letter EA, Clauwaert KM, Lambert WE, Van Bocxlaer JF, De Leenheer AP, Piette MH. Distribution study of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine in a fatal overdose. J Anal Toxicol 2002;26:113–118.

195. Rohrig TP, Prouty RW. Tissue distribution of methylenedioxymethamphetamine. J Anal Toxicol 1992;16:52–53.

196. Elliott SP. MDMA and MDA concentrations in antemortem and postmortem specimens in fatalities following hospital admission. J Anal Toxicol 2005;29:296–300.

197. Zhao H, Brenneisen R, Scholer A, McNally AJ, ElSohly MA, Murphy TP, Salamone SJ. Profiles of urine samples taken from ecstasy users at rave parties: analysis by immunoassays, HPLC, and GC-MS. J Anal Toxicol 2001;25:258–269.

198. Hsu J, Liu C, Hsu CP, Tsay W-I, Li J-H, Lin D-L, Liu RH. Performance characteristics of selected immunoassays for preliminary test of 3,4-methylenedioxymethampheta

mine,methamphetamine, and related drugs in urine specimens. J Anal Toxicol 2003;27:471–478.

199. Poklis A, Fitzgerald RL, Hall KV, Saady JJ. EMIT-d.a.u. monoclonal amphetamine/methamphetamine assay. II. Detection of methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA). Forensic Sci Int 1993;59:63–70.

200. De Letter EA, De Paepe P, Clauwaert KM, Belpaire FM, Lambert WE, Van Bocxlaer JF, Piette MH. Is vitreous humour useful for the interpretation of 3,4-methylenedioxymethamphetamine (MDMA) blood levels? Experimental approach with rabbits. Int J Legal Med 2000;114:29–35.

201. De Letter EA, Bouche M_P, van Bocxlaer JF, Lambert WE, Piette MH. Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: discussion by means of a distribution study in 2 fatalities. Forensic Sci Int 2004;141:85–90.

202. Dams R, De Letter EA, Mortier KA, Cordonnier JA, Lambert WE, Piette MH, et al. Fatality due to combined use of the designer drugs MDMA and PMA: a distribution study. J Anal Toxicol 2003;27:318–322.

203. Pichini S, Poudevida S, Pujadas M, Menoyo E, Pacifici R, Farré M, de la Torre R. Assessment of chronic exposure to MDMA in a group of consumers by segmental hair analysis. Ther Drug Monit 2006;28:106–109.

204. Martins LF, Yegles M, Samyn N, Ramaekers JG, Wennig R. Time-resolved hair analysis of MDMA enantiomers by GC/MS-NCI. Forensic Sci Int 2007;172:150–155.

205. Han E, Park Y, Yang W, Lee J, Lee S, Kim E, et al. The study of metabolite-to-parent drug ratios of methamphetamine and methylenedioxymethamphetamine in hair. Forensic Sci Int 2006;161:124–129.

206. Ravina P, Quiroga JM, Ravina T. Hyperkalemia in fatal MDMA ("ecstasy") toxicity. Int J Cardiol 2004;93: 307–308.

207. Kish SJ. How strong is the evidence that brain serotonin neurons are damaged in human users of ecstasy? Pharmacol Biochem Behav 2002;71:845–855.

208. Gouzoulis-Mayfrank E, Daumann J. The confounding problem of polydrug use in recreational ecstasy/MDMA users: a brief overview. Psychopharmacology 2006;20: 188–193.

209. Gouzoulis-Mayfrank E, Daumann J. Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: how strong is the evidence for persistent brain damage? Addiction 2006;101:348–361.

210. Zakzanis KK, Campbell Z, Jovanovski D. The neuropsychology of ecstasy (MDMA) use: a quantitative review. Hum Psychopharmacol Clin Exp 2007;22:427–435.

211. Rogers J. Cognitive performance amongst recreational users of "ecstasy". Psychopharmacology 2000;151:19–24.

212. Gouzoulis-Mayfrank E, Daumann J, Tuchtenhagen F, Pelz S, Becker S, Kunert H-J, et al. Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). J Neurol Neurosurg Psychiatry 2000;68: 719–725.

213. Brown J, McKone E, Ward J. Deficits of long-term memory in ecstasy users are related to cognitive complexity of the task. Psychopharmacology 2010;209: 51–67.

214. Hoshi R, Mullins K, Boundy C, Brignell C, Piccini P, Curran HV. Neurocognitive function in current and ex-users of ecstasy in comparison to both matched polydrug-suing controls and drug-naïve controls. Psychopharmacology 2007;194:371-379.

215. Laws KR, Kokkalis J. Ecstasy (MDMA) and memory function: a meta-analytic update. Hum Psychopharmacol 2007;22:381–388.

216. Jager G, de Win MM, van der Tweel I, Schilt T, Kahn RS, van den Brink W, et al. Assessment of cognitive brain function in ecstasy users and contributions of other drugs of abuse: results from an fMRI study. Neuropsychopharmacology 2008;33:247–258.

217. Parrott AC. Human research on MDMA (3,4-methylenedioxymethamphetamine) neurotoxicity: cognitive and behavioural indices of change. Neuropsychobiology 2000;42:17–24.

218. Hooft PJ, van de Voorde HP. Reckless behaviour related to the use of 3,4-methylenedioxymethamphetamine (ecstasy): apropos of a fatal accident during car-surfing. Int J Legal Med 1994;106:328-329.

219. Hausken AM, Skurtveit S, Christophersen AS. Characteristics of drivers testing positive for heroin or ecstasy in Norway. Traffic Inj Prev 2004;5:107–111.

220. Augsburger M, Donze N, Menetrey A, Brossard C, Sporkert F, Giroud C, Mangin P. Concentration of drugs in blood of suspected impaired drivers. Forensic Sci Int 2005;153:11–15.

221. Senna M-C, Augsburger M, Aebi B, Briellmann TA, Donze N, Dubugnon J-L, et al. First nationwide study on driving under the influence of drugs in Switzerland. Forensic Sci Int 2010; 198:11–16.

222. Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of central stimulant amines: age and gender differences in concentrations of amphetamine, methamphetamine, and ecstasy in blood. J Stud Alcohol Drugs 2008;69:202–208.

223. Ramaekers JG, Kuypers KP, Samyn N. Stimulant effects of 3,4-methylenedioxymethamphetamine (MDMA) 75 mg and methylphenidate 20 mg on actual driving during intoxication and withdrawal. Addiction 2006;101: 1614–1621.

224. Kuypers KP, Samyn N, Ramaekers JG. MDMA and alcohol effects, combined and alone, on objective and subjective measures of actual driving performance and psychomotor function. Psychopharmacology (Berl) 2006;187:467–475.

225. Brookhuis KA, de Waard D, Samyn N. Effects of MDMA (ecstasy), and multiple drugs use on (simulated) driving performance and traffic safety. Psychopharmacology 2004;173:440–445.

226. Lamers CT, Ramaekers JG, Muntjewerff ND, Sikkema KL, Samyn N, Read NL, et al. Dissociable effects of a

single dose of ecstasy (MDMA) on psychomotor skills and attentional performance. J Psychopharmacol 2003; 17:379–387.

227. Liechti ME, Vollenweider FX. Acute psychological and physiological effects of MDMA ("Ecstasy") after haloperidol pretreatment in healthy humans. Eur Neuropsychopharmacol 2000;10:289–295.

228. Singarajah C, Lavies NG. An overdose of ecstasy. A role for dantrolene. Anaesthesia 1992;47:686–687.

229. Duffy MR, Ferguson C. Role of dantrolene in treatment of heat stroke associated with ecstasy ingestion. Br J Anaesth 2007;99:148–149.

Chapter 10

PSYCHOACTIVE PHENETHYLAMINE, PIPERAZINE, and PYRROLIDINOPHENONE DERIVATIVES

Brandon Wills, DO, MS
Timothy Erickson, MD

PHENETHYLAMINE COMPOUNDS

The term "phenethylamine or amphetamine (α-methyl phenethylamine) designer drugs" refers to drugs with varying stimulant and psychotomimetic properties that were originally synthesized to avoid legal restriction by altering the structure of known illicit drugs.[1] These synthetic drugs are usually substituted phenethylamine analogs based on variations of the basic phenethylamine structure (benzene ring with an ethylamine group on the first carbon). The 3 main classes of substances of abuse derived from phenethylamine as identified in Table 10.1 include the following: 1) amphetamine stimulants (amphetamine, methamphetamine), 2) single methylenedioxy-substituted amphetamine entactogens (e.g., 3,4-methylenedioxyamphetamine [MDA], 3,4-methylenedioxymethamphetamine [MDMA], 3,4-methylenedioxyethamphetamine [MDEA], N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine [MBDB]), and 3) 3-fold ring substituted phenethylamine psychedelics (e.g., 2,5-dimethoxy-4-bromoamphetamine [DOB], 2,5-dimethoxy-4-ethylamphetamine [DOET], 4-methyl-2,5-dimethoxyamphetamine [DOM], 4-iodo-2,5-dimethoxyamphetamine [DOI]). Although originally applied to MDMA and MBDB,[2] amphetamine or phenethylamine designer drugs now refer to other methylenedioxy-substituted amphetamine compounds (e.g., MDEA, MBDB) that reportedly assist individuals experience a "touching from within." The phenethylamine molecule is also the basic chemical structure for endogenous catecholamines, neurotransmitters, and many therapeutic drugs (phenylpropanolamine, amphetamine, ephedrine).

The prototype psychoactive phenethylamine compound is mescaline (3,4,5-trimethoxyphenylethylamine), which is a natural constituent of the peyote cactus [*Lophophora williamsii* (Lem. ex Salm-Dyck) Coult.]. Although mescaline was a model for the studies of the structure-activity relationships of hallucinogenic drugs,[3] most of the synthetic compounds contained the 3-carbon chain of amphetamine.[4] Hence, the term "designer amphetamine drugs." In the early 1950s, Peretz et al. first described the hallucinogenic properties of the mescaline analog, 3,4,5-trimethoxyamphetamine (TMA).[5] In the 1960s and 1970s, Shulgin et al. investigated the clinical effects of a variety of methoxylated amphetamine compounds derived from mescaline.[6] He synthesized the mescaline analog, TMA from elemicin, and he reported that this drug produced changes in sensory perception at about half the usual dose of mescaline. Other drugs investigated by Shulgin et al. during the 1960s included 3-methoxy-4,5-methylenedioxyamphetamine (derived from myristicin) and ethyl homologs of 2,4,5-trimethoxyamphetamine (DOM, DOET).[7,8]

During the 1990s, the illicit drug market for hallucinogenic drugs changed considerably.[9] Newly marketed *para*-substituted methoxy street drugs included

TABLE 10.1. Phenethylamine and Other Designer Amphetamine Drugs.*

Chemical Type	Compound	Abbreviation; Street Name	CAS RN
Phenethylamines			
Methylenedioxy-Entactogens			
	3,4-Methylenedioxyamphetamine	MDA; Love	4764-17-4
	3,4-Methylenedioxyethamphetamine	MDEA, MDE; Eve	82801-81-8
	3,4-Methylenedioxymethamphetamine	MDMA; Ecstasy	42542-10-9
	3,4-Methylenedioxy-*N*-*N*-dimethylamphetamine	MDMMA	
	N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine	MBDB	103818-46-8
p-Methoxy-Series	4-Methoxyamphetamine (*p*-methoxyamphetamine)	PMA	64-13-1
	4-Methoxymethamphetamine (*p*-methoxymethamphetamine)	PMMA	22331-70-0
Thio-Substituted	4-Methylthioamphetamine	4-MTA	14116-06-4
Dimethoxy-Hallucinogens			
	2,5-Dimethoxyamphetamine	DMA	2801-68-5
	2,5-Dimethoxy-4-bromoamphetamine	DOB, 4-Bromo-DMA, PBR	32156-26-6
	2,5-Dimethoxy-4-chloroamphetamine	DOC	
	2,5-Dimethoxy-4-ethylamphetamine	DOET	22004-32-6
	4-Iodo-2,5-dimethoxyamphetamine	DOI	64584-34-5
	4-Methyl-2,5-dimethoxyamphetamine	DOM (STP)	15588-95-1
	3,4,5-Trimethoxyamphetamine†	TMA	1082-88-8
2C-Designer Series (2,5-phenethylamines)			
	2,5-Dimethoxy-4-bromophenethylamine	2C-B, MFT, BDMPEA	66142-81-2
	1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b:4,5-b′]difuran-4-yl)-2-aminoethane	2C-B-fly	
	2,5-Dimethoxy-4-methyl-phenethylamine	2C-D	24333-19-5
	4-Ethyl-2,5-dimethoxy-*β*-phenethylamine	2C-E	
	2-(2,5-Dimethoxyphenyl)ethanamine	2C-H	
	4-Iodo-2,5-dimethoxy-*β*-phenethylamine	2C-I	
	2-(2,5-Dimethoxy-4-propylhenyl)ethanamine	2C-P	
	2,5-Dimethoxy-4-ethylthio-*β*-phenethylamine	2C-T-2	
	2-[4-(Isopropylthio)-2,5-dimethoxyphenyl]ethanamine		
	2,5-Dimethoxy-4-propylthio-*β*-phenethylamine	2C-T-7	207740-26-9
Piperazines			
	N-Benzylpiperazine	BZP, A2	2759-28-6
	1-Piperonylpiperazine [1-(3,4-Methylenedioxybenzyl)piperazine]	MDBP	32231-06-4
	1-(4-Methoxyphenyl)piperazine	MeOPP	38212-30-5
	1-(3-Chlorophenyl)piperazine	mCPP	6640-24-0
	1-(3-Trifluoromethylphenyl)piperazine	TFMPP; Molly	15532-75-9
	1-(4-Methoxyphenyl)piperazine	MeOPP	38212-30-5
Pyrrolidinophenones			
	α-Pyrrolidinopropiophenone	PPP	19134-50-0
	4′-Methoxy-α-pyrrolidinopropiophenone	MOPPP	—
	3′,4′-Methylenedioxy-α-pyrrolidinopropiophenone	MDPPP	—
	4′-Methyl-α-pyrrolidinopropiophenone	MPPP	—
	4′-Methyl-α-pyrrolidinohexanophenone	MPHP	—

*This list is not exhaustive—there are at least 200 potential substituted phenethylamine stimulant and psychedelic drugs including fluorinated derivatives.

†The family of trimethylamphetamine compounds includes at least 5 other isomers of TMA including TMA-2, TMA-3, TMA-4, TMA-5, and TMA-6.

4-methoxyamphetamine (PMA) and 4-methoxymeth-amphetamine (PMMA) as well as the *para*-substituted methylthio compound, 4-methylthioamphetamine (4-MTA). The newer designer amphetamines are often marketed on the street as MDMA (ecstasy), but these compounds may also be sold as the actual compound or as a street drug that publicize specific properties.[10,11] Table 10.1 classifies some of the most common amphet-amine designer drugs groups based on location and type of substituents. The psychotomimetic properties of

phenethylamine compounds are usually several-fold more potent for the *R* (−)-enantiomers than for the *S* (+)-enantiomers.[12] Substituents often enhance potency, particularly bromine at the *para* position of the benzene ring of phenethylamine compounds.[13] Figure 10.1 compares the chemical structures of various substituted amphetamine designer drugs. Fluoromethoxyamphetamine derivatives are not listed in this figure as these compounds are uncommon and little is known about their properties.[14]
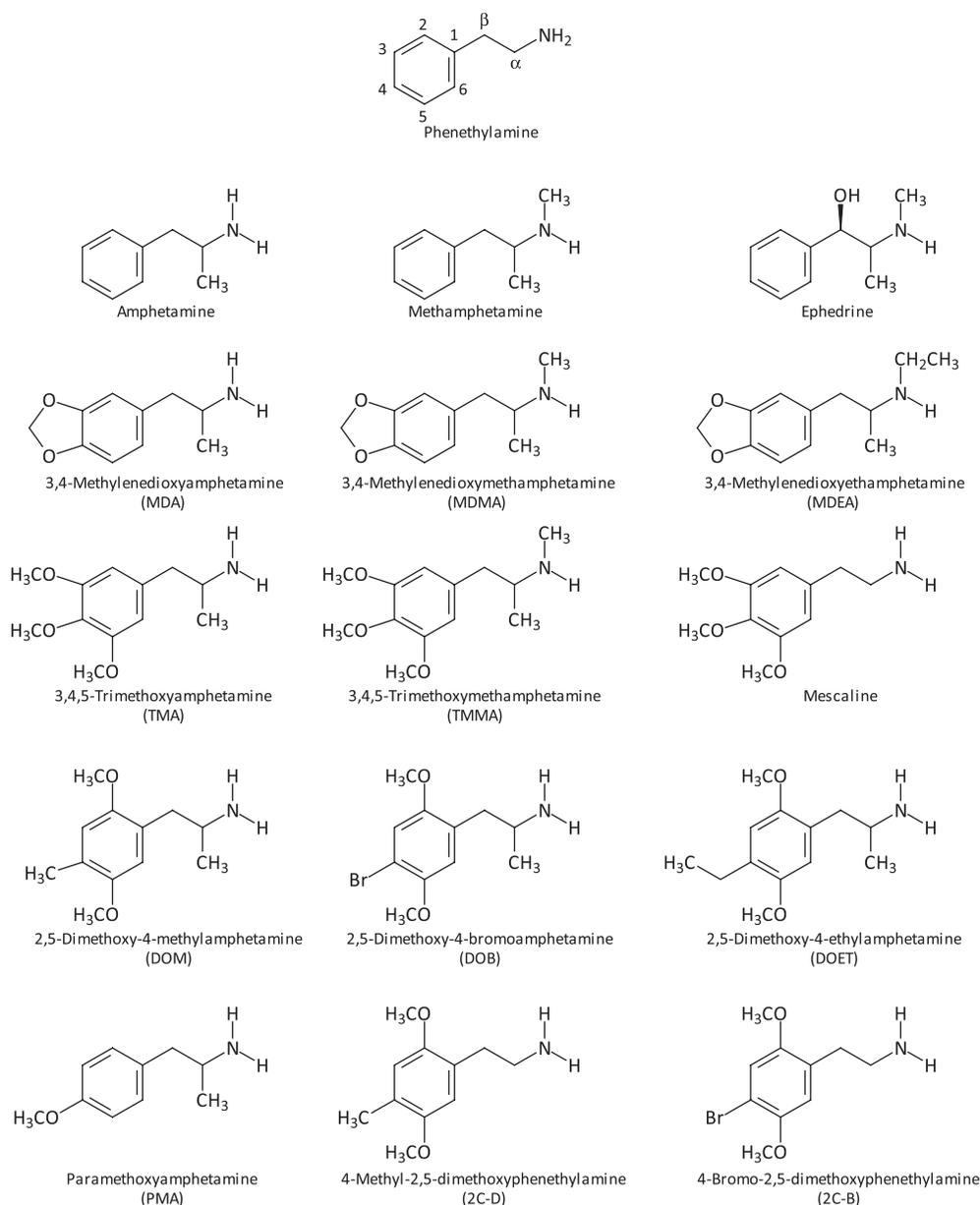
FIGURE 10.1. Comparative chemical structures of phenethylamine and related hallucinogenic compounds.

3,4-Methylene-dioxyamphetamine (MDA)

German scientists synthesized MDA in the early 1910s. In the late 1950s and early 1960s, MDA was patented as a cough suppressant, a tranquilizer, and an appetite suppressant; however, MDA was never marketed for medical purposes.[15] During the 1970s, MDA and MDMA were pharmaceutical adjuncts to psychotherapy;[16] however, the US Drug Enforcement Administration (DEA) added both these drugs to schedule I after reports of the abuse of these drugs in the 1980s.[17] Some controversy surrounded this decision as a result of the subsequent limitations to research, primarily from therapists using MDMA to enhance self-disclosure and promote trust in patients undergoing psychotherapy.[18]

IDENTIFYING CHARACTERISTICS

Like most designer amphetamines, MDA (tenamfetamine, [INN]) exists as the free base or as the salt of various acids. These salts are water soluble; therefore, potential routes of administration include ingestion, insufflation, and intravenous (IV) injection.[19] The high boiling point and low vapor pressure of MDA and other methylenedioxyamphetamine designer drugs limit the use of these drugs via inhalation of vapors.

Table 10.2 lists some physiochemical properties of MDA. In contrast to most amphetamine designer drugs, the S (+)-enantiomer of MDA is more potent than the R (−)-enantiomer.[20]

EXPOSURE

Synthesis of MDA involves the intermediate, nitropropane, which is prepared from 4-methylthiobenzaldehyde, nitroethane, and n-butylamine.[21] Tablets sold as ecstasy

TABLE 10.2. Some Physiochemical Properties of 3,4-Methylenedioxyamphetamine (MDA)

Physical Property	Value
pKa Dissociation Constant	9.67 (25°C/77°F)
log P (Octanol-Water)	1.64
Water Solubility	2.25E+04 mg/L (25°FC/77°F)
Vapor Pressure	1.69E-03 mm Hg (25°FC/77°F)

(MDMA) usually contain a single substance, but analysis of street drugs sold as ecstasy may contain MDA or other structurally similar drugs (MDEA, PMA, MBDB).[22]

TOXICOKINETICS

There are few toxicokinetic data on amphetamine designer drugs other than MDMA. MDA is a metabolite of MDMA biotransformation in humans.[23] The absorption of MDA is relatively rapid with time to peak plasma concentrations of approximately 3 hours.[24] MDA is the parent drug of MDMA and a N-demethylated metabolite of MDMA as well as MDEA.[23] Biotransformation of MDA occurs by demethylenation to the corresponding catecholamine via primarily CYP2D6 based on *in vitro* studies.[25] Postmortem analyses suggest that the bile contains significant amounts of MDA.[26] There are several potential sources of drug-drug interactions with amphetamine designer drugs, but the clinical relevance of these potential drug interactions remains unclear. Although human polymorphism of CYP2D6 or the concomitant ingestion of CYP2D6 inhibitors (e.g., fluoxetine, paroxetine, quinidine) potentially alters the metabolism of amphetamine designer drugs, there is no definite evidence that this potential effect produces clinically significant effects. Theoretically, polymorphism of CYP2D6 may cause decreased metabolism of MDA and MDEA and relatively higher drug concentrations.

HISTOPATHOLOLGY AND PATHOPHYSIOLOGY

In general, animal studies indicate that phenethylamine designer drugs are indirect sympathomimetic agents that increase the release of biogenic amines by binding and blocking the transport of these neurotransmitters including serotonin, norepinephrine, and to a lesser extent, dopamine.[27] Modulation of serotonergic neurotransmission probably mediates the thermoregulatory and altered perceptions associated with the use of these drugs, whereas dopaminergic neurotransmission modulates stimulant effects (e.g., muscle tone). The ability of these drugs to alter neurotransmitter release and clearance varies with both the drug and the receptor density in the particular area of the brain examined.[28] MDA has both central stimulant effects and psychotomimetic properties.[29]

Substituted amphetamines are indirect sympathomimetics with similar pathophysiology as amphetamine and methamphetamine. Psychotomimetic amphetamine designer drugs (e.g., MDMA, MDA) affect serotonergic pathways more than dopaminergic pathways. There are

limited human data on the individual differences between amphetamine designer drugs other than MDMA; however, the pathophysiology of these amphetamine designer drugs is probably similar to MDMA.

CLINICAL RESPONSE

There is very little data regarding tolerance, addition, and abstinence following the use of amphetamine designer drugs including MDA. Presumably, the complications of MDA and MDMA use are similar. Muscle stiffness, depression, fatigue, anxiety, and difficulty concentrating are common complaints within 1 to 2 days after use of amphetamine designer drugs like MDMA.[30] Semistructured interviews of ecstasy users indicate that a majority of individuals feel that they develop tolerance to the effects of drugs purported to be MDMA.[31]

DIAGNOSTIC TESTING

Analytic Methods

SCREENING

The enzyme-linked immunosorbent assays (ELISA) are rapid methods for the detection of methylenedioxy-amphetamine designer drugs in the urine based on using d-amphetamine equivalents as the cutoff.[32] The cross-reactions of MDA with urine amphetamine immunoassays are variable,[96] and screening biologic samples with chromatographic methods (e.g., liquid chromatography/tandem mass spectrometry, gas chromatography/mass spectrometry [GC/MS]) is necessary to confirm the presence of amphetamine designer drugs.[33] The Abbott Abuscreen® ONLINE amphetamine immunoassay (Abbott Laboratories, Abbott Park, IL) tested positive for urine MDA concentrations exceeding 3,000 ng/mL.[34] The cross-reactivity of MDA with the reagents of the Abbott TDx® fluorescence polarization immunoassay (FPIA) amphetamine/methamphetamine II is high (136–170%);[35] additionally, MDA is a minor metabolite of MDEA biotransformation. The cross-reactivity of the other MDA metabolites [3,4-dihydroxyethylamphetamine (DHE), 4-hydroxy-3-methoxyethylamphetamine (HME), 3,4-dihydroxyamphetamine (DHA), 4-hydroxy-3-methoxyamphetamine (HMA) ranged between 0.03% and 0.4%. The presence of MDA concentrations of approximately 10–15% of the MDMA concentration is consistent with MDMA metabolism to MDA;[36] these MDA concentrations do not necessarily indicate the ingestion of MDA.[37] A negative amphetamine immunoassay screen does not exclude the recent use of ring-substituted phenethylamine drugs including MDA. The multiplex cloned enzyme donor immunoassay (CEDIA® Amphetamines/Ecstasy, Microgenics Corp., Fremont, CA) has greater sensitivity and specificity for MDA and other ring-substituted phenethylamine drugs.[38] At the 500-ng/mL cutoff, this immunoassay detects most of these compounds. Table 10.3 lists the cross-reactivity of various ring-substituted phenethylamine compounds with the CEDIA® Amphetamines/Ecstasy assay.

CONFIRMATION

Methods for the confirmation and quantitation of amphetamine designer drugs in biologic samples include thin layer chromatography, capillary electrophoresis,[39] GC/MS,[40,41] liquid chromatography/tandem mass spectrometry,[42] and liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry.[43] The use of liquid chromatography/tandem mass spectrometry allows the detection of tryptamine, piperazine, pyrrolidinophenone, phenylcyclohexyl, and other amphetamine derivates with a limit of detection (LOD) ranging between 1–5 ng/mL. The LOD for methylenedioxy-amphetamine designer drugs using liquid chromatography/atmospheric pressure chemical ionization/

TABLE 10.3. Cross-reactivity of compounds by CEDIA® Amphetamines/Ecstasy Assay. Adapted from Reference 38.

Compound	Concentration (ng/mL)	Cross-reactivity (%)
d-Amphetamine	500	100
d-Methamphetamine	500	100
3,4-Methylenedioxyamphetamine (MDA)	500	113
3,4-Methylenedioxymethamphetamine (MDMA)	250	199
3,4-Methylenedioxyethylamphetamine (MDEA)	250	207
N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)	500	123
1-(3,4-methylenedioxyphenyl)-2-butanamine (BDB)	500	72*
p-Methoxymethamphetamine (PMMA)	300	100
p-Methoxyamphetamine (PMA)	2,000	24†

*Concentration for a positive result, 694 ng/mL.
†Concentration for positive result, 2,174 ng/mL.

mass spectrometry ranges between 1–2 ng/mL compared with 10 ng/mL for GC/MS.[44] The lower limit of quantitation (LLOQ) for GC/MS is 25 ng/mL. Following propylchloroformate derivatization and automated solid-phase microextraction with capillary GC, the LOD for MDA urine samples is about 15 ng/mL.[45] Liquid chromatography using derivatization with 9-fluorenyl-methyl chloroformate allows the relatively rapid quantitation of MDA in the range of 10 ng/mL compared with the more time-consuming method, GC/MS.[46] The analysis time using liquid chromatography/tandem mass spectrometry for the quantitation of MDA in biologic materials is about 20 minutes with a LLOQ in the range of 2 ng/mL.[47] The use of gas chromatography/negative-ion chemical ionization/mass spectrometry allows the quantitation of the enantiomers of MDA, and MDEA in plasma samples with LLOQ for the latter 2 compounds near 5 ng/mL.[48] Analytic methods to profile samples of amphetamine designer drugs (e.g., MDA) include HPLC with fluorometric detection,[49] capillary electrochromatography,[50] GC/MS, and thin-layer chromatography with solid-phase extraction.[51]

MDA is stable in serum, whole blood, and urine when stored under frozen conditions (-20°C/-4°F) for at least 21 weeks.[52] This compound is also stable under refrigeration (4°C/39.2°F) and room temperature (20°C/68°F); however, degradation of the biologic matrix may limit analysis of these samples. MDA is detectable in hair samples utilizing liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry with a LOD of 0.05 ng/mg.[53]

Biomarkers

Animal studies indicate that MDA concentrates in erythrocytes with a mean erythrocyte/plasma ratio of 1.45 ± 0.08, similar to MDMA.[54] A case report suggests that the ratios of vitreous humor/femoral blood for MDA are relatively similar after complete absorption and distribution.[26] Postmortem redistribution of MDA may occur, particularly following prolonged postmortem interval. In postmortem blood drawn over 2 days after death related to MDMA intoxication, samples of the heart and femoral blood contained MDA concentrations of 1.33 mg/L and 0.19 mg/L, respectively, with heart/femoral blood ratio of 7.[55]

TREATMENT

The management of intoxication with amphetamine designer drugs including MDA is similar to the treatment of methamphetamine toxicity. The majority of patients presenting with amphetamine designer drug-induced toxicity require only reassurance, a quiet environment, and benzodiazepines as needed.[135]

Stabilization

Clinical features of serious intoxication with amphetamine designer drugs include coma, shock, acute renal failure, severe hyperthermia (i.e., temperature >40–42°C/104–107.6°F), and seizures. Severely intoxicated patients require IV access, cardiac monitoring, pulse oximetry, an electrocardiogram (ECG), and supplemental oxygen. Respiratory depression does not usually occur during mild to moderate intoxication, but respiratory failure requiring intubation may develop during serious intoxication. All patients with altered consciousness should be evaluated with a rapid blood glucose test (e.g., Accu-Chek®, Roche, Nutley, NJ). Core temperatures must be measured in any agitated patient with initiation of aggressive cooling measures (evaporative cooling, ice water baths) for core temperatures exceeding 40°C (104°F). Hypotension is frequently associated with substantial fluid deficits and these patients require aggressive fluid resuscitation. Lidocaine and amiodarone are drugs of choice for stable monomorphic or polymorphic ventricular tachycardia, particularly in patients with underlying impaired cardiac function. Unstable ventricular rhythms may require cardioversion. Although there are few data on amphetamine designer drugs, the administration of IV sodium bicarbonate is a therapeutic option for wide complex QRS tachycardia, similar to cocaine-induced cardiotoxicity.[56] Shock is a poor prognostic sign that indicates the need for continuous invasive or noninvasive hemodynamic monitoring to optimize the combination of fluid infusion and vasopressors.

HYPERTENSION/TACHYCARDIA

Most patients tolerate sinus tachycardia and hypertension associated with the ingestion of amphetamine designer drugs without pharmacologic intervention, unless elevated blood pressure and/or tachycardia contribute to myocardial ischemia or end organ failure. Both hypertension and tachycardia typically respond to IV benzodiazepines (adults: lorazepam 2 mg or diazepam 5 mg IV bolus titrated to effect).

If hypertension persists and is associated with adverse clinical effects, the use of nitroprusside or a calcium channel blocker is appropriate. Although β-adrenergic blocking agents (e.g., esmolol, labetalol) can reduce clinically significant tachycardia, these drugs potentially exacerbate hypertension by the vasoconstriction caused by unopposed alpha agonist stimulation following blockade of the vasodilating properties of

beta$_2$-adrenergic receptors. Consequently, beta-blockers are usually considered only for very high heart rates because of concern about the development of vasospasm and worsening hypertension. If beta-blockers are administered, then the concurrent administration of a vasodilator (e.g., nitroprusside) should be considered.[57] Intravenous nitroprusside (0.5–8 μg/kg/min) is the drug of choice for hypertensive emergencies with evidence of end organ injury (central nervous system [CNS], cardiac, or renal dysfunction). Intravenous nitroglycerin and phentolamine (2.5–5 mg initial IV bolus) are alternatives to the use of IV nitroprusside.

AGITATION

As a result of the sympathomimetic and psychotomimetic properties of amphetamine designer drugs, the patient often presents with agitation, restlessness, tachypnea, and tachycardia. Treatment options for agitation include diazepam (Adults: 5–10 mg IV, Children: 0.1–0.3 mg/kg), lorazepam (Adults: 1–2 mg IV) or midazolam; these drugs should be titrated to the desired clinical effect.[135] Phenothiazine compounds (chlorpromazine) are not routinely recommended due to potential undesirable side effects (orthostatic hypotension, seizures) even though chlorpromazine has some protective effects against lethal doses of MDA when administered prophylactically in animal models.[58,59] The presumed mechanism of action is dopaminergic blockade, but there are no human clinical data to confirm the efficacy of phenothiazine compounds in humans. Haloperidol is an option for the treatment of delirium in critically ill patients. A calm environment and familiar faces may also help alleviate agitation.

SEIZURES

Seizures are usually self-limited and respond to benzodiazepines (lorazepam, diazepam). Therapeutic options for the treatment of status epilepticus include phenobarbital and anesthetic agents (e.g., propofol, midazolam IV infusion). Hyperthermia, acidosis, hypoxemia, hyponatremia, or rhabdomyolysis may complicate the clinical course during status epilepticus; seriously intoxicated patients should be evaluated for the presence of these complications.

HYPERTHERMIA

Hyperthermia (i.e., core temperature >40–42°C/104–107.6ºF) should be treated aggressively because this complication is frequently associated with a fatal outcome and signs of end-organ failure (hypotension,

hyperkalemia, metabolic acidosis, rhabdomyolysis, elevated serum creatine kinase, coagulopathy).[60] Cooling measures include removal of clothing; cool rooms; cool mist spray to the skin; liberal use of fans, ice baths, and sedation (benzodiazepines). Ice-bath immersion produces the most rapid response, but technical difficulties frequently limit the use of this modality. The use of hypothermic blankets or the application of ice packs often does not produce sufficient cooling for these patients. For refractory hyperthermia, neuromuscular paralysis, sedation, and mechanical ventilation may be necessary to reduce muscle rigidity and hyperactivity. Vital signs should be followed closely (i.e., every 15–20 min) and cooling measures continued until rectal temperatures are below 38.5°C (101.3ºF). Several case reports suggest that IV dantrolene (total dose: 1–10 mg/kg) may reduce muscular hyperactivity and hyperthermia associated with amphetamine designer drug toxicity in patients unresponsive to conventional measures to treat hyperthermia.[61,62] Although many of the clinical features of amphetamine designer drug intoxication resemble the serotonin syndrome, the use of nonselective serotonin antagonists (methysergide, cyproheptadine) has not been well defined because of the lack of clinical data.[63,64] Animal studies suggest that carvedilol (α_1-, $\beta_{1,2,3}$-adrenergic receptor antagonist) is a potential treatment for hyperthermia associated with amphetamine designer drugs.[65,66] However, there are inadequate clinical data to determine the efficacy of this drug for hyperthermia in this setting.

Gut Decontamination

There are few data on the effect of decontamination procedures on the clinical outcome of toxicity associated with amphetamine designer drugs. Most patients present more than 1 hour after ingestion; the use of activated charcoal in these patients is not expected to improve outcome. Additionally, the administration of activated charcoal is difficult and aspiration is a significant risk because many of these patients are agitated upon presentation to the emergency department (ED). In a study of mice, the administration of 1g activated charcoal/kg 1 minute after gavage with 100 mg methamphetamine/kg delayed the onset of toxicity, but did not alter mortality.[67]

Elimination Enhancement

There are few, if any clinical data on the efficacy of hemodialysis, forced acid diuresis, and hemoperfusion during amphetamine designer drug intoxication. The large volume of distribution of these drugs suggests that

these measures will not significantly increase the elimination rate of these compounds. Acidification of the urine potentially increases the incidence of rhabdomyolysis and renal dysfunction.

Antidotes

There are no specific antidotes for the complications associated with the ingestion of amphetamine designer drugs.

Supplemental Care

ANCILLARY TESTS

Depending on clinical judgment and severity of poisoning, laboratory examination for severe intoxications should include complete blood count, serum tests (electrolytes, calcium, phosphorus, uric acid, creatine kinase, hepatic transaminases, creatinine, glucose, blood urea nitrogen), coagulation profile (platelet count, fibrin split products, fibrinogen, prothrombin time, partial thromboplastin time), urinalysis including myoglobin, arterial blood gases, chest x-ray, ECG, cardiac monitoring, and appropriate diagnostic neurologic examinations (i.e., computed tomography [CT], cerebral angiography, lumbar puncture). The use of the urine dipstick is a convenient method to detect the presence of hemoglobin or myoglobin at concentrations above 5–10 mg/L.[68] After the cessation of the myoglobin formation, hepatic metabolism and renal excretion clear plasma myoglobin within 1–6 hours. The presence of myoglobinuria suggests rhabdomyolysis, which necessitates generous fluid replacement; urinary alkalinization is not necessary, particularly because the excretion of amphetamine designer drugs potentially decreases in alkaline urine.

COMPLICATIONS

Severe amphetamine designer drug intoxication may be complicated by acute renal failure, rhabdomyolysis, acute compartment syndrome, subarachnoid hemorrhage, intracerebral hematoma, cerebral edema with transtentorial herniation, disseminated intravascular coagulation (DIC), and adult respiratory distress syndrome. Laboratory examinations and repeat physical examinations are necessary to diagnose these conditions early. Management is primarily supportive, but surgery may be required for intracranial lesions or compartment syndromes. Supportive care for compartment syndrome includes analgesics, elevation of extremities,

elimination of exposure to amphetamine designer drugs, low-molecular-weight dextran, and consideration of the use of anticoagulant or antiplatelet drugs. Fasciotomies are usually unnecessary unless there is clear evidence of high compartment pressures and associated distal ischemia.

Hyponatremia may result from syndrome of inappropriate antidiuretic hormone hypersecretion (SIADH); therefore, hyponatremia may not respond to the IV administration of 0.9% NaCl. Fluid restriction after the replacement of fluid losses is generally the treatment for hyponatremia secondary to SIADH. If severe or life-threatening hyponatremia develops, slow correction with 3% NaCl should be considered.[69] Alterations in the urinary sodium may require up to 24 hours to manifest; hence, urinary sodium concentrations can be misleading within this period.[70]

Patients exhibiting hemodynamic compromise, end-organ injury, hyperthermia, cardiotoxicity, or recurrent seizure activity should be admitted to a monitored bed or intensive care setting. Stable patients who respond to supportive care and sedation may be observed in the emergency department setting and discharged with proper drug counseling referral if there are no significant psychiatric issues.

3,4-Methylenedioxyethamphetamine (MDEA)

Alexander T. Shulgin first reported the psychedelic effects of 3,4-methylenedioxyethamphetamine (MDEA; CAS RN: 82801-81-8) in the late 1970s.[71] MDEA entered the street drug market in the late 1980s as a legal substitute for MDMA. However, MDEA became a DEA controlled schedule I drug in 1987 based on new US laws prohibiting analogs of controlled substances (e.g., MDA, MDMA). In the early 1990s, MDEA became a controlled substance in Germany and the Netherlands. Iwersen and Schmoldt reported the first fatal case of MDEA intoxication in 1996.[72]

EXPOSURE

Although MDMA or ecstasy tablets usually contain a single substance, MDEA is one of several ring-substituted amphetamine compounds (e.g., MDA, MBDB) frequently sold as ecstasy. Analysis of 12 different sets containing 10 confiscated ecstasy tablets each demonstrated that 97% of the tablets contained a single

substance as measured by HPLC and near infrared spectroscopy in reflectance mode.[73] About 47% of the tablets were MDMA, whereas approximately 43% were MDEA. The term "ecstasy" now includes other ring-substituted amphetamine compounds beside MDMA including MDEA, MDA, and MBDB. Besides ecstasy, street names for MDEA include Eve and Intellect. The typical recreational dose of MDEA is 1–3 tablets with the amount ranging from 60–175 mg per tablet.[74] The frequent substitution of MDMA for MDEA or the adulteration of MDEA with MDMA complicates the interpretation of reports on the effects of MDEA that do not include analytic confirmation of the exposure.

TOXICOKINETICS

Absorption

The absorption of MDEA is relatively rapid with time to peak plasma MDEA concentration (C_{max}) ranging between 1.5–3 hours.[24] In a study of 14 healthy volunteers receiving 100–140 mg MDEA hydrochloride, peak plasma MDEA concentrations ranged from 235–465 ng/mL (mean, 332 ng/mL) 1.8–5.0 hours (mean, 2.9 h) after ingestion.[75] The peak plasma MDA concentration (i.e., as a result of MDEA metabolism) occurred 4–7 hours (mean, 5.5 h) after ingestion with a range from 7–33 ng/mL (mean, 23 ng/mL). In a study of 5 healthy volunteers, the mean C_{max} of the $R(-)$-enantiomer of MDEA following the ingestion of 70 mg MDEA was greater than the $S(+)$-enantiomer (127 ± 34 ng/mL vs. 80 ± 30 ng/mL) with similar mean time to maximum concentrations (2.8 ± 0.9 h vs. 2.6 ± 0.6 h).[76]

Biotransformation

The metabolism of amphetamine designer drugs primarily involves O-demethylation and, to a lesser extent, N-dealkylation.[77] *In vitro* studies indicate that humans metabolize methylenedioxy-substituted amphetamine entactogens by 1) demethylenation followed by catechol-O-methyl transferase-catalyzed methylation and/or conjugation with glucuronide or sulfate; and 2) N-dealkylation, deamination, and oxidation (MDMA, MDA, MDEA only) to the corresponding benzoic acid derivatives along with glycine conjugation. The cytochrome P450 isoenzymes, CYP2D1/6 or CYP3A2/4 catalyze the demethylenation pathway along with other cytosolic enzymes. N-Dealkylation occurs via cytochrome P450 isoenzymes CYP2D6 and CYP3A4 with lesser contributions from CYP1A2 and CYP2B6.[78] These reactions are enantioselective with a preference for the S-enantiomers based on *in vitro* studies.[79] N-

Deethylation of MDEA is catalyzed predominately by CYP3A2/4 and to a minor extent by CYP1A2 and CYP2D1.[80] Figure 10.2 compares the metabolic pathways and cytochrome P450 isoenzymes involved with the biotransformation of MDEA and MDA with MDMA. The deethylation of MDEA produces the active metabolite, MDA.

R,S-Methylenedioxyethamphetamine (MDEA) undergoes ring degradation by O-dealkylation to the corresponding 3,4-dihydroxy metabolites followed by methylation of the hydroxyl group at position 3 of the aromatic ring. Degradation of the side chain by N-dealkylation produces the corresponding primary amines, and oxidative N-deamination forms the substituted phenylacetone compounds that are degraded to the corresponding benzoic acids and conjugated with glycine to form substituted hippurates. Following ingestion of 140 mg MDEA by 9 healthy volunteers, the main metabolites detectable in the urine were 3,4-dihydroxyethylamphetamine (DHE), 3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxyethylamphetamine (HME).[81] Although MDA is an active metabolite, there are inadequate data to determine the concentrations of MDA or other metabolites that are sufficient to contribute to the clinical effects of MDEA. Minor metabolites included 3,4-dihydroxyamphetamine (DHA), 4-hydroxy-3-methoxyamphetamine (HMA), piperonyl acetone, 3,4-dihydroxyphenyl acetone, and 4-hydroxy-3-methoxyphenyl acetone. In this study, the peak plasma MDA concentrations (7–33 ng/mL) were relatively low compared with peak plasma HME concentrations (67–673 ng/mL).

Elimination

The major urinary metabolite of MDEA is 4-hydroxy-3-methoxyethlamphetamine (HME). In the above study of 9 human volunteers receiving 140 mg MDEA, urinary HME accounted for elimination of about 32% of the dose during the first 32 hours after ingestion.[81] The mean percentage of the MDEA dose excreted unchanged in the urine during this period was 19%.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

MDEA is an indirect serotonergic agonist, similar to MDMA; additionally, MDEA (and MDMA) release smaller amounts of dopamine and norepinephrine from intracellular stores. The primary site of action is probably the serotonin transporter and the presynaptic plasmalemma where MDEA and MDMA are substrate-type serotonin releasers.[74] Similar to other methylenedioxy-substituted entactogens, direct agonist effects on the
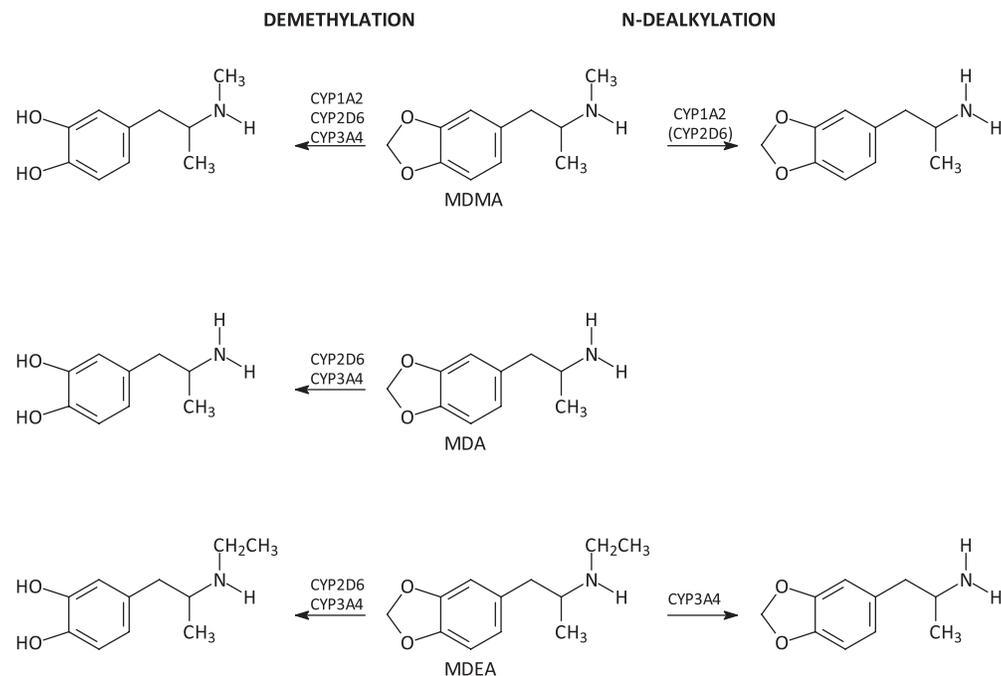
FIGURE 10.2. Human cytochrome P450 (CYP) pathways of the biotransformation of methylenedioxyamphetamine designer drugs, MDMA, MDA and MDEA. Minor CYP isoenzymes are listed in parentheses.

$5-HT_2$ receptors probably account for the psychedelic alterations of perception.

The postmortem examination of fatalities from amphetamine designer drugs including MDEA reveals abnormalities related to hyperthermia, seizures, rhabdomyolysis, DIC, and cardiac arrest associated with fatal intoxications involving MDEA.[82] Thus, autopsy results from of these patients resemble the examination of cases of heat stroke.[83] These histologic changes include evidence of coagulopathy with scattered subcutaneous bruises, hemorrhagic pleural effusion, epicardial petechiae, pulmonary hemorrhage, hemorrhagic gastritis, focal pericardial hemorrhage, and renal tubular necrosis.[84] Other findings include intracerebral hemorrhage and cerebral edema.[137,141] Pulmonary and cerebral congestion are common postmortem finding in deaths related to overdoses. Full rigor mortis may be present with elevated body temperature in the period shortly after death.[85]

CLINCAL RESPONSE

MDEA has both stimulant effects (increased endurance, alertness, sexual arousal) similar to amphetamine as well as psychedelic effects (euphoria, perceptual alterations, sociability, increased sense of empathy and tolerance) similar to MDMA and 3-fold ring-substituted phenethylamine psychedelics (e.g., DOB, DOM). Undesirable side effects following recreational use and during clinical trials include restlessness, muscular tension, agitation, bruxism, restless leg syndrome, muscle stiffness, headache, nausea, anorexia, insomnia, dry mouth, depersonalization, mydriasis, tachycardia, and elevated blood pressure.[74,86] There are inadequate data to determine the prevalence of these adverse effects in patients ingesting MDEA. Most individuals using MDEA or MDMA at dance parties do not develop serious clinical complications, and individual susceptibility may contribute to the occurrence of fatalities after the use of these drugs.[87] Manifestations of serious MDEA intoxication include coma, seizures, hyperthermia, severe rhabdomyolysis, hepatorenal failure, and arrhythmias, similar to severe MDMA toxicity.[88] Vivid auditory and visual hallucinations, dysphoria, agitation, disorientation, and paranoia may occur following the ingestion of typical recreational doses (e.g., 140 mg) of MDEA.[89] Fatalities associated with MDEA involve hyperthermia, DIC, and multiorgan failure, similar to MDA and MDMA.[90]

Although there are few data on single methylenedioxy-substituted amphetamine entactogens other than MDMA, there is concern about the potential neurotoxicity of MDEA and similar compounds based on animal studies, clinical studies, and indirect evidence

from postmortem examination of chronic MDMA users.[91,92] Polydrug use in these individuals limits conclusions regarding the potential causal role of amphetamine designer drugs in the development of these sequelae.

DIAGNOSTIC TESTING

Analytic Methods

Similar to MDMA and 4-substituted amphetamines (PMA, PMMA, PMEA, 4-MTA), MDEA cross-reacts with immunoassays for amphetamines and methamphetamines. In a study of the Bio-Quant Direct® ELISA kit (Bio-Quant, San Diego, CA) for amphetamine, the cross-reactivity of MDEA with a concentration equivalent to 50 ng/mL amphetamine was 18% compared with MDA (282%), PMA (265%), 4-MTA (280%), and MDMA (73%).[93] Following propylchloroformate derivatization and automated solid-phase microextraction with capillary gas chromatography, the LOD for MDEA in urine samples is approximately 5 ng/mL.[45] Liquid chromatography using derivatization with 9-fluorenylmethyl chloroformate allows the relatively rapid quantitation of MDEA in the range of 25 ng/mL compared with the more time-consuming method, GC-MS.[46] The use of gas chromatography/negative-ion chemical ionization/mass spectrometry allows the quantitation of enantiomers of MDA and MDEA in plasma samples with LLOQ for the latter 2 compounds near 5 ng/mL.[48] Analysis of hydrolyzed urine samples with GC/MS allows the quantitation of MDEA metabolites (HMA, HMMA) with an LLOQ in the range of 25 μg/L and coefficient of variation (CV) <15%.[94] Separation of MDEA from MBDB and other regioisomeric 3,4-methylenedioxyphenethylamines requires GC/MS after formation of perfluoroacyl derivatives, heptafluorobutrylamides, and pentafluoropropionylamides.[95] MDEA is detectable in hair samples utilizing liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry with a LOD of 0.05 ng/mg.[53]

High performance liquid chromatography with UV detection is primarily used to analyze methylenedioxyamphetamine designer drugs (e.g., MDEA) in street samples rather than in biologic samples because of the low absorptivity of amphetamines.[49] MDA, MDMA, and MDEA are stable in serum, whole blood, and urine when stored under frozen conditions (–20ºC/–4ºF) for at least 21 weeks.[52] This compound is also stable under refrigeration (4ºC/39.2ºF) and room temperature (20ºC/68ºF); however, degradation of the biologic matrix may limit analysis of these samples.

Biomarkers

BLOOD

A 20-year-old man was arrested for speeding and driving under the influence. His blood MDEA and ethanol concentrations were 0.59 mg/L and nondetectable, respectively.[96] The postmortem blood (source not identified) from a 27-year-old woman, who was found in cardiopulmonary arrest, contained a MDEA concentration of 1.2 mg/L along with diazepam and caffeine.[97] A 21-year-old man was found in cardiopulmonary arrest and resuscitation efforts were unsuccessful.[98] The postmortem examination demonstrated concentric left ventricular hypertrophy and an absence of significant coronary atherosclerosis. The postmortem blood MDEA concentration (site not reported) was 2.0 mg/L along with 0.26 mg propoxyphene/L and 1.0 mg norpropoxyphene/L. A 19-year-old man died despite resuscitative efforts after developing diaphoresis, agitation, hallucinations, muscle spasms, respiratory failure, and coma.[82] A postmortem femoral blood sample contained only 12 mg MDEA/L. Postmortem redistribution of MDEA may occur, particularly following a prolonged postmortem interval.

URINE

An analysis of the performance of 2 different amphetamine reagents (amphetamine/methamphetamine II, amphetamine class) utilizing the Abbott TDx™ immunoassay demonstrated marked variability in the reaction of 22 phenethylamine compounds to the reagents with MDA, PMA, and MDEA demonstrating the highest reactivity.[99,100] At concentrations of 1 mg/L, crossreactivity with other designer drugs (e.g., DOM, DOB, TMA, 2C-B) is unlikely. The Abbott Abuscreen® ONLINE amphetamine immunoassay tested positive for urine MDEA concentrations exceeding 50 mg/L.[34] In urine samples from 9 volunteers ingesting a single dose of 140 mg MDEA, the Abbott TDx® fluorescence polarization immunoassay (FPIA) amphetamine/methamphetamine II was positive for 33–62 hours after ingestion, using the recommended cutoff value of 0.3 mg/L, and confirmation by GC/MS.[35] A negative amphetamine immunoassay screen does not exclude the recent use of amphetamine designer drugs including MDEA.

TREATMENT

Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)

N-Methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) is the *N*-methyl homologue of BDB and the α-ethyl analogue of MDMA. Illicit samples of MBDB contain a racemic mixture (enantiomer ratio (–/+) = 1). Analysis of illicit samples of MDMA suggest that MBDB is an uncommon substituent for MDMA in ecstasy tablets;[101] recent ecstasy tablets contain primarily MDMA.[102] Analysis of confiscated ecstasy tablets in the United Kingdom from 1994–2003 indicated that MBDB was present in some ecstasy tablets in the late 1990s, but not later.[103] The street name for MBDB is Methyl J. MBDB is a controlled substance in the United States and France. MBDB is a selective serotonin-releasing agent that is less potent than MDMA. In animal studies, MBDB has less dopaminergic and serotonergic activity than other ring-substituted phenethylamine drugs (e.g., MDMA, MDA, MDEA).[2] The principal metabolic pathway for the biotransformation of MBDB is *N*-demethylation to 3,4-methylenedioxybutanamine (BDB); cytochrome P450 isoenzymes involved in this pathway include CYP1A2, and to a lesser extent, CYP2D6 and CYP3A2/4.[104,105] Renal excretion of unchanged MBDB probably accounts for the majority of the elimination of MBDB.[106] The slightly increased renal excretion of the (–) isomer results in some reduction of the enantiomer ratios (–/+) during the elimination phase. Recommended recreational doses of MBDB are higher (i.e., 180–210 mg) than MDMA (80–150 mg).[107] Although the effect of MDMA and MBDB on serotonin receptors is similar, the action of MBDB on dopamine receptors is substantially less than MDMA as a result of less interaction of MBDB with the dopamine transporter.

The clinical effects of MBDB are similar, but less potent than MDMA. Analytic methods for the quantitation of MBDB and other ring-substituted phenethylamine drugs include GC/MS,[108] gas chromatography/tandem mass spectrometry with electron impact and chemical ionization,[109] liquid chromatography/electrospray ionization/tandem mass spectrometry,[110] and LC/MS.[111] The LOD and LLOQ for the latter method are 0.5–1 ng/mL and 2 ng/mL, respectively, along with a CV <15%. MBDB is a relatively rare ring-substituted phenethylamine compound in urine drug screens.[112] In a convenience sample of 10 individuals screening positive for MBDB, the urine MBDB concentration ranged between 0.1 and 24 mg/L as measured by GC/MS with trifluoroacetic anhydride as the derivatizing agent.[113] The sensitivity of most commercial amphetamine immunoassays for MBDB and the metabolite, BDB is relatively low unless the concentrations of these compounds are very high.[114] The exception is sensitivity of the CEDIA® Amphetamines/Ecstasy immunoassay for MBDB and BDB. The treatment of MBDB and MDA intoxication is similar.

4-Methylthioamphetamine (4-MTA)

Nichols et al. first reported the synthesis of 4-MTA in 1992.[115] Identification of 4-MTA in confiscated street drugs first occurred in 1997; the European Union placed 4-MTA on the schedule I of controlled substances in 1999.

IDENTIFYING CHARACTERISTICS

This compound is the methylthio analogue of PMA and a sulfur (thio-substituted) analogue of amphetamine. Typically, 4-MTA ($C_{10}H_{15}NS$) exists in tablets as the hydrochloride salt.

EXPOSURE

Street names for 4-MTA include Flatliner, MK, and S5. Occasionally, tablets sold as ecstasy contain 4-MTA.[116] Synthesis of 4-MTA involves the Leuckart process and the nitropropene route.[117] Precursors of the latter route include 4-methyltiobenzaldehyde, nitroethane, and *n*-butylamine. By-products of the Leuckart synthesis of 4-MTA include 4-methylthiophenyl-2-propanone, 4-methylthiophenyl-2-propanol, and 4-methylthiobenzyl alcohol.[118] *N*-alkyl homologues of 4-MTA include 4-methylthiomethampheamine, 4-methylthioethylamphetamine, 4-methylthiodimethamhpetamine, 4-methylthiopropylamphetamine, and 4-methylthiobutylamphetamine; there are few data on illicit use and effects of these homologues.[119]

DOSE EFFECT

Typical doses of 4-MTA in tablets sold on the street range from approximately 100–140 mg.[120] The ingestion of 6 tablets of 4-MTA was associated with cardiac arrest in a 27-year-old man.[90] *In vitro* studies suggest that substantial interindividual variation in 4-MTA may occur as a result of differences in individual toxicokinetics.
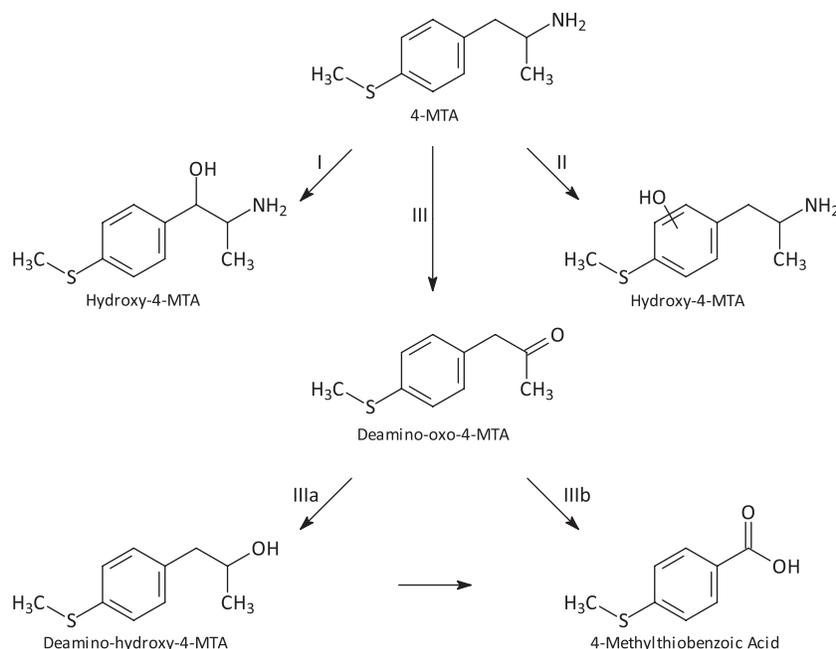
FIGURE 10.3. Proposed phase-I metabolic pathways of 4-MTA in humans. The pathways include β-hydroxylation of the side chain (I), ring hydroxylation (II), oxidative deamination (III) followed by reduction into the corresponding alcohol (IIIa), or degradation of the side chain (IIIb).

TOXICOKINETICS

There are few toxicokinetic data on 4-MTA in humans. 4-MTA undergoes limited biotransformation and substantial renal excretion of unchanged 4-MTA occurs. Figure 10.3 displays the proposed metabolic pathways for the biotransformation of 4-MTA including oxidative deamination, ring hydroxylation, and β-oxidation of the side chain. *In vitro* studies indicate that 4-methylthiobenzoic acid is the major metabolite via oxidative deamination.[121] Although there are few data on the cytochrome P450 isoenzymes involved with the metabolism of 4-MTA, *in vitro* studies suggest that CYP2D6 is an important isoenzyme in the ring hydroxylation of 4-MTA.[122] These studies suggest that CYP2D6 rapid metabolizers are more susceptible to 4-MTA intoxication than CYP2D6 poor metabolizers; however, the clinical significance of this potential effect remains undetermined. Limited kinetic data in an intubated patient with 4-MTA intoxication suggested that the plasma elimination half-life of 4-MTA during poisoning is approximately 7 hours.[123]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

In rodent studies, 4-MTA is a selective serotonin agonist (increases serotonin release, inhibits serotonin uptake)

and an inhibitor of monoamine oxidase A.[124,125] The affinity of 4-MTA for dopamine and norepinephrine receptors is low. The administration of 4-MTA also causes a dose-dependent increase in plasma concentrations of ACTH, corticosterone, prolactin, and renin.[126] Animal studies suggest that 4-MTA intoxication may cause hyperthermia, hypertension, and the serotonin syndrome.[127] The postmortem examinations of patients dying from 4-MTA intoxication are typically nonspecific with the primary finding of visceral congestion. Postmortem examination of a 27-year-old man, who collapsed and died suddenly demonstrated no evidence of hyperthermia, cardiac anomalies, or anatomic causes of death.[128] The postmortem blood contained 4-MTA and trace amounts of MDMA.

CLINICAL RESPONSE

The clinical effects of 4-MTA are similar to PMA and MDMA with the exception that the slower onset of action of 4-MTA compared with the latter 2 drugs.[116,129] Adverse clinical effects associated with the illicit use of 4-MTA include headache, stomach pain, diaphoresis, tachycardia, and tremors. The clinical features of serious intoxication associated with 4-MTA include seizures and respiratory failure.[129]

DIAGNOSTIC TESTING

Similar to MDMA, MDA, and other 4-subsitituted amphetamines (PMA, PMMA, PMEA), 4-MTA cross-reacts with immunoassays for amphetamines and meth-amphetamines. In a study of the Bio-Quant Direct® ELISA kit for amphetamine, the cross-reactivity of 4-MTA with a concentration equivalent to 50 ng/mL amphetamine was 280% compared with MDA (282%), PMA (265%), and MDMA (73%).[93] 4-MTA is one of 31 designer amphetamines, tryptamines, and pipera-zines in serum samples detected by screening with liquid chromatography/tandem mass spectrometry after mixed-mode solid-phase extraction; the limit of detec-tion (LOD) with this method is 2.5 ng/mL.[130] Methods for the quantitation of 4-MTA include gas chromatography/nitrogen phosphorus detection, high performance liquid chromatography with diode array UV detection (HPLC/DAD),[123] GC/MS,[131] and liquid chromatography/tandem mass spectrometry.[128] The limit of 4-MTA detection using GC/MS in electron ion-ization and positive-ion chemical ionization mode is 30 ng/mL.[132]

In a case series of 6 individuals in various stages of intoxication, the 4-MTA concentrations in blood samples ranged from 0.43–2.08 mg/L.[129] The blood 4-MTA concentration did not correlate to the level of intoxication; however, the blood samples from these individuals also contained other drugs (e.g., marijuana). In a case series of 3 patients requiring assisted ventila-tion for 4-MTA intoxication, the plasma 4-MTA con-centration ranged from 0.13–0.76 mg/L.[123]

The postmortem femoral and right heart blood from a 27-year-old man, who died suddenly, contained 4-MTA concentrations of 5.23 mg/L and 7.60 mg/L, respectively.[128] The femoral blood also contained a minor concentration (0.001 mg/L) of MDMA. 4-MTA was the only drug detected in the postmortem blood of a 22-year-old man dying about 5 hours after developing coma and seizures.[131] Reportedly, he had hot skin, diaphoresis, dyspnea, and shaking prior to admission. The postmortem femoral blood contained 4.6 mg 4-MTA/L. Analysis of perimortem blood (i.e., drawn during cardiopulmonary resuscitation) demon-strated 4-MTA concentrations of 4.0 mg/L, respectively, as measured by GC with nitrogen phosphorus detection.

TREATMENT

Treatment of 4-MTA intoxication is supportive, similar to MDA and MDMA intoxication. Potential life-threatening effects include hyperthermia, seizures, coma, respiratory failure, and serotonin syndrome.

4-Methoxyamphetamine and 4-Methoxymethamphetamine (PMA/PMMA)

IDENTIFYING CHARACTERISTICS

4-Methoxyamphetamine (*p*-methoxyamphetamine, PMA) tablets are illegally sold in a variety of shapes and sizes including white tablets with a Mitsubishi symbol on one side.[133] Analysis of 6 tablets found with a deceased man contained the logo "xTc"; analysis of the tablets demonstrated approximately 50 mg of PMA.[134] PMA tablets typically contain between 40–70 mg compared with a mean dose of about 60–70 mg (range, 20–110 mg) in MDMA tablets.[135,136]

EXPOSURE

Epidemiology

Epidemiologic data on PMA and *p*-methoxymetham-phetamine (PMMA) are limited primarily to case reports, postmortem analysis, or drug seizures. Most epi-demiologic data on amphetamine designer drugs refer-ring to MDMA use rather specific phenethylamine derivatives; however, PMA and PMMA may be adulter-ants in street samples of MDMA.[143] The abuse of the various phenethylamine derivatives (e.g., PMA, PMMA) is sporadic and probably much less common than MDMA. Medical reports on PMA exposure include street drugs purportedly sold as ecstasy in Australia,[137,138] the midwestern United States,[133] Canada,[139] Belgium,[134] and Norway.[140] Case reports from Denmark document fatal cases resulting from the ingestion of both PMA and PMMA along with other drugs of abuse.[141]

Impurities

There are no approved medical uses for PMA or PMMA. By-products of the older Leuckart synthesis of PMA using reductive amination include 4-(4-methoxybenzyl)pyrimidine and 4-methyl-5-(4-methoxyphenyl)pyrimidine.[118] Another method for the production of PMA using the Leuckart reaction involves the use of anethole (i.e., main constituent of anise oil).[142] Peracid oxidation converts this precursor to its phenyl acetone analogue. A specific impurity for the use of anethole as a precursor in the Leuckart synthesis of PMA is 4-methoxyphenol. Other impurities in PMA synthesized from this process include 4-methyl-5-(4-methoxyphenyl)pyrimidine,

N-(β-4-methoxyphenylisopropyl)-4-methoxybenzyl methyl ketimine, 1-(4-methoxyphenyl)-*N*-(2-(4-methoxyphenyl)-1-methylethyl-2-propanamine, 1-(4-methoxyphenyl)-*N*-methyl-*N*-(2-(4-methoxyphenyl)-1-methylethyl-2-propanamine, and *N*-(*β*-4-methoxyphenylisopropyl)-4-methoxybenzaldimine. Analysis of PMA tablets demonstrated the presence of by-products of the chemical synthesis of PMA including 4-methoxydimethylamphetamine, 4-methoxyethylamphetamine, and 4-hydroxymethamphetamine from the Leuckart process.[143] The chemical precursors of PMA and MDMA are different; therefore, PMA is an adulterant of MDMA rather than a contaminant. Nicotinamide was the only adulterant in *p*-methoxyamphetamine (PMA) samples confiscated in Australia over 6 years.[135] Substrates of the Leuckart synthesis of PMMA include formic acid, *N*-methylformamide, and *p*-methoxyphenylacetone, whereas 4-methoxybenzaldehyde is a substrate for the synthesis of 4-methoxyamphetamine (PMA).[144,145] *N*,*N*-dimethyl-*p*-methoxyamphetamine and *N*-formyl-*p*-methoxymethamphetamine are by-products of this process.

Methods of Abuse

Similar to other phenethylamine compounds associated with club drugs, PMA and PMMA typically are ingested as tablets, capsules, or powder. Frequently, users believe they are ingesting MDMA rather than PMA or PMMA.[146] Insufflation and IV injection are uncommon, but case reports associate fatalities with the injection of PMA.[137,139] Typically, users ingest 1–2 tablets that contain varying amounts of the purported drug.

DOSE EFFECT

Desired effects from the ingestion of PMA or PMMA involve the alteration of perception. The term "entactogen" refers to drugs that induce euphoria, heighten sexual arousal, and/or promote an empathetic disposition.[135,147] There are limited data in the medical literature on the response to specific doses of PMA or PMMA. Animal studies suggest that the toxicity of PMA and PMMA is similar.[148] In mice, the IV LD_{50} of PMA is about 25 mg/kg compared with 31 mg/kg for MDA (3,4-methylenedioxyamphetamine).[149] Several case series associate the ingestion of 1–5 tablets of PMA with fatalities.[22,137,146] Analysis of tablets from 4 fatal cases of PMA intoxication demonstrated that the amount of PMA in these tablets ranged between 50–90 mg/tablet.[137] Extrapolation of this study suggests PMA doses of 50 mg – 450 mg are potentially fatal. Fatalities from the purported ingestion of typical recreational doses of amphetamine designer drugs suggest the occurrence of idiosyncratic responses.

TOXICOKINETICS

In vitro studies indicate that PMA undergoes *O*-demethylation to 4-hydroxyamphetamine via CYP2D6 (debrisoquine hydroxylase) followed by conjugation.[150,151] Differences in the polymorphic expression of CYP2D6 account for differences in *O*-demethylation between human subjects.[152] CYP1A2, CYP3A4, and catechol-*O*-methyl transferase may also catalyze the demethylation of PMA. Animal studies indicate that the primary metabolite, 4-hydroxyamphetamine, is an active metabolite, but the concentration of this metabolite in the brain after the administration of PMA is probably insufficient to contribute to PMA toxicity.[153] Alternate metabolic pathways of PMA biotransformation in animal models include oxidation to *N*-hydroxy-paramethoxyamphetamine followed by conjugation, *N*-hydroxylation, or deamination.[77]

Parametohoxymethamphetamine (PMMA) undergoes *O*-demethylation by CYP2D6 followed by hydroxylation, catechol-*O*-methyltransferase-catalyzed methylation, and then conjugation.[154] Very little paramethoxymethamphetamine (PMMA) undergoes *N*-demethylation to paramethoxyamphetamine (PMA).[77] *p*-Methoxyethylamphetamine (PMEA) is the *N*-ethylated analogue of PMA that is a relatively recently recognized drug of abuse. Potential metabolites of PMEA include *p*-hydroxyamphetamine (POHAP), *p*-hydroxyethylamphetamine (POHEA), and *p*-methoxyamphetamine (PMA) with *O*-demethylation to POHEA accounting for most of the metabolites.[155]

Marked interindividual variation in the elimination of PMA occurs between individuals, in part as a result of the interindividual variation in CYP2D6 activity. Poor metabolizers of CYP2D6 excrete substantially increased amounts of unchanged PMA in the urine. In a study of 3 volunteers, the 2 volunteers who were extensive metabolizers of CYP2D6 excreted a majority of the PMA dose as the free or conjugated 4-hydroxyamphetamine, whereas the poor metabolizer of CYP2D6 excreted about 50% of the dose as unchanged PMA.[152] Postmortem analyses suggest that the bile contains significant amounts of PMA.[26]

Potential drug interactions include the coadministration of CYP2D6 inhibitors. A case report associated fatal PMA intoxication with a modest blood PMA concentration (0.24 mg/L) in a patient on therapeutic doses of the CYP2D6 inhibitor, fluoxetine.[22] *In vitro* studies indicate that PMA is a weak inhibitor of multidrug resistance transporter, P-glycoprotein.[156]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

In vitro studies indicate that paramethoxyamphetamine (PMA) enhances the effect of serotonin more than MDMA.[20,157] In studies of rats using *in vivo* chronoamperometry, PMA was a potent inhibitor of serotonin uptake and a relatively weak releasing agent of serotonin and dopamine.[158] PMA did not increase locomotor activity in rats, which is dependent on dopamine uptake, in contrast to the effect of MDMA. *In vitro* studies of PMA in mouse brain also indicate that *p*-methoxyamphetamine is an inhibitor of serotonin oxidation by monoamine oxidase A.[159] Other methylenedioxyamphetamine compounds probably do not share this property with PMA, and potentially the increase in extracellular serotonin is greater than after the use of MDMA.

Animal models suggest that paramethoxyamphetamine may be relatively more toxic than other substituted phenethylamine derivatives as a result of the greater enhancement of serotonin release and the delayed onset of action allowing higher doses.[149] In rodent studies, PMMA and PMA in equivalent doses produce dose-related reduction in presynpatic serotonergic markers in the brain after twice-daily injections for 4 consecutive days.[160] Severe toxicity and fatalities resulting from PMA or PMMA share many similar features with MDMA and other phenethylamine entactogens (MDA, MDEA). Postmortem examination of PMA abusers found dead from PMA intoxication typically demonstrate evidence of hyperthermia, DIC (epicardial petechiae, pulmonary hemorrhage, hemorrhagic peritoneal/pleural effusions), skeletal muscle edema/necrosis, and renal tubular necrosis.[84]

CLINICAL RESPONSE

Case reports associate PMA and PMMA with fatalities despite the uncommon use of these drugs, primarily as an adulterant or substitute for MDMA.[140,146,161] Fatalities associated with PMA and PMMA typically involve hyperthermia, seizures, rhabdomyolysis, electrolyte abnormalities, DIC, multiorgan failure, and terminal dysrhythmias.[137,141] This clinical pattern resembles severe serotonin syndrome. Hyperthermia is the most important prognostic factor for potentially fatal cases.[135] Serious complications include intracerebral hemorrhage, cerebral edema,[141] intracerebral hemorrhage with midline shift,[137] and tonsillar herniation. Acute tubular necrosis and acute renal failure may occur, particularly in association with rhabdomyolysis, myoglobinuria, or multiorgan failure.[84,137]

DIAGNOSTIC TESTING

Analytic Methods

Similar to MDMA, MDA, and other 4-subsitituted amphetamines (PMMA, PMEA, 4-MTA), PMA cross-reacts with immunoassays for amphetamines and methamphetamines. In a study of the Bio-Quant Direct® ELISA kit for amphetamine, the cross-reactivity of PMA with a concentration equivalent to 50 ng/mL amphetamine was 265% compared with MDA (282%), 4-MTA (280%), and MDMA (73%).[93] 4-Methoxyamphetamine (PMA) along with other methylenedioxy-phenethylamine drugs are detectable in blood, urine, and postmortem tissue by liquid chromatography/sonic spray ionization/mass spectrometry with a validated calibration curve in the range of 10–1,000 ng/mL (blood and urine) and 20–2,000 ng/g (tissue).[162] The LOD for PMA and MDA in blood and urine for this method is 2.5 ng/mL and 5 ng/mL, respectively, based on a signal-to-noise ratio of 3. Other methods for the quantitation of PMA in biologic samples include GC/MS,[153] HPLC with fluorescence detection,[146] and capillary electrophoresis with diode array detection.[163] The LOD for the latter method is between 50–60 ng/mL. PMA is detectable in hair samples utilizing liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry with a LOD of 0.20 ng/mg.[53]

Analysis of illicit drugs for the presence of synthetic substrates and by-products yields information about the source of the illicit material. Minor variations in reaction conditions (e.g., molar ratio of reagents, temperature) cause characteristic concentrations of by-products. Compounds suggestive of the preparation of PMA from 4-methoxybenzaldehyde via 4-methoxyphenyl-2-propanone using a Leuckart reductive amination include 4-methoxyphenol, 4-methoxybenzaldehyde, 4-methoxyphenyl-2-propanone, and 4-methoxyphenyl-2-propanol, 4-methoxyphenyl-propene as detected by GC/MS after solid-phase extraction.[164] By-products of this older Leuckart synthesis of PMA include 4-(4-methoxybenzyl)pyrimidine and 4-methyl-5-(4-methoxyphenyl)pyrimidine.[118] The absence of these by-products in illicit PMA tablets indicates the use of a synthetic process other than the Leuckart reaction.

Biomarkers

A case report suggests that the postmortem vitreous humor/femoral blood ratios for PMA, MDA, and MDMA are near unity after complete absorption and distribution.[26] Case reports of individuals dying with

PMA and/or PMMA in their postmortem blood usually involve polydrug abuse. Postmortem PMA concentrations in blood samples for PMA abusers dying at home with evidence of hyperthermia from PMA intoxication typically exceed 1 mg/L.[22] In a case series of 6 fatal PMA intoxications, the postmortem femoral blood of 2 individuals found dead contained PMA concentrations of 1.7 mg/L and 4.9 mg/L, whereas the postmortem femoral blood of 2 individuals dying shortly after hospital admission contained PMA concentrations of 2.2 mg/L and 3.7 mg/L.[137] The postmortem blood in these cases also contained lesser amounts of MDMA and/or methamphetamine, as well as other drugs of abuse (cannabinoids, cocaine). In another case series, the PMA concentrations in postmortem peripheral blood of 2 men dying a few hours after hospital admission were 0.78 mg/L (0.78 mg/kg) and 3.4 mg/L (3.4 mg/kg).[141] These blood samples also contained PMMA concentrations of 0.68 mg/L and 3.3 mg/L, as well as MDMA and cocaine metabolites. In a case series of 9 fatal PMA intoxications, the postmortem PMA blood concentration (source unspecified) ranged between 0.3 mg/L and 1.9 mg/L.[139] All died on the scene or within 3 hours of admission to the hospital. Some postmortem redistribution of PMA may occur. Two men and 1 woman presented to the emergency department in extremis, and they died shortly thereafter of PMA intoxication. The postmortem femoral blood of these 3 individuals contained PMA concentrations of 0.6 mg/L, 0.6 mg/L, and 1.3 mg/L.[146] The corresponding heart blood concentrations were 0.7 mg/L, 0.8 mg/L, and 2.3 mg/L, respectively. Limited clinical and postmortem data suggest that PMA concentrations exceeding 0.5 mg/L may be toxic,[137] but the occurrence of idiopathic responses and limited data on patients with PMA intoxication do not permit conclusions based only on the PMA or PMMA concentrations in postmortem blood samples.

In a case series of 8 fatal PMMA intoxications, the PMMA concentration in postmortem heart blood ranged from 1.208–15.824 mg/L as measured by GC/MS.[165] All specimens contained smaller amounts of PMA as well as variable amounts of other drugs (e.g., MDA, MDMA, ketamine, methamphetamine). Postmortem redistribution of PMMA also occurs. The postmortem PMMA and PMA concentrations in femoral blood from a 22-year-old man who died after ingesting ecstasy were 0.85 mg/L and 0.61 mg/L, respectively.[166] The heart blood contained PMMA and PMA concentrations of 2.12 mg/L and 1.94 mg/L, respectively, with corresponding heart/femoral blood ratios of 2.49 and 3.18. A 27-year-old man developed agitation, seizures, altered consciousness, hyperthermia, and cardiac arrest after ingesting an unidentified liquid. The postmortem examination revealed pulmonary congestion and moderate cerebral edema with herniation; the postmortem heart blood sample contained *p*-methoxyethylamphetamine (PMEA) at a concentration of 12.2 mg/L along with another metabolite, *p*-hydroxyethylamphetamine (POHEA).[155] A negative amphetamine immunoassay screen does not exclude the recent use of PMA or PMMA.

Abnormalities

In case reports of confirmed PMA intoxications, electrocardiographic abnormalities include sinus tachycardia, anterolateral ST depression, T wave inversion, QRS prolongation, atrial fibrillation, multifocal PVCs, and supraventricular tachycardia.[135,138] Occasional case reports associate severe hypoglycemia, hypocalcemia, and hyperkalemia with PMA intoxication (with and without hepatorenal dysfunction).[138,140]

TREATMENT

Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

2,5-Dimethoxyamphetamine (DMA) and the 2,5-Dimethoxyamphetamine-Derived Designer Drugs

2,5-Dimethoxyamphetamine (DMA) is approximately 5 times as potent as mescaline based on human dosing studies;[167] DMA may be substituted for mescaline in illicit street drugs.[168] The dimethoxy-hallucinogens include 4-methyl-2,5-dimethoxyamphetamine (DOM), 4-iodo-2,5-dimethoxyamphetamine (DOI), 4-chloro-2,5-dimethoxyamphetamine (DOC),[169] 4-bromo-2,5-dimethoxyamphetamine (DOB), 4-bromo-2,5-dimethoxymethamphetamine (MDOB), and 2,4,5-trimethoxyamphetamine (TMA-2) These drugs are potent serotonin 5HT$_2$ receptor agonists. The metabolism of these drugs occurs primarily by *O*-demethylation or in case of DOM by hydroxylation of the methyl moiety.[170] Limited experimental clinical data suggest that the effects of 2,5-dimethoxyamphetamine (DMA) persist approximately 7 hours after the ingestion of DMA doses over 75 mg. DMA has the same molecular weight and electron ionization mass spectra as the 5 other positional ring isomers (2,3-dimethoxy-

amphetamine, 2,4-dimethoxyamphetamine, 2,6-dimethoxyamphetamine, 3,4-dimethoxyamphetamine, 3,5-dimethoxyamphetamine). Perfluoroacylation of the amine group allows the identification of the 6 regioisomeric dimethoxyamphetamine compounds by capillary GC.[171]

4-BROMO-2,5-DIMETHOXYAMPHETAMINE (DOB)

EXPOSURE

The synthesis of DOB is relatively simple. Substrates for the synthesis of DOB include 2,5-dimethoxybenzaldehyde and hydroquinone.[13] Intermediates of the former process are 2,5-dimethoxy-β-nitrostyrene and 2,5-dimethoxyamphetamine. DOB hydrochloride is synthesized as a white crystalline powder, but this compound is sold as paper squares similar to LSD.[172] In Australia, DOB is frequently sold as LSD.[13] Classically, DOB is sold as 1-cm^2 pieces of colored paper that are embossed with various figures (bird, beaver, unicorn). These pieces of paper are bonded to a thinner, nonabsorbent sheet, and the DOB concentration of each 1-cm^2 dose usually ranges between 1.4 mg and 4.6 mg.[13] As a result of the diffusion of DOB within the sheet of DOB during the production process, the concentration of DOB in individual pieces may vary depending on the location.

DOSE EFFECT

The ingestion of pieces with high DOB concentrations may cause serious toxicity.[173] DOB is approximately 100–150 times as potent as mescaline based on human dosing studies.[167] In a volunteer study, DOB doses ≤2 mg caused stimulation without perceptual distortion when administered as the hydrochloride salt.[174]

TOXICOKINETICS

DOB (4-bromo-2,5-dimethoxyamphetamine) is another 4-position substituted phenethylamine that probably undergoes O-demethylation followed by conjugation and urinary elimination.[175] There are few data on the cytochrome P450 isoenzymes involved with the metabolism of DOB and other similar dimethoxy-phenethylamine compounds; CYP2D6 probably does not contribute significantly to the biotransformation of DOB.[176]

CLINICAL RESPONSE

DOB is a potent psychotomimetic compound that is the longest-acting substituted amphetamine. Although the effects DOB and LSD are similar, the onset of intoxication following the ingestion of DOB is slower and the duration of action is longer than LSD. Typically, the onset of action of DOB begins within 1 hour of ingestion and peak effects occur about 3–4 hours after ingestion; these peak effects persist 4–10 hours after ingestion, and symptoms of intoxication resolve within 24–36 hours based on experimental studies by Shulgin.[13] Several case reports associated DOB with mydriasis, frightening visual hallucinations, dysphoria, labile emotions, extreme agitation, disorientation, and seizures. Rare case reports associate the ingestion of DOB with fatalities. A 21-year-old woman was found dead with a package of DOB nearby and DOB in her postmortem blood.[177] Severe symptomatic arterial vasospasm developed after the ingestion of DOB, as confirmed by angiography.[178]

Other synthetic, ring-substituted phenethylamine amphetamine derivatives (e.g., 2,5-dimethoxy-4-chloroampheteamine [DOC], 2,5-dimethoxy-4-iodoamphetamine [DOI]) have similar toxic effects including altered consciousness, mydriasis, sinus tachycardia, seizures, temperature elevation, and increased serum creatine kinase based on case reports.[179]

DIAGNOSTIC TESTING

Methods for the identification of DOB in street drug samples involve capillary zone electrophoresis/diode array detection, Fourier-transform infrared spectroscopy (FTIR), and GC/MS.[180] Unlike lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-bromoamphetamine (DOB) is neither light nor heat sensitive; therefore, DOB does not require refrigeration or storage in dark environments.[13]

Two comatose patients were admitted to the hospital after the ingestion of 4-bromo-2,5-dimethoxyamphetamine (DOB) at an unknown time prior to admission.[181] The patient with a serum DOB concentration of 0.013 mg/L on admission survived after developing seizures. The other patient had a serum DOB concentration of 0.019 mg/L on admission as measured by GC/MS; he died 6 days later after developing seizures and severe metabolic acidosis (pH = 6.6). The duration and temperature of storage were not reported. The postmortem blood (source unidentified) of a 21-year-old woman found dead at the scene contained 0.91 mg DOB/L.[177]

TREATMENT

Treatment is supportive, similar to the treatment of MDA or MDMA intoxication.

4-METHYL-2,5-DIMETH-OXYAMPHETAMINE (DOM)

Methyl-2,5-dimethoxyamphetamine (DOM; STP, Serenity-Tranquility-Peace) is approximately 50–80 times more potent than mescaline based on human dosing studies.[182] DOM in doses of 2–3 mg causes mild sympathetic stimulation, euphoria, and perceptual distortions with changes in physiologic parameters (pulse, blood pressure, temperature, pupil size),[183] whereas doses exceeding 5 mg causes excessive sympathetic stimulation and hallucinations based on clinical studies.[184] Higher doses (10–20 mg) of DOM produce unpleasant effects that limit the acceptance of this illicit drug.[185] Biotransformation of DOM primarily involves hydroxylation of the 4-methyl moiety rather than *O*-demethylation of other dimethoxy-phenethylamine compounds (e.g., DOC, DOI, DOC). Volunteer studies suggest that tolerance develops to some of the dysphoric effects of amphetamine designer drugs. In a study of 5 men receiving 6 mg DOM daily for 3 consecutive days, marked attenuation of subjective effects of DOM occurred over the 3-day study.[186] Only 1 participant continued to experience moderately strong effects by the third day, whereas 4 of 5 of the participants experienced extremely intense effects on the first day.

2,5-DIMETHOXY-4-ETHYLAMPHETAMINE (DOET)

2,5-Dimethoxy-4-ethylamphetamine (DOET) is the ethyl homologue of DOM. The oral administration of low doses (1.5 mg hydrochloride salt) of DOET to paid volunteers produced mild euphoria and enhanced self-awareness without perceptual distortions or psychotomimetic effects.[187] Subjective effects began 1–1.5 hours after ingestion with peak effects occurring about 3–4 hours after ingestion. Resolution of effects occurred within 5–6 hours. Doses of DOET up to 4 mg are associated with mydriasis, anxiety, and restlessness.[188] A volunteer study suggested that a 4-mg dose of racemic DOET produces psychedelic effects only when the participants eyes were closed.[8] A pilot study of paid volunteers ingesting up to 4 mg DOET suggest the kidney excretes approximately 10–40% of the DOET dose in the urine within the first 24 hours after ingestion.[189]

4-Bromo-2,5-dimethoxyphenethylamine (2C-B) and the 2C-Designer Series

HISTORY

Shulgin synthesized most of the known members of the 2C-phenethylamine series in the 1970s and 1980s including the production of 2C-B by elemental bromination of 2,5-dimthoxyphenethylamine in acetic acid.[190] In 1975, Shulgin et al. documented the sensory enhancement associated with 2C-B intoxication during experiments with volunteers. Ragan et al. first reported 2C-B in illicit US street samples in 1985.[191] In the late 1990s and early 2000s, analysis of tablets sold in Dutch "smart shops" demonstrated the presence of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and several other phenethylamine compounds in the 2C-series (2C-T-2, 2C-T-7).[192,193] As 2C-B became a schedule I drug, other members of the 2C-series appeared in illicit drug samples.

IDENTIFYING CHARACTERISTICS

The "2C" refers to the 2 carbon atoms separating the primary amine functionality from the phenyl ring; these drugs also contain methoxy groups at positions 2 and 5 of the aromatic ring and a lipophilic substituent (e.g., alkyl, alkylthio, halogen) in position 4 of the aromatic ring. Street names for 2C-B include Venus, Bromo, Erox, XTC, Synergy, and Nexus.

EXPOSURE

2C-B is the most common illicit drug from the 2C series (2C-B, 2C-I, 2C-D, 2C-E, 2C-P, 2C-T-2, 2C-T-7). 2C-B and other members of the 2C-series are frequently ingested with other club drugs (MDMA, ketamine, MBDB).

DOSE EFFECT

Anecdotal data suggests that recreational doses of 2C-B range from 4–30 mg with lower doses (4–10 mg) producing entactogenic effects, whereas high doses (10–20 mg) cause psychedelic and sympathomimetic effects. In self-reported experimental studies, psychedelic effects began following the ingestion of 2C-B doses from 8–10 mg, and these effects persisted approximately 4–8 hours.[190] Doses of 2C-B exceeding 30 mg may cause frightening hallucinations, agitation, dysphoria, tachycardia, hypertension, and, hyperthermia.

TOXICOKINETICS

There are few human data on the toxicokinetics of 2C-series drugs; existing data suggest that these drugs primarily undergo *O*-demethylation at the 2-position by deamination followed by oxidation to the corresponding acid or and by reduction of the 5-position to the corresponding alcohol.[194] Other metabolic reactions include side chain hydroxylation, sulfoxidation of sulfur containing 2C-compounds, and phase II reactions (glucuronidation, sulfation, *N*-acetylation). *In vitro* studies indicate that the metabolism of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) occurs via oxidative deamination by monoamine oxidase (MAO-A, MAO-B) followed by demethylation, or to a lesser extent, by oxidative deamination catalyzed by cytochrome P450 isoenzymes (CYP2D6).[195,196] Most 2C-series phenethylamine drugs have slightly greater affinity for MAO-A than MAO-B. There is substantial interspecies variation in the biotransformation of 2C-B as well as interindividual variation in humans. Major metabolites of oxidative deamination include 2-(4-bromo-2,5-dimethoxyphenyl)-ethanol (BDMPE), 4-bromo-2,5-dimethoxyphenylacetic acid (BDMPAA), and 4-bromo-2,5-dimethoxybenzoic acid (BDMBA). Urinary metabolites found in a urine sample from a man abusing 2C-B included BDMBA, BDMPAA, and 4-bromo-2-hydroxy-5-methoxyphenethylamine.[197] In rodent studies, the plasma elimination of 2C-B is rapid (i.e., t½ about 1 h) and the volume of distribution is large (16 L/kg).[198]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

In vitro studies indicate that the 2C-series phenethylamine drugs act primarily at the 5-HT$_2$ receptor, displaying agonist and antagonist activity at specific subreceptor sites.[199] 2C-B also demonstrates significant affinity to 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ serotonin receptors.[200]

CLINICAL RESPONSE

The few medical data on the medical effects of 2C-B and other members of the 2C-B series are primarily from the experimental studies by Shulgin in the 1970s.[107,190] The frequent concomitant ingestion of other illicit drugs complicates the interpretation of anecdotal reports of the effect of these entactogens. 2C-B produces euphoria and increased sensory receptiveness (auditory, olfactory, tactile, visual), whereas adverse reactions include frightening hallucinations, tachycardia, elevated blood pressure, and potentially hyperthermia.

DIAGNOSTIC TESTING

The cross-reactivity of 2C-B and other members of the 2C-series with commercial amphetamine immunoassays is relatively low. Methods for the quantitation of 2C-B and other phenethylamine designer drugs include LC/MS,[201] capillary electrophoresis coupled with electrospray ionization/mass spectrometry,[202] HPLC with UV detection,[203] and GC/MS.[204,205] The LOD and LLOQ for 2C-series phenethylamine drugs are approximately 8–16 ng/mL and 27–53 ng/mL, respectively, with CV <20% using LC/MS.

TREATMENT

The treatment of 2C-B intoxication is supportive, similar to MDMA and MDA toxicity.

Bromo-Dragonfly

In 1998, Parker et al. synthesized a potent hallucinogen, 1-(8-bromobenzo[1,2-b;   4,5-b′]difuran-4-yl)-2-aminopropane.[206] This compound (bromobenzodifuranylisopropylamine) is a 5-HT$_{2A}$ agonist that is structurally similar to several phenethylamine compounds (e.g., 2C-B, DOB) as demonstrated in Figure 10.4. The name *bromo-dragonfly* derives from the similarity of the structure of this compound to a dragonfly. *In vivo* studies indicate that bromo-dragonfly demonstrate high affinity for 5-HT$_2$ receptors as indicated by the following K$_i$ values: 5-HT$_{2A}$, 0.04 ± 0.01 nM; 5-HT$_{2B}$, 0.19 ± 0.10 nM; 5-HT$_{2C}$, 0.02 ± 0.01.[206] These values indicate that bromo-dragonfly is a more potent ligand for the 5-HT$_{2A}$ receptor than LSD. Bromo-dragonfly has a single stereocenter with the *R*-(−)-enantiomer being relatively higher affinity for the 5-HT$_{2A}$ receptors than the *S*-(+)-enantiomer.[207]
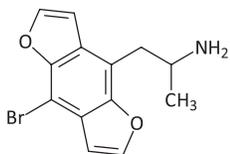
FIGURE 10.4. Chemical structure of bromo-dragonfly.

Peak psychedelic effects of this compound are delayed up to 6 hours; adverse effects from the illicit use of bromo-dragonfly based on anecdotal data include headache, fever, and dysphoria, particularly in inexperienced users. A case report associated the delayed onset (i.e., 8 h) of seizures, severe agitation, hallucinations, mydriasis, and apnea requiring intubation with the use of bromo-dragonfly and ketamine.[208] He recovered without sequelae after developing aspiration pneumonia. Recently, several deaths were attributed to the use of bromo-dragonfly. An 18-year-old woman was found dead in her home the morning after ingesting bromo-dragonfly with her boyfriend.[209] The postmortem examination demonstrated only nonspecific findings (e.g., pulmonary edema, mild cerebral edema, inflammation of the gastric mucosa, renal ischemic changes). Two analyses of femoral blood yielded bromo-dragonfly concentrations of 4.7 ± 0.7 μg/kg as measured by liquid chromatography/tandem mass spectrometry. This concentration was approximately 8-fold higher than the bromo-dragonfly concentration in blood from 2 men hospitalized after ingesting bromo-dragonfly during a techno party as measured by the same analytic method (LOD, 0.2 μg/kg). The bromo-dragonfly concentrations in postmortem urine samples from this 18-year-old woman before and after the addition of β-glucuronidase were 22 ± 2 μg/kg and 33 ± 3 μg/kg, respectively. This difference indicates the presence of the conjugation of some metabolites with glucuronic acid. Desorption atmospheric pressure photoionization is a direct analysis technique rapid detection of hallucinogens in illicit drug samples using a microchip-heated nebulizer with mass spectrometry.[210]

PIPERAZINES

Piperazines are a broad class of chemical compounds that contain a piperazine moiety composed of a 6-membered ring with 2 opposing nitrogen atoms. Many of these compounds are biologically active with therapeutic uses as antidepressants, serotonin receptor antagonists/agonists, and as antifungal and antiviral drugs.

1-Benzylpiperazine (BZP)

HISTORY

The Wellcome Research Laboratories synthesized *N*-benzylpiperazine (BZP) as an anthelminthic agent in 1943. Animal studies in the 1970s suggested that BZP had antidepressant activity, but side effects (stereotypic behavior, hyperactivity) and addictive properties similar to amphetamine prevent the clinical use of this drug in humans.[211] BZP is an active constituent of rave (dance party) drugs originally marketed over the Internet during the late 1990s. These pills were promoted as an herbal high because of the structural similarity between BZP and piperine (1-piperoylpiperidine), the amide of the alkaloid piperidine in black pepper. A variety of benzylpiperazine and α-pyrrolidinophenone compounds rapidly spread in the club drug scene as legal alternatives to MDMA with both stimulant and psychoactive properties.[212] However, the US Drug Enforcement Agency (DEA) added BZP and related drugs to schedule I in 2004 as a result of fatalities associated with the use of these drugs.[213] Australia classified BZP as a restricted substance in 2008.

IDENTIFYING CHARACTERISTICS

BZP is a piperazine derivative that contains a 6-membered heterocycle comprised of 2 nitrogen atoms linked by 2 ethyl chains. Derivatives of piperazine are constituents of many synthetic compounds including antimicrobials, sildenafil, pesticides, plastics, medical radiolabeled tracers, and resins. In the freebase form, BZP is a pale yellow liquid that reacts with air and light; however, the hydrochloride salt is more stable. Benzylpiperazine tablets are sold in dosages of 125 mg BZP dihydrochloride (88 mg free base) and as mixtures of BZP and other piperazines [e.g., 1-(3-trifluoromethylphenyl)piperazine].[214] Street names for BZP and other piperazines include A2, Bliss, BNZ, Bolts, Charge, Exodus, Frenzy, Goodstuff, Grins, Herbal Ecstasy, Herbal Party Pill, Jumps, Kandy, Legal E, Legal X, Nemesis, Red Hearts, and Silver Bullet.[215] The street name "X4" applies to a mixture of 4 piperazine compounds.

EXPOSURE

Piperazine designer drugs are frequently consumed as combinations (party pills, herbal highs) with the most prevalent mixture being BZP and 1-(3-trifluoromethylphenyl)piperazine (TFMPP).[216] BZP is frequently promoted as a legal stimulant to improve vitality, energy, and cognition. In New Zealand, the United Kingdom,

and other European countries, piperazine-based "party pills" are popular dance party drugs for the induction of euphoria and wakefulness. Although BZP is a restricted, schedule I drug in the United States, BZP remains legal in the United Kingdom, Canada, and New Zealand.

DOSE EFFECT

The clinical effects of BZP and *d*-amphetamine are similar. In a double-blind, controlled study of 18 former amphetamine addicts receiving either 10 mg *d*-amphetamine or 100 mg BZP hydrochloride, the physical parameters (i.e., blood pressure, pulse rate, pupil size) of the 2 treatment groups were statistically similar with the exception of a slightly larger pupil size in the BZP group.[211] The 2 groups liked both drugs equally, but they disliked the placebo (lactose). The median number of BZP pills ingested by a convenience sample of women

and men presenting to an emergency department with BZP intoxication was 3 and 4, respectively, with a range of 1–34.[217]

TOXICOKINETICS

The main isoenzyme catalyzing the metabolism of piperazine-type designer drugs is the cytochrome P450 isoenzyme, CYP2D6, with lesser contributions from CYP1A2 and CYP3A4. The biotransformation of BZP is limited, primarily involving hydroxylation of the aromate to 3-OH-BZP and 4-OH-BZP.[77] *N*-dealkylation to piperazine and the degradation of the piperazine heterocycle to the corresponding ethylenediamine or aniline derivative is another pathway.[77] Phase II reactions include partial glucuronidation or sulfation of the phenolic metabolites, methylation of the catechols, and partial acetylation of the aniline derivatives. Figure 10.5 displays the metabolic pathways of common piperazine derivatives along with
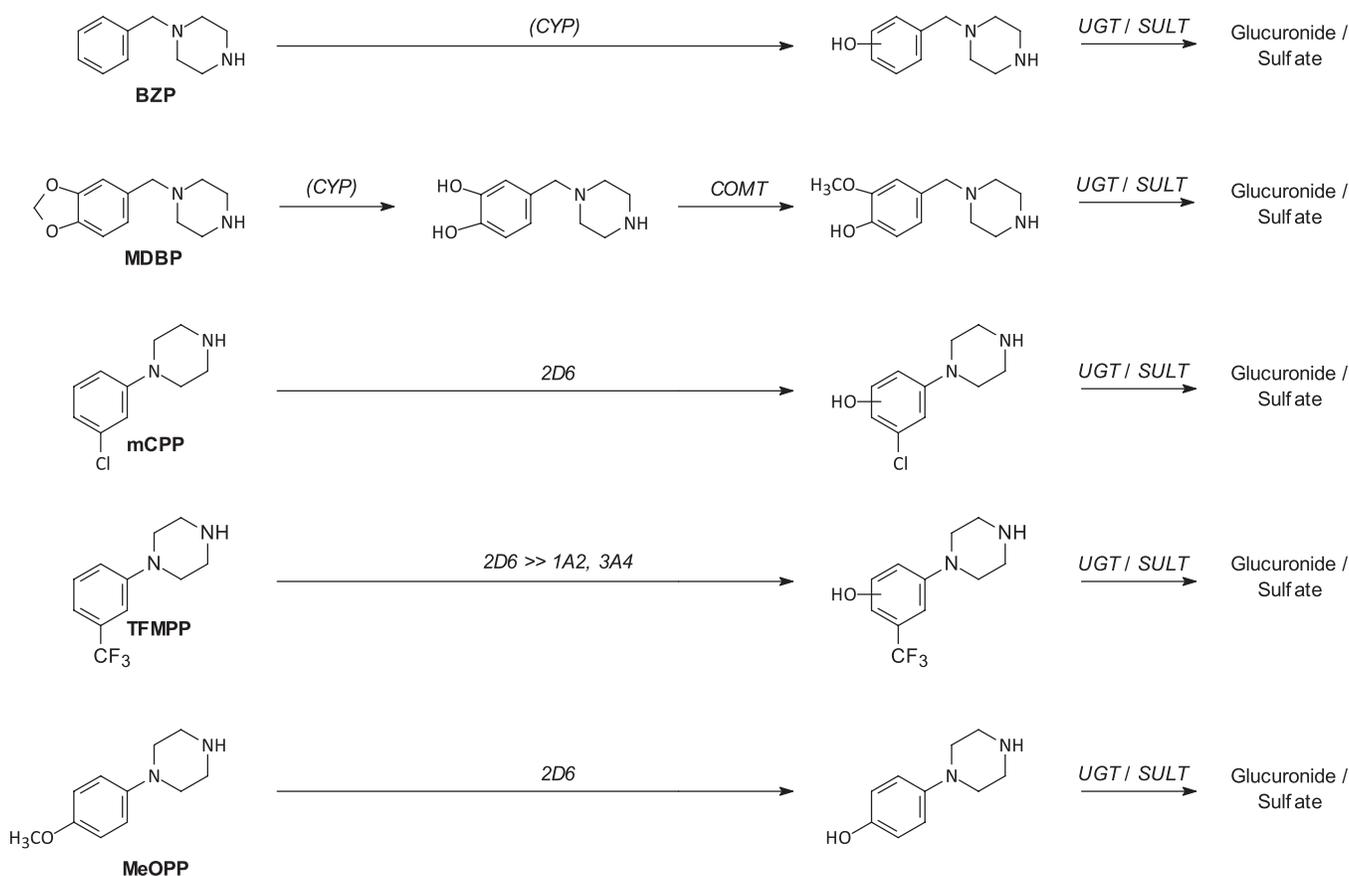
FIGURE 10.5. Major metabolic pathways of common piperazine derivatives. UGT = UDP glucuronyl transferase; SULT = sulfotransferase; COMT = catechol *O*-methyl transferase; BZP = *N*-benzyl piperazine; MDBP = 1-(3,4-methylenedioxybenzyl)piperazine; mCPP = 1-(3-chlorophenyl)piperazine; TFMPP = 1-(3-trifluoromethylphenyl)piperazine; MeOPP = 1-(4-methoxyphenyl)piperazine.

the cytochrome P450 isoenzymes involved with the pathways. The kidneys excrete a substantial amount of BZP unchanged along with the excretion of sulfate conjugates of the hydroxylated metabolites. In a study of 7 healthy adults ingesting 200 mg BZP, the mean maximum plasma concentration (C_{max}) was about 260 ± 19 ng/mL (range, 222–344 ng/mL) with a plasma elimination half-life of about 5.5 ± 0.4 hours.[218] The mean time to the maximum plasma concentration was 75 minutes. The plasma concentration of the major metabolites, 3-OH-BZP and 4-OH-BZP were relatively low ($C_{max} = 13 \pm 1$ ng/mL and 7 ± 1 ng/mL, respectively) as measured by LC/MS. Phenylpiperazine compounds are almost exclusively metabolized prior to renal excretion.[77]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

BZP has both direct and indirect sympathomimetic properties that produce improvement in performance tasks and auditory vigilance based on volunteer studies in healthy adults.[219] In chronically treated rats, BZP increases synaptic concentrations of norepinephrine in the hypothalamus, dopamine in the striatum, and serotonin in the hippocampus.[220] *In vitro* studies indicate that BZP enhances the release of dopamine from the dopamine transporter;[221] BZP has no effect on the release of serotonin from synaptosomes in these studies. *In vivo* microdialysis studies in conscious rats indicate that benzylpiperazine increases the transporter-mediated efflux of dopamine transporter substrate, whereas 1-(3-trifluoromethylphenyl)piperazine (TFMPP) is a selective releaser of serotonin in synaptosomes.[222] In this study, the combination of these 2 drugs facilitated serotonin and dopamine release similar to MDMA; however, several of the 7 rats receiving high doses (10 mg/kg) of BZP and TFMPP developed seizures.

CLINICAL RESPONSE

Toxicity associated with 1-benzylpiperazine (BZP) resembles a sympathomimetic toxidrome with tachycardia, anxiety, agitation, mydriasis, headache, nausea, vomiting, palpitations, confusion, and seizures based on case reports.[217] Symptoms during recreational use typically resolve within 4–6 hours. Figure 10.6 displays the clinical features associated with a convenience sample of 88 patients (96 visits) presenting to an Australian emer-
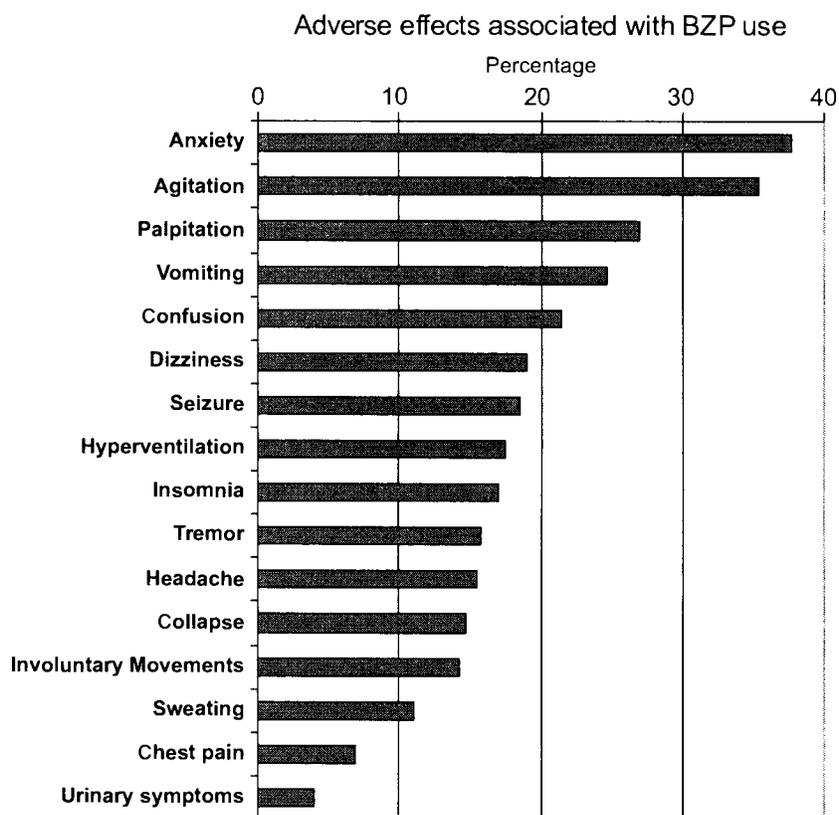
FIGURE 10.6. Clinical features of 1-benzylpiperazine intoxication in a convenience sample of 88 patients presenting to an emergency department. (Reprinted with permission from P Gee, M Gilbert, S Richardson, G Moore, S Paterson, P Graha, Toxicity from the recreational use of 1-benzylpiperazine, Clinical Toxicology, Vol. 46, Issue 9, p. 804, copyright 2008.)

gency department with BZP intoxication confirmed by the presence of BZP in plasma samples.[217] About 30% of the patients had plasma samples positive for only BZP, whereas about 57% of the patients ingested ethanol along with BZP. Two of the patients in this study survived after developing hyperthermia and multiorgan failure. There were no fatalities in this case series during the period of study. Case reports associate the use of BZP with seizures, hyperthermia, DIC, rhabdomyolysis, and hepatorenal dysfunction both as a single drug of abuse and in combination with MDMA.[223]

Toxic effects associated with the combination of BZP and TFMPP include urinary retention, nausea, vomiting, anxiety, insomnia, visual hallucinations, paranoia, depression, chest pain, and seizures based on case reports.[224] An intense hangover may develop from the use of the blended BZP and TFMPP pills. Status epilepticus with severe respiratory and metabolic acidosis may occur following the ingestion of these combination pills.[225] Occasional case reports associate the use of piperazine-type designer drugs with cerebral edema and death, usually in combination with MDMA.[226]

DIAGNOSTIC TESTING

Analytic Methods

Amphetamine immunoassays do not reliably detect BZP and commercial amphetamine immunoassays demonstrate little or no cross-reactivity with BZP, the BZP metabolite, *N*-benzylethylenediamine, or structurally related compounds (e.g., TFMPP, MeOPP).[227] In an analytic study, BZP did not cross-react with a fluorescence polarization immunoassay (AxSYM® amphetamine/methamphetamine II assay, Abbott Laboratories, Abbott Park, IL) at a BZP concentration of 100,000 ng/mL. The reaction of BZP to the reagent in an enzyme-multiple immunoassay (EMIT d.a.u.®, Dade Behring Inc., Deerfield, IL) was weak with 0.4% and 1.3% cross-reactivity at BZP concentrations of 300 ng/mL, and 12,000 ng/mL, respectively. Methods for the quantitation of piperazine designer drugs in biologic samples include gas chromatography/nitrogen phosphorous detection,[212] GC/MS in the selected-ion monitoring mode,[228,229] capillary electrophoresis with GC/MS,[230] HPLC/DAD,[231] and GC/MS with high performance liquid chromatography/electrospray ionization/mass spectrometry.[232] The LOD for BZP, TFMPP, and their main metabolites in urine samples using the latter method ranges between 5–40 ng/mL (scan mode) and between 0.2–1 ng/mL (SIM mode) following enzymatic hydrolysis, solid-phase extraction, and trifluoroacetyl anhydride-ethyl acetate derivatization. For hair samples, methods of quantitation for BZP include GC/MS with

mixed-mode solid-phase extraction and *p*-tolylpiperazine as the internal standard.[233] The LLOQ of the latter method is 0.05 ng/mg.

Biomarkers

An 18-year-old woman developed a seizure after ingesting 5 pills containing BZP. On arrival at the ED, she was agitated, but oriented (Glasgow Coma Scale [GCS] = 15) with mild hypertension and sinus tachycardia (156 bpm).[234] Her serum sample contained only 2.5 mg BZP/L and no other drugs. She was discharged 12 hours after admission with no sequelae. There are few postmortem data on the piperazine compounds. Analysis of postmortem blood (site unreported) from a 17-year-old Swedish man with a history of ecstasy and BZP (A2) abuse contained 1.7 mg BZP/L along with detectable concentrations of MDMA, MDA, and THC.[213] The case report did not provide clinical details. A 17-year-old adolescent died after falling through a roof. The postmortem blood (site unreported) contained BZP and TFMPP concentrations of 1.39 mg/L and 0.15 mg/L, respectively, along with 140 mg ethanol/dL.[231]

Abnormalities

Potentially serious laboratory abnormalities associated with BZP intoxication include hypoglycemia, hyponatremia, and QT_c prolongation; however, ventricular dysrhythmias are unusual despite the presence of multiorgan failure.[223]

TREATMENT

Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

meta-Chlorophenylpiperazine (mCPP)

meta-Chlorophenylpiperazine (mCPP) is a relatively new synthetic drug that became a drug of abuse throughout Europe during the mid-2000s. Although mCPP can be injected or insufflated in powdered form, the usual route of abuse is oral. Street names for the multicolored tablets include Rolls Royce, Harlequin, Smarties, Rainbow, and X4. The amount of mCPP in illicit tablets ranges from 22–80 mg; these tablets may contain cocaine, MDMA, or the contaminant, *p*-chlorophenylpiperazine.[235]

mCPP is a probe for serotonin function in psychiatric research as well as a potential anorexic drug. Typical oral doses of mCPP used for clinical investigations are 0.5 mg/kg.[236] Case reports associate clinical doses of mCPP with the development of a serotonin-like syndrome.[237] Methods for the quantitation of mCPP in biologic samples include MS with separation by LC or GC. For hair samples, methods of quantitation for mCPP involve GC/MS with mixed-mode solid-phase extraction and *p*-tolylpiperazine as the internal standard.[233] The LLOQ of the latter is 0.05 ng/mg.

TOXICOKINETICS

The absolute bioavailability and clearance of mCPP is highly variable with large interindividual differences that complicate the therapeutic administration of this drug.[238] The absolute bioavailability ranged from 12–84% (mean, 39%) in a study of 12 healthy men ingesting 0.4 mg mCPP/kg.[239] The mean time to peak mCPP plasma concentrations was 2.19 ± 0.70 hours with a range from 1.40–4.08 hours. The biotransformation of piperazine derivatives is greater for phenylpiperazine (mCPP, TFMPP) than benzylpiperazine derivatives (e.g., BZP). The major metabolic pathway for the biotransformation of mCPP is hydroxylation of the aromatic ring via the cytochrome P450 isoenzyme, CYP2D6. Alternate metabolic pathways involve the degradation of the piperazine ring to *p*-hydroxy-mCPP and then conjugation to glucuronide or sulfate. Phenylpiperazine compounds are almost exclusively metabolized prior to renal excretion.[77] In a study of 12 healthy men, the plasma elimination half-life of mCPP ranged from 2.6–6.1 hours after ingestion.[239] mCPP is a minor metabolite of nefazodone and the main active metabolite of trazodone;[240] additionally mCPP is a metabolite of etoperidone and mepiprazole.[241] The co-administration of these drugs and mCPP increases the steady-state plasma mCPP concentration in poor metabolizers of CYP2D6, particularly in combination with the CYP2D6 inhibitor, fluoxetine.[242]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The binding profile of *m*-chlorophenylpiperazine (mCPP) is complex; mCPP is a central serotonin agonist and a vascular serotonin antagonist with affinity for most serotonin receptors (1A, 1B, 1D, 2C) and for α_2-adrenergic receptors. The affinity of mCPP for 5-HT$_{2C}$ receptor subtypes is high, whereas the affinity of mCPP for serotonin 1A, 1B, and 1D receptor subtypes is substantially less. This drug is also an antagonist of 5-HT$_3$ and 5-HT$_{2A}$ receptor subtypes.[243]

CLINICAL RESPONSE

mCPP is a stimulant and psychedelic compound with effects similar to MDMA. In clinical studies of patients receiving 0.5 mg mCPP/kg, 3 patients developed symptoms of a serotonin-like syndrome.[237] Other adverse effects reported from clinical trials include heightened sensitivity toward light and noise, fear of losing control, shivering, lightheadedness, and anxiety.[239] Peak subjective and physiologic effects typically occur about 1–2 hours after ingestion and resolve within 4–8 hours. The clinical effects associated with mCPP intoxication include nausea, diaphoresis, agitation, restlessness, myoclonus, and hypomania based on clinical studies and case reports. A 29-year-old woman developed nausea, anxiety, agitation, and visual hallucinations a half hour after ingesting her third tablet of mCPP. Vital signs were stable and there were no ECG abnormalities. She was discharged after 8 hours of observation. Laboratory testing revealed the presence of cocaine metabolites and ethanol (70 mg/dL).

DIAGNOSTIC TESTING

Analytic methods for the quantitation of mCPP include HPLC[244] and liquid chromatography/atmospheric pressure chemical ionization/tandem mass spectrometry. In a study of 12 healthy volunteers receiving 0.4 mg mCPP/kg, the mean peak plasma mCPP concentration was 53 ± 35 ng/mL with a range between 12.9 and 104.6 ng/mL.[239] The presence of mCPP in biologic samples does not necessarily imply the ingestion of this drug because mCPP is a metabolite of some antidepressants (e.g., trazodone, etoperidone, mepiprazole, nefazodone). A plasma sample from a 29-year-old woman with mCPP intoxication (nausea, anxiety, agitation, hallucinations) contained 320 ng mCPP/mL.[235] Her urine sample contained 2,300 ng mCPP/mL.

TREATMENT

Treatment is supportive, similar to the treatment of MDMA or MDA intoxication.

1-Piperonylpiperazine (MDBP)

1-Piperonylpiperazine (MDBP) is a relative weak inhibitor of serotonin uptake.[245] The primary metabolic pathway of 1-piperonylpiperazine [1-(3,4-methylenedioxybenzyl)piperazine, MDBP] is demethylenation of

the methylenedioxy moiety and subsequent methylation to N-(4-hydroxy-3-methoxybenzyl)piperazine followed by partial glucuronidation or sulfation.[246] The kidneys excrete a substantial amount of MDBP unchanged. Methods for the quantitation of MDBP in biologic samples include GC/MS.[247] Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

1-(4-Methoxyphenyl) piperazine (MeOPP)

The biotransformation of piperazine derivatives is greater for MeOPP than BZP. MeOPP and most other phenylpiperazine compounds are almost exclusively metabolized prior to renal excretion. The major metabolic pathway for the biotransformation of MeOPP is O-demethylation of the methoxy moiety. CYP2D6 is the major cytochrome P450 isoenzyme involved in the O-demethylation of MeOPP.[248] In rodent studies, metabolites of MeOPP include 1-(4-hydroxy phenyl) piperazine and 4-hydroxyaniline.[249] Amphetamine immunoassays do not reliably detect MeOPP or related compounds (e.g., BZP, TFMPP). Methods for the quantitation of MeOPP in biologic samples include MS with separation by LC or GC. Reliable detection of MeOPP and related drugs (BZP, TFMPP) in urine samples requires GC with nitrogen phosphorus detection or GC/MS in selected-ion monitoring mode after mixed-mode solid-phase extraction and derivatization with heptafluorobutyric anhydride.[229] The LLOQ for the latter method is 5 ng/mL. For hair samples, methods of quantitation for MeOPP involve GC/MS with mixed-mode solid-phase extraction and p-tolylpiperazine as the internal standard.[233] The LLOQ of the latter is 0.05 ng/mg. Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

1-(3-trifluoromethylphenyl) piperazine (TFMPP)

In 2002, TFMPP was temporarily added to the schedule I controlled substances list under the US Controlled Substances Act; however, TFMPP was removed from this list in 2004 after further review.[250] The sale of TFMPP is banned in Australia, Belgium, Denmark, Greece, New Zealand, and Sweden. The nonselective serotonergic agonist, 1-(3-trifluoromethylphenyl)piperazine (TFMPP) is usually ingested as a mixture with BZP.[251] The combination of BZP and TFMPP enables drug users to reproduce the dopaminergic and serotoninergic effects of MDMA.[252]

TOXICOKINETICS

The major metabolic pathways for the biotransformation of TFMPP is alteration of the aromate by hydroxylation and by degradation of the piperazine moiety to N-(3-trifluoromethylphenyl)ethylenediamine, N-(hydroxy-3-trifluoromethylphenyl)ethylenediamine, 3-trifluoromethylaniline, and hydroxy-3-trifluoromethylaniline.[253] Phase II reactions included glucuronidation, sulfonation, and acetylation of phase I metabolites. In rodent and human studies, p-hydroxy-TFMPP (4-OH-TFMPP) is the main urinary metabolite.[254,255] Studies of human liver microsomes indicate that the CYP2D6 is also the major isoenzyme catalyzing the hydroxylation of TFMPP along with minor contributions by CYP1A2 and CYP3A4.[256] TFMPP is almost exclusively (i.e., >98–99%) metabolized prior to renal excretion.[77] In a study of volunteers ingesting 60 mg TFMPP, the mean peak plasma TFMPP concentration (24 ng/mL) occurred about 1½ hours after ingestion; the mean plasma elimination half-life was 2 and 6 hours.[254] In vitro studies suggest that potential drug interactions may result from the ingestion of TFMPP with drugs that inhibit CYP2D6, CYP1A2, and CYP3A4 similar to benzylpiperazine.[257] Potential drug interactions occur between BZP and TFMPP because of similar metabolic pathways; however, the clinical significance of these potential interactions is not well defined.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

TFMPP is a centrally active, aryl piperazine compound with serotoninergic properties.[258] In vivo microdialysis studies in conscious rats indicate that TFMPP is a selective releaser of serotonin in synaptosomes, whereas benzyl piperazine increases the transporter-mediated efflux of serotonin and dopamine transporter substrate.[221] The action of TFMPP on various serotonin receptor types is complex. Rodent studies suggest that 5-HT$_{1B}$, 5-HT$_{1C}$, and possibly σ-receptors mediate the effects of TFMPP, whereas this compound does not bind strongly to 5-HT$_{1A}$, 5-HT$_2$, dopaminergic, or adrenergic receptors.[259]

CLINICAL RESPONSE

The combination of BZP and TFMPP produces sympathomimetic and dissociative symptoms. Clinical effects

associated with the ingestion of this combination by 3 patients presenting to an emergency department were nausea, vomiting, malaise, anxiety, agitation, mydriasis, ataxia, clonus, and sinus tachycardia.[260] All recovered without sequelae with supportive care.

DIAGNOSTIC TESTING

Methods for the quantitation of TFMPP and related drugs (MeOPP, BZP) in urine samples include GC with nitrogen phosphorus detection, chiral capillary electrophoresis,[261] liquid chromatography/electrospray ionization/mass spectrometry,[254] and GC/MS.[262] The ranges of BZP and TFMPP concentrations in serum samples from 3 patients intoxicated with these drugs ranged from 260–270 ng/mL and 30–60 ng/mL, respectively. They recovered without sequelae. Amphetamine immunoassays do not reliably detect TFMPP and related compounds (e.g., BZP, MeOPP). For hair samples, methods of quantitation for TFMPP involve GC/MS with mixed-mode solid-phase extraction and

p-tolylpiperazine as the internal standard.[233] The LLOQ of the latter is 0.05 ng/mg.

TREATMENT

Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

PYRROLIDINO-PHENONES

α-Pyrrolidinophenone compounds along with a variety of benzylpiperazine compounds rapidly spread as drugs of abuse in the club drug scene during the late 1990s. These drugs gained popularity as legal alternatives to MDMA with both stimulant and psychoactive properties.[212] Extensive metabolism of pyrrolidinophenone
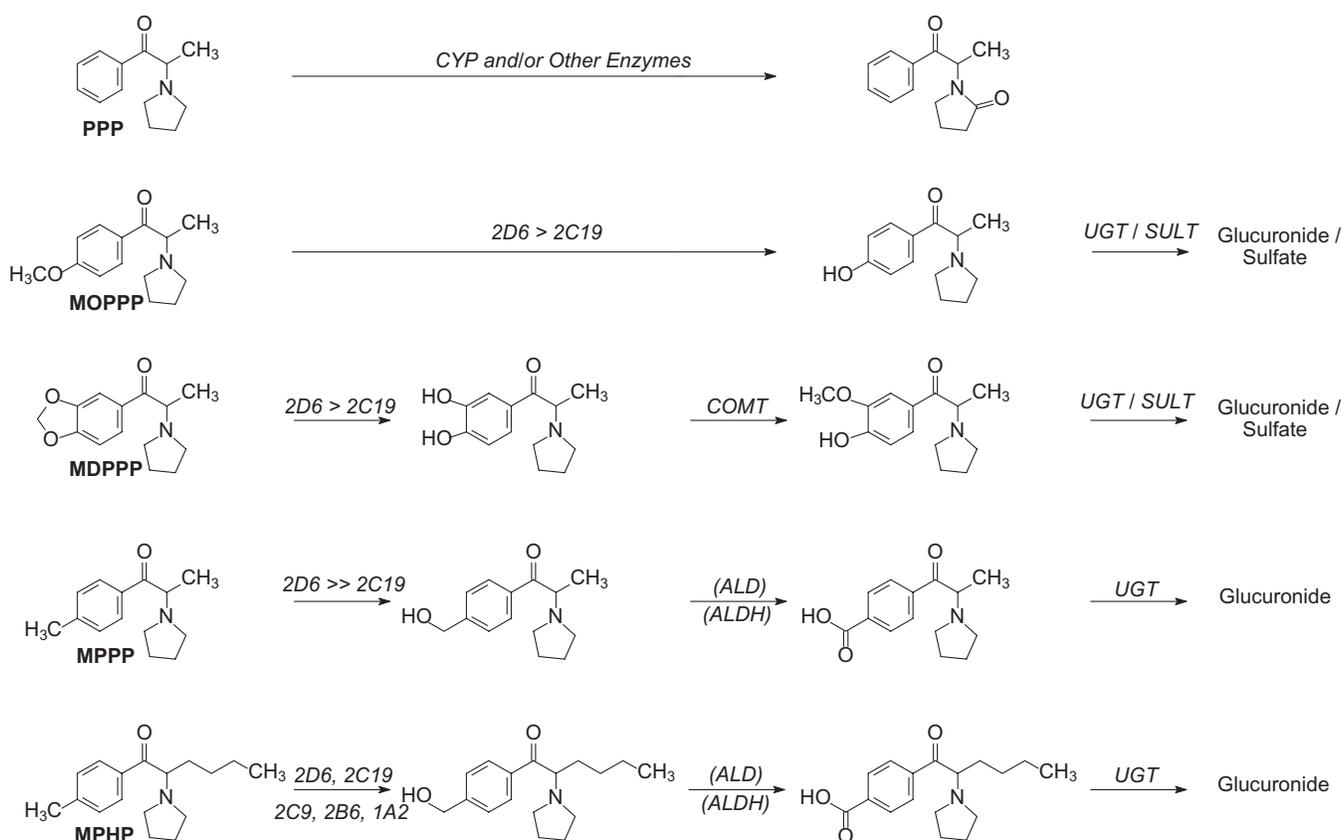
FIGURE 10.7. Major metabolic pathways of common pyrrolidinophenone derivatives. UGT = UDP glucuronyl transferase; SULT = sulfotransferase; COMT = catechol *O*-methyl transferase; ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; PPP = pyrrolidinopropiophenone; MOPP = R,S-4′-methoxy-α-pyrrolidinopropiophenone; MDPPP = R,S-3′,4′-methylenedioxy-α-pyrrolidinopropiophenone; MPPP = R,S-4′-methyl-α-pyrrolidinopropiophenone; MPHP = R,S-4′-methyl-α-pyrrolidinohexanophenone.

derivatives occurs in humans, primarily as a result of hydroxylation catalyzed by the cytochrome P450 isoenzyme, CYP2D6, and to a lesser extent by CYP2C19.[263] Pyrrolidinopropiophenone (PPP) undergoes ring oxidation to the corresponding lactam or degradation by double dealkylation to cathinone and subsequent keto reduction.[246] Although 4′-hydroxy-PPP is a common metabolite of PPP and MeOPP, 2″-oxo-PPP is a unique metabolite of PPP biotransformation; therefore, the presence of this latter metabolite indicates the ingestion of PPP.[264] *R*,*S*-3′, 4′-methylenedioxy-α-pyrrolidinopropiopenone (MDPPP) undergoes demethylenation of the methylenedioxy group followed by partial 3′-methylation of the resulting catechol, oxidative deamination to the corresponding diketo compounds and/or hydroxylation of the pyrrolidine ring with dehydrogenation to the corresponding lactam; the main metabolite is 4′-hydroxy-PPP.[265] Common metabolites of *R*,*S*-4′-methoxy-α-pyrrolidinopropiophenone (MOPPP) and MDPPP include 3′,4′-dihydroxy-PPP, 3′-methoxy-4′-hydroxy-PPP, and 3′-methoxy-4′-hydroxy-2-oxo-propiophenone. In addition to CYP2D6, CYP2C19 is potentially involved with the *O*-demethylation of MOPPP and MDPPP.[77] Figure 10.7 displays the major metabolic pathways of common pyrrolidinophenone compounds. Treatment is supportive and similar to the treatment of MDMA or MDA intoxication.

References

1. Henderson GL. Designer drugs: past history and future prospects. J Forensic Sci 1988;33:569–575.
2. Nichols DE. Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J Psychoactive Drugs 1986;18:305–313.
3. Shulgin AT. Mescaline: the chemistry and pharmacology of its analogs. Lloydia 1973;36:45–58.
4. Jacob P III, Shulgin AT. Structure-activity relationships of the classic hallucinogens and their analogs. NIDA Res Monogr 1994;146:74–91.
5. Peretz DI, Smythies JR, Gibson WC. A new hallucinogen: 3,4,5-trimethoxyphenyl-beta-aminopropane with notes on the stroboscopic phenomenon. J Ment Sci 1955;101:317–329.
6. Shulgin AT. The six trimethoxyphenylisopropylamines (trimethoxyamphetamines). J Med Chem 1966;9:445–446.
7. Shuglin AT. The ethyl homologs of 2,4,5-trimethoxyphenylisopropylamine. J Med Chem 1968;11:186–187.
8. Shulgin AT. 3-Methoxy-4,5-methylenedioxyamphetamine, a new psychotomimetic agent. Nature 1964;201:1120–1121.
9. Randall T. Ecstasy-fueled "rave" parties become dances of death for English youths. JAMA 1992;268:1505–1506.
10. Landry MJ. MDMA: a review of epidemiologic data. J Psychoactive Drugs 2002;34:163–169.
11. Pentney AR. An exploration of the history and controversies surrounding MDMA and MDA. J Psychoactive Drugs 2001;33:213–221.
12. Snyder SH, Unger S, Blatchley R, Barfknecht CF. Stereospecific actions of DOET (2,5-dimethoxy-4-ethylamphetamine) in man. Arch Gen Psychiatry 1974;31:103–106.
13. Delliou D. 4-bromo-2,5-dimethoxyamphetamine: psychoactivity, toxic effects and analytical methods. Forensic Sci Int 1983;21:259–267.
14. Rosner P, Quednow B, Girreser U, Junge T. Isomeric fluoro-methoxy-phenylalkylamines: a new series of controlled-substance analogues (designer drugs). Forensic Sci Int 2005;148:143–156.
15. Climko RP, Roehrich H, Sweeney DR, Al-Razi J. Ecstasy: a review of MDMA and MDA. Int J Psychiatry Med 1986–1987;16:359–372.
16. Naranjo C, Shulgin AT, Sargent T. Evaluation of 3,4-methylenedioxyamphetamine (MDA) as an adjunct to psychotherapy. Med Pharmacol Exp 1967;17:359–364.
17. Drug Enforcement Agency, Department of Justice. Schedule of controlled substances: scheduling of 3,4-methylenedioxymethamphetamine (MDMA) into schedule I of the Controlled Substances Act. Fed Regist 1986;51: 36552-36560.
18. Grinspoon L, Bakalar JB. Can drugs be used to enhance the psychotherapeutic process? Am J Psychother 1986; 40:393–404.
19. Kalant H. The pharmacology and toxicology of "ecstasy" (MDMA) and related drugs. CMAJ 2001;165:917–928.
20. Nichols DE, Lloyd DH, Hoffman AJ, Nichols MB, Yim GK. Effects of certain hallucinogenic amphetamine analogues on the release of [3H]serotonin from rat brain synaptosomes. J Med Chem 1982;25:530–535.
21. Dal Cason TA. An evaluation of the potential for clandestine manufactured of 3,4-methylenedioxyamphetamine (MDA) analogs and homologs. J Forensic Sci 1990; 35:675–697.
22. Byard RW, Gilbert J, James R, Lokan RJ. Amphetamine derivative fatalities in South Australia—is "ecstasy" the culprit? Am J Forensic Med Pathol 1998;19:261–265.
23. de la Torre R, Farré M, Ortuño J, Mas M, Brenneisen R, Roset PN, et al. Non-linear pharmacokinetics of MDMA ("ecstasy") in humans. Br J Clin Pharmacol 2000;49: 104–109.
24. Maurer HH, Bickeboeller-Friedrich J, Kraemer T, Peters FT. Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ('ecstasy'). Toxicol Lett 2000;112–113:133–142.
25. Meyer MR, Peters FT, Maurer H. Investigations on the human hepatic cytochrome P450 isozymes involved in the metabolism of 3,4-methylendioxy-amphetamine

(MDA) and benzodioxolyl-butanamine (BDB) enantiomers. Toxicol Lett 2009;190:54–60.

26. Dams R, De Letter EA, Mortier KA, Cordonnier JA, Lambert WE, Piette MH, et al. Fatality due to combined use of the designer drugs MDMA and PMA: a distribution study. J Anal Toxicol 2003;27:318–322.

27. Johnson M, Letter AA, Merchant K, Hanson GR, Gibb JW. Effects of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine isomers on central serotonergic, dopaminergic and nigral neurotensin systems of the rat. J Pharmacol Exp Ther 1988;244: 977–982.

28. Callaghan PD, Irvine RJ, Daws LC. Differences in the *in vivo* dynamics of neurotransmitter release and serotonin uptake after acute *para*-methoxyamphetamine and 3,4-methylenedioxymethamphetamine revealed by chronoamperometry. Neurochem Int 2005;47:350–361.

29. Glennon RA, Young R. MDA: an agent that produces stimulus effects similar to those of 3,4-DMA, LSD and cocaine. Eur J Pharmacol 1984;99:249–250.

30. Cohen RS. Subjective reports on the effects of the MDMA ("ecstasy") experience in humans. Prog Neuropsychopharmacol Biol Psychiatry 1995;19: 1137–1145.

31. Verheyden SL, Henry JA, Curran HV. Acute, sub-acute and long-term subjective consequences of "ecstasy" (MDMA) consumption in 430 regular users. Hum Psychopharmacol 2003;18:507–517.

32. Laloup M, Tilman G, Maes V, De Boeck G, Wallemacq P, Ramaekers J, Samyn N. Validation of an ELISA-based screening assay for the detection of amphetamine, MDMA and MDA in blood and oral fluid. Forensic Sci Int 2005;153:29–37.

33. Nordgren HK, Beck O. Multicomponent screening for drugs of abuse: direct analysis of urine by LC-MS-MS. Ther Drug Monit 2004;26:90–97.

34. Lekskulchai V, Mokkhavesa C. Evaluation of Roche Abuscreen ONLINE Amphetamine Immunoassay for screening of new amphetamine analogues. J Anal Toxicol 2001;25:471–475.

35. Ensslin HK, Kovar KA, Maurer HH. Toxicological detection of the designer drug 3,4-methylenedioxyethylamphetamine (MDE, "Eve") and its metabolites in urine by gas chromatography-mass spectrometry and fluorescence polarization immunoassay. J Chromatogr B Biomed Appl 1996;683:189-197.

36. Garcia-Repetto R, Moreno E, Soriano T, Jurado C, Gimenez MP, Menendez M. Tissue concentrations of MDMA and its metabolite MDA in three fatal cases of overdose. Forensic Sci Int 2003;135:110-114.

37. Kunsman GW, Levine B, Kuhlman JJ, Jones RL, Hughes RO, Fujiyama CI, Smith ML. MDA-MDMA concentrations in urine specimens. J Anal Toxicol 1996;20: 517-521.

38. Loor R, Lingenfelter C, Wason PP, Tang K, Davoudzadeh D. Multiplex assay of amphetamines, methamphetamine, and ecstasy drug using CEDIA® technology. J Anal Toxicol 2002;26:267–273.

39. Kraemer T, Maurer HH. Determination of amphetamine, methamphetamine and amphetamine-derived designer drugs or medicaments in blood and urine. J Chromatogr B Biomed Sci Appl 1998;713:163–187.

40. Fitzgerald RL, Blanke RV, Glennon RA, Yousif MY, Rosecrans JA, Poklis A. Determination of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine enantiomers in whole blood. J Chromatogr 1989;490:59–69.

41. Valentine JL, Middleton R. GC-MS identification of sympathomimetic amine drugs in urine: rapid methodology applicable for emergency clinical toxicology. J Anal Toxicol 2000:211–222.

42. Wohlfarth A, Weinmann W, Dresen S. LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum. Anal Bioanal Chem 2010;396: 2403–2414.

43. Bogusz MJ, Kruger K-D, Maier R-D. Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 2000;24:77–84.

44. Pirnay SO, Abraham TT, Huestis MA. Sensitive gas chromatography-mass spectrometry method for simultaneous measurement of MDEA, MDMA, and metabolites HMA, MDA, and HMMA in human urine. Clin Chem 2006;52:1728–1734.

45. Ugland HG, Krogh M, Rasmussen KE. Automated determination of "Ecstasy" and amphetamines in urine by SPME and capillary gas chromatography after propylchloroformate derivatization. J Pharm Biomed Anal 1999;19:463–475.

46. Herraez-Hernandez R, Campins-Falco P, Verdu-Andres J. Sensitive determination of methylenedioxylated amphetamines by liquid chromatography. Analyst 2001; 126:581–586.

47. Wood M, De Boeck G, Samyn N, Morris M, Cooper DP, Maes RA, De Bruijn EA. Development of a rapid and sensitive method for the quantitation of amphetamines in human plasma and oral fluid by LC-MS-MS. J Anal Toxicol 2003;27:78–87.

48. Peters FT, Samyn N, Lamers CT, Riedel WJ, Kraemer T, de Boeck G, Maurer HH. Drug testing in blood: validated negative-ion chemical ionization gas chromatographic-mass spectrometric assay for enantioselective measurement of the designer drugs MDEA, MDMA, and MDA and its application to samples from a controlled study with MDMA. Clin Chem 2005;51:1811–1822.

49. Sadeghipour F, Veuthey JL. Sensitive and selective determination of methylenedioxylated amphetamines by high-performance liquid chromatography with fluorometric detection. J Chromatogr A 1997;787:137–143.

50. Lurie IS, Bailey CG, Anex DS, Bethea MJ, McKibben TD, Casale JF. Profiling of impurities in illicit methamphetamine by high-performance liquid chromatography and

capillary electrochromatography. J Chromatogr A 2000; 870:53–68.

51. Kochana J, Wilamowski J, Parczewski A. Profiling of impurities in *p*-methoxymethamphetamine (PMMA) by means of SPE/TLC method examination of the influence of experimental conditions according to 2(4) factorial. Forensic Sci Int 2003;134:214–218.

52. Clauwaert KM, van Bocxlaer JF, de Leenheer AP. Stability study of the designer drugs "MDA, MDMA and MDEA" in water serum whole blood, and urine under various storage temperatures. Forensic Sci Int 2001; 124:36–42.

53. Stanaszek R, Piekoszewski W. Simultaneous determination of eight underivatized amphetamines in hair by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS). J Anal Toxicol 2004;28:77–85.

54. Garrett ER, Seyda K, Marroum P. High performance liquid chromatographic assays of the illicit designer drug "ecstasy," a modified amphetamine, with applications to stability, partitioning and plasma protein binding. Acta Pharm Nord 1991;3:9–13.

55. Elliott SP. MDMA and MDA concentrations in antemortem and postmortem specimens in fatalities following hospital admission. J Anal Toxicol 2005;29:296–300.

56. Kerns SW, Garvey L, Owens J. Cocaine-induced wide complex dysrhythmias. J Emerg Med 1997;15:321–329.

57. Shannon M. Methylenedioxymethamphetamine (MDMA, "Ecstasy"). Ped Emerg Care 2000;16:377–380.

58. Davis WM, Catravas JD, Waters IW. Effects of an IV lethal dose of 3,4-methylenedioxyamphetamine (MDA) in the dog and antagonism by chlorpromazine. Gen Pharmacol 1986;17:179–183.

59. Davis WM, Borne RF. Pharmacologic investigation of compounds related to 3,4 methylenedioxyamphetamine. Subst Alcohol Actions Misuse 1984;5:105–110.

60. Henry JA, Jeffreys KJ, Dawling S. Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). Lancet 1992;340:384–387.

61. Webb C, Williams V. Ecstasy intoxication: appreciation of complications and the role of dantrolene. Anaesthesia 1993;18:542–543.

62. Barrett PJ. Ecstasy and dantrolene. Br Med J 1992;305:1225.

63. Ames D, Wirshing WC. Ecstasy, the serotonin syndrome, and neuroleptic malignant syndrome—a possible link? JAMA 1993;269:869–870.

64. Friedman R. Ecstasy, the serotonin syndrome, and neuroleptic malignant syndrome—a possible link? JAMA 1993;269:869–870.

65. Sprague JE, Moze P, Caden D, Rusyniak DE, Holmes C, Goldstein DS, Mills EM. Carvedilol reverses hyperthermia and attenuates rhabdomyolysis induced by 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) in an animal model. Crit Care Med 2005;33:1311–1316.

66. Jackson WL Jr. Toward directed therapy for amphetamine-mediated hyperthermia: is carvedilol worth raving about? Crit Care Med 2005;33:1443–1445.

67. McKinney PE, Tomaszewski C, Phillips S, Brent J, Kulig K. Methamphetamine toxicity prevented by activated charcoal in a mouse model. Ann Emerg Med 1994;24: 220–223.

68. Curry SC, Chang D, Connor D. Drug and toxin-induced rhabdomyolysis. Ann Emerg Med 1989;18:1068–1084.

69. Ajaelo I, Koenig K, Snoey E. Severe hyponatremia and inappropriate antidiuretic hormone secretion following ecstasy use. Acad Emerg Med 1998;5:839–840.

70. Zenenberg R, Goldfarb DS. Evaluation of hyponatremia associated with use of methylenedioxymethamphetamine (MDMA). Int J Med Toxicol 2000;3:30.

71. Braun U, Shulgin AT, Braun G. Centrally active N-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine). J Pharm Sci 1980;69:192–195.

72. Iwersen S, Schmoldt A. Two very different fatal cases associated with the use of methylenedioxyethylamphetamine (MDEA). Eve as deadly as Adam. J Toxicol Clin Toxicol 1996;34:241–244.

73. Sondermann N, Kovar KA. Screening experiments of ecstasy street samples using near infrared spectroscopy. Forensic Sci Int 1999;106:147–156.

74. Freudenmann RW, Spitzer M. The neuropsychopharmacology and toxicology of 3,4-methylenedioxy-N-ethylamphetamine (MDEA). CNS Drug Rev 2004;10: 89–116.

75. Brunnenberg M, Lindenblatt H, Gouzoulis-Mayfrank E, Kovar KA. Quantitation of *N*-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma by high-performance liquid chromatography and fluorescence detection. J Chromatogr B Biomed Sci Appl 1998;719:79–85.

76. Spitzer M, Franke B, Walter H, Buechler J, Wunderlich AP, Schwab M, Kovar KA, Hermle L, Grön G. Enantioselective cognitive and brain activation effects of *N*-ethyl-3,4-methylenedioxyamphetamine in humans. Neuropharmacology 2001;41:263–271.

77. Staack RF, Maurer HH. Metabolism of designer drugs of abuse. Curr Drug Metab 2005;6:259–274.

78. Kreth K, Kovar K, Schwab M, Zanger UM. Identification of the human cytochromes P450 involved in the oxidative metabolism of "ecstasy"-related designer drugs. Biochem Pharmacol 2000;59:1563–1571.

79. Meyer MR, Peters FT, Maurer HH. The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxyethylamphetamine and its single enantiomers. Drug Metab Disp 2009;37: 1152–1156.

80. Kraemer T, Maurer HH. Toxicokinetics of amphetamines: metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine, and their *N*-alkyl derivatives. Ther Drug Monit 2002;24:277–289.

81. Ensslin HK, Maurer HH, Gouzoulis E, Hermle L, Kovar KA. Metabolism of racemic 3,4-methylenedioxyethylamphetamine in humans. Isolation, identification, quantification, and synthesis of urinary metabolites. Drug Metab Dispos 1996;24:813–820.

82. Weinmann W, Bohnert M. Lethal monointoxication by overdosage of MDEA. Forensic Sci Int 1998;91:91–101.

83. Milroy CM, Clark JC, Forrest AR. Pathology of deaths associated with "ecstasy" and "Eve" misuse. J Clin Pathol 1996;49:149–153.

84. Byard RW, Rodgers NG, James RA, Kostakis C, Camilleri AM. Death and paramethoxyamphetamine—an evolving problem. Med J Aust 2002;176:496.

85. James RA, Dinan A. 2. Hyperpyrexia associated with paramethoxyamphetamine (PMA) abuse. Med Sci Law 1998;38:83–85.

86. Gouzoulis E, von Bardeleben U, Rupp A, Kovar KA, Hermle L. Neuroendocrine and cardiovascular effects of MDE in healthy volunteers. Neuropsychopharmacology 1993;8:187–193.

87. Irvine RJ, Keane M, Felgate P, McCann UD, Callaghan PD, White JM. Plasma drug concentrations and physiological measures in "dance party" participants. Neuropsychopharmacology 2006;31:424–430.

88. Schwartz RH, Miller NS. MDMA (ecstasy) and the rave: a review. Pediatrics 1997;100:705–708.

89. Gouzoulis E, Borchardt D, Hermle L. A case of toxic psychosis induced by "Eve" (3,4-methylenedioxyethylamphetamine). Arch Gen Psychiatry 1993;50:75.

90. de Letter EA, Piette MH, Lambert WE, Cordonnier JA. Amphetamines as potential inducers of fatalities: a review in the district of Gent from 1976–2004. Med Sci Law 2006;46:37–65.

91. Gouzoulis-Mayfrank E, Daumann J. Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: how strong is the evidence for persistent brain damage? Addiction 2006;101:348–361.

92. Kish SJ, Furukawa Y, Ang L, Vorce SP, Kalasinsky KS. Striatal serotonin is depleted in brain of a human MDMA (ecstasy) user. Neurology 2000;55:294–296.

93. Apollonio LG, Whittall IR, Pianca DJ, Kyd JM, Maher WA. Matrix effect and cross-reactivity of select amphetamine-type substances, designer analogues, and putrefactive amines using the Bio-Quant direct ELISA presumptive assays for amphetamine and methamphetamine. J Anal Toxicol 2007;31:208–213.

94. Pirnay SO, Abraham TT, Huestis MA. Sensitive gas chromatography-mass spectrometry method for simultaneous measurement of MDEA, MDMA, and metabolites HMA, MDA, and HMMA in human urine. Clin Chem 2006;52:1728–1734.

95. Thigpen AL, DeRuiter J, Clark CR. GC-MS studies on the regioisomeric 2,3- and 3,4-methylenedioxyphenethlamines related to MDEA, MDMMA, and MBDB. J Chromatogr Sci 2007;45:229–235.

96. Bost RO. 3,4-Methlenedioxymethamphetamine (MDMA) and other amphetamine derivatives. J Forensic Sci 1988;33:576–587.

97. Arimany J, Medallo J, Pujol A, Vingut A, Borondo JC, Valverde JL. Intentional overdose and death with 3,4-methylenedioxyethamphetamine (MDEA; "Eve"). Case report. Am J Forensic Med Pathol 1998;19:148–151.

98. Dowling GP, McDonough ET III, Bost RO. "Eve" and "ecstasy" a report of five deaths associated with the use of MDEA and MDMA. JAMA 1987;257:1615–1617.

99. Cody JT. Cross-reactivity of amphetamine analogues with Roche Abuscreen radioimmunoassay reagents. J Anal Toxicol 1990;14:50–53.

100. Cody JT, Schwarzhoff R. Fluorescence polarization immunoassay detection of amphetamine, methamphetamine, and illicit amphetamine analogues. J Anal Toxicol 1993;17:26–30.

101. Mitrevski B, Zdravkovski Z. Rapid and simple method for direct determination of several amphetamines in seized tablets by GC-FID. Forensic Sci Int 2005;152:199–203.

102. Furnari C, Ottaviano V, Rosati F, Tondi V. Identification of 3,4-methylenedioxyamphetamine analogs encountered in clandestine tablets. Forensic Sci Int 1998;92:49–58.

103. Schifano F, Corkery J, Deluca P, Oyefeso A, Ghodse AH. Ecstasy (MDMA, MDA, MDEA, MBDB) consumption, seizures, related offences, prices, dosage levels and deaths in the UK (1994–2003). J Psychopharmacol 2006;20:456–463.

104. Kintz P. Excretion of MBDB and BDB in urine, saliva, and sweat following single oral administration. J Anal Toxicol 1997;21:570–575.

105. Kraemer T, Maurer HH. Toxicokinetics of amphetamines: metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine, and their N-alkyl derivatives. Ther Drug Monit 2002;24:277–289.

106. Nagai T, Matsushima K, Suzuki A, Saotome A, Kurosu A, Nihei H, et al. Enantiomer analysis of a new street drug, 3,4-methylenedioxy-N-methyl-butanamine, in rat urine. J Anal Toxicol 2002;26:104–109.

107. Shulgin A, Shulgin A. PIHKAL. A chemical love story. Berkeley CA: Transform Press; 1991.

108. Maresova V, Chadt J, Prikryl L. Simultaneous determination of amphetamines and amphetamine-derived designer drugs in human urine by GC-MS. Neuro Endocrinol Lett 2006;27(suppl 2):S121–S124.

109. Borth S, Hansel W, Rosner P, Junge T. Synthesis of 2,3- and 3,4-methylenedioxyphenylalkylamines and their regioisomeric differentiation by mass spectral analysis using GC-MS-MS. Forensic Sci Int 2000;114:139–153.

110. Concheiro M, dos Santos Sadler Simoes S, Quintela O, de Castro A, Rodrigues Dias MJ, Cruz A, et al. Fast LC-MS/MS method for the determination of amphetamine, methamphetamine, MDA, MDMA, MDEA,

MBDB and PMA in urine. Forensic Sci Int 2007; 171:44–51.

111. Concheiro M, de Castro A, Quintela O, Lopez-Rivadulla M, Cruz A. Determination of drugs of abuse and their metabolites in human plasma by liquid chromatography-mass spectrometry an application to 156 road fatalities. J Chromatogr B 2006;832:81–89.

112. Carter N, Rutty GN, Milroy CM, Forrest AR. Deaths associated with MBDB misuse. Int J Legal Med 2000; 113:168–170.

113. Kronstrand R. Identification of *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) in urine from drug users. J Anal Toxicol 1996;20:512–516.

114. Kintz P, Giroud C. Immunoassay responses of MBDB. J Anal 1997;21:589–590.

115. Huang X, Marona-Lewicka D, Nichols DE. *p*-Methylthioamphetamine is a potent new non-neurotoxic serotonin-releasing agent. Eur J Pharmacol 1992;229: 31–38.

116. Winstock AR, Wolff K, Ramsey J. 4-MTA. a new synthetic drug on the dance scene. Drug Alcohol Depend 2002;67:111–115.

117. Poortman AJ, Lock E. Analytical profile of 4-methylthioamphetamine (4-MTA), a new street drug. Forensic Sci Int 1999;100:221–233.

118. Kirkbride KP, Ward AD, Jenkins NF, Klass G, Coumbaros JC. Synthesis of 4-methyl-5-arylpyrimidines and 4-aryl-pyrimidines: route specific markers for the Leuckart preparation of amphetamine, 4-methoxyamphetamine, and 4-methylthioamphetamine. Forensic Sci Int 2001;115: 53–67.

119. Blachut D, Wojtasiewicz K, CCzarnocki Z, Szukalski B. The analytical profile of some 4-methylthioamphetamine (4-MtA) homologues. Forensic Sci Int 2009;192:98–114.

120. Groombridge C. The identification of 4-methylthioamphetamine in a drug seizure. Microgram 1998;31: 150–159.

121. Carmo H, Hengstler JG, de Boer D, Ringel M, Carvalho F, Fernandes E, et al. Comparative metabolism of the designer drug 4-methylthioamphetamine by hepatocytes from man, monkey, dog, rabbit, rat and mouse. Naunyn Schmiedebergs Arch Pharmacol 2004;369:198–205.

122. Carmo H, Brulport M, Hermes M, Oesch F, de Boer D, Remiao F, et al. CYP2D6 increases toxicity of the designer drug 4-methylthioamphetamine (4-MTA). Toxicology 2007;229:236–244.

123. Elliott SP. Analysis of 4-methylthioamphetamine in clinical specimens. Ann Clin Biochem 2001;38:339–347.

124. Scorza MC, Carrau C, Silveira R, Zapata-Torres G, Cassels BK, Reyes-Parada M. Monoamine oxidase inhibitory properties of some methoxylated and alkylthio amphetamine derivatives: structure-activity relationships. Biochem Pharmacol 1997;54:1361–1369.

125. Murphy J, Flynn JJ, Cannon DM, Guiry PJ, McCormack P, Baird AW, et al. *In vitro* neuronal and vascular responses to 5-hydroxytryptamine: modulation by 4-methylthioamphetamine, 4-methylthiomethamphetamine and 3,4-methylenedioxymethamphetamine. Eur J Pharmacol 2002;444:61–67.

126. Li Q, Murakami I, Stall S, Levy AD, Brownfield MS, Nichols DE, Van de Kar LD. Neuroendocrine pharmacology of three serotonin releasers: 1-(1, 3-benzodioxol-5-yl)-2-(methylamino)butane (MBDB),5-methoxy-6-methyl-2-aminoindan (MMAi) and *p*-methylthioamphetamine (MTA). J Pharmacol Exp Ther 1996; 279:1261–1267.

127. Carmo H, Remião F, Carvalho F, Fernandes E, de Boer D, dos Reys LA, de Lourdes Bastos M. 4-Methylthioamphetamine-induced hyperthermia in mice: influence of serotonergic and catecholaminergic pathways. Toxicol Appl Pharmacol 2003;190:262–271.

128. Decaestecker T, De Letter E, Clauwaert K, Bouche MP, Lambert W, Van Bocxlaer J, et al. Fatal 4-MTA intoxication: development of a liquid chromatographic-tandem mass spectrometric assay for multiple matrices. J Anal Toxicol 2001;25:705–710.

129. De Letter EA, Coopman VA, Cordonnier JA, Piette MH. One fatal and seven non-fatal cases of 4-methylthioamphetamine (4-MTA) intoxication: clinico-pathological findings. Int J Legal Med 2001;114:352–356.

130. Wohlfarth A, Weinmann W, Dresen S. LC-MS/MS screening method for designer amphetamines, tryptamines, and piperazines in serum. Anal Bioanal Chem 2010;396: 2403–2414.

131. Elliott SP. Fatal poisoning with a new phenylethylamine: 4-methylthioamphetamine (4-MTA). J Anal Toxicol 2000;24:85–89.

132. Ewald AH, Peters FT, Weise M, Maurer H. Studies on the metabolism and toxicological detection of the designer drug 4-methylthioamphetamine (4-MTA) in human urine using gas chromatography-mass spectrometry. J Chromatogr B 2005;824:123–131.

133. Kraner JC, McCoy DJ, Evans MA, Evans LE, Sweeney BJ. Fatalities caused by the MDMA-related drug paramethoxyamphetamine (PMA). J Anal Toxicol 2001; 25:645–648.

134. Voorspoels S, Coucke V, Covaci A, Maervoet J, Schepens P, De Meyere C, Jacobs W. Resurgence of a lethal drug: paramethoxyamphetamine deaths in Belgium. J Toxicol Clin Toxicol 2002;40:203–204.

135. Caldicott DG, Edwards NA, Kruys A, Kirkbride KP, Sims DN, Byard RW, et al. Dancing with "death": *p*-methoxyamphetamine overdose and its acute management. J Toxicol Clin Toxicol 2003;41:143–154.

136. Cole JC, Bailey M, Sumnall HR, Wagstaff GF, King LA. The content of ecstasy tablets: implications for the study of their long-term effects. Addiction 2002;97:1531–1536.

137. Felgate HE, Felgate PD, James RA, Sims DN, Vozzo DC. Recent paramethoxyamphetamine deaths. J Anal Toxicol 1998;22:169–172.

138. Ling LH, Marchant C, Buckley NA, Prior M, Irvine RJ. Poisoning with the recreational drug paramethoxyamphetamine ("death"). Med J Aust 2001;174:453–455.

139. Cimbura G. PMA deaths in Ontario. Can Med Assoc J 1974;110:1263–1267.

140. Refstad S. Paramethoxyamphetamine (PMA) poisoning; a "party drug" with lethal effects. Acta Anaesthesiol Scand 2003;47:1298–1299.

141. Johansen SS, Hansen AC, Muller IB, Lundemose JB, Franzmann MB. Three fatal cases of PMA and PMMA poisoning in Denmark. J Anal Toxicol 2003;27:253–256.

142. Waumans D, Bruneel N, Tytgat J. Anise oil as paramethoxyamphetamine (PMA) precursor. Forensic Sci Int 2003;133:159–170.

143. Blachut D, Wojtasiewicz K, Czarnocki Z. Identification and synthesis of some contaminants present in 4-methoxyamphetamine (PMA) prepared by the Leuckart method. Forensic Sci Int 2002;127:45–62.

144. Błachut D, Wojtasiewicz K, Czarnocki Z. Some pyridine derivatives as "route-specific markers" in 4-methoxyamphetamine (PMA) prepared by the Leuckart Method Studies on the role of the aminating agent in their distribution in the final product. Forensic Sci Int 2005;152: 157–173.

145. Kochana J, Wilamowski J, Parczewski A, Surma M. Synthesis of standards of the most important markers of Leuckart p-methoxymethamphetamine (PMMA) examination of the influence of experimental conditions and a drug diluent on SPE/TLC profiling. Forensic Sci Int 2003;134:207–213.

146. Martin TL. Three cases of fatal paramethoxyamphetamine overdose. J Anal Toxicol 2001;25:649–651.

147. Nichols DE. Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. J Psychoactive Drugs 1986;18:305–313.

148. Glennon RA, Ismaiel AE, Martin B, Poff D, Sutton M. A preliminary behavioral investigation of PMMA, the 4-methoxy analog of methamphetamine. Pharmacol Biochem Behav 1988;31:9–13.

149. Paton DM, Bell JI, Yee R, Cook DA. Pharmacology and toxicity of 3,4-methylenedioxyamphetamine, paramethoxyamphetamine and related dimethoxyamphetamines. Proc West Pharmacol Soc 1975;18:229–231.

150. Wu D, Otton SV, Inaba T, Kalow W, Sellers EM. Interactions of amphetamine analogs with human liver CYP2D6. Biochem Pharmacol 1997;53:1605–1612.

151. Bach MV, Coutts RT, Baker GB. Involvement of CYP2D6 in the in vitro metabolism of amphetamine, two N-alkylamphetamines and their 4-methoxylated derivatives. Xenobiotica 1999;29:719–732.

152. Kitchen I, Tremblay J, André J, Dring LG, Idle JR, Smith RL, Williams RT. Interindividual and interspecies variation in the metabolism of the hallucinogen 4-methoxyamphetamine. Xenobiotica 1979;9:397–404.

153. Kaminskas LM, Irvine RJ, Callaghan PD, White JM, Kirkbride P. The contribution of the metabolite p-hydroxyamphetamine to the central actions of p-methoxyamphetamine. Psychopharmacology (Berl) 2002;160:155–160.

154. Staack RF, Theobald DS, Paul LD, Springer D, Kraemer T, Maurer HH. Identification of human cytochrome P450 2D6 as major enzyme involved in the O-demethylation of the designer drug p-methoxymethamphetamine. Drug Metab Dispos 2004;32:379–381.

155. Zaitsu K, Katagi M, Kamata T, Kamata H, Shima N, Tsuchihashi H, et al. Determination of a newly encountered designer drug "p-methoxyethylamphetamine" and its metabolites in human urine and blood. Forensic Sci Int 2008;177:77–84.

156. Ketabi-Kiyanvash N, Weiss J, Haefeli WE, Mikus G. P-glycoprotein modulation by the designer drugs methylenedioxymethamphetamine, methylenedioxyethylamphetamine and paramethoxyamphetamine. Addict Biol 2003;8:413–418.

157. Menon MK, Tseng LF, Loh HH. Pharmacological evidence for the central serotonergic effects of monomethoxyamphetamines. J Pharmacol Exp Ther 1976; 197:272–279.

158. Daws LC, Irvine RJ, Callaghan PD, Toop NP, White JM, Bochner F. Differential behavioral and neurochemical effects of para-methoxyamphetamine and 3,4-methylenedioxymethamphetamine in the rat. Prog Neuropsychopharmacol Biol Psychiatry 2000;24: 955–977.

159. Green AL, El Hait MA. p-Methoxyamphetamine, a potent reversible inhibitor of type-A monoamine oxidase in vitro and in vivo. J Pharm Pharmacol 1980;32: 262–266.

160. Steele TD, Katz JL, Ricaurte GA. Evaluation of the neurotoxicity of N-methyl-1-(4-methoxyphenyl)-2-aminopropane (para-methoxymethamphetamine, PMMA). Brain Res 1992;589:349–352.

161. Byard RW, James RA, Gilbert JD, Felgate PD. Another PMA-related fatality in Adelaide. Med J Aust 1999; 170:139–140.

162. Mortier KA, Dams R, Lambert WE, De Letter EA, Van Calenbergh S, De Leenheer AP. Determination of para-methoxyamphetamine and other amphetamine-related designer drugs by liquid chromatography/sonic spray ionization mass spectrometry. Rapid Commun Mass Spectrom 2002;16:865–870.

163. Nieddu M, Boatto G, Sini L, Dessi G. Determination of p-methoxyamphetamine by capillary electrophoresis with diode array detection from urine and plasma samples. J Liquid Chromatogr Rel Technol 2007;30: 431–438.

164. Coumbaros JC, Kirkbride KP, Klass G. Application of solid-phase microextraction to the profiling of an illicit drug: manufacturing impurities in illicit 4-methoxyamphetamine. J Forensic Sci 1999;44: 1237–1242.

165. Lin D-L, Liu H-C, Yin H-L. Recent paramethoxymethamphetamine (PMMA) deaths in Taiwan. J Anal Toxicol 2007;31:109–113.

166. Becker J, Neis P, Rohrich J, Zorntlein S. A fatal parame-thoxymethamphetamine intoxication. Leg Med 2003;5 (Suppl 1):S138–S141.

167. Cassels BK, Gomez-Jeria JS. A reevaluation of psychoto-mimetic amphetamine derivatives in humans. J Psychoactive Drugs 1985;17:129–130.

168. Shaler RC, Padden JJ. Identification of hallucinogens in illicit seizures I: 2,5-dimethoxyamphetamine. J Pharm Sci 1972;61:1851–1855.

169. Ovaska H, Viljoen A, Puchnarewicz M, Button J, Ramsey J, Holt DW, et al. First case report of recreational use of 2,5-dimethoxy-4-chloroamphetamine confirmed by toxi-cological screening. Eur J Emerg Med 2008;15:354–356.

170. Ewald AH, Maurer HH. 2,5-Dimethoxyamphetamine-derived designer drugs: Studies on the identification of cytochrome P450 (CYP) isoenzymes involved in forma-tion of their main metabolites and on their capability to inhibit CYP2D6. Toxicol Lett 2008;183:52–57.

171. Maher HM, Awad T, DeRuiter J, Clark CR. GC-MS and GC-IRD studies on dimethoxyamphetamines (DMA): regioisomers related to 2,5-DMA. Forensic Sci Int 2009; 192:115–125.

172. Ragan FA Jr, Hite SA, Samuels MS, Garey RE. 4-Bromo-2,5-dimethoxyphenethylamine: identification of anew street drug. J Anal Toxicol 1985;9:91–93.

173. Buhrich N, Morris G, Cook G. Bromo-DMA: the Australasian hallucinogen? Aust N Z J Psychiatry 1983; 17:275–279.

174. Shulgin AT, Sargent T, Naranjo C. 4-Bromo-2,5-dimethoxyphenylisopropylamine, a new centrally active amphetamine analog. Pharmacology 1971;5:103–107.

175. Berankova K, Balikova M. Study on metabolites of 2,5-dimethoxy-4-bromamphetamine (DOB) in human urine using gas chromatography-mass spectrometry. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2005;149:465–468.

176. Ewald AH, Maurer HH. 2,5-dimethoxyamphetamine-derived designer drugs: studies on the identification of cytochrome P450 (CYP) isoenzymes involved in forma-tion of their main metabolites and on their capability to inhibit CYP2D6. Toxicol Lett 2008;182:52–57.

177. Winek CL, Collom WD, Bricker JD. A death due to 4-bromo-2,5-dimethoxyamphetamine. Clin Toxicol 1981; 18:267–271.

178. Bowen JS, Davis GB, Kearney TE, Bardin J. Diffuse vas-cular spasm associated with 4-bromo-2,5-dimethoxyam-phetamine ingestion. JAMA 1983;249:1477–1479.

179. Ovaska H, Viljoen A, Puchnarewicz M, Button J, Ramsey J, Holt DW, et al. First case report of recreational use of 2,5-dimethoxy-4-chloroamphetamine confirmed by toxi-cological screening. Eur J Emerg Med 2008;15:354–356.

180. da Costa JL, Wang AY, Micke GA, Maldaner AO, Romano RL, Martins-Junior HA, et al. Chemical identi-fication of 2,5-dimethoxy-4-bromoamphetamine (DOB). Forensic Sci Int 2007;173:130–136.

181. Balikova M. Nonfatal and fatal DOB (2,5-dimethoxy-4-bromoamphetamine) overdose. Forensic Sci Int 2005; 153:85–91.

182. Shulgin AT, Sargent T, Naranjo C. Structure–activity rela-tionships of one-ring psychotomimetics. Nature 1969; 221:537–541.

183. Faillace LA, Snyder SH, Weingartner H. 2,5-Dimethoxy-4-methylampheamine: clinical evaluation of a new hal-lucinogenic drug. J Nerv Ment Dis 1970;15:119–126.

184. Snyder SH, Faillace LA, Weingartner H. DOM (STP), a new hallucinogenic drug, and DOET: effects in normal subjects. Am J Psychiatry 1968;125:113–120.

185. Snyder SH, Faillace L, Hollister L. 2,5-dimethoxy-4-methyl-amphetamine (STP): a new hallucinogenic drug. Science 1967;158:669–670.

186. Angrist B, Rotrosen J, Gershon S. Assessment of tolerance to the hallucinogenic effects of DOM. Psychopharmacologia 1974;36:203–207.

187. Snyder SH, Faillace LA, Weingartner H. DOM (STP), a new hallucinogenic drug, and DOET: effects in normal subjects. Am J Psychiatry 1968:125:357–364.

188. Snyder SH, Weingartner H, Faillace LA. DOET (2,5-dimethoxy-4-ethylamphetamine), a new psychotro-pic drug. Effects of varying doses in man. Arch Gen Psychiatry 1971;24:50–55.

189. Snyder SH, Faillace LA, Weingartner H. A new psycho-tropic agent. Psychological and physiological effects of 2,5-dimethoxy-4-ethyl amphetamine (DOET) in man. Arch Gen Psychiatry 1969;21:95–101.

190. Shulgin AT, Carter MF. Centrally active phenethylamines. Psychopharmacol Commun 1975;1:93–98.

191. Ragan FA Jr, Hite SA, Samuels MS, Garey RE. 4-Bromo-2,5-dimethoxyphenethylamine: identification of a new street drug. J Anal Toxicol 1985;9:91–93.

192. Giroud C, Augsburger M, Rivier L, Mangin P, Sadeghipour F, Varesio E, et al. 2C-B: a new psychoactive phenylethyl-amine recently discovered in ecstasy tablets sold on the Swiss black market. J Anal Toxicol 1998;22:345–354.

193. de Boer D, Bosman I. A new trend in drugs-of-abuse; the 2C-series of phenethylamine designer drugs. Pharm World Sci 2004;26:110–113.

194. Maurer HH. Chemistry, pharmacology, and metabolism of emerging drugs of abuse. Ther Drug Monit 2010;32: 544–549.

195. Theobald DS, Maurer HH. Identification of monoamine oxidase and cytochrome P450 isoenzymes involved in the deamination of phenethylamine-derived designer drugs (2C-series). Biochem Pharmacol 2007;73:287–297.

196. Carmo H, Hengstler JG, de Boer D, Ringel M, Remião F, Carvalho F, et al. Metabolic pathways of 4-bromo-2,5-dimethoxyphenethylamine (2C-B): analysis of phase I metabolism with hepatocytes of six species including human. Toxicology 2005;206:75–89.

197. de Boer D, Reys LA, Pylon N, Gijzels M, Bosman IJ, Maes RA. Preliminary results on the urinary excretion

of 2C-B (4-bromo-2,5-dimethoxyphenethylamine) and its metabolites in humans. Br J Pharmacol 1999;127: 41.

198. Rohanova M, Palenicek T, Balikova M. Deposition of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and its metabolite 4-bromo-2-hydroxy-5-methoxyphenethyl-amine in rats after subcutaneous administration. Toxicol Lett 2008;178:29–36.

199. Villalobos CA, Bull P, Sáez P, Cassels BK, Huidobro-Toro JP. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) and structurally related phenylethylamines are potent 5-HT2A receptor antagonists in *Xenopus laevis* oocytes. Br J Pharmacol 2004;141:1167–1174.

200. Glennon RA, Titeler M, Lyon RA. A preliminary investigation of the psychoactive agent 4-bromo-2,5-dimethoxyphenethylamine: a potential drug of abuse. Pharmacol Biochem Behav 1988;30:597–601.

201. Pichini S, Pujadas M, Marchei E, Pellegrini M, Fiz J, Pacifici R, et al. Liquid chromatography-atmospheric pressure ionization electrospray mass spectrometry determination of "hallucinogenic designer drugs" in urine of consumers. J Pharm Biomed Anal 2008;47: 335–342.

202. Boatto G, Nieddu M, Dessì G, Manconi P, Cerri R. Determination of four thiophenethylamine designer drugs (2C-T-series) in human plasma by capillary electrophoresis with mass spectrometry detection. J Chromatogr A 2007;1159:198–202.

203. Soares ME, Carvalho M, Carmo H, Remião F, Carvalho F, Bastos ML. Simultaneous determination of amphetamine derivatives in human urine after SPE extraction and HPLC-UV analysis. Biomed Chromatogr 2004;18: 125–131.

204. Habrdova V, Peters FT, Theobald DS, Maurer HH. Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. J Mass Spectrom 2005;40:785–795.

205. Theobald DS, Staack RF, Puetz M, Maurer HH. New designer drug 2,5-dimethoxy-4-ethylthio-β-phenethylamine (2C-T-2): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. J Mass Spectrom 2005;40:1157–1172.

206. Parker MA, Marona-Lewicka D, Lucaites VL, Nelson DL, Nichols DE. A novel (benzodifuranyl)aminoalkane with extremely potent activity at the 5-HT2A receptor. J Med Chem 1998;41:5148–5149.

207. Chambers JJ, Kurrasch-Orbaugh DM, Parker MA, Nichols DE. Enantiospecific synthesis and pharmacological evaluation of a series of super-potent, conformationally restricted 5-HT(2A/2C) receptor agonists. J Med Chem 2001;44:1003–1010.

208. Wood DM, Looker JJ, Shaikh L, Buttton J, Puchnarewicz M, Davies S, et al. Delayed onset of seizures and toxicity associated with recreational use of bromo-dragonfly. J Med Toxicol 2009;5:226–229.

209. Andreasen MF, Telving R, Birkler RI, Schumacher B, Johannsen M. A fatal poisoning involving bromo-dragonfly. Forensic Sci Int 2009;183:91–96.

210. Kauppila TJ, Arvola V, Haapala M, Pol J, Aalberg L, Saarela V, et al. Direct analysis of illicit drugs by desorption atmospheric pressure photoionization. Rapid Commun Mass Spectrom 2008;22:979–985.

211. Campbell H, Cline W, Evans M, Lloyd J, Peck AW. Comparison of the effects of dexamphetamine and 1-benzylpiperazine in former addicts. Eur J Clin Pharmacol 1973;6:170–176.

212. de Boer D, Bosman IJ, Hidvégi E, Manzoni C, Benkö AA, dos Reys LJ, Maes RA. Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. Forensic Sci Int 2001;121:47–56.

213. Wikström M, Holmgren P, Ahlner J. A2 (*N*-benzylpiperazine) a new drug of abuse in Sweden. J Anal Toxicol 2004;28:67–70.

214. Johnstone AC, Lea RA, Brennan KA, Schenk S, Kennedy MA, Fitzmaurice PS. Benzylpiperazine: a drug of abuse? J Psychopharmacol 2007;21:888–894.

215. Green SL, Kerr F, Braitberg G. Review article: amphetamines and related drugs of abuse. Emerg Med Australasia 2008;20:391–402.

216. Staack RF. Piperazine designer drugs of abuse. Lancet 2007;369:1411–1413.

217. Gee P, Gilbert M, Richardson S, Moore G, Paterson S, Graham P. Toxicity from the recreational use of 1-benzylpiperazine. Clin Toxicol 2008;46:802–807.

218. Antia U, Lee HS, Kydd RR, Tingle MD, Russell BR. Pharmacokinetics of "party pill" drug *N*-benzylpiperazine (BZP) in healthy human participants. Forensic Sci Int 2009;186:63–67.

219. Bye C, Munro-Faure AD, Peck AW, Young PA. A comparison of the effects of 1-benzylpiperazine and dexamphetamine on human performance tests. Eur J Clin Pharmacol 1973;6:163–169.

220. Tekes K, Tóthfalusi L, Malomvölgyi B, Hermán F, Magyar K. Studies on the biochemical mode of action of EGYT-475, a new antidepressant. Pol J Pharmacol Pharm 1987; 39:203–211.

221. Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, Rothman RB. Effects of "Legal X" piperazine analogs on dopamine and serotonin release in rat brain. Ann N Y Acad Sci 2004;1025:189–197.

222. Baumann MH, Clark RD, Budzynski AG, Partilla JS, Blough BE, Rothman RB. *N*-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, or "Ecstasy"). Neuropsychopharmacology 2005;30:550–560.

223. Gee P, Jerram T, Bowie. Multiorgan failure from 1-benzylpiperazine ingestion—legal high or lethal high? Clin Toxicol 2010;48:230–233.

224. Wilkins C, Girling M, Sweetsur P. The prevalence of use, dependency, and harms of legal "party pills" containing benzylpiperazine (BZP) and trifluorophenylmethylpi-

perazine (TFMPP) in New Zealand. J Subst Use 2007; 12:213–224.

225. Gee P, Richardson S, Woltersdorf W, Moore G. Toxic effects of BZP-based herbal party pills in humans: a prospective study in Christchurch, New Zealand. N Z Med J 2005;118(1227):U1784.

226. Balmelli C, Kupferschmidt H, Rentsch K, Schneemann M. [Fatal brain edema after ingestion of ecstasy and benzylpiperazine]. Dtsch Med Wochenschr 2001;126:809-811. [German]

227. Peters FT, Martinez-Ramirez JA. Analytical toxicology of emerging drugs of abuse. Ther Drug Monit 2010; 32:532–539.

228. Wikstrom M, Holmgren P, Ahlner J. A2 (N-benzylpiperazine) a new drug of abuse in Sweden. J Anal Toxicol 2004;28:67–70.

229. Peters FT, Schaefer S, Staack RF, Kraemer T, Maurer HH. Screening for and validated quantification of amphetamines and of amphetamine- and piperazine-derived designer drugs in human blood plasma by gas chromatography/mass spectrometry. J Mass Spectrom 2003;38:659–676.

230. Bishop SC, McCord BR, Gratz SR, Loeliger JR, Witkowski MR. Simultaneous separation of different types of amphetamine and piperazine designer drugs by capillary electrophoresis with a chiral selector. J Forensic Sci 2005;50:326–335.

231. Elliott S, Smith C. Investigation of the first deaths in the United Kingdom involving the detection and quantitation of the piperazines BZP and 3-TFMPP. J Anal Toxicol 2008;32:172–177.

232. Tsutsumi H, Katagi M, Miki A, Shima N, Kamata T, Nishikawa M, et al. Development of simultaneous gas chromatography-mass spectrometric and liquid chromatography-electrospray ionization mass spectrometric determination method for the new designer drugs, N-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl) piperazine (TFMPP) and their main metabolites in urine. J Chromatogr B Analyt Technol Biomed Life Sci 2005;819:315–322.

233. Barroso M, Costa S, Dias M, Vieira DN, Queiroz JA, Lopez-Rivadulla M. Analysis of phenylpiperazine-like stimulants in human hair as trimethylsilyl derivatives by gas chromatography-mass spectrometry. J Chromatogr A 2010;1217:6274–6280.

234. Wood DM, Dargan PI, Holt DW, Ramsey J, Jones AL. Collapse, reported seizure—and an unexpected pill. Lancet 2007;369:1490–1491.

235. Koveleva J, Devuyst E, De Paepe P, Verstraete A. Acute chlorophenylpiperazine overdose: a case report and review of the literature. Ther Drug Monit 2008;30: 394–398.

236. Stein DJ, Hollander E, DeCaria CM, Simeon D, Cohen L, Aronowitz B. m-Chlorophenylpiperazine challenge in borderline personality disorder: relationship of neuroendocrine response, behavioral response, and clinical measures. Biol Psychiatry 1996;40:508–513.

237. Klaassen T, Ho Pian KL, Westenberg HG, den Boer JA, van Praag HM. Serotonin syndrome after challenge with the 5-HT agonist meta-chlorophenylpiperazine. Psychiatry Res 1998;79:207–212.

238. Gijsman HJ, Van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, et al. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. J Clin Psychopharmacol 1998;18:289–295.

239. Feuchtl A, Bagli M, Stephan R, Frahnert C, Kölsch H, Kühn KU, Rao ML. Pharmacokinetics of m-chlorophenylpiperazine after intravenous and oral administration in healthy male volunteers: implication for the pharmacodynamic profile. Pharmacopsychiatry 2004;37:180–188.

240. Mercolini L, Colliva C, Amore M, Fanali S, Raggi MA. HPLC analysis of the antidepressant trazodone and its main metabolite m-CPP in human plasma. J Pharm Biomed Anal 2008;47:882–887.

241. Mayol RF, Cole CA, Luke GM, Colson KL, Kerns EH. Characterization of the metabolites of the antidepressant drug nefazodone in human urine and plasma. Drug Metab Dispos 1994;22:304–311.

242. Maes M, Westenberg H, Vandoolaeghe E, Demedts P, Wauters A, Neels H, Meltzer HY. Effects of trazodone and fluoxetine in the treatment of major depression: therapeutic pharmacokinetic and pharmacodynamic interactions through formation of meta-chlorophenylpiperazine. J Clin Psychopharmacol 1997;17: 358–364.

243. Robertson DW, Bloomquist W, Wong DT, Cohen ML. mCPP but not TFMPP is an antagonist at cardiac 5HT3 receptors. Life Sci 1992;50:599–605.

244. Mercolini L, Colliva C, Amore M, Fanali S, Raggi MA. HPLC analysis of the antidepressant trazodone and its main metabolite m-CPP in human plasma. J Pharm Biomed Anal 2008;47:882–887.

245. Hashimoto K. Effects of benzylpiperazine derivatives on the acute effects of 3,4-methylenedioxymethamphetamine in rat brain. Neurosci Lett 1993;152:17–20.

246. Maurer HH, Kraemer T, Springer D, Staack RF. Chemistry, pharmacology, toxicology, and hepatic metabolism of designer drugs of the amphetamine (ecstasy), piperazine, and pyrrolidinophenone types. A synopsis. Ther Drug Monit 2004;26:127–131.

247. Staack RF, Maurer HH. New designer drug 1-(3,4-methylenedioxybenzyl) piperazine (MDBP): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. J Mass Spectrom 2004;39:255–261.

248. Staack RF, Theobald DS, Paul LD, Springer D, Kraemer T, Maurer HH. In vivo metabolism of the new designer drug 1-(4-methoxyphenyl)piperazine (MeOPP) in rat and identification of the human cytochrome P450 enzymes responsible for the major metabolic step. Xenobiotica 2004;34:179–192.

249. Staack RF, Maurer HH. Toxicological detection of the new designer drug 1-(4-methoxyphenyl)piperazine and its metabolites in urine and differentiation from an intake of structurally related medicaments using gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003;798:333–342.

250. Drug Enforcement Administration. Schedules of controlled substances; placement of 2,5-dimethoxy-4-(n)-propyl-thiophenethylamine and *N*-benzylpiperazine into schedule I of the Controlled Substances Act. Final rule. Fed Regist 2004;69:12794–12797.

251. Nicholson TC. Prevalence of use, epidemiology and toxicity of "herbal party pills" among those presenting to the emergency department. Emerg Med Australas 2006;18: 180–184.

252. Lecompte Y, Roussel O, Perrin M. [1-Benzylpiperazine (BZP) and 1-(3-trifluorométhylphényl)pipérazine (TFMPP): emergence of two agents which lead to misuse]. Ann Pharm Fr 2008 Mar;66:85–91. [French]

253. Staack RF, Fritschi G, Maurer HH. New designer drug 1-(3-trifluoromethylphenyl) piperazine (TFMPP): gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry studies on its phase I and II metabolism and on its toxicological detection in rat urine. J Mass Spectrom 2003;38:971–981.

254. Antia U. "Party pill" drugs – BZP and TFMPP. N Z Med J 2009;122:55–68.

255. Tsutsumi H, Katagi M, Miki A, Shima N, Kamata T, Nakajima K, et al. Isolation, identification and excretion profile of the principal urinary metabolite of the recently banned designer drug 1-(3-trifluoromethylphenyl)piperazine (TFMPP) in rats. Xenobiotica 2005;35:107–116.

256. Staack RF, Paul LD, Springer D, Kraemer T, Maurer HH. Cytochrome P450 dependent metabolism of the new designer drug 1-(3-trifluoromethylphenyl)piperazine (TFMPP). *In vivo* studies in Wistar and Dark Agouti rats as well as *in vitro* studies in human liver microsomes. Biochem Pharmacol 2004;67:235–244.

257. Antia U, Tingle MD, Russell BR. Metabolic interactions with piperazine-based "party pill" drugs. J Pharm Pharmacol 2009;61:877–882.

258. Murphy DL, Lesch KP, Aulakh CS, Pigott TA. Serotonin-selective arylpiperazines with neuroendocrine, behavioral, temperature, and cardiovascular effects in humans. Pharmacol Rev 1991;43:527–552.

259. Herndon JL, Pierson ME, Glennon RA. Mechanistic investigation of the stimulus properties of 1-(3-trifluoromethylphenyl)piperazine. Pharmacol Biochem Behav 1992;43:739–48.

260. Wood DM, Button J, Lidder S, Ramsey J, Holt DW, Dargan PI. Dissociative and sympathomimetic toxicity associated with recreational use of 1-(3-trifluoromethyl-phenyl)piperazine (TFMPP) and 1-benzylpiperazine (BZP). J Med Toxicol 2008;4:254–257.

261. Bishop SC, McCord BR, Gratz SR, Loeliger JR, Witkowski MR. Simultaneous separation of different types of amphetamine and piperazine designer drugs by capillary electrophoresis with a chiral selector. J Forensic Sci 2005;50:326–335.

262. Vorce SP, Holler JM, Levine B, Past MR. Detection of 1-benzylpiperazine and 1-(3-trifluoromethylphenyl)-piperazine in urine analysis specimens using GC-MS and LC-ESI-MS. J Anal Toxicol 2008;32:444–450.

263. Springer D, Staack RF, Paul LD, Kraemer T, Maurer HH. Identification of cytochrome P450 enzymes involved in the metabolism of 4′-methoxy-alpha-pyrrolidinopropio-phenone (MOPPP), a designer drug, in human liver microsomes. Xenobiotica 2003;33:989–998.

264. Springer D, Fritschi G, Maurer HH. Metabolism of the new designer drug α-pyrrolidinopropiophenone (PPP) and the toxicological detection of PPP and 4′methyl-α-pyrrolidinopropiophenone (MPPP) studied in rat urine using gas chromatography-mass spectrometry. J Chromatogr B 2003;796:253–266.

265. Springer D, Fritschi G, Maurer HH. Metabolism and toxicological detection of the new designer drug 3′,4′-methylenedioxy-α-pyrrolidinopropiophenone studied in urine using gas chromatography-mass spectrometry. J Chromatogr 2003;793:377–388.

Chapter 11

TRYPTAMINE DESIGNER DRUGS

N,*N*-DIETHYL-TRYPTAMINE (DET)

N,*N*-Diethyltryptamine (DET) produces psychedelic effects similar to dimethyltryptamine (DMT) or mescaline. In contrast to DMT, DET is a synthetic substance that has not been found in the plant kingdom. *In vitro* studies indicate that DET significantly inhibits monoamine oxidase ($I_{50} = 5 \times 10^{-3}$M).[1] Rodent studies suggest that DET is about one-half as potent as DMT following intraperitoneal administration as measured by alteration of behavior.[2] The intravenous (IV) injection of 10 mg DET/kg into rodents was associated with clonic convulsions.[3] DET undergoes 6-hydroxylation to 6-hydroxy-*N*,*N*-diethyltryptamine (6-HDET) and *N*-deethylation to *N*-ethyltryptamine and indole-3-acetic acid (free and conjugated 3-IAA). Human studies suggest that elimination of 6-HDET and 3-IAA in the urine account for <20% of the administered dose of DET.[4] In a study of volunteers, the administration of 1 mg DET/kg was associated with elevated blood pressure, mydriasis, disinhibition, and visual distortions.[4] Adverse effects included anxiety, tremors, nausea, vomiting, and a feeling of unpleasantness. The intramuscular administration of 0.65 to 0.85 mg DET/kg to normal test participants produced the onset of vertigo, mydriasis, nausea, sweating, tremor, and elevated heart rate and blood pressure within 8 to 15 minutes.[3] Within 30 minutes after injection, elation, distortions of time and vision, loosening of associations, and a feeling of intoxication replaced earlier effects. These effects resolved within 3 hours. Some volunteers experienced unpleasant after-effects (e.g., mild fatigue, headache, depres-

sion, insomnia). These adverse effects resolved within 24 hours. Treatment of DET intoxication is supportive, similar to the treatment of 5-methoxy-diisopropyltryptamine (5-MeO-DIPT; Foxy) intoxication.

5-METHOXY-*N*,*N*-DIISOPROPYL-TRYPTAMINE (FOXY)

Shulgin and Carter synthesized 5-methoxy-diisopropyltryptamine (5-MeO-DIPT) in 1980.[5] Their report documented the hallucinogenic properties of this compound following oral administration, similar to *N*,*N*-dimethyltryptamine (DMT). Reports of the abuse of this hallucinogen appeared in the late 1990s and early 2000s.[6] In 2003, this compound was amended into schedule I of the US Controlled Substances Act; Japan banned the use of 5-MeO-DIPT in 2005.

IDENTIFYING CHARACTERISTICS

5-Methoxy-diisopropyltryptamine is one of several designer tryptamine compounds that also include 5-methoxy-α-methyltryptamine, α-methyltryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine, *N*,*N*-diethyltryptamine, and *N*,*N*-dipropyltryptamine. 5-MeO-DIPT is a potent hallucinogen that is structurally related to DMT and bufotenine (5-hydroxy-*N*,*N*-dimethyltryptamine, CAS RN: 487-93-4). Popular street names for this synthetic substance include Foxy, Foxy Methoxy, and 5MEO. Synthesis of 5-MeO-DIPT involves the

conversion of 5-methoxy indole via the corresponding indole-3-glyoxylamide. All these compounds are agonists for serotonin 5-HT$_2$ receptors; their hallucinogenic properties depend on their affinity for these receptors.

DOSE EFFECT

The administration of oral doses of up to 6–10 mg 5-MeO-DIPT (Foxy) to healthy volunteers produced relaxation, mild auditory illusions (i.e., lowering of tone), and talkativeness that peaked about 1–1.5 hours after administration and resolved within 4 hours.[5] Adverse effects included nausea, vomiting, diarrhea, muscle spasms, muscle hyperactivity, and agitation. The intense sensory disturbances commonly associated with dimethyltryptamine and psilocybin use were absent.

TOXICOKINETICS

The relatively good oral bioavailability of 5-methoxy-diisopropyltryptamine is unusual among tryptamine compounds because of the resistance of this drug to degradation by gastrointestinal monoamine oxygenase.[5] Figure 11.1 displays the metabolic pathways of 5-methoxy-diisopropyltryptamine biotransformation. As suggested by the analysis of urine from volunteers

ingesting 5-MeO-DIPT, the major metabolites probably result from demethylation (5-hydroxy-diisopropyltryptamine, 5-OH-DIPT) and hydroxylation (6-hydroxy-5-methoxy-N,N-diisopropyltryptamine, 6-OH-5-MeO-DIPT).[7,8] Side chain degradation by N-deisopropylation to the corresponding secondary amine (5-methoxy-N-isopropyltryptamine, 5-MeO-NIPT) is probably a minor pathway, and the formation of the N-oxide is minimal. Presumed metabolites of 5-methoxy-diisopropyltryptamine include 5-methoxy-isopropyltryptamine, 5-methoxy-isopropyltryptamine-N-oxide, 5-methoxy-indole acetic acid, and 5-hydroxy-indole acetic acid. In rat liver microsomal fractions, kinetic and inhibition studies indicate that CYP2C11 and CYP3A2 mediates side-chain N-dealkylation, whereas CYP2D2 and CYP2C6 mediate the aromatic ring O-demethylation.[9]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

In vitro studies indicate that 5-MeO-DIPT has high affinity for 5-HT$_{1A}$ receptors and somewhat less affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors; however, animal studies suggest that 5-HT$_{2A}$ receptors are the important sites of action for the hallucinogenic effects of 5-MeO-DIPT.[10] *In vitro* studies also suggest that 5-MeO-DIPT
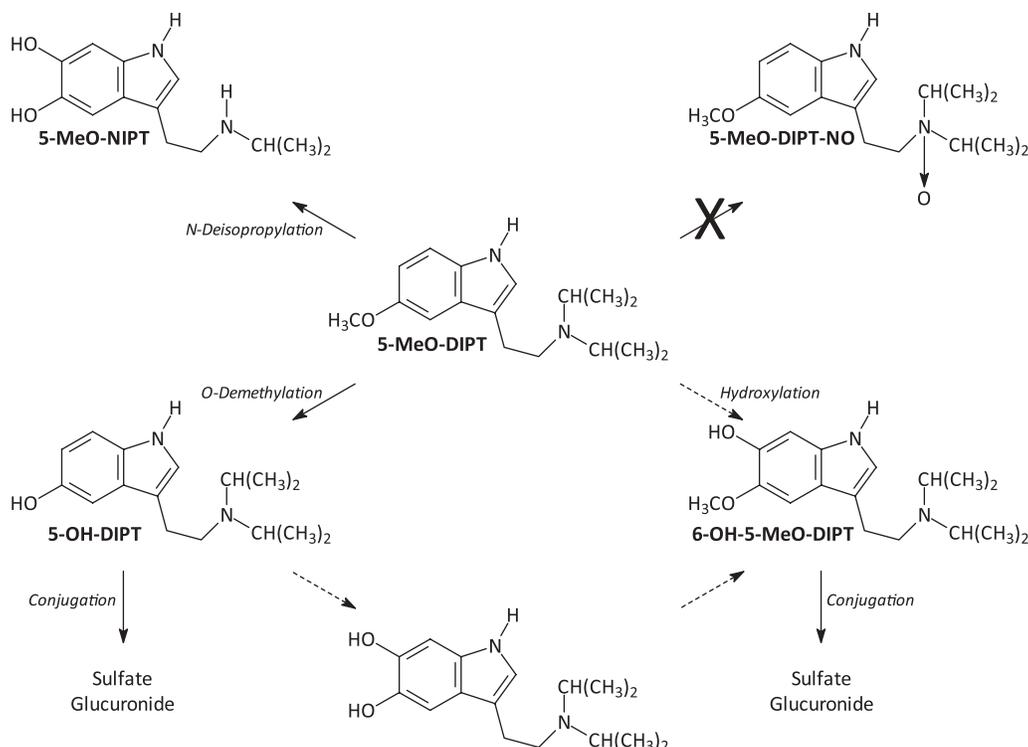
FIGURE 11.1. Metabolic pathways of 5-methoxy-diisopropyltryptamine (5-Meo-DIPT) in humans. 5-Meo-DIPT is metabolized mainly by demethylation and hydroxylation. Most of the 6-OH-5-Meo-DIPT formed by hydroxylation is excreted as its conjugates.

acts as a competitive serotonin transporter (SERT) inhibitor.[11] SERT belongs to the Na$^+$-dependent transporter family that is responsible for the uptake of serotonin (5-HT) following the release of 5-HT into the synaptic cleft. The hallucinogenic effects of 5-MeO-DIPT are probably mediated by multiple molecular targets in the serotonergic system.

CLINICAL RESPONSE

Adverse effects associated with the use of Foxy include nausea, vomiting, mydriasis, auditory and visual hallucinations, formication, tachycardia, hypertension, confusion, echolalia, and paranoia based on case reports.[12,13] Tremors and seizures are rare. Other adverse effects associated with the recreational use of 5-MeO-DIPT include restlessness, agitation, and muscle tension. Case reports associate the use of 5-MeO-DIPT with rhabdomyolysis and transient acute renal failure.[14] Recurrent seizures are more common following intoxication with 5-methoxy-α-methyltryptamine than with 5-MeO-DIPT. The onset of action following the ingestion of Foxy is relatively rapid (<2 h) with symptoms persisting approximately 3–6 hours. Case reports suggest that flashbacks can occur following the chronic use of 5-methoxy-*N*,*N*-diisopropyltryptamine.[15]

DIAGNOSTIC TESTING

Methods for quantification of 5-MeO-DIPT metabolites include liquid chromatography/mass spectrometry, liquid chromatography/tandem mass spectrometry, and gas chromatography/mass spectrometry (GC/MS). Methods for the simultaneous detection and quantitation of designer tryptamine, β-carboline, and other phenethylamine compounds including 5-MeO-DIPT involves micellar electrokinetic chromatography (MEKC),[16] GC/MS, liquid chromatography/electrospray ionization/mass spectrometry, and mass spectral fragmentation.[17,18] The limit of detection (LOD) for DET using cation-selective exhaustive injection-sweep-MEKC was about 2 ng/mL. A 23-year-old man presented to the emergency department (ED) with nausea, vomiting, formication (tactile hallucinations), and paranoia after ingesting Foxy 3 hours earlier.[13] A serum sample drawn 1 h after admission to the emergency department contained 0.14 mg 5-MeO-DIPT/L as measured by GC/MS. Analysis by GC/MS detected 5-MeO-DIPT concentrations of 1.7 mg/L in a urine sample collected from a 21-year-old man 4 hours after the ingestion of a homemade capsule containing 5-MeO-DIPT. He developed visual hallucinations and mydriasis, but his symptoms resolved by the time of the sample collection.

A 29-year-old man developed intense agitation after the rectal administration of 5-MeO-DIPT; he died a few hours later in the hospital. Postmortem blood (site not reported) contained the following concentrations: 5-MeO-DIPT, 0.412 mg/L; 5-OH-DIPT, 0.327 mg/L; and 5-MeO-NIPT, 0.020 mg/L.[19] The urine sample from a 21-year-old man contained 1.7 mg 5-MeO-DIPT/L as measured by GC/MS.[20] Clinical features of intoxication at the time of presentation included mydriasis, mild hallucinations, and difficulty moving his extremities. At the time of the sampling 4 hours after ingestion, the patient was asymptomatic. Analysis of a urine sample collected 35 hours after the ingestion of 50 mg 5-MeO-DIPT detected the presence of 5-MeO-DIPT as measured by liquid chromatography/mass spectrometry (LOD, 3 ng/mL).[7] Urine drug screens used in the clinical setting do not commonly detect the various tryptamine designer drugs.[21]

TREATMENT

The treatment of 5-MeO-DIPT is supportive. Life-threatening medical reactions following exposure to tryptamine compounds are extremely rare. All patients should be evaluated for organic disease with vital signs, physical examination, and a thorough history that includes current symptoms, premorbid function, and prior medical and psychiatric conditions. Vital signs should include an accurate rectal temperature that is repeated if the patient deteriorates. The sympathomimetic effects of tryptamines are usually mild and transient, except in patients with underlying cardiovascular disease. Important aspects of the management of tryptamine-related panic attacks include reduction of sensory stimulation and reassurance that the frightening images are drug-related, similar to the treatment of harmala alkaloid intoxication. Continuous interpretations of sensory misperceptions and pseudo-hallucination are necessary to reduce the anxiety associated with a "bad trip."

α-METHYLTRYPT-AMINE (AMT) and α-ETHYLTRYPTAMINE (AET)

α-Methyltryptamine (AMT) and α-ethyltryptamine (AET) are synthetic drugs derived from tryptamine

with *N,N*-dimethyltryptamine (DMT) being the prototypical hallucinogen in this group. During the early 1960s, α-ethyltryptamine (Monase®, Upjohn Company) was marketed in the United States as an antidepressant and stimulant, but the drug was removed shortly after introduction because of the occurrence of serious blood disorders. Since the 1980s, AET has been sold illicitly as a substitute for MDMA (ecstasy).[22] Clinical trials and animal studies during the early 1960s evaluated the efficacy of α-ethyltryptamine acetate (Monase®) and α-methyltryptamine as neuroleptic agents for schizophrenia and depression. AMT (Indopan) was marketed in the Soviet Union during the 1960s as an antidepressant. AMT was one of the hallucinogenic drugs used by Ken Kesey during the 1960s as recorded by Alexander Shulgin.[23] In 2003, the US Drug Enforcement Administration (DEA) added α-methyltryptamine and 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT, Foxy) to schedule I of the Controlled Substances Act.[24,25] Previously, α-ethyltryptamine and *N,N*-dimethyltryptamine were included in this list.

IDENTIFYING CHARACTERISTICS

Both α-ethyltryptamine (etryptamine, CAS RN: 2235-90-7, $C_{12}H_{16}N_2$) and α-methyltryptamine (indopan, CAS RN: 299-26-3, $C_{11}H_{14}N_2$), possess central stimulant and hallucinogenic activity.[26] Both optical isomers of α-ethyltryptamine are active; the stimulant properties reside primarily in the (−)-enantiomer, whereas the (+) enantiomer has hallucinogenic properties. Street names for α-ethyltryptamine include Love Pearls and ET; α-methyltryptamine is known as Day Tripper and IT-290. Table 11.1 lists some physiochemical properties of α-ethyltryptamine, which is a reversible monoamine oxidase inhibitor and monamine-releasing substance (i.e., serotonin, dopamine).

DOSE EFFECT

Humans

The administration of up to 300 mg α-ethyltryptamine acetate daily for 2 months to psychiatric inpatients and outpatients was associated with occasional side effects including facial flushing, headache, gastrointestinal dis-

TABLE 11.1. Some Physiochemical Properties of α-Ethyltryptamine.

Physical Property	Value
Melting Point	97°C (206.6°F)
log P (Octanol-Water)	2.170
Water Solubility	510 mg/L

tress, insomnia, and irritability.[27] A case report associated the ingestion of 700 mg α-ethyltryptamine with fatal hyperthermia and agitated delirium.[22]

In studies of volunteer inmates, α-methyltryptamine was a more powerful psychotomimetic agent than α-ethyltryptamine with effects similar to LSD. The most common adverse effects associated with the ingestion of 20 mg α-methyltryptamine were mydriasis, nervous tension, and restlessness.[28] The most prominent effects following the ingestion of 150 mg α-ethyltryptamine by these volunteers were anorexia and the feeling of intoxication. The ingestion of an estimated 100 mg AMT was associated with agitation, confusion, disorientation, hallucination, diaphoresis, mydriasis, and sinus tachycardia.[29] The patient recovered with supportive care.

Animals

In animal studies, the oral administration of up 30 mg α-ethyltryptamine/kg body weight and up to 20 mg α-methyltryptamine/kg body weight daily for 28 days produced no obvious clinical effects or histologic abnormalities.[30] Chronic oral administration of α-methyltryptamine and α-ethyltryptamine in daily doses up to 10 mg/kg body weight for 1 year produced some transient salivation, muscle tension in a group of rats, but no histologic changes were detected.[31] The administration of daily doses up to 30 mg α-ethyltryptamine/kg to dogs caused transient sign of intoxication, but no permanent changes or histologic evidence of tissue damage.

TOXICOKINETICS

Early pharmacokinetic studies suggest the absorption of AET is rapid with a relatively wide volume of distribution; intoxication may occur following ingestion as well as insufflation. AET undergoes 6-hydroxylation to the inactive metabolite, 3-(2-aminobutyl)-6-hydroxyindole. Elimination occurs primarily via the kidney with a plasma half-life of approximately 8 hours.[32] In rodent studies, elimination of AMT occurs primarily by renal excretion of unchanged AMT.[33] The primary urinary metabolites in these studies were 2-oxo-AMT, 6-OH-AMT, 7-OH-AMT, and 1′-OH-AMT. Limited data in humans suggests that liver excretes little AET in the bile, whereas a majority of the dose of AET appears in the urine within 12–24 hours.[32]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

In rodent studies, multiple doses of AET (8 × 30 mg/kg/sc) produce reductions in serotonin and 5-hydroxy-

indole acetic acid (5-HIAA) concentrations in the hippocampus and frontal cortex similar to MDMA.[34]

CLINICAL RESPONSE

Clinical features associated with the ingestion of AET or AMT include nausea, agitation, restlessness, lethargy, euphoria, confusion, distortion of images (color, shape), and visual hallucinations.[29] Mild elevations of blood pressure and temperature typically occur; other effects include mydriasis, salivation, diaphoresis, clenching of the jaw, and tachycardia. Depersonalization may also occur, but alteration of visual perception is uncommon. In volunteer studies, the onset of action of α-methyltryptamine is relatively slow with an onset of action being 3–4 hours and the duration of action being about 12–24 hours.[28] The onset of action of α-ethyltryptamine is about 30–90 minutes.[28] The duration of intoxication following ingestion of AET was approximately 6 hours with subsequent feelings of lethargy and sedation.

DIAGNOSTIC TESTING

Analytic Methods

A sensitive (LOD, 1 ng/mL) and selective method for the determination for α-methyltryptamine and 5-methoxy-*N*,*N*-diisopropyltryptamine involves extraction using Extrelut® column (Merck & Co., Inc., Whitehouse Station, NJ), with an internal standard, bupivacaine, followed by derivatization with acetic anhydride and analysis by gas chromatography/electrospray ionization/mass spectrometry in selected ion monitoring.[35]

Biomarkers

A 19-year-old woman became disoriented and vomited after ingesting α-ethyltryptamine.[36] Later, she developed cardiac arrest; she could not be resuscitated by bystanders or by the paramedics. She was pronounced dead at the scene; postmortem examination demonstrated pulmonary edema, generalized visceral congestion, and some epicardial petechiae. Analysis of postmortem samples demonstrated the following α-ethyltryptamine concentrations: heart blood, 5.6 mg/L; vitreous humor, 2.4 mg/L; bile, 22.0 mg/L; and gastric contents, 52.9 mg. α-Ethyltryptamine can cross-react with some immunoassays for amphetamines. Analysis of postmortem blood from a fatality involving hyperthermia after the use of α-ethyltryptamine demonstrated 1.1 mg α-ethyltryptamine/L.[22] Postmortem blood from the iliac vein of a young college student found unre-

sponsive after ingesting α-methyltryptamine about 12 hours previously contained 2.0 mg α-methyltryptamine/L.[37] The amount of α-methyltryptamine in the gastric contents was 9.6 mg.

TREATMENT

Treatment of AMT or AET intoxication is supportive, similar to 5-MeO-DIPT intoxication. Although life-threatening medical reactions following exposure to tryptamine compounds are extremely rare, case reports associate the use of these drugs with severe hyperthermia. All patients should be evaluated for organic disease with vital signs, physical examination, and a thorough history that includes current symptoms, premorbid function and prior medical and psychiatric conditions. Vital signs should include accurate rectal or core temperatures that are repeated if the patient deteriorates. As measured by rectal thermocouple, a core temperature exceeding 40.5°C (105°F) indicates the need to cool the patient (e.g., ice water bath, evaporative cooling) and to monitor vital signs every 10–15 minutes after stabilization. Ice packs and the use of hypothermic blankets are alternative measures when an ice bath is not available. The patient should be removed when the core rectal temperature drops to 39°C (102°F). Less severe temperature elevations (38–40°C/100.4–104°F) can be treated by placement in a cool room, removal of clothes, minimization of physical activity, sedation with benzodiazepines as needed, tepid sponging, or evaporative methods (cool mist spray, fans). These patients should receive aggressive fluid resuscitation to maintain adequate urine output and to minimize the effects of rhabdomyolysis.

References

1. Huszti Z, Borsy J. The effect of diethyltryptamine and its derivatives on monamine oxidase. Biochem Pharmacol 1964;13:1151–1156.
2. Cohen I, Vogel WH. Determination and physiological disposition of dimethyltryptamine and diethyltryptamine in rat brain, liver and plasma. Biochem Pharmacol 1972; 21:1214–1216.
3. Boszormenyi Z, Der P, Nagy T. Observations on the psychotogenic effect of *N*,*N*-diethyltryptamine, a new tryptamine derivative. J Ment Sci 1959;105:171–181.
4. Szara S, Rockland LH, Rosenthal D, Handlon JH. Psychological effects and metabolism of *N*,*N*-diethyltryptamine in man. Arch Gen Psychiatr 1966;1: 320–329.
5. Shulgin AT, Carter MF. *N*,*N*-Diisopropyltryptamine (DIPT) and 5-methoxy-*N*,*N*-diisopropyltryptamine

(5-MeO-DIPT). Two orally active tryptamine analogs with CNS activity. Commun Psychopharmacol 1980;4:363–369.

6. Katagi M, Tsutsumi H, Miki A, Nakajima K, Tsuchihashi H. Analysis of clandestine tablets of amphetamines and their related designer drugs encountered in recent Japan. Jpn J Forensic Toxicol 2002;20:303–319.

7. Katagi M, Kamata T, Zaitsu K, Shima N, Kamata H, Nakanishi K, et al. Metabolism and toxicologic analysis of tryptamine-derived drugs of abuse. Ther Drug Monit 2010; 32:328–331.

8 Kamata T, Katagi M, Kamata HT, Miki A, Shima N, Zaitsu K, et al. Metabolism of the psychotomimetic tryptamine derivative 5-methoxy-N,N-diisopropyltryptamine in humans: identification and quantification of its urinary metabolites. Drug Metab Dispos 2006;34:281–287.

9. Narimatsu S, Yonemoto R, Masuda K, Katsu T, Asanuma M, Kamata T, et al. Oxidation of 5-methoxy-N,N-diisopropyltryptamine in rat liver microsomes and recombinant cytochrome P450 enzymes. Biochem Pharmacol 2008;75: 752–760.

10. Fantegrossi WE, Harrington AW, Kiessel CL, Eckler JR, Rabin RA, Winter JC, et al. Hallucinogen-like actions of 5-methoxy-N,N-diisopropyltryptamine in mice and rats. Pharmacol Biochem Behav 2006;83:122–129.

11. Sogawa C, Sogawa N, Tagawa J, Fujino A, Ohyama K, Asanuma M, et al. 5-Methoxy-N,N-diisopropyltryptamine (Foxy), a selective and high affinity inhibitor of serotonin transporter. Toxicol Lett 2007;170:75–82.

12. Itokawa M, Iwata K, Takahashi M, Sugihara G-I, Sasaki T, Abe Y-I, et al. Acute confusional state after designer tryptamine abuse. Psychiatry Clin Neurosci 2007;61:196–199.

13. Wilson JM, McGeorge F, Smolinske S, Meatherall R. A foxy intoxication. Forensic Sci Int 2005;148:31–36.

14. Alatrash G, Majhail NS, Pile JC. Rhabdomyolysis after ingestion of "foxy," a hallucinogenic tryptamine derivative. Mayo Clin Proc 2006;81:550–551.

15. Ikeda A, Sekiguchi K, Fujita K, Yamadera H, Koga Y. 5-Methoxy-N,N-diisopropyltryptamine-induced flashbacks. Am J Psychiatry 2005;162:815.

16. Wang M-J, Tsai C-H, Hsu W-Y, Liu J-T, Lin C-H. Optimization of separation and online sample concentration of N,N-dimethyltryptamine and related compounds using MEKC. J Sep Sci 2009;32:441–445.

17. Chen BH, Liu JT, Chen WX, Chen HM, Lin CH. A general approach to the screening and confirmation of tryptamines and phenethylamines by mass spectral fragmentation. Talanta 2008;74:512–517.

18. Kikura-Hanajiri R, Hayashi M, Saisho K, Goda Y. Simultaneous determination of nineteen hallucinogenic tryptamines/beta-carbolines and phenethylamines using gas chromatography-mass spectrometry and liquid chromatography-electrospray ionisation-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005;825: 29–37.

19. Tanaka E, Kamata T, Katagi M, Tsuchihashi H, Honda K. A fatal poisoning with 5-methoxy-N,N-diisopropyltryptamine, Foxy. Forensic Sci Int 2006;163:152–154.

20. Meatherall R, Sharma P. Foxy, a designer tryptamine hallucinogen. J Anal Toxicol 2003;27:313–317.

21. Shimizu E, Watanabe H, Kojima T, Hagiwara H, Fujisaki M, Miyatake R, et al. Combined intoxication with methylone and 5-MeO-MIPT. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:288–291.

22. Daldrup T, Heller C, Matthiesen U, Honus S, Bresges A, Haarhoff K. [Etryptamine, a new designer drug with a fatal effect.] Z Rechtsmed 1986;97:61–68. [German]

23. Shulgin A, Shulgin A. Tryptamines I Have Known and Loved: The Continuation. Berkeley, CA: Transform Press, 1997:566–568.

24. Drug Enforcement Administration (DEA), Department of Justice. Schedules of controlled substances: Placement of alpha-methyltryptamine and 5-methoxy-N,N-diisopropyltryptamine into Schedule I of the Controlled Substances Act. Fed Regist 2004;69:58050–58053.

25. Drug Enforcement Administration (DEA), Department of Justice. Schedules of controlled substances: temporary placement of alpha-methyltryptamine and 5-methoxy-N,N-diisopropyltryptamine into Schedule I. Final rule. Fed Regist 2003;68:16427–16430.

26. Hong SS, Young R, Glennon RA. Discriminative stimulus properties of alpha-ethyltryptamine optical isomers. Pharmacol Biochem Behav 2001;70:311–316.

27. Turner WJ, Merlis S. Clinical studies with ethyltryptamine. J Neuropsychiatr 1961;2(Suppl 1):73–76.

28. Murphree HB, Dippy GR, Jenney EH, Pfeiffer CC. Effects in normal man of α-methyltryptamine and α-ethyltryptamine. Clin Pharmacol Ther 1961;2:722–726.

29. Long H, Nelson LS, Hoffman RS. Alpha-methyltryptamine revisited via easy internet access. Vet Hum Toxicol 2003; 45:149.

30. Gray JE, McWade DH, Johnston RL, Larson EJ, Freyburger WA. Toxicopathologic studies of α-methyl- and α-ethyltryptamine acetates (Monase). 1. Acute, subacute, and miscellaneous studies. Toxicol Appl Pharmacol 1962;4:547–560.

31. Keller JG, Viguera C, Kundzins W. Studies on the toxicology of α-methyl- and α-ethyltryptamine acetates (Monase). II. Chronic studies. Toxicol Appl Pharmacol 1962;4: 697–709.

32. Eberts FS Jr. Metabolic studies with 3-(2-aminobutyl-1-C14) indole acetate [monase-C14]. I. Distribution and excretion in rat, dog, and man. J Neuropsychiatr 1961;2 (Suppl 1):146S–150S.

33. Kanamori T, Kuwayama K, Tsujikawa K, Miyaguchi H, Iwata YT, Inoue H. In vivo metabolism of α-methyltryptamine in rats: identification of urinary metabolites. Xenobiotica 2008;38:1476–1486.

34. Huang X, Johnson MP, Nichols DE. Reduction in brain serotonin markers by α-ethyltryptamine (Monase). Eur J Pharmacol 1991;200:187–190.

35. Ishida T, Kudo K, Kiyoshima A, Inoue H, Tsuji A, Ikeda N. Sensitive determination of alpha-methyltryptamine (AMT) and 5-methoxy-N,N-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine using gas chromatography-mass spectrometry. J Chromatogr B 2005; 823:47–52.

36. Morano RA, Spies C, Walker FB, Plank SM. Fatal intoxication involving etryptamine. J Forensic Sci 1993;38:721–725.

37. Boland DM, Andollo W, Hime GW, Hearn WL. Fatality due to acute α-methyltryptamine intoxication. J Anal Toxicol 2005;29:394–397.

Chapter 12

DIURETICS, IPECAC, and LAXATIVES

Cyrus Rangan, MD

Patients with anorexia nervosa (i.e., purge) and bulimia nervosa (i.e., binge/purge) frequently experience recurrent episodes of attempted weight loss by calorie restriction, self-induced vomiting, and/or the surreptitious use of pharmaceutic drugs (diuretics, syrup of ipecac, laxatives). Bulimic patients couple binge eating with behavior intended to promote weight loss (diuretics/laxatives/emetics, self-induced vomiting, strenuous exercise, fasting), whereas anorexic patients control their dietary intake much more than bulimics. Both anorexic and bulimic patients often abuse other drugs including ethanol and various types of diet pills.[1] Chronic abuse of syrup of ipecac, diuretics, and laxatives may cause tolerance to the anorexic effects of the drugs along with a desire to increase dosing to control weight.[2] The combination of dietary restrictions and abuse of over-the-counter preparations may result in electrolyte imbalance (hypokalemia, hypocalcemia, hypomagnesemia), protein-calorie malnutrition, and cardiac dysrhythmias.[3] The psychologic impact of this abuse is consistent with the pre-existing eating disorder rather than a direct toxic effect of the abused pharmaceutic agents. These patients appear preoccupied with weight-control, calorie intake, or continuous exercise, even with apparently average or thin body habitus. They hide their body shape with baggy clothes, and surreptitiously use frequent trips to the bathroom for purging and catharsis. Often, they express self-defeating statements after food consumption and display low self-esteem, mood swings, and depression. A perfectionist personality frequently accompanies eating disorders, which may intensify after self-imposed escalation of dosage regimens of diuretics, syrup of ipecac, and/or laxatives.

DIURETICS

Classification of diuretics include the following: 1) the loop diuretics or inhibitors of the $Na^+/K^+/2Cl^-$ symporter (e.g., bumetanide, ethacrynic acid, furosemide), 2) inhibitors of the Na^+/Cl^- symporter (thiazide diuretics), 3) carbonic anhydrase inhibitors (e.g., acetazolamide), 4) osmotic diuretics (e.g., glycerin, mannitol, urea), 5) inhibitors of renal epithelial Na^+ channels (e.g., amiloride, triamterene), and 6) mineralocorticoid receptor antagonists (e.g., spironolactone). Abuse of diuretics causes net weight loss and multiple systemic toxicities by promoting the excretion of water and electrolytes; however, these drugs neither prevent the absorption of food nor accelerate the metabolism or excretion of nutrients. The World Anti-Doping Agency (WADA) added diuretics to the list of prohibited substances in 1988. During 2008, diuretics accounted for 7.9% (436 cases) of all adverse analytic findings reported by WADA laboratories with hydrochlorothiazide (137 cases) and furosemide (104 cases) representing the most common abused diuretics.[4] Abuse of diuretics in the competitive sports world results from the use of these drugs to induce rapid weight loss to meet a weight category and to mask the administration of other doping agents by reducing urine

drug concentrations. There is no evidence that the use of diuretics improves athletic performance as the dehydration associated with diuretic use impairs maximal exercise capacity and muscular strength.[5]

Furosemide

IDENTIFYING CHARACTERISTICS

Furosemide [5-(aminosulfonyl)-4-chloro-2-((furanylmethyl)amino)benzoic acid, $C_{12}H_{11}ClN_2O_5S$] is a commonly abused diuretic. Figure 12.1 displays the chemical structure of furosemide (CAS RN: 54-31-9). Table 12.1 lists some physiochemical properties of furosemide.

EXPOSURE

Although many individuals with eating disorders use diuretics, relatively few of these individuals continue the use of diuretics on a regular basis.[2] A commonly reported use for diuretics in these patients is the control of weight gain secondary to premenstrual water retention. The prevalence of diuretic use in patients with bulimia nervosa is particularly high. In a study of 275 bulimic women, approximately one-third of these patients reported the use of diuretics for weight control and about 10% in this series used diuretics daily.[6] Reported weekly diuretic use in 14 regular diuretic users ranged from 1–14 times.[7] Furosemide is a synthetic benzoic-
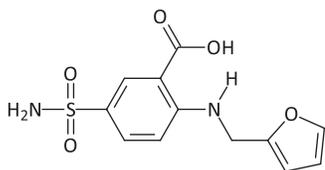
FIGURE 12.1. Chemical structure of furosemide.

TABLE 12.1. Some Physiochemical Properties of Furosemide.

Physical Property	Value
Molecular Weight	330.7441 g/mol
Melting Point	295°C (563°F)
log P (Octanol-Water)	2.03
Water Solubility	73.1 mg/L (30°C/86°F)
Vapor Pressure	3.23E-10 mm Hg (25°C/77°F)

sulfonamide-furan "loop" diuretic and saluretic for treatment of edema, congestive heart failure, and chronic renal insufficiency.

DOSE EFFECT

Typical daily doses of furosemide are 1–3 mg/kg body weight in children and 20–600 mg in adults depending on the severity of the underlying illness (lower doses for hypertension and edema, higher doses for chronic renal failure). A 25-year-old woman developed syncope, nausea, vomiting, dizziness, hyponatremia, and hypokalemia after taking 200 mg of furosemide daily for 3 days while fasting to reach a specific weight target.[8] Interpretation of clinical data on toxicity associated with chronic abuse of diuretics is complicated by variable intake of fluid and electrolytes as well as the unreliability of histories for the surreptitious use of diuretics and other drugs (diet pills, herbs, ipecac, laxatives). Chronic abuse of 12.5 mg furosemide/kg daily and 375 mg 18 hours prior to admission resulted in muscle weakness, carpopedal spasms, myalgias, and tetany secondary to electrolyte depletion (calcium, magnesium, potassium).[9] Abuse of furosemide may reach 2 g daily for extended periods with relatively minor changes in serum electrolytes, depending on dietary habits and the extent of abuse of other weight control measures.[10]

TOXICOKINETICS

Kinetics

Furosemide is rapidly absorbed after ingestion with about 60% bioavailability.[11] The volume of distribution is small (0.11 L/kg) with high protein binding (~98–99%). The liver conjugates about 35% of the absorbed dose of furosemide with glucuronic acid. The kidney excretes the remainder as unchanged furosemide. Following therapeutic doses of furosemide, the serum half-life averages approximately 1.5 hours, but serum half-life may exceed 20 hours after chronic ingestion of high doses of furosemide.[11] Total systemic clearance of furosemide is about 2 mL/min/kg body weight.

Furosemide crosses the placenta during the peripartum period with similar furosemide concentrations in umbilical cord and maternal blood 8–10 hours after daily oral doses of 25–40 mg.[12] In general, diuretics are considered safe for use in lactating mothers despite the secretion of furosemide into breast milk.[13]

Tolerance

Gradual tachyphylaxis to diuretics develops after long-term administration; repeat use of loop diuretics may

initiate and perpetuate alternating diuresis and reflex water retention, leading to weight fluctuations.[14] Hypertrophy occurs in the distal tubule in response to elevated tubular sodium concentrations and the enhanced reabsorption of sodium and water. Coadministration of thiazide restores diuresis in therapeutic settings by blocking distal tubule sodium reabsorption.[15] Diuretic abuse may involve dual diuretic administration when loop diuretics fail to control weight.

Drug Interactions

The concomitant administration of furosemide with ceftazidime increases the serum concentrations of the latter; however, there are few data on the clinical significance of this potential interaction.[16] Case reports suggest that the administration of an aminoglycoside (e.g., gentamicin) followed by furosemide may increase the risk of ototoxicity.[17] The concomitant administration of phenytoin or phenobarbital with furosemide may decrease serum furosemide concentration by about 50–65% as a result of a reduction in the gastrointestinal (GI) absorption of furosemide.[18] Case reports associate the ingestion of chloral hydrate within 24 hours prior to the administration of furosemide with diaphoresis, flushing, anxiety, and hypertension similar to degreaser's flush.[19,20] The combination of furosemide and corticosteroids may result in severe potassium depletion, possibly augmented by steroid-induced overproduction of endogenous aldosterone.[21] Potassium depletion by furosemide may potentiate myocardial sensitivity to digoxin toxicity.[22] The concomitant administration of herbal diuretic preparations (e.g., licorice, gossypol) with furosemide may potentiate the electrolyte abnormalities associated with chronic furosemide abuse.[23]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Furosemide binds reversibly to the sodium-potassium-chloride symporter complex in the thick ascending loop of Henle, blocking the reabsorption of sodium and chloride; consequently, interstitial hypertonicity decreases along with water reabsorption. The persistence of these electrolytes in the lumen of the thick ascending limb abolishes the 10-millivolt transepithelial potential gradient, which is the driving force for calcium and magnesium efflux into the interstitial space. Subsequently, reabsorption of calcium and magnesium decreases. Furosemide significantly enhances urinary potassium excretion both by reducing potassium reabsorption in

the loop of Henle and by enhancing distal tubular potassium secretion.[24]

Mechanism of Toxicity

Furosemide induces ototoxicity by altering the electrolyte composition of endolymph, and inducing edema of the stria vascularis, presumably by inhibition of electrolyte symporters similar to those found in the ascending limb of the loop of Henle.[25] Hyponatremia and hypokalemia are common complications of furosemide abuse. Diminished sodium and chloride reabsorption from overuse or abuse of diuretics causes hyponatremia. The resulting increased delivery of sodium to the distal tubule activates the renin-angiotensin system; subsequently, production of aldosterone increases along with increased secretion of potassium and hydrogen ions into the distal tubule.

Case reports associate the rapid correction of severe hyponatremia in patients chronically abusing furosemide with central pontine myelinolysis.[26] During prolonged hyponatremia from chronic furosemide abuse, the brain minimizes cerebral edema by extruding osmotically active particles (e.g., amino acids, sodium, potassium) from the intracellular compartment. The rapid expansion of extracellular fluid during the quick correction of chronic hyponatremia with hyperosmotic saline rapidly reduces brain size; damage of myelin sheaths subsequently occurs.[27,28] Although the central pons is the most sensitive area of the brain, other areas of the brain may undergo myelinolysis. A mutation in the gene (SLC12A3) for the thiazide-sensitive NaCl coporter in the distal convoluted tubule is responsible for hypokalemic metabolic alkalosis and hypomagnesemia associated with Gitelman syndrome that simulates chronic diuretic abuse.[29]

CLINICAL RESPONSE

Illicit Use

Overdoses of diuretics rarely cause acute toxicity; however, significant dehydration and electrolyte imbalance occurs following the chronic abuse of diuretics by patients with eating disorders. The abuse of furosemide is associated with signs of dehydration including thirst, lethargy, poor skin turgor, postural hypotension, syncope, dry mucous membranes, tachycardia, and fatigue. Delirium, rhabdomyolysis, and renal dysfunction (acute tubular necrosis) may result from severe dehydration and hypokalemia. Tinnitus with temporary or irreversible deafness may occur following chronic abuse of high doses of furosemide.

Central Pontine Myelinolysis

Case reports associate the rapid correction of chronic hyponatremia secondary to anorexia nervosa and diuretic abuse with the development of confusion, delirium, oculomotor abnormalities, dysarthria, dysphagia, spastic weakness, and quadriparesis or pseudobulbar palsy. The onset of symptoms of this disorder may occur several hours to a week after the correction of the serum sodium concentration. A 35-year-old woman became severely hyponatremic (serum sodium, 91 mEq/L) and hypokalemic (serum potassium, 1.6 mEq/L) following the chronic abuse of furosemide (400 mg daily) and the ingestion of large volumes of water.[26] The serum sodium was corrected rapidly within the first 32 hours (1 mEq/L/h); she became agitated, labile, and tremulous within 2–3 days. Four days later, she was dysphagic, ataxic, and dysarthric; initially, she had a flaccid quadriplegia that subsequently became spastic. Several months of physical therapy were necessary to regain her gait and speech. Pancreatitis, nephrocalcinosis, ureteral stones, and hypercalciuria may occur following long-standing diuretic abuse.[30]

Pseudo-Bartter Syndrome

Case series associate the surreptitious use of diuretics with hyperreninemic hypokalemic metabolic alkalosis in the presence of normal blood pressure and normal urinary excretion of sodium and chloride, similar to Bartter syndrome. The latter syndrome is a rare genetic abnormality that causes chronic hypokalemic alkalosis in adults. Distinguishing these 2 syndromes may be difficult when the patient does not admit the use of diuretics. In a study of 5 patients with pseudo-Bartter syndrome and 6 patients with Bartter syndrome, the plasma sodium potassium, chloride, bicarbonate, renin, and aldosterone concentrations of the 2 groups were similar, whereas the serum magnesium and uric acid concentrations were greater in the pseudo-Bartter syndrome group.[31] Gitelman syndrome is the hypocalciuric variant of Bartter syndrome that causes hypomagnesemia in adults. This syndrome causes hypokalemic metabolic alkalosis and hyperreninemic hyperaldosteronism with normal blood pressure similar to Bartter syndrome and diuretic abuse.

Abstinence Syndrome

Discontinuation of chronic diuretic use may cause rebound edema and weight gain that requires days to weeks to resolve.[32] Fluid retention with paradoxical diuresis may be particularly prominent during the first several days after cessation of diuretic abuse. This transient weight gain may be associated with bloating, abdominal distention, and ankle edema. The perception of increased weight gain after cessation of diuretic use encourages resumption of diuretic abuse by anorectic and bulimic women.

Reproductive Abnormalities

Diuretics are not recommended during pregnancy, secondary to neonatal kidney and electrolyte abnormalities, especially during the third trimester. The US Food and Drug Administration classifies furosemide as category C (little or no adverse animal data, no controlled human studies) in pregnancy.

DIAGNOSTIC TESTING

Analytic Methods

Analytic techniques for the quantitation of furosemide in biologic samples include high performance liquid chromatography (HPLC) with spectrofluorometric detection,[30] gas chromatography/electron capture negative ionization/mass spectrometry,[33] and solid-phase extraction followed by high pressure liquid chromatography/electrospray ionization/tandem mass spectrometry.[34] This latter method detects 35 diuretics and metabolites at urinary concentrations significantly below 100 ng/mL, whereas the limit of detection (LOD) for gas chromatography/electron capture negative ionization/mass spectrometry is typically <5–10 ng/mL. WADA established a single minimum required performance level (MRPL) of 250 ng/mL for the detection for diuretics in urine by accredited laboratories, typically by gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), or liquid chromatography/tandem mass spectrometry with electrospray ionization.[35] Similar to hydrochlorothiazide and triamterene, the short half-life of furosemide limits detection of this diuretic in urine samples collected 24–48 hours after administration.

Biomarkers

Detection of surreptitious diuretic use involves sequential measurements of urine chloride concentrations and the analytic confirmation of diuretics in urine samples. Patients abusing diuretics typically develop hypokalemic metabolic alkalosis with normal urine chloride excretion during periods of abstinence. Wide variation in the urinary excretion of potassium, sodium, and chloride suggests diuretic use. Urine concentrations of these

ions increase shortly after ingestion of diuretics, whereas maximum reduction in the urinary concentration of these ions occurs during the postdiuretic phase. Consequently, measurement of urinary chloride excretion does not necessarily distinguish between Bartter syndrome and the use of furosemide because furosemide impairs tubular reabsorption of chloride similar to Bartter syndrome. Diuretic use causes contraction of extracellular fluid and increases proximal tubule solute reabsorption. The presence of reduced uric acid and fractional lithium clearances (i.e., markers of postproximal solute delivery) along with reduced renal response to a standard infusion of furosemide separate diuretic use (pseudo-Bartter syndrome) from Bartter syndrome.[31]

Abnormalities

The characteristic electrolyte profile associated with furosemide abuse is a hyponatremic, hypochloremic, and hypokalemic metabolic alkalosis. However, the presenting serum and urinary electrolyte abnormalities associated with diuretic abuse may be complicated by the concomitant use of laxatives, self-induced vomiting, or dietary restrictions. The hypokalemia associated with diuretic abuse may be severe (e.g., 1.3 mEq/L) in association with normal serum chloride concentrations and elevated serum bicarbonate.[36] Although serum sodium and magnesium concentrations are often normal, occasionally mild to moderate hyponatremia and hypomagnesemia develop along with hypokalemia.[37] Hyponatremic hypotonic dehydration along with hypokalemia following chronic furosemide abuse typically occurs in the setting of excessive water ingestion and/or the abuse of other diuretics (e.g., hydrochlorothiazide).[38] Rarely, hyperglycemia and hyperuricemia may result from the chronic abuse of diuretics.[38] Excessive diuresis and dehydration may cause a transient decrease in glomerular filtration rate and elevation of serum blood urea nitrogen. A paradoxical diuresis may continue for several days after drug cessation. Magnetic resonance imaging (MRI) of the brain in patients with central pontine myelinolysis demonstrates ventricular dilation and extensive central pontine lesions. The size of the MRI lesion does not correlate well with the initial serum sodium concentration or the severity of neurologic abnormalities.[39]

TREATMENT

Initial medical management should be directed at the correction of fluid and electrolyte imbalance along with the restoration of a normal diet. These patients should

be evaluated for electrolyte (sodium, potassium, chloride, magnesium, calcium) and acid–base abnormalities, anemia, pancreatitis, rhabdomyolysis, volume depletion, renal dysfunction, and electrocardiographic abnormalities. Fluid replacement typically involves replacement of sodium and chloride losses with saline. Rare complications include cardiac dysrhythmias, hearing loss, hyperuricemia, and pancreatitis. Severe chronic hyponatremia should be corrected relatively slowly (<0.55 mEq/L/h) to prevent the development of central pontine myelinolysis.[26] The serum sodium should be <135 mEq/L with the first 48 hours of treatment; hypernatremia should be avoided. Peripheral edema may occur during the first few weeks after cessation of diuretic abuse, which usually responds to salt restriction. Hospitalization may be necessary to correct hypokalemic metabolic alkalosis and to stop the use of diuretics and other methods to control weight.

Hydrochlorothiazide

IDENTIFYING CHARACTERISTICS

Hydrochlorothiazide [2H-1,2,4-benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-1,1-dioxide, $C_7H_8ClN_3O_4S_2$] is another commonly abused diuretic. Figure 12.2 displays the chemical structure hydrochlorothiazide (CAS RN: 58-93-5). In conditions within the body, hydrochlorothiazide is moderately soluble in water. Table 12.2 lists some physiochemical properties of hydrochlorothiazide.

EXPOSURE

Hydrochlorothiazide is a synthetic compound available as monotherapy for the treatment of hypertension or edema, and more frequently in combination therapy with other pharmaceuticals (e.g., angiotensin converting enzyme inhibitors, potassium sparing diuretics). This diuretic is distributed as a pill or capsule; however, this drug is not available commercially in a parenteral form.
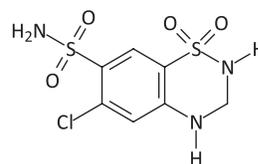
FIGURE 12.2. Chemical structure of hydrochlorothiazide.

TABLE 12.2. Some Physiochemical Properties of Hydrochlorothiazide.

Physical Property	Value
Molecular Weight	297.7391 g/mol
Melting Point	274°C (525.2°F)
pKa Dissociation Constant	7.9
log P (Octanol-Water)	–0.07
Water Solubility	722 mg/L (25°C/77°F)
	1,080 mg/L (37°C/98.6°F, pH 7.4)
Vapor Pressure	1.32E-09 mm Hg (25°C/77°F)

TOXICOKINETICS

The oral bioavailability of hydrochlorothiazide is relatively high (i.e., about $70 \pm 15\%$). In a study of 10 healthy volunteers receiving 25 mg hydrochlorothiazide daily for 45 days, the mean time to peak hydrochlorothiazide concentration was 2.8 ± 0.40 hours.[40] The apparent volume of distribution and mean terminal plasma elimination half-life vary several-fold among pharmacologic studies of clinical doses of hydrochlorothiazide (i.e., 25–100 mg), at least partially as a result of differences in the postdose sampling times between studies. The mean apparent volume of distribution in the studies with longer monitoring periods are in the range of approximately 3–4 L/kg.[41] In a study of 4 healthy adults receiving a single dose of 100 mg hydrochlorothiazide and monitored for 48 hours, the mean V_d was 3.48 L/kg.[42] The mean apparent volume of distribution (V_d) was 2.58 ± 0.30 L/kg in a study of 12 healthy volunteers receiving hydrochlorothiazide doses up to 200 mg.[43] The mean terminal plasma elimination half-lives of the studies with longer monitoring periods are in the range of approximately 9–12 hours.[41] A study of 12 healthy volunteers receiving a single dose of 25–200 mg hydrochlorothiazide demonstrated a mean terminal plasma half-life of 9.75 hours.[43] In a study of 10 healthy volunteers receiving 25 mg hydrochlorothiazide daily for 45 days, the plasma elimination half-life was 8.00 ± 2.50 hours.[40] Elimination of hydrochlorothiazide occurs almost exclusively by renal excretion of unchanged drug. Although hydrochlorothiazide is excreted in breast milk, case reports suggest that the daily ingestion of 50 mg hydrochlorothiazide by lactating mothers does not produce detectable hydrochlorothiazide concentration (i.e., <20 ng/mL) in breast feeding infants.[44]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Thiazide diuretics inhibit sodium and chloride reabsorption in the cortical diluting segment of the proximal segments of the distal tubule and the distal portion of the ascending loops of Henle; however, hydrochlorothiazide does not limit the reabsorption of calcium or magnesium. The increased amount of sodium leaving the proximal tubule results in a reflex increase in aldosterone secretion that enhances sodium reabsorption from the distal tubule. The exchange of sodium for potassium causes an increased loss of potassium in the urine. Hydrochlorothiazide is a relatively weak diuretic compared with furosemide, but hydrochlorothiazide produces a dose-dependent loss of potassium in the urine that is greater than furosemide. Marked hypokalemia is uncommon unless the dose of hydrochlorothiazide is large or the patient has Cushing syndrome. The inhibition of the renal tubular excretion of uric acid may cause an attack of gout in susceptible patients.

CLINICAL RESPONSE

Similar to furosemide, chronic abuse of hydrochloride may cause severe electrolyte and fluid imbalance, pseudo-Bartter syndrome, and central pontine myelinolysis (i.e., after the rapid correction of hyponatremia). Complications of the therapeutic use of hydrochlorothiazide include hemorrhagic pancreatitis, acute gout, and hyperglycemia in diabetic patients.[45]

DIAGNOSTIC TESTING

Analytic Methods

Analytic techniques for the quantitation of hydrochlorothiazide in biologic samples include thin layer chromatography (TLC),[46] high performance liquid chromatography (HPLC) with UV detection,[47] HPLC with electrochemical detection,[48] liquid chromatography/tandem mass spectrometry,[49] and GC/MS. The LOD for TLC is approximately 5,000 ng/mL compared with 300 ng/mL for GC/MS and approximately 1 ng/mL for liquid chromatography/tandem mass spectrometry.[50] *In vitro* studies indicate that hydrochlorothiazide concentrates in erythrocytes. The concentration of hydrochlorothiazide is about 10-fold higher in erythrocytes than plasma;[51] limited pharmacokinetic data suggests that whole blood hydrochlorothiazide concentrations are about 2.5-fold higher than plasma hydrochlorothiazide concentrations.[52] Degradation products of hydrochlorothiazide in aqueous media (e.g., 4-amino-6-chloro-1,3-benzenedisulphonamide) allows the detection of

diuretic use up to 120 hours after administration when analyzed by liquid chromatography/tandem mass spectrometry.[53]

Biomarkers

In a study of 10 healthy volunteers receiving 25 mg hydrochlorothiazide daily for 45 days, the mean peak plasma hydrochlorothiazide concentration was 200 ± 40 ng/mL.[40] Limited pharmacokinetic data suggests that peak hydrochlorothiazide concentrations following therapeutic doses are higher in elderly patients compared with healthy young adults. In a study of 6 healthy young and 6 elderly volunteers receiving 25 mg hydrochlorothiazide daily, the mean peak plasma hydrochlorothiazide concentrations on day 1 were 298 ± 27 ng/mL and 484 ± 32 ng/mL, respectively.[54] On day 8, these 2 plasma concentrations (356 ± 18 ng/mL and 615 ± 43 ng/mL, respectively) were not significantly different than day 1. The mean peak plasma hydrochlorothiazide concentration in 4 healthy adults ingesting 100 mg hydrochlorothiazide was about 657 ng/mL.[42]

Abnormalities

The use of hydrochlorothiazide inhibits the urinary excretion of calcium with associated development of increased serum total and ionized calcium concentrations.[55] Abnormalities associated with the use and abuse of hydrochlorothiazide include pancreatitis, volume contraction and alkalosis, hypokalemia, hyponatremia, hyperuricemia, and reduced carbohydrate tolerance.[56,57]

TREATMENT

Treatment of complications associated with hydrochlorothiazide abuse is supportive, similar to furosemide abuse.

SYRUP OF IPECAC

HISTORY

The naturalist, Georg Marggraf and the physician Willem Pison first reported the use of ipecacuanha root (Rio ipecac) based on their travels in Brazil during the early 1900s.[58] Le Gras imported ipecacuanha root from South America in the latter 17th century as a treatment for flux (dysentery) and the ague (fever).[59] Jean-Adrien Helvetius used ipecacuanha root to treat members of the French Royalty for dysentery; both the root and powder became widely available in Europe during the 18th century. Pelletier isolated emetine from the mixture of alkaloids in extracts of ipecac, while working at the School of Chemistry in Paris in 1817. Later, the major methoxylated alkaloid retained the name emetine, whereas the other principal alkaloid with a free phenol group was named, cephaeline.[60] In 1908, Harrison first described ipecac poisoning when his stable boy developed severe vomiting and died soon after the incidental ingestion of fluid extract of ipecac (vinum ipecacuanha).[61] The pathology of muscle damage caused by the administration of emetine as an anthelminthic was described in the 1920s.[62] Although multiple deaths were attributed to the use of emetine during the first half of the 19th century, the lack of sufficient clinical details in these case reports limits conclusions regarding the contribution of toxic myocarditis to these deaths.[63] In the late 1950s, dehydroemetine was introduced as a more efficacious and less toxic treatment for amebiasis than emetine. Emetine was investigated as a chemotherapeutic agent for cancer treatment in the early and mid-19th century.[64] The more potent fluid extract of ipecac was removed from the US Pharmacopeia in the mid-1960s because of the severe toxicity associated with use of the extract instead of the syrup. In the United States, the use of emetine for the treatment of amebiasis ceased in the 1960s. The use of syrup of ipecac as an emetic for the treatment of poisonings has declined dramatically over the last 2 decades.

IDENTIFYING CHARACTERISTICS

Structure

The active ingredients in syrup of ipecac are emetine (CAS RN: 483-18-1) and cephaeline (CAS RN: 483-17-0). Figure 12.3 and Figure 12.4 display the chemical structures of emetine and cephaeline, respectively. Table 12.3 and Table 12.4 list some physiochemical properties of emetine (methyl cephaeline, $C_{29}H_{40}N_2O_4$) and cephaeline ($C_{28}H_{38}N_2O_4$), respectively.
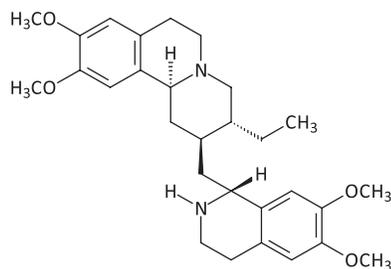
FIGURE 12.3. Chemical structure of emetine.
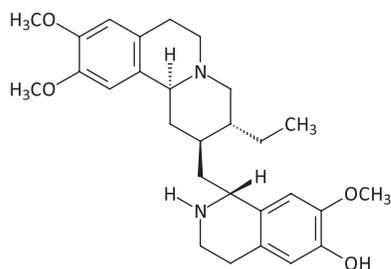
FIGURE 12.4. Chemical structure of cephaeline.

TABLE 12.3. Some Physiochemical Properties of Emetine.

Physical Property	Value
Molecular Weight	480.6389 g/mol
Melting Point	74°C (165.2°F)
log P (Octanol-Water)	5.200
Water Solubility	984 mg/L (15°C/59°F)
Vapor Pressure	2.47E-12 mm Hg
	(25°C/77°F)

TABLE 12.4. Some Physiochemical Properties of Cephaeline.

Physical Property	Value
Molecular Weight	466.6123 g/mol
Melting Point	115.5°C (239.9°F)
log P (Octanol-Water)	4.890

Form

Syrup of ipecac is available in liquid form. Ipecac fluid extract is 14-fold more potent than syrup of ipecac. The United States Pharmacopeia (USP) guidelines state that syrup of ipecac should contain 1.23–1.57 mg/mL total ether soluble alkaloids (minimum 90% cephaeline and emetine).[65] However, the content of these alkaloids varies with manufacturing processes; the actual content of cephaeline and emetine may not meet the USP standards. Typically, the amount of cephaeline exceeds the amount of emetine in a 30-mL bottle of syrup of ipecac with a ratio ranging from approximately 1.5–3.

EXPOSURE

Although eating disorders occur in either gender and in various age groups, young women comprise the vast majority of patients with eating disorders. In a 5-year study of outpatients presenting to a suburban eating disorder clinic, there were 815 females (98.1%) and 16 males (1.9%) ranging in age from 11–74 years.[66] Of the 851 outpatients, 3.1% had a history of chronically abusing ipecac and 1.1% admitted the current abuse of ipecac. Anorexic and bulimic patients also use digital stimulation to induce vomiting.

The principal alkaloids in syrup ipecac are emetine and cephaeline; the sources of these compounds are the ground roots of *Psychotria ipecacuanha* (Brot.) Standl. (*Cephaelis ipecacuanha*, *Cephaelis acuminata*, *Uragoga acuminata*) from the Rubiaceae family. This small perennial shrub reaches 20–40 cm in height with opposite decussate leaves and white flowers (compact cymes). The twisted, fragmented roots are 6–15 cm in diameter. Although the structure of cephaeline and emetine are similar, emetine is the focus of the majority of toxicologic research on syrup of ipecac because of its relatively greater toxicity.[67]

DOSE EFFECT

Medical Use

Syrup of ipecac is no longer recommended for the standard treatment of poisonings. Previous single-dosing recommendations were 5–10 mL (120–240 mL water) in children aged 6–12 months, 15 mL (120–240 mL water) in children aged 1–12 years, and 15–30 mL (240 mL water) in adolescents and adults. The dose may be repeated in all age groups if vomiting has not occurred within 20–30 minutes after initial administration. There are limited data on the threshold doses and dose response for muscle damage following abuse of ipecac. In phase I dose-ranging studies of emetine as an intravenous (IV) chemotherapeutic agent, severe muscle weakness began at cumulative IV doses of approximately 15 mg/kg.[64] The suggested emetine dose for chemotherapy was 1.5 mg/kg intravenously weekly for a minimum total dose of 15 mg/kg.

Toxicity

In the older medical literature, a toxic cumulative emetine dose during therapeutic use was approximated at 20 mg/kg;[68] however, this toxic threshold is difficult to evaluate because of the potential effect of unreported medical conditions (e.g., parasitic infections, liver abscess, pneumonia, sepsis) associated with patients treated for amebiasis during this period. A 4-year-old boy received 60 mL ipecac fluid extract after ingesting commercial antacid tablets containing oil of peppermint, calcium carbonate, magnesium carbonate, and

magnesium trisilicate.[60] He was discharged home; he continued to vomit and developed diarrhea and fever. About 36 hours after the initial visit, he was hospitalized in profound shock with a heart rate of 200 beats per minute; he died 8 hours later. Death was ascribed to ipecac poisoning, but the actual cause of death was unclear because of multiple potential causes.

The surreptitious use of syrup of ipecac and the lack of laboratory confirmation of chronic abuse limit the estimation of the cumulative toxic dose necessary to cause damage. Consequently, the total emetine dose required to produce the onset of myopathy varies widely (i.e., 500–36,000 mg). [69] The escalating weekly abuse of syrup of ipecac from 30–210 mL over 6 months by a 19-year-old woman was associated with moderate to marked muscle weakness, waddling gait, dyspnea, palpitations, left atrial enlargement, and a reduced left ventricular ejection fraction (40%).[70] She also had mild elevation of serum muscle enzymes and hepatic aminotransferases along with normal electrolytes except mild hyperkalemia (5.4 mEq/L). She improved markedly with abstinence. A 26-year-old woman developed mild generalized motor weakness, dyspnea, QT_c prolongation (480 ms), and mild global ventricular dysfunction (ejection fraction, 45%).[71] She admitted the use of approximately 1,300–2,000 mL syrup of ipecac, and she improved following cessation of ipecac use. A prospective review of cases of chronic syrup of ipecac abuse revealed a range of 100–2,000 estimated lifetime doses.[72] A severe skeletal myopathy developed in the patient who had ingested 2,000 doses.

TOXICOKINETICS

Absorption

Both emetine and cephaeline are rapidly absorbed from the GI tract after ingestion, but the amount absorbed is highly variable depending primarily on the amount of emesis produced by syrup of ipecac. In a study of 12 volunteers receiving 30 mL syrup of ipecac (~25 mg cephaeline, ~15 mg emetine), all volunteers vomited and the recovery of both alkaloids was $76 \pm 14\%$ of the administered dose.[73] Peak plasma alkaloid concentrations occurred within 1 hour as measured by HPLC with fluorescence detection. The LOD for cephaeline and emetine was 1 ng/mL and 2.5 ng/mL, respectively. In a study of 10 healthy volunteers receiving 30 mL syrup of ipecac (45 mg cephaeline, 13.8 mg emetine), the mean peak plasma cephaeline concentration was 16.5 ± 13.5 ng/mL at 20.5 ± 10.9 minutes, whereas the mean peak plasma emetine concentration was 9.6 ± 4.1 ng/mL at 19.0 ± 8.1 minutes.[74] In a study of 10 emergency department patients

receiving 30 mL syrup of ipecac as a gastric decontamination measure, the range of plasma concentrations at 30 minutes after administration were as follows: cephaeline, nondetectable–73 ng/mL; emetine, nondetectable–68 ng/mL.[75] All patients vomited within 30 minutes after administration of syrup of ipecac.

Distribution

Volume of distribution (V_d) of emetine is probably large based on the prolonged excretion of emetine from the body during chronic use; however, determination of the V_d is limited by few data on pharmacokinetics in volunteer studies or patients abusing syrup of ipecac.

Biotransformation

There are few *in vivo* data on the biotransformation of emetine and cephaeline in humans. *In vitro* studies in human hepatic microsomal enzyme systems indicate that CYP2D6 converts emetine to cephaeline and 9-*O*-demethylemetine, whereas CYP3A4 catalyzes the conversion of emetine to cephaeline, 9-*O*-demethylemetine and 10-*O*-demethylemetine.[76] In rats, cephaeline (6′-*O*-demethylemetine) undergoes rapid conjugation with glucuronide (cephaeline-6′-*O*-glucuronide) and primarily biliary excretion, whereas emetine is demethylated to cephaeline and 9-*O*-demethylemetine before glucuronidation.[77] The biliary excretion of emetine metabolites is substantially lower than cephaeline metabolites (i.e., 57% vs. 7% of the administered dose, respectively).

Elimination

The elimination of cephaeline and emetine from the plasma following absorption is relatively rapid (i.e., 12–24 h), depending on the absorbed amount of these alkaloids. In a study of 12 volunteers receiving a single dose of 30 mL syrup of ipecac (~25 mg cephaeline, ~15 mg emetine), neither alkaloid was detectable in plasma 6 hours after administration as measured by HPLC with fluorescence detection.[73] All of these volunteers vomited after administration of syrup of ipecac. Two volunteers receiving 20 mL syrup of ipecac (~17 mg cephaeline, ~10 mg emetine) did not vomit, and their plasma contained detectable concentrations of these 2 alkaloids 24 hours after ingestion. The persistence of these alkaloids in the urine suggests that they may accumulate after chronic administration, particularly if vomiting does not occur after use; however, there are few data confirming or quantitating the accumulation of these alkaloids. The plasma elimination half-life of these 2 compounds in rodent studies follows a biexponential

decrease with a half-life of approximately 3–9 hours (cephaeline) and 65–163 hours (emetine).[77]

Maternal and Fetal Kinetics

The P-glycoprotein transport protein on the placental syncytiotrophoblast prevents maternal–fetal transfer of emetine;[78] however, forceful vomiting induced by syrup of ipecac may cause premature uterine contractions in pregnant mothers.[79] The alkaloids of syrup of ipecac are not known to transfer into breast milk.

Tolerance

Chronic abuse of syrup of ipecac can lead to physiologic tolerance, prompting the user to increase dosing to induce vomiting. Although tolerance to the emetic effects may develop, escalation of the dose of ipecac may cause increased adverse effects (diarrhea, tachycardia, myopathy).[80]

Drug Interactions

There are few data on the drug interactions associated with the use of syrup of ipecac.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Emetine and cephaeline in syrup of ipecac irritate stomach epithelium; stimulation of gastric mucosal sensory receptors activates the vomiting center in the brain. These alkaloids also directly stimulate the chemoreceptor trigger zone in the area postrema of the medulla.[81]

Mechanism of Toxicity

Accumulation of emetine following chronic syrup of ipecac ingestion causes damage to both skeletal and cardiac muscle, but the injury pattern is not unique to emetine or the abuse of syrup of ipecac. In animal studies, emetine affects phase 0 depolarization, similar to quinidine.[82] Emetine blocks cardiac L-type calcium channels and fast sodium channels *in vitro*,[83] suggesting an electrochemical contribution to the cardiotoxicity of emetine and the development of cardiac dysrhythmias.[84,85] The use of emetine as an anthelminthic is associated with a variety of electrocardiographic changes including PR and QT_c prolongation, nonspecific ST-T changes, and T-wave inversions, but serious cardiomy-

opathies following the use of emetine as an anthelminthic are rare.[86]

Muscle

Isolated necrotic fibers and granular basophilic fibers appear in microscopic examination of muscle biopsy from patients with myopathies associated with emetine use; however, hypertrophic fibers are usually absent.[87] There is a predominance of type I fibers and a slight decrease in the average diameter of muscle fibers along with swollen core-targetoid fibers and intracytoplasmic inclusion bodies in the type I fibers. Type II-A and II-B fibers occasionally may be affected.[88] Perifascicular atrophy and inflammatory cell infiltrates are usually absent.[70] Electron microscopy reveals sarcomeric and sarcotubular abnormalities including Z-line streaming, myofibrillar disorganization, and increased lysosomal activity.[89] Expansion of electron dense material extends into the A- and I-bands; occasionally, the entire sarcomere or adjacent sarcomeres become involved. The granular breakdown of myofilaments and sarcomeres results in the formation of cytoplasmic bodies, which include the Z-band material. These cytoplasmic bodies appear as central granular electron-dense cores of various shapes and sizes surrounded by electron-lucent halos containing 7- to 10-nm filaments and glycogen granules in the marginal zone.[90] The necrotizing myopathy associated with syrup of ipecac abuse differs from the vacuolar myopathy associated with hypokalemia in bulimic patients abusing diuretics and laxatives.

There is substantial variation in the toxicity of emetine between animal species with dogs being particularly sensitive.[91] In rat studies, emetine causes a necrotizing myopathic process with vacuolar degeneration, disruption of myofibrils, and fine cytoplasmic body formation, probably as a result of direct toxicity at the subcellular level.[92] These pathologic changes in muscle are not associated with recognizable morphologic or physiologic alterations in the neurons innervating the muscle units.[93] Examination of myocardial biopsies from dogs administered 3.3–32 mg/kg over 5–32 days demonstrated partial to complete loss of mitochondrial cristae with the rest of the myocardial fine structure intact.[94] These changes occur in both type 1 and type 2 fibers. Electron microscopy reveals degenerative changes primarily in type 1 fibers including Z-band streaming, paucity of mitochondria, dense body formation in the sarcoplasm, and sarcotubular lesions.[95] The severity and distribution of these abnormalities increase with increasing exposure time. Muscle tissue exhibits anaerobic glycolysis with lactate formation; the loss of

myosin ATPase and myosin dehydrogenase resolve spontaneously after cessation of syrup of ipecac abuse.[90]

HOMEOSTASIS

Repeated vomiting from chronic ingestion of syrup of ipecac may cause hypochloremic metabolic alkalosis and hypokalemia resulting from chloride depletion, contraction of extracellular volume, secondary hyperaldosteronism, increased renal tubular sodium absorption, and increased urinary excretion of potassium.[96] Metabolic alkalosis results from the loss of hydrogen ion from the gastric secretions and the renal tubular secretion of hydrogen ion with the absorption of bicarbonate. The transcellular potassium redistribution from the extracellular to the intracellular compartments maintains the alkalosis. Low urinary chloride reflects the contraction of intravascular volume, and to a lesser extent, loss of chloride in gastric secretions.

Postmortem Examination

Dilated cardiomyopathy and evidence of cardiac muscle injury occur in cases of chronic syrup of ipecac abuse. In a case of Munchausen syndrome by proxy with documented postmortem cephaeline and emetine concentrations, the postmortem examination of a 47-month-old child revealed ascites, bilateral pleural effusions, and biventricular dilation.[97] The skeletal muscle examination by light microscopy was normal, but examination of cardiac muscle demonstrated myofibrillar degeneration and myocytolysis in the outer third of the left ventricular free wall without inflammation or fibrosis. Electron microscopy revealed local zones of myofibrillar lysis with periodic clumps of Z-band material resembling cytoplasmic bodies. Rare case reports on the administration of emetine for amebiasis associated therapeutic emetine doses with interstitial myocarditis; however, the contribution of emetine to these deaths is unclear.[98]

CLINICAL RESPONSE

Illicit Use

Clinical features of chronic ipecac abuse include diarrhea, vomiting, abdominal cramps, muscle stiffness and weakness, mild tremor, fatigue, and peripheral edema. Frequent vomiting may cause erosion of the tooth enamel (perimolysis), particularly of the posterior occlusal surfaces of the maxillary incisors.[99] These changes may result in excessive sensitivity to temperature changes in the mouth, particularly when eating hot or cold food. Old amalgams may rise above the surface of the tooth as a result of the erosion of the enamel. Self-induced vomiting may cause trauma to the dorsum of the dominant hand, manifest by lesions ranging from superficial ulcerations to hyperpigmented scars.[100] Other abnormalities of the upper GI tract include chronic pharyngitis, benign parotid enlargement, and esophagitis.[101,102] Gastrointestinal complications of the abuse of ipecac include esophageal rupture (Boerhaave syndrome), mucosal tear at the gastroesophageal junction (Mallory-Weiss syndrome), hemorrhagic gastritis, reflux esophagitis, and ileus.[103] Malnutrition may cause dermatologic abnormalities similar to dermatomyositis; however, the lack of inflammation of muscle tissue on biopsy distinguishes emetine myopathy from dermatomyositis.[104] Follicular hyperkeratosis may result from vitamin C deficiency. Repeated vomiting from chronic ingestion of syrup of ipecac may cause aspiration pneumonitis.

BEHAVIORAL ABNORMALITIES

Behavioral and mental status changes are not usually associated with emetine toxicity.

MEDICAL COMPLICATIONS

In studies of emetine for the treatment of amebiasis, the most common adverse effects other than local pain were general weakness and electrocardiographic changes in about one-half of the patients along with precordial chest pain in about one-third of the patients.[105] Other toxic effects included diarrhea, nausea, and less commonly tachycardia.

PERIPHERAL MYOPATHY.   Case reports of patients with anorexia nervosa or bulimia nervosa associate the chronic abuse of ipecac with reversible myopathy, manifest by proximal muscle weakness, waddling gait secondary to extensor muscle weakness in the hips, winged scapula, dysphagia, and weakness of the neck flexors. Typically, severely affected patients have difficulty arising from a chair or horizontal position, lifting their arm over their neck, climbing stairs, and crossing their legs. Often, Gower's sign (standing by the patient, bring the hands proximally up the legs) is positive. Proximal muscles are more affected than distal muscles. Examination of mental status, cranial nerves, muscle bulk, coordination, and sensation are usually normal. Although deep tendon reflexes (DTRs) may be sluggish, reflexes typically are normal. The neuromuscular abnormalities associated with chronic abuse of syrup of ipecac are similar to the myopathy associated with the

administration of emetine.[106] Occasionally, these patients may develop mild sensory disturbances, tremors, and myalgias. Muscle strength typically returns to baseline within several weeks to 5 months.

CARDIOMYOPATHY. Rarely, congestive heart failure is associated with anorexia nervosa and bulimia, particularly during the initial phase of nutritional rehabilitation.[107] Although mild global left ventricular dysfunction and nonspecific electrocardiographic changes are common in patients with marked skeletal myopathy from ipecac abuse, fulminant heart failure does not usually occur in patients abusing ipecac. A 26-year-old woman developed skeletal muscle weakness, dyspnea on exertion, mild basilar rales, QT_c prolongation (480 ms), and mild left ventricular dysfunction (ejection fraction, 45%) after abusing syrup of ipecac for 5 months.[71] She did not develop any dysrhythmias, and she improved with cessation of ipecac use. Other cardiac abnormalities associated with the abuse of syrup of ipecac include left atrial enlargement and mitral valve prolapse.[70] Clinical signs of congestive heart failure occur rarely following the use of emetine as an anthelminthic, primarily following IV administration or the ingestion of concentrated liquid extract.

Fatalities

Sudden death is a complication of anorexia nervosa and bulimia (e.g., refeeding phase, liquid-protein diets, starvation-induced QT_c prolongation), even in the absence of ipecac abuse.[108,109] Although case reports associate sudden death with the abuse of ipecac in the clinical setting of anorexia nervosa and bulimia, the contribution of ipecac is not clearly defined. Several of these case reports document complications (myocarditis, pericarditis, pulmonary embolism, pneumonia) not usually associated with the alkaloids in syrup of ipecac.[110,111] A 17-year-old adolescent developed generalized myalgias, bilateral ankle swelling, progressive skeletal muscle weakness, hypotension, intractable congestive heart failure, and cardiac arrest unresponsive to medical management.[112] Subsequently, 12 empty bottles of syrup of ipecac were found under her bed. The postmortem examination demonstrated vacuolar degeneration and focal myocardial necrosis. The urine screen was positive for emetine, but emetine apparently was not detected in the postmortem blood.

Abstinence Syndrome

An abstinence syndrome has not been associated with the cessation of syrup of ipecac abuse.

Reproductive Abnormalities

Abuse of syrup of ipecac causes maternal volume depletion with potential effects on fetal homeostasis, amniotic fluid volume, and uterine and placental perfusion; however, these effects are not well reported in the medical literature.

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantitation of emetine and cephaeline in biologic samples include spectrophotometry,[113] thin layer chromatography (TLC),[114] high performance liquid chromatography (HPLC) with fluorescence detection,[115] reverse-phase liquid chromatography with UV detection at 254 nm,[116] and HPLC with photodiode array detection.[117] The LOD for these 2 alkaloids in urine as measured by liquid chromatography with fluorescence detection was 5 ng/mL compared with 1 ng/mL and 2.5 ng/mL for plasma samples. The LOD and the lower limit of quantitation (LLOQ) using liquid chromatography with UV detection for analyzing emetine was 50 ng/mL and 100 ng/mL, respectively. Emetine and cephaeline are stable in frozen (−20°C/−4°F) plasma or urine for at least 90 days.[118]

Biomarkers

BLOOD

Figure 12.5 displays the mean plasma cephaeline and emetine concentrations during the first 3 hours after administration of 30 mL syrup of ipecac (45 mg cephaeline, 13.8 mg emetine) to 10 healthy volunteers.[74] A 14-year-old adolescent was admitted to the hospital with nausea, vomiting, diarrhea, and abdominal pain after head trauma from an automobile accident. Subsequently, his surreptitious use of the syrup of ipecac was discovered when analysis of admission blood yielded a serum emetine concentration of >10-fold higher than the upper limit of normal.[119] A 34-month-old child was hospitalized for intractable vomiting, myopathy, and elevated serum creatine kinase.[120] The diagnosis of Munchausen syndrome by proxy was made after LC/MS analysis of 2 serum samples revealed emetine concentrations of 21 ng/mL and 26 ng/mL.

A 26-year-old woman complained of dyspnea, palpitations, and fatigue after consuming 3–4 bottles of ipecac syrup daily for 3 months.[121] In the hospital, she developed biventricular cardiac failure and intractable ventricular fibrillation. Postmortem examination revealed pericardial effusions, a small pulmonary emboli, and
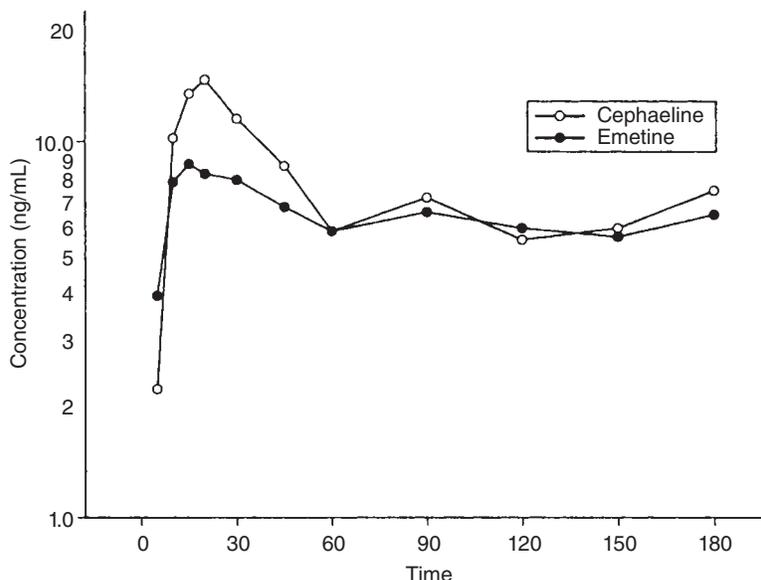
FIGURE 12.5. Mean cephaeline and emetine concentrations during first 3 hours after the administration of 30 mL syrup of ipecac to 10 healthy volunteers. (Reprinted with permission from EJ Scharman, MJ Hutzler, GJ Rosencrance, TS Tracy, Single dose pharmacokinetics of syrup of ipecac, Therapeutic Drug Monitoring, Vol. 22, Issue 5, p. 438, copyright 2000.)

diffuse interstitial edema of the heart. As measured by fluorescence spectrophotometry, emetine was detected in the following tissues: liver, 14 µg/g; kidney, 7.4 µg/g; blood, 2.4 µg/g; bile, 1.9 µg/g; pericardial fluid, 0.7 µg/g; and cerebrospinal fluid, 0.1 µg/g.

URINE

The kidney excretes relatively small amounts of cephaeline and emetine in the urine following the ingestion of syrup of ipecac depending in part on the amount expelled in the vomitus. In a study of 12 volunteers receiving a single 30-mL dose of syrup of ipecac, the urinary excretion of cephaeline and emetine during the first 48 hours after dosing accounted for $0.73 \pm 0.30\%$ and $0.79 \pm 0.24\%$, respectively, of the administered dose.[73] However, these 2 alkaloids were detectable in urine of the volunteers for at least 2 weeks, and in 1 individual up to 12 weeks after administration as measured by HPLC with fluorescence detection. The urine emetine concentration in a child hospitalized for Münchausen syndrome by proxy was 100 ng/mL.[120] One week later, the urine emetine concentration declined to 78 ng/mL.

Abnormalities

Chest X-ray may reveal aspiration pneumonitis in patients with chronic abuse of syrup of ipecac or pneumomediastinum from protracted vomiting. Two-

dimensional echocardiography in patients with marked skeletal myopathy may demonstrate reduced left ventricular ejection fraction, which often is asymptomatic.

ELECTRODIAGNOSTIC STUDIES

Electromyographic features of the myopathy associated with chronic abuse of ipecac include occasional fibrillations, complex repetitive discharges, myopathic motor-unit potentials, and increased insertional activity, particularly in proximal muscles.[69] These abnormalities are usually reversible after cessation of the use of syrup of ipecac. The amplitude of evoked action potentials and nerve conduction velocities are typically normal.

ELECTROCARDIOGRAM

The most common electrocardiographic changes in patients undergoing cancer treatment with emetine were ST-T wave abnormalities, QT prolongation, and premature ventricular contractions.[64,105] Electrocardiographic abnormalities frequently occur in patients with skeletal muscle weakness and evidence of emetine toxicity. The changes associated with the chronic abuse of ipecac include non-specific ST-T wave changes, T-wave inversions, QRS and QT_c prolongation, and sinus tachycardia. These changes are non-specific and may occur without laboratory evidence of electrolyte abnormalities. These changes usually resolve with abstinence from syrup of ipecac.

Blood

The chronic abuse of syrup of ipecac by bulimic patients is frequently associated with mild elevation of serum hepatic aminotransferases (alanine aminotransferase, aspartate aminotransferase), lactate dehydrogenase, and serum muscle enzymes (creatine phosphokinase, aldolase).[122] Hyponatremia, hypochloremia, hypokalemia, hypocalcemia, and hypomagnesemia may be observed with chronic abuse of syrup of ipecac, particularly in combination with diuretics and laxatives.

TREATMENT

Similar to the abuse of diuretics, initial medical management should be directed at the correction of fluid and electrolyte imbalance along with the restoration of a normal diet after an initial evaluation of the adequacy of respiration and cardiac function. These patients should be evaluated for electrolyte (sodium, potassium, chloride, magnesium, calcium), and acid-base abnormalities, muscular weakness, anemia, rhabdomyolysis, volume depletion, renal dysfunction, cardiomyopathy, and electrocardiographic abnormalities. Fluid replacement typically involves replacement of sodium and chloride losses with saline. Rare complications include cardiac dysrhythmias. Vasopressors and invasive monitoring with central venous, pulmonary wedge pressure or noninvasive hemodynamic monitoring may be necessary for severe hypotension associated with severe cardiomyopathy.

Severe hyponatremia should be corrected relatively slowly (<0.55 mEq/L/h) to prevent the development of central pontine myelinolysis. Peripheral edema may occur during the first few weeks after cessation of purging behaviors, which usually responds to salt restriction. Hospitalization may be necessary to correct hypokalemic metabolic alkalosis and to stop the use of ipecac or other surreptitious methods to control weight. Emetine-induced myopathy is usually reversible and resolves spontaneously including return of normal cardiac function.[3]

LAXATIVES

The 5 major classes of laxatives include the following: 1) lubricant (liquid paraffin, mineral oil), 2) bulk (psyllium), 3) stool softeners, 4) osmotic (lactulose, magnesium sulfate/citrate, sodium sulfate, sorbitol), and 5) stimulant (aloes, bisacodyl, buckthorn, cascara, castor oil, danthron, frangula, phenolphthalein, rhubarb,

sagrada, senna, sodium picosulfate).[123] Danthron (synthetic anthraquinone) and phenolphthalein (diphenylmethane derivative) are banned from sale in the United States as a result of animal studies suggesting an increased risk of cancer.[124] Plant preparations (e.g., aloe, cascara, frangula, rhubarb, senna) contain anthranoid compounds, which are alternatives to other stimulant laxatives (bisacodyl, castor oil, phenolphthalein). The abuse of laxatives involves 1) patients with psychiatric disorders (anorexia nervosa, bulimia nervosa) who surreptitiously use laxatives to lose weight, 2) individuals desiring a specific stool regimen, and 3) patients with Munchausen syndrome who surreptitiously abuse laxatives themselves or administer laxatives to their children (Munchausen syndrome by proxy). The Munchausen syndrome involves the appearance of chronic factitious illness resulting from a desire to manipulate and deceive the medical profession for secondary gain (attention, sympathy, removal from conflict).

In a study of patients with eating disorders, laxative abusers demonstrated more perfectionism and avoidant personality features compared with nonlaxative abusers.[125] Laxative-abusing anorexia nervosa patients had high scores on the histrionic scale, whereas laxative-abusing bulimia nervosa patients had the higher pathologic scores on drive for thinness, body dissatisfaction, passive-aggressive and borderline personality, ineffectiveness, and lack of introspection. In a study of 5,355 young women, individuals abusing laxatives were older, had poor perceived health, and were less likely to seek treatment compared with women engaged in self-induced vomiting.[126] Additionally, laxative-abusing patients with eating disorders also display higher levels of depression and self-directed hostility compared with individuals self-inducing vomiting.[127] Stimulant laxatives are the most commonly abused laxatives by individuals with eating disorders including diphenylmethane compounds (bisacodyl), castor oil, and anthraquinones (aloe, cascara, senna).

Bisacodyl

IDENTIFYING CHARACTERISTICS

Bisacodyl (CAS RN: 603-50-9, $C_{22}H_{19}NO_4$) is a diphenylmethane stimulant laxative used to treat constipation and to promote bowel evacuation; this compound [2-(4,4'-diacetoxydiphenylmethyl)pyridine] is the acetic acid diester of the laxative, diphenol 2-(4,4'-dihydroxy-diphenyl)methyl-pyridine. Table 12.5 lists some physiochemical properties of bisacodyl. Figure 12.6 displays

TABLE 12.5. Some Physiochemical Properties of Bisacodyl.

Physical Property	Value
Molecular Weight	361.3906 g/mol
Melting Point	133.5°C/272.3°F
log P (Octanol-Water)	3.370
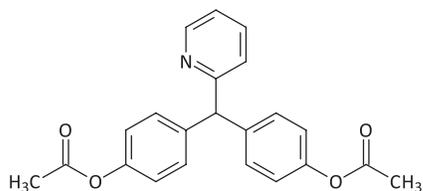
FIGURE 12.6. Chemical structure of bisacodyl.

the chemical structure of bisacodyl. The active metabolite is deacetylbisacodyl or bisacodyl diphenol (dihydroxydiphenylpyridyl methane, CAS RN: 603-41-8). Sodium picosulfate is the sulfate conjugate of bisacodyl that also has laxative properties.

EXPOSURE

Bisacodyl is available as pills, capsules, and rectal suppositories, usually as over-the-counter preparations. Trade names include Bisac-Evac®, Correctol® (Bristol-Myers, Squibb, Princeton, NJ), Dulcolax® (Boehringer Ingelheim, Ingelheim am Rheim, Germany), Ex-Lax Ultra® (Pharmaceutical Corp., East Hanover, NJ) , Feen-a-Mint® (Schering Plough Healthcare Products, Memphis, TN), and Fleet Laxative® (Fleet Laboratories, Lynchburg, VA), Typically, these preparations are available as 5 mg enteric coated tablets or 10 mg suppositories.

DOSE EFFECT

The typical therapeutic dose of bisacodyl for bowel evacuation prior to medical procedures or the treatment of constipation is 5–15 mg orally or rectally. Thresholds for toxic doses of laxatives depend on nutritional and fluid intake, which varies significantly among patients with eating disorders. A single therapeutic dose of bisacodyl may cause diarrhea; however, the cumulative dose to produce dehydration and electrolyte disturbances is highly variable.

TOXICOKINETICS

Absorption

The absorption of bisacodyl depends on the formulation (e.g., enteric coated tablet, solution, dragée, suppository). Following administration of suppositories, the absorption of bisacodyl is minimal despite the relatively prompt onset of laxative effects (15–60 min). In a study of 16 volunteers receiving a single 10 mg bisacodyl suppository, the mean time to maximum serum concentration of the active deacetylated metabolite, bis-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM, bisacodyl diphenol) was 0.67 ± 0.11 hours with a mean total serum BHPM concentration (free plus conjugate) of 35.48 ± 2.60 ng/mL (range, 10–55 ng/mL).[128] Following ingestion of a solution containing 10 mg bisacodyl, the mean peak total bisacodyl concentration (free + conjugated) in plasma samples from 12 healthy volunteers was 236 ± 59 ng/mL with a mean time to peak concentration of 1.7 hours.[129] Plasma BHPM concentrations were nondetectable in 6 of these 12 volunteers after administration of a single 10 mg bisacodyl suppository despite the rapid onset of catharsis (mean, 20 ± 10 minutes).

Biotransformation

Endogenous esterases in the wall of the large intestine rapidly hydrolyze bisacodyl to the active metabolite [bis-(p-hydroxyphenyl)-pyridyl-2-methane, BHPM].[130] In contrast to the formation of BHPM, bacterial action is necessary to convert sodium picosulfate or sennosides to their respective active metabolites. Following hydrolysis, BHPM undergoes conjugation with glucuronide before excretion in the urine. Glucuronidation of BHPM occurs in the intestinal wall rather than the liver. BHPM appears in the stool as the free form.

Elimination

The elimination of bisacodyl depends on the dosage form. In a study of 16 volunteers receiving 10 mg bisacodyl suppository, urinary BHPM accounted for $3.4 \pm 0.5\%$ of the administered dose.[128] Following the ingestion of 10 mg bisacodyl enteric-coated tablets by 5 healthy volunteers, urinary excretion of BHPM glucuronide accounted for approximately 10% of the bisacodyl dose during the first 48 hours after ingestion.[130] A majority of the administered dose of bisacodyl appears in the stool as free BHPM. Following ingestion of a solution containing 10 mg bisacodyl, approximately $43 \pm 15\%$ of the administered dose appeared in the urine as BHPM gluc-

uronide compared with approximately $9 \pm 3\%$ for the dragée and $3 \pm 1\%$ for the suppository.[129] In a study of 5 healthy volunteers receiving two 5-mg coated bisacodyl tablets, the mean percentage of BHPM glucuronide in the urine was $10.5 \pm 1.1\%$.[130] Analysis of bile from patients receiving bisacodyl indicates that the glucuronide conjugate of deacetylbisacodyl, but not free bisacodyl or deacetylbisacodyl appears in the bile.[131] The liver excretes an estimated 10–30% of the bisacodyl dose in the bile.

Maternal and Fetal Kinetics

The transfer of bisacodyl to breast milk is minimal, primarily because of the poor absorption of this compound from the GI tract.[132]

Tolerance

Although some laxative abusers report the necessity of higher doses of laxatives to achieve the desired effects, the development of tolerance to the effects of stimulant laxatives is not well documented in humans.[133]

Drug Interactions

Major drug interactions between laxatives and pharmaceutic drugs have not been identified. Stimulant laxatives may increase intestinal transit, theoretically reducing drug absorption; however, there are insufficient data to conclude that the use of cathartics significantly alters the absorption of pharmaceutic drugs.[134]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Following hydrolysis of bisacodyl by endogenous esterases, the active metabolite (BHPM) promotes secretion of electrolytes and water along with stimulation of bowel motility by inducing low-grade endothelial inflammation, mediated by activation of the cyclic AMP and cyclic GMP pathways. Chronic use of stimulant laxatives causes dehydration, hyponatremia, and hypokalemia, which are exacerbated by poor mineral intake in patients with eating disorders.[133] Cathartic (atonic) colon is a historic term initially described in 1943 by Heilbrun from case reports of anatomic alteration of the colon secondary to chronic stimulant laxative use, manifest by loss of haustral folds, pseudostrictures (sandglass formed spasms), dilated lumen, and gaping of the ileocecal valve with a predilection for the terminal ilium and right side of the colon.[135] The major histopathologic changes involve mononuclear infiltration of the mucosa

and submucosa of the colon and terminal ileum. Typically, the atrophy, thinning, and mild ulceration of the mucosa and muscular layers of the terminal ileum and ascending colon develop with normal mucosal patterns usually, but not always in the remainder of the colon.[136] In a retrospective review of a convenience series of 29 chronically constipated women with stimulant use (>3 times weekly for >1 y) and 26 women without stimulant laxative use, colonic redundancy and dilation were common in both groups; however, loss of haustral folds was more common in the stimulant laxative group (27.6% vs 0%, $P \leq .005$).[137] Loss of haustral markings occurred in 15 (40.5%) of the stimulant laxative users in the following locations: 1) left colon, 6 patients, 2) right colon, 2 patients, 3) transverse colon, 5 patients, and 4) entire colon, 2 patients. Most case reports of cathartic colon involve middle-aged women using therapeutic doses of laxatives for several decades prior to 1960, raising issues regarding the presence of preexisting abnormalities leading to laxative use and the potential complication of the concomitant ingestion of other toxins (e.g., podophyllin) as a result of the lack of prospective studies.[138,139] A case-control study of 11 matched pairs of chronically constipated women did not detect pathophysiologically significant differences in the electron microscopic examination of endoscopic biopsies of the left colon and rectum between the women chronic stimulant laxative use (>1 y) and those without laxative use.[140]

CLINICAL RESPONSE

Catharsis occurs within 1 hour of the rectal administration of therapeutic doses (i.e., 10 mg) of bisacodyl as a result of the direct action of the active metabolite (BHPM) on intestinal mucosa; however, following ingestion of a bisacodyl solution, the onset of catharsis is delayed approximately 6 hours after administration.[129] Clinical features of laxative abuse in general are nonspecific including abdominal and/or rectal pain, large volume chronic diarrhea alternating with constipation, bloating, nausea, vomiting, edema, myalgias, dehydration, and weight loss.[141] The typical range of bowel movements in healthy adults is 3 times daily to 3 times weekly,[123] whereas stool frequency often exceeds 10 bowel movements daily in chronic laxative abusers. A majority of chronic laxative abuser report nocturnal bowel movements in contrast to most organic causes of diarrhea.[142] Stool volume usually exceeds 1 L daily, and the stool may contain blood.[143] Additionally, these patients may develop peripheral edema, protein-losing gastroenteropathies, malabsorption, fat soluble vitamin deficiencies (A, D E, K), and mild steatorrhea

(>10 g/24 h) as a result of chronic laxative abuse.[144,145] Rarely, case reports associate the chronic abuse of bisacodyl with recurrent renal stones (uric acid/ammonium acid urate) as a result of acidification of the urine during chronic laxative abuse.[146] Historical case reports of cathartic colon primarily involve chronically constipated women with long-term use of therapeutic doses of stimulant cathartics rather than women with eating disorders and stimulant laxative abuse (e.g., bisacodyl).

Clinical features of hypokalemia associated with laxative abuse include muscle cramps, generalized weakness, fatigue, headache palpitations, and abdominal pain (ileus). The differential diagnosis of chronic diarrhea includes inflammatory bowel disease (e.g., Crohn disease), malabsorption syndrome, hormone-related secretory diarrhea (e.g., thyrotoxicosis), and primary or secondary lactase deficiency.

The International Agency for Research on Cancer (IARC) and the US National Toxicology Program do not list bisacodyl as a suspected carcinogen. In a 26-week study of p53(+/-) mice receiving up to 8,000 mg bisacodyl daily by gavage, bisacodyl did not induce drug-related neoplasms.[147] Additionally, bisacodyl did not increase micronuclei in polychromatic erythrocytes or cause transformations in the *in vitro* Syrian hamster embryo cell transformation assay. Although drug tolerance may moderate many of the GI symptoms, case reports associate the development of colonic ischemia following long-term abuse of bisacodyl susceptible patients.[148] Withdrawal symptoms from chronic laxative abuse include rebound edema, constipation, and drug craving.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the detection and quantitation of bisacodyl and BHPM (bisacodyl diphenol) in biological samples include high performance thin layer chromatography,[149] high performance liquid chromatography with diode array detection,[150] GC/MS after trimethylsilylation,[151] capillary GC/MS,[152] and GC/MS with bisphenol A as the internal standard and derivatization of bisacodyl diphenol as a methylated compound. The LOD for BHPM using the latter method in serum is 5 ng/mL compared with 10 ng/mL for capillary GC/MS after enzymatic hydrolysis in the full-scan mode with a coefficient of variation <15%, similar to GC/MS analysis of urine samples after trimethylsilylation.[151] Simple thin layer chromatography techniques can identify bisacodyl and bisacodyl diphenol by blue/light purple coloration after the addition of acetate buffer and glucuronidase;[153]

however, both thin layer chromatography and gas chromatography with flame ionization detection lack specificity in the detection of bisacodyl and other laxatives in urine samples. High performance thin layer chromatography (HPTLC) is the primary commercial screening method to determine the presence of diphenolic (bisacodyl, phenolphthalein) and anthraquinone (cascara, senna) laxatives in biologic samples (urine, stool) after pretreatment of the sample with β-glucuronidase and subsequent column extraction.[154,155] In a study of 11 volunteers receiving 20 mg bisacodyl (standard dose, 5 mg), the sensitivity and specificity for detecting bisacodyl in urine samples using this method is 73% and 91%, respectively.[156] The positive predictive value of these results indicate that the false-positive rate is high (i.e., about 30%) when the prevalence of bisacodyl use in the tested population is <10%. The window of detection of bisacodyl in urine is <24–48 hours after use when screening with HPTLC.

High performance liquid chromatography with UV spectrophotometric diode array detection (225 nm) identifies bisacodyl in urine samples with a LOD of 100 ng/mL.[157] The LOD for bisacodyl diphenol in urine samples using this method is 0.04 µg/mL. Gas chromatography/mass spectrometry is highly specific for bisacodyl in urine after enzymatic cleavage of conjugates. In a study of 8 volunteers ingesting 10 mg bisacodyl, hydrolyzed urine samples collected 12 hours after ingestion from 7 volunteers were positive for bisacodyl as measured by GC/MS.[158] Bisacodyl was not detectable in urine samples collected 24 hours or 48 hours after ingestion. The major bisacodyl metabolite (BHPM, bisacodyl diphenol) is detectable in urine samples by HPLC with a LLOQ in the range of 0.5 µg/mL after hydrolysis.[159] Maximum excretion of the urinary metabolite occurs on the second day after ingestion of bisacodyl. In a study of 3 volunteers ingestion 10 mg bisacodyl, hydrolyzed urine samples collected within 48 hours after ingestion contained 1,000–5,000 ng bisacodyl diphenol/mL compared with 17,000 ng/mL in a random urine sample collected from a 43-year-old woman surreptitiously abusing bisacodyl.[159]

Biomarkers

URINE

Following the ingestion of single doses of laxatives, the parent compounds are detectable only for a few days after use. Following hydrolysis of urine samples with glucuronidase, bisacodyl is detectable by HPTLC up to 32 hours after ingestion of a 5-mg dose.[149] However, this method produces false-positives, and the sensitivity is substantially less for senna than bisacodyl.[156] Bisacodyl

and phenolphthalein are detectable in urine samples up to 4 days after the ingestion of single doses of these laxatives, when measured by electron beam ionization GC/MS with computer-controlled multiple-ion detector (LOD, 10–500 ng/mL).[151] Gas chromatography/mass spectrometry after enzymatic cleavage of conjugates followed by extractive methylation, detects bisacodyl and other stimulant laxatives (anthraquinone, phenolphthalein) and their metabolites in human urine with greater than 96% specificity.[152] As measured by GC/MS, bisacodyl was detectable in hydrolyzed urine in 7 of the 8 volunteers 12 hours after ingestion of 10 mg bisacodyl.[158] The bisacodyl concentrations in stool samples obtained 24 and 48 hours after ingestion were not detectable (level of quantitation not reported).

STOOL

The daily stool weight on a Western diet does not usually exceed 225 g, whereas the volume and weight of the stool increases during laxative abuse.[160] As stool volume reaches 3 L daily, the fecal sodium concentrations approach serum sodium concentrations. In secretory diarrhea secondary to stimulant laxative use (bisacodyl, phenolphthalein, senna), the stool osmolal gap is usually small (<30–40 mOsmol/L).[161] Stool osmolality below plasma osmolality suggests the addition of water to the stools sample; the presence of stool sodium concentrations exceeding plasma suggests the addition of urine to the stool.

Abnormalities

Diarrhea is typically defined as both increased frequency (>3 stools/d) and liquidity; however, patients often do not consider increased frequency of defecation alone as diarrhea.[162] The classification of diarrhea based on stool appearance and clinical characteristics includes the following: 1) watery (liquid stool without fat/blood cells), 2) fatty (positive Sudan stain), and inflammatory (leukocytes on Wright's stain or positive fecal lactoferrin assay).[163] Watery diarrhea is further divided into osmotic diarrhea (fecal osmotic gap >50 mOsm/kg) and secretory diarrhea (malabsorption of fecal electrolytes). The clinical evaluation of chronic diarrhea (>4 weeks duration) is extensive with an infectious etiology being less likely than acute diarrhea (typically, <2 weeks duration). Table 12.6 lists the major causes of chronic diarrhea. Laxative abuse usually causes watery diarrhea as result of the abuse of osmotic laxatives (osmotic diarrhea) or nonosmotic laxatives (secretory diarrhea). Fecal leukocytes may appear following chronic laxative abuse in the absence of fever and bloody diarrhea; consequently, the presence of fecal leukocytes does not exclude the diagnosis of laxative abuse.

The effect of chronic laxative abuse on serum electrolytes and acid-base status is variable and multifactorial as a result of differences in dietary intake and compensatory mechanisms. Metabolic disturbances associated with chronic laxative abuse include fecal sodium and water loss, hypokalemia, metabolic alkalosis with secondary hyperaldosteronism, and renal retention of sodium. Resulting electrolyte abnormalities include hyponatremia, hypokalemia, and hypochloremia. The amount of sodium depletion depends on stool volume, dietary salt intake, and the renin-angiotensin-aldosterone system; however, hyponatremia does not usually occur because of concomitant water loss. The severity of the potassium deficit depends on the severity of the laxative abuse, dietary potassium intake, and plasma aldosterone concentrations; hypokalemia may be severe and associated with rhabdomyolysis. Case reports indicate that ventricular dysrhythmias (e.g., *torsades de pointes*) may occur in the setting of hypokalemia and bisacodyl abuse. A 45-year-old woman required cardioversion for 2 episodes of *torsade de pointes* while hospitalized for the evaluation of chronic diarrhea and hypokalemia ($K^+ = 2.4$ mEq/L).[164] Analytic testing revealed bisacodyl in her urine and she admitted laxative abuse. Hypokalemia facilitates the development of hypochloremic metabolic alkalosis.

TREATMENT

Stabilization

Medical management of the complications associated with chronic stimulant abuse including bisacodyl should be directed at the correction of fluid and electrolyte imbalance along with the restoration of a normal diet after an initial evaluation of the adequacy respirations and cardiac function. These patients should be evaluated for electrolyte (sodium, potassium, chloride, magnesium, calcium) and acid-base abnormalities, anemia, rhabdomyolysis, volume depletion, renal dysfunction, cardiomyopathy, and electrocardiographic abnormalities. Fluid replacement typically involves replacement of sodium and chloride losses with saline; initial treatment begins with isotonic crystalloid solutions, 1–2 L in the adult patient or 10–20 mL/kg in the pediatric patient, followed by maintenance IV infusion and concurrent treatment of electrolyte disturbances (hypokalemia) depending on renal and myocardial function. Subsequent therapy includes volume repletion and gradual correction of hyponatremia and other electrolyte disturbances. Severe hyponatremia should be corrected relatively slowly (<0.55 mEq/L/h) to prevent the development of central pontine myelinolysis. Blood gas analysis may be necessary in more severely ill patients with acid-base

TABLE 12.6. Major Causes of Chronic Diarrhea.[163]

Secretory Diarrhea	Osmotic Diarrhea	Inflammatory Diarrhea	Fatty Diarrhea
Nonosmotic laxative abuse	Osmotic laxative abuse (Mg^{++}, SO_4^{-2}, PO_4^{-3}, lactulose, polyethylene glycol)	Inflammatory bowel disease (Ulcerative colitis, Crohn disease, diverticulitis, ulcerative jejunoileitis)	Malabsorption syndromes (Mucosal diseases, short bowel syndrome, postresection diarrhea, small bowel bacterial overgrowth, mesenteric ischemia)
Congenital electrolyte malabsorption	Carbohydrate malabsorption	Invasive bacterial infection (*Clostridium, Escherichia coli,* tuberculosis)	Maldigestion (Pancreatic insufficiency, reduced luminal bile acid)
Bacterial toxins		Ulcerating viral infection (*Cytomegalovirus, Herpes simplex*)	
Ileal bile acid malabsorption		Invasive parasites (Amebiasis)	
		Ischemic colitis	
Inflammatory bowel disease (Ileal Crohn disease, lymphocytic colitis, collagenous colitis, diverticulitis)		Radiation enterocolitis	
Vasculitis		Neoplasia (Carcinoma of colon, lymphoma)	
Drugs and poisons			
Disordered regulation (Postvagotomy, postsympathectomy, diabetic neuropathy, hyperthyroidism, Addison disease, irritable bowel syndrome, functional diarrhea)			
Neuroendocrine tumors (Gastrinoma, VIPoma, somatostatinoma, mastocytosis, carcinoid syndrome, medullary carcinoma of the thyroid)			
Neoplasia (Carcinoma of colon, lymphoma, villous adenoma)			
Epidemic secretory diarrhea (Brainerd)			
Idiopathic secretory diarrhea (Sporadic)			

disturbances. Rare complications of chronic laxative abuse include cardiac dysrhythmias (*torsade de pointes* or simply "torsade"). The treatment of torsade includes IV magnesium sulfate and, if necessary, overdrive pacing.

Supplemental Care

Psychologic dependence on laxatives occurs in patients with eating disorders; however, physical dependence does not develop other than some bloating and weight gain following cessation of use. Supportive care for patients with chronic laxative abuse include high-calorie, high fiber diet, gradual regularization of stools habits (bulk preparations, saline enemas), and potassium supplements and/or potassium sparing drugs (spironolactone) as needed.[165] Constipation may complicate the recovery period; the use of bulk-type laxatives, bran, regular exercise and adequate hydration are preferable to recurrent use of stimulant or osmotic laxatives. Peripheral edema during the first few weeks after cessation of laxative abuse usually responds to salt restriction. Hospitalization may be necessary to correct severe electrolyte imbalance and to stop the use of laxatives or other inappropriate methods to control weight. Psychotic behavior may occur as a manifestation of pre-existing psychopathology associated with the eating disorder.

Cascara

IDENTIFYING CHARACTERISTICS

Cascara fluid extract contains anthranoid compounds, primarily anthrone *C*-glycosides including cascaroside A [glycoside of barbaloin, 8-*O*-(β-D-glucopyranosyl) barbaloin] and the following free dihydroanthraquinone derivatives: aloe-emodin (CAS RN: 481-72-1), emodin (CAS RN: 518-82-1), and chrysophanol (chrysophanic acid, CAS RN: 481-74-3).[166] Cascaroside A has a molecular weight of 580.53 g/mol with a chemical formula of $C_{27}H_{32}O_{14}$; cascaroside B is the diasteroisomer of cascaroside A. Figure 12.7 displays the chemical structures of these anthraquinone compounds. Anthranoid compounds refer to compounds with sub-

stitutions at C_{10} in the basic anthrace ring as follows: anthrones, C_{10}-H_2; anthraquinones, C_{10} = O; dianthrones, C_{10}-additional anthrace ring. These substances occur as an anthranoid moiety (aglycon) linked with a sugar (e.g., glucose). The hydrophilic properties of these glycosides limit GI absorption.

EXPOSURE

The botanic source of cascara sagrada (cascara) is the bark from the erect shrub, *Frangula purshiana* Cooper (*Rhamnus purshiana* DC.). Various preparations of cascara include casanthranol, cascara sagrada bark, cascara fluid extract, cascara sagrada extract, and cascara sagrada fluid extract. Dried bark of cascara sagrada contains approximately 8–10% hydroxyanthraquinone glycosides; these hydroxyanthracene derivatives have hydroxy groups at the C_1 and C_8 positions and sugar groups at the hydroxyl moiety (*O*-glycosides) or at the C_{10} position (*C*-glycosides). Fingerprint chromatography by HPLC with ultraviolet (UV) and evaporative light scattering (ELS) detection allows the identification and origin of botanic samples of cascara.[167] In 2002, the US Food and Drug Administration (US FDA) concluded that cascara is not generally recognized as safe and effective because of the lack of information submitted to the US FDA during a review of over-the-counter (OTC) laxatives; consequently, the US FDA banned the use of cascara along with aloe in OTC preparations.[168]

DOSE EFFECT

The daily recommended dose for cascara for children is 150–500 mg (~10.5–50 mg hydroxyanthracenes) and 300–1,000 mg (~21–100 mg hydroxyanthracenes). The toxic dose of cascara in humans is not well defined. Although melanosis coli is indicative of cascara abuse over at least 4–12 months, patients abusing these laxatives do not invariably develop this condition even after years of anthraquinone laxative use.

TOXICOKINETICS

Anthrone *C*-glycosides (Cascaroside A–D) in cascara are prodrugs. The absorption of these large molecules is
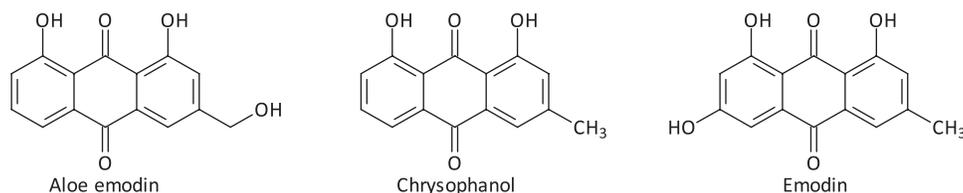
FIGURE 12.7. Chemical structures of 3 common anthraquinone compounds in cascara.

limited until glucosidases in colonic bacteria catalyze the cleavage of the sugar moiety and the formation of free compounds (aglycones). In contrast to the dianthrone *O*-glycosides (e.g., sennosides A and B in senna), bacterial flora in the GI tract do not easily metabolize the anthrone *C*-glycosides; therefore, the laxative response of different animal species to cascara varies substantially.[169] The released anthranoid aglycones (free anthrones) diffuse to the intestinal wall where they alter intestinal secretions and motility.[170] Animal studies suggest that some hydroxylation of these aglycones occurs after absorption in addition to the conjugation of the anthrone compounds to glucuronides and sulfates. Prolonged action of cascara results from the biliary excretion and enterohepatic recirculation of these compounds. The maternal use of cascara is compatible with breastfeeding.[171] Most laxative abusers report the necessity of higher doses of laxatives to achieve the desired effects.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Melanosis coli is a complication of anthraquinone laxative abuse (e.g., cascara), marked by intestinal macrophages filled with a lipofuscin pigment in the lamina propria of the colon. Electron microscopy of these pigmented lesions reveals apoptosis of surface epithelial cells in the colon and phagocytosis of resulting apoptotic bodies by intraepithelial macrophages. These macrophages migrate to the lamina propria where intracellular degradation of the apoptotic bodies results in the formation of lipofuscin.[172] Lipofuscin pigmentation may spread to the submucosa and into pericolonic lymph nodes.[173]

CLINICAL RESPONSE

The onset of action of the laxative effects of cascara is about 6–8 hours. Most of the clinical features of chronic laxative abuse are nonspecific with the exception of melanosis coli. Chronic anthraquinone laxative abuse (cascara, senna) may cause the benign condition (i.e., melanosis coli), manifest by the asymptomatic accumulation of dark brown-black discoloration (lipofuscin-like, pigment-laden macrophages) in the submucosa of the colon in a pattern similar to alligator skin. This process occurs primarily following chronic ingestion of anthraquinone laxatives, but occasionally melanosis coli is associated with bisacodyl or phenolphthalein abuse as well as lead and mercurous chloride poisoning.[174] Extensive melanosis coli may be difficult to distinguish from inflammatory or ischemic bowel disease during endoscopic visualization. Confirmatory diagnosis of melanosis coli is made via biopsy.[175] Rarely, the abuse of cascara is associated with gastric melanosis.[176] Pathologic changes associated with melanosis coli typically appear after 4–12 months of laxative abuse, and these abnormalities disappear over a similar period. Rare case reports associate the use short term use of cascara (3 times daily for 3 d) with the development of portal hypertension and severe cholestatic hepatitis that resolved following cessation of cascara use.[177] The liver biopsy demonstrated moderately severe acute and chronic portal inflammation including a large number of eosinophils suggesting an immune-mediated hepatitis. Experimental studies indicate that cascara lacks long term carcinogenic potential as defined by the absence of initiating properties and weak or absent promoting activity.[178,179]

DIAGNOSTIC TESTING

Analytic methods for the quantitation of anthranoid compounds in cascara sagrada and cascara sagrada fluid extract include thin layer chromatography,[180] capillary electrophoresis,[181] and HPLC with UV detection (254 nm).[166] The use HPLC with fluorescence detection (excitation 435 nm and emission 515 nm) allows the quantitation of aloe-emodin, rhein, emodin, and chrysophanol in plasma samples with a LLOQ of 6.5 ng/mL, 20 ng/mL, 40 ng/mL, and 15 ng/mL, respectively.[182] Rare case reports associate chronic cascara use with severe hypokalemia, hypocalcemia with tetany, metabolic acidosis, and renal dysfunction.[183]

TREATMENT

Medical management of the complications associated with chronic stimulant abuse including cascara should be directed at the correction of fluid and electrolyte imbalance along with the restoration of a normal diet after an initial evaluation of the adequacy of respirations and cardiac function. Symptomatic patients should be evaluated for electrolyte (sodium, potassium, chloride, magnesium, calcium) and acid-base abnormalities, anemia, rhabdomyolysis, volume depletion, renal dysfunction, cardiomyopathy, and electrocardiographic abnormalities. Treatment is supportive, similar to bisacodyl abuse.

Castor Oil

IDENTIFYING CHARACTERISTICS

Castor oil (CAS RN: 8001-79-4) is a colorless to pale yellow viscous liquid with a slightly acrid taste and a

specific gravity of 0.945–0.965 at 25°C (77°F).[184] This compound is poorly soluble in water (<1 mg/mL at 20°C/68°F) with a viscosity of 283 cP at 37°C (98.6°F). The boiling point of castor oil is 313°C (595.4°F); the maximum absorbance of this compound is 270 nm. Other names for castor oil include ricinus communis seed oil, castor seed oil, oleum ricini, ricinol, and ricinus oil. The major constituent in castor oil is the unsaturated fatty acid, ricinoleic acid (CAS RN: 141-22-0, $C_{18}H_{34}O_3$). This C_{18} aliphatic fatty acid is structurally similar to some dietary fats (e.g., oleic acid) and to hydroxylated C_{18} fatty acids that occur in the gut as a result of enzymatic hydration of unabsorbed dietary fats by colonic bacteria. Ricinoleic acid is a colorless liquid that is insoluble in water. Table 12.7 lists some physiochemical properties of ricinoleic acid. Ricinine is a piperidine alkaloid (3-cyano-4-methoxy-N-methyl-2-pyridone, CAS RN: 524-40-3) that occurs in both castor beans and castor oil. Table 12.8 lists some physiochemical properties of ricinine. Figure 12.8 displays the chemical structures of ricinoleic acid and ricinine.

EXPOSURE

The seeds of *Ricinus comminus* L are the source of this ancient cathartic known to early Egyptians, Greeks, and Romans.[185] The castor bean contains ricin, ricin agglutinin, and ricinine. Ricinine is a biomarker for ricin because both compounds share a similar plant source in the castor bean and leaves. Methods of extraction include the use of solvents or mechanical techniques (crushing, grinding, cold pressing). The latter process leaves a residue (pomace) that contains most of the ricin and an allergen. Ricin is water soluble and this toxin does not partition easily into the oil extract; however, castor oil does contain some ricinine. Ricinoleic acid is the major constituent of castor oil, accounting for about 80–90% of the fatty acid content. As measured by GC,

a sample of castor oil contained the following fatty acids: 1) ricinoleic acid, 82%; 2) C_{18} acids (linoleic oleic, stearic acids), 16%; and 3) palmitic acid, 2%.[186] This seed oil is a fragrance ingredient and skin-conditioning agent in cosmetics (e.g., emollient, surfactant), a folk remedy for the induction of labor, and an industrial lubricant; the many hydroxylated triacylglycerols in the seed oil are a raw material for surface coatings and polymer formulations. The main medicinal use of castor oil is as a purgative. The oil seed extract is a popular lay drug for the induction of labor in a nonmedical setting; however, there are inadequate clinical data to support this indication.[187] Castor oil is classified by the US Food and Drug Administration as generally recognized as safe and effective for use as a stimulant laxative. Finished food may contain castor oil (<500 ppm) as a diluent, release/antisticking agent, or flavoring agent; castor oil is also a plasticizer in rubber food containers.[184] Castor oil was a component of some mummification balms detected in Egyptian mummies (e.g., Ptolemaic era, circa 100 BC).[188]

DOSE EFFECT

The acceptable daily castor oil intake is 0.7 mg/kg body weight as established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee.

TABLE 12.8. Some Physiochemical Properties of Ricinine.

Physical Property	Value
Molecular Weight	164.16132 g/mol
Melting Point	201.5°C (394.7°F)
log P (Octanol-Water)	−0.450
Water Solubility	2700 mg/L (10°C/50°F)
Vapor Pressure	4.57E-05 mm Hg (25°C/77°F)

TABLE 12.7. Some Physiochemical Properties of Ricinoleic Acid.

Physical Property	Value
Molecular Weight	298.4608 g/mol
Melting Point	5.5°C (41.9°F)
Boiling Point	245°C (473°F)
Density	0.9236 (22°C/71.6°F)
log P (Octanol-Water)	6.190
Water Solubility	3460 mg/L (25°C/77°F)
Vapor Pressure	2.63E-09 mm Hg (25°C/77°F)
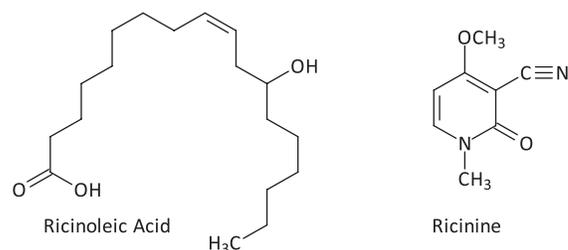
FIGURE 12.8. Chemical structure of ricinoleic acid.

TOXICOKINETICS

There are few clinical data on the toxicokinetics of castor oil and ricinoleic acid in humans. Animal studies indicate that pancreatic enzymes hydrolyze castor oil to glycerol and ricinoleic acid. Esterification of ricinoleic acid occurs in the intestinal epithelial cells, followed by entry into the chyle and distribution into adipose tissue similar to other fatty acids.[189] Potential metabolites from the cyclization of a hydroxylated β-oxidation intermediate include epoxydicarboxylic acids, primarily 3,6-epoxyoctanedioic and 3,6-epoxydodecanedioic acids with lesser amounts of 3,6-epoxydecanedioic acid. 3,6-Epoxyoctanedioic acid is a normal constituent of urine; however, the source of this fatty acid is unknown.[190] Figure 12.9 displays the proposed biotransformation of ricinoleic acid.
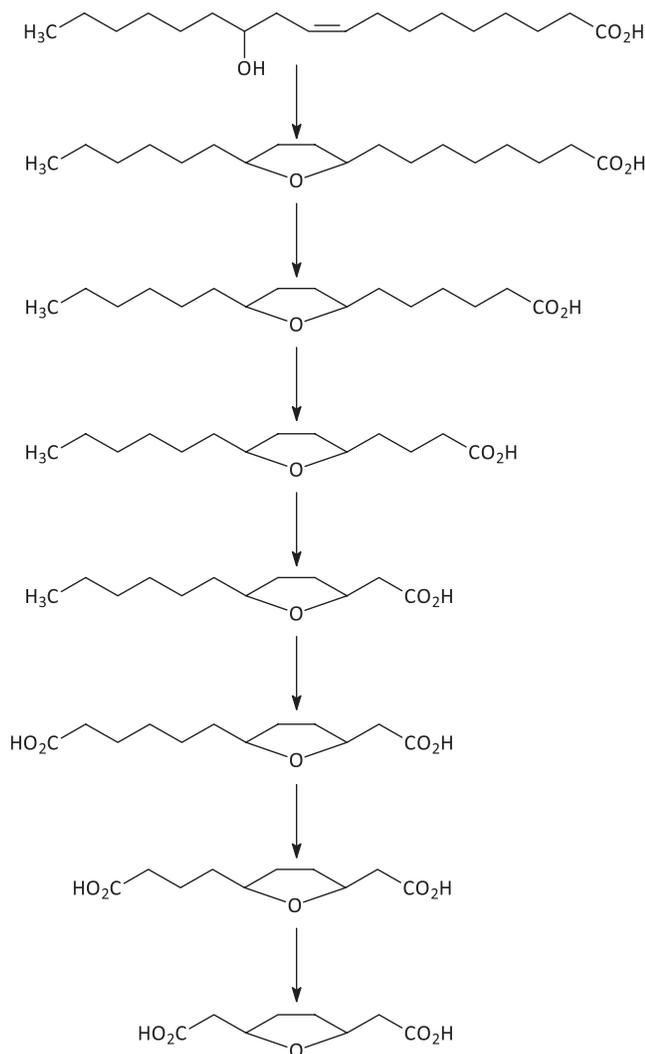
FIGURE 12.9. Proposed metabolism of ricinoleic acid.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Long chain fatty acid in castor oil including ricinoleic acid inhibits net water and electrolyte absorption at the luminal surface, resulting in the secretion of electrolytes and water. The presence of hydroxyl groups on these fatty acids delays absorption and increases the purgative effect by prolonging exposure of the colon to these compounds. Although potassium losses exceed sodium or chloride losses in healthy patients, the loss of electrolytes in the stool is typically negligible; however, laxative abuse increases the loss of sodium in relation to the potassium. Subsequently, activation of the renin–angiotensin–aldosterone system reduces sodium losses by increasing the excretion of potassium in the urine and stool. Electron scanning studies of the morphology of rabbit colon during perfusion studies with sodium ricinoleate suggest that this compound may cause desquamation of surface epithelial cells;[191] however, the relevance of these *in vitro* studies to doses of castor oil ingested by humans is unclear.

CLINICAL RESPONSE

Similar to other stimulant laxatives, adverse reactions associated with the abuse of castor oil include electrolyte and fluid imbalance. The subcutaneous (SC) injection of a large dose (i.e., 500 mL) of castor oil by a 28-year-old transsexual was associated with immediate local pain, abdominal and chest pain, headache, vomiting, tinnitus, hematuria, and jaundice.[192] She developed fever, tachycardia, renal failure, respiratory distress with pulmonary infiltrates, hypoxemia requiring intubation, hepatitis, hemolysis, and thrombocytopenia. She recovered from the multiorgan failure, but she required hemodialysis for 1½ months. Characteristic features of patients chronically abusing castor oil include very watery stool with a large amount of oil drops floating on the fecal surface that contain mostly ricinoleic acid. Castor oil and hydrogenated castor oil (castor wax) contain several allergens that produce a variety of allergic reactions including contact dermatitis,[193] fixed drug eruption,[194] and cheilitis.[195]

DIAGNOSTIC TESTING

Analytic methods for the detection and quantitation of components in castor oil include thin layer chromatography,[196] GC,[190,197] liquid chromatography/mass spectrometry with evaporative light scattering,[198] refractive index, flame ionization,[199] negative ion atmospheric pressure chemical ionization or positive ion matrix-

assisted laser desorption ionization,[200] and matrix-assisted laser desorption/ionization time-of-flight MS.[201] Ricinine is a biomarker for ricin formed after the ingestion of castor bean because both substances share the same plant source; additionally, ricinine is a minor constituent of castor oil. The use of solid phase extraction followed by liquid chromatography/tandem mass spectrometry, LC/MS and matrix-assisted laser desorption/ionization time-of-flight/mass spectrometry,[202] or isocratic high performance liquid chromatography/electrospray ionization/tandem mass spectrometry[203] allows the quantitation of ricinine in urine samples. The LLOQ for the latter method is 0.083 ng/mL. The initial urine from a 28-year-old transsexual with multiorgan failure after the SC injection of an estimated 500 mL castor oil contained 41 ng ricinine/mL.[192] Ricinine is stable in human urine when stored at 25°C (77°F) or 5°C (41°F) for 3 weeks.

TREATMENT

Medical management of the complications associated with chronic stimulant abuse including castor oil should be directed at the correction of fluid and electrolyte imbalance along with the restoration of a normal diet after an initial evaluation of the adequacy of respirations and cardiac function. Toxicity associated with the abuse of castor oil typically involves GI symptoms. Symptomatic patients should be evaluated for electrolyte (sodium, potassium, chloride, magnesium, calcium) and acid-base abnormalities, anemia, rhabdomyolysis, volume depletion, hepatorenal dysfunction, cardiomyopathy, and electrocardiographic abnormalities.

The SC or IV injection of castor oil has been associated with multiorgan failure similar to ricin toxicity.[192] These patients require a careful evaluation of their respiratory and cardiac function along with hemodynamic monitoring in an intensive care setting for the development of hepatorenal failure, respiratory distress, hemolysis, and disseminated intravascular coagulation. Treatment is supportive; there are no antidotes or effective methods to enhance the elimination of the toxin(s).

Senna

IDENTIFYING CHARACTERISTICS

The main active ingredients in senna are dianthrone compounds (sennosides A–D) from the anthraquinone family. The most common sennosides are the highly hydrophilic R,R-isomer sennoside A (CAS RN: 81-27-6, $C_{42}H_{38}O_{20}$, MW: 862.7391 g/mol) and the R,S-isomer sennoside B (CAS RN: 128-57-4); these compounds hydrolyze to the corresponding aglycone (sennidin) and 2 molecules of glucose.[204] The metabolite, rhein is the main active ingredient of senna. Senna contains a variety of rhein compounds including rhein-8-glucoside, rhein-8-diglucoside, and rhein anthrone-8-glucoside as well as aloe emodin dianthrone diglucoside, aloe emodin glucoside, and chrysophanol glucosides. Trade names include Ex-Lax® regular strength (Novartis Consumer Health, Parsippany, NJ), Senokot® (Purdue Products, L.P., Stamford, CT), Castoria®, and Black Draught.

EXPOSURE

The purgative properties of senna were known to the Ayurvedic and allopathic systems of medicine. The sources of senna are the dried leaves and fruits of *Senna alexandrina* P. Mill. (formerly, *Cassia acutifolia* or *C. angustifolia*). In a convenience sample of market formulations of senna leaf from several commercial sources, the mean percentage of total sennosides was $2.15 \pm 0.06\%$ w/w with the following distribution: A—$0.62 \pm 0.05\%$; B—$0.81 \pm 0.09\%$; C—$0.25 \pm 0.03\%$; and D—$0.46 \pm 0.07\%$, as measured by HPTLC.[205] Senna is available as pills, capsules, and rectal suppositories, usually in over-the-counter preparations.

DOSE EFFECT

In rodent studies, the acute toxicity of the active ingredients of senna (sennosides A and B) is relatively low compared with other fractions (rhein-8-glucoside, fraction IV) with low laxative activity; the estimated lethal oral dose of these laxative drugs exceeds 5,000 mg/kg.[206] The regular use of anthracene laxatives over 4–12 months is associated with the development of melanosis coli.[141] Case reports associate the chronic abuse of senna tablets with finger clubbing, hypercalcemia, nephrocalcinosis, and renal dysfunction. The chronic use of 100–200 Senokot® tablets daily was associated with finger clubbing that resolved after cessation of use and recurred after resuming Senokot® abuse for 2–3 months.[207] A 36-year-old woman with anorexia nervosa and a 6-year history of ingesting 50–100 senna tablets (12.5 mg/tablet) daily developed postural hypotension, dehydration, hypercalcemia, finger clubbing, hypertrophic osteoarthropathy, nephrocalcinosis, and renal dysfunction.[208] Chronic dehydration from the senna and the large amount of daily calcium (i.e., calcium sennosides) probably accounted for the clinical features of hypercalcemia rather than a direct toxic effect of senna.

TOXICOKINETICS

The isomers sennosides A and B are natural prodrugs. In humans, these hydrophilic compounds pass through the upper intestinal tract to the lower GI tract where intestinal bacteria hydrolyze these dianthrone-*O*-glucosides to the corresponding anthraquinone aglycone followed by the reduction of these compounds to the corresponding pharmacologically active anthrone (i.e., rhein anthrone). Physiochemical factors and increased peristalsis limit the absorption of rhein anthrone by the large intestine; therefore, most of the rhein anthrone appears in the feces.[209] Rhein is the oxidized product of rhein anthrone. Peak rhein concentrations occur about 3–5 hours and 10–11 hours after administration, probably as a result of the absorption of free rhein in the senna and the absorption of rhein after the degradation of sennosides.[210] The mean elimination half-life of rhein is about 7 hours.[210] Animal studies indicate that excretion of absorbed anthraquinone aglycone compounds occurs in the bile and urine after conjugation to glucuronide or sulfate.[211]

The transfer of senna to breast milk is minimal, primarily because of the poor absorption of this compound from the GI tract.[132] In particular, the excretion of rhein in breast milk following the use of senna is small. After the ingestion of a senna laxative containing 15 mg sennosides daily for 3 days, the concentration of rhein in breast milk was <10 ng/mL in 94 of 100 breast milk samples from 20 lactating women.[212] The stools of the breastfeeding infants remained normal during maternal use of senna. Most laxative abusers report the necessity of higher doses of laxatives to achieve the desired effects.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Senna-based alkaloids are composed of dianthrone diglucosides (sennosides A and B) and an aglycone (rhein anthrone). Intestinal bacteria hydrolyze the glucosides from the aglycone, after which both substances interact with inflammatory cells in the colon.[213] Sennosides increase paracellular permeability of small molecules by stimulating chloride secretion, which is electrochemically and osmotically balanced by an efflux of sodium and water via the paracellular pathway.[214] Rhein directly irritates colonic epithelial cells and indirectly stimulates secretion via neurohumoral mechanisms,[215] thereby enhancing intestinal transit and water secretion.[216] The rarity of the association between hepatitis and chronic senna use/abuse suggests an idiopathic reaction.

CLINICAL RESPONSE

Illicit Use

Case reports associated chronic abuse of senna tablets (e.g., calcium sennosides) with finger clubbing, hypercalcemia, bilateral medullary renal calcifications, and renal dysfunction.[207,208] Several case reports suggest a possible association between the use and abuse of tea from senna fruits and leaves and liver dysfunction.[217] A 77-year-old man developed cholestasis and hepatitis after ingesting senna daily for 3 months.[218] The liver biopsy demonstrated bridging hepatocellular necrosis and canalicular cholestasis. Fatigue, abdominal pain, and jaundice resolved within 1 month of the cessation of senna use; the serum bilirubin and hepatic aminotransferases also returned to normal values during this period. There was no rechallenge. Other case reports document the recurrence of a toxic hepatitis following reintroduction of senna in a 26-year-old nurse ingesting sennoside B and large amounts of folia sennae tea[219] and in a 28-year-old poor metabolizer (homozygous for CYP2D6*4 variant) drinking an herbal tea from senna leaves.[220]

Abstinence Syndrome

Withdrawal symptoms from chronic laxative abuse include rebound edema, constipation, and drug craving. Abdominal cramping, constipation, bloating, mood swings, fatigue, and malaise may persist for several weeks after cessation of senna abuse. Case reports suggest that rebound edema and congestive heart failure may occur in susceptible patients within the first 2 weeks after cessation of stimulant laxative abuse.[221]

Reproductive Abnormalities

Senna is generally considered safe for use during pregnancy with animal studies failing to demonstrate fetal risks when administered during pregnancy. Case-control studies also do not demonstrate increased risk of congenital malformations from the use of senna during pregnancy. In an analysis of the Hungarian Case-Control Surveillance System of Congenital Abnormalities, the adjusted odds ratio (OR) for the congenital abnormalities for pregnant women ingestion 10–30 mg senna daily was 1.0 (95% CI: 0.9–1.1).[222] There were no statistically significant associations between any congenital abnormality and senna use during pregnancy, although the OR for neural tube deficits was marginal (OR, 1.8, 95% CI: 1.0–3.0).

Carcinogenesis

Although a retrospective study of 3,049 patients undergoing diagnostic colorectal endoscopy suggested an increased risk of colorectal cancer (RR, 3.04; 95% CI: 1.18–4.90) in patients chronically using anthranoid laxatives,[223] most experimental and epidemiologic data do not support a causal link between chronic laxative use (cascara, senna) and colorectal cancer.[224,225] The International Agency for Research on Cancer (IARC) and the US National Toxicology Program do not list senna as a suspected carcinogen.

DIAGNOSTIC TESTING

Analytic methods for the detection and quantitation of sennosides and rhein in solid samples include spectrophotometry,[226] fluorometry, high performance thin layer chromatography,[227] HPLC with UV detection,[228] ion pair HPLC,[229] and GC/MS after hydrolysis and extractive methylation.[152]

TREATMENT

Medical management of the complications associated with chronic stimulant abuse including senna should be directed at the correction of fluid and electrolyte imbalance along with the restoration of a normal diet after an initial evaluation of the adequacy of respirations and cardiac function. Symptomatic patients should be evaluated for electrolyte (sodium, potassium, chloride, magnesium, calcium) and acid-base abnormalities, anemia, rhabdomyolysis, volume depletion, hepatorenal dysfunction, cardiomyopathy, and electrocardiographic abnormalities. Treatment is supportive.

Osmotic Laxatives

Osmotic laxatives include lactulose, magnesium sulfate/citrate, sodium sulfate, and sorbitol. The adult dose of 70% sorbitol is 1–2 mL/kg.[2] The recommended adult dose of 10% magnesium citrate is 250 mL. The administration of a single, large therapeutic dose of magnesium sulfate (13.9 g) to 7 healthy volunteers was not associated with a significant increase in the serum magnesium concentration.[230] Magnesium and sodium increase intestinal water by retention as sulfate or phosphate ions; additionally, magnesium induces the release of cholecystokinin and subsequent intraluminal accumulation of water and electrolytes. Lactulose increases the presence of osmotically active molecules in the colon along with flatulence and intestinal colic.

Typical laxative screens detect stimulant laxatives, but not osmotic laxatives. Methods to detect the abuse of osmotic diuretics include determination of stool osmolal gap, fecal sulfate (sodium sulfate containing laxatives), fecal phosphate (phosphate containing laxatives), and fecal magnesium (magnesium containing laxatives).[231] The sum of the sodium and potassium concentrations multiplied by 2 approximates the sum of ionic constituents in the stool; the difference between this estimation and the stool osmolality is the osmolal gap. The presence of a large stool osmolal gap and normal stool osmolality suggests the use of saline laxatives. The osmolar gap following abuse of saline cathartics usually exceeds 100 mOsmol/L.[143] The chronic use of sodium sulfate (Glauber's salt) or magnesium sulfate (Epsom salt) increases the sulfate concentration in urine. Typically, the daily urinary excretion of magnesium and sulfate is <16 mEq and <60 mEq, respectively.[143] As measured by atomic absorption spectrophotometry, diarrheal stools should contain <24 mEq magnesium/L unless the patient is ingesting magnesium containing compounds. Hypocalcemia occasionally results from chronic laxative abuse, particularly in association with hyperphosphatemia from chronic abuse of phosphorous containing osmotic cathartics; complications of hypocalcemia include tetany and osteomalacia in association with vitamin D deficiency.[232]

Phenolphthalein

Phenolphthalein was originally a colorant for wine. Following absorption, limited excretion of phenolphthalein occurs in the urine. Enterohepatic recirculation results in the excretion of phenolphthalein glucuronide in the bile and subsequent laxative action. Dermatologic changes associated with phenolphthalein use include Steven-Johnson syndrome, bullous photodermatitis, toxic epidermal necrolysis, and fixed drug eruption with hyperpigmentation.[233,234] Free phenolphthalein in urine turns pink following the addition of alkalizing agents; however, most phenolphthalein in urine exists as conjugates. These conjugated forms (sulfates, glucuronides) do not become pink unless the conjugates are hydrolyzed by β-glucuronidase or acid hydrolysis. These methods are not specific because these procedures produce pink urine following the ingestion of beet (*Beta vulgaris* L.), rhubarb (*Rheum* spp.), and bromsulfophthalein. Pink discoloration in the diapers of a child with unexplained chronic diarrhea and weight loss suggests the diagnosis of Münchausen syndrome by proxy. Treatment is supportive.

References

1. Harris RT. Bulimarexia and related serious eating disorders with medical complications. Ann Intern Med 1983;99:800–807.

2. Bulik CM. Abuse of drugs associated with eating disorders. J Subst Abuse 1992;4:69–90.

3. Casiero D, Frishman WH. Cardiovascular complications of eating disorders. Cardiol Rev 2006;14:227–231.

4. WADA. The 2008 WADA Laboratory statistics. Available at http://www.wada-ama.org/rtecontent/document/WADA_2008_LaboratoryStatisticsReport_Final.pdf. Accessed 2010 Dec 4.

5. Cadwallader AB, de la Torre X, Tieri A, Botre F. The abuse of diuretics as performance-enhancing drugs and masking agents in sport doping: pharmacology, toxicology and analysis. Br J Pharmacol 2010;161:1–16.

6. Mitchell JE, Hatsukami D, Eckert ED, Pyle RL. Characteristics of 275 patients with bulimia. Am J Psychiatry 1985;142:482–485.

7. Mitchell JE, Pomeroy C, Seppala M, Huber M. Diuretic use as a marker for eating problems and affective disorders among women. J Clin Psychiatry 1988;49:267–270.

8. Niezgoda JA, Walter MC, Jarrard MR. Furosemide overdose and maximal allowable weight standards. Mil Med 1989;154:608–609.

9. Brucato A, Bonati M, Gaspari F, Colussi G, Giachetti M, Zoppi F, et al. Tetany and rhabdomyolysis due to surreptitious furosemide–importance of magnesium supplementation. J Toxicol Clin Toxicol 1993;31:341–344.

10. Pomeroy C, Mitchell JE, Seim HC, Seppala M. Prescription diuretic abuse in patients with bulimia nervosa. J Fam Pract 1988;27:493–496.

11. Ponto LL, Schoenwald RD. Furosemide (frusemide). A pharmacokinetic/pharmacodynamic review (Part I). Clin Pharmacokinet 1990;18:381–408.

12. Beermann B, Groschinsky-Grind M, Fåhraeus L, Lindström B. Placental transfer of furosemide. Clin Pharmacol Ther 1978;24:560–562.

13. Berlin CM, Briggs GG. Drugs and chemicals in human milk. Semin Fetal Neonatal Med 2005;10:149–159.

14. Sjöström P. Mechanisms of reduced effects of loop diuretics in healthy volunteers and in patients with renal disease. Scand J Urol Nephrol 1988;111(suppl):S1–S66.

15. Wilcox CS New insights into diuretic use in patients with chronic renal disease. J Am Soc Nephrol 2002;13:798–805.

16. Chrysos G, Gargalianos P, Lelekis M, Stefanou J, Kosmidis J. Pharmacokinetic interactions of ceftazidime and frusemide. J Chemother 1995;7:107–110.

17. Bates DE, Beaumont SJ, Baylis BW. Ototoxicity induced by gentamicin and furosemide. Ann Pharmacother 2002;36:446–451.

18. Richens A. Interactions with antiepileptic drugs. Drugs 1977;13:266–275

19. Dean RP, Rudinsky BF, Kelleher MD. Interaction of chloral hydrate and intravenous furosemide in a child. Clin Pharm 1991;10:385–387.

20. Malach M, Berman N. Furosemide and chloral hydrate. Adverse drug interaction. JAMA 1975;232:638–639.

21. Guthrie GP Jr, Kotchen TA. Hypertension and aldosterone overproduction without renin suppression in Cushing's syndrome from an adrenal adenoma. Am J Med 1979;67:524–528.

22. Macoliç V, Vrhovac B. Pharmacokinetics and interactions of digoxin, theophylline and furosemide in diseases with edema. Int J Clin Pharmacol Ther Toxicol 1993;31:6–11

23. Wright CI, Van-Buren L, Kroner CI, Koning MM. Herbal medicines as diuretics: a review of the scientific evidence. J Ethnopharmacol 2007;114:1–31.

24. Wittner M, Di Stefano A, Wangemann P, Greger R. How do loop diuretics act? Drugs 1991;41:1–13.

25. Humes HD. Insights into ototoxicity. Analogies to nephrotoxicity. Ann N Y Acad Sci 1999;884:15–18.

26. Copeland PM. Diuretic abuse and central pontine myelinolysis. Psychother Psychosom 1989;52:101–105.

27. Verbalis JG, Martinez AJ. Neurological and neuropathological sequelae of correction of chronic hyponatremia. Kidney Int 1991;39:1274–1282.

28. Norenberg MD, Papendick RE. Chronicity of hyponatremia as a factor in experimental myelinolysis. Ann Neurol 1984;15:544–547.

29. Schepkens H, Hoeben H, Vanholder R, Lameire N. Mimicry of surreptitious diuretic ingestion and the ability to make a genetic diagnosis. Clin Nephrol 2001;55:233–237.

30. Tajiri J, Nakayama M, Sato T, Isozaki S, Uchino K. Pseudo-Bartter's syndrome due to furosemide abuse: report of a case and an analytical review of Japanese literature. Jap J Med 1981;20:216–221.

31. Colussi G, Rombola G, Airaghi C, De Ferrari ME, Minetti L. Pseudo-Bartter's syndrome from surreptitious diuretic intake: differential diagnosis with true Bartter's syndrome. Nephrol Dial Transplant 1992;7:896–901.

32. de Wardener HE. Idiopathic edema: role of diuretic abuse. Kidney Int 1981;19:881–892.

33. Morra V, Davit P, Capra P, Vincenti M, Di Stilo A, Botrè F. Fast gas chromatographic/mass spectrometric determination of diuretics and masking agents in human urine: Development and validation of a productive screening protocol for antidoping analysis. J Chromatogr A 2006;1135:219–229.

34. Goebel C, Trout GJ, Kazlauskas R. Rapid screening method for diuretics in doping control using automated solid phase extraction and liquid chromatography-electrospray tandem mass spectrometry. Anal Chim Acta 2004;502:65–74.

35. Thieme D, Grosse J, Lang R, Mueller RK, Wahl A. Screening, confirmation and quantification of diuretics in urine for doping control analysis by high-performance liquid chromatography-atmospheric pressure ionisation

tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 2001;757:49–57.

36. Jamison RL, Ross JC, Kempson RL, Sufit CR, Parker TE. Surreptitious diuretic ingestion and pseudo-Bartter's syndrome. Am J Med 1982;73:142–147.

37. Simpson IJ, Black PN, Couch RA. Surreptitious diuretic ingestion mimicking Bartter's syndrome. N Z Med J 1986;99;945–946.

38. Spratt DI, Pont A. The clinical features of covert diuretic use. West J Med 1982;137:331–335.

39. Amann B, Schafer M, Sterr A, Arnold S, Grunze H. Central pontine myelinolysis in a patient with anorexia nervosa. Int J Eat Disord 2001;30:462–466.

40. Giudicelli JF, Richer C, Mattei A. Pharmacokinetics and biological effects of captopril and hydrochlorothiazide after acute and chronic administration either alone or in combination in hypertensive patients. Br J Clin Pharmacol 1987;23:S51–S63.

41. Chen T-M, Chiou WL. Large differences in the biological half-life and volume of distribution of hydrochlorothiazide in normal subjects from eleven studies. Int J Clin Pharmacol Ther Toxicol 1992;30:34–37.

42. Redalieu E, Chan KK, Tipnis V, Zak SB, Gilleran TG, Wagner WE Jr, LeSher AR. Kinetics of hydrochlorothiazide absorption in humans. J Pharm Sci 1985;74:765–767.

43. Patel RB, Patel UR, Rogge MC, Shah VP, Prasad VK, Selen A, Welling PG. Bioavailability of hydrochlorothiazide from tablets and suspensions. J Pharm Sci 1984;73:359–361.

44. Miller ME, Cohn RD, Burghart PH. Hydrochlorothiazide disposition in a mother and her breast-fed infant. J Pediatr 1982;101:789–791.

45. Gray MJ. Use and abuse of thiazides in pregnancy. Clin Obstet Gynecol 1968;11:568–578.

46. Stumph MJ, Noall MW. Simplified detection of hydrochlorothiazide in urine by thin-layer chromatography. J Anal Toxicol 1984;8:170–172.

47. de Vries JX, Voss A. Simple determination of hydrochlorothiazide in human plasma and urine by high performance liquid chromatography. Biomed Chromatogr 1993;7:12–14.

48. Richter K, Oertel R, Kirch W. New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection. J Chromatogr A 1996;729:293–296.

49. Liu F, Xu Y, Gao S, Zhang J, Guo Q. Determination of hydrochlorothiazide in human plasma by liquid chromatography/tandem mass spectrometry. J Pharmaceut Biomed Anal 2007;44:1187–1191.

50. Tutunji MF, Ibrahim HM, Khabbas MH, Tutunji LF. Simultaneous determination of bisoprolol and hydrochlorothiazide in human plasma by HPLC coupled with tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:1689–1697.

51. Yamazaki M, Itoh S, Okuda T, Tanabe K, Nakahama H, Fukuhara Y, Orita Y. Binding of hydrochlorothiazide to erythrocytes. J Pharmacobiodyn 1980;12:423–428.

52. Redalieu E, Tipnis VV, Wagner WE Jr. Determination of plasma hydrochlorothiazide levels in humans. J Pharm Sci 1978;67:726–728.

53. Deventer K, Pozo OJ, Van Eenoo P, Delbeke FT. Detection of urinary markers for thiazide diuretics after oral administration of hydrochlorothiazide and altizide-relevance to doping control analysis. J Chromatogr A 2009;1216:2466–2473.

54. Sabanathan K, Castleden CM, Adam HK, Ryan J, Fitzsimons TJ. A comparative study of the pharmacokinetics and pharmacodynamics of atenolol, hydrochlorothiazide and amiloride in normal young and elderly subjects and elderly hypertensive patients. Eur J Clin Pharmacol 1987;32:53–60.

55. Duarte CG, Winnacker JL, Becker KL, Pace A. Thiazide-induced hypercalcemia. N Engl J Med 1971;284:828–830.

56. Gjonnaess H. Thiazide treatment in pregnancy with special reference to maternal and foetal electrolyte. Acta Obstet Gynecol Scand 1968;47:404–419.

57. Lindheimer MD, Katz AI. Sodium and diuretics in pregnancy. N Engl J Med 1973;288:891–894.

58. Editorial. The history of ipecacuanha in dysentery. Br Med J 1915;2:728.

59. Lee MR. Ipecacuanha: the South American vomiting root. J R Coll Physicians Edinb 2008;38:355–360.

60. Smith RP, Smith DM. Report of a fatal case and review of the literature. N Engl J Med 1961;265:523–525.

61. Harrison RT. A case of ipecacuanha poisoning? Lancet 1908;2:536.

62. Young WA, Tudhope GR. The pathology of prolonged emetine administration. Trans Roy Soc Trop Med Hyg 1926;20:93–99.

63. Kent L, Kingsland RC. Effects of emetine hydrochloride on the electrocardiogram in man. Am Heart J 1950;39:576–587.

64. Panettiere F, Coltman CA Jr. Phase I experience with emetine hydrochloride (NSC 33669) as an antitumor agent. Cancer 1971;27:835–841.

65. The United States Pharmacopeia. 21st Ed. Rockville, MD: United States Pharmacopeial Convention; 1985:556.

66. Greenfeld D, Mickley D, Quinlan DM, Roloff P. Ipecac abuse in a sample of eating disordered outpatients. Int J Eat Disord 1993;13:411–414.

67. Manno BR, Manno JE. Toxicology of ipecac: a review. Clin Toxicol 1977;10:221–242.

68. Brem TH, Konwaler BE. Fatal myocarditis due to emetine hydrochloride. Am Heart J 1955;50:476–481.

69. Palmer EP, Guay AT. Reversible myopathy secondary to abuse of ipecac in patients with major eating disorders. N Engl J Med 1985;313:1457–1459.

70. Friedman AG, Seime RJ, Roberts T, Fremouw WJ. Ipecac abuse: a serious complication in bulimia. Gen Hosp Psychiatr 1987;9:225–228.

71. Ho PC, Dweik R, Cohen MC. Rapidly reversible cardiomyopathy associated with chronic ipecac ingestion. Clin Cardiol 1998;21:780–783.

72. Pope HG, Hudson JI, Nixon RA, Herridge PL. Epidemiology of ipecac abuse. N Engl J Med 1986;314:245–246.

73. Yamashita M, Yamashita M, Azuma J. Urinary excretion of ipecac alkaloids in human volunteers. Vet Hum Toxicol 2002;44:257–259.

74. Scharman EJ, Hutzler JM, Rosencrance JG, Tracy TS. Single dose pharmacokinetics of syrup of ipecac. Ther Drug Monit 2000;22:566–573.

75. Moran DM, Crouch DJ, Finkle BS. Absorption of ipecac alkaloids in emergency patients. Ann Emerg Med 1984;13:1100–1102.

76. Asano T, Kushida H, Sadakane C, Ishihara K, Wakui Y, Yanagisawa T. Metabolism of ipecac alkaloids cephaeline and emetine by human hepatic microsomal cytochrome P450s, and their inhibitory effects on P450 enzyme activities. Biol Pharm Bull 2001;24:678–682.

77. Asano T, Watanabe J, Sadakane C, Ishihara K, Hirakura K, Wakui Y, et al. Biotransformation of the ipecac alkaloids cephaeline and emetine from ipecac syrup in rats. Eur J Drug Metab Pharmacokinet 2002;27:29–35.

78. Ganapathy V, Prasad PD, Ganapathy ME, Leibach FH. Placental transporters relevant to drug distribution across the maternal-fetal interface. Pharm Exp Ther 2000;294:413–420.

79. Lovejoy FH Jr, Shannon M, Woolf AD. Recent advances in clinical toxicology. Curr Probl Pediatr 1992;22:119–129.

80. Palmer EP, Guay AT. Reversible myopathy secondary to abuse of ipecac in patients with major eating disorders. N Engl J Med 1985;313:1457–1459.

81. Manoguerra AS, Cobaugh DJ, Guidelines for the Management of Poisoning Consensus Panel. Guideline on the use of ipecac syrup in the out-of-hospital management of ingested poisons. Clin Toxicol 2005;43:1–10.

82. Yang WC, Dubick M. Mechanism of emetine cardiotoxicity. Pharmacol Ther 1980;10:15–26.

83. Lemmens-Gruber R, Karkhaneh A, Studenik C, Heistracher P. Cardiotoxicity of emetine dihydrochloride by calcium channel blockade in isolated preparations and ventricular myocytes of guinea-pig hearts. Br J Pharmacol 1996;117:377–383.

84. Lemmens-Gruber R, Studenik C, Karkhaneh A, Heistracher P. Mechanism of sodium channel blockade in the cardiotoxic action of emetine dihydrochloride in isolated cardiac preparations and ventricular myocytes of guinea pigs. J Cardiovasc Pharmacol 1997;30:554–561.

85. Sugie H, Russin R, Verity MA. Emetine myopathy: two case reports with pathobiochemical analysis. Muscle Nerve 1984;7:54–59.

86. Turner PP. The effects of emetine on the myocardium. Br Heart J 1963;25:81–88.

87. Thyagarajan D, Day BJ, Wodak J, Gilligan B, Dennett X. Emetine myopathy in a patient with an eating disorder. Med J Aust 1993;159:757–760.

88. Mateer JE, Farrell BJ, Chou SS, Gutmann L. Reversible ipecac myopathy. Arch Neurol 1985;42:188–190.

89. Kuntzer T, Bogousslavsky J, Deruaz JP, Janzer R, Regli F. Reversible emetine-induced myopathy with ECG abnormalities: a toxic myopathy. J Neurol 1989; 236:246–248.

90. Halbig L, Gutmann L, Goebel HH, Brick JF, Schochet S. Ultrastructural pathology in emetine-induced myopathy. Acta Neuropathol (Berl) 1988;75:577–582.

91. Gimble AI, Davison C, Smith PK. Studies on the toxicity, distribution and excretion of emetine. J Pharmacol Exp Ther 1948;94:431–418.

92. Bradley WG, Fewings JD, Harris JB, Johnson MA. Emetine myopathy in the rat. Br J Pharmacol 1976;57:29–41.

93. Duane DD, Engel AG. Emetine myopathy. Neurology 1970;20:733–739.

94. Pearce MB, Bulloch TR, Murphy ML. Selective damage of myocardial mitochondria due to emetine hydrochloride. Arch Pathol 1971;91:8–18.

95. Bindoff L, Cullen MJ. Experimental (-) emetine myopathy. Ultrastructural and morphometric observations. J Neurol Sci 1978;39:1–15.

96. Mars DR, Anderson NH, Riggall FC. Anorexia nervosa: a disorder with severe acid-base derangements. South Med J 1982;75:1038–1042.

97. Schneider DJ, Perez A, Knilans TE, Daniels SR, Bove KE, Bonnell H. Clinical and pathologic aspects of cardiomyopathy from ipecac administration in Münchhausen syndrome by proxy. Pediatrics 1996;97:902–906.

98. Brem TH, Konwaler BE. Fatal myocarditis due to emetine hydrochloride. Am Heart J 1955;50:476–481.

99. House RC, Grisius R, Bliziotes MM, Licht JH. Perimolysis: unveiling the surreptitious vomiter. Oral Surg Oral Med Oral Pathol 1981;51:152–155.

100. Mitchell JE, Seim HC, Colon E, Pomeroy C. Medical complications and medical management of bulimia. Ann Intern Med 1987;107:71–77.

101. Buchanan JA, Fortune F. Bilateral parotid enlargement as a presenting feature of bulimia nervosa in a postadolescent male. Postgrad Med J 1994;70:27–30.

102. Stege P, Visco-Dangler L, Rye L. Anorexia nervosa: review including oral and dental manifestations. J Am Dent Assoc 1982;104:648–652.

103. Timberlake GA. Ipecac as a cause of the Mallory-Weiss syndrome. South Med J 1984;77:804–805.

104. Bennett HS, Spiro AJ, Pollack MA, Zucker P. Ipecac-induced myopathy simulating dermatomyositis. Neurology 1982;32:91–94.

105. Klatskin G, Friedman H. Emetine toxicity in man; studies on the nature of early toxic manifestations, their relation to the dose level, and their significance in determining safe dosage. Ann Intern Med 1948;28:892–915.

106. Ratnesar VC, Pobee J. Emetine toxicity with predominant neuromuscular manifestations. Postgrad Med J 1962;38:586–588.

107. Powers PS. Heart failure during treatment of anorexia nervosa. Am J Psychiatry 1982;139:1167–1170.

108. Bruch H. Death in anorexia nervosa. Psychosom Med 1971;33:135–144.

109. Isner JM, Roberts WC, Heymfield SB, Yager J. Anorexia nervosa and sudden death. Ann Intern Med 1985;102:49–52.

110. Friedman EJ. Death from ipecac intoxication in a patient with anorexia nervosa. Am J Psychiatry 1984;141:702–703.

111. Dawson JA, Yager J. A case of abuse of syrup of ipecac resulting in death. J Am Coll Health 1986;34:280–282.

112. Schiff RJ, Wurzel CL, Brunson SC, Kasloff I, Nussbaum MP, Frank SD. Death due to chronic syrup of ipecac use in a patient with bulimia. Pediatrics 1986;78:412–416.

113. Seth RK, Ray GK. Method for the estimation of emetine. Indian J Pharm 1967;29:130–132.

114. Habib MS, Harkiss KJ. Quantitative determination of emetine and cephaeline in ipecacuanha root and its preparations. Planta Med 1970;18:270–274.

115. Crouch DJ, Moran DM, Finkle BS, Peat MA. Quantitative analysis of emetine and cephaeline by reversed-phase high performance liquid chromatography with fluorescence detection. J Anal Toxicol 1984;8:63–65.

116. Eldawy MA, Mabrouk MM, El-Barbary FA. Determination of chlorpheniramine maleate and tincture ipecac in dosage form by liquid chromatography with ultraviolet detection. J AOAC Int 2003;86:675–680.

117. Lachman MF, Romeo R, McComb RB. Emetine identified in urine by HPLC, with fluorescence and ultraviolet/diode array detection, in a patient with cardiomyopathy. Clin Chem 1989;35:499–502.

118. Asano T, Sadakane C, Ishihara K, Yanagisawa T, Kimura M, Kamei H. High-performance liquid chromatographic assay with fluorescence detection for the determination of cephaeline and emetine in human plasma and urine. J Chromatogr B Biomed Sci Appl 2001;757:197–206.

119. Rashid N. Medically unexplained myopathy due to ipecac abuse. Psychosomatics 2006;47:167–169.

120. Bader AA, Kerzner B. Ipecac toxicity in "Münchhausen syndrome by proxy." Ther Drug Monit 1999;12:259–260.

121. Adler AG, Walinsky P, Krall RA, Cho SY. Death resulting from ipecac syrup poisoning. JAMA 1980;243:1927–1928.

122. Moldawsky RJ. Myopathy and ipecac abuse in a bulimic patient. Psychosomatics 1985;26:448–449.

123. Rutter K, Maxwell D. Diseases of the alimentary system. Constipation and laxative abuse. Br Med J 1976;2:997–1000.

124. van Gorkom BA, de Vries EG, Karrenbeld A, Kleibeuker JH. Review article: anthranoid laxatives and their potential carcinogenic effects. Aliment Pharmacol Ther 1999;13:443–452.

125. Pryor T, Wiederman MW, McGilley B. Laxative abuse among women with eating disorders: an indication of psychopathology? Int J Eat Disord 1996;20:13–18.

126. Mond JM, Hay PJ, Rodgers B, Owen C, Mitchell JE. Correlates of self-induced vomiting and laxative misuse in a community sample of women. J Nerv Ment Dis 2006;194:40–46.

127. Bryant-Waugh R, Turner H, East P, Gamble C, Mehta R. Misuse of laxatives among adult outpatients with eating disorders: prevalence and profiles. Int J Eat Disord 2005;39:404–409.

128. Flig E, Hermann TW, Zabel M. Is bisacodyl absorbed at all from suppositories in man? Int J Pharm 2000;196:11–20.

129. Roth W, Beschke K. [Pharmacokinetics and laxative effect of bisacodyl following administration of various dosage forms]. Arzneimittelforschung 1988;38:570–574. [German]

130. Jauch R, Hankwitz R, Beschke K, Pelzer H. Bis-(p-hydroxyphenyl)-pyridyl-2-methane: the common laxative principle of bisacodyl and sodium picosulfate. Arzneimittelforschung 1975;25:1796–1800.

131. Sund RB, Roland M, Kristiansen S, Salvesen B. Biliary excretion of bisacodyl and picosulphate in man: studies in gallstone patients after biliary tract surgery. Acta Pharmacol Toxicol 1982;50:50–57.

132. Ostrea EM Jr, Mantaring JB 3rd, Silvestre MA. Drugs that affect the fetus and newborn infant via the placenta or breast milk. Pediatr Clin North Am 2004;51:539–579.

133. Müller-Lissner S. Classification, pharmacology, and side-effects of common laxatives. Ital J Gastroenterol Hepatol 1999;31(Suppl 3):S234–S237.

134. Barceloux D, McGuigan M, Hartigan-Go K. Position statement: cathartics. American Academy of Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists. J Toxicol Clin Toxicol 1997;35:743–752.

135. Heilbrun N. Roentgen evidence suggesting enterocolitis associated with prolonged cathartic abuse. Radiology 1943;41:486–491.

136. Urso FP, Urso MJ, Lee CH. The cathartic colon: pathological findings and radiological/pathological correlation. Radiology 1975;116:557–559.

137. Joo JS, Ehrenpreis ED, Gonzalez L, Kaye M, Breno S, Wexner SD. Alterations in colonic anatomy induced by chronic stimulant laxatives: the cathartic colon revisited. J Clin Gastroenterol 1998;26:283–286.

138. Wald A. Is chronic use of stimulant laxatives harmful to the colon? J Clin Gastroenterol 2003;36:386–389.

139. Muller-Lissner S. What has happened to the cathartic colon? Gut 1996;39:486–488.

140. Riecken EO, Zeitz M, Emde C, Hopert R, Witzel L, Hintze R, et al. The effect of an anthraquinone laxative on colonic nerve tissue: a controlled trial in constipated women. Z Gastroenterol 1990;28:660–664.

141. Oster JR, Materson BJ, Rogers AI. Laxative abuse syndrome. Am J Gastroenterol 1980;74:451–458.

142. Slugg PH, Carey WD. Clinical features and follow-up of surreptitious laxative users. Cleve Clin Q 1984;51: 167–171.

143. Ewe K, Karbach U. Factitious diarrhoea. Clin Gastroenterol 1986;15:723–740.

144. Baker EH, Sandle GI. Complications of laxative abuse. Annu Rev Med 1996;47:127–134.

145. Heizer WD, Warshaw AL, Waldmann TA, Laster L. Protein-losing gastroenteropathy and malabsorption associated with factitious diarrhea. Ann Intern Med 1968;68:839–852.

146. Wu W-J, Huang C-H, Chiang C-P, Huang C-H, Wang C-N. Urolithiasis related to laxative abuse. J Formos Med Assoc 1993;92:1004–1006.

147. Stoll RE, Blanchard KT, Stoltz JH, Majeska JB, Furst S, Lilly PD, Mennear JH. Phenolphthalein and bisacodyl: assessment of genotoxic and carcinogenic responses in heterozygous p53 (+/–) mice and Syrian hamster embryo (SHE) assay. Toxicol Sci 2006;90:440–450.

148. Lopez Morra HA, Fine SN, Dickstein G. Colonic ischemia with laxative use in young adults. Am J Gastroenterol 2005;100:2134–2136.

149. Perkins SL, Livesey JF. A rapid high-performance thin-layer chromatographic urine screen for laxative abuse. Clin Biochem 1993;25:179–181.

150. Stolk LM, Hoogtanders K. Detection of laxative abuse by urine analysis with HPLC and diode array detection. Pharm World Sci 1999;21:40–43.

151. Kok RM, Faber DB. Qualitative and quantitative analysis of some synthetic, chemically acting laxatives in urine by gas chromatography-mass spectrometry. J Chromatogr 1981;222:389–398.

152. Beyer J, Peters FT, Maurer HH. Screening procedure for detection of stimulant laxatives and/or their metabolites in human urine using gas chromatography-mass spectrometry after enzymatic cleavage of conjugates and extractive methylation. Ther Drug Monit 2005;27: 151–157.

153. Morton J. The detection of laxative abuse. Ann Clin Biochem 1987;24:107–108.

154. Bytzer P, Stokholm M, Andersen I, Klitgaard NA, Schaffalitzky de Muckadell OB. Prevalence of surreptitious laxative abuse in patients with diarrhoea of uncertain origin: a cost benefit analysis of a screening procedure. Gut 1989;30:1379–1384.

155. de Wolff FA, de Haas EJ, Verweij M. A screening method for establishing laxative abuse. Clin Chem 1981;27: 914–917.

156. Shelton JH, Santa Ana CA, Thompson DR, Emmett M, Fordtran JS. Factitious diarrhea induced by stimulant laxatives: accuracy of diagnosis by a clinical reference laboratory using thin layer chromatography. Clin Chem 2007;53:85–90.

157. Fullinfaw RO, Bury RW, Moulds RF. Screening procedure for stimulant laxatives in urine using high perfor-

mance liquid chromatography with diode array detection. J Chromatogr 1988;433:131–140.

158. Kacere RD, Srivatsa SS, Tremaine WJ, Ebnet LE, Batts KP. Chronic diarrhea due to surreptitious use of bisacodyl: case reports and methods for detection. Mayo Clin Proc 1993;68:355–357.

159. Lööf L, Hartvig P, Lanbeck-Vallén K, Lindström B. Quantitation of a bisacodyl metabolite in urine for the diagnosis of laxative abuse. Ther Drug Monit 1980;2: 345–349.

160. Moriarty KJ, Silk DB. Laxative abuse. Dig Dis 1988;6:15–29.

161. Phillips S, Donaldson L, Geisler K, Pera A, Kochar R. Stool composition in factitial diarrhea: a 6-year experience with stool analysis. Ann Intern Med 1995;123: 97–100.

162. American Gastroenterological Association Clinical Practice and Practice Economics Committee. AGA technical review on the evaluation and management of chronic diarrhea. Gastroenterology 1999;116:1464–1486.

163. Schiller LR. Management of diarrhea in clinical practice: strategies for primary care physicians. Rev Gastroenterol Disord 2007;7(suppl 3):S27–S38.

164. Krahn LE, Lee J, Richardson JW, Martin MJ, O'Connor MK. Hypokalemia leading to *torsades de pointes*. Munchausen's disorder or bulimia nervosa? Gen Hosp Psychiatry 1997;19:370–377.

165. Colton P, Woodside DB, Kaplan AS. Laxative withdrawal in eating disorders: treatment protocol and 3 to 20-month follow-up. Int J Eat Disord 1999;25:311–317.

166. Quercia V. HLPC in the determination of some anthraquinone glycosides. Pharmacology 1980:20(suppl 1): S76–S80.

167. Alaerts G, Matthijs N, Smeyers-Verbeke J, Vander Heyden Y. Chromatographic fingerprint development for herbal extracts: a screening and optimization methodology on monolithic columns. J Chromatogr A 2007; 1172:1–8.

168. Food and Drug Administration, HHS. Status of certain additional over-the-counter drug category II and III active ingredients. Final rule. Fed Regist 2002;67: 31125–31127.

169. Fairbairn JW. Biological assay and its relation to chemical structure. Pharmacology 1976;14(Suppl 1):S48–S61.

170. Rosengren JE, Aberg T. Cleansing of the colon without enemas. Radiologe 1975;15:421–426.

171. American Academy of Pediatrics Committee on Drugs. Transfer of drugs and other chemicals into human milk. Pediatrics 2001;108:776–789.

172. Walker NI, Smith MM, Smithers BM. Ultrastructure of human melanosis coli with reference to its pathogenesis. Pathology 1993;25:120–123.

173. Ewing CA, Kalan M, Chucker F, Ozdemirli M. Melanosis coli involving pericolonic lymph nodes associated with the herbal laxative Swiss Kriss: a rare and incidental

finding in a patient with colonic adenocarcinoma. Arch Pathol Lab Med 2004;128:565–567.

174. Muller-Lissner SA. Adverse effects of laxatives: fact and fiction. Pharmacology 1993;47(suppl 1):S138–S145.

175. Chaudhary BN, Sharma H, Nadeem M, Niayesh MH. Ischemic colitis or melanosis coli: a case report. World J Emerg Surg 2007;2:25.

176. Mitty RD, Wolfe GR, Cosman M. Initial description of gastric melanosis in a laxative-abusing patient. Am J Gastroenterol 1997;92:707–708.

177. Nadir A, Reddy D, van Thiel DH. Cascara sagrada-induced intrahepatic cholestasis causing portal hypertension: case report and review of herbal hepatotoxicity. Am J Gastroenterol 2000;95:3634–3637.

178. Mereto E, Ghia M, Brambilla G. Evaluation of the potential carcinogenic activity of Senna and Cascara glycosides for the rat colon. Cancer Lett 1996;101:79–83.

179. Borrelli F, Mereto E, Capasso F, Orsi P, Sini D, Izzo AA, et al. Effect of bisacodyl and cascara on growth of aberrant crypt foci and malignant tumors in the rat colon. Life Sci 2001;69:1871–1877.

180. Gyanchandani ND, Yamamoto M, Nigam IC. Anthraquinone drugs. I. Thin-layer chromatographic identification of aloes, cascara, senna, and certain synthetic laxatives in pharmaceutical dosage forms. J Pharm Sci 1969;58:197–200.

181. Koyama J, Morita I, Kawanishi K, Tagahara K, Kobayashi N. Capillary electrophoresis for simultaneous determination of emodin, chrysophanol, and their 8-beta-D-glucosides. Chem Pharm Bull (Tokyo) 2003;51:418–420.

182. Yan D, Ma Y. Simultaneous quantification of five anthraquinones in rat plasma by high-performance liquid chromatography with fluorescence detection. Biomed Chromatogr 2007;21:502–507.

183. Houghton BJ, Pears MA. Chronic potassium depletion due to purgation with cascara. Br Med J 1958;1(5083):1328–1330.

184. Johnson W Jr and Cosmetic Ingredient Review Expert Panel. Final report on the safety assessment of ricinus communis (castor) seed oil, hydrogenated castor oil, glyceryl ricinoleate, glyceryl ricinoleate se, ricinoleic acid, potassium ricinoleate, sodium ricinoleate, zinc ricinoleate, cetyl ricinoleate, ethyl ricinoleate, glycol ricinoleate, isopropyl ricinoleate, methyl ricinoleate, and octyldodecyl ricinoleate. Int J Toxicol 2007;26(Suppl 3):S31-S77.

185. Gaginella TS, Phillips SF. Ricinoleic acid: current view of an ancient oil. Am J Dig Dis 1975;20:1171–1177.

186. Kato A, Yamaura Y. A rapid gas chromatographic method for the determination of fatty acid compositions of soybean oil and castor oil. Chem Ind 1970;39:1260.

187. Boel ME, Lee SJ, Rijken MJ, Paw MK, Pimanpanarak M, Tan SO, et al. Castor oil for induction of labour: not harmful, not helpful. Aust N Z J Obstet Gynaecol 2009;49:499–503.

188. Tchapla A, Méjanelle P, Bleton J, Goursaud S. Characterisation of embalming materials of a mummy of the Ptolemaic era. Comparison with balms from mummies of different eras. J Sep Sci 2004;27:217–234.

189. Watson WC, Gordon RS Jr. Studies on the digestion, absorption and metabolism of castor oil. Biochem Pharmacol 1962;11:229–236.

190. Hagenfeldt L, Blomquist L, Midtvedt T. Epoxydicarboxylic aciduria resulting from the ingestion of castor oil. Clin Chim Acta 1986;161:157–163.

191. Gaginella TS, Chadwick VS, Debongnie JC, Lewis JC, Phillips SF. Perfusion of rabbit colon with ricinoleic acid: dose-related mucosal injury, fluid secretion, and increased permeability. Gastroenterology 1977;73:95–101.

192. Smith SW, Graber NM, Johnson RC, Barr JR, Hoffman RS, Nelson LS. Multisystem organ failure after large volume injection of castor oil. Ann Plast Surg 2009;62:12–14.

193. Taghipour K, Tatnall F, Orton D. Allergic axillary dermatitis due to hydrogenated castor oil in a deodorant. Contact Dermatitis 2008;58:168–169.

194. Di Berardino L, Della Torre F. Side effects to castor oil. Allergy 2003;58:826.

195. Le Coz C-J, Ball C. Recurrent allergic contact dermatitis and cheilitis due to castor oil. Contact Dermatitis 2000;42:114–115.

196. Srinivasulu C, Mahapatra SN. A rapid method for detecting groundnut oil in castor oil. J Chromatogr 1973;86:261–262.

197. Ramsey JD, Lee TD, Osselton MD, Moffat AC. Gas-liquid chromatographic retention indices of 296 non-drug substances on SE-30 or OV-1 likely to be encountered in toxicological analyses. J Chromatogr 1980;184:185–206.

198. Palmer AJ, Palmer FJ. Rapid analysis of triacylglycerols using high-performance liquid chromatography with light scattering detection. J Chromatogr 1989;465:369–377.

199. Neff WE, List GR, Byrdwell WC. Analysis of triacylglycerol positional isomers in food products as brominated derivatives by high-performance liquid chromatography coupled with a flame ionization detection. J Chromatogr A 2001;912:187–190.

200. Stübiger G, Pittenauer E, Allmaier G. Characterisation of castor oil by on-line and off-line non-aqueous reverse-phase high-performance liquid chromatography-mass spectrometry (APCI and UV/MALDI). Phytochem Anal 2003;14:337–346.

201. Ayorinde FO, Garvin K, Saeed K. Determination of the fatty acid composition of saponified vegetable oils using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2000;14:608–615.

202. Darby SM, Miller ML, Allen RO. Forensic determination of ricin and the alkaloid marker ricinine from castor bean extracts. J Forensic Sci 2001;46:1033–1042.

203. Johnson RC, Lemire SW, Woolfitt AR, Ospina M, Preston KP, Olson CT, Barr JR. Quantification of ricinine in rat and human urine: a biomarker for ricin exposure. J Anal Toxicol 2005;29:149–155.

204. Grimminger W, Witthohn K. Analytics of senna drugs with regard to the toxicological discussion of anthranoids. Pharmacol 1993;47(Suppl 1):S98–S109.

205. Shah SA, Ravishankara MN, Nirmal A, Shishoo CJ, Rathod IS, Suhagia BN. Estimation of individual sennosides in plant materials and marketed formulations by an HPTLC method. J Pharm Pharmacol 2000;52:445–449.

206. Hietala P, Marvola M, Parviainen T, Lainonen H. Laxative potency and acute toxicity of some anthraquinone derivatives, senna extracts and fractions of senna extracts. Pharmacol Toxicol 1987;61:153–156.

207. Silk DB, Gibson JA, Murray CR. Reversible finger clubbing in a case of purgative abuse. Gastroenterology 1975; 68:790–794.

208. Lim AK, Hooke DH, Kerr PG. Anorexia nervosa and senna misuse: nephrocalcinosis, digital clubbing and hypertrophic osteoarthropathy. Med J Aust 2008; 188:121–122.

209. de Witte P. Metabolism and pharmacokinetics of anthranoids. Pharmacol 1993;47(Suppl 1):S86–S97.

210. Krumbiegel G, Schulz HU. Rhein and aloe-emodin kinetics from senna laxatives in man. Pharmacol 1993;47(Suppl 1):S120–S124.

211. de Witte P, Lemli L. The metabolism of anthranoid laxatives. Hepatogastroenterology 1990;37:601–605.

212. Faber P, Strenge-Hesse A. Relevance of rhein excretion in to breast milk. Pharmacology 1988;36(Suppl 1): S212–S220.

213. Lemli J. Mechanism of action of sennosides. Bull Acad Natl Med 1995;179:1605–1611.

214. Leng-Peschlow E. Sennoside-induced secretion is not caused by changes in mucosal permeability or Na+, K(+)-ATPase activity. J Pharm Pharmacol 1993;45:951–954.

215. Goerg KJ, Wanitschke R, Schwarz M, Meyer zum Büschenfelde KH. Rhein stimulates active chloride secretion in the short-circuited rat colonic mucosa. Pharmacology 1988;36:111–119.

216. Yagi T, Yamauchi K. Synergistic effects of anthraquinones on the purgative activity of rhein anthrone in mice. J Pharm Pharmacol 1999;51:93–95.

217. Vanderperren, Rizzo M, Angenot L, Haufroid V, Jadoul M, Hantson P. Acute liver failure with renal impairment related to the abuse of senna anthraquinone glycosides. Ann Pharmacother 2005;39:1353–1357.

218. Sonmez A, Yilmaz MI, Mas R, Ozcan A, Celasun B, Dogru T, et al. Subacute cholestatic hepatitis likely related to the use of senna for chronic constipation. Acta Gastroenterol Belg 2005;68:385–387.

219. Beuers U, Spengler U, Pape GR. Hepatitis after chronic abuse of senna. Lancet 1991;337:372–373.

220. Seeybold U, Landauer N, Hillebrand S, Goebel F-D. Senna-induced hepatitis in a poor metabolizer. Ann Intern Med 2004;141:650–651.

221. Riley JA, Brown AR, Walker BE. Congestive cardiac failure following laxative withdrawal. Postgrad Med J 1996;72:491–492.

222. Acs N, Banhidy f, Puho EH, Czeizel AE. Senna treatment in pregnant women and congenital abnormalities in their offspring—a population-based case-control study. Reprod Toxicol 2009;28:100–104.

223. Siegers CP, von Hertzberg-Lottin E, Otte M, Schneider B. Anthranoid laxative abuse—a risk for colorectal cancer? Gut 1993;34:1099–1101.

224. Lyden-Sokolowski A, Nilsson A, Sjoberg P. Two-year carcinogenicity study with sennosides in the rat: emphasis on gastro-intestinal alterations. Pharmacology 1993; 47(suppl 1):S209–S215.

225. Nusko G, Schneider B, Schneider I, Wittekind C, Hahn EG. Anthranoid laxative use is not a risk factor for colorectal neoplasia: results of a prospective case control study. Gut 2000;46:651–655.

226. Habib A-A, El-Sebakhy NA. Spectrophotometric estimation of sennosides and rhein glycosides in senna and its preparations. J Nat Prod 1980;43:452–458.

227. Komolafe OO. High pressure liquid chromatographic analysis of sennosides A and B purgative drugs. J Pharm Sci 1981;70:727–730.

228. Hayashi S-I, Yoshida A, Tanaka H, Mitani Y, Yoshizawa K. Analytical studies on the active constituents in crude drugs. IV. Determination of sennosides in senna and formulations by high-performance liquid chromatography. Chem Pharm Bull 1980;28:406–412.

229. Sun S-W, Su H-T. Validated HPLC method for determination of sennosides A and B in senna tablets. J Pharm Biomed Anal 2002;29:881–894.

230. Morris ME, LeRoy S, Sutton SC. Absorption of magnesium from orally administered magnesium sulfate in man. J Toxicol Clin Toxicol 1987;25:371–382.

231. Duncan A, Forrest JA. Surreptitious abuse of magnesium laxatives as a cause of chronic diarrhoea. Eur J Gastroenterol Hepatol 2001;13:599–601.

232. Goldfinger P. Hypokalemia, metabolic acidosis, and hypocalcemic tetany in a patient taking laxatives. A case report. J Mt Sinai Hosp N Y 1969;36:113–116.

233. Artymowicz RJ, Childs AL, Paolini L. Phenolphthalein-induced toxic epidermal necrolysis. Ann Pharmacother 1997;31:1157–1159.

234. Wyatt E, Greaves M, Sondergaard J. Fixed drug eruption (phenolphthalein) evidence for a blood-borne mediator. Arch Dermatol 1972;106:671–673.

Chapter 13

NORADRENERGIC AGENTS

Cyrus Rangan, MD

During the late 1930s, studies involving narcoleptic patients demonstrated that amphetamines suppress appetite and promote weight loss; by the 1940s, amphetamines were marketed as both single therapy and combination treatment for the control of appetite and loss of weight. Subsequently, numerous case reports associated the use of amphetamine-containing appetite suppressants ("rainbow pills") containing thyroid hormones, digitalis, laxatives, diuretics, and sedatives, with addictive behavior, hypertension, cardiotoxicity, and death.[1] In 1961, scientists at McNeil Laboratories (Fort Washington, PA) synthesized the oxazoline derivative, aminorex; in the following year, these scientists discovered the appetite suppressant properties of aminorex. This drug became available as an appetite suppressant in Switzerland, Austria, and Germany for over-the-counter (OTC) sale in 1965. Between 1965 and 1972, the incidence of primary pulmonary hypertension in these 3 countries increased 10-fold.[2] A majority of the new patients with pulmonary hypertension had taken aminorex for weight loss; the female to male ratio among these patients was 4:1. The first epidemic of pulmonary hypertension related to appetite suppressants ended when aminorex was withdrawn from the market in 1972.[3] Since the 1960s, researchers have developed numerous structurally related amphetamine-type anorexiants including ephedrine, diethylpropion, phentermine, phenmetrazine, phendimetrazine, and phenylpropanolamine; however, the US Food and Drug Administration (FDA) has banned several diet-control preparations (dexfenfluramine, fenfluramine, phenmetrazine, phenylpropanolamine) amid reports of serious adverse reactions.

A small proportion of patients using amphetamine-based appetite suppressants may escalate dosage voluntarily as tolerance develops to the anorectic effects; drug-seeking behavior may begin because of a physiologic addiction to amphetamine compounds.

Abusers of noradrenergic appetite suppressants usually prefer oral preparations; however, some abusers with highly addictive behavior may seek alternate routes of recreational abuse (insufflation, injection).

DIETHYLPROPION

IDENTIFYING CHARACTERISTICS

Diethylpropion (CAS RN: 90-84-6) is an *N,N*-diethyl cathinone analog [2-(diethylamino)-1-phenyl-1-propanone] with a structural formula of $C_{13}H_{19}NO$. Synonyms include amfepramone, α-benzoyltriethylamine, and propiophenone, whereas trade names include Adiposon, Anorex® (Klein-Becker, Salt Lake City, UT), Obesitex, Silutin, Tenuate, Tepanil, and Tylinal. Figure 13.1 displays the chemical structure of the phenylethylamine compound, diethylpropion. Diethylpropion displays minor sympathomimetic properties with stimulant activity much less than dextroamphetamine. The pharmaceutic preparation of diethylpropion is a racemate; experimental studies suggest that the (+) and (−) isomers of diethylpropion undergo spontaneous racemization at physiologic pH.[4] Under microscopic analysis, diethylpropion appears as very small prisms
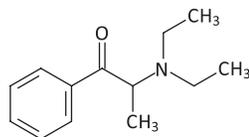
FIGURE 13.1. Chemical structure of diethylpropion.

that crystalize to forms ranging from thin, elongated laths to long rods.[5]

EXPOSURE

Diethylpropion was introduced in 1962 as an anorexiant for the short-term treatment of obesity in conjunction with diet and exercise; this sympathomimetic drug was an alternative to dextroamphetamine with fewer cardiovascular and central nervous system (CNS) side effects. In a meta-analysis of 13 randomized clinical trials of diethylpropion and weight loss, the mean weight loss of obese patients receiving 75 mg daily for 6–52 weeks was 3.0 kg (95% CI: –1.6 to 11.5 kg) when compared with placebo (lifestyle modification only).[6] The weight loss effect was modest and some studies did not demonstrate a statistically significant difference between the treatment and placebo groups. Diethylpropion is available as tablets and capsules under US Drug Enforcement Administration (DEA) schedule IV.

The abuse of diethylpropion is relatively rare;[7,8] the euphoria associated with diethylpropion is substantially less than dextroamphetamine or phenmetrazine.[9] These studies indicate that intravenous (IV) abuse of diethylpropion is unlikely because of the large number of tablets required to simulate the effects of dextroamphetamine. Most case reports of diethylpropion abuse involve individuals with polydrug abuse and previous experience with other amphetamine compounds (e.g., dextroamphetamine, phenmetrazine).[10] Although the use of diethylpropion initially may produce a feeling of well-being and talkativeness, the development of marked anxiety and agitation after 24–72 hours limits the continued use of this drug.[11]

DOSE EFFECT

Diethylpropion is formulated as 25 mg tablets or capsules for oral administration up to 3 times per day, or as 75-mg sustained-release tablets. The acute toxic dose of diethylpropion depends on tolerance; limited clinical data suggests that toxicity may develop in nontolerant adults at diethylpropion doses exceeding 750 mg.[12] A case report associated the development of anxiety, depression, labile mode, anorexia, insomnia, phobias,

ideas of reference with the chronic abuse of diethylpropion by a 27-year-old woman; she reported the use of 50–100 75-mg tablets daily (range up to 300 tablets).[13]

TOXICOKINETICS

The gastrointestinal (GI) tract absorbs diethylpropion well, with peak plasma diethylpropion concentrations occurring within 2 hours.[14] Diethylpropion undergoes extensive biotransformation with de-alkylation being the main metabolic pathway. The liver metabolizes diethylpropion via 1) N-deethylation to N-ethylaminopropiophenone and aminopropiophenone, 2) carbonyl reduction to N,N-diethylnorephedrine, and 3) deamination to benzoic acid with subsequent conjugation to glycine and the formation of hippuric acid.[15,16] N,N-Diethylnorephedrine undergoes de-alkylation to N-ethylnorephedrine. Norephedrine (phenylpropanolamine) is a minor metabolite of diethylpropion; p-hydroxylation is probably an insignificant pathway for the biotransformation of diethylpropion. All metabolites of diethylpropion are metabolically active. The kidney excretes minor amounts (i.e., <2%) of diethylpropion as unchanged drug depending on urinary pH.[17] The terminal plasma elimination half-life of diethylpropion is approximately 8 hours.[14] Limited pharmacologic data suggests that saturation kinetics may occur at supratherapeutic diethylpropion doses (e.g., 400 mg).[18]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Augmentation of norepinephrine and dopamine activity in the hypothalamus suppresses appetite, mildly dampens olfactory and gustatory acuity, and produces anorexia.[19] Amphetamine compounds may increase levels of leptin, a satiety-signaling neuromodulator and decrease concentrations of the neuromodulator (neuropeptide Y) that stimulates hunger, decreases energy expenditure, and increases fat storage.[20,21] Diethylpropion and phentermine induce appetite suppression via similar mechanisms.

CLINICAL RESPONSE

Dry mouth and insomnia were the most frequent adverse events in a clinical trial involving the administration of 50-mg diethylpropion twice daily for 6 months; there were no significant differences in blood pressure, pulse rate, electrocardiogram, echocardiography, or clinical chemistries when comparing the values at the end of the treatment phase with the baseline of 37 obese, healthy volunteers.[22] Occasional case reports

associate the use of diethylpropion with the development of acute psychosis, paranoia, and less-common affective symptoms.[23] These case reports often involve patients with histories of prior drug abuse, excessive diethylpropion use, or prior psychiatric disorders (e.g., depression, neurosis). Clinical features of the psychosis include auditory and visual hallucinations, paranoid delusions, feeling of influence (by telepathy), ideas of surveillance, ideas of reference, and thought broadcasting.[24,25] Although most of these patients did not have a prior history of psychotic disorders, follow-up of some of these patients suggests a typical clinical course of chronic paranoid schizophrenia in the absence of continued diethylpropion abuse.[26] The dose, time course, and duration of diethylpropion use is highly variable with some psychotic episodes occurring within a few weeks of the initiation of therapeutic diethylpropion doses while other episodes occur within a week of the cessation of chronic diethylpropion use.[27,28] Abrupt discontinuance following chronic diethylpropion abuse may result in apathy, depression, lethargy, anxiety, myalgias, abdominal pain, and sleep disturbances marked by diminished rapid eye movement (REM) that requires several weeks to resolve.

Rare case reports associate the therapeutic use of diethylpropion with transient ischemic attacks and possible cerebral vasospasm,[29] *torsade de pointes* in a patient with QTc prolongation (0.52 msec) and marked hypokalemia (2.4 mEq/L),[30] and primary pulmonary hypertension in a patient with BMPR2 gene mutation.[31] However, the causal role of diethylpropion in the etiology of these abnormalities is unclear because of the presence of multiple etiologic factors and the lack of known pathologic mechanisms.

DIAGNOSTIC TESTING

Analytic methods for the detection and quantitation of diethylpropion include gas chromatography (GC) with flame ionization,[16,32] high performance liquid chromatography (LC) with UV detection (254 nm),[4] gas chromatography/electron impact/mass spectrometry,[15] and GC/MS with heptafluorobutyric derivation.[33] The use of high performance capillary electrophoresis with confirmation by GC/MS in electron impact ionization mode allows the detection of diethylpropion in solid samples (e.g., Chinese herbs).[34] The limit of detection (LOD) with this method is 0.4 µg/mL with intraday precision <3%. Diethylpropion is unstable in alkaline media and relatively stable in neutral to acidic conditions. In refrigerated (4°C/39.2°F) plasma samples, diethylpropion was undetectable within 1 week under alkaline conditions (pH = 11), whereas little deterioration in diethylpropion occurred during the same period in samples stored under neutral (pH = 7) and acidic (pH = 5) conditions.[32] The peak plasma diethylpropion concentration after a single oral dose of 400 mg is ~300 ng/mL 2 hours after ingestion.[18]

TREATMENT

The treatment of diethylpropion is supportive, similar to phenylpropanolamine intoxication.

EPHEDRINE

IDENTIFYING CHARACTERISTICS

Ephedrine is β-hydroxylated methamphetamine [(1R,2S)-2-methylamino-1-phenylpropan-1-ol, CAS RN: 299-42-3], which has a structural formula of $C_{10}H_{15}NO$. Figure 13.2 displays the chemical structure of ephedrine. Table 13.1 lists some physiochemical properties of ephedrine.

Do-do tablets were OTC cough and cold preparations, which contained 222 mg ephedrine hydrochloride, 30 mg caffeine, and 50 mg theophylline sodium glycinate. Rare case reports associated the abuse of this medication as a substitute for amphetamine.[35]

EXPOSURE

Ephedrine has less addicting potential than amphetamine or methamphetamine; however, occasional case
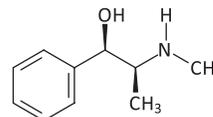
FIGURE 13.2. Chemical structure of ephedrine.

TABLE 13.1. Physiochemical Properties of Ephedrine.

Physical Property	Value
Melting Point	34°C (93.2°F)
Boiling Point	255°C (491°F)
pKa Dissociation Constant	10.252
log P (Octanol-Water)	1.13
Water Solubility	6.36E+04 mg/L (30°C/86°F)
Vapor Pressure	8.30E-04 mm Hg (25°C/77°F)

reports appear in the English, Russian, and Japanese medical literature documenting ephedrine abuse and addiction, usually in polydrug abusers.[36] A 33-year-old woman was admitted for an 18-month history of ephedrine abuse that began with the use of 50 mg daily for appetite suppression.[37] Her ephedrine use escalated to 1,500 mg daily as a result of tolerance, rebound somnolence and fatigue, and an inability to abstain despite her job loss for stealing the drug at work. On presentation for inpatient treatment, she was disheveled and emotionally labile with poor cognition; she had a sinus tachycardia and normal systolic blood pressure. Her only other known addiction was nicotine.

Ephedrine is distributed as a synthetic pharmaceutic preparation or as a herbal dietary supplement (e.g., ma huang) derived from plants (e.g., central Asian evergreen shrub, *Ephedra sinica*).[38] The typical ma huang preparation contains 1–2% total alkaloids with ephedrine and pseudoephedrine comprising ~90% of the total alkaloid content;[39] however, the total ephedra alkaloid content may range up to 8%.[40] In a study of 35 commercial oral ephedrine-based diet-control agents and bronchodilators, the total ephedra alkaloid content ranged from 5.97–29.3 mg per dose for administration 1–3 times daily.[41] In the United States, ephedrine was sold as a tablet, capsule, or constituent in an intranasal spray until the FDA banned the commercial sale of this drug as an OTC agent in 2004 based on an unreasonable risk of illness or injury under the conditions of use recommended or suggested in labeling.[42] Clinical uses for ephedrine included the treatment of spinal anesthesia-induced hypotension, bronchoconstriction, upper respiratory tract symptoms, obesity, depression, narcolepsy, and Stokes-Adams attacks.[43]

DOSE EFFECT

Maximal daily therapeutic doses of ephedrine range up to 120 mg. Tolerance to the effects of ephedrine develops during ephedrine abuse, resulting in reported daily ephedrine doses ranging up to 10–20 times the therapeutic dose. In a case series of 5 patients admitted for inpatient treatment of ephedrine addiction, the mean reported ephedrine dose was 1,450 mg with a range up to 2,500 mg.[37] Following the ingestion of 50–100 mg ephedrine daily for 10 days to improve athletic performance, a 19-year-old woman developed hemodynamically stable ventricular tachycardia that was resistant to cardioversion and amiodarone.[44] Sixty hours after admission when the ingested ephedrine would be expected to be eliminated (no plasma ephedrine concentrations reported), electrophysiology studies revealed an reproducible ventricular tachycardia; she underwent successful ablation of the dysrhythmia. She

remained in normal sinus rhythm at her 1-year follow-up examination.

TOXICOKINETICS

The absorption of therapeutic ephedrine doses from the GI tract and nasal mucosa is relatively complete (i.e., >90%) with peak plasma ephedrine concentrations occurring within ~2 hours.[45] The approximate volume of distribution of ephedrine is approximately 3 ± 1 L/kg. About 8–20% of the absorbed ephedrine dose undergoes N-demethylation to norephedrine, whereas oxidative deamination of ephedrine to 1-phenylpropan-1,3-diol followed by side-chain oxidation to benzoic acid and hippuric acid accounts for approximately 4–13% of the absorbed dose.[46] The liver metabolizes approximately 4% of ephedrine to norephedrine via N-demethylation and converts a minor proportion to inactive, renally excreted metabolites via conjugation, deamination, and p-hydroxylation. The kidneys excrete the majority of the ephedrine dose unchanged in urine along with a small proportion of inactive conjugates, depending upon urine pH and species.[47] The plasma half-life of ephedrine ranges from ~5.7–7.1 hours.

The combination of ephedrine and other stimulants (caffeine, theophylline) with a monoamine oxidase inhibitor (phenelzine) can produce serious drug interactions. A 28-year-old woman developed hypotension, sinus tachycardia, neuromuscular irritability, rhabdomyolysis, encephalopathy, and hyperthermia after ingesting a combination tablet (18.31 mg ephedrine hydrochloride, 30 mg caffeine, 100 mg theophylline) 8 hours prior to admission.[48] She had stopped her 60-mg daily intake of phenelzine the preceding day. She recovered after a complicated 3-week hospitalization that included 17 days on a respirator for acute respiratory distress syndrome.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Ephedrine is both a direct and indirect sympathomimetic compound. Catecholamine reuptake complexes on adrenergic nerve membranes transport ephedrine from intercellular spaces into the nerve cell. The nerve cell packages ephedrine into presynaptic storage vesicles together with endogenous norepinephrine, dopamine, or serotonin. Ephedrine increases the alkalinity of the vesicle, thus lowering the threshold for action potentials to induce vesicle exocytosis. As a result, incoming action potentials release elevated molar concentrations of neurotransmitters, along with ephedrine, into the synapse, thus characterizing the indirect sympathomimetic effect of ephedrine.[49] Once in the synaptic space, ephedrine is a weak direct agonist of postsynaptic

adrenergic receptors with an affinity higher for β-receptors than for α-receptors.[49] Augmentation of peripheral norepinephrine activity mildly stimulates thermogenesis in brown adipose tissue.[50] In ephedrine-caffeine combination preparations, caffeine antagonizes adenosine and inhibits cellular phosphodiesterase, thus enhancing adipose tissue thermogenesis.[51] In the lung, ephedrine produces bronchodilation through stimulation of bronchial β$_2$-adrenergic receptors.[52]

Hyperthermia from ephedrine poisoning is mediated in part by effects on the hypothalamus, caudate, and putamen.[53] The combination of ma huang or ephedrine with caffeine enhances cardiotoxicity compared with ephedrine alone.[54] Chronic abuse of ephedrine may cause dilated left ventricular hypertrophy with impaired systolic function without evidence of myocardial infarction or fibrosis.[55] Hyperventilation may occur via direct bronchial adrenergic receptor stimulation, and via indirect response to increased cardiac output induced by ephedrine.[56]

Patients with ephedrine intoxication may exhibit left ventricular hypertrophy and coronary artery disease in up to 33% of cases; however, ephedrine deaths are commonly associated with chronic stimulant abuse including the abuse of other drugs associated with coronary disease (e.g., cocaine). Heat stroke and rhabdomyolysis are uncommon findings in postmortem examinations of cases where postmortem blood samples contain detectable concentrations of ephedrine, when compared with amphetamine or methamphetamine abuse deaths.[57]

CLINICAL RESPONSE

Potential adverse effects associated with the use and abuse of ephedrine include mydriasis, nausea, vomiting, CNS hyperstimulation, headache, tremor, excess sweating, chest pain, tachycardia, palpitations, chest pain, hypertension, constipation, urinary retention, and euphoria.[58,59] Case reports associate ephedrine use and abuse with ischemic and hemorrhagic stroke,[60] intestinal ischemia,[61] and myocardial infarction,[62] frequently in patients with underlying disease (atherosclerosis, berry aneurysm).[63] Rare case reports associate ephedrine abuse with emergence of bipolar symptoms[64] and paranoid psychosis manifest by delusions, auditory hallucinations, and clear consciousness.[65] Some of these patients had predisposing factors (family history, abnormal premorbid personality, polydrug abuse); the role of ephedrine in these cases was not well defined. Potentially, abrupt cessation of high doses of ephedrine may cause fatigue, depression, and suppressed REM sleep; however, an abstinence syndrome following ephedrine abuse is not well defined. Fatalities following ephedrine abuse

may result from intracerebral hemorrhagic stroke, myocardial infarction, or cardiomyopathy.[63,66] These individuals often have underlying vascular disease along with polydrug abuse.[67] Consequently, the contribution of ephedrine abuse to these deaths is unclear.

DIAGNOSTIC TESTING

Methods for the quantitation of ephedrine in biologic samples include HPLC with UV detection,[68] HPLC with fluorometry and derivatization with 9-fluroenylmethyl chloroformate,[69] liquid chromatography/tandem mass spectrometry,[70] and GC/MS. The lower limit of quantitation (LLOQ) for ephedrine using HPLC with fluorometry is 2 ng/mL with a coefficient of variation in the range of 12% near the LLOQ. Ephedrine is stable in urine samples stored at 37°C (98.6°F) for 1 week and in refrigerated (4°C/39.2°F) and frozen (−20°C/-4°F), sterile urine samples for up to 2 years.[71] There was no significant loss of ephedrine in frozen samples undergoing 3 cycles of freezing and thawing to room temperature.

Dosing for weight control at 45–50 mg per day results in steady-state ephedrine concentrations of 95–135 ng/mL. The ingestion of 22 mg (−)-ephedrine hydrochloride by 10 hospital outpatients produced a mean peak plasma ephedrine concentration of 79.4 ng/mL (range, 52.7–138.8 ng/mL) compared with a mean peak plasma ephedrine concentration of 87.4 ng/mL (range, 44.0–118.6 ng/mL) following the ingestion of 11 mg 3 times daily for 2 weeks.[72] The mean peak plasma ephedrine concentration following the ingestion of 50 mg ephedrine by 16 healthy volunteers was 137.8 ± 33.1 ng/mL (range, 80.2–206.9 ng/mL).[45] After ingesting over twice the daily recommended dose of a combination tablet containing ~10 mg ephedrine and 100 mg caffeine for 2 days, a 22-year-old woman developed palpitations, nausea, tremulousness, abdominal pain, vomiting, S-T segment depression, and inverted T-waves.[73] The serum ephedrine concentration ~8 hours after the last dose was 150 ng/mL; the serum caffeine concentration was not reported. She was discharged after 2 days without sequelae. The postmortem blood ephedrine concentrations in a series of nontrauma-related fatalities ranged from 20–12,350 ng/mL with a blood ephedrine concentration below 490 ng/mL in 50% of the cases.[67] A majority of these decedents also tested positive for other drugs (e.g., cocaine, opiates); therefore, the role of ephedrine in these deaths is difficult to determine. Associated morbidities included left ventricular hypertrophy with coronary artery disease (33%), hepatic steatosis (21%), and nephrosclerosis (17%). Limited postmortem data suggest that ephedrine does not undergo significant postmortem redistribution.[74]

TREATMENT

The treatment of ephedrine intoxication is similar to the treatment of phenylpropanolamine toxicity.

PHENDIMETRAZINE

IDENTIFYING CHARACTERISTICS

Phendimetrazine is 3,4-dimethyl-2-phenylmorpholine (CAS RN: 634-03-7, $C_{12}H_{17}NO$) as displayed in Figure 13.3; the salt, phendimetrazine tartrate is the active ingredient of Adipost, Bontril, Obezine, Plegine, Prelu-2, Statobex, and Obex-LA (South Africa). The latter product is a sustained release preparation (105 mg *d*-phendimetrazine bitartrate). Under microscopic analysis, phendimetrazine appears as thin, square plates to square prismatic forms.[5] Phendimetrazine exists as *d*- and *l*-diastereoisomers.[75] Table 13.2 lists some physiochemical properties of phendimetrazine.

EXPOSURE

Phendimetrazine is a synthetic amphetamine derivative. Both phendimetrazine and phenmetrazine were introduced in the 1950s as sympathomimetic weight-control medications inducing less euphoria, insomnia, and tachycardia than other amphetamine derivatives; however, the current use of phendimetrazine is infrequent. The abuse of phendimetrazine is also uncommon, even among drug abusers.[76] Phendimetrazine is avail-
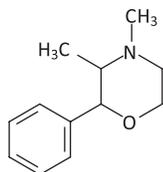
FIGURE 13.3. Chemical structure of phendimetrazine.

TABLE 13.2. Some Physiochemical Properties of Phendimetrazine.

Physical Property	Value
Molecular weight	
Phendimetrazine	191.2694 g/mol
Phendimetrazine tartrate	341.3563 g/mol
log P (Octanol-Water)	1.700

able in the United States as a DEA schedule III drug in both tablet and capsule forms.

DOSE EFFECT

Typical doses of phendimetrazine for the short-term treatment of obesity are 35 mg tablets or capsules 3 times per day or 105 mg sustained-release tablets.

TOXICOKINETICS

Peak serum phendimetrazine concentrations occur ~1 hour after the ingestion of immediate-release preparations and 1–2 hours after the ingestion of sustained release preparation.[77] Phendimetrazine undergoes extensive biotransformation including *N*-demethylation to phenmetrazine[78] as well as the formation of *N*-hydroxyphenmetrazine and the *N*-oxide of phendimetrazine.[79] The kidney excretes only ~10–30% of the phendimetrazine dose as unchanged drug.[78,79] The approximate plasma half-life of immediate-release phendimetrazine is approximately 2 hours, whereas the plasma half-life of the sustained-release preparation is ~9 hours.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Phendimetrazine is an indirect-acting sympathomimetic drug that causes mild CNS stimulation and blood pressure elevation, similar to other noradrenergic appetite suppressants. Therapeutic doses of phendimetrazine cause early satiety and decreased appetite.

CLINICAL RESPONSE

Phendimetrazine and phenmetrazine are associated with similar physiologic and psychological effects, similar to other noradrenergic appetite suppressants including anxiety, agitation, nervousness, paranoia, psychosis, and hallucinations. A case report associates the use of phendimetrazine, phentermine, and an unknown "thyroid" medication over 3 days with the development of diffuse peripheral arterial spasm that responded to nifedipine.[80] The patient remained asymptomatic 6 months later with no further use of these medications. Rare case reports associate chronic phendimetrazine use with dilated cardiomyopathy that resolved with cessation of phendimetrazine use[81] and acute interstitial nephritis (in combination with phentermine) that also resolved following cessation of these 2 drugs.[82] Rare case reports associate therapeutic use and abuse of phendimetrazine with rhabdomyolysis. A 23-year-old woman developed nausea, vomiting, chest pain, myal-

gias, and dark urine after ingesting 75 mg phendimetrazine daily for 3 days.[83] Her serum creatine phosphokinase (now creatine kinase, CK) peaked at 7,224 U/L on the second hospital day; her kidney function remained normal and she was discharged on the fifth hospital day without sequelae.

DIAGNOSTIC TESTING

Analytic methods for the quantitation of phendimetrazine in biologic samples include thin layer chromatography, gas chromatography with flame ionization detection[84] or nitrogen phosphorous detection,[85] and GC/MS with acetic acid derivation.[86] Phendimetrazine does not concentrate in erythrocytes; therefore, whole blood and serum concentrations of phendimetrazine are similar. Peak serum phendimetrazine concentrations following therapeutic doses of phendimetrazine range from ~0.05–0.24 mg/L, whereas peak serum phendimetrazine concentrations associated with the ingestion of 35 mg immediate-release phendimetrazine range up to 0.09–0.10 mg/L.[77,87] A 21-year-old man collapsed while working at a restaurant; resuscitation at the workplace and the hospital was unsuccessful.[86] Postmortem blood (site unspecified) contained 0.3 mg phendimetrazine/L as measured by gas chromatography/flame ionization detection. A 38-year-old man was found dead 4 days after disappearing during a desert race.[88] His postmortem blood contained 0.67 mg phendimetrazine/L; no other drugs were detected as measured by gas chromatography/flame ionization detection.

TREATMENT

The treatment of phendimetrazine toxicity is supportive, similar to phenylpropanolamine intoxication.

PHENMETRAZINE

IDENTIFYING CHARACTERISTICS

Phenmetrazine is a synthetic morpholine amphetamine derivative (3-methyl-2-phenylmorpholine, CAS RN: 134-49-6). Figure 13.4 displays the chemical structure of phenmetrazine ($C_{11}H_{15}NO$). Phenmetrazine hydrochloride was the active ingredient of Preludin. Under microscopic analysis, phenmetrazine is an elongated lath-like crystal in stellate arrangement.[5] Table 13.3 lists some physiochemical properties of phenmetrazine.
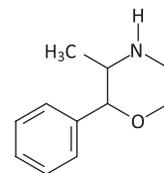
FIGURE 13.4. Chemical structure of phenmetrazine.

TABLE 13.3. Some Physiochemical Properties of Phenmetrazine.

Physical Property	Value
Molecular Weight	
Phenmetrazine	177.2429 g/mol
Phenmetrazine hydrochloride	213.7038 g/mol
log P (Octanol-Water)	1.490

EXPOSURE

Phenmetrazine was introduced in the 1950s along with phendimetrazine as sympathomimetic weight-control medications with less euphoria, insomnia, and tachycardia than other amphetamine anorexiants;[89] however, phenmetrazine was subsequently classified as a schedule II drug in 1971 as a result of reports of psychotic reactions and high addiction potential. Methods of phenmetrazine abuse involve both oral and IV administration. Phenmetrazine remained a popular drug of abuse during the 1970s that replaced amphetamine as a stimulant in some IV polydrug users.[90] This drug was a substitute for cocaine and amphetamine in heroin abusers; after dissolution of phenmetrazine in water, the solution was used to "cook" the heroin prior to injection. The use of phenmetrazine as a primary drug of abuse is uncommon.[91] Eventually, phenmetrazine was withdrawal from the US market.

DOSE EFFECT

The recommended therapeutic dose of phenmetrazine is 25 mg 3 times daily. The lethal dose of phenmetrazine is not well defined; the LD_{50} is 15–20 mg/kg for adult monkeys and ~5 mg/kg for young monkeys. The chronic abuse of 750–900 mg daily was associated with the development of acute paranoid psychosis that resolved with hospitalization and chlorpromazine therapy.[92]
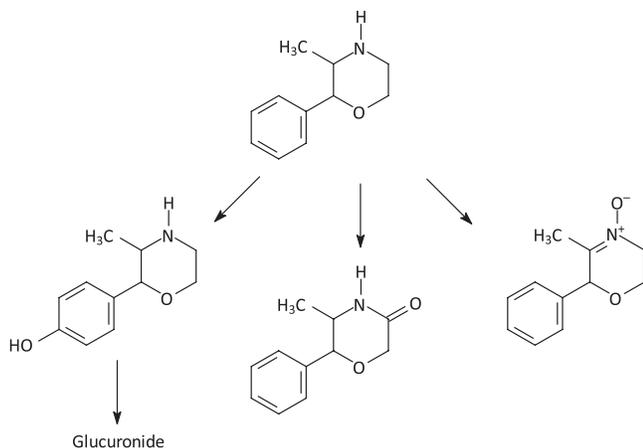
FIGURE 13.5. Biotransformation of phenmetrazine.

TOXICOKINETICS

Phenmetrazine is well absorbed by the GI tract with immediate- and sustained-release preparations reaching peak concentrations within ~2 hours and 5 hours, respectively.[93] This drug undergoes *p*-hydroxylation followed by glucuronide conjugation and oxidation via CYP3A and CYP2D6 to lactam metabolites [5-methyl-3-oxo-6-phenylmorpholine, 3-methyl-2-(4'-hydroxyphenyl)morpholine]. Figure 13.5 displays the biotransformation of phenmetrazine. In a study of urine samples from volunteers receiving 25 mg [^{14}C] phenmetrazine hydrochloride, lactam metabolites accounted for a mean 19% (range, 17–21%) and the total (free plus conjugated) *p*-hydroxylated metabolite accounted for a mean 22% (range, 16–26%) of the administered dose within the first 24 hours.[94] *N*-hydroxyphenmetrazine nitrone (5–7%) was a minor metabolite. The kidney excreted a mean 19% (range, 11–25%) as unchanged phenmetrazine during the first 24 hours. Over 72 hours, a mean of 92% (range, 85–100%) of the administered dose appeared in the urine. There is substantial intraspecies variation in phenmetrazine biotransformation, similar to amphetamine and methamphetamine. The plasma elimination half-life of phenmetrazine following ingestion of single therapeutic doses of immediate release phenmetrazine is ~8 hours.[93]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Phenmetrazine primarily blocks the reuptake of dopamine and to a lesser extent, norepinephrine into presynaptic adrenergic nerve cells; additionally, phenmetrazine inhibits monoamine oxidase, leading to an increased prevalence of synaptic catecholamines. Centrally, phenmetrazine exerts this effect on the tuberoinfundibular system, leading to appetite suppression.[95] Postmortem examination of fatal cases of phenmetrazine are nonspecific, common to individuals dying from drug abuse. Autopsy examination of a 17-year-old adolescent found dead with fresh track marks and only phenmetrazine in postmortem blood revealed pulmonary edema and aspiration of material deep into the bronchi.[96]

CLINICAL RESPONSE

Phenmetrazine is a CNS stimulant that produces clinical effects similar to other amphetamine-type appetite suppressants. Toxic effects associated with phenmetrazine abuse include stereotyped activity, agitation, anxiety, insomnia, anxiety, and restlessness. Case reports associate phenmetrazine abuse with a psychosis similar to amphetamine-induced psychosis and with the exacerbation of underlying schizophrenia with paranoid ideations. Clinical features include auditory hallucinations, labile affect, agitation, elation, delusions, ideas of reference, fear, and paranoia.[97] Typically, these psychotic symptoms occur following the ingestion of large doses of phenmetrazine (i.e., 10–30 tablets in the setting of continue abuse) and resolve within 1–2 days of abstinence.[98] Case reports associate the IV administration of phenmetrazine with fever, diaphoresis, diffuse myalgias, headache, GI distress, hypotension, rhabdomyolysis, and intravascular coagulation.[99] This clinical pattern simulates septic shock, but the blood cultures from these patients were negative. Fatalities associated with the abuse of phenmetrazine include patients with hyperthermia, agitation, and bizarre behavior (excited delirium-type clinical presentation), trauma/suicide, sudden death (i.e., found dead at home), and underlying cardiovascular disease.[100] Most of these fatalities involve polydrug abuse, primarily IV drug use.

DIAGNOSTIC TESTING

Analytic methods for the quantitation of phenmetrazine in biologic samples include gas chromatography with electron capture detection,[101] gas chromatography/flame ionization detection,[94,102] gas chromatography/mass spectrometry (GC/MS) with derivatization involving 2,2,2-trichloroethyl chloroformate or isooctane and methyl chloroformate,[103,104] and GC/MS with selected ion monitoring, chemical ionization, and 4-carbethoxyhexafluorobutyryl chloride derivatization.[105] The LOD for phenmetrazine in urine samples using the latter method is 0.2 mg/L with between-run precision of ~3%. The LLOQ for the latter method in urine samples is

0.05 mg/L with an intraday coefficient of variation <9%. Ephedrine may interfere with the quantitation of phenmetrazine by GC/MS during the derivation process as a result of the reaction of ephedrine and formaldehyde under high-temperature injection.[106] The use of gas chromatography/flame ionization detection and GC with electron capture detection allows comparison of seized drugs for the determination of common sources of illicit phenmetrazine samples.[107] Phenmetrazine cross-reacts with urinary amphetamine immunoassays (e.g., EMIT) only at high phenmetrazine concentrations (e.g., >6 mg/L).[108]

The therapeutic plasma phenmetrazine concentrations range from 0.07–0.13 mg/L; the mean plasma phenmetrazine concentration following ingestion of a single 75 mg phenmetrazine tablet was approximately 0.13 mg/L as measured by radioimmunoassay (tritiated acetic anhydride).[93] In a convenience series of 7 arrested drivers (car theft, drug addiction), the blood phenmetrazine concentrations ranged from 0.2–4 mg/L as measured by GC/MS.[103] Most of these drivers failed field sobriety tests. Postmortem blood from a 17-year-old adolescent found dead with fresh track marks contained a phenmetrazine concentration of 4 mg/L, whereas a liver sample contained 5 mg/L as measured by GC/MS.[96] No other drugs were detected in the postmortem blood. A 29-year-old woman was found dead at her home with evidence of recent IV drug abuse; the postmortem blood (site not reported) contained 16 mg phenmetrazine/L and no other detectable drugs.[100] A 25-year-old man developed hyperthermia, tachycardia, hypotension, and paranoid psychosis after the IV injection of phenmetrazine.[100] He died 3 hours after admission to the hospital; postmortem blood contained 5 mg phenmetrazine/L and no other detectable drugs as measured by GC/MS.

TREATMENT

The treatment of phenmetrazine toxicity is supportive with initial attention directed toward assessment and stabilization of respiration, circulation (myocardial ischemia, hypotension), and temperature, similar to phenylpropanolamine intoxication. Seriously intoxicated IV phenmetrazine abusers may develop hypotension, hyperthermia, agitation, and disseminated intravascular coagulation. The presence of hyperthermia (i.e., core body temperature >40°C/104°F) requires aggressive treatment of agitation with benzodiazepines and evaporative cooling measures. Treatment of hypotension includes IV saline and vasopressor infusions (e.g., dopamine) for patient unresponsive to fluid therapy. Patients unresponsive to dopamine may respond to α-receptor agonists (e.g., norepinephrine).

PHENTERMINE

IDENTIFYING CHARACTERISTICS

Phentermine (CAS RN: 122-09-8) is an α-methyl amphetamine derivative (1,1-dimethyl-2-phenylethylamine, $C_{10}H_{15}N$). Figure 13.6 displays the chemical structure of phentermine. Trade names for this compound include Adipex-P (phentermine hydrochloride, 37.5 mg), Ionamin (15 mg and 30 mg phentermine as the cationic exchange resin complex), and Omnibex. Table 13.4 lists some physiochemical properties of phentermine.

EXPOSURE

Phentermine is a sympathomimetic amine approved by the FDA in 1959 for short-term weight loss in combination with diet and exercise. Phentermine and fenfluramine were part of the popular off-label anorexiant, Fen-Phen until 1997, when the FDA banned fenfluramine because of the association of this combination with cardiac valvular disease. Phentermine remains on the US market for the treatment of obesity as a single agent and as combination capsule with fluoxetine.[109] Phentermine is available as a sustained-release resin complex (37.5 mg) and immediate release capsules (15 mg, 30 mg) under DEA schedule IV. In a meta-analysis of 9 randomized clinical trials of phentermine and weight loss, the mean weight loss of obese patients receiving 15–30 mg daily for 2–24 weeks was 3.6 kg
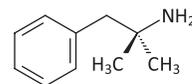
FIGURE 13.6. Chemical structure of phentermine.

TABLE 13.4. Some Physiochemical Properties of Phentermine.

Physical Property	Value
Molecular Weight	
Phentermine	149.2328 g/mol
Phentermine hydrochloride	185.6937 g/mol
Boiling Point	205°C (401°F)
log P (Octanol-Water)	1.9
Water Solubility	1.86E + 04 mg/L (25°C/77°F)
Vapor Pressure	0.096 mm Hg (25°C/77°F)

241

(95% CI: 0.6–6.0 kg) when compared with placebo (life-style modification only).[110]

DOSE EFFECT

The therapeutic dose of phentermine base is 15–30 mg daily. The ingestion of approximately 560 mg phentermine over 8 hours was associated with pressured speech, tactile and visual hallucinations, and refractory seizures; she recovered without sequelae after treatment with benzodiazepines (diazepam, lorazepam), fosphenytoin, propofol infusion, and supportive care in the intensive care unit.[111]

TOXICOKINETICS

When ingested as a sustained release resin, peak phentermine concentrations occur within ~8 hours. Phentermine undergoes minimal p-hydroxylation, N-oxidation, and N-hydroxylation, followed by conjugation.[112] The approximate volume of distribution of phentermine is approximately 3–4 hours. The plasma half-life of phentermine in the resin complexes of pharmaceutic preparations ranges from ~19–24 hours; however, in a study of 5 adults receiving phentermine in an aqueous solution with urine pH <5, the plasma elimination half-life was approximately 7–8 hours. Within the first 24 hours, the kidney excreted 71.7–83.6% of the phentermine dose unchanged with 0.7% p-hydroxylated conjugates, and 5% N-hydroxylated and N-oxidized conjugates.[112]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Phentermine is a weak sympathomimetic amine that induces appetite suppression similar to diethylpropion by increasing norepinephrine release and inhibiting uptake, resulting in increased sympathetic activity. Phentermine is also a weak monoamine oxidase (MAO) inhibitor that typically does not produce significant MAO activity at therapeutic doses. In rat brain tissue, the IC_{50} of phentermine for MAO_A and MAO_B was 143 μM and 285 μM, respectively, compared with 2 nM and 18 nM, respectively, for the potent MAO inhibitor, harmaline.[113]

CLINICAL RESPONSE

Adverse effects associated with the therapeutic use of phentermine for obesity include headache, irritability, anxiety, insomnia, palpitations, hypertension, depression, and breathlessness.[114] Several case reports associate high doses of phentermine (60–90 mg daily) with the

restlessness, irritability, pressured speech with flight of ideas, paranoid delusions, and auditory hallucinations with clear consciousness. Predisposing factors include a strong family history of affective disorder.[115] Repeat dosing of phentermine against medical advice resulted in the recurrence of the psychosis.[116] A 37-year-old woman developed the subacute onset of severe left parieto-occipital headache along with left-sided facial and extremity numbness that resolved within 1 week.[117] She had recently begun the use of phentermine for weight loss; her cerebral magnetic resonance imaging (MRI) scan and conventional 4-vessel cerebral angiography were normal 7 days after the onset of symptoms. A 48-year-old woman developed chest pain and a subsequent witnessed cardiac arrest (ventricular tachycardia/fibrillation) requiring defibrillation and intubation.[118] Her peak serum creatine phosphokinase (now creatine kinase or CK) and fraction were elevated at 716 U/L and 36.1 U/L, respectively; coronary angiography demonstrated a left ventricular ejection fraction of 35% with mild diffuse nonobstructive coronary artery disease. Her only medication was phentermine (dose not reported); 5 days after admission when the expected phentermine concentration would be nondetectable, the electrophysiology study demonstrated inducible sustained ventricular tachycardia. There were no reported plasma phentermine concentrations. Marked lethargy, syncope, QTc prolongation (512 msec), and polymorphic ventricular tachycardia developed in a 23-year-old woman who ingested illicit diet pills containing phentermine and chlorpheniramine based on a urine drug screen.[119] She was successfully treated with IV magnesium sulfate infusion and recovered without sequelae. No plasma phentermine concentrations were reported. Another case report associated the development of chest pain and subsequent ventricular fibrillation responsive to cardioversion in a moderately obese 37-year-old woman on phentermine, thyroid preparation, and trichlormethiazide.[120] Her cardiac workup was normal including coronary angiogram and electrophysiology testing with the exception of mild hypokalemia (2.9 meq/L). Case reports associate daily phentermine therapy with acute interstitial nephritis,[121] ruptured superior mesenteric artery aneurysm,[122] and peripheral vascular disease;[123] however, the role of phentermine in causing these diseases is unclear.

DIAGNOSTIC TESTING

Analytic Methods

Short retention times along with poor peak shape and resolution complicate analysis of small molecules (e.g., phentermine) with GC; consequently, most quantitative

methods include derivatization. Analytic methods for the quantitation of phentermine in biologic samples include gas chromatography/flame ionization detection and N-trifluoroacetamide derivatization,[124] gas chromatography with nitrogen phosphorus detection and amantadine as the internal standard,[125] GC/MS with N-trifluoroacetamide derivatization,[126] high performance liquid chromatography with fluorescence detection (excitation, 325 nm; emission, 430 nm),[127] ultra performance LC/MS,[128] and GC/MS using pentafluoropropionic anhydride derivatization and N-propylamphetamine as the internal standard.[129] The LOD for the latter method is 500 ng/mL, whereas the LOD and LLOQ for gas chromatography with nitrogen phosphorus detection is 5 ng/mL and 20 ng/mL, respectively (precision and accuracy <10%). Potential interference with the quantitation of phentermine may occur a as result of the partial co-elution of phentermine (1.72 min) and methamphetamine (1.81) at m/z 150 in polydrug samples; the use of an optimized gradient helps alleviate this problem.

Biomarkers

Following therapeutic doses of phentermine, the plasma phentermine concentration varies substantially between individuals. Plasma phentermine concentrations do not correlate well to weight loss or failure to lose weight in obese adults. In a 20-week study of obese adults receiving phentermine resinate, the mean steady-state plasma phentermine concentration in 13 adults with sustained weight loss was 203.0 ± 67.6 ng/mL compared with 212.9 ± 114.1 ng/mL in 11 adults without significant weight loss as measured by gas chromatography with nitrogen specific detection (LOD, 2 ng/mL).[114] Headache and palpitations developed in a 25-year-old woman ingesting 60 mg phentermine daily during this study. She withdrew from the study when her steady-state plasma phentermine concentration was 381 ng/mL. In a study of 21 healthy adults receiving 30 mg phentermine hydrochloride (24.1 mg base) daily for 14 days, the mean peak plasma phentermine concentration on day 14 was 116 ± 31.7 ng/mL (range, 65.3–192.0 ng/mL) compared with 142 ± 40 ng/mL (range, 65.7–234 ng/mL) for the ingestion of the 30 mg phentermine base.[130] Phentermine fatalities were associated with postmortem phentermine concentrations of 1,500 ng/mL and 7,600 ng/mL; the latter case involved a 32-year-old man found dead with also 12 mg ethchlorvynol/L and 10 mg amobarbital/L (toxic concentration) as measured by gas chromatography with nitrogen phosphorus detection.[131] Limited postmortem data suggests that phentermine undergoes postmortem redistribution. The central/peripheral blood phentermine ratio from 1 postmortem case was 1.7:1.[74]

TREATMENT

The treatment of phentermine toxicity is supportive, similar to phenylpropanolamine intoxication.

PHENYL-PROPANOLAMINE

IDENTIFYING CHARACTERISTICS

Phenylpropanolamine (CAS RN: 14838-15-4) is the β-hydroxylated amphetamine, 1-alpha-(1-aminoethyl) benzyl alcohol (norephedrine). This weakly basic compound is an indirect-acting sympathomimetic amine that is structurally related to ephedrine. Figure 13.7 displays the chemical structure of l-phenylpropanolamine [(−)-norephedrine, $C_9H_{13}NO$]. This structure has 4 potential stereoisomers with phenylpropanolamine comprising the enantiomeric forms, d-phenylpropanolamine and l-phenylpropanolamine. The other 2 potential stereoisomers are the enantiomeric forms of the diastereoisomer norpseudoephedrine. Table 13.5 lists some physiochemical properties of phenylpropanolamine. Pharmaceutic preparations contain a racemic mixture of phenylpropanolamine hydrochloride (CAS
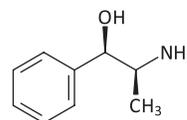
FIGURE 13.7. Chemical structure of phenylpropanolamine.

TABLE 13.5. Some Physiochemical Properties of Phenylpropanolamine.

Physical Property	Value
Molecular Weight	
Phenylpropanolamine	151.2056 g/mol
Phenylpropanolamine hydrochloride	187.6665 g/mol
pKa Dissociation Constant	9.44 (20°C/68°F)
log P (Octanol-Water)	0.67
Water Solubility	1.49E+05 mg/L (25°C/77°F)
Vapor Pressure	8.67E-04 mm Hg (25°C/77°F)

RN: 154-41-6). The melting point of phenylpropanolamine hydrochloride is 194°C (381.2°F). Most of the sympathomimetic activity of the racemic mixture resides in the *l*-enantiomer.

EXPOSURE

The medical uses of phenylpropanolamine included the treatment of nasal congestion, priapism, and control of urinary incontinence. Phenylpropanolamine was a popular OTC treatment for upper respiratory tract symptoms and for weight loss (e.g., Acutrim®, J.B. Laboratories, Holland, MI; Dexatrim®, Chattem, Inc., Chattanooga, TN; Prolamine™, Super Odrinex™) in combination with exercise and dietary restriction. The latter preparations frequently contained ephedrine and/ or caffeine in addition to phenylpropanolamine. In 2000, the FDA requested drug companies to discontinue all OTC phenylpropanolamine-containing products as a result of studies demonstrating an increased risk of hemorrhagic stroke in women using phenylpropanolamine for weight-control.[132] Phenylpropanolamine analogs are present in certain plants; however, medicinal phenylpropanolamine is manufactured synthetically.[133]

DOSE EFFECT

The recommended daily dose of phenylpropanolamine for weight loss is 25–75 mg immediate-release or 75 mg sustained-release capsules up to a daily maximum of 150 mg. In volunteer studies, the administration of therapeutic doses of phenylpropanolamine (e.g., 75 mg sustained release) does not cause euphoria or stimulant activity.[134] Cardiovascular and CNS complications following the use and abuse of phenylpropanolamine are rare events in comparison to the large number of individuals exposed to phenylpropanolamine. These adverse events are not clearly dose-related as these events occur following therapeutic use, abuse, and overdose. A majority of hemorrhagic strokes in 2 case-control studies of phenylpropanolamine in appetite suppressants and OTC preparations involved the use of daily phenylpropanolamine doses ≤ 75 mg, suggesting that many of these complications are idiosyncratic events in susceptible patients.[135,136] In a case series of 16 strokes associated with the use of phenylpropanolamine, the phenylpropanolamine dose ranged from 75–675 mg.[137] In this case series, a 19-year-old woman developed a right frontal hemorrhage after intentionally ingesting 675 mg phenylpropanolamine. She had uncontrolled hypertension on admission (180/110); moderate disability remained after discharge from the hospital. Most patients in this case series ingested phenylpropanol-

amine in combination with other decongestants for 1 to several days prior to the onset of symptoms. The average therapeutic dose of phenylpropanolamine produces a small, but statistically significant increase in systolic blood pressure that is more prominent with higher doses, shorter administration, and immediate release formulations.[138,139] In a meta-analysis of 33 clinical trials involving 2,165 patients, the average increase in systolic blood pressure was 5.5 mm Hg (95% CI: 3.1–8.0), whereas the diastolic blood pressure increased an average of 4.1 mm Hg (95% CI: 2.2–6.0) with no effect on heart rate. The phenylpropanolamine doses ranged from 25–150 mg with the high-dose studies (n = 7) demonstrating higher posttreatment changes in systolic (mean pooled difference, 9.46 mm Hg; 95% CI: −19.17–38.09) and diastolic (mean pooled difference, 6.54 mm Hg; 95% CI: −14.27–27.35) blood pressures than pooled values of the 33 trials as a whole.

TOXICOKINETICS

Absorption

The GI absorption of phenylpropanolamine is relatively good. In a study of 24 healthy male volunteers receiving a single dose of 25 mg phenylpropanolamine as a syrup, the mean calculated absolute bioavailability was $61.9 \pm 6.5\%$ (range, 49.1–73.2%).[140]

Distribution

In a study of 24 healthy male volunteers receiving a single dose of 25 mg phenylpropanolamine as a syrup, the mean steady-state volume of distribution was approximately 1.7 L/kg (range, 1.5–2.2 L/kg). The mean volume of distribution following the administration of a single 25 mg phenylpropanolamine solution to 5 healthy volunteers was 3.26 ± 0.75 L/kg compared with a steady-state volume of distribution of 4.08 ± 0.91 L/kg following the ingestion of a 25-mg phenylpropanolamine solution every 4 hours for 7 doses.[141]

Biotransformation

Phenylpropanolamine undergoes minimal *p*-hydroxylation and conjugation in the liver with approximately 4% of the dose excreted as urinary metabolites of phenylpropanolamine.

Elimination

The kidney excretes most of the absorbed dose of phenylpropanolamine as unchanged drug. In a study of 16

healthy volunteers receiving 6 doses of 25 mg phenyl-propanolamine over 36 hours, the mean total urinary phenylpropanolamine excretion within 36 hours after the last dose was 75%.[142] The mean maximum phenyl-propanolamine concentration in urine samples (mean urine pH = 6.3 ± 0.3) was 112 ± 50 mg/L 4 hours after the last dose. Urinary alkalinization decreases the urinary clearance of phenylpropanolamine and increases the plasma phenylpropanolamine elimination half-life. Alkalization of the urine (mean pH ~7.5) in 4 healthy male volunteers resulted in an approximate mean reduction of the urinary phenylpropanolamine clearance of ~33%, when compared with placebo (no sodium bicarbonate).[143]

The serum half-life of phenylpropanolamine is ~3–4 hours. In a study of 5 healthy volunteers receiving single doses of 25 mg and 100 mg phenylpropanolamine, the mean serum half-lives were 3.8 hours and 4.3 hours, respectively.[141] Following alkalization in the above study, the mean plasma phenylpropanolamine half-life increased from 4.03 ± 0.41 hours to 5.39 ± 0.34 hours.

Tolerance

There are few data on the issue of tolerance following the chronic use of phenylpropanolamine. Animal studies suggest that short term use (<2 weeks) of phenylpropanolamine does not reduce the weight loss associated with the administration of 20 mg *d,l*-phenylpropanolamine as measured by interscapular brown adipose tissue thermogenesis in adult male Sprague-Dawley rats.[144]

Drug Interactions

Case reports associate the ingestion of phenylpropanol-amine by patients on monoamine oxidase inhibitors (MAOIs) with the development of hypertensive crisis similar to the association with structurally similar drugs (phenylephrine, pseudoephedrine). Adverse effects in these case reports include malignant hypertension, headache, fever, and intracranial hemorrhage.[145] The use of other drugs (e.g., meperidine) associated with hyper-tensive crises and MAOIs complicate the determination of causality in some of these cases.[146] The concomitant ingestion of caffeine and phenylpropanolamine may increase blood pressure more than the ingestion of the same dose of phenylpropanolamine alone; however, the effect is relatively small and the clinical significance of this difference is unclear on a population basis.[139] Case reports associate severe hypertension in a 27-year-old woman ingesting indomethacin while receiving 85 mg *d*-phenylpropanolamine daily for several months[147] and in a 31-year-old man ingesting 75 mg phenylpropanol-

amine daily for 2 days while treated with methyldopa and oxprenolol for hypertension.[148] In the former case, rechallenge reproduced the same blood pressure eleva-tions associated with indomethacin. Both these patients had headaches, but neither patient had clinically signifi-cant end organ dysfunction.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Phenylpropanolamine is a prominent peripheral and weak CNS adrenergic stimulant. Phenylpropanolamine enhances the release of norepinephrine, resulting in the direct stimulation of α-adrenergic receptors. Although phenylpropanolamine also has some α_2-adrenergic activity, rodent studies indicate that the anorectic effects of phenylpropanolamine result primarily from the stim-ulation of α_1-adrenergic receptors in the paraventricular hypothalamus.[149,150] Animal studies suggest that the ano-rectic effects of phenylpropanolamine differ from dex-troamphetamine, which stimulates dopaminergic and β-adrenergic receptors.[151] Although *in vitro* studies suggest that phenylpropanolamine has some dopami-nergic activity, this dopaminergic effect is substantially less potent than dextroamphetamine.[152] Rodent studies indicate that phenylpropanolamine causes little presynaptic release of serotonin.[153] Peripheral stimula-tion of α-adrenergic receptors produces vasoconstric-tion, reduces tissue hyperemia, edema, and nasal congestion, and increases nasal airway patency. Phenylpropanolamine blunts the release of synaptic norepinephrine associated with standing as a result of dose-dependent α_1-agonist, α_2-agonist, and indirect sympathomimetic effects.[149,154]

CLINICAL RESPONSE

Use/Abuse

Adverse effects associated with the use of phenylpropa-nolamine include severe headache, hypertension, intra-cranial hemorrhage, and rarely fatal strokes.[155] Most of these cases involve women under 30 years of age ingest-ing therapeutic doses of phenylpropanolamine, often in combination with other drugs (e.g., caffeine, antihista-mines). Cutaneous reactions include fixed drug eruptions.[156]

Mental Disorders

Psychiatric manifestations associated with the therapeutic use of phenylpropanolamine include mania, paranoid schizophrenia, hallucinations, paranoid delusions, depres-sion, agitation, and anxiety.[157,158] Frequently, these patients

ingest several additional medications and have previous histories of mood disorders or family histories of psychosis. In several patients, recurrent psychotic symptoms developed following rechallenge with phenylpropanolamine.[159] No single set of symptoms describes the psychiatric abnormalities associated with phenylpropanolamine use is these case series; furthermore, these abnormalities appear idiopathic as the dose of phenylpropanolamine ranges from therapeutic doses to large overdoses.

Medical Complications

CENTRAL NERVOUS SYSTEM

Case reports associate the use and abuse of both diet pills and OTC cough and cold preparations containing phenylpropanolamine in the preceding 24 hours with the development of intracranial hemorrhages.[160,161] About one-third of these cases involve supratherapeutic doses of phenylpropanolamine. The vast majority of these intracranial lesions are hemorrhagic strokes with and without evidence of vasculitis; only rarely are these strokes ischemic. The presence of cerebral aneurysm, tumor, or arteriovenous malformation increases the risk of hemorrhage stroke following the use of phenylpropanolamine. Most of the hemorrhage strokes are intracerebral; occasionally, subarachnoid hemorrhages occur. In a case series of 15 strokes associated with the use of phenylpropanolamine as a cough and cold preparation, there were 12 cases of intracerebral hemorrhage, 2 cases of subarachnoid hemorrhage, and 1 case of mild subarachnoid combined with a small fronto-orbital hemorrhage.[137] Most, but not all patients presented with hypertension. None of these cases involved the use of phenylpropanolamine as an appetite suppressant.

The Hemorrhagic Stroke Project was a case-control study of 702 patients with subarachnoid or intracerebral hemorrhage within 30 days before enrollment that demonstrated an increased risk of hemorrhagic stroke in women using appetite suppressants containing phenylpropanolamine.[135] For women using appetite suppressants containing phenylpropanolamine with 3 days of developing symptoms of a stroke, the adjusted odd ratio was 16.58 (95% CI: 1.51–182.21; $P = .02$). Although this study suggested that the use of phenylpropanolamine-containing OTC cough and cold preparations also were associated with an increased risk of stroke (adjusted OR = 3.13, 95% CI: 0.86–11.46) this association was not statistically significant ($P = .06$); there was no association of stroke and phenylpropanolamine use in men. Subsequent discovery suggests that chance, recall, selection bias, and confounding may contribute to the positive findings in this study.[162] A Mexican case-control study of 177 patients with intracranial hemorrhage and no structural intracranial lesions did not confirm the Hemorrhagic Stroke Project when comparing each case with 3–4 controls matched for age, sex, and place of residence.[163] The OR was 0.95 (95% CI: 0.68–1.34, $P = .77$).

CARDIOVASCULAR SYSTEM

Phenylpropanolamine may increase blood pressure depending on dose and individual sensitivity. In a study of 37 healthy young adults receiving a single dose of 85 mg phenylpropanolamine, 12 participants developed supine diastolic blood pressures exceeding 100 mm Hg in comparison to 4 of 34 participants receiving 50 mg phenylpropanolamine.[164] Although higher phenylpropanolamine doses are associated with higher blood pressure, experimental studies suggest that the serum phenylpropanolamine concentration does not correlate directly to systolic or diastolic blood pressure.[165] Furthermore, adverse effects associated with these blood pressure changes are typically mild.

Myocardial injury following the use or abuse of phenylpropanolamine is rare. Case reports associate both the ingestion of single therapeutic (50 mg) and overdoses (2 g with 8 g caffeine) with severe hypertension, ST-T wave depression, and mildly elevated CK-MB fractions; symptoms included headache, dyspnea, nausea, blurred vision, and chest pain.[166,167] The M-mode and 2-dimensional echocardiography along with technetium pyrophosphate cardiac scintigraphic scanning were normal; all symptoms and the electrocardiographic abnormalities resolved within 36–48 hours. Coronary angiography may be normal; cardiac isoenzymes elevations are typically mild and return to normal values within a few days.[168] The use of phenylpropanolamine as an appetite suppressant is not clearly associated with pulmonary hypertension or cardiac valvular disease. Associated findings include ventricular extrasystoles, ventricular tachycardia, and tingling in the extremities.[149]

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantitation of phenylpropanolamine in biologic fluids include gas chromatography with nitrogen specific detection[169] or electron capture detector,[170] capillary GC with trifluoroacetic anhydride derivatization and electron capture detection,[171] radioenzymatic assay,[172] HPLC with ultraviolet detection (220 nm)[173] and (205 nm),[174] high performance liquid chromatography with postcolumn fluorescent derivatization,[175] liquid chromatography with fluorescence

detection,[176] high performance liquid chromatography/ atmospheric pressure chemical ionization/mass spectrometry,[177] and GC/MS. High performance liquid chromatography with the use of the chiral derivatizing agent, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate to form diastereomeric thiourea derivatives followed by reverse-phase chromatography and UV detection (254 nm) allows the quantitation of the enantiomers of phenylpropanolamine in plasma.[178] The LLOQ for this method is 10 ng/mL with an interday coefficient of variation <5%. The LLOQ with high pressure liquid chromatography is 4 ng/mL with an interday coefficient of variation below 15%. The LOD for HPLC with UV detection (205 nm) in plasma is 0.4 ng/mL, whereas the LOD for liquid chromatography/ atmospheric pressure chemical ionization/mass spectrometry is ~1 ng/mL. High performance liquid chromatography with UV detection at 220 nm is less sensitive with a LOD for phenylpropanolamine in serum samples of ~ 25 ng/mL.

Biomarkers

The mean peak serum phenylpropanolamine concentration following the ingestion of a single 100 mg phenylpropanolamine solution was approximately 397 ng/mL (range, 310–480 ng/mL).[141] Figure 13.8 displays the time course of serum phenylpropanolamine concentrations following the administration of a syrup containing 25 mg, 50 mg, or 100 mg phenylpropanolamine. In a study of 8 healthy male volunteers receiving a single dose of 100 mg phenylpropanolamine, the mean peak serum phenylpropanolamine concentration was 474 ± 176 ng/mL as measured by HPLC.[179] In a study of the effect of phenylpropanolamine on patients with neurogenic bladder dysfunction, the mean steady-state plasma phenylpropanolamine concentration in 4 patients receiving 100 mg daily was 263 ng/mL compared with 312 ng/mL in plasma samples from 5 patients receiving 200 mg daily.[180] The maximum plasma phenylpropanolamine (642 ng/mL) in this study was not associated with adverse effects.

Fatal phenylpropanolamine concentrations are not well defined because of the lack of documentation of postmortem blood concentrations in individuals dying as a result of phenylpropanolamine intoxication. Although case reports document postmortem concentrations ranging from 2,000–48,000 ng/mL,[181] the circumstances surrounding these deaths are not well described and the contribution of phenylpropanolamine to these deaths remains unclear. In a case series of 12 pediatric deaths with detectable phenylpropanolamine concentrations, the blood phenylpropanolamine concentration (site not reported) ranged from 40–840 ng/mL.[182] Phenylpropanolamine was not an official cause of death in any of these cases. In a study of postmortem redistribution, the mean heart/femoral whole blood ratio in 3 blood samples containing phenylpropanolamine was 2.4 (range, 0.8–4.6).[74]

Abnormalities

In a cases series of 12 patients undergoing cerebral angiography after developing intracranial lesions following the ingestion of phenylpropanolamine, 8 patients (67%) had evidence of vasculitis.[137] Four of these patients had follow-up cerebral digital subtraction angiography with complete resolution of the signs of vasculitis.

FIGURE 13.8. Serum phenylpropanolamine Concentrations following the administration of a syrup containing 25 mg, 50 mg, or 100 mg phenylpropanolamine. (From SS Scherzinger, R Dowse, I Kanfer, Journal of Clinical Pharmacology, Steady state pharmacokinetics and dose-proportionality of phenylpropanolamine in healthy subjects, Vol. 30 Issue 4, p. 373, copyright © 1990 SAGE Publications, Reprinted by permission of SAGE Publications.)

TREATMENT

Stabilization

The treatment of phenylpropanolamine toxicity is supportive with initial attention directed toward assessment and stabilization of respiration, circulation (myocardial ischemia, hypotension), and temperature. Agitation and combativeness may complicate the initial evaluation; rapid control of behavioral abnormalities may require benzodiazepines (Adult: lorazepam 1–2 mg IV or intramuscular [IM], or diazepam 2–5 mg IV); repeat doses

may be necessary. Continued agitation despite adequate benzodiazepine therapy may respond to the addition of butyrophenones (Adult: haloperidol 2–5 mg IV/IM or droperidol 1.25–5 mg IV/IM); butyrophenones should be used with caution in patients with elevated body temperature, QT_c prolongation, or seizures. Consequently, benzodiazepines are the drug of choice for sedation. Additional supportive measures as indicated by the clinical situation include IV fluid therapy, supplemental oxygen, electrocardiographic monitoring, monitoring of urine output, and intubation for respiratory failure. Catecholamine depletion may cause hypotension in severe phenylpropanolamine intoxication. Treatment of hypotension includes IV saline and vasopressor infusions (e.g., dopamine) for patients unresponsive to fluid therapy. Patients unresponsive to dopamine may respond to α-receptor agonists (e.g., norepinephrine). Patients with chest pain or dyspnea should be evaluated for myocardial ischemia (electrocardiogram, cardiac enzymes) and treated with nitroglycerin, aspirin, and morphine as indicated. Central nervous system depression may indicate the presence of intracerebral bleeding and the need for imaging studies of the brain.

Hyperthermia

The presence of hyperthermia (i.e., core body temperature >40°C/104°F) requires aggressive treatment of agitation with benzodiazepines and evaporative cooling measures. These measures involve spraying or sponging the patient with tepid water, accompanied by evaporation with an electric fan. Applying small ice packs to the axillae and groin may augment cooling. Intubation and chemical paralysis may be necessary in patients with severe agitation and persistent hyperthermia. Cooling blankets, antipyretics, ice baths, and alcohol baths are not typically effective for patients with core temperatures above 40°C/104°F. There are inadequate clinical data to determine of efficacy of dantrolene for the treatment of stimulant-induced hyperthermia. Patients with hyperthermia should be monitored with rectal or esophageal probes, if available.

Hypertension

Patients with phenylpropanolamine generally tolerate elevated blood pressures as long as there is no evidence of end organ failure. Severe, persistent hypertension should be treated with the IV administration of a short-acting antihypertensive agent (e.g., Adults: nitroprusside 0.3–10 μg/kg/min IV; nitroglycerin 5–100 μg/min IV titrated to effect). β-Adrenergic blockers should be used with caution because of the theoretical potential for unopposed α-adrenergic stimulation and worsening hypertension. The safest β-blockers in this situation are short-acting drugs (e.g., esmolol 500 μg/kg/minute IV bolus followed by 50 μg/kg/minute titrated to effect up to 500 μg/kg/min) and β-blockers with α-adrenergic blocking properties (e.g., labetalol 20 mg IV over 2 minutes, followed by 40 mg at 10-min intervals as needed). Marked sustained hypertension may respond to an α-blocking agent (e.g., Adult: phentolamine 1–10 mg IV).

Dysrhythmias

Sinus tachycardia is the most common dysrhythmia during phenylpropanolamine toxicity. Typically, the presence of sinus tachycardia without hemodynamic compromise does not require treatment other than benzodiazepines for sedation. Ventricular dysrhythmias are treated with standard therapeutic measures (amiodarone, lidocaine). The use of β-adrenergic blocking agents should be used with caution because of the potential of unopposed α-adrenergic stimulation and subsequent hypertension.

Gut Decontamination

Gut decontamination measures are unlikely to benefit patients presenting over 1 hour after the ingestion of phenylpropanolamine or patients using other routes of administration (injection, insufflation). Induction of emesis is contraindicated for phenylpropanolamine ingestion; there are inadequate data to recommend the use of gastric lavage following phenylpropanolamine ingestion. The administration of single-dose activated charcoal is a therapeutic option for patients presenting within 1 hour of ingestion, particularly for sustained-release products; however, there are limited clinical data to confirm the efficacy of this decontamination measure.

Elimination Enhancement

Although the urinary elimination of weakly basic drugs such as phenylpropanolamine increases in acidic urine, the risk of urine acidification in this setting outweighs the potential benefits. There are no data to support the use of forced diuresis or hemodialysis to enhance the renal elimination of phenylpropanolamine.

Antidotes

There are no specific antidotes to phenylpropanolamine intoxication.

Supplemental Care

Serum electrolytes, creatinine, blood urea nitrogen, CK, transaminases, and myoglobin should be monitored in

patients with serious phenylpropanolamine toxicity. Patients with hyperthermia should be monitored for the development of disseminated intravascular coagulation with complete blood count, coagulation studies, fibrinogen, and fibrin degradation products. Excessive muscle overactivity and hyperthermia may cause rhabdomyolysis, hyperkalemia, and renal dysfunction. Treatment of patients with rhabdomyolysis (i.e., serum CK >1,000–2,000 U/L) includes generous fluid replacement and correction of metabolic acidosis. Alkalinization of the urine to prevent the renal deposition of myoglobin and subsequent renal damage may reduce the renal clearance of phenylpropanolamine and similar phenethylamine compounds. Chronic abusers of dieting agents including phenylpropanolamine may require long-term treatment for psychosis, anxiety, or depression with antipsychotics and antidepressants. Treatment for addiction to phenylpropanolamine or similar drugs includes drug counseling, cognitive-behavioral therapy, and motivational interviewing.[183]

References

1. Bagchi D, Preuss HG (Ed). Obesity: epidemiology, pathophysiology, and prevention. Boca Raton, FL: CRC Press; 2006.

2. Greiser E. Epidemiologische untersuchungen zum zusammenhang swischen appetitzueglere innahme und primaer vasculaer pulmonaler hypertonie. Internist 1973;14:437–442

3. Gurtner HP. Pulmonary hypertension, "plexogenic pulmonary arteriopathy" and the appetite depressant drug aminorex: post or propter? Bull Eur Physiopathol Respir 1979;15:897–923.

4. Mey B, Paulus H, Lamparter E, Blaschke G. Kinetics of racemization of (+)- and (−)-diethylpropion: studies in aqueous solution, with and without the addition of cyclodextrins, in organic solvents and in human plasma. Chirality 1998;10:307–315.

5. Julian EA, Plein EM. Microcrystalline identification of drugs of abuse: stimulant street drugs. J Forensic Sci 1983;28:992–999.

6. Haddock CK, Poston WS, Dill PL, Foreyt JP, Ericsson M. Pharmacotherapy for obesity: a quantitative analysis of four decades of published randomized clinical trials. Int J Obes Relat Metab Disord 2002;26:262–273.

7. Caplan J. Habituation to diethylpropion (Tenuate). Can Med Assoc J 1963;88:943–944.

8. Clein LJ, Benady DR. Case of diethylpropion addiction. Br Med J 1962;2(5302):456.

9. Jasinski DR, Nutt JG, Griffith JD. Effects of diethylpropion and d-amphetamine after subcutaneous and oral administration. Clin Pharmacol Ther 1974;16:645–652.

10. Cohen S. Diethylpropion (Tenuate): an infrequently abused anorectic. Psychosomatics 1977;18:28–33.

11. Allmark J, Rylander G. [Still one form of drug addiction]. Lakartidningen 1968;65:1530–1538. [Swedish]

12. Jouglard J, Brun A, Arditti J, Boyer J, Deveze JL, Michela G, et al. Acute poisoning by anorexigenics. Experience of the Poison Control Center of Marseille, 1973 to 1978. Therapie 1979;34:205–219.

13. Caplan J. Habituation to diethylpropion (Tenuate). Can Med Assoc J 1963;88:943–944.

14. Silverstone T. Appetite suppressants a review. Drugs 1992;43:820–836.

15. Beckett AH, Stanojcic M. Re-evaluation of the metabolism and excretion of diethylpropion in non-sustained and sustained release formulations. J Pharm Pharmacol 1987;39:409–415.

16. Testa B, Beckett AH. Metabolism and excretion of diethylpropion in man under acidic urine conditions. J Pharm Pharmacol 1973;25:119–124.

17. Testa B, Beckett AH. The metabolism of diethylpropion in man; influence of changes in drug formulation and urinary pH. Pharm Acta Helv 1974;49:21–27.

18. Wright GJ, Lang JF, Lemieux RE, Goodfriend MJ Jr. The objective and timing of drug disposition studies, appendix III. Diethylpropion and its metabolites in the blood plasma of the human after subcutaneous and oral administration. Drug Metab Rev 1975;4:267–276.

19. Temmel AF, Quint C, Toth J, Herneth A, Hummel T. Topical ephedrine administration and nasal chemosensory function in healthy human subjects. Arch Otolaryngol Head Neck Surg 1999;125:1012–1014.

20. Wilding JP. Neuropeptides and appetite control. Diabet Med 2002;19:619–627.

21. Brunner L, Levens N. The regulatory role of leptin in food intake. Curr Opin Clin Nutr Metab Care 1998;1:565–571.

22. Cercato C, Roizenblatt VA, Leança CC, Segal A, Lopes Filho AP, Mancini MC, Halpern A. A randomized double-blind placebo-controlled study of the long-term efficacy and safety of diethylpropion in the treatment of obese subjects. Int J Obes (Lond) 2009;33:857–865.

23. Carney MW. Diethylpropion and psychosis. Clin Neuropharmacol 1988;11:183–188.

24. Martin CA, Iwamoto ET. Diethylpropion-induced psychosis reprecipitated by an MAO inhibitor: case report. J Clin Psychiatry 1984;45:130–131.

25. Deveaugh-Geiss J, Pandurangi A. Confusional paranoid psychosis after withdrawal from sympathomimetic amines: two case reports. Am J Psychiatry 1982;139:1190–1191.

26. Hoffman BF. Diet pill psychosis: follow-up after 6 years. Can Med Assoc J 1983;129:1077–1078.

27. Little JD, Romans SE. Psychosis following readministration of diethylpropion: a possible role for kindling? Int Clin Psychopharmacol 1993;8:67–70.

28. Fookes BH. Schizophrenia-like reaction to diethylpropion. Lancet 1976;2(7996):1206.

29. Crols R, Dierckx R, Saerens J, De Deyn PP. Transient ischemic attacks associated with amfepramone therapy: a case report. Funct Neurol 1993;8:351–354.

30. O'Keefe JC, Butrous GS, Dymond DS, Littlejohns P, Peters N, Banim SO. Ventricular arrhythmias complicating weight reduction therapy in a patient with a prolonged QT interval. Postgrad Med J 1985;61:419–421.

31. Abramowicz MJ, Van Haecke P, Demedts M, Delcroix M. Primary pulmonary hypertension after amfepramone (diethylpropion) with BMPR2 mutation. Eur Respir J 2003;22:560–562.

32. Dangor CM, Beckett AH, Veltman AM. Simultaneous determination of amfepramone and its two major metabolites in biological fluids by gas liquid chromatography. Arzneimittelforschung 1986;36:1307–1310.

33. Valentine JL, Middleton R. GC-MS identification of sympathomimetic amine drugs in urine: rapid methodology applicable for emergency clinical toxicology. J Anal Toxicol 2000;24:211–222.

34. Ku Y-R, Chang Y-S, Wen K-C, Ho L-K. Analysis and confirmation of synthetic anorexics in adulterated traditional Chinese medicines by high-performance capillary electrophoresis. J Chromatogr A 1999;848:537–543.

35. Loosmore S, Armstrong D. Do-do abuse. Br J Psychiatry 1990;157:278–281.

36. Miller SC. Psychiatric effects of ephedra: addiction. Am J Psychiatry 2005;162:2198.

37. Tinsley JA, Watkins DD. Over-the-counter stimulants: abuse and addiction. Mayo Clin Proc 1998;73:977–982.

38. Mehendale SR, Bauer BA, Yuan CS. Ephedra-containing dietary supplements in the US versus ephedra as a Chinese medicine. Am J Chin Med 2004;32:1–10.

39. Betz JM, Gay ML, Mossoba MM, Adams S, Portz BS. Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing Má Huáng. J AOAC Int 1997;80:303–315.

40. Hurlbut JA, Carr JR, Singleton ER, Faul KC, Madson MR, Storey JM, Thomas TL. Solid-phase extraction cleanup and liquid chromatography with ultraviolet detection of ephedrine alkaloids in herbal products. J AOAC Int 1998;81:1121–1127.

41. Haller CA, Duan M, Benowitz NL, Jacob P 3rd: Concentrations of ephedra alkaloids and caffeine in commercial dietary supplements. J Anal Toxicol 2004;28:145–151.

42. Food and Drug Administration, HHS. Final rule declaring dietary supplements containing ephedrine alkaloids adulterated because they present an unreasonable risk. Final rule. Fed Reg 2004; 69:6787–6854.

43. Meersschaert K, Brun L, Gourdin M, Mouren S, Bertrand M, Riou B, et al. Terlipressin-ephedrine versus ephedrine to treat hypotension at the induction of anesthesia in patients chronically treated with angiotensin converting-enzyme inhibitors: a prospective, randomized, double-blinded, crossover study. Anesth Analg 2002; 94: 835–840.

44. Rakovec P, Kozak M, Sebestjen M. Ventricular tachycardia induced by abuse of ephedrine in a young healthy woman. Wien Klin Wochenschr 2006;118:558–561.

45. Berlin I, Warot D, Aymard G, Acquaviva E, Legrand M, Labarthe B, et al. Pharmacodynamics and pharmacokinetics of single nasal (5 mg and 10 mg) and oral (50 mg) doses of ephedrine in healthy subjects. Eur J Clin Pharmacol 2001;57:447–455.

46. Sever PS, Dring LG, Williams RT. The metabolism of (−)-ephedrine in man. Eur J Clin Pharmacol 1975;9: 193–198.

47. Wilkinson GR, Beckett AH. Absorption, metabolism, and excretion of the ephedrines in man. II. Pharmacokinetics. J Pharm Sci 1968;57:1933–1938.

48. Dawson JK, Earnshaw SM, Graham CS. Dangerous monoamine oxidase inhibitor interactions are still occurring in the 1990s. J Accid Emerg Med 1995;12:49–51.

49. Kobayashi S, Endou M, Sakuraya F, Matsuda N, Zhang XH, Azuma M, et al. The sympathomimetic actions of *l*-ephedrine and *d*-pseudoephedrine: direct receptor activation or norepinephrine release? Anesth Analg 2003;97: 1239–1245.

50. Levitsky DA, Strupp BJ. Direct and indirect thermogenic effects of anorectic drugs. Adv Nutr Res 1985;7: 187–201.

51. Dulloo AG, Seydoux J, Girardier L. Peripheral mechanisms of thermogenesis induced by ephedrine and caffeine in brown adipose tissue. Int J Obes 1991;15: 317–326.

52. Nelson HS. Stepwise therapy of bronchial asthma: the role of beta-adrenergic agonists. Ann Allergy 1985;54: 289–293.

53. Bowyer JF, Newport GD, Slikker W Jr, Gough B, Ferguson SA, Tor-Agbidye J. An evaluation of *l*-ephedrine neurotoxicity with respect to hyperthermia and caudate/putamen microdialysate levels of ephedrine, dopamine, serotonin, and glutamate. Toxicol Sci 2000;55:133–142.

54. Dunnick JK, Kissling G, Gerken DK, Vallant MA, Nyska A. Cardiotoxicity of Ma Huang/caffeine or ephedrine/caffeine in a rodent model system. Toxicol Pathol 2007;35: 657–664.

55. Mark PB, Watkins S, Dargie HJ. Cardiomyopathy induced by performance enhancing drugs in a competitive bodybuilder. Heart 2005;91:888.

56. Dekhuijzen PN, Machiels HA, Heunks LM, van der Heijden HF, van Balkom RH. Athletes and doping: effects of drugs on the respiratory system. Thorax 1999;54: 1041–1046.

57. Blechman KM, Karch SB, Stephens BG. Demographic, pathologic, and toxicological profiles of 127 decedents testing positive for ephedrine alkaloids. Forensic Sci Int 2004;139:61–69.

58. James LP, Farrar HC, Komoroski EM, Wood WR, Graham CJ, Bornemeier RA, Valentine JL. Sympathomimetic

drug use in adolescents presenting to a pediatric emergency department with chest pain. J Toxicol Clin Toxicol 1998;36:321–328.

59. Shekelle PG, Hardy ML, Morton SC, Maglione M, Mojica WA, Suttorp MJ, et al. Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis. JAMA 2003;289:1537–1545.

60. Chen C, Biller J, Willing SJ, Lopez AM. Ischemic stroke after using over the counter products containing ephedra. J Neurol Sci 2004;217:55–60.

61. Greller HA, Flomenbaum M, Nelson LS, Hoffman RS. Intestinal ischemia from an ephedra containing "smoothie." J Toxicol Clin Toxicol 2004;42:486

62. Forte RY, Precoma-Neto D, Chiminacio Neto N, Maia F, Faria-Neto JR. Myocardial infarction associated with the use of a dietary supplement rich in ephedrine in a young athlete. Arq Bras Cardiol 2006;87:179–181.

63. Bruno A, Nolte KB, Chapin J. Stroke associated with ephedrine use. Neurology 1993;43:1313–1316.

64. Conway CR, Ziaee L, Langenfeld SJ. Ephedrine-induced emergence of bipolar symptoms. Bipolar Disord 2006;8:204–205.

65. Whitehouse AM, Duncan JM. Ephedrine psychosis rediscovered. Br J Psychiatry 1987;150:258–261.

66. Naik SD, Freudenberger RS. Ephedra-associated cardiomyopathy. Ann Pharmacother 2004;38:400–403.

67. Blechman KM, Karch SB, Stephens BG. Demographic, pathologic, and toxicological profiles of 127 decedents testing positive for ephedrine alkaloids. Forensic Sci Int 2004;139:61–69.

68. Kaddoumi A, Kubota A, Nakashima MN, Takashashi M, Nakashima K. High performance liquid chromatography with UV detection for the simultaneous determination of sympathomimetic amines using 4-(4,5-diphenyl-1H-imidazole-2-yl)benzoyl chloride as a label. Biomed Chromatogr 2001;15:379–388.

69. Aymard G, Labarthe B, Warot D, Berlin I, Diquet B. Sensitive determination of ephedrine and norephedrine in human plasma samples using derivatization with 9-fluorenylmethyl chloroformate and liquid chromatography. J Chromatogr B 2000;744:25–31.

70. Trujillo WA, Sorenson WR. Determination of ephedrine alkaloids in human urine and plasma by liquid chromatography/tandem mass spectrometry: collaborative study. J AOAC Int 2003;86:643–656.

71. Jiménez C, de la Torre R, Ventura M, Segura J, Ventura R. Stability studies of amphetamine and ephedrine derivatives in urine. J Chromatogr B Analyt Technol Biomed Life Sci 2006;843:84–93.

72. Pickup ME, May CS, Ssendagire R, Paterson JW. The pharmacokinetics of ephedrine after oral dosage in asthmatics receiving acute and chronic treatment. Br J Clin Pharmacol 1976;3:123–134.

73. Schier JG, Traub SJ, Hoffman RS, Nelson LS. Ephedrine-induced cardiac ischemia: exposure confirmed with a serum level. J Toxicol Clin Toxicol 2003;41:849–853.

74. Dalpe-Scott M, Degouffe M, Garbutt D, Drost M. A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. Can Soc Forensic Sci J 1995;28:113–121.

75. Dvornik D, Schilling G. Stereochemistry of d-3,4-dimethyl-2-phenylmorpholine (phendimetrazine). J Med Chem 1965;8:466–469.

76. Jain NC, Budd RD, Sneath TC. Frequency of use or abuse of amphetamine-related drugs. Am J Drug Alcohol Abuse 1979;6:53–57.

77. Muller FO, Hundt HK, Gosling JA. Availability of phendimetrazine from sustained and non-sustained action formulations. S Afr Med J 1975;49:135–139.

78. Rudolph GR, Miksic JR, Levitt MJ. GLC determination of phendimetrazine in human plasma, serum, or urine. J Pharm Sci 1983;72:519–521.

79. Beckett AH, Salami MA. A note on the identification of N-hydroxyphenmetrazine as a metabolic product of phendimetrazine and phenmetrazine. J Pharm Pharmacol 1974;24:900–902.

80. Kim I, Whitsett TL. Acute vasospasm associated with anorexiant use. J Okla State Med Assoc 1988;81:395–398.

81. Rostagno C, Caciolli S, Felici M, Gori F, Serneri GG. Dilated cardiomyopathy associated with chronic consumption of phendimetrazine. Am Heart J 1996;131:407–409.

82. Markowitz GS, Tartini A, Agati VD. Acute interstitial nephritis following treatment with anorectic agents phentermine and phendimetrazine. Clin Nephrol 1998;50:252–254.

83. Kwiker D, Godkar D, Lokhandwala N, Yakoby M. Rare case of rhabdomyolysis with therapeutic doses of phendimetrazine tartrate. Am J Ther 2006;13:175–176.

84. Hundt HK, Clark EC, Muller FO. GLC determination of phendimetrazine in serum. J Pharm Sci 1975;64:1041–1043.

85. Rdolph GR, Miksic JR, Levitt MJ. GLC determination of phendimetrazine in human plasma, serum, or urine. J Pharm Sci 1983;72:519–521.

86. Hood I, Monforte J, Gault R, Mirchandani H. Fatality from illicit phendimetrazine use. J Toxicol Clin Toxicol 1988;26:249–255.

87. Hadler AJ. Sustained-action phendimetrazine in obesity. J Clin Pharmacol J New Drugs 1968;8:113–117.

88. Kintz P, Tracqui A, Mangin P, Lugnier AA, Chaumont AJ. A simple gas chromatographic identification and determination of 11 CNS stimulants in biological samples. Application on a fatality involving phendimetrazine. Forensic Sci Int 1989;40:153–159.

89. Hampson, J. Phenmetrazine and dexamphetamine in the management of obesity. Lancet 1960;275:1265.

90. Mellar J, Hollister LE. Phenmetrazine: an obsolete problem drug. Clin Pharmacol Ther 1982;32:671–675.

91. Chambers CD, White OZ. A study of phenmetrazine (Preludin) abuse. Chem Depend 1980;4:101–111.

92. Ananth JV. Repeated episodes of phenmetrazine psychosis. Can Med Assoc J 1971;105:1280–1281.

93. Quinn GP, Cohn MM, Reid MB, Greengard P, Weiner M. The effect of formulation on phenmetrazine plasma levels in man studied by a sensitive analytic method. Clin Pharmacol Ther 1967;8:369–373.

94. Franklin RB, Dring LG, Williams RT. The metabolism of phenmetrazine in man and laboratory animals. Drug Metab Dispos 1977;5:223–233.

95. Brown GR, Forster G, Foubister AJ, Stribling D. Synthesis and resolution of the novel appetite suppressant 2-benzylmorpholine, a nonstimulant isomer of phenmetrazine. J Pharm Pharmacol 1990;42:797–799.

96. Norheim G. A fatal case of phenmetrazine poisoning. J Forensic Sci Soc 1973;13:287–289.

97. Nemetz PS. Phenmetrazine psychosis. Br Med J 1968;3(5621):803–804.

98. Jaroszynski J, Spasowicz E, Ulasinska-Rubach D. Phenmetrazine psychoses. Pol Med J 1971;10:253–257.

99. Kendrick WC, Hull AR, Knochel JP. Rhabdomyolysis and shock after intravenous amphetamine administration. Ann Intern Med 1977;86:381–387.

100. Holmgren P, Lindquist O. Lethal intoxication with centrally stimulating amines in Sweden 1966–1973. Z Rechtsmed 1975;75:265–273.

101. Anggård E, Hankey A. Derivatives of sympathomimetic amines for gas chromatography with electron capture detection and mass spectrometry. Acta Chem Scand 1969;23:3110–3119.

102. Beckett AH, Tucker GT, Moffat AC. Routine detection and identification in urine of stimulants and other drugs, some of which may be used to modify performance in sport. J Pharm Pharmacol 1967;19:273–294.

103. Dasgupta A, Handler MS, Nine JS. Convenient derivatization method for gas chromatography/mass spectrometric determination of phenmetrazine in urine using 2,2,2-trichloroethyl chloroformate. J Forensic Sci 1998; 43:630–635.

104. Kronstrand R, Hatanpaa M, Jonsson JA. Determination of phenmetrazine in urine by gas chromatography-mass spectrometry. J Anal Toxicol 1996;20:277–280.

105. Dasgupta A, Hart A, Humphrey P, Blackwell W. Gas chromatography-electron ionization and chemical ionization mass spectrometric analysis of urinary phenmetrazine after derivatization with 4-carbethoxyhexalfuorobutyryl chloride—a new derivative. J Forensic Sci 1998;43:636–640.

106. Wille SM, Lambert WE. Phenmetrazine or ephedrine? Fooled by library search. J Chromatogr Anal 2004; 1045:259–262.

107. Stromberg L, Maehly AC. Comparative gas chromatographic analysis of narcotics III. Phenmetrazine hydrochloride. J Chromatogr 1975;109:67–72.

108. Dasgupta A, Mahle CE. Determination of phenmetrazine in urine by gas chromatography-mass spectrometry after liquid-liquid extraction and derivatization with perfluorooctanoyl chloride. J Forensic Sci 1997;42:937–941.

109. Callahan BT, Yuan J, Ricaurte GA. Fluoxetine increases the anorectic and long-term dopamine-depleting effects of phentermine. Synapse 2000;38:471–476.

110. Li Z, Maglione M, Tu W, Mojica W, Arterburn D, Shugarman LR, et al. Meta-analysis: pharmacologic treatment of obesity. Ann Intern Med 2005;142: 532–546.

111. Takeshita J. Internet pharmacy prescription and phentermine overdose. J Clin Psychiatry 2003;64:215.

112. Beckett AH, Brookes LG. The metabolism and urinary excretion in man of phentermine, and the influence of N-methyl and p-chloro-substitution. J Pharm Pharmacol 1971;23:288–294.

113. Kilpatrick IC, Traut M, Heal DJ. Monoamine oxidase inhibition is unlikely to be relevant to the risks associated with phentermine and fenfluramine: a comparison with their abilities to evoke monoamine release. Int J Obes Relat Metab Disord 2001;25:1454–1458.

114. Douglas A, Douglas JG, Robertson CE, Munro JF. Plasma phentermine levels, weight loss and side-effects. Int J Obes 1983;7:591–595.

115. Cleare AJ. Phentermine, psychosis, and family history. J Clin Psychopharmacol 1996;16:470–471.

116. Devan GS. Phentermine and psychosis. Br J Psychiatry 1990;156:442–443.

117. Kokkinos J, Levine SR. Possible association of ischemic stroke with phentermine. Stroke 1993;24:310–313.

118. Makaryus JN, Makaryus AN. Cardiac arrest in the setting of diet pill consumption. Am J Emerg Med 2008;26: 732.e1–732.e3.

119. Hung Y-M, Chang J-C. Weight-reducing regimen associated with polymorphic ventricular tachycardia. Am J Emerg Med 2006;24:714–716.

120. Bibbs HR. Diet pills and sudden death. N Engl J Med 1988;318:1127.

121. Markowitz GS, Tartini A, Agati VD. Acute interstitial nephritis following treatment with anorectic agents phentermine and phendimetrazine. Clin Nephrol 1998;50:252–254.

122. Sobel RM. Ruptured retroperitoneal aneurysm in a patient taking phentermine hydrochloride. Am J Emerg Med 1999;17:102–103.

123. Jefferson HJ, Jayne DR. Peripheral vasculopathy and nephropathy in association with phentermine. Nephrol Dial Transplant 1999;14:1761–1763.

124. O'Brien JE, Zazulak W, Abbey V, Hinsvark O. Determination of amphetamine and phentermine in biological fluids. J Chromatogr Sci 1972;10:336–341.

125. Dadgar D, Climax J, Lambe R, Darragh A. Gas-liquid chromatographic determination of phentermine in human plasma following oral administration to healthy subjects. J Chromatogr 1985;337:136–141.

126. Cho AK, Hodshon BJ, Lindeke B, Miwa GT. Application of quantitative GC-mass spectrometry to study of

pharmacokinetics of amphetamine and phentermine. J Pharm Sci 1973;62:1491–1494.

127. Kaddoumi A, Nakashima MN, Maki T, Matsumura Y, Nakamura J, Nakashima K. Liquid chromatography studies on the pharmacokinetics of phentermine and fenfluramine in brain and blood microdialysates after intraperitoneal administration to rats. J Chromatogr B 2003; 791:291–303.

128. Apollonio LG, Pianca DJ, Whittall IR, Maher WA, Kyd JM. A demonstration of the use of ultra-performance liquid chromatography-mass spectrometry [UPLC/MS] in the determination of amphetamine-type substances and ketamine for forensic and toxicological analysis. Analyt Technol Biomed Life Sci 2006;836:111–115.

129. Palmer RB, Kim NH, Dasgupta A. Simultaneous determination of fenfluramine and phentermine in urine using gas chromatography mass spectrometry with pentafluoropropionic anhydride derivatization. Ther Drug Monit 2000;22:418–422.

130. Groenewoud G, Schall R, Hundt HK, Müller FO, van Dyk M. Steady-state pharmacokinetics of phentermine extended-release capsules. Int J Clin Pharmacol Ther Toxicol 1993;31:368–372.

131. Levine B, Caplan YH, Dixon AM. A fatality involving phentermine. J Forensic Sci 1984;29:1242–1245.

132. Lewis JD, Strom BL. Balancing safety of dietary supplements with the free market. Ann Intern Med 2002; 136:616–618.

133. Krizevski R, Dudai N, Bar E, Lewinsohn E. Developmental patterns of phenylpropylamino alkaloids accumulation in khat (*Catha edulis* Forsk.). J Ethnopharmacol 2007; 114:432–438.

134. Morgan JP, Funderburk FR, Blackburn GL, Noble R. Subjective profile of phenylpropanolamine: absence of stimulant or euphorigenic effects at recommended dose levels. J Clin Psychopharmacol 1989;9:33–38.

135. Edwards M, Russo L, Harwood-Nuss A. Cerebral infarction with a single oral dose of phenylpropanolamine. Am J Emerg Med 1987;5:163–164.

136. Kernan WN, Viscoli CM, Brass LM, Broderick JP, Brott T, Feldmann E, et al. Phenylpropanolamine and the risk of hemorrhagic stroke. N Engl J Med 2000 343: 1826–1832.

137. Cantu C, Arauz A, Murillo-Bonilla LM, López M, Barinagarrementeria F. Stroke associated with sympathomimetics contained in over-the-counter cough and cold drugs. Stroke 2003;34:1667–1672.

138. McEwen J. Phenylpropanolamine-associated hypertension after the use of "over-the-counter" appetite-suppressant products. Med J Aust 1983;2:71–73.

139. Lake CR, Zaloga G, Clymer R, Quirk RM, Chernow B. A double dose of phenylpropanolamine causes transient hypertension. Am J Med 1988;85:339–343.

140. Schran HF, Petryk L, Chang C-T, O'Connor R, Gelbert MB. The pharmacokinetics and bioavailability of clemastine and phenylpropanolamine in single-component and combination formulation. J Clin Pharmacol 1996; 36:911–922.

141. Scherzinger SS, Dowse R, Kanfer I. Steady state pharmacokinetics and dose-proportionality of phenylpropanolamine in healthy subjects. J Clin Pharmacol 1990;30: 372–377.

142. Chester N, Mottram DR, Reilly T, Powell M. Elimination of ephedrines in urine following multiple dosing: the consequences for athletes, in relation to doping control. Br J Clin Pharmacol 2003;57:62–67.

143. Zimmerman CL, O'Connell MB, Soria I. The effects of urine pH modification on the pharmacokinetics and pharmacodynamics of phenylpropanolamine. Pharm Res 1990;7:96–102.

144. Wellman PJ, Sellers TL. Weight loss induced by chronic phenylpropanolamine: anorexia and brown adipose tissue thermogenesis. Pharmacol Biochem Behav 1986; 24:605–11.

145. Harrison WM, McGrath PJ, Stewart JW, Quitkin F. MAOIs and hypertensive crises: the role of OTC drugs. J Clin Psychiatry 1989;50:64–65.

146. Smookler S, Bermudez AJ. Hypertensive crisis resulting from an MAO inhibitor and an over-the-counter appetite suppressant. Ann Emerg Med 1982;11:482–484.

147. Lee KY, Beilin LJ, Vandongen R. Severe hypertension after ingestion of an appetite suppressant (phenylpropanolamine) with indomethacin. Lancet 1979; 1(8126):1110–1111.

148. McLaren EH. Severe hypertension produced by interaction of phenylpropanolamine with methyldopa and oxprenolol. Br Med J 1976;2(6030):283–284.

149. Samanin R, Garattini S. Neurochemical mechanism of action of anorectic drugs. Pharmacol Toxicol 1993;73: 63–68.

150. Davies BT, Wellman PJ, Morien A. An assessment of the involvement of paraventricular hypothalamic alpha 2-adrenoceptors in phenylpropanolamine anorexia. Physiol Behav 1993;54:121–128.

151. Wellman PJ. The pharmacology of the anorexic effect of phenylpropanolamine. Drugs Exp Clin Res 1990;16: 487–495.

152. Hoebel BG, Hernandez L. Microdialysis studies of psychostimulants. NIDA Res Monogr 1989;95:343–344.

153. McMahon LR, Wellman PJ. Effects of systemic phenylpropanolamine and fenfluramine on serotonin activity within rat paraventricular hypothalamus. Physiol Behav 1996;59:63–69.

154. Lake CR, Rosenberg DB, Gallant S, Zaloga G, Chernow B. Dose-dependent response to phenylpropanolamine: inhibition of orthostasis. J Clin Pharmacol 1991;31: 624–635.

155. Lake CR, Gallant S, Masson E, Miller P. Adverse drug effects attributed to phenylpropanolamine: a review of 142 case reports. Am J Med 1990;89:195–208.

156. Heikkila H, Kariniemi A-L, Stubb S. Fixed drug eruption due to phenylpropanolamine hydrochloride. Br J Dermatol 2000;142:845–847.

157. Boffi BV, Klerman GL. Manic psychosis associated with appetite suppressant medication, phenylpropanolamine. J Clin Psychopharmacol 1989;9:308–309.

158. Lake CR, Masson EB, Quirk RS. Psychiatric side effects attributed to phenylpropanolamine. Pharmacopsychiatry 1988;21:171–181.

159. Marshall RD, Douglas CJ. Phenylpropanolamine-induced psychosis. Potential predisposing factors. Gen Hosp Psychiatry 1994;16:358–360.

160. Sloan MA, Kittner SJ, Rigamonti D, Price RT. Occurrence of stroke associate with use/abuse of drugs. Neurology 1991;41:1358–1364.

161. Forman HP, Levin S, Stewart B, Patel M, Feinstein S. Cerebral vasculitis and hemorrhage in an adolescent taking diet pills containing phenylpropanolamine: case report and review of literature. Pediatrics 1989;83: 737–741.

162. Stier BG, Hennekens CH. Phenylpropanolamine and hemorrhagic stroke in the Hemorrhagic Stroke Project: a reappraisal in the context of science, the Food and Drug Administration, and the law. Ann Epidemiol 2006; 16:49–52.

163. Arauz A, Velasquez L, Cantu C, Nader J, Lopez M, Murillo L, Aburto Y. Phenylpropanolamine and intracranial hemorrhage risk in a Mexican population. Cerebrovasc Dis 2003;15:210–214.

164. Horowitz JD, Lang WJ, Howes LG, Fennessy MR, Christophidis N, Rand MJ, Louis WJ. Hypertensive responses induced by phenylpropanolamine in anorectic and decongestant preparations. Lancet 1980;1(8159): 60–61.

165. Dowse R, Scherzinger SS, Kanfer I. Serum concentrations of phenylpropanolamine and associated effects on blood pressure in normotensive subjects: a pilot study. Int J Clin Pharmacol Ther Toxicol 1990;28:205–210.

166. Pilsczek FH, Karcic AA, Freeman I. Case report: Dexatrim (phenylpropanolamine) as a cause of myocardial infarction. Heart Lung 2003;32:100–104.

167. Pentel PR, Mikell FL, Zavoral JH. Myocardial injury after phenylpropanolamine ingestion. Br Heart J 1982; 47:51–54.

168. Leo PJ, Hollander JE, Shih RD, Marcus SM. Phenylpropanolamine and associated myocardial injury. Ann Emerg Med 1996;28:359–362.

169. Kinsun H, Moulin MA, Savini EC. Simultaneous GLC determination of phenylpropanolamine and chlorpheniramine in urine using a nitrogen selective detector. J Pharm Sci 1978;67:118–119.

170. Neelakantan L, Kostenbauder HB. Electron-capture GLC determination of phenylpropanolamine as a pentofluorophenyloxazolidine derivative. J Pharm Sci 1976; 65:740–742.

171. Crisologo N, Dye D, Bayne WF. Electron-capture capillary gas chromatographic determination of phenylpropanolamine in human plasma following derivatization with trifluoroacetic anhydride. J Pharm Sci 1984;73: 1313–1315.

172. Reid A, Fleming PJ, Lake CR. Radioenzymatic determination of phenylpropanolamine in plasma. Anal Biochem 1987;165:275–286.

173. Dowse R, Haigh JM, Kanfer I. Determination of phenylpropanolamine in serum and urine by high-performance liquid chromatography. J Pharm Sci 1983;72:1018–1020.

174. Yamashita K, Motohashi M, Yashiki T. High-performance liquid chromatographic determination of phenylpropanolamine in human plasma and urine, using column switching combined with ion-pair chromatography. J Chromatogr 1990;527:103–114.

175. O'Connell MB, Pentel PR, Zimmerman CL. Individual variability in the blood pressure response to intravenous phenylpropanolamine: a pharmacokinetic and pharmacodynamic investigation. Clin Pharmacol Ther 1989;45:252–259.

176. Al-Dirbashi O, Kuroda N, Akiyama S, Nakashima K. High-performance liquid chromatography of methamphetamine and its related compounds in human urine following derivatization with fluorescein isothiocyanate. J Chromatogr B Biomed Sci Appl 1997;695:251–258.

177. Bogusz JM, Kruger K-D, Maier R-D. Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 2000;24:77–84.

178. Stockley CS, Wing LM, Miners JO. Stereospecific high-performance liquid chromatographic assay for the enantiomers of phenylpropanolamine in human plasma. Ther Drug Monit 1991;13:332–338.

179. Rao VV, Rambhau D, Rao BR, Srinivasu P. Time dependent pharmacokinetic interaction between phenylpropanolamine and chlorpheniramine maleate in human subjects. Drug Metab Drug Interact 1999;15:259–268.

180. Amark P, Beck O. Effect of phenylpropanolamine on incontinence in children with neurogenic bladders. A double-blind crossover study. Acta Paediatr 1992;81: 345–350.

181. Baselt RC. Disposition of toxic drugs and chemicals in man. 8th ed. Foster City, CA: Biomedical Publications; 2008.

182. Hanzlick R, Davis G. National Association of Medical Examiners Pediatric Toxicology Registry Report 1: phenylpropanolamine. Am J Forensic Med Pathol 1992; 13:37–41.

183. Baker A, Lee NK. A review of psychosocial interventions for amphetamine use. Drug Alcohol Rev 2003;22: 323–335.

Chapter 14

SEROTONINERGIC and MIXED AGENTS

FENFLURAMINE WITH AND WITHOUT PHENTERMINE

HISTORY

Fenfluramine was developed in the early 1960s as an anorexic drug with less stimulating side effects than existing amphetamines. Beginning in France in 1963, fenfluramine and dexfenfluramine became a commonly prescribed appetite suppressants for the short-term treatment of obesity. Between 1963 and 1996, about 50 million European patients received dexfenfluramine or fenfluramine as an appetite suppressant.[1] The US Food and Drug Administration (FDA) approved the use of fenfluramine for the short-term treatment of obesity in 1973. In 1984, Weintraub and associates proposed the use of the combination fenfluramine and phentermine as a means to reduce the dose and adverse effects of these 2 drugs while maintaining weight loss from appetite suppression.[2] Between 1996 and 1998, approximately 5 million US patients used prescription appetite suppressants (i.e., fenfluramine, dexfenfluramine with or without phentermine) with a 4:1 ratio of women to men.[3] In the early 1990s, French investigators reported a cluster of 10 patients with primary pulmonary hypertension and a close temporal relationship between fenfluramine use and the development of exertional dyspnea.[4] Connolly et al published the first case series of 24 women (18 from Fargo, ND) with the development of valvular heart disease during the use of the combination of fenfluramine and phentermine for 1–28 months. Most of these patients had mitral regurgitation (92%) and aortic regurgitation (79%); 5 patients required valve replacement. On September 15, 1997, the manufacturers of fenfluramine and dexfenfluramine withdrew these medications from the US market.

IDENTIFYING CHARACTERISTICS

Fenfluramine is a trifluoromethyl derivative of amphetamine. Figure 14.1 compares the chemical structures of fenfluramine, phentermine, and amphetamine. The drug fenfluramine is a racemic mixture of the active dextro-isomer, dexfenfluramine and the inactive isomer, *l*-fenfluramine. Fenfluramine was marketed under the trade name of Pondimin.

EXPOSURE

Epidemiology

The use of appetite suppressants is common among the approximately 44% of women and 29% of men in the United States trying to lose weight.[5] Surveys of US adults suggest that up to one-third of adults that use prescription weight loss pills are not obese.[6] Additionally, up to one-third of adults continued using fenfluramine or dexfenfluramine after these drugs had been withdrawn from the US market. About 10% of the users of prescription appetite suppressants obtained these medications from illegitimate sources (e.g., friends, family).
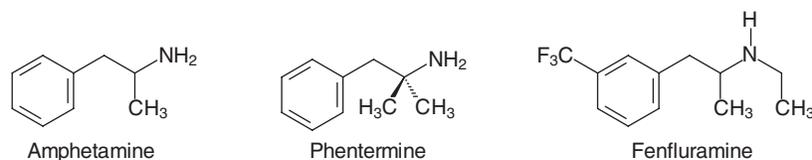
FIGURE 14.1. Chemical structures of fenfluramine, phentermine, and amphetamine.

Sources

Fenfluramine is a serotonin-releasing drug that suppresses food intake centrally. The administration of fenfluramine and other appetite suppressants produces limited weight loss (i.e., average <10% baseline weight) with a plateau in weight loss occurring by 4–6 months.[7] Withdrawal of the drug typically results in return of the patient to the baseline weight; the administration of fenfluramine does not result in permanent reduction of appetite.

DOSE EFFECT

Illicit/Medical Use

Originally, fenfluramine was marketed as an antidepressant; fenfluramine dosages for the treatment of depression ranged up to 120 mg.[8] Subsequently, the use of fenfluramine primarily involved the treatment of obesity. Current clinical data suggests that the prevalence of valvular heart disease in patient receiving fenfluramine increases with increasing dose and duration of use. In a case series of 14 women undergoing valve replacement for valvular disease associated with anorexic agents, 13 women received a mean dose of 58.5 ± 22.3 mg fenfluramine and 32.1 ± 11.4 mg phentermine daily for an average of 12.1 ± 7.3 month prior to presentation.[9] None of these patients had echocardiograms prior to the initiation of fenfluramine-phentermine therapy. Although the combination of these 2 drugs allows the use of lower individual doses, the combination also enhances pulmonary vasoconstriction and valvular heart disease.[10] Clinical studies suggest that the prevalence of valvular regurgitation increases with duration of therapy; however, the relationships of dose and duration of fenfluramine use to the development of valvulopathy are not well defined because of the presence of confound variables in many studies. In a multicenter, controlled investigation of valvular heart disease in fenfluramine/phentermine users, the prevalence of aortic regurgitation (i.e., primarily mild aortic regurgitation) occurred only in the group of patients receiving

TABLE 14.1. Comparison of Adjusted Odds Ratios, *P* values, and Duration of Treatment in Study by Jollis JG et al. [12]

Duration of Treatment (Days)	Adjusted Odds Ratio	*P* Value
90–180	1.5	.23
180–360	2.4	.002
361–720	4.6	<.001
>720	6.2	<.001

this drug combination for >3 months.[11] The prevalence of moderate or severe mitral or aortic regurgitation was not statistically different in the fenfluramine/phentermine users and the control group (no fenfluramine/phentermine use in the last 5 years). In a study of 1,163 fenfluramine-phentermine users and 672 control patients (no fenfluramine/phentermine within 5 years), the adjusted odds ratio of mild or more severe aortic regurgitation compared with controls increased with duration of treatment as listed in Table 14.1.[12] Fenfluramine/phentermine-associated valvular disease primarily involved aortic regurgitation in patients receiving these drugs for over 6 months. The prevalence of moderate or severe mitral regurgitation was not statistically different between the 2 groups.

Many of the earlier reports on valvular heart disease and fenfluramine use involved the combination of fenfluramine and phentermine. In a single-center, retrospective cohort study designed to evaluate the prevalence of cardiovascular abnormalities in patients randomized to treatment with either fenfluramine alone or placebo, there was no increased risk of valvulopathy.[13] The duration of fenfluramine treatment was up to 3 months; the median posttreatment follow-up was 4.4 years (range, 4.1–4.9 years).

Toxicity

The ingestion of an estimated 300 mg fenfluramine by a 13-year-old girl was associated with drowsiness, vomiting, and mydriasis.[14] Her vital signs were stable and she recovered uneventfully. A seizure and cardiopulmonary arrest occurred 2 hours after the ingestion of 1,600 mg
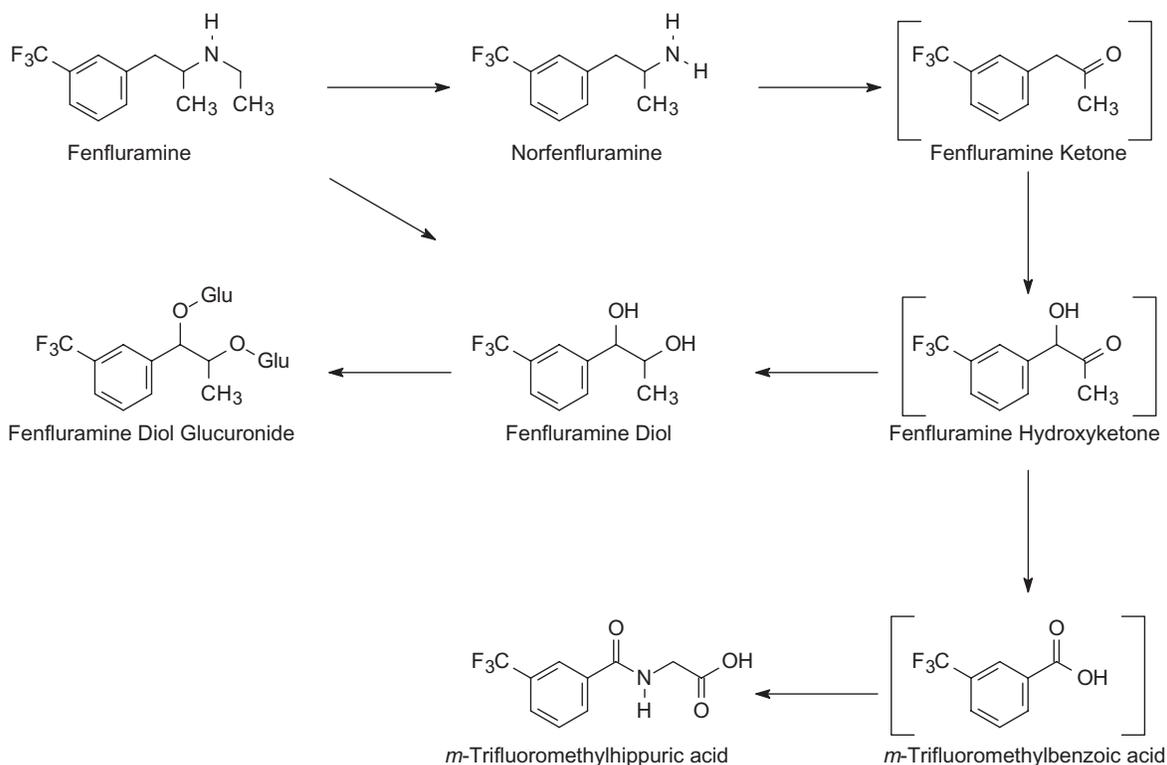
FIGURE 14.2. Proposed metabolic pathway for racemic fenfluramine.[16]

fenfluramine by a 17-year-old girl.[15] She failed to respond to 1 hour of cardiopulmonary resuscitation and she was pronounced dead. The body temperature was not reported.

TOXICOKINETICS

Absorption

Radioactive tracer studies in humans indicate that the absorption of fenfluramine from the gastrointestinal tract (GI) is relatively complete. In a study of 4 volunteers receiving 1 mg [^{14}C] *d,l*-fenfluramine hydrochloride, the average recovery of radioactivity in the urine exceeded 90%.[16] Peak plasma concentration of fenfluramine and norfenfluramine occur approximately 4 hours and 4–6 hours after ingestion, respectively.[17]

Biotransformation

Fenfluramine undergoes de-ethylation to norfenfluramine. Subsequent potential pathways involve oxidative deamination to fenfluramine ketone and *C*-oxidation to the fenfluramine diol and fenfluramine hydroxyketone.

The latter compound undergoes biotransformation to *m*-trifluoromethylbenzoic acid. Conjugation with glycine produces fenfluramine diol glucuronide and the main urinary metabolite, *m*-trifluoromethylhippuric acid. Under normal urinary pH, this latter compound accounts for a majority of the fenfluramine dose excreted by the kidneys.[18] Figure 14.2 displays the proposed metabolic pathways for racemic fenfluramine.

Elimination

Elimination of fenfluramine results primarily from hepatic metabolism and the renal excretion of urinary metabolites. The mean plasma elimination half-life of *d*-fenfluramine after the ingestion of 60 mg racemic fenfluramine by 8 healthy volunteers daily for 15 days was 19 ± 2 hours compared with 25 ± 3 hours for *l*-fenfluramine.[19] The metabolite, *d*-norfenfluramine persists about 1.5–2 times longer in the plasma than *d*- fenfluramine. Furthermore, the mean plasma elimination half-life is longer for *l*-norfenfluramine than *d*-norfenfluramine. In the same study, the mean plasma elimination half-life of *l*-norfenfluramine was 50 ± 5 hours compared with 34 ± 4 for *d*-norfenfluramine.

257

The elimination of fenfluramine and norfenfluramine in the urine is pH dependent. In alkaline urine unchanged fenfluramine and norfenfluramine account for approximately 2% of the dose excreted by the kidney; whereas in acidic conditions (pH ~5), unchanged fenfluramine and norfenfluramine account for approximately 35–50% of the dose excreted in the urine.[20] Under normal conditions with alteration of urinary pH, these 2 compounds accounted for 6–23% of the excreted dose. The urinary elimination half-life of fenfluramine is pH-dependent. At urinary pH of 5.0, the elimination half-life of fenfluramine is about 11 hours. Within the first 24 hours after an estimated fenfluramine overdose of 30 mg/kg by child, the plasma elimination half-life was approximately 8–9 hours.[21]

Tolerance

Although fenfluramine is an efficacious appetite suppressant, the effectiveness of appetite suppressants decreases over time, particularly after 3 months.[22] In a 34-week, double-blind clinical trial of 121 overweight patients (mean body weight, 154% ± 1.2% ideal body weight), the treatment group received 60 mg extended-release fenfluramine plus 15 mg phentermine.[23] The mean initial weight loss in the treatment group during the first 18 weeks was approximately 10%, however, during the last 10 weeks of the study, there was no significant weight loss in the treatment group.

Drug Interactions

There are limited data on the synergistic action of phentermine and fenfluramine. Although some epidemiology studies suggest synergism, the existing studies lack adequate experimental design and power to yield definitive conclusions regarding the effect of the addition of phentermine on the prevalence of fenfluramine-induced valvular disease. In a reader-blinded, controlled, multicenter study, the prevalence rates and relative risk (RR) of mild or greater aortic regurgitation were 8.9% in the dexfenfluramine group (RR = 2.18; 95% CI: 1.32–3.59), 13.7% in the phentermine/fenfluramine group (RR = 3.34; 95% CI: 2.09–5.35), and 4.1% in the untreated group.[11] The differences in the prevalence rates for the dexfenfluramine and fenfluramine/phentermine groups compared with the control group were statistically significant ($P < .001$). In a study of 257 obese patients with a history of appetite suppressant use and 239 control participants, the odds ratio (OR) for cardiac-valve abnormalities based on the FDA criteria was as follows: dexfenfluramine only, 12.7 (95% CI: 2.9–56.4); dexfenfluramine and phentermine, 24.5 (95%

CI: 5.9–102.2); and fenfluramine and phentermine, 26.3 (95% CI: 7.9–87.1).[24] Comparisons between the groups was limited because the duration of treatment differed between the 3 groups.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Histopathology

The valvulopathy associated with fenfluramine involves regurgitation with plaques on the valve resulting from the proliferation of myofibroblastic cells with myxoid stroma.[25] Stenosis is not associated with this valvulopathy. Pathologic examination of diseased valves from patients undergoing valve replacement demonstrate a glistening white appearance without the yellow discoloration or calcification associated with rheumatic heart disease. The presence of thick valves with numerous onlays composed primarily glycosaminoglycans and increased vascularity and inflammation of the underlying valve helps separate fenfluramine-induced valvular disease from normal and rheumatic valves.[26] Reduced valve mobility results from diffuse leaflet thickening and tethering of the chordae, particularly the posterior leaflet resulting in central regurgitation. Microscopic findings include smooth muscle cell hyperplasia along with abundant extracellular matrix and focal proliferation and fibrosis on the surface of the leaflet without obvious signs of inflammation or necrosis. The resulting formation of plaques has a "stuck-on" appearance (valve onlays) to normal-appearing underlying structures. Rare case reports also associated fenfluramine/phentermine use with the development of fibroproliferative plaques in pulmonary arteries of patients with pulmonary hypertension.[27] The proliferation of smooth-muscle cells and fibroblasts occurs without elastic fibers or destruction of underlying structures. The pathology associated with fenfluramine-associated valvular heart disease is similar to the cardiac pathology found with the carcinoid syndrome and valvulopathy associated with ergot and methysergide.

Mechanism of Action

Fenfluramine is a serotoninergic agent that increases the release of serotonin and inhibits the reuptake of serotonin from nerve terminals. This drug modulates the normal appetite by decreasing the desire to eat and the eating rate. In volunteer studies, 60 mg fenfluramine slows the local rate of eating and eliminates the characteristic decline in the rate of feeding across the course of a meal with a subsequent mean reduced caloric intake

of 26%.[28] The mediators of serotonin-induced satiety include the 5-HT$_{2C}$ and 5-HT$_{1B}$ receptors.[29] *In vitro* studies indicate that fenfluramine and norfenfluramine have low affinity for 5-HT$_{1B}$ receptors, moderate affinity for 5-HT$_{2C}$ receptors, and high affinity for 5-HT$_{2B}$ receptors.[30]

Mechanism of Toxicity

The pathophysiologic mechanism of fenfluramine-associated valvular disease remains unknown. Potential mechanisms of toxicity include 1) direct vasoconstriction and inhibition of nitric oxide production by valvular endocardium,[31] and 2) activation of mitogenic effects of the 5-HT$_{2B}$ receptor via protein kinase cascade, resulting in proliferation of fibromyxoid plaques.[32] The latter action may result from the effects of norfenfluramine rather than fenfluramine because the binding of this active metabolite for the 5-HT$_{2B}$ receptor if much greater than the parent compound, fenfluramine.[33] Furthermore, the concentration of 5-HT$_{2B}$ and 5-HT$_{2A}$ receptor transcripts in heart valves is at least 300-fold higher than 5-HT$_{2C}$ receptor transcripts.[34]

Postmortem Examination

Autopsy findings following fatal fenfluramine overdose are usually nonspecific with the presence of pulmonary edema, mild cerebral congestion, and occasionally right ventricular dilation. Cardiac abnormalities are typically absent.

CLINICAL RESPONSE

Illicit/Medical Use

In clinical trials, adverse effects include drowsiness, lightheadedness, dry mouth, nausea, vomiting, diarrhea, and urinary frequency. These effects are dose-related and usually mild.

Valvular Heart Disease

Valvular regurgitation and heart disease occurs in patients receiving fenfluramine or dexfenfluramine with or without phentermine as well as ergot alkaloids (ergotamine, methysergide), dopamine agonists (pergolide, cabergoline) and possibly 3,4-methylnedioxy-methamphetamine (MDMA, ecstasy).[35] Most of these cases involve mild or moderate valvulopathy with the primary long-term complication being aortic insufficiency in a minority of these individuals.[36] Valve repair or replacement is usually unnecessary. In an observa-

tional study of 5,743 fenfluramine users examined by echocardiography from 1997–2004, 38 (0.66%) of these patients required cardiac surgery.[37] Of these 38 patients, 25 had clear evidence of fenfluramine-related valvular damage. Although initial case series and uncontrolled surveys suggested that the prevalence of cardiac valvular abnormalities ranged up to 30–40%,[38] acquisition and interpretation biases probably caused an overestimation of the true prevalence of valvular disease in patient receiving fenfluramine or the combination of fenfluramine and phentermine. Most controlled studies report prevalence rates for FDA case definitions (moderate or greater mitral regurgitation and/or mild or greater aortic regurgitation) in the range of 5–15% compared with 3–6% in control groups.[39,40] However, the risk estimates vary widely between studies. Analysis of 6 controlled cohort studies of fenfluramine, dexfenfluramine and/or phentermine yielded a RR = 2.32 (95% CI: 1.79–3.01, $P < .00001$) for aortic regurgitation with an attributable rate of 4.9%.[41] The relative risk ratio for mitral regurgitation was 1.55 (95% CI: 1.06–2.25, $P = .02$) with an attributable rate of 1.0%. In contrast, another analysis of published studies with echocardiographic assessment of cardiac valve regurgitation in patients exposed to fenfluramine or dexfenfluramine and suitable controls reported the following summary of relative risks: mild or greater aortic regurgitation, 19.6 (95% CI: 16.3–23.5, $P < .00001$); moderate or greater mitral regurgitation, 5.9 (95% CI: 4.0–8.6, $P < .00001$).[42]

Potential factors affecting the prevalence of valvular regurgitation include advancing age, obesity, gender, rheumatic fever, and other drugs (e.g., ergotamine, methylsergide). Although aortic root diameter at the sinuses of Valsalva is proportional to body size and increases with age, a population-based sample indicated that the use of fenfluramine or dexfenfluramine with or without phentermine is associated with aortic regurgitation independent of aortic dilation or fibrocalcification.[43]

Following cessation of fenfluramine use, the valvulopathy associated with these appetite suppressants do not usually progress, and many patients experience a regression of the valvulopathy over time.[39] Improvement in the regurgitation is more common than worsening of regurgitation; even moderate to severe mitral or aortic regurgitation may improve over the first few years after cessation of fenfluramine use, particularly in the first 6 months.[44,45] In a follow-up study of the original cohort of patients from Fargo, North Dakota with appetite suppressant-induced valvulopathy, serial echocardiograms were obtained a mean of about 1 year apart for 38 patients with at least mild mitral regurgitation and 43 patients with at least mild aortic regurgitation.[46]

Mitral regurgitation improved by at least 1 grade in 17 patients (P = .001) and aortic regurgitation improved by at least 1 grade in 19 patients (P = .004). No change in severity of mitral and aortic regurgitation occurred in 19 and 22 patients, respectively. Two patients in each group experienced worsening of regurgitation by at least 1 grade. Regression usually occurs within 6 months of the cessation of drug use; typically the regression involves multiple affected valves rather than a single valve. Rare case reports associate the development of valvular disease several years after the discontinuation of prolonged treatment with fenfluramine and dexfenfluramine.[47] To date, an excess prevalence of valvular heart disease has not been reported for phentermine when used as monotherapy for obesity.

Clinical features of patients with valvular disease include atypical chest pain, murmur, palpitations, and evidence of congestive heart failure (peripheral edema, dyspnea on exertions, reduced exercise tolerance, orthopnea, paroxysmal nocturnal dyspnea).

PULMONARY HYPERTENSION

Primary pulmonary hypertension (i.e., mean pulmonary arterial pressure >25 mm Hg at rest) is a rare, often fatal disease that is particularly common in women in their third or fourth decade of life. Anorexic drugs (e.g., fenfluramine, dexfenfluramine, diethylpropion) are suspected factors in the development of primary pulmonary hypertension along with cocaine use, human immunodeficiency virus, oral-contraceptive use, and aminorex fumarate;[48] however, current data does not support a definite causal connection between fenfluramine and primary pulmonary hypertension because of the presence of confounding variables and genetic susceptibility (e.g., germline bone morphogenetic protein receptor).[1] The prevalence of severe anorexic drug-associated pulmonary hypertension varies substantially between countries with prevalence relatively low in the United Kingdom compared with France. Exposure to fenfluramine compounds occurred in 15 (20%) of patients with primary pulmonary hypertension referred to a French speciality center compared with 2 (4%) of 55 patients referred to an English Heart Lung Transplant center with primary pulmonary hypertension.[4,49] An additional patient in the latter group was exposed to diethylpropion. In a retrospective review of patients referred to the French National Reference Center for Pulmonary Hypertension from 1986–2004, approximately 18% had a history of fenfluramine use (mean, 6 months; range, 3–12 months).[50] The clinical course, median survival rates, and genetic features of the fenfluramine-associated cases were not different than the patient with idiopathic pulmonary hypertension. Review of newly diagnosed patients at 13 tertiary pulmonary hypertension centers in the United States from 1998–2001demonstrated an increased prevalence of appetite suppressant use in the referral consistent with prior studies, but there was no dramatic increase in referrals in the period of time immediately after the withdrawal of fenfluramine and dexfenfluramine from the US market.[51]

Overdose

Clinical features of fenfluramine intoxication include facial flushing, diaphoresis, mydriasis, agitation, sinus tachycardia, hypertension, muscle rigidity, trismus, muscle fasciculations, and clonus. In serious overdoses, seizures, hyperthermia, coma, respiratory depression, intractable ventricular fibrillation, and death.[52] The onset of symptoms typically is rapid with serious cardiorespiratory complications occurring within the first 1–4 hours after ingestion.[53] Drowsiness, sinus tachycardia, and mydriasis may persist 24–48 hours after large fenfluramine overdoses.

Abstinence Syndrome

Case reports suggest that abrupt cessation of large doses of fenfluramine may result in depression, insomnia, and anorexia reaching a maximum about 4 days after withdrawal. In a study of 11 patients receiving 80 mg fenfluramine daily for 28 days, self-reported rating indicated a depression of mood following cessation of fenfluramine use.[8] Peak scores occurred 4 days after fenfluramine use stopped. A similar decline in mood developed in 20 patients receiving 60 mg fenfluramine daily for 28 days following cessation of fenfluramine.[54] These effects typically are mild and resolve spontaneously.

Reproductive Abnormalities

There are few data on the reproductive outcomes of women using fenfluramine and phentermine during pregnancy. Existing data provides no clear evidence that the use of these drugs during pregnancy causes adverse reproductive outcomes. In a controlled prospective cohort study of women contacting the California Teratogen Information Service during pregnancy, 233 women were controls and 98 women were in the first trimester fenfluramine/phentermine exposure group.[55] The study detected no significant difference in prematurity, spontaneous pregnancy loss, or the overall frequency of minor or major anomalies between the 2 groups.

DIAGNOSTIC TESTING

SI Units

$$1 \ ng/mL = 4.32 \ nM$$
$$1 \ nM = 0.231 \ ng/mL$$

Analytic Methods

Short retention times, poor peak shapes, and low resolution complicate the analysis of small molecules, such as phentermine and fenfluramine. Methods for the quantitation of fenfluramine include gas chromatography (GC),[56] high performance liquid chromatography with UV detection (HPLC/UV, 210 nm),[57] isocratic HPLC/UV with derivation by 4-(4,5-diphenyl-1H-imidazsole-2-yl)benzoyl chloride,[58] and gas chromatography/mass spectrometry (GC/MS) with pentafluoropropionic anhydride derivatization.[59] The limit of detection (LOD) for the latter 2 methods is 10 ng/mL and 500 ng/mL, respectively. Following headspace-solid phase microextraction and derivations with heptafluorobutyric acid, the LOD for fenfluramine using GC/MS is 5 ng/g.[60] The use of chiral HPLC allows the quantitation of the enantiomers of fenfluramine and the metabolite, norfenfluramine.[61] The use of GC/MS in selected ion monitoring mode allows the quantitation of numerous phenylalkylamine derivatives in hair samples including fenfluramine (LLOQ = 0.02 ng/mg).[62]

Biomarkers

Blood

THERAPEUTIC. Following the administration of therapeutic doses (e.g., 60 mg daily) of fenfluramine, the peak plasma fenfluramine concentrations range from about 50–150 ng/mL. In a volunteer study of 5 fasting men receiving 60 mg fenfluramine, the mean peak plasma fenfluramine concentration was 63 ± 4.5 ng/mL at 2–4 hours after ingestion as measured by gas chromatography with flame ionization detection.[63] The mean peak plasma norfenfluramine concentration was about 16 ng/mL at 4–6 hours after ingestion. In a convenience sample of 13 obese adult women, the mean daily intake of fenfluramine was 149 ± 24 mg between the 4th and 16th week of the study.[64] The mean plasma fenfluramine and norfenfluramine concentrations in samples drawn during evaluations at the study site every 2 weeks were 239 ± 32 ng/mL and 93 ± 29 ng/mL, respectively. In a study of 8 healthy volunteers receiving 60 mg racemic fenfluramine daily for 15 days, the mean steady state d-fenfluramine and d-norfenfluramine concentrations

were 43 ± 6 ng/mL and 16 ± 2 ng/mL, respectively, compared with 67 ± 11 ng/mL for l-fenfluramine and 24 ± 3 ng/mL for l-norfenfluramine.[19] Maximum concentrations of these 2 compounds during this study were 65 ± 9 ng/mL and 21 ± 3 ng/mL, respectively.

OVERDOSE. Case reports associated plasma fenfluramine concentrations ranging from 240–850 ng/mL with tachycardia, mydriasis, confusion, agitation, and muscle stiffness. A 3½-year-old girl developed diaphoresis, facial flushing, muscle stiffness, mydriasis, shallow, irregular respirations, hypertension, and altered consciousness after the ingestion of a large dose of fenfluramine.[21] The plasma fenfluramine concentration 7½ hours after ingestion was 1,140 ng/mL as measured by gas chromatography. At that time, the patient was improving, and the plasma norfenfluramine concentration was 280 ng/mL.

POSTMORTEM. Case reports associate fatal fenfluramine intoxication with postmortem fenfluramine blood concentrations ranging from 650–28,000 ng/mL.[65,66] The case at the lower end of the range involved a severely hyperthermic 13-year-old child, who died 3½ hours after ingestion of fenfluramine. The body temperature was not reported in the 17-year-old girl whose postmortem fenfluramine blood concentration was 28,000 ng/mL. She failed to respond to 1 hour of cardiopulmonary resuscitation and was pronounced dead 3 hours after ingesting a large dose of fenfluramine.[15] Several hours after ingestion, the fenfluramine concentrations in postmortem liver samples typically are several fold higher than blood. A 36-year-old woman was found dead, and autopsy examination did not reveal any anatomic causes of death.[67] Postmortem samples from heart blood and brain contained a fenfluramine concentration of 7.27 mg/L and 155 mg/L, respectively.

Abnormalities

The echocardiographic abnormalities associated with appetite suppressants are not unique. The morphology of these changes may mimic other diseases, presenting as degenerative, myxomatous, nonspecific, rheumatic valve morphology.[68]

TREATMENT

Treatment for fenfluramine overdose is supportive. Immediate life-threatening effects include progressive obtundation and respiratory depression following severe overdose. Serious complications of fenfluramine overdose usually present within the first few hours of

ingestion. These patients require cardiac monitoring, pulse oximetry, and careful observation for the development of seizures and ventricular dysrhythmias. Sinus tachycardia, hypertension, and agitation are common complications of mild to moderate fenfluramine intoxication; these effects usually require only benzodiazepine therapy and a calm environment. There are few data on the efficacy of decontamination procedures for fenfluramine intoxication; the administration of activated charcoal is a therapeutic option if the patient presents to the healthcare facility within 1 hour of ingestion. However, there are inadequate clinical data to conclude the administration of activated charcoal improves outcome. There are no effective antidotes for the treatment of fenfluramine intoxication.

Treatment for appetite suppressant-associated valvulopathy (fenfluramine or dexfenfluramine with or without phentermine) include discontinuation of the anorectic drug, cardiac physical examination, and echocardiography in symptomatic patients (heart murmurs, associated physical findings and/or symptoms). Doppler echocardiogram is an alternative to standard echocardiography in obese patients with very large body habitus.

DEXFENFLURAMINE

HISTORY

In 1996, the FDA approved the use of dexfenfluramine as an appetite suppressant; Interneuron Pharmaceuticals (Lexington, MA) manufactured dexfenfluramine and Wyeth-Ayerst Laboratories marketed this drug under the trade name, Redux. Previously, dexfenfluramine and fenfluramine were marketed extensively in Europe for the treatment of obesity. Both Wyeth-Ayerst Laboratories and their parent company American Home Products Corporation (Madison, NJ) agreed to voluntarily withdraw their drugs on September 17, 1997 as a result of reports on the association of this drug with cardiac valvular insufficiency. The latter company also withdrew their drug, Pondimin (fenfluramine).

IDENTIFYING CHARACTERITICS

Dexfenfluramine (CAS RN: 3239-44-9) is the *d*-isomer of fenfluramine that is distributed as the hydrochloride salt (MW 267.7 g/mol). This drug has most of the same anorectic properties as the racemic mixture of fenfluramine. Dexfenfluramine was marketed under the trade name, Redux (Interneuron Pharmaceuticals, Inc.).

EXPOSURE

Dexfenfluramine is no longer available in the United States or in most global markets.

DOSE EFFECT

In a case series of 14 women undergoing cardiac surgery for valvular disease associated with anorexic agents, 1 woman received dexfenfluramine (30 mg daily for 13 months) and phentermine (60 mg daily for 6 months, concomitantly).[10] The estimated ingestion of 1,800 mg dexfenfluramine was associated with clonus, coma, hypertension, sinus tachycardia, seizures, and respiratory failure.[69] She recovered within 48 hours after intensive supportive care including endotracheal intubation.

TOXICOKINETICS

Absorption

The average bioavailability of dexfenfluramine in obese and nonobese individuals is approximately 60% and 70%, respectively.[70] Peak plasma concentrations of dexfenfluramine occur about 3–5 hours after ingestion, whereas peak nordexfenfluramine concentrations occur about 6–8 hours after ingestion. The peak plasma concentrations of nordexfenfluramine are approximately one-half of the peak fenfluramine concentrations.

Distribution

Dexfenfluramine is highly lipophilic and has an extensive volume of distribution. In a study of 10 obese volunteers (mean $145 \pm 13\%$ ideal body weight) and 10 healthy, nonobese volunteers ($93 \pm 8\%$ ideal body weight), the steady-state volume of distribution was 11.2 ± 6.2 L/kg and 13.7 ± 2.7 L/kg, respectively.[70] These findings are consistent with the similar distribution of fenfluramine into lean tissue and excess body fat.

Biotransformation

Dexfenfluramine undergoes extensive hepatic metabolism with first-pass metabolism accounting for about 20% of the administered dose.[71] Figure 14.3 displays proposed pathways for the metabolism of dexfenfluramine. *In vivo* studies indicate that the biotransformation of dexfenfluramine to the active metabolite, nordexfenfluramine involves CYP2D6 isoforms; however, CYP2D6 does not catalyze the metabolism of nordexfenfluramine.[72]
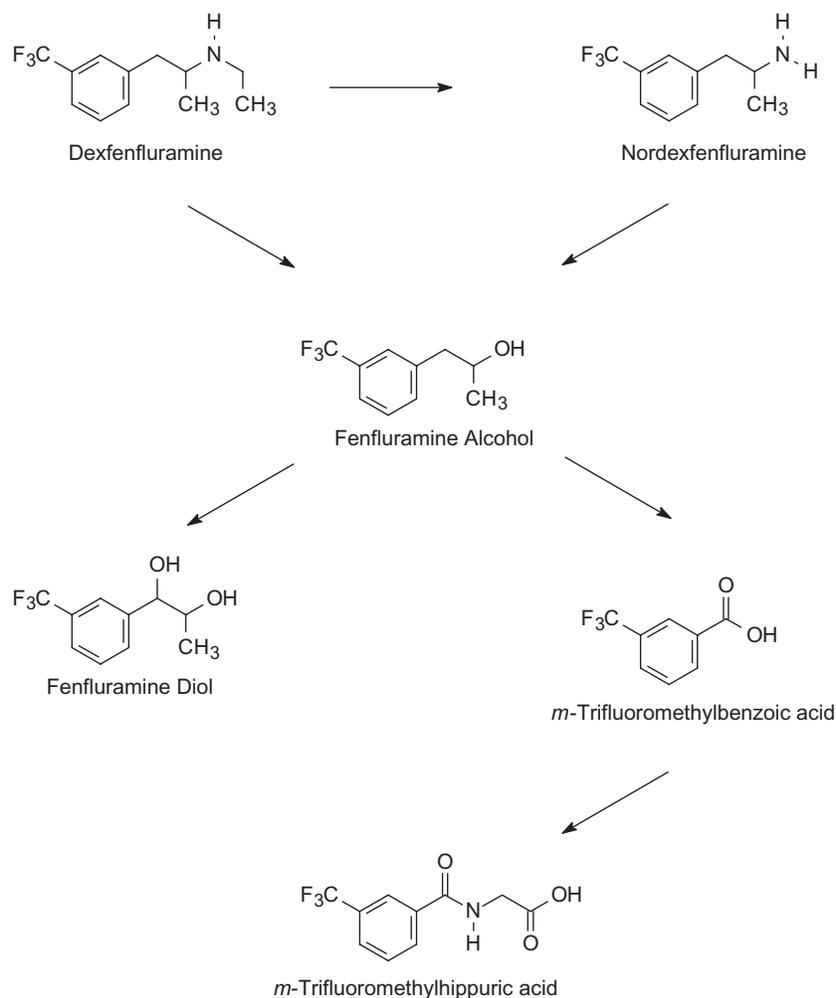
FIGURE 14.3. Proposed metabolic pathways of dexfenfluramine.[72]

Elimination

Following oral administration, the terminal plasma elimination half-lives of obese and nonobese healthy volunteers are similar. After the ingestion of 30 mg dexfenfluramine hydrochloride (25.9 mg dexfenfluramine base) by 5 obese and 5 nonobese volunteers, the mean terminal plasma elimination half-lives were 16.5 ± 7.1 hours and 14.5 ± 2.6 hours, respectively.[70] The appearance of the dealkylated active metabolite (nordexfenfluramine) in the plasma were slower than dexfenfluramine; however, this metabolite persisted much longer in the plasma than dexfenfluramine.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Pathologic examination of diseased valves associated with dexfenfluramine use reveals glistening white valve leaflets, diffuse, irregular cusp thickening, lack of commissural fusion, and shortened and fused chordae tendineae.[35] Histopathologic examination demonstrates an avascular collagenous matrix surrounding the valve with preservation of underlying valve structure along with proliferation of smooth muscle cells and myofibroblasts.

Mechanism of Action

Like fenfluramine, dexfenfluramine decreases the normal appetite by reducing the desire to eat (i.e., premeal hunger ratings) and decreasing the eating rate.[73] The other isomer of fenfluramine, *l*-fenfluramine, is inactive. Unlike fenfluramine, dexfenfluramine is a pure serotonin agonist. Selective serotonin receptor antagonist (5-HT$_1$ and 5-HT$_2$) studies in rodents indicate that the hypophagia associated with dexfenfluramine administration results from 5-HT$_{1B}$ or 5-HT$_{2C}$ activity.[74,75]

Mechanism of Toxicity

Dexfenfluramine inhibits neuronal serotonin reuptake via inhibition of the serotonin transporter, increased release of serotonin, and the subsequent stimulation of serotonin receptors.[76] Inhibition of the serotonin transporter also occurs in smooth muscle cells, pulmonary endothelium, and platelets. In isolated tissue preparations, dexfenfluramine causes pulmonary vasoconstriction by the inhibition of potassium channels and membrane depolarization, and increases the resistance in pulmonary arteries by an influx of extracellular calcium into the smooth muscle cells. The major metabolite, nordexfenfluramine produces a relatively greater degree of vasoconstriction in these preparations than dexfenfluramine.[77] Activation of 5-HT_2 receptors by nordexfenfluramine causes the influx of extracellular calcium and the release of calcium from the sarcoplasmic reticulum of the pulmonary artery smooth muscle cells, resulting in pulmonary constriction. The development of drug-induced valvular disease probably involves a complex interaction between various factors including serotonin transporter, serotonin, 5HT_{2B} receptors, and individual susceptibility with subsequent activation of protein kinases and potentiation of transforming growth factor β.[35]

CLINICAL RESPONSE

Illicit/Medical Use

In clinical trials, common adverse effects include diarrhea, malaise, dry mouth, and somnolence.[78] These symptoms typically are transient and mild. Similar to fenfluramine, the use of dexfenfluramine is associated with an increased prevalence of valvular regurgitation compared with control populations (no appetite suppressant use).[79] The prevalence of cardiac valvular disease in patients receiving dexfenfluramine varies between studies as a result of the presence of methodologic differences including ages of groups, concomitant treatment with phentermine, prior treatment with other appetite suppressants, dose, duration of treatment, and time since discontinuation of treatment. In a study of 172 dexfenfluramine patients, 172 unexposed controls matched for age, sex, and body mass index, and 68 unmatched participants (51 dexfenfluramine patients, 17 controls), the prevalence of FDA-grade regurgitation in the dexfenfluramine patients was 7.6% compared with 2.1% for controls (OR = 3.82, P = .01). The prevalence of FDA-grade regurgitation in other studies of dexfenfluramine patients ranged from 6.9% (P = NS)[80] to 17% (P < .01),[24] when compared with unexposed controls. This difference resulted primarily from the increased frequency of mild aortic regurgitation in the dexfenfluramine group. Studies of the valvulopathy associated with fenfluramine and dexfenfluramine have not detected a significant difference in the prevalence of cardiac disease or the type of pathology following the use of these 2 drugs.[81] Heart murmurs associated with dexfenfluramine-induced valvular disease typically are systolic murmurs, grade I–II/VI.[82]

Similar to fenfluramine, short-term use (i.e., <3 months) of dexfenfluramine is not usually associated with clinically significant cardiac valvular disease. In a clinical trial prior to the discontinuation of dexfenfluramine, there was a small increase in the prevalence of minor degrees of aortic and mitral regurgitation following short-term dexfenfluramine use (mean, 72.5 ± 22.5 days). The natural history of dexfenfluramine and fenfluramine/phentermine-associated valvular disease is similar including medical history, physical findings, ejection fraction, pulmonary artery systolic pressures, and cardiovascular events.[83] Following cessation of therapy, progression of aortic valvular regurgitation is unlikely and regression of aortic regurgitation occurs in a substantial number of previously treated patients.[84] A follow-up study of these patients within 3–5 months after discontinuation of dexfenfluramine no longer detected a difference between the treatment and placebo group.[85] Rare case reports associate dexfenfluramine use with severe regurgitation that requires valve replacement.[86]

Overdose

Fenfluramine and dexfenfluramine produce similar effects following an overdose including mydriasis, sinus tachycardia, hypertension, agitation, elevated body temperature, muscle stiffness and rigidity, clonus, and hyperreflexia.[87] The clinical features of serious intoxications include coma, seizures, respiratory failure, and hyperthermia.

DIAGNOSTIC TESTING

Analytic methods for the quantitation of dexfenfluramine and fenfluramine are similar. Following the administration of therapeutic doses (e.g., 30 mg daily) of dexfenfluramine, the peak plasma dexfenfluramine concentrations average approximately 20 ng/mL. In a study of 18 healthy, young adults, the mean concentrations of dexfenfluramine following single 15 mg and 30 mg doses of fenfluramine were 10.04 ± 3.36 ng/mL and 23.46 ± 6.51 ng/mL, respectively.[88] In a study of 8 healthy volunteers receiving 30 mg dexfenfluramine daily for 15 days, the mean steady state *d*-fenfluramine and *d*-

norfenfluramine concentrations were 45 ± 5 ng/mL and 19 ± 3 ng/mL, respectively.[19] Maximum concentrations of these 2 compounds during this study were 70 ± 5 ng/mL and 26 ± 5 ng/mL, respectively. Echocardiographic changes of cardiac valves associated with dexfenfluramine or fenfluramine are similar. Thickening of the valvular leaflets and subvalvular apparatus occurs with variable degrees of leaflet retraction and reduced leaflet mobility. In more severe cases, variable degrees of valvular regurgitation appear but significant obstruction is not usually present.

TREATMENT

Treatment is supportive.

SIBUTRAMINE

HISTORY

Sibutramine originally was developed as an antidepressant in the 1980s,[89] later the use of this compound was limited to the treatment of obesity as a result of the ability of sibutramine to decrease food intake and increase satiety. In 1997, the FDA approved the use of sibutramine for the long-term treatment of obesity, and by 2001 this drug was marketed in Italy, United Kingdom, and Australia. In 2002, sibutramine was withdrawn from the Italian market because of 2 deaths and 50 adverse events (e.g., tachycardias, dysrhythmias, hypertension) reported in sibutramine users. The European Committee for Proprietary Medicinal Products assessed the risk-benefit ratio of sibutramine and this drug subsequently was reinstated in Italy. Similar assessments by the FDA and Health Canada resulted in favorable conclusions regarding the safety of sibutramine; however, sibutramine was withdrawn from the US markets in 2010 when the Sibutramine Cardiovascular Outcomes Trial (SCOUT) demonstrated an increased risk of nonfatal myocardial infarction and nonfatal stroke in patients with preexisting cardiovascular disease receiving sibutramine compared with placebo.[90] Sibutramine was withdrawn from Europe in early 2010.

IDENTIFYING CHARACTERISTICS

Sibutramine (CAS RN: 106650-56-0, C_{17}-H_{26}ClN) is a tertiary amine compound with an asymmetrical carbon as demonstrated in Figure 14.4. Meridia® (Abbott Laboratories, Abbott Park, IL) and Reductil® (Abbott Laboratories) are trade names for the racemic mixture
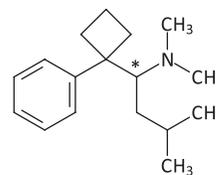
FIGURE 14.4. Chemical structure of sibutramine. Asterisk denotes stereocenter.

of sibutramine (*N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N*,*N*-dimethyl amine) hydrochloride monohydrate.

EXPOSURE

Sibutramine had been the most commonly used appetite suppressant until withdrawal from the US and European markets in 2010. The primary action of sibutramine is satiety. Similar to fenfluramine and dexfenfluramine, sibutramine reduces food intake and appetite in humans. In a double-blind, placebo-controlled, 14-day study of obese participants, 30 mg sibutramine reduced caloric intake by 23% at day 7 and by 26% on day 14 compared with placebo.[91] The World Anti-Doping Agency (WADA) added sibutramine to the list of prohibited substances in 2006. Some "herbal" weight loss products (e.g., Chinese slimming capsules, Chinese slimming tea) may contain sibutramine, which may be added without listing the content of sibutramine on the label.[92,93]

Postmarketing surveillance suggests that the abuse potential of sibutramine is substantially less than phentermine. In a study of 8,780 questionnaires completed on intake to 58 drug abuse treatment programs, the percentage of respondents indicating that they knew of the use of sibutramine to "get high" was 0.9% compared with 2.8% for phentermine ($P < .001$). Experimental human studies indicate that sibutramine lacks the amphetamine-type abuse liability when administered acutely. In a volunteer study comparing 25 mg and 75 mg sibutramine with 20 mg *d*-amphetamine and placebo, 25 mg sibutramine produced subjective effects indistinguishable from placebo.[94] The 75 mg sibutramine dose was poorly tolerated as a result of unpleasant side effects (e.g., anxiety, confusion, decreased vigor) in contrast to the positive mood associated with 20 mg *d*-amphetamine.

DOSE EFFECT

Sibutramine is a selective monamine reuptake inhibitor with established efficacy in weight loss along with modification of lifestyle and diet. At therapeutic doses

(10–20 mg daily), sibutramine reduces food intake by increasing satiety and attenuates the fall in metabolic rate associated with weight loss. A meta-analysis of sibutramine with lifestyle modification reported a mean pooled difference in weight loss of 4.45 kg (95% CI: 3.62–5.29 kg) at 12 months, when compared with placebo (lifestyle modification only).[95]

TOXICOKINETICS

Absorption

The absorption of sibutramine is rapid with bioavailabilities in the range of 75%. Sibutramine undergoes extensive first-pass metabolism.

Biotransformation

Sibutramine is a tertiary amine that undergoes extensive biotransformation in the liver to the active amine metabolites, M1 (mono-desmethyl sibutramine, norsibutramine) and M2 (di-desmethyl sibutramine, nor-norsibutramine). These demethylated metabolites are hydroxylated on the cyclobutane ring or the isopropyl chain, followed by conjugation with glucuronide to inactive metabolites. Figure 14.5 displays the proposed met-

abolic pathways for the biotransformation of sibutramine. *In vitro* studies indicate that CYP2B6 is the primary catalyst for the *N*-demethylation of sibutramine to M1 and M2.[96] Although CYP2C19, CYP3A4, and CYP 3A5 catalyze the formation of M1 at high substrate concentrations (>5 μM), the contribution of these isoforms to sibutramine metabolism probably is minor because these high substrate concentrations are not usually present following therapeutic doses. The demethylation of sibutramine to M1 and M2 is rapid with M2 being the major metabolite in human plasma. Following the ingestion of 15 mg sibutramine by 12 young volunteers, the mean peak plasma M1 concentration was 3.2 ± 1.3 ng/mL at 2.5 ± 0.9 hours after ingestion compared with 7.8 ± 1.8 ng/mL at 3.2 ± 1.4 hours for M2.[97]

Elimination

The main urinary metabolites are the carbamoyl glucuronides formed from M1 and M2 along with their hydroxylated analogs.[98] The kidney excretes little unchanged sibutramine in the urine; M1 and M2 are minor metabolites in the urine. The elimination of sibutramine in similar in young adults and the elderly. The mean plasma elimination half-lives in 12 young and 12 elderly adults following the ingestion of 15 mg
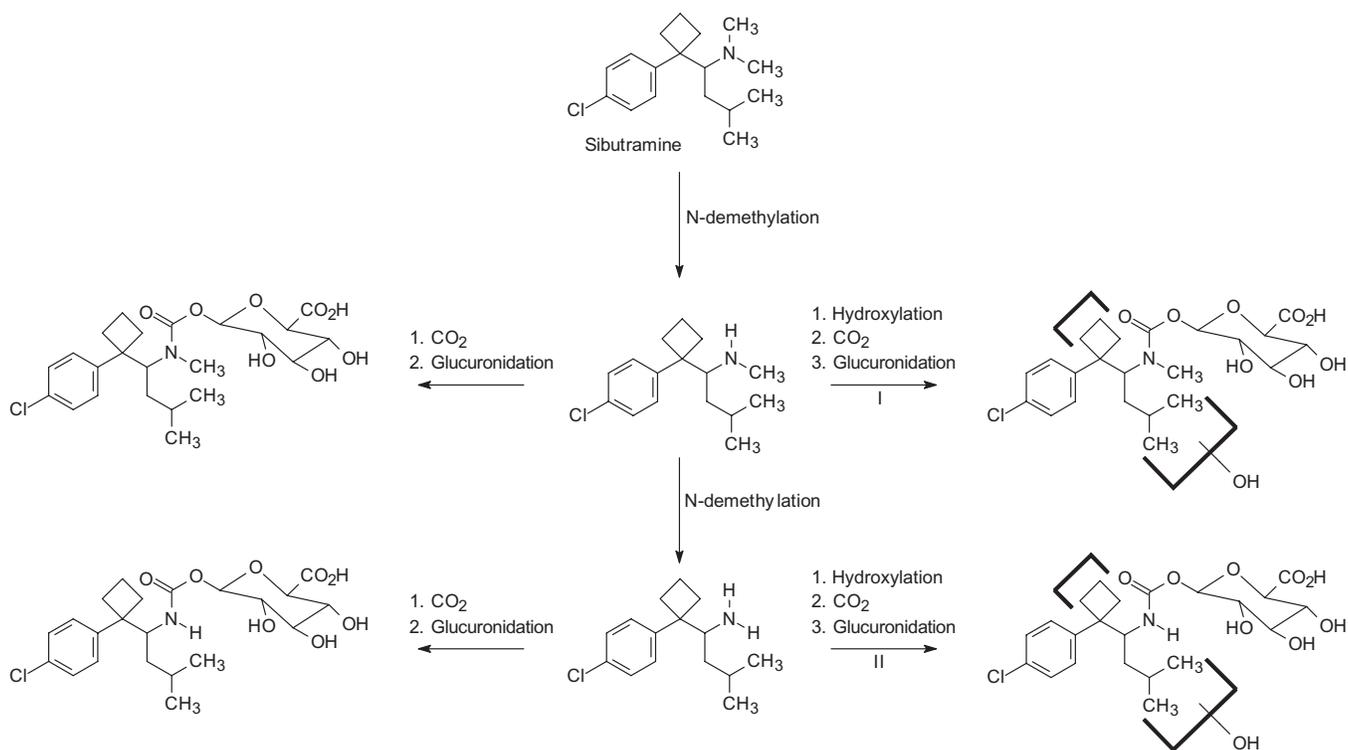
FIGURE 14.5. Proposed main pathways for the biotransformation of sibutramine. The reaction order indicated for hydroxylation and glucuronidation in pathways I and II is tentative.[98]
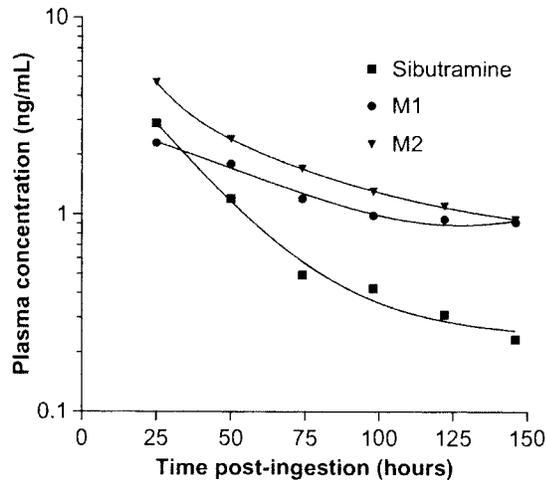
FIGURE 14.6. Plasma concentrations of sibutramine (solid square), mono-desmethyl sibutramine (M1, solid circle), and di-desmethyl sibutramine (M2, solid inverted triangle) following a sibutramine overdose by a 4-year-old girl. (Reprinted with permission from F Bucaretchi, EM de Capitani, SM Mello, R Lanaro, RF Barros, LCR Fernandes, JL Da Costa, S Hyslop, Serotonin syndrome following sibutramine poisoning in a child, with sequential quantification of sibutramine and its primary and secondary amine metabolites in plasma, Clinical Toxicology, Vol. 47, Issue 6, p. 599, copyright 2009.)

sibutramine were 19.0 ± 5.4 hours and 22.4 ± 6.2 hours, respectively.[97] Case reports suggest that the elimination of sibutramine and the metabolites is nonlinear following a sibutramine overdose. Figure 14.6 displays the plasma concentrations of sibutramine and reactive metabolites (M1, M2) beginning 25 hours after a sibutramine overdose (23 mg/kg) by a 4-year-old girl.

Tolerance

In short-term studies, tolerance does not develop to the hypophagia induced by sibutramine. Follow-studies of initial responders indicate that response to sibutramine remains after 10 months of therapy. In a randomized, double-blind, placebo-controlled study of 36 obese men and women, the amount food consumed following 15 mg sibutramine daily for 14 days was approximately 16% less than placebo.[99] After a 10-month, open-label treatment with 15 mg sibutramine and dietary advice, mean weight loss in responders was significantly less than nonresponders (11.8 ± 6.2 kg vs. 6.8 ± 2.7 kg, respectively, $P < .05$). The Sibutramine Trial of Obesity Reduction and Maintenance (STORM) trial detected little evidence of tolerance to the use of sibutramine over the 24 months of treatment.[100]

Drug Interactions

Potential drug interactions include the concomitant use of CY3A4 inhibitors (e.g., ketoconazole, erythromycin) and highly protein-bound drugs (e.g., warfarin, phenytoin); however, there are few clinical data and the clinical significance of these potential interactions is unclear. Additionally, sibutramine inhibits the reuptake of serotonin; the concomitant administration of selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (MAOIs) potentially increases the risk of serotonin syndrome.[101]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Sibutramine is a selective inhibitor of the presynaptic reuptake of serotonin, norepinephrine, and to a lesser extent, dopamine.[102,103] The rise in plasma dopamine is small compared with the increase in plasma norepinephrine. *In vivo* studies in rodents with monoamine receptor antagonists indicate that hypophagia associated with sibutramine involves α_1-adrenergic, α_2-adrenergic, 5-HT$_{2A}$, 5-HT$_{2C}$ receptors.[104] In rodent studies, sibutramine increases energy expenditure (i.e., thermogenesis) with M2 being much more potent than the parent compound, sibutramine.[105,106] Sibutramine has little affinity for the 5-HT$_{2A}$ receptor. This drug inhibits norepinephrine reuptake at least 10-fold more than serotonin reuptake.[107]

Mechanism of Toxicity

Sibutramine-induced inhibition of norepinephrine reuptake potentially increases blood pressure and heart rate. The therapeutic use of sibutramine may cause a slight increase in blood pressure in both normotensive and hypertensive patients, particularly during the initial phase of treatment. A meta-analysis of 21 placebo-controlled, double-blind, randomized trials of sibutramine demonstrated an increase in systolic (1.6 ± 10 mm Hg) and diastolic (1.8 ± 7 mm Hg) blood pressures in treated patients. The large standard deviation resulted from the interaction of the direct effect of sibutramine on blood pressure and weight-loss induced reduction of blood pressure. In studies of high-risk obese older women with cardiovascular disease and diabetes mellitus, the therapeutic use of sibutramine is associated with small median increases in pulse rate and small median decreases in systolic and diastolic blood pressure.[108] Elevation of blood pressure in obese patients treated with sibutramine does not usually result in

cessation of treatment, and blood pressure typically is controlled with the usual antihypertensive agents.[109]

CLINICAL RESPONSE

Illicit/Medical Use

Common adverse effects associated with the therapeutic use of sibutramine include anorexia, nausea, constipation, dry mouth, headache, palpitations, tachyarrhythmias, and insomnia.[110,111] In a retrospective review of California Poison Control System data on sibutramine exposures, the most common clinical features of symptomatic individuals were tachycardia, chest pain, agitation, irritation, dizziness, vertigo, nausea, and vomiting.[112] Most of the reported exposures to sibutramine were unintentional; 40% of the 62 exposures were asymptomatic.

Valvular Disease

To date, an increased prevalence of cardiac valvulopathy has not been associated with the use of sibutramine. In a double-blind, placebo-controlled, parallel-arm study of 210 obese type II diabetic patients, the prevalence of left-sided cardiac valve dysfunction was similar for the sibutramine group (2.3%, 3/133) and the placebo group (2.6%, 2/77).[113] The duration of the study was 12 months with a 12-month open-label extension. In a study of 106 obese patients with minimal tricuspid regurgitation on echocardiographic examination, there was slight, statistically significant increase in systolic blood pressure and heart rate at follow-up compared with baseline.[114] There was no statistically significant difference in aortic or mitral valve function, and no significant increase in pulmonary artery pressures. The mean duration of follow-up was 24 months.

Dysrhythmias/Myocardial Infarction

Case reports and drug monitoring programs document a variety of adverse cardiac effects in patients receiving sibutramine including atrial fibrillation, supraventricular tachycardia, QT_c prolongation,[115] *torsade de pointes*, ventricular tachycardia, myocardial infarction in a women with normal coronary arteries,[116] QT_c prolongation with ventricular fibrillation and cardiac arrest in a women with normal coronary arteries,[117] cardiomyopathy,[118] and heart block.[119] However, a causal connection between sibutramine and these events was not clearly established. In some of these case reports, QT prolongation and ventricular dysrhythmias occurred in susceptible patients with predisposing genetic abnormalities

(e.g., cardiac potassium channel subunit mutation).[120] In clinical studies of sibutramine, small increases in heart rate and systolic blood pressure occur in the treatment group compared with baseline; however, these changes are balanced by reduction in BMI.[121] Additionally, some clinical trials suggest that the use of sibutramine in patients with cardiovascular disease increases the risk of adverse cardiac events. In a study of 10,744 overweight or obese patients with preexisting cardiovascular disease, the risk of a primary outcome event was 11.4% in the sibutramine group compared with 10.0% in the placebo group (hazard ratio [HR], 1.16; 95% CI: 1.03–1.31; P = .02).[90] Although the rates of cardiovascular deaths were similar in the 2 groups, the rates of nonfatal myocardial infarction in the sibutramine and placebo groups were 4.1% and 2.6%, respectively (HR, 1.28; 95% CI: 1.04–1.57; P = .02).

Behavioral Abnormalities/Mental Disorders

Psychiatric complications associated with sibutramine therapy are rare, primarily involving patients with preexisting psychiatric disorders. Case reports associate the development of acute delirium in a patient with delusional disorder on sibutramine for 2 weeks,[122] acute psychosis in an adolescent with Kleine-Levin syndrome,[123] panic attacks in women with a history of a stable panic disorder,[124] an affective psychosis (agitation, irritability, hugging strangers) in a woman without a history of abuse or psychiatric disorders,[125] and a manic episode in a patient with a bipolar disorder following the initiation of sibutramine therapy.[126] Other case reports associated the development of acute psychosis in patients without prior documented psychiatric disorders.[127,128] A 26-year old woman developed catatonia and psychosis while taking excessive doses of sibutramine (up to 60 mg daily) and following a restrictive diet.[129] Symptoms included mutism, flat affect, immobility, paranoid ideations, and auditory hallucinations. She improved dramatically following the cessation of sibutramine and the administration of risperidone. The psychosis resolved within 1 month of cessation of sibutramine therapy. The use of sibutramine is not associated with hepatotoxicity in clinical trials.

Overdose

There are few data on the clinical effects of sibutramine during an overdose. The clinical features of sibutramine intoxication probably are an exacerbation of the pharmacologic properties of this drug. A case report associated the development of serotonin syndrome (diaphoresis, tachycardia, hypertension, agitation, insomnia, incoordination, hypertonia, hallucinations)

with the ingestion of approximately 400 mg sibutramine (23 mg/kg) 25 hours prior to admission.[130] She developed mild rhabdomyolysis, but there were no significant abnormalities in the electrocardiogram or other blood chemistries. She was discharged with no sequelae after 6 days of supportive care.

Reproductive Abnormalities

There are few data on the reproductive effects of sibutramine. Case series suggest that this drug is not a major teratogen.[131] There were no malformation in 10 women exposed to sibutramine during their pregnancy.[132] In a convenience series of 52 women exposed to sibutramine during their first semester, there were no congenital abnormalities detected in their neonates within the first month of life.[133] Although there were 7 cases of hypertensive complication, the lack of a control group limits conclusions regarding the clinical significance of these complications.

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantitation of sibutramine and active metabolites in biologic samples include HPLC,[134] isocratic LC with UV detection,[135] gas chromatography/electron ionization/mass spectrometry with methyl and trimethylsilyl derivation,[136] and liquid chromatography/electrospray ionization/tandem mass spectrometry.[137,138] Analysis with liquid chromatography/tandem mass spectrometry using a Chiralcel AGP chiral stationary-phase column allows the quantitation of sibutramine enantiomers.[139] The LOD for sibutramine metabolites using gas chromatography/electron ionization/mass spectrometry ranges from 10–50 ng/mL. The lower limit of quantitation (LLOQ) for liquid chromatography/electrospray ionization/tandem mass spectrometry ranges from about 0.1–0.3 ng/mL, whereas the coefficient variation ranges from about 2–5%. Gas chromatography/mass spectrometry methods are available to detect banned stimulants in urine including sibutramine (LOD, 10 ng/mL) that meet the standards of WADA.[140] Using liquid-liquid extraction, liquid chromatography/electrospray ionization/tandem mass spectrometry, and collision-induced dissociation, sibutramine and desmethyl metabolites in urine samples are detectable in the range of 6–40 ng/mL.[141] Sibutramine and related metabolites are not found in saliva with GC/MS, probably because extensive binding of conjugated metabolites to proteins limits diffusion of these compounds into saliva.

Biomarkers

In a study of fasted volunteers, mean peak plasma concentrations of sibutramine and the major metabolite, M2 were 2.98 ± 1.63 ng/mL and 7.65 ± 1.22 ng/mL, respectively.[142] The time to mean peak plasma concentrations were 1.14 ± 0.29 hours and 3.75 ± 1.78 hours, respectively. There are few data on the blood concentrations associated with sibutramine intoxication. Following ingestion of 10 mg sibutramine hydrochloride (8.37 mg free sibutramine), the hydroxylated metabolites are detectable for up to 60 hours as measured by GC/MS (LOD, 10 ng/mL).[136]

Abnormalities

Rare case reports document severe hyponatremia with sibutramine therapy, manifest by headache, nausea, generalized weakness, and confusion.[143] Although the etiology of the hyponatremia was ascribed to syndrome of inappropriate antidiuretic hormone (SIADH), there were no measurements of antidiuretic hormone concentrations. Severe hypoglycemia and coma developed in a patient receiving sibutramine for obesity and lorazepam for insomnia.[144]

TREATMENT

Treatment is supportive.

References

1. Tellier P. Fenfluramines, idiopathic pulmonary primary hypertension and cardiac valve disorders: facts and artifacts. Ann Med Interne (Paris) 2001;152:429–436.
2. Weintraub M, Hasday JD, Mushlin AI, Lockwood DH. A double-blind clinical trial in weight control. Use of fenfluramine and phentermine alone and in combination. Arch Intern Med 1984;144:1143–1148.
3. Khan LK, Serdula MK, Bowman BA, Williamson DF. Use of prescription weight loss pills among U.S. adults in 1996–1998. Ann Intern Med 2001;134:282–286.
4. Brenot F, Herve P, Petitpretz P, Parent F, Duroux P, Simonneau G. Primary pulmonary hypertension and fenfluramine use. Br Heart J 1993;70:537–541.
5. Serdula MK, Mokdad AH, Williamson DF, Galuska DA, Mendlein JM, Heath GW. Prevalence of attempting weight loss and strategies for controlling weight. JAMA 1999;282:1353–1358.
6. Blanck HM, Khan LK, Serdula MK. Prescription weight loss pill use among Americans: patterns of pill use and lessons learned from the fen-phen market withdrawal. Prev Med 2004;39:1243–1248.

7. Fernstrom JD, Choi S. The development of tolerance to drugs that suppress food intake. Pharmacol Ther 2008; 117:105–122.

8. Oswald I, Lewis SA, Dunleavy DL, Brezinova V, Briggs M. Drugs of dependence though not of abuse: fenfluramine and imipramine. Br Med J 1971;3(5766):70–73.

9. Caccitolo JA, Connolly HM, Rubenson DS, Orszulak TA, Schaff HV. Operation for anorexigen-associated valvular heart disease. J Thorac Cardiovasc Surg 2001;122: 656–664.

10. Wellman PJ, Maher TJ. Synergistic interactions between fenfluramine and phentermine. Int J Obes Relat Metab Disord 1999;23:723–732.

11. Gardin JM, Schumacher D, Constantine G, Davis KD, Leung C, Reid CL. Valvular abnormalities and cardiovascular status following exposure to dexfenfluramine or phentermine/fenfluramine. JAMA 2000;283:1703–1709.

12. Jollis JG, Landolfo CK, Kisslo J, Constantine GD, Davis KD, Ryan T. Fenfluramine and phentermine and cardiovascular findings: effect of treatment duration on prevalence of valve abnormalities. Circulation 2000;101: 2071–2077.

13. Davidoff R, McTiernan A, Constantine G, Davis KD, Balady GJ, Mendes LA, et al. Echocardiographic examination of women previously treated with fenfluramine. Long-term follow-up of a randomized, double-blind, placebo-controlled trial. Arch Intern Med 2001;161: 1429–1436.

14. White AG, Beckett AH, Brookes LG. Fenfluramine overdosage. Br Med J 1967;1(5542):740.

15. Veltri JC, Temple AR. Fenfluramine poisoning. J Pediatr 1975;87:119–121.

16. Brownsill R, Wallace D, Taylor A, Campbell B. Study of human urinary metabolism of fenfluramine using gas chromatography-mass spectrometry. J Chromatogr 1991; 562:267–277.

17. Richards RP, Gordon BH, Ings RM, Campbell DB, King LJ. The measurement of *d*-fenfluramine and its metabolite, *d*-norfenfluramine in plasma and urine with an application of the method to pharmacokinetic studies. Xenobiotica 1989;19:547–553.

18. Bruce RB, Maynard WR Jr. Fenfluramine metabolism. J Pharm Sci 1968;57:1173–1176.

19. Caccia S, Conforti I, Duchier J, Garattini S. Pharmacokinetics of fenfluramine and norfenfluramine in volunteers given *d*- and *d*,*l*-fenfluramine for 15 days. Eur J Clin Pharmacol 1985;29:221–224.

20. Beckett AH, Brookes LG. The absorption and urinary excretion in man of fenfluramine and its main metabolite. J Pharm Pharmacol 1967;19(suppl):S41–S52.

21. Darmady JM. Diazepam for fenfluramine intoxication. Arch Dis Child 1974;49:328–330.

22. Carvajal A, Garcia del Pozo J, Martin de Diego I, Rueda de Castro AM, Velasco A. Efficacy of fenfluramine and dexfenfluramine in the treatment of obesity: a meta-analysis. Methods Find Exp Clin Pharmacol 2000;22: 285–290.

23. Weintraub M, Sundaresan PR, Madan M, Schuster B, Balder A, Lasagna L, Cox C. Long-term weight control study. I (weeks 0 to 34). The enhancement of behavior modification, caloric restriction, and exercise by fenfluramine plus phentermine versus placebo. Clin Pharmacol Ther 1992;51:586–594.

24. Khan MA, Herzog CA, St Peter JV, Hartley GG, Madlon-Kay R, Dick CD, et al. The prevalence of cardiac valvular insufficiency assessed by transthoracic echocardiography in obese patients treated with appetite-suppressant drugs. N Engl J Med 1998;339:713–718.

25. Volmar KE, Hutchins GM. Aortic and mitral fenfluramine-phentermine valvulopathy in 64 patients treated with anorectic agents. Arch Pathol Lab Med 2001;125: 1555–1561.

26. McDonald PC, Wilson JE, Gao M, McNeill S, Spinelli JJ, Williams OD, et al. Quantitative analysis of human heart valves does anorexigen exposure produce a distinctive morphological lesion? Cardiovasc Pathol 2002;11: 251–262.

27. Tomita T, Zhao Q. Autopsy findings of heart and lungs in a patient with primary pulmonary hypertension associated with use of fenfluramine and phentermine. Chest 2002;121:649–652.

28. Rogers PJ, Blundell JE. Effect of anorexic drugs on food intake and the micro-structure of eating in human subjects. Psychopharmacology (Berl) 1979;66:159–165.

29. Halford JC, Harrold JA, Boyland EJ, Lawton CL, Blundell JE. Serotonergic drugs effects on appetite expression and use for the treatment of obesity. Drugs 2007;67:27–55.

30. Rothmann RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ, et al. Evidence for possible involvement of 5-HT2B receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medication. Circulation 2000;102:2836–2841.

31. Archer SL, Djaballah K, Humbert M, Weir EK, Fartoukh M, Dall'Ava-Santucci J et al. Nitric oxide deficiency in fenfluramine- and dexfenfluramine-induced pulmonary hypertension. Am J Respir Crit Care Med 1998;158: 1061–1067.

32. Seghatol Ff, Rigolin VH. Appetite suppressants and valvular heart disease. Curr Opin Cardiol 2002;17:486–492.

33. Setola V, Dukat M, Glennon RA, Roth BL. Molecular determinants for the interaction of the valvulopathic anorexigen norfenfluramine with the 5-HT2B receptor. Mol Pharmacol 2005;68:20–33.

34. Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, et al. Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. Mol Pharmacol 2000;57:75–81.

35. Bhattacharyya S, Schapira AH, Mikhailidis DP, Davar J. Drug-induced fibrotic valvular heart disease. Lancet 2009;374:577–585.

36. Fleming RM, Boyd LB. The longitudinal effects of fenfluramine-phentermine use. Angiology 2007;58:353–359.

37. Dahl CF, Allen MR, Urie PM, Hopkins PN. Valvular regurgitation and surgery associated with fenfluramine use: an analysis of 5743 individuals. BMC Med 2008:6:34.

38. Li R, Serdula MK, Williamson DR, Bowman BA, Graham DJ, Green L. Dose-effect of fenfluramine use on the severity of valvular heart disease among fen-phen patients with valvulopathy. Int J Obes 1999;23:926–928.

39. Burger AJ, Charlamb MJ, Singh S, Notarianni M, Blackburn GL, Sherman B. Low risk of significant echocardiographic valvulopathy in patients treated with anorectic drugs. Int J Cardiol 2001;79:159–165.

40. Weissman NJ. Appetite suppressants and valvular heart disease. Am J Med Sci 2001;321:285–291.

41. Loke YK, Derry S, Pritchard-Copley A. Appetite suppressants and valvular heart disease—a systematic review. BMC Clin Pharmacol 2002;2:6.

42. Hopkins PN, Polukoff GI. Risk of valvular heart disease associated with use of fenfluramine. BMC Cardiovasc Disord 2003;3:5.

43. Palmieri V, Arnett DK, Roman MJ, Liu JE, Bella JN, Oberman A, et al. Appetite suppressants and valvular heart disease in a population-based sample: the HyperGEN Study. Am J Med 2002;112:710–715.

44. Hensrud DD, Connolly HM, Grogan M, Miller FA, Bailey KR, Jensen MD. Echocardiographic improvement over time after cessation of use of fenfluramine and phentermine. Mayo Clin Proc 1999;74:1191–1197.

45. Vagelos R, Jacobs M, Popp RL, Liang D. Reversal of phen-fen associated valvular regurgitation documented by serial echocardiography. J Am Soc Echocardiogr 2002;15:653–657.

46. Mast ST, Jollis JG, Ryan T, Anstrom KJ, Crary JL. The progression of fenfluramine-associated valvular heart disease assessed by echocardiography. Ann Intern Med 2001;134:261–266.

47. Greffe G, Chalabreysse L, Mouly-Bertin C, Lantelme P, Thivolet F, Aulangner G, Obadia J-F. Valvular heart disease associated with fenfluramine detected 7 years after discontinuation of treatment. Ann Thorac Surg 2007;82:1541–1543.

48. Abenhaim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. N Engl J Med 1996;335:609–616.

49. Thomas SH, Butt AY, Corris PA, Egan JJ, Higenbottam TW, Madden BP, Waller PC. Appetite suppressants and primary pulmonary hypertension in the United Kingdom. Br Heart J 1994;74:660–663.

50. Souza R, Humbert M, Sztrymf B, Jais X, Yaici A, Le Pavec J, et al. Pulmonary arterial hypertension associated with fenfluramine exposure: a report of 109 cases. Eur Respir J 2008;31:343–348.

51. Walker AM, Langleben D, Korelitz JJ, Rich S, Rubin LJ, Strom BL, et al. Temporal trends and drug exposures in pulmonary hypertension: an American experience. Am Heart J 2006;152:521–526.

52. Haines AP, Shoenberg PJ. Hyperpyrexia and overdose. Br Med J 1972; 1(5800):632–633.

53. von Muhlendahl KE, Krienke EG. Fenfluramine poisoning. Clin Toxicol 1979;14:97–106.

54. Steel JM, Briggs M. Withdrawal depression in obese patients after fenfluramine treatment. Br Med J 1972;3:26–27.

55. Jones KL, Johnson KA, Dick LM, Felix RJ, Kao KK, Chambers CD. Pregnancy outcomes after first trimester exposure to phentermine/fenfluramine. Teratology 2002;65:125–130.

56. Morris RG, Reece PA. Improved gas-liquid chromatographic method for measuring fenfluramine and norfenfluramine in heparinised plasma. J Chromatogr 1983;278:434–438.

57. Gross AS, Phillips AC, Boutagy J, Shenfield GM. Determination of dexfenfluramine and nordexfenfluramine in urine by high-performance liquid chromatography using ultraviolet detection. J Chromatogr 1993;621:115–120.

58. Kaddoumi A, Kubota A, Nakashima MN, Takahashi M, Nakashima K. High performance liquid chromatography with UV detection for the simultaneous determination of sympathomimetic amines using 4-(4,5-diphenyl-1*H*-imidazole-2-yl)benzoyl chloride as a label. Biomed Chromatogr 2001;15:379–388.

59. Palmer RB, Kim NH, Dasgupta A. Simultaneous determination of fenfluramine and phentermine in urine using gas chromatography mass spectrometry with pentafluoropropionic anhydride derivatization. Ther Drug Monit 2000;22:418–422.

60. Namera A, Yashiki M, Liu J, Okajima K, Hara K, Imamura T, Kojima T. Simple and simultaneous analysis of fenfluramine, amphetamine and methamphetamine in whole blood by gas chromatography-mass spectrometry after headspace-solid phase microextraction and derivatization. Forensic Sci Int 2000;109:215–223.

61. Kaddoumi A, Nakashima MN, Nakashima K. Fluorometric determination of DL-fenfluramine, DL-norfenfluramine and phentermine in plasma by achiral and chiral high-performance liquid chromatography. J Chromatogr B 2001;763:79–90.

62. Kim JY, Jung KS, Kim MK, Lee JL, In MK. Simultaneous determination of psychotropic phenylalkylamine derivatives in human hair by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 2007;21:1705–1720.

63. Campbell DB, Turner P. Plasma concentrations of fenfluramine and its metabolite, norfenfluramine, after single and repeated oral administration. Br J Pharmacol 1971;43:465P–466P.

64. Innes JA, Watson ML, Ford MJ, Munro JF, Stoddart ME, Campbell DB. Plasma fenfluramine levels, weight loss, and side effects. Br Med J 1977;2:1322–1325.

65. Fleisher MR, Campbell DB. Fenfluramine overdosage. Lancet 1969;2(7633):1306–1307.

66. Gold RG, Gordon HE, Da Costa RW, Porteous IB, Kimber KJ. Fenfluramine overdosage. Lancet 1969; 2(7633):1306.

67. Kintz P, Mangin P. Toxicological findings after fatal fenfluramine self-poisoning. Hum Exp Toxicol 1992;11: 51–52.

68. Roldan CA, Gelgand EA, Decker P, Prasad A, Shively BK. Morphology of anorexigen-associated valve disease by transthoracic and transesophageal echocardiography. Am J Cardiol 2002;90:1269–1273.

69. LoVecchio F, Curry SC. Dexfenfluramine overdose. Ann Emerg Med 1998;32:102–103.

70. Cheymol G, Weissenburger J, Poirier JM, Gellee C. The pharmacokinetics of dexfenfluramine in obese and nonobese subjects. Br J Clin Pharmacol 1995;39:684–687.

71. Marchant NC, Breen MA, Wallace D, Bass S, Taylor AR, Ings RM, et al. Comparative biodisposition and metabolism of ^{14}C-(+/−)-fenfluramine in mouse, rat, dog and man. Xenobiotica 1992;22:1251–1266.

72. Gross AS, Phillips AC, Rieutord A, Shenfield GM. The influence of the sparteine/debrisoquine genetic polymorphism on the disposition of dexfenfluramine. Br J Clin Pharmacol 1996;41:311–317.

73. McGuirk J, Goodall E, Silverstone T, Willner P. Differential effects of *d*-fenfluramine, *l*-fenfluramine and *d*-amphetamine on the microstructure of human eating behaviour. Behav Pharmacol 1991;2:113–119.

74. Neill JC, Cooper SJ. Evidence that *d*-fenfluramine anorexia is mediated by 5-HT1 receptors. Psychopharmacology (Berl) 1989;97:213–218.

75. Vickers SP, Dourish CT, Kennett GA. Evidence that hypophagia induced by *d*-fenfluramine and *d*-norfenfluramine in the rat is mediated by 5-HT2C receptors. Neuropharmacology 2001;41:200–209.

76. Rothman RB, Ayestas MA, Dersch CM, Baumann MH. Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates. Implications for primary pulmonary hypertension. Circulation 1999;100:869–875.

77. Hong Z, Olschewski A, Reeve HL, Nelson DP, Hong F, Weir EK. Nordexfenfluramine causes more severe pulmonary vasoconstriction than dexfenfluramine. Am J Physiol Lung Cell Mol Physiol 2004;286:L531–L538.

78. Davis R, Faulds D. Dexfenfluramine. An updated review of its therapeutic use in the management of obesity. Drugs 1996;52:696–724.

79. Ko GT, Chan HC, Chow CC. Dexfenfluramine and heart-valve regurgitation in Chinese patients with type 2 diabetes. Hong Kong Med J 2003;9:243–246.

80. Weissman NJ, Tighe JF Jr, Gottdiener JS, Gwynne JT. An assessment of heart-valve abnormalities in obese patients taking dexfenfluramine, sustained-release dexfenflura-mine, or placebo. Sustained-Release Dexfenfluramine Study Group. N Engl J Med 1998;339:725–732.

81. Sachdev M, Miller WC, Ryan T, Jollis MG. Effect of fenfluramine-derivative diet pills on cardiac valves: a meta-analysis of observational studies. Am Heart J 2002;144:1065–1071.

82. Roldan CA, Gill EA, Shively BK. Prevalence and diagnostic value of precordial murmurs for valvular regurgitation in obese patients treated with dexfenfluramine. Am J Cardiol 2000;86:535–539.

83. Gardin JM, Weissman NJ, Leung C, Panza JA, Fernicola D, Davis KD, et al. Clinical echocardiographic follow-up of patients previously treated with dexfenfluramine or phentermine/fenfluramine. JAMA 2001;286:2011–2014.

84. Weissman NJ, Panza JA, Tighe JF Jr, Gwynne JT. Natural history of valvular regurgitation 1 year after discontinuation of dexfenfluramine therapy a randomized, double-blind, placebo-controlled trial. Ann Intern Med 2001; 134:267–273.

85. Weissman NJ, Tighe JF Fr, Gottdiener JS, Gwynne JT. Prevalence of valvular-regurgitation associated with dexfenfluramine three to five months after discontinuation of treatment. J Am Coll Cardiol 1999;34: 2088–2095.

86. Aurigemma GP, Ronen A, Cuenoud H, Folland ED, Meyer TE. Severe mitral valve regurgitation associated with dexfenfluramine use. Cardiology 2002;98:215–217.

87. Genné D, De Torrenté A, Humair L. [Voluntary poisoning with dexfenfluramine (Isomeride)]. Schweiz Med Wochenschr 1994;124:2217–2219. [French]

88. Saletu B, Barbanoj MJ, Anderer P, Sieghart W, Grunberger J. Clinical-pharmacological study with the two isomers (*d*-, *l*-) of fenfluramine and its comparison with chlorpromazine and *d*-amphetamine: blood levels, EEG mapping and safety evaluation. Meth Find Exp Clin Pharmacol 1993;15:291–312.

89. Buckett WR, Thomas PC, Luscombe GP. The pharmacology of sibutramine hydrochloride (BTS 54 524), a new antidepressant which induces rapid noradrenergic down-regulation. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:575–584.

90. James WP, Caterson ID, Coutinho W, Finer N, Van Gaal LF, Maggioni AP, et al; SCOUT Investigators. Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. N Engl J Med 2010;363:905–917.

91. Rolls BJ, Shide DJ, Thorwart ML, Ulbrecht JS. Sibutramine reduces food intake in non-dieting women with obesity. Obes Res 1998;6:1–11.

92. Vidal C, Quandte S. Identification of a sibutramine-metabolite in patient urine after intake of a "pure herbal" Chinese slimming product. Ther Drug Monit 2006;28: 690–692.

93. Jung J, Hermanns-Clausen M, Weinmann W. Anorectic sibutramine detected in a Chinese herbal drug for weight loss. Forensic Sci Int 2006;161:221–222.

94. Schuh LM, Schuster CR, Hopper JA, Mendel CM. Abuse liability assessment of sibutramine, a novel weight control agent. Psychopharmacology 2000;147:339–346.

95. Li Z, Maglione M, Tu W, Mojica W, Arterburn D, Shugarman LR, et al. Meta-analysis: pharmacologic treatment of obesity. Ann Intern Med 2005;142:532–546.

96. Bae SK, Cao S, Seo K-A, Kim H, Kim M-J, Shon J-H, et al. Cytochrome P450 2B6 catalyzes the formation of pharmacologically active sibutramine (*N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N*,*N*-dimethylamine) metabolites in human liver microsomes. Drug Metab Disp 2008;36:1679–1688.

97. Hind ID, Mangham JE, Ghani SP, Haddock RE, Garratt CJ, Jones RW. Sibutramine pharmacokinetics in young and elderly healthy subjects. Eur J Clin Pharmacol 1999;54:847–849.

98. Link M, Hakala KS, Wsol V, Kostiainen R, Ketola RA. Metabolite profile of sibutramine in human urine: a liquid chromatography-electrospray ionization mass spectrometric study. J Mass Spectrom 2006;41:1171–1178.

99. Barkeling B, Elfhag K, Rooth P, Rössner S. Short-term effects of sibutramine (Reductil) on appetite and eating behaviour and the long-term therapeutic outcome. Int J Obes Relat Metab Disord 2003;27:693–700.

100. James WP, Astrup A, Finer N, Hilsted J, Kopelman P, Rössner S, et al. Effect of sibutramine on weight maintenance after weight loss: a randomised trial. STORM Study Group. Sibutramine Trial of Obesity Reduction and Maintenance. Lancet 2000;356(9248):2119–2125.

101. Giese SY, Neborsky R. Serotonin syndrome: potential consequences of Meridia combined with Demerol or fentanyl. Plast Reconstr Surg 2001;107:293–294.

102. Lechin F, van der Dijs B, Hernandez G, Orozco B, Rodriguez S, Baez S. Neurochemical, neuroautonomic and neuropharmacological acute effects of sibutramine in healthy subjects. Neurotoxicology 2006;27:184–191.

103. Rowley HL, Butler SA, Prow MR, Dykes SG, Aspley S, Kilpatrick IC, Heal DJ. Comparison of the effects of sibutramine and other weight-modifying drugs on extracellular dopamine in the nucleus accumbens of freely moving rats. Synapse 2000;38:167–176.

104. Jackson HC, Bearham MC, Hutchins LJ, Mazurkiewicz SE, Needham AM, Heal DJ. Investigation of the mechanisms underlying the hypophagic effects of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in the rat. Br J Pharmacol 1997;121:1613–1618.

105. Liu YL, Heal DJ, Stock MJ. Mechanism of the thermogenic effect of Metabolite 2 (BTS 54 505), a major pharmacologically active metabolite of the novel anti-obesity drug, sibutramine. Int J Obes Relat Metab Disord 2002;26:1245–1253.

106. Connoley IP, Liu YL, Frost I, Reckless IP, Heal DJ, Stock MJ. Thermogenic effects of sibutramine and its metabolites. Br J Pharmacol 1999;126:1487–1495.

107. Heal DJ, Aspley S, Prow MR, Jackson HC, Martin KF, Cheetham SC. Sibutramine: a novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from *d*-amphetamine and *d*-fenfluramine. Int J Obes Relat Metab Disord 1998;22(Suppl 1):S18–S28.

108. Coutinho WF. The obese older female patient: CV risk and the SCOUT study. Int J Obes 2007;31(Suppl 2):S26–S30.

109. Sramek JJ, Leibowitz MT, Weinstein SP, Rowe ED, Mendel CM, Levy B, et al. Efficacy and safety of sibutramine for weight loss in obese patients with hypertension well controlled by beta-adrenergic blocking agents: a placebo-controlled, double-blind, randomized trial. J Hum Hypertens 2002;16:13–19.

110. Maggioni AP, Caterson I, Coutinho W, Finer N, van Gaal L, Sharma AM, et al. Tolerability of sibutramine during a 6-week treatment period in high-risk patients with cardiovascular disease and/or diabetes: a preliminary analysis of the Sibutramine Cardiovascular outcomes (SCOUT) Trial. J Cardiovasc Pharmacol 2008;52:393–402,

111. Padwal RS, Majumdar SR. Drug treatments for obesity: orlistat, sibutramine, and rimonabant. Lancet 2007;369(9555):71–77.

112. Minns AB, Ebrahimi S, Te L, Ly B, Clark RF, Cantrell FL. A retrospective review of California poison control system data of sibutramine exposures. Clin Toxicol 2009;47:814–817.

113. Bach DS, Rissanen AM, Mendel CM, Shepherd G, Weinstein SP, Kelly F, et al. Absence of cardiac valve dysfunction in obese patients treated with sibutramine. Obes Res 1999;7:363–369.

114. Guven A, Koksal N, Cetinkaya A, Sokmen G, Ozdemir R. Effects of the sibutramine therapy on pulmonary artery pressure in obese patients. Diabetes Obes Metab 2004;6:50–55.

115. Harrison-Woolrych M, Clark DW, Hill GR, Rees MI, Skinner JR. QT interval prolongation associated with sibutramine treatment. Br J Clin Pharmacol 2006;61:464–469.

116. Yim K-M, Ng HW, Chan C-K, Yip G, Lau FL. Sibutramine-induced acute myocardial infarction in a young lady. Clin Toxicol 2008;46:877–879.

117. Ernest D, Gershenzon A, Corallo CE, Nagappan R. Sibutramine-associated QT interval prolongation and cardiac arrest. Ann Pharmacother 2008;42:1514–1517.

118. Sayin T, Güldal M. Sibutramine: possible cause of a reversible cardiomyopathy. Int J Cardiol 2005;99:481–482.

119. Coulter DM. The New Zealand intensive medicines monitoring programme in pro-active safety surveillance. Pharmacoepidemiol Drug Saf 2000;9:273–280.

120. Harrison-Woolrych M, Clark DW, Hill GR, Rees MI, Skinner JR. QT interval prolongation associated with sibutramine treatment. Br J Clin Pharmacol 2006;61:464–469.

121. Daniels SR, Long B, Crow S, Styne D, Sothern M, Vargas-Rodriguez I, et al. Cardiovascular effects of sibutramine in the treatment of obese adolescents: results of a randomized, double-blind, placebo-controlled study. Pediatrics 2007;120:e147–e157.

122. Fernández P, Peiró AM. A sibutramine-induced delusional disorder relapse. J Neuropsychiatry Clin Neurosci 2007;19:88–89.

123. Dogangun B, Bolat N, Rustamov I, Kayaalp L. Sibutramine-induced psychotic episode in an adolescent. J Psychosom Res 2008;65:505–506.

124. Binkley K, Knowles SR. Sibutramine and panic attacks. Am J Psychiatry 2002;159:1793–1794.

125. Naik S, Khoo CL, Lua r, Chai SB, Liew A, Sim K. Recurrent episodes of brief affective psychosis induced by sibutramine. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:1359–1360.

126. Cordeiro Q, Vallada H. Sibutramine-induced mania episode in a bipolar patient. Int J Neuropsychopharmacol 2002;5:283–284.

127. Litvan L, Alcoverro-Fortuny O. Sibutramine and psychosis. J Clin Psychopharmacol 2007;27:726–727.

128. Rosenbohm A, Bux CJ, Connemann BJ. Psychosis with sibutramine. J Clin Psychopharmacol 2007;27:315–317.

129. Lee J, Teoh T, Lee T-S. Catatonia and psychosis associated with sibutramine: a case report and pathophysiologic correlation. J Psychosom Res 2008;64:107–109.

130. Bucaretchi F, De Capitani EM, Mello SM, Lanaro R, Barros RF, Fernandes LC, et al. Serotonin syndrome following sibutramine poisoning in a child, with sequential quantification of sibutramine and its primary and secondary amine metabolites in plasma. Clin Toxicol 2009; 47:598–601.

131. Kadioglu M, Ulku C, Yaris F, Kesim M, Kalyoncu NI, Yaris E. Sibutramine use in pregnancy: report of two cases. Birth Defects Res A Clin Mol Teratol 2004; 70:545–546.

132. Einarson A, Bonari L, Sarkar M, McKenna K, Koren G. Exposure to sibutramine during pregnancy: a case series. Eur J Obstet Gynecol Reprod Biol 2004;116:112.

133. De Santis M, Straface G, Cavaliere AF, Carducci B, Caruso A. Early first-trimester sibutramine exposure: pregnancy outcome and neonatal follow-up. Drug Saf 2006;29:255–259.

134. Hind ID, Mangham JE, Ghani SP, Haddock RE, Garratt CJ, Jones RW. Sibutramine pharmacokinetics in young and elderly healthy subjects. Eur J Clin Pharmacol 1999; 54:847–849.

135. Link M, Novotná R, Suchanová B, Skálová L, Wsól V, Szotáková B. The stereoselective biotransformation of the anti-obesity drug sibutramine in rat liver microsomes and in primary cultures of rat hepatocytes. J Pharm Pharmacol 2005;57:405–410.

136. Strano-Rossi S, Colamonici C, Botre F. Detection of sibutramine administration: a gas chromatography/mass spectrometry study of the main urinary metabolites. Rapid Commun Mass Spectrom 2007;21:79–88.

137. Jain DS, Subbaiah G, Sanyal M, Shrivastav PS, Pal U, Ghataliya S, et al. Liquid chromatography/electrospray ionization tandem mass spectrometry validate method for simultaneous quantification of sibutramine and its primary and secondary amine metabolites in human plasma and its application to a bioequivalence study. Rapid Commun Mass Spectrom 2006;20:3509–3521.

138. Chen J, Lu W, Zhang Q, Jiang X. Determination of the active metabolite of sibutramine by liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003;785:197–203.

139. Kang W, Bae K, Noh K. Enantioselective determination of sibutramine and its active metabolites in human plasma. J Pharm Biomed Anal 2010;51:264–267.

140. Strano-Rossi S, Colamonici C, Botre F. Parallel analysis of stimulants in saliva and urine by gas chromatography/ mass spectrometry: perspectives for "in competition" anti-doping analysis. Anal Chim Acta 2008;606:217–222.

141. Thevis M, Sigmund G, Schiffer A-K, Schanzer W. Determination of *N*-desmethyl- and *N*-bidesmethyl metabolites of sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry. Eur J Mass Spectrom 2006;12:129–136.

142. Abolfathi Z, Couture J, Vallée F, LeBel M, Tanguay M, Masson E. A pilot study to evaluate the pharmacokinetics of sibutramine in healthy subjects under fasting and fed conditions. J Pharm Pharm Sci 2004;7:345–349.

143. Esposito P, Rampino T, Gregorini M, Soccio G, Piotti G, Bedino G, et al. Severe symptomatic hyponatremia during sibutramine therapy: a case report. Am J Kidney Dis 2008;52:137–139.

144. Lin Y-Y, Hsu C-W, Chu S-J, Tsai S-H. Another dangerous combination for hypoglycemic coma: concurrent use of sibutramine and lorazepam. QJ Med 2008;101:243–244.

Chapter 15

ANABOLIC-ANDROGENIC STEROIDS

HISTORY

The use of drugs to enhance physical performance has occurred since the beginning of recorded time. Ancient Greeks ate mushrooms and sesame seeds to enhance performance, and Roman gladiators used stimulants to increase endurance.[1] In modern sports, documentation of the abuse of performance-enhancing drugs appeared in the early 1900s, when athletes ingested stimulants (cocaine, amphetamines, ephedrine, strychnine) to alleviate fatigue and increase focus.[2] Anabolic-androgenic steroids (AASs) are now the most common illicit drugs used to enhance performance at the modern Olympic Games along with stimulants, primarily by weightlifters and athletes in track and field.[3] The AASs are a group of synthetic derivatives of testosterone with both skeletal muscle-building (anabolic) and masculinizing (androgenic) effects. In 1889, physiologist Charles E. Brown-Sequard reported improvement in a variety of his body functions (strength, intellect, force of urine stream) following the injection of an extract of testicles from the dog and guinea pig. The primary natural male hormone, testosterone was first isolated from the testis of bulls in 1935 by David et al.[4] Butenandt[5] and Ruzicka[6] independently synthesized testosterone in the same year, and both chemists received the Nobel Prize in 1939 for their work. Most of the AASs were developed during the 1950s when chemists attempted unsuccessfully to separate the anabolic and androgenic properties of these testosterone derivatives.[7] Nandrolone, the 19-nor analog of testosterone was the first anabolic steroid with sufficient dissociation of androgenic and anabolic prop-

erties to justify introduction into clinical practice during the 1950s.[8] Dr. John Ziegler, an American physician-weightlifter, administered AASs to 3 future American weightlifting champions after learning of the success of AAS-using Russian weightlifters at the 1954 World Championships.[9] In 1958, the US Food and Drug Administration (FDA) approved the use of methandrostenolone (Dianabol) for the treatment of hypogonadism, resulting in the increased availability of this steroid. By the mid-1960s, the use of AASs to enhance performance in sports spread, particularly among weightlifters and other strength athletes. An estimated one-third of the US Track and Field athletes in the 1968 pre-Olympic training camp were using AAS.[10] From 1966 until the collapse of the German Democratic Republic (GDR) in 1990, hundreds of East German physicians and scientists performed doping research and administered prescription drugs as well as unapproved experimental drug preparations to adult and adolescent athletes of both sexes.[11]

In 1963, the Council of Europe defined doping in sports as a result of the death of a Danish cyclist at the 1960 Olympics, the death of a UK cyclist at the Tour de France, and the prevalence of potentially life-threatening drugs in sports.[2] In 1964, the International Olympic Committee (IOC) unanimously voted to ban doping in sports. By 1967, the IOC established a Medical Commission with responsibilities to prohibit doping, to develop the Olympic Movement Anti-Doping Code, and to formulate a list of prohibited substances. In 1974, the IOC banned the use of AASs and testing for AASs by immunoassay screening and gas chromatography/

mass spectrometry confirmation began in 1976.[12] In 1984, the use of testosterone was also banned. From the 1960s through the 1980s, the German Democratic Republic established a systematic doping program for thousands of their athletes that included the use of parenteral preparations of epitestosterone propionate to avoid detection of illicit AASs.[11]

In 1988, the IOC stripped Ben Johnson of his Olympic gold medal and world record in the 100-meter dash for using an AAS. In the same year, the distribution or possession of AASs with intent to distribute without a valid prescription became a felony, when US Federal Food, Drug and Cosmetic Act (FFDCA) was amended as part of the Anti-Drug Abuse Act. In 1990, the Anabolic Steroids Control Act defined an AAS as any drug or hormonal substance chemically and pharmacologically related to testosterone (other than estrogens, progestins, and corticosteroids) that promotes muscle growth. These synthetic compounds became DEA schedule III drugs as defined by the US Controlled Substances Act. Later, this act was amended by the Anabolic Steroid Control Act of 2004; on January 20, 2005, the amended Controlled Substance Act added both anabolic steroids and prohormones to the list of controlled substances, making possession of the banned substances a federal crime. In response to continuing demand for illicit AASs, designer AAS appeared as a means to avoid detection of these illicit drugs. An example was the synthesis of tetrahydrogestrinone from the palladium-charcoal catalyzed hydrogenation of gestrinone by the Bay Area Laboratory Cooperative, an American nutritional supplement company. However, analyses and legal action resulted in the banning of several athletes as a result of the use of these synthetic steroids. Subsequently, major league baseball revamped their AAS policy calling for a 50-game ban for first-time offenders (up from 10 days), a 100-game penalty for second-time offenders (up from 30 days), and a lifetime ban for a third positive test. Previously, a baseball player could be suspended for life only after the fifth positive test.

IDENTIFYING CHARACTERISTICS

Steroids are organic molecules with a tetracyclic ring system; all steroids with the exception of retinoic acid are derived from cholesterol. There are 4 major classes of natural steroid hormones (androgens, corticoids, estrogens, progestogens) with testosterone being the principal male androgenic steroid. AASs are synthetic compounds similar in chemical structure to testosterone (MW 288 g/mol). Figure 15.1 displays the chemical structures of common AASs. There are 3 major classes of AASs (oral, injectable oil-based, injectable water-
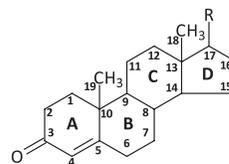
Compound	R
Testosterone	—OH
Testosterone Propionate	—O—OCOCH$_2$CH$_3$
Testosterone Enantate	—O—CO(CH$_2$)$_5$CH$_3$
Testosterone Cipionate	—O—COCH$_2$CH$_2$— (cyclopentyl)
Nandrolone Decanoate	—O—CO(CH$_2$)$_8$CH$_3$ *(no methyl group at position 19)*
Nandrolone Phenpropionate	—O—CO(CH$_2$)$_2$— (phenyl) *(no methyl group at position 19)*
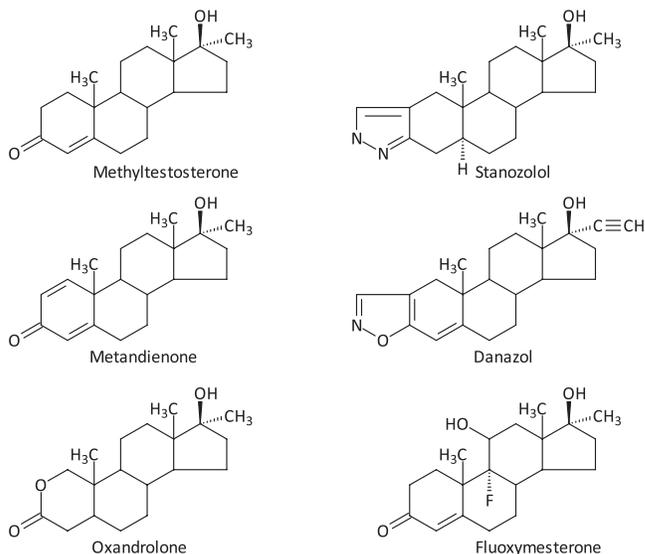
FIGURE 15.1. Chemical structures of some common anabolic-androgenic steroids including testosterone. The compounds in the upper table are for parenteral administration, whereas the 6 compounds in the lower portion of the figure are for oral use. The 4 rings of the testosterone molecule are labeled A–D as indicated in the structure at the top.[122]

based) and at least 30 anabolic-androgenic steroid compounds. Table 15.1 lists the common oral and injectable anabolic-androgenic steroids. Abuse of other forms of AASs includes the use of buccal (Striant), sublingual (tetrahydrogestrinone), and transdermal (testosterone cream) preparations; these short-acting preparations

are typically testosterone-based. The advantage of the buccal, sublingual, and transdermal preparations is the rapid clearance within 1 week after even large doses compared with 2–14 days for oral preparations and 4 weeks for water-soluble parenteral preparations. Nonsteroidal selective androgen receptor modulators (SARMs) are experimental substances (e.g., bicyclic hydantoin, analogues of aryl propionamide, quinoline, tetrahydroquinoline) that may offer better dissociation of the biologic and anabolic effects of steroids; although these substances are not routinely available, the World Anti-Doping Agency (WADA) added these substances to the prohibited list in 2008. Up-to-date information on the list of banned substances is available on the WADA Web site (http://www.wada-ama.org/en/).

TABLE 15.1. Common Oral and Injectable Anabolic-Androgenic Steroids.

Oral AAS	Injectable AAS
Ethylestrenol	Boldenone undecylenate
Fluoxymesterone	Clostebol
Mesterolone	Drostanolone propionate
Metandienone	Isocaproate, decanoate
Metenolone	Methenolone enanthate
Methandrostenolone	Nandrolone decanoate
Methyltestosterone	Nandrolone phenpropionate
Methenolone acetate	Testosterone cypionate
Mibolerone	Testosterone enanthate
Norethandrolone	Testosterone propionate
Oxandrolone	Trenbolone*
Oxymetholone	Trenbolone acetate†
Stanozolol†	
Testosterone undecanoate	

*Veterinary steroid.
†Intramuscular use less common.

Testosterone (CAS RN: 58-22-0, 17-beta-hydroxyandrost-4-en-3-one) is the prototype for all AASs. Figure 15.2 displays modifications of the basic testosterone structure. Modification of testosterone during the synthesis of AASs involves the following three methods: alkylation of the 17β-hydroxyl group, esterification at the 17α-position, or modification of the steroid nucleus to enhance anabolic properties. Although all currently available anabolic steroids have androgenic properties, the anabolic properties are greater for synthetic AASs than for testosterone. Some structural modifications improve bioavailability and prolong the duration of action, whereas other modifications reduce anabolic effects while enhancing androgenic effects. Esterification at the 17β-hydroxy position increases lipophilic and androgenic properties while improving intramuscular bioavailability. In general, alkylation of the 17α-hydroxy position retards hepatic degradation and improves oral bioavailability, but these compounds typically are less potent than 17β-esters.[13] Weaker formulations of AASs are often marketed as prohormones in dietary supplements, particularly dehydroepiandrosterone (DHEA), 19-norandrostenediol, androstenedione, 19-norandrostenedione, 1-testosterone, and prostanozol.[14] The lack of the 17α-alkyl moiety results in extensive first-pass metabolism, and the anabolic effect of DHEA and androstenedione are limited by their weak binding to the androgen receptor despite some conversion to testosterone. The effect of these AASs are substantially greater on women than men because of the relatively larger increase in testosterone in the former. Tetrahydrogestrinone (THG; 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4,9,11-trien-3-one), norbolethone (CAS RN: 1235-15-0; 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-3-one) and madol
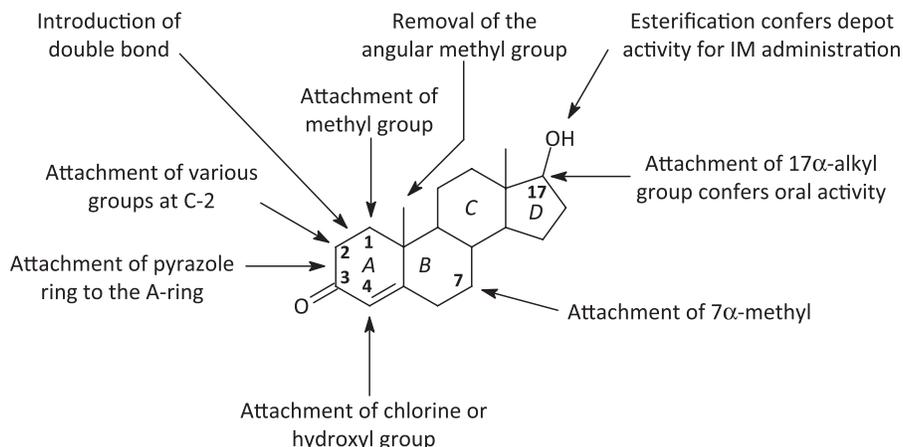
FIGURE 15.2. Basic structure of testosterone with an overview of structure–activity relationships.[51]

(17 α-methyl-5α-androst-2-en-17β-ol) are designer anabolic steroids, which were synthesized to avoid detection during use.[15,16] However, THG is a nonspecific androgen agonist that binds many steroid hormone receptors, particularly the glucocorticoid receptors.[17]

EXPOSURE

Epidemiology

Data on the actual prevalence of AAS use are limited by recall and reporting biases associated with illicit drug use. Many studies of AASs use focus on adolescents. In a nationwide study of 3,403 12th-grade male students in 46 private and public US high schools during the 1980s, 6.6% of the respondents admitted to ever using AASs based on their response to questionnaires.[18] The individual participation rate was low (50.3%). Almost 40% of the respondents admitted to the administration of 5 or more cycles of AASs. Further studies performed at local and statewide levels have confirmed similar findings indicating that 3–12% of high school male adolescents use AASs at some time in their lives. These studies indicate that about one-third of the high school students use AASs for appearance rather than athletic performance. In 1999, the National Institute on Drug Abuse Monitoring the Future study indicated that approximately 2.7% of US 8th and 10th graders and 2.9% of 12th graders admitted to the use of AASs at least once.[19] For 10th graders, the prevalence of AAS use increased to 2.7% in 1999 from 2.0% in 1998. For all 3 grades, the 1999 levels represented a significant increase from 1991, when 1.9% of 8th graders, 1.8% of 10th graders, and 2.1% of 12th graders admitted AAS use at least once. The data on adolescent use of AAS in 2004 and 1999 were similar with 2.5% of 12th graders reporting the use of AASs at least once.[20] Risk factors for the use of AASs among adolescents include male sex, participation in strength-related sports, and use of other illicit drugs.[21] In college and professional athletics, the prevalence of AAS abuse is less defined because of the ramification of AAS use. However, the prevalence of AAS use among these groups is probably substantially higher than high school students, particularly in football players and male track and field athletes.[22] Between 1972 and 2000, there were 29 athletes disciplined for positive AAS drug tests.[3] However, the abuse of AASs is probably more widespread among these athletes because of the difficulty detecting all AAS use and the small number of doping charges compared with the number of positive drug tests. Current trends suggest that most AAS abusers are nonathletes using these drugs for cosmetic purposes.[23] The most common steroids involved with doping are testosterone and nandrolone based on WADA data[24] and retrospective autopsy data.[25] In 2006, 34 WADA-accredited laboratories analyzed almost 200,000 urine samples with positive findings in approximately 2%.[26] Of the positive samples, AASs accounted for 45% of the adverse findings with the most common AASs being testosterone, nandrolone, stanozolol, and methandienone.

Sources

Anabolic-androgenic steroids were developed to treat hypogonadism, a condition in which the testes do not produce sufficient testosterone for normal growth, development, and sexual functioning. Clinical studies suggest that the use of AASs (e.g., nandrolone) or testosterone improves lean body mass in patients with mild to moderate cachexia secondary to chronic illness (e.g., human immunodeficiency virus [HIV]).[27] The use of testosterone and AAS has not been effective for the treatment of the catabolic states associated with severe burns or muscle-wasting diseases. Historical uses of AASs include anemia, hereditary angioedema, metastatic breast cancer, protein deficiencies following trauma or severe infections, and certain psychiatric disorders (involutional psychoses, depression).[28]

Origin

In the United States, AASs are DEA schedule III drugs of the Controlled Substances Act. The primary sources for illicit AASs in the United States are manufacturing facilities in Mexico.[23] Traffickers from the United States enter Mexico and purchase AASs at pharmacies in Baja, California, where AASs are available by prescription. They subsequently smuggle the AASs back into the United States. Other sources of illicit AAS in the United States include Russia, Poland, Hungary, Spain, Italy, Greece, Canada, and the Netherlands as well as some clandestine labs in the United States. The diversion of legitimate pharmaceutic preparations provides a much smaller portion (i.e., 10–15%) of illicit AASs compared with other illicit sources.[29] Information on the administration and procurement of AASs is widely available on the Internet.[30]

Impurities

There are few data on the purity of illicit samples of AASs as a result of the lack of regulation. Consequently, there are no assurances that the chronic AAS abuser knows the dose or type of AAS. The difficulty determining doses used by AAS abusers limits the ability of

studies to elucidate the effect of AAS abuse. Frequently, illicit samples of AASs do not contain declared ingredients or concentrations of ingredients. Analysis of 70 products confiscated from illegal sources demonstrated 17 (35.4%) of the 48 steroidal compounds did not contain labeled ingredients as measured by liquid chromatography/tandem mass spectrometry, gas chromatography/mass spectrometry with nitrogen phosphorus detection, gel electrophoresis, and immunological tests.[31] Visual inspection did not distinguish original products from counterfeits.

Methods of Abuse

The pattern of AAS abuse among athletes is quite variable, and the dosing intervals are not usually regular. These patterns include stacking, tapering, plateauing, cycling, and pyramiding. Many AAS abusers are polydrug users including the abuse of traditional recreational drugs and misuse of prescription drugs.[32] Athletes typically administer AAS intramuscularly (IM) with or without oral preparations in cycles lasting 6–12 weeks with periods of abstinence between these cycles as a means to reduce adverse effects.[29] The abuse of transdermal patches, sublingual tablets, dermatologic gels, and nasal sprays is rare. In an attempt to maximize anabolic gains and minimize side effects, some AAS users start with low doses at the beginning of a cycle and then steadily increase the dose until a gradual "tapering" phase at the end of a cycle (i.e., "pyramid" regimen). Frequently, these athletes use more than 1 steroid simultaneously (i.e., "stacking") or use several AASs in overlapping patterns to avoid the development of tolerance (i.e., "plateauing"). In an Internet study of 207 weightlifters and bodybuilders using AASs, the steroid regimens included a mean of 3.1 agents with cycles ranging from 5–10 weeks and AAS doses 5–29 times above physiologic replacement doses.[33] Often these athletes ingest other drugs (i.e., "array") as a means to reduce adverse effects and enhance the effect of AASs. These additional medications include human chorionic gonadotrophin, anti-acne drugs, oral hypoglycemic agents, analgesics, ketoconazole shampoo, stimulant amino acids, erythropoietin, aminoglutethimide, diuretics, and estrogen antagonists.[34] The abuse of AASs continues, in part, because of the effectiveness of these regimens with training programs and the perceived difficulty detecting AAS use, particularly in weightlifters.[35] Commonly abused steroid supplements (i.e., precursors of testosterone and related hormones) include androstenedione and DHEA. The latter compound is an endogenous hormone secreted by the adrenal cortex in response to adrenocorticotropin (ACTH).[36]

DOSE EFFECT

The dose of AAS used by athletes depends on individual needs and the athletic requirements of the particular sport. Endurance athletes use AAS doses near or slightly below physiologic replacement concentrations (i.e., about 7 mg testosterone daily) as a means to block catabolism, while sprinters typically use 1.5–2 times replacement concentrations.[29] Traditional strength athletes use much higher doses (i.e., 10–100 times replacement concentrations) to "bulk up." Generally, the dose of AASs is lower in women than men.

Data from human test participants indicate that AAS produce a dose-dependent and sex-dependent increase in lean body mass and strength, but the changes are highly variable and relatively small without an accompanying conditioning and strength program.[37] The administration of AAS to men participating in weight training consistently produces increased strength when compared with controls (i.e., weight training alone).[28] The endogenous testosterone production during male adolescence produces a sex differential in lean body mass similar to the increment in lean body mass caused by the administration of exogenous AAS to adults.[38] However, different androgen-dependent processes have different testosterone dose-response relationships. In a study of 61 eugonadal men receiving a long-acting gonadotropin-releasing hormone agonist to suppress endogenous testosterone secretion, changes in leg press strength, leg power, thigh and quadriceps muscle volumes, hemoglobin, and insulin-like growth factor 1 (somatomedin C) positively correlated to testosterone concentrations.[39] Changes in fat mass and plasma high-density lipoprotein (HDL) cholesterol were negatively correlated to the testosterone dose.

Although adverse effects following AAS administration are usually dose related, there are few data on the long-term physiologic effects of chronic AAS use, particularly in women. In addition to dose and duration of use, long-term toxicity depends on the age of initiation, gender, steroid structure, and concurrent illicit use of other drugs. The daily production of testosterone in healthy men is about 4–10 mg compared with about 1 mg in healthy women.[40] Psychotic symptoms associated with AAS abuse typically occur in individuals using >1 g testosterone weekly, but the development of psychologic changes is highly variable. In a randomized, placebo-controlled, crossover trial of 56 healthy men aged 20–50 years, the administration of testosterone cypionate for 6 weeks in doses increasing to 600 mg/wk caused little psychologic change in most participants (i.e., 84%).[41] The regimen produced mild hypomania in 12% and marked hypomania in 4% of the men.

TOXICOKINETICS

Most of the data on the kinetics of testosterone and AASs is derived from the pharmacokinetics of these compounds in animals or in hypogonadal males receiving therapeutic doses of AASs.[42] There are few data on the toxicokinetics of AASs in individuals abusing AASs at doses up to 10–100 times the therapeutic dose.

Absorption

Despite the rapid absorption of testosterone, the systemic bioavailability of oral testosterone is low as a result of extensive first-pass hepatic metabolism. Structural modifications of testosterone produce synthetic testosterone derivative (anabolic-androgenic steroids), which increase bioavailability and prolong the duration of action. Alkylation of the 17-α position of testosterone produces oral AAS, whereas esterification of the 17-β position results in injectable AAS (e.g., lipid-soluble cypionate or enanthate). The duration of action of these esters depends on the rate of absorption from the site of administration as determined by the chain length of the acid moiety and the formulation. Hydrolysis of these esters *in vivo* prolongs the duration of action compared with testosterone. Anabolic-androgenic steroids can diffuse across the skin and mucous membranes, allowing other delivery modes including transdermal patches, nasal sprays, and buccal tablets. Following oral administration of 120 mg testosterone undecanoate, volunteer studies indicate that plasma concentrations of testosterone are detectable for about 1–6 hours after administration using gas chromatography/tandem mass spectrometry.[43] There are dramatic individual variations (i.e., 10-fold) in the peak total plasma testosterone concentrations. In a study of 61 eugonadal men receiving long-acting gonadotropin-releasing hormone agonist to suppress endogenous testosterone secretion, the mean nadir testosterone concentrations ranged from 2.53–23.7 ng/mL following weekly injections of testosterone enanthate doses of 25–600 mg for 20 weeks.[39]

Distribution

Anabolic-androgenic steroids are bound in the plasma to sex-hormone-binding globulins. Although testosterone is highly protein bound (i.e., 98%) in plasma, the binding of AAS to sex-hormone-binding globulins is highly variable based on animal studies.[44]

Biotransformation

The metabolism of endogenous testosterone involves the conversion to the estrogenic compound, estradiol via steroid aromatase and the androgenic compound, 5α-dihydrotestosterone via 5α–steroid-reductase. Comparatively, the biotransformation of AASs is quite complex.[45] Table 15.2 lists the principal urinary metabolites of common AASs. The initial and rate-liming step in testosterone metabolism is reduction of the C4-C5 double bond on the A ring with 5α-reductase and 5β-reductase. Hydroxylation of testosterone by CYP450 isoenzymes results in the formation of a variety of minor urinary metabolites of testosterone. Single-dose human excretion studies indicate that 6-β-hydroxylation is also a minor pathway for the biotransformation of boldenone (17β-hydroxyandrosta-1,4-dien-3-one) and 17α-methyltestosterone.[46] However, 6β-hydroxylation of the B-ring is the major metabolic pathway for 4-chloro-1,2-dehydro-17-α-methyltestosterone, methandienone, and fluoxymesterone because the presence of a C1-C2 double

TABLE 15.2. Major Urinary Metabolites of Common Anabolic Androgenic Steroids.

Compound	Major Urinary Metabolite
17α-Alkyl Esters (*Oral*)	
Methandienone	17α-Methyl-5β-(α)-androstan-3α,17β-diol; 17α-methyl-5β-androst-1-en-3α,17β-diol; 17α-methyl-1,4-androstadien-6β,17β-diol-3-one
17α-Methyltestosterone	17α-Methyl-5β-(α)-androstan-3α,17β-diol
Oxandrolone	17-Epimer *
Oxymesterone	17α-Methyl-5-androstan-3,4,17-triol isomers
Stanozolol	3′-Hydroxystanozolol, 4β-hydroxystanozolol
17β-OH Esters (*Parenteral*)	
Boldenone	5β-Androst-1-en-17β-ol-3-one
Drostanolone	2α-methyl-5α-androstan-3-ol-17-one
Methenolone	18-Hydroxylated and several metabolites
Nandrolone (19-nortestosterone)	19-Norandrosterone (19-noretiocholanolone)

*Urinary excretion of unchanged oxandrolone also occurs.

bond in the former 2 steroids and the C9 α-fluorine atom in the latter compound blocks A-ring reduction. Metabolic changes (e.g., 12-hydroxylation) of AASs at the C-ring are minor. D-ring metabolism by the enzymatic oxidation of 17β-hydroxysteroid dehydrogenase to form the corresponding 17-keto steroid is a major metabolic pathway for testosterone and all AASs with secondary 17β-hydroxy groups (e.g., boldenone, clostebol, drostanolone, mesterolone, methenolone, nandrolone, norclostebol, stenbolone).[47] The main urinary metabolites of testosterone are androsterone (3α-hydroxy-5α-androstan-17-one), etiocholanolone (3α-hydroxy-5β-androstan-17-one), epiandrosterone (3β-hydroxy-5α-androstan-17-one), 5α-androstane-3α, 17β-diol, and 5β-androstane-3α,17β-diol.

Elimination

In individuals without AAS use, only small amounts (i.e., about 1%) of endogenous testosterone appears unchanged in the urine. Phase II conjugation reactions couple AASs and associated metabolites with glucuronic acid or sulfate before excretion in the urine. The vast majority (i.e., about 90%) of the absorbed dose of testosterone appears in the urine as glucuronide or sulfate conjugates. In a study of 8 hypogonadal males, the terminal elimination half-lives of 500 mg and 1,000 mg IM doses of testosterone undecanoate were 18.3 ± 2.3 days and 23.7 ± 2.7 days, respectively.[48] The mean residence times were 21.7 ± 1.1 days and 23.0 ± 0.8 days, respectively. Not all anabolic steroids undergo phase II reactions. Unconjugated AASs in human urine include oxandrolone, fluoxymesterone, 4-chloro-1,2-dehydro-17α-methyltestosterone, and formebolone along with metabolites of oxandrolone, methandienone, and stanozolol.[45] There is very limited (i.e., about 5%) enterohepatic recirculation of testosterone.

Maternal and Fetal Kinetics

Anabolic-androgenic steroids readily cross the placenta. Abnormal urogenital virilization occurs in females secondary to prenatal exposure to exogenous or endogenous androgens. In rodent studies, the parenteral administration of testosterone on gestational days 14–18 causes urogenital sinus elongation in a female mouse fetus.[49]

Drug Interactions

There are few data on the drug interactions of AASs. Rare case reports associate enhancement of anticoagulant effects with the use of AASs (e.g., oxymetholone).[50]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Testosterone is both an active hormone and a prohormone for the formation of the more active androgen, 5α-dihydrotestosterone (DHT) via 5α-reductase. Figure 15.3 displays the synthesis and metabolism of testosterone and other endogenous steroids. The production of testosterone occurs predominantly (i.e., 95%) in the Leydig cells of the testes and, to a lesser, extent in the adrenal glands. The secretion of androgens from the adrenal cortex is insufficient to maintain male sexuality. Women also secrete small amounts of testosterone from the ovaries and adrenal glands including DHEA and androstenedione. Testosterone acts as an androgen either directly by binding to the androgen receptor or indirectly by conversion to DHT. This latter compound binds more avidly to the androgen receptor than testosterone. Dihydrotestosterone amplifies the action of androgen and conveys specific function to the androgen-androgen receptor complex.[51] The conversion of testosterone to DHT is especially important for the appearance of virilization in female AAS users because high levels of 5α-reductase activity in the male accessory sex glands limits the effect of DHT. Activation of the intracellular, ligand-dependent androgen receptor complex by testosterone and AAS results in the production of RNA, DNA, and the subsequent enhancement of protein synthesis including increased amounts of actin and myosin in skeletal muscles. The androgen receptor belongs to the nuclear receptor family that contains a DNA-binding domain, a ligand-binding domain, and at least 2 transcriptional activation domains. The enzyme, aromatase controls the androgen/estrogen ratio by catalyzing the conversion of testosterone to estradiol in other tissues (e.g., adipose tissue, brain). Anabolic-androgenic steroids (e.g., methandienone, nandrolone, stanozolol) with the most potent anabolic effects also possess the relatively greatest androgenic effects. The interactions of AAS with the androgen receptors in various tissues vary between compounds in this group, and these variations account for differences in the anabolic and androgenic effects of these compounds. The use of AAS increases skeletal muscle mass and strength when used in combination with intensive strength training and high-protein, high-calorie diets.[52]

Endogenous testosterone is responsible for sexual maturation at all stages of development throughout the life of males. Increased AAS plasma concentrations suppress gonadotropin-releasing hormone, endogenous testosterone secretion, luteinizing hormone, and follicle-stimulating hormone by a negative-feedback
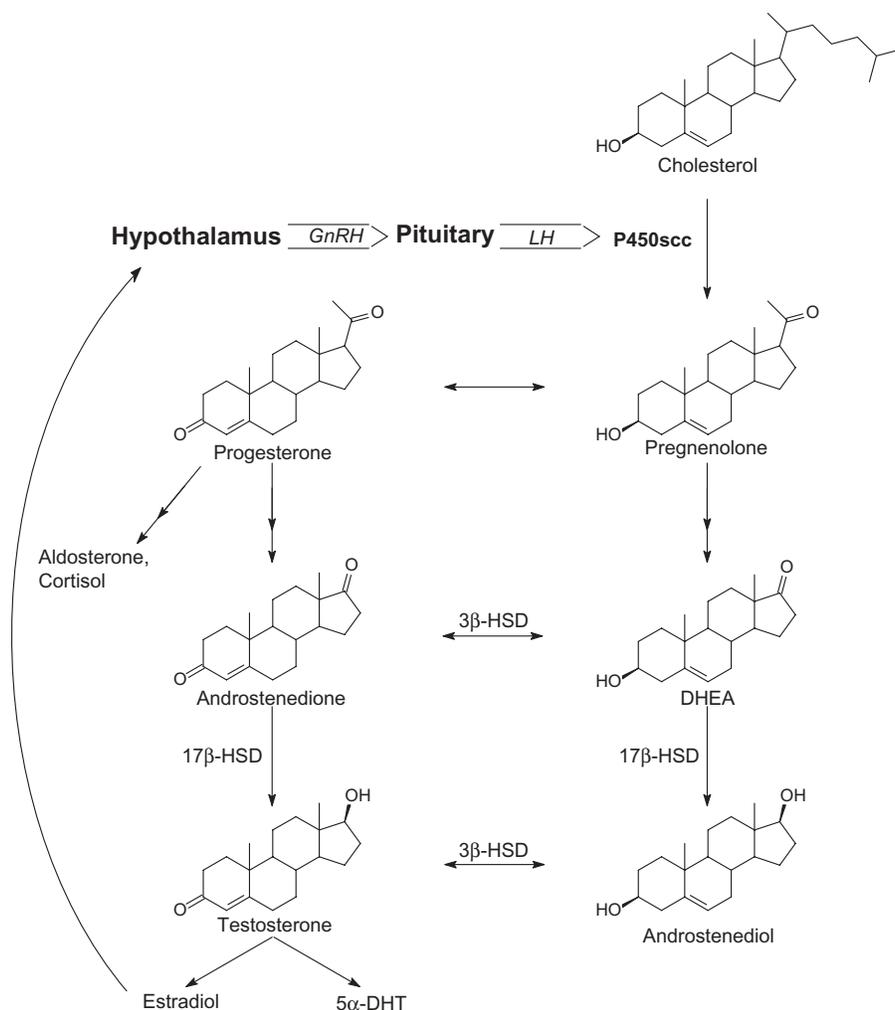
FIGURE 15.3. Biochemical synthesis and metabolism of endogenous steroids. The administration or formation of testosterone causes a downregulation of luteinizing hormone and a reduction in the endogenous formation of steroids via a negative feedback mechanism. GnRH = gonadotropin releasing hormone; LH = leuteinizing hormone; P450scc = cholesterol-side-chain-cleavage enzyme; 3β-HSD = 3β-hydroxysteroid dehydrogenase; 17β-HSD = 17β-hydroxysteroid dehydrogenase; DHEA = dehydroepi-androsterone; 5α-DHT = 5α-dihydrotestosterone.

mechanism. Endogenous androgens stimulate RNA polymerase, resulting in an increased production of protein. These proteins are responsible for normal male sexual development, including the growth and maturation of the prostate, seminal vesicle, penis, and scrotum. During puberty, androgens cause a sudden increase in growth and development of muscle along with redistribution of body fat and deepening of the voice. Continued endogenous testosterone secretion produces increased hair (beard, body hair), fusion of the epiphyses, termination of growth, and the maintenance of spermatogenesis. Testosterone also affects the formation of erythropoietin, the balance of calcium, and blood glucose concentrations.

Mechanism of Toxicity

Most of the adverse effects following the use of AASs result from the enhancement of normal physiologic response to testosterone by either direct receptor agonist activity or suppression of steroid biosynthesis. In general, toxic effects associated with AAS abuse involve the following: 1) anabolic side effects, 2) enhanced androgenic effects, 3) estrogenic side effects, 4) antiandrogenic effects from the suppression of the hypothalamus–pituitary–adrenal/gonadal axes, 5) hepatotoxicity, and 6) neuropsychiatric effects.[53] Methodologic issues limit the determination of the toxic effects of illicit AAS use including the extraordinary doses and

types of AASs used by athletes compared with medical use, reporting bias of self-reports, the paucity of well-documented pathologic findings, and the lack of well-defined postmortem markers of AAS use.[53] Most medical data on the toxic effects of AAS abuse involve case reports rather than epidemiologic studies. Pathologic abnormalities from AAS abuse are best-documented in the cardiovascular system, reproductive system, liver, and serum lipids. Animals studies suggest that AAS can cause dysplasia of collagen fibrils and decreased tensile strength, and potentially the use of these drugs could cause disruption of connective tissue.[54]

CARDIOVASCULAR SYSTEM

Potential adverse effects of AAS on the cardiovascular system include atherogenesis, thrombosis, vasospasm, myocarditis, concentric left ventricular hypertrophy, myocardial fibrosis, hypertrophic cardiomyopathy with ventricular dysrhythmias, and direct myocardial injury.[55] However, the contribution of AAS use to these potential adverse cardiovascular effects remains unclear.[56] Chronic AAS use enhances hepatic triglyceride lipase activity, resulting in reduction of high-density lipoproteins and elevation of low-density lipoproteins.[57,58] Although these changes are reversible within several months of cessation of AAS use, chronic AAS use theoretically increases the risk of cardiac disease.[30] Potentially, the chronic abuse of AAS enhances coagulability and thrombosis, but the clinical importance of this potential adverse effect also remains unclear. Studies of chronic AAS abuse in weightlifters suggest that some anabolic-androgenic steroid-using weightlifters have accelerated activation of their hemostatic system as evidenced by increased generation of both thrombin and plasmin. A study of AAS-positive steroid using weightlifters indicated that these individuals had a higher percentage of abnormally high plasma thrombin–antithrombin complexes along with elevated plasma concentrations of prothrombin fragment 1, antithrombin II, and protein S, when compared with non-AAS-using controls.[59] Additionally, the plasma concentrations of tissue plasminogen activator and its inhibitor were lower in AAS users than in controls.

Clinical studies on bodybuilders suggest that chronic AAS use impairs vascular reactivity independent of the smooth muscle hypertrophy and vascular stiffness associated with bodybuilding.[60] Anabolic-androgenic steroids decrease the production of cyclic guanosine monophosphate (cGMP) by inhibiting guanylyl transferase. As a result, AASs potentially inhibit the ability of nitric oxides to relax smooth muscles in the coronary arteries resulting in coronary artery vasospasm and potentially sensitizing AAS users to sudden death.[55]

Case reports associate the chronic use of anabolic steroids with sudden death and contraction band necrosis in the myocardium.[61] In these cases, no other cause of death was apparent, but the role of chronic, high-dose anabolic steroid use in these deaths remains unclear. Athletes with certain genetic mutations and structural abnormalities may be particularly vulnerable to the use of anabolic steroids including athletes with accessory AV pathways, latent structural heart diseases (dilated cardiomyopathy, arrhythmogenic right ventricular dysplasia type II, myocarditis, segmental arrhythmogenic ventricular cardiomyopathy, coronary artery anomalies), latent Brugada syndrome, mutations of the long QT syndrome genes, and other genetic mutations of ion channels (cardiac ryanodine receptor gene defects, calsequestrin gene defects).[62,63] Pathologic evidence of some of these abnormalities may not appear on postmortem examination. The use of diuretics to mask the use of anabolic steroids may predispose these athletes to serious ventricular dysrhythmias from hypokalemia and dehydration.

LIVER

Cholestatic jaundice with intrahepatic cholestasis and variable degrees of hepatocellular necrosis on liver biopsy is the most commonly reported serious pathologic abnormality of the liver associated with AASs abuse.[64] Rarely, case reports associate the presence of multiple, dilated liver cysts filled with blood in the liver (peliosis hepatitis) with chronic use of AASs.[65] The pathogenesis of peliosis hepatis is unknown. Other pathologic abnormalities of the liver detected in the autopsy of AAS abusers include focal nodular hyperplasia and adenomas.[66,67]

REPRODUCTIVE SYSTEM

Although the same androgen receptor mediates both the anabolic and androgenic effects of steroids, the specific structural characteristics of individual AASs determines the balance between anabolic and androgenic effects. Masculinization results from the presence of high doses of AASs in women. The aromatase cytochrome P450 (CYP19) enzyme complex converts testosterone to estradiol, which binds to the estrogen receptor. Although this conversion usually accounts for a small percentage of testosterone biotransformation at physiologic doses, excess amounts of testosterone increase the formation of estradiol. Gynecomastia is a consequence of excessive estradiol concentrations in chronic male AAS abusers that result from the peripheral conversion of excess testosterone to estradiol. Drugs used by male AAS abusers to prevent the feminizing effects

of estradiol include the use of aromatase inhibitors (e.g., anastrozole, aminoglutethimide) and drugs that block estrogen receptor (clomiphene, tamoxifen).

Postmortem

Concentric left ventricular myocardial hypertrophy is a common pathologic finding following the chronic use of AAS. A 21-year-old, previously healthy weightlifter collapsed during a bench press workout, and paramedics found him in ventricular fibrillation. For the preceding several months, he used parenteral AASs (nandrolone, 19-nortestosterone). Postmortem findings included marked cardiac hypertrophy, regional myocardial fibrosis, and focal myocardial necrosis along with hepatosplenomegaly and renal hypertrophy.[68] There was no evidence of recent myocardial inflammation. Other autopsies of AAS abusers have not demonstrated cardiac hypertrophy, but histologic examination of cardiac tissue also detected focal myocardial necrosis. The postmortem examination of 2 cases of sudden death in 2 previously healthy chronic AAS abusers (i.e., bodybuilders) demonstrated focal myocardial necrosis (contraction band necrosis) without evidence of significant coronary artery disease or myocardial hypertrophy.[61] Other pathologic changes associated with cardiac arrest in previously healthy athletes following AAS use (e.g., oxymesterone) include hypertrophic cardiomyopathy, acute cellular necrosis,[69] interstitial fibrosis, and myocarditis.[70] Typically, there is no evidence of coronary thrombosis in these cases of sudden death. However, evidence of recent (i.e., 2 weeks) myocardial infarction may occur in these cases without evidence of coronary artery disease.[71] Rarely, the abuse of androgenic-anabolic steroids are associated with the development of blood-filled cysts (peliosis) involving the liver, spleen, bone marrow, lymph nodes, and lung. A 29-year-old bodybuilder was found dead at home; an autopsy demonstrated peliosis of the lung with the left pleural cavity filled with blood.[72]

CLINICAL RESPONSE

Illicit Use

There are limited data on the short-term complication of AAS use with most data involving case reports. The long-term health effects of chronic AAS abuse are not well defined because of the difficulty studying illicit drug use and the highly variable dosages involved with AAS abuse. Clinical and laboratory studies indicate that the administration of AASs causes physiologic changes, primarily in the liver, reproductive system, and serum lipids.[64,73] The use of AAS produces an unfavorable change in the blood lipid profile (i.e., elevated low-density lipoprotein, reduced high-density lipoprotein) with potentially increased risk of coronary heart disease; although conflicting, some data suggest left ventricular hypertrophy may persist after cessation of AAS use as a result of elevated blood pressure during use.[74,75] Other potential adverse effects include glucose intolerance (i.e., increased peripheral insulin resistance), hyperinsulinism, behavioral and mood changes, cerebrovascular accidents, prostate abnormalities, edema, and immune dysfunction. Most physiologic changes associated with AAS use are reversible within several months of the cessation of AAS use.[29]

Behavioral Abnormalities

Individual behavioral and psychiatric responses to chronic AAS abuse are extremely variable, depending on pre-existing psychiatric conditions, personality, and type and dose of AAS. Case reports and some epidemiology studies associate chronic AAS use with changes in behavior, mood, and somatic perceptions in a small subset of chronic AAS abusers.[76,77] Psychiatric complication associated with chronic AAS use include mania, aggression, and agitation, but no large-scale epidemiologic studies have confirmed a causal relationship between chronic AAS abuse and severe psychiatric complications (e.g., psychosis).[78,79] Depression may occur, particularly in the immediate period after cessation of use, along with fatigue, decreased libido, insomnia, anorexia, and headaches.[80,81] Studies suggest that male AAS users have more frequent and prolonged periods of anger, aggression, irritability, and hostility than nonusers.[82,83] Multivariate models based on data from the National Longitudinal Study of Adolescent Health ($N = 6,823$) indicate that young men using anabolic-androgenic steroids report greater involvement in violent behaviors than nonusers after control for the effects of key demographic variables, previous violent behavior, and polydrug use.[84] In a study of 2 sets of male monozygotic twins with only 1 twin using AASs, the use of AASs was associated with anxiety, hostility, aggressiveness, and paranoid ideations as determined by comparing responses to a Symptoms Check list-90 and the Hostility and Direction of Hostility Questionnaire.[85] Behavioral abnormalities include distractibility, delirium, delusions, irritability, paranoia, impulsivity, insomnia, hostility, anxiety, agitation, aggression, violence, and mood lability.[86,87] These behavioral effects are dose dependent with effects ranging from mild effects (increased confidence, enhanced self-esteem, euphoria) to serious behavioral abnormalities

(mood swings, grandiose thinking, paranoia, impulsivity, hostility, violence, and antisocial behavior).[80]

MOOD DISORDERS

The true prevalence of these neuropsychiatric disorders is difficult to determine, but relatively few individuals develop these adverse effects; they are found primarily in high-dose chronic AAS abusers. In a study of 88 AAS-using athletes and 68 nonusers, the incidence of major mood disorders (mania, hypomania, major depression) was significantly more frequent ($P < .01$) in steroid users compared with nonusers.[88] Additionally, these mood disorders were more frequent ($P < .001$) in current AAS users than in abstinent AAS users.

MEDICAL COMPLICATIONS

CARDIOPULMONARY. Several case reports associate the chronic use of AAS with serious cardiovascular complications including acute myocardial infarction, cardiac arrest, and hypertrophic cardiomyopathy without significant cardiac valvular or coronary artery disease.[68,70] Case reports associate chronic AAS abuse with myocardial infarctions in young men with and without evidence of coronary artery occlusion; the presence of coronary artery disease in these athletes occurs despite the lack of known risk factors for coronary artery disease.[89,90] The development of an acute myocardial infarction was associated with high-dose AAS abuse (e.g., 6 weeks daily, 2 years intermittently) by a 44-year-old recreational bodybuilder with diffuse coronary artery disease and multiple myocardial risk factors including polycythemia, smoking, and family history of early coronary artery disease.[91] Left ventricular hypertrophy is a common structural abnormality in bodybuilders with AAS abuse. A study of 21 bodybuilders with reported AAS abuse suggests that concentric hypertrophy of the left ventricular wall and impaired diastolic function are common complications of steroid abuse.[92] Echocardiographic studies of these athletes demonstrated increased left ventricular posterior wall thickness and end-diastolic volumes as well as decreased ratios of ventricular end-diastolic diameter to body mass. A case report of 2 previous healthy bodybuilders associated sudden cardiac death with chronic AAS abuse.[61] There was evidence of focal myocardial necrosis without clinically significant coronary artery disease, but the role of chronic AAS abuse in the cardiac arrest remains unclear. Ventricular dysrhythmias are not commonly associated with chronic AAS abuse. Several case reports associate persistent atrial fibrillation with chronic AAS use.[93] A 22-year-old man developed generalized weakness, diaphoresis, anxiety, and dyspnea.[94] The electrocardiogram revealed rapid atrial fibrillation and the echocardiogram indicated an early cardiomyopathy. He had gynecomastia, and he admitted to the recent injection of anabolic steroids. Although there is no direct evidence that AASs are thrombogenic in humans, case reports suggest a possible causal relationship between AAS use and thrombogenic events (e.g., massive pulmonary embolus, cerebral thrombosis, cardiomyopathy with congestive heart failure, biventricular thrombi, hepatorenal dysfunction).[95,96] Studies on the association between chronic AAS use and hypertension or left ventricular hypertrophy are inconsistent.[97]

CENTRAL NERVOUS SYSTEM. Several case reports associated chronic AAS abuse with the development of seizures, ischemic midbrain lesions with residual dysfunction (hemiparesis, aphasia, dysarthria),[98] and thrombotic strokes,[99] but the causal link between cerebrovascular accidents and chronic AAS abuse is unproven. A 34-year-old bodybuilder developed an acute right hemiparesis and dysarthria after using various AAS for 4 years.[100] He had a simple partial seizure. At discharge, he had mild motor weakness in the right upper extremity with no sensory changes. A 21-year-old man developed a generalized tonic-clonic seizure left-sided hemiparesis after using 6–8 mg ethylestrenol daily for 6 weeks.[101] Angiography demonstrated an occlusion of the right anterior cerebral and right middle cerebral arteries. In a retrospective cohort study of 248 patients testing positive for the presence of AAS in connection with receiving medical care, the incidence of unspecified convulsions was increased in the AAS-positive group (RR = 53.9, 95% CI = 7.0–415.7) when compared with controls (i.e., patients testing negative for AAS at the same institution).[102] One of the AAS-positive patients with seizures died. However, there are few data from cohort or case-control studies to confirm the causal link between AAS abuse and seizures.

IMMUNE SYSTEM. Anabolic-androgenic steroids are potentially immunosuppressive based on a study suggesting that chronic AAS abuse reduces immunoglobulins (i.e., IgA), increases natural killer cell activity, and increases mitogen response to staphylococcal antigens.[103] There were no significant differences in T-cell subsets among steroid users and nonusers in this study. However, the clinical relevance of these potential immunosuppressive effects is unclear. Sporadic case reports associated infectious complications from the parenteral use of AASs including local abscess at the site of injection,[104] septic arthritis,[105] HIV,[106] and hepatitis,[107] usually as a result of sharing contaminated needles.

285

LIVER.   Case reports associate AAS abuse with hepatic enlargement, peliosis hepatis, serious cholestatic jaundice [2,17-dimethyldihydrotestosterone (methasterone)],[108,109] and hepatorenal failure, particularly following the use of oral 17α-alkylated AAS (e.g., methandrostenolone, methyltestosterone, oxymetholone, oxandrolone, stanozolol).[66,110] Typically, non-17-alkylated anabolic steroids are not associated with hepatocellular damage.[111] Jaundice typically resolves several months after cessation of AAS use, and liver transplantation is not usually necessary. Case reports also associate the use of high doses of AAS with the development of hepatocellular necrosis. A 26-year-old male bodybuilder reportedly used testosterone enanthate (500 mg IM twice weekly), stanozolol (40 mg orally/day), and methylandrostenediol (30 mg/day orally for 5 weeks). He subsequently developed evidence of severe hepatitis with hepatic dysfunction that required a prolonged hospitalization.[112] Peliosis hepatis is a rare illness characterized by blood-filled spaces within the hepatic parenchyma that typically occurs in association with a variety of chronic wasting diseases (e.g., tuberculosis, malignancy). This condition has been associated with both the chronic use of pharmacologic doses of AAS and the chronic, intermittent abuse of AAS by bodybuilders.[65] The appearance of peliosis hepatis is not related to the dose of AAS or duration of use.[66] Some athletes using AAS take polyunsaturated phospholipids and vitamin B complex (Compound N) to reduce the elevation of hepatic aminotransferases often associated with the use of AAS;[113] however, there are inadequate data to determine the efficacy of this combination supplement.

MUSCULOSKELETAL SYSTEM.   The use of supraphysiologic doses of testosterone increases muscle mass and maximal voluntary strength in a dose-dependent manner, but there is no definitive evidence that testosterone improves performance in endurance events.[114,115] Rare case reports associate a general necrotizing myopathy with the chronic use of AAS. A 23-year-old man developed diffuse myalgias and severe rhabdomyolysis with anuria.[116] Although he developed a viral upper respiratory tract infection 1 week prior to the development of rhabdomyolysis, muscle biopsy and serum testing revealed no evidence of inflammation or immune disease. He had a history of chronic AAS abuse. Other case reports associate localized rhabdomyolysis of the deltoid muscles with chronic AAS injections in the same location.[117] There are few data in the medical literature regarding the effect of chronic AAS abuse on the function of connective tissue. Several case reports associate the chronic abuse of AAS with disruption of connective tissues including spontaneous rupture of ligaments and muscles.[118,119] In adolescents, premature closure of the epiphyseal plates during chronic AAS use can stunt bone growth.[120]

SKIN.   The most common dermatologic effects of AAS abuse are alopecia, male pattern baldness, and hirsutism, particularly in women. Other adverse dermatologic effects include keloid formation, sebaceous cysts, comedones, seborrheic furunculosis, folliculitis, striae, and acne. A common triad of chronic AAS abuse is the combination of acne, striae, and gynecomastia. Hyperplasia of the pilosebaceous glands and increased sebum production cause a high incidence of acne in chronic AAS abusers. These effects become prominent about 1 month after initiation of AAS abuse, depending on the dose and frequency of use.[121] Gynecomastia in males is a common adverse effect of chronic AAS abuse. These effects are not readily reversible, particularly in adolescents.[122] Adverse effects in women following chronic AAS use include masculinization (male pattern baldness, hirsutism), acne, oily skin, and breast atrophy.[123] The virilizing effects of AAS use by women are similar to the clinical features of the virilizing syndrome associated with congenital adrenal hyperplasia and adrenal carcinoma.

Reproductive Abnormalities

All AASs suppress gonadotropin secretion and therefore suppress endogenous testicular function. Adverse effects of AASs on the male reproductive system include reduced hormone levels (gonadotrophic hormones, endogenous testosterone, sex-hormone-binding globulin), impotence (erectile dysfunction),[124] alterations in sperm morphology, and reductions in sperm count, sperm motility, and the size of the testicles.[125] These changes are not responsive to the administration of human chorionic gonadotropin, and resolution of the alterations in sperm count occurs several months after cessation of AAS use with some individuals requiring up to 30 weeks of abstinence.[126] Although chronic AAS abuse produces hypogonadism, decreased serum testosterone and impaired spermatogenesis, these changes are reversible within a few months to 1 year following cessation of AAS use.[127,128] Abnormalities of the female reproductive system following chronic AAS abuse include menstrual irregularity, deep voice, increased libido, and increased clitoris size along with elevated sex-hormone-binding globulin. Anabolic-androgenic steroids are contraindicated in pregnant women (i.e., Pregnancy Category X) with potential effects including masculinization of the female fetus, clitoral hypertrophy of the female fetus, decreased birth weight, and premature bone maturation.[126]

Carcinogenesis

Rarely, case reports associate chronic *oral* AAS abuse with the development of hepatocellular adenomas, which usually regress along with minor elevations of serum hepatic aminotransferase concentrations after cessation of AAS abuse.[129] These tumors are not malignant, but occasionally surgical removal is necessary because of liver dysfunction or intrahepatic bleeding.[130] Other case reports associate chronic AAS abuse with the following cancers: kidney,[131] prostate (adenocarcinoma),[132] testes (leiomyosarcoma),[133] and liver (hepatocellular carcinoma, angiosarcoma).[134,135] However, there are inadequate data (i.e., cohort or case-control studies) to causally link chronic AAS use with cancer.

DIAGNOSTIC TESTING

Most clinical laboratories in healthcare facilities do not routinely perform analysis of urine samples for AASs. Clinically, abuse of AASs is suspect in male bodybuilders and weightlifters with small testes, low sperm counts, high hematocrit and hemoglobin values, and low serum sex-hormone-binding globulin concentrations. The presence of hirsutism, balding, and acne in a women athlete also suggests the use of AASs.

Analytic Methods

Analysis of steroids is a complicated process because of the large number of compounds (i.e., >7,000), thermal lability, poor volatility, and low UV extinction coefficients. Because of the rapid metabolism of AAS, detection times for determining the use of these drugs increase by analyzing urine samples for AAS metabolites, primarily by gas chromatography/mass spectrometry (GC/MS). Increasing useful, newer analytic methods to supplement gas chromatography/mass spectrometry include liquid chromatography/mass spectrometry and carbon isotope mass spectrometry for the detection of testosterone. Confirmation of AAS use involves the comparison of the electron impact mass spectrum with GC retention times of the trimethylsilyl derivatives of the steroid and/or the corresponding metabolites to synthesized or isolated reference standards. Analytic methods for qualitative and/or quantitative determinations of AAS typically employ gas or liquid chromatography combined with electron, chemical, or atmospheric pressure ionization followed by analyses using quadrupole, ion trap, linear ion trap, or hyphenated techniques.[136] The 2 common atmospheric-pressure ionization modes are electrospray ionization and atmospheric-pressure chemical ionization. The limit of detection for liquid chromatography/tandem mass spec-

trometry is approximately 25–50 pg/mL.[137,138] Other methods for the detection of AAS in urine samples include micellar electrokinetic capillary chromatography, high performance liquid chromatography, capillary gas chromatography, and liquid chromatography with electrochemical detection.[139,140] Analysis by liquid chromatography/electrospray/tandem mass spectrometry with simultaneous acquisition of precursor ion scan (m/z 105, 91, 77) allows the detection of prohormones derived from nutritional supplements even in the presence of impurities and/or degradation products in unconjugated urine samples.[141] Liquid chromatography/tandem mass spectrometry is more specific than radioimmunoassay for the quantitation of testosterone and dihydrotestosterone with the latter method typically reporting higher concentrations as a result of interfering serum steroids.[142] Compared with gas chromatography/mass spectrometry, liquid chromatography/tandem mass spectrometry after double extraction by solid-phase extraction allows the quantitation of AASs (e.g., nandrolone) without the hydrolysis and derivatization steps.[143] Measurements of testosterone and dihydrotestosterone in fluoride-containing blood tubes (e.g., gray-top) are usually approximately 15–20% lower than blood collected in plain containers (e.g., red-top).

The diagnosis can be confirmed by one of several tests, depending upon the compound to be tested. Storage of urine samples at 37°C (98.6°F) for several days results in the deconjugation of most steroid glucuronide and sulfate compounds.[144] Anabolic-androgenic steroids are not thermally labile, but small elevations of testosterone may result from the contamination of urine samples with *Candida albicans*. However, these changes are unlikely to cause false-positive tests.[145]

Biomarkers

Human urinary steroid profiles contain several statistically stable parameters, including the testosterone/epitestosterone ratio, and testosterone metabolites (androsterone, etiocholanolone, 5β-androstane-3α,17β-diol, 5α-androstane-3α,17β-diol).[137] Testosterone is one of the few compounds in athletic drug-testing programs that have a threshold. Analytic methods cannot distinguish between testosterone administered exogenously and testosterone produced endogenously. Initially, these programs used a testosterone to epitestosterone ratio of 6:1,[146] but now the WADA lowered the ratio to 4. Individuals using testosterone suppress the production of both testosterone and epitestosterone, resulting in replacement with only testosterone and elevated testosterone/epitestosterone ratio. The distribution of this ratio for both male and female athletes is approximately log-normal with a mean of about 1.0.[147]

Limitations of this ratio result from the effects of microbial degradation, excessive ethanol consumption, pharmacogenetic factors, pathologic conditions, and alteration of the ratio during puberty.[148] This ratio returns to baseline after cessation of testosterone use, whereas the ratio remains elevated for the rare athlete with a naturally increased ratio. Serial testing of the testosterone/epitestosterone ratio over at least 1 month after an event help determine the use of doping if the individual coefficient of variation exceeds 60% in 3 or more urine samples during this period.

Efforts to escape detection include the administration of epitestosterone or human chorionic gonadotropin to increase epitestosterone concentrations to produce a more favorable ratio; thus, the WADA established a threshold of 200 ng/mL for urinary epitestosterone to detect attempts to alter this ratio.[149] The ratio of testosterone to luteinizing hormone is also high (i.e., >30) in the urine of men using testosterone as a result of the testosterone-induced suppression of the secretion of luteinizing hormone.[150] This ratio is not useful in women. An alternative method for the detection of doping with AASs involves analysis of the ratio of the 2 stable carbon isotopes, $^{13}C/^{12}C$ with gas chromatography/combustion/isotope-ratio mass spectrometry. Exogenous testosterone or precursors contain less ^{13}C than endogenous homologues; consequently, urinary steroids with low $^{13}C/^{12}C$ ratios indicate pharmaceutic sources.[151] Urinary reference ranges for the $^{13}C/^{12}C$ ratio require correction for diet.[152] Similarly, recommended confirmatory procedure for the detection of 5α-dihydrotestosterone (DHT) abuse in male athletes uses the DHT/epitestosterone ratio in urine.[153] These ratios exceed reference standards within 24 hours of IM injection of DHT and return to baseline values within 1 month of the cessation of DHT use. Case reports suggest that the ingestion of AAS-contaminated meat (e.g., clostebol) can produce false-positive test results for AAS abuse.[154]

Norandrosterone is a human urinary metabolite of nandrolone (19-nortestosterone) that occurs naturally in trace amounts, particularly in pregnant women.[155] In a study of 30 healthy men without a history of AAS use, the range of the urinary excretion rates were 0.03–0.25 µg/24 hours or 0.01–0.32 ng/mL in nonfractionated 24-hour urine. To account for interindividual variability and the log-normal distribution, a threshold of 19-norandrosterone endogenous concentration of 2 ng/mL was established that represented the geometric mean plus 4 standard deviations. The maximum norandrosterone concentration in this group of urine samples was 0.63 ng/mL with a mean concentration of 0.078 ng/mL.[156] Almost all (i.e., about 95%) urine samples from healthy men have nondetectable concentrations of norandrosterone, and only very rarely do nondoping urine samples contain norandrosterone concentrations above the IOC-accredited laboratory cutoff of 2 ng/mL when corrected for urine specific gravity.[2] Both norandrostenedione and norandrostenediol are dietary supplements available in the United States, but all major sports organizations prohibit the use of these supplements. Potentially, these substances could be accidentally ingested by individuals, who subsequently could test positive for norandrosterone.

Abnormalities

Although some studies of athletes who chronically use AASs demonstrate asymptomatic elevation of serum hepatic aminotransferases,[66] most longitudinal studies of these athletes do no report changes in the common liver enzymes.[159] When present, the elevations of liver enzymes resolve without evidence of permanent pathologic changes following cessation of AAS use. Rarely, case reports associate evidence of severe hepatocellular necrosis (e.g., elevated serum hepatic aminotransferases, hyperbilirubinemia, hypoprothrombinemia) with chronic, intermittent AAS abuse.[112]

The total cholesterol concentration remains relatively unchanged during AAS use, but the concentrations of low density lipoprotein and apolipoprotein B increase about 30–50%, while the concentrations of high-density lipoprotein and apolipoprotein A1 decrease about the same amount.[157,158] There is substantial variation in the effect of different AAS on the cholesterol profile.[159,160] These alterations typically resolve within about 5 months after cessation of steroid use.

TREATMENT

The treatment of acute or chronic AAS abuse is supportive, and there are no specific measures directed to the medical complication of AAS abuse. There are no clinical data to guide the treatment of AAS overdose including the efficacy of decontamination measures. The acute symptoms associated with AAS typically are transitory (nausea, vomiting) and should respond to general treatment regimens (antiemetics). The most important aspect of treating AAS abuse is the cessation of AAS use.[161] Coordination of efforts to stop the use of AAS may benefit from therapy with a psychiatrist or an addiction specialist as well as medical treatment by an endocrinologist for the endocrine effects associated with chronic AAS abuse.[162] The treatment of agitation during AAS use and agitation during the use of other substances of abuse is similar; treatment includes the administration of benzodiazepines (lorazepam) and

antipsychotic agents (haloperidol, atypical antipsychotics) when associated with psychotic features.[86]

References

1. Prokop L. The struggle against doping and its history. J Sports Med Phys Fitness 1970;10:45–48.

2. Bowers LD. Abuse of performance-enhancing drugs in sport. Ther Drug Monit 2002;24:178–181.

3. Prendergast HM, Bannen T, Erickson TB, Honore KR. The toxic torch of the modern Olympic Games. Vet Hum Toxicol 2003;45:97–102.

4. David K, Dingemanse E, Freud J, Lacquer E. Uber kristallines männliches hormon aus hoden (testosteron), wirksamer als aus harn oder cholesterin bereitetes androsterone. Hoppe-Seylers Z Physiol Chem 1935;233:281–282.

5. Butenandt A, Hanisch G. Uber testosteron. Umwandlung des dehydroandrosterons in androstendiol und testosteron, ein weg zur darstellung des testosterons aus cholesterin. Hoppe-Seylers Z Physiol Chem 1935;237:89–97.

6. Ruzicka L, Goldberg NW, Rosenberg HR. Sexualhormone X. herstellung des 17-methyltestosteron und anderer androstenund androstanderivate. Zusammenhänge zwischen chemischer konstitution und männlicher hormonwirkung. Helv Chim Acta 1935;18:1487–1498.

7. Hoberman JM, Yesalis CE. The history of synthetic testosterone. Sci Am 1995;272:76–81.

8. Hershberger LG, Shipley EG, Meyer RK. Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. Proc Soc Exp Biol Med 1953;83:175–180.

9. Todd T. A history of the use of anabolic steroids in sport. In: Berryman JW, Park RJ, eds. Sport and exercise science: essays in the history of sports medicine. Urbana, IL: University of Illinois Press, 1992:326–327.

10. Todd T. Anabolic steroids: the gremlins of sport. J Sport Hist 1987;14:87–107.

11. Franke WW, Berendonk B. Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government. Clin Chem 1997;43:1262–1279.

12. Brooks RV, Firth RG, Sumner NA. Detection of anabolic steroids by radioimmunoassay. Br J Sports Med 1975;9:89–92.

13. Bagatell CJ, Bremner WJ. Androgens in men–uses and abuses. N Engl J Med 1996;334:707–714.

14. Van Eenoo P, Delbeke FT. Metabolism and excretion of anabolic steroids in doping control—new steroids and new insights. J Steroid Biochem Mol Biol 2006;101:161–178.

15. Catlin DH, Sekera MH, Ahrens BD, Starcevic B, Chang YC, Hatton CK. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. Rapid Commun Mass Spectrom 2004;18:1245–1249.

16. Sekera MH, Ahrens BD, Chang YC, Starcevic B, Georgakopoulos C, Catlin DH. Another designer steroid: discovery, synthesis, and detection of "madol" in urine. Rapid Commun Mass Spectrom 2005;19:781–784.

17. Friedel A, Geyer H, Kamber M, Laudenbach-Leschowsky U, Schanzer W, Thevis M. Tetrahydrogestrinone is a potent but unselective binding steroid and affects glucocorticoid signalling in the liver. Toxicol Lett 2006;164:16–23.

18. Buckley WE, Yesalis CE, Friedl KE, Anderson WA, Streit AL, Wright JE. Estimated prevalence of anabolic steroid use among male high school seniors. JAMA 1988;260:3441–3445.

19. Johnston LD, O'Malley PM, Bachman JG. Monitoring the Future national survey results on adolescent drug use: overview of key findings, 1999 (NIH Publication No. 00-4690). Bethesda, MD: National Institute on Drug Abuse; 2000.

20. Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the Future national results on adolescent drug use: Overview of key findings, 2004 (NIH Publication 05-5726). Bethesda, MD: National Institute on Drug Abuse; 2005.

21. Bahrke MS, Yesalis CE, Brower KJ. Anabolic-androgenic steroid abuse and performance-enhancing drugs among adolescents. Child Adolesc Psychiatr Clin N Am 1998;7:821–838.

22. Yesalis CE, Anderson WA, Buckley WE, Wright JE. Incidence of the nonmedical use of anabolic-androgenic steroids. NIDA Res Monogr 1990;102:97–112.

23. Parkinson AB, Evans NA. Anabolic androgenic steroids: a survey of 500 users. Med Sci Sports Exerc 2006;38:644–651.

24. Saudan C, Baume N, Robinson N, Avois L, Mangin P, Saugy M. Testosterone and doping control. Br J Sports Med 2006;40(suppl 1):i22–i24.

25. Petersson A, Garle M, Holmgren P, Druid H, Krantz P, Thiblin I. Toxicological findings and manner of death in autopsied users of anabolic androgenic steroids. Drug Alcohol Depend 2006;81:241–249.

26. Kicman AT. Pharmacology of anabolic steroids. Br J Pharmacol 2008;154:502–521.

27. Storer TW, Woodhouse LJ, Sattler F, Singh AB, Schroeder ET, Beck K, Padero M, et al. A randomized, placebo-controlled trial of nandrolone decanoate in human immunodeficiency virus-infected men with mild to moderate weight loss with recombinant human growth hormone as active reference treatment. J Clin Endocrinol Metab 2005;90:4474–4482.

28. Haupt HA, Rovere GD. Anabolic steroids: a review of the literature. Am J Sports Med 1984;12:469–484.

29. Yesalis CE, Bahrke MS. Anabolic-androgenic steroids current issues. Sports Med 1995;19:326–340.

30. Sullivan ML, Martinez CM, Gennis P, Gallagher EJ. The cardiac toxicity of anabolic steroids. Prog Cardiovasc Dis 1998;41:1–15.

31. Thevis M, Schrader Y, Thomas A, Sigmund G, Geyer H, Schanzer W. Analysis of confiscated black market drugs using chromatographic and mass spectrometric approaches. J Anal Toxicol 2008;32:232–242.

32. Skårberg K, Nyberg F, Engström I. The development of multiple drug use among anabolic-androgenic steroid users: six subjective case reports. Subst Abuse Treat Prev Policy 2008;3:24.

33. Perry PJ, Lund BC, Deninger MJ, Kutscher EC, Schneider J. Anabolic steroid use in weightlifters and bodybuilders: an internet survey of drug utilization. Clin J Sport Med. 2005;15:326–330.

34. Hall RC, Hall RC. Abuse of supraphysiologic doses of anabolic steroids. South Med J 2005;98:550–555.

35. Curry LA, Wagman DF. Qualitative description of the prevalence and use of anabolic androgenic steroids by United States powerlifters. Percept Mot Skills 1999;88:224–233.

36. Kroboth PD, Salek FS, Pittenger AL, Fabian TJ, Frye RF. DHEA and DHEA-S: a review. J Clin Pharmacol 1999;39:327–348.

37. Friedl KE, Dettori JR, Hannan CJ Jr, Patience TH, Plymate SR. Comparison of the effects of high dose testosterone and 19-nortestosterone to a replacement dose of testosterone on strength and body composition in normal men. J Steroid Biochem Mol Biol 1991;40:607–612.

38. Forbes GB. The effect of anabolic steroids on lean body mass: the dose response curve. Metabolism 1985;34:571–573.

39. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, et al. Testosterone dose-response relationships in healthy young men. Am J Physiol Endocrinol Metab 2001; 281: E1172–E1181.

40. Hough DO. Anabolic steroids and ergogenic aids. Am Family Physician 1990;41:1157–1164.

41. Pope HG Jr, Kouri EM, Hudson JI. Effects of supraphysiologic doses of testosterone on mood and aggression in normal men: a randomized controlled trial. Arch Gen Psychiatry 2000;57:133–140.

42. Snyder PJ, Lawrence DA. Treatment of male hypogonadism with testosterone enanthate. J Clin Endocrinol Metab 1980;51:1335–1339.

43. Peng S-H, Segura J, Farre M, Gonzalez JC, del al Torre X. Plasma and urinary markers of oral testosterone undecanoate misuse. Steroids 2002;67:39–50.

44. Saartok T, Dahlberg E, Gustafsson JA. Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. Endocrinology 1984;114:2100–2106.

45. Schanzer W. Metabolism of anabolic androgenic steroids. Clin Chem 1996;42:1001–1020.

46. Schanzer W, Horning S, Donike M. Metabolism of anabolic steroids in humans: synthesis of 6 beta-hydroxy metabolites of 4-chloro-1,2-dehydro-17 alpha-methyltestosterone, fluoxymesterone, and metandienone. Steroids 1995;60:353–366.

47. Masse R, Goudreault D. Studies on anabolic steroids—11,18-hydroxylated metabolites of mesterolone, methenolone and stenbolone: new steroids isolated from human urine. J Steroid Biochem Mol Biol 1992;42:399–410.

48. Zhang GY, Gu YQ, Wang XH, Cui YG, Bremner WJ. A pharmacokinetic study of injectable testosterone undecanoate in hypogonadal men. J Androl 1998;19:761–768.

49. Yucel S, Cavalcanti AG, Wang Z, Baskin LS. The impact of prenatal androgens on vaginal and urogenital sinus development in the female mouse. J Urol 2003;170:1432–1436.

50. Edwards MS, Curtis JR. Decreased anticoagulant tolerance with oxymetholone. Lancet 1971;2:221.

51. Graham MR, Davies B, Grace FM, Kicman A, Baker JS. Anabolic steroid use patterns of use and detection of doping. Sports Med 2008;38:505–525.

52. Council on Scientific Affairs. Medical and nonmedical uses of anabolic-androgenic steroids. JAMA 1990;264:2923–2927.

53. Buttner A, Thieme D. Side effects of anabolic androgenic steroids: pathological findings and structure—activity relationships. Handb Exp Pharmacol 2010;195:459–484.

54. Laseter JT, Russell JA. Anabolic steroid-induced tendon pathology: a review of the literature. Med Sci Sports Exerc 1991;23:1–3.

55. Melchert RB, Welder AA. Cardiovascular effects of androgenic-anabolic steroids. Med Sci Sports Exerc 1995;27:1252–1262.

56. Dhar R, Stout CW, Link MS, Homoud MK, Weinstock J, Estes NA 3rd. Cardiovascular toxicities of performance-enhancing substances in sports. Mayo Clin Proc 2005;80:1307–1315.

57. Webb OL, Laskarzewski PM, Glueck CJ. Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-administered exogenous testosterone and anabolic-androgenic steroids. Metabolism 1984;33:971–975.

58. Parssinen M, Seppala T. Steroid use and long-term health risks in former athletes. Sports Med 2002;32:83–94.

59. Ferenchick GS, Hirokawa S, Mammen EF, Schwartz KA. Anabolic-androgenic steroid abuse in weight lifters: evidence for activation of the hemostatic system. Am J Hematol 1995;49:282–288.

60. Lane HA, Grace F, Smith JC, Morris K, Cockcroft J, Scanlon MF, Davies JS. Impaired vasoreactivity in bodybuilders using androgenic anabolic steroids. Eur J Clin Invest 2006;36:483–488.

61. Fineschi V, Riezzo I, Centini F, Silingardi E, Licata M, Beduschi G, Karch SB. Sudden cardiac death during ana-

bolic steroid abuse: morphologic and toxicologic findings in two fatal cases of bodybuilders. Int J Legal Med 2007; 121:48–53.

62. Furlanello F, Bentivegna S, Cappato R, De Ambroggi L. Arrhythmogenic effects of illicit drugs in athletes. Ital Heart J 2003;4:829–837.

63. Furlanello F, Serdoz LV, Cappato R, De Ambroggi L. Illicit drugs and cardiac arrhythmias in athletes. Eur J Cardiovasc Prev Rehabil 2007;14:487–494.

64. Sánchez-Osorio M, Duarte-Rojo A, Martínez-Benítez B, Torre A, Uribe M. Anabolic-androgenic steroids and liver injury. Liver Int 2008;28:278–282.

65. Cabasso A. Peliosis hepatis in a young adult bodybuilder. Med Sci Sports Exerc 1994; 26:2–4.

66. Soe KL, Soe M, Gluud C. Liver pathology associated with the use of anabolic-androgenic steroids. Liver 1992;12:73–79.

67. Ishak KG, Zimmerman HJ. Hepatotoxic effects of the anabolic/androgenic steroids. Semin Liver Dis 1987; 7:230–236.

68. Luke JL, Farb A, Virmani R, Sample RH. Sudden cardiac death during exercise in a weight lifter using anabolic androgenic steroids: pathological and toxicological findings. J Forensic Sci 1990;35:1441–1447.

69. Hausmann R, Hammer S, Betz P. Performance enhancing drugs (doping agents) and sudden death—a case report and review of the literature. Int J Legal Med 1998; 111, 261–264.

70. Kennedy MC, Lawrence C. Anabolic steroid abuse and cardiac death. Med J Aust 1993;158:346–348.

71. Fineschi V, Baroldi G, Monciotti F, Paglicci Reattelli L, Turillazzi E. Anabolic steroid abuse and cardiac sudden death: a pathologic study. Arch Pathol Lab Med 2001;125: 253–255.

72. Vougiouklakis T, Mitselou A, Batistatou A, Boumba V, Charalabopoulos K. First case of fatal pulmonary peliosis without any other organ involvement in a young testosterone abusing male. Forensic Sci Int 2009;186:e13–e16.

73. Bonetti A, Tirelli F, Catapano A, Dazzi D, Dei Cas A, Solito F, et al. Side effects of anabolic androgenic steroids abuse. Int J Sports Med 2008;29:679–687.

74. Achar S, Rostamian A, Narayan SM. Cardiac and metabolic effects of anabolic-androgenic steroid abuse on lipids, blood pressure, left ventricular dimensions, and rhythm. Am J Cardiol 2010;106:893–901.

75. Thiblin I, Petersson A. Pharmacoepidemiology of anabolic androgenic steroids: a review. Fund Clin Pharmacol 2004;19:27–44.

76. Dean CE. Prasterone (DHEA) and mania. Ann Pharmacother 2000;34:1419–1422.

77. Hall RC, Hall RC, Chapman MJ. Psychiatric complications of anabolic steroid abuse. Psychosomatics 2005;46: 285–290.

78. Thiblin I, Runeson B, Rajs J. Anabolic androgenic steroids and suicide. Ann Clin Psychiatry 1999;11:223–231.

79. Uzych L. Anabolic-androgenic steroids and psychiatric-related effects: a review. Can J Psychiatry 1992;37: 23–28.

80. Brower KJ. Anabolic steroid abuse and dependence. Curr Psychiatry Rep 2002;4:377–387.

81. Corrigan B. Anabolic steroids and the mind. MJA 1996; 165:222–226.

82. Tricker R, Casaburi R, Storer TW, Clevenger B, Berman N, Shirazi A, Bhasin S. The effects of supraphysiological doses of testosterone on angry behavior in healthy eugonadal men–a clinical research center study. J Clin Endocrinol Metab 1996;81:3754–3758.

83. Lefavi RG, Reeve TG, Newland MC. Relationship between anabolic steroid use and selected psychological parameters in male bodybuilders. J Sport Behav 1990;13: 157–166.

84. Beaver KM, Vaughn MG, Delisi M, Wright JP. Anabolic-androgenic steroid use and involvement in violent behavior in a nationally representative sample of young adult males in the United States. Am J Public Health 2008;98: 2185–2187.

85. Pagonis TA, Angelopoulos NV, Koukoulis GN, Hadjichristodoulou CS, Toli PN. Psychiatric and hostility factors related to use of anabolic steroids in monozygotic twins. Eur Psychiatry 2006;21:563–569.

86. Trenton AJ, Currier GW. Behavioral manifestations of anabolic steroid use. CNS Drugs 2005;19:571–595.

87. Parrott AC, Choi PY, Davies M. Anabolic steroid use by amateur athletes: effects upon psychological mood states. J Sports Med Phys Fitness 1994;34:292–298.

88. Pope HG Jr, Katz DL. Psychiatric and medical effects of anabolic-androgenic steroid use. A controlled study of 160 athletes. Arch Gen Psychiatry 1994;51:375–382.

89. Hourigan LA, Rainbird AJ, Dooris M. Intracoronary stenting for acute myocardial infarction (AMI) in a 24-year-old man using anabolic androgenic steroids. Aust NZ J Med 1998;28:838–839.

90. Wysoczanski M, Rachko M, Bergmann SR. Acute myocardial infarction in a young man using anabolic steroids. Angiology 2008;59:376–378.

91. Stergiopoulos K, Brennan JJ, Mathews R, Setaro JF, Kort S. Anabolic steroids, acute myocardial infarction and polycythemia: a case report and review of the literature. Vasc Health Risk Manage 2008;4:1475–1480.

92. Urhausen A, Holpes R, Kindermann W. One- and two-dimensional echocardiography in bodybuilders using anabolic steroids. Eur J Appl Physiol Occup Physiol 1989; 58:633–640.

93. Lau DH, Stiles MK, Shashidhar BJ, Young GD, Sanders P. Atrial fibrillation and anabolic steroid abuse. Int J Cardiol 2007;117:e86–e87.

94. Sullivan ML, Martinez CM, Gallagher EJ. Atrial fibrillation and anabolic steroids. J Emerg Med 1999;17: 851–857.

95. Bispo M, Valente A, Maldonado R, Palma R, Gloria H, Nobrega J, Alexandrino P. Anabolic steroid-induced

cardiomyopathy underlying acute liver failure in a young bodybuilder. World J Gastroenterol 2009;15:2920–2922.

96. Gaede JT, Montine TJ. Massive pulmonary embolus and anabolic steroid abuse. JAMA 1992;267:2328–2329.

97. Nottin S, Nguyen L-D, Terbah M, Obert P. Cardiovascular effects of androgenic anabolic steroids in male bodybuilders determined by tissue Doppler imaging. Am J Cardiol 2006;97:912–915.

98. Santamarina RD, Besocke AG, Romano LM, Ioli PL, Gonorazky SE. Ischemic stroke related to anabolic abuse. Clin Neuropharmacol 2008;31:80–85.

99. Laroche GP. Steroid anabolic drugs and arterial complications in an athlete–a case history. Angiology 1990;41:964–969.

100. Frankle MA, Eichberg R, Zachariah SB. Anabolic androgenic steroids and a stroke in an athlete: case report. Arch Phys Med Rehabil 1988;69:632–633.

101. Akhter J, Hyder S, Ahmed M. Cerebrovascular accident associated with anabolic steroid use in a young man. Neurology 1994;44:2405–2406.

102. Petersson A, Garle M, Granath F, Thiblin I. Morbidity and mortality in patients testing positively for the presence of anabolic androgenic steroids in connection with receiving medical care: A controlled retrospective cohort study. Drug Alcohol Depend. Aug 2006;81:215–220.

103. Calabrese LH, Kleiner SM, Barna BP, Skibinski CI, Kirkendall DT, Lahita RG, Lombardo JA. The effects of anabolic steroids and strength training on the human immune response. Med Sci Sports Exerc 1989;21:386–392.

104. Rich JD, Dickinson BP, Flanigan TP, Valone SE. Abscess related to anabolic-androgenic steroid injection. Med Sci Sports Exerc 1999;31:207–209.

105. Evans NA. Local complications of self administered anabolic steroid injections. Br J Sports Med 1997;31:349–350.

106. Scott MJ, Scott MJ. HIV infection associated with injections of anabolic steroids. JAMA 1989;262:207–208.

107. Rich JD, Dickinson BP, Merriman NA, Flanigan TP. Hepatitis C virus infection related to anabolic-androgenic steroid injection in a recreational weight lifter. Am J Gastroenterol 1998;93:1598.

108. Sanchez-Osorio M, Duarte-Rojo A, Martinez-Benitez B, Torre A, Uribe M. Anabolic-androgenic steroids and liver injury. Liver Int 2007;28:278–282.

109. Kafrouni MI, Anders RA, Verma S. Hepatotoxicity associated with dietary supplements containing anabolic steroids. Clin Gastroenterol Hepatol 2007;5:809–812.

110. Gurakar A, Caraceni P, Fagiuoli S, Van Thiel DH. Androgenic/anabolic steroid-induced intrahepatic cholestasis: a review with four additional case reports. J Okla State Med Assoc 1994;87:399–404.

111. Marquardt GH, Logan CE, Tomhave WG, Dowben RM. Failure of non-17-alkylated anabolic steroids to produce abnormal liver function tests. J Clin Endocrinol Metab 1964;24:1334–1336.

112. Stimac D, Milic S, Dintinjana RD, Kovac D, Ristic S. Androgenic/anabolic steroid-induced toxic hepatitis. J Clin Gastroenterol 2002; 35:350–352.

113. Pagonis TA, Koukoulis GN, Hadjichristodoulou CS, Toli P, Angelopoulos NV. Multivitamins and phospholipids complex protects the hepatic cells from androgenic-anabolic-steroids-induced toxicity. Clin Toxicol 2008;46:57–66.

114. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. J Endocrinol 2001;170:27–38.

115. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996;335:1–7.

116. Hughes M, Ahmed S. Anabolic androgenic steroid induced necrotizing myopathy. Rheumatol Int 2011;31:915–917.

117. Farkash U, Shabshin N, Pritsch Perry M. Rhabdomyolysis of the deltoid muscle in a bodybuilder using anabolic-androgenic steroids: a case report. J Athl Train 2009;44:98–100.

118. Freeman BJ, Rooker GD. Spontaneous rupture of the anterior cruciate ligament after anabolic steroids. Br J Sports Med 1995;29:274–275.

119. Liow RY, Tavares S. Bilateral rupture of the quadriceps tendon associated with anabolic steroids. Br J Sports Med 1995;29:77–79.

120. Wilson JD. Androgen abuse by athletes. Endocr Rev 1988;9:181–199.

121. Kiraly CL, Collan Y, Alen M. Effect of testosterone and anabolic steroids on the size of sebaceous glands in power athletes. Am J Dermatopathol 1987;9:515–519.

122. Lukas SE. Current perspectives on anabolic-androgenic steroid abuse. TIPS 1993;14:61–68.

123. Malarkey WB, Strauss RH, Leizman DJ, Liggett M, Demers LM. Endocrine effects in female weight lifters who self-administer testosterone and anabolic steroids. Am J Obstet Gynecol 1991;165:1385–1390.

124. Bickelman C, Ferries L, Eaton RP. Impotence related to anabolic steroid use in a body builder response to clomiphene citrate. West J Med 1995;162:158–160.

125. Alen M, Hakkinen K: Androgenic steroid effects on serum hormones and on maximal force development in strength athletes. J Sports Med Phys Fitness 1987;27:38–46.

126. Graham S, Kennedy M. Recent developments in the toxicology of anabolic steroids. Drug Saf 1990;5:458–476.

127. Knuth UA, Maniera H, Nieschlag E. Anabolic steroids and semen parameters in bodybuilders. Fertil Steril 1989;52:1041–1047.

128. Boyadjiev NP, Georgieva KN, Massaldjieva RI, Gueorguiev SI. Reversible hypogonadism and azoospermia as a result of anabolic-androgenic steroid use in a bodybuilder with personality disorder. A case report. J Sports Med Phys Fitness 2000;40:271–274.

129. Socas L, Zumbado M, Perez-Luzardo O, Ramos A, Perez C, Hernandez JR, Boada LD. Hepatocellular adenomas associated with anabolic androgenic steroid abuse in bodybuilders: a report of two cases and a review of the literature. Br J Sports Med 2005;39:e27.

130. Martin NM, Abu Dayyeh BK, Chung RT. Anabolic steroid abuse causing recurrent hepatic adenomas and hemorrhage. World J Gastroenterol 2008;14:4573–4575.

131. Rosner F, Khan MT. Renal cell carcinoma following prolonged testosterone therapy. Arch Intern Med 1992;152: 426, 429.

132. Roberts JT, Essenhigh DM. Adenocarcinoma of prostate in 40-year-old body-builder. Lancet 1986;2:742.

133. Froehner M, Fischer R, Leike S, Hakenberg OW, Noack B, Wirth MP. Intratesticular leiomyosarcoma in a young man after high dose doping with Oral-Turinabol: a case report. Cancer 1999;86:1571–1575.

134. Falk H, Thomas LB, Popper H, Ishak KG. Hepatic angiosarcoma associated with androgenic-anabolic steroids. Lancet 1979;4:1120–1123.

135. Johnson FL, Lerner KG, Siegel M, Feagler JR, Majerus PW, Hartmann JR, Thomas ED. Association of androgenic-anabolic steroid therapy with development of hepatocellular carcinoma. Lancet 1972;2:1273–1276.

136. Thevis M, Schanzer W. Mass spectrometry in sports drug testing: Structure characterization and analytical assays. Mass Spectrom Rev 2007;26:79–107.

137. Guan F, Uboh CE, Soma LR, Luo Y, Rudy J, Tobin T. Detection, quantification ad confirmation of anabolic steroids in equine plasma by liquid chromatography and tandem mass spectrometry. J Chromatogr B 2005;829: 56–68.

138. Thevis M, Geyer H, Mareck U, Schanzer W. Screening for unknown synthetic steroids in human urine by liquid chromatography-tandem mass spectrometry. J Mass Spectrom 2005;40:955–962.

139. Wintersteiger R, Sepulveda MJ. Electrochemical detection of anabolics in human plasma and urine. Anal Chim Acta 1993;273:383–390.

140. Lurie IS, Sperling AR, Meyers RP. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC. J Forensic Sci 1994;39:74–85.

141. Pozo OJ, Deventer K, Van Eenoo P, Delbeke FT. Efficient approach for the comprehensive detection of unknown anabolic steroids and metabolites in human urine by liquid chromatography-electrospray-tandem mass spectrometry. Anal Chem 2008;80:1709–1720.

142. Wang C, Shiraishi S, Leung A, Baravarian S, Hull L, Goh V, t al. Validation of testosterone and dihydrotestosterone liquid chromatography tandem mass spectrometry assay: interference and comparison with established methods. Steroids 2008;73:1345–1352.

143. Buiarelli F, Giannetti L, Jasionowska R, Cruciani C, Neri B. Determination of nandrolone metabolites in human urine: comparison between liquid chromatography/tandem mass spectrometry and gas chromatography/ mass spectrometry. Rapid Commun Mass Spectrom 2010; 24:1881–1894.

144. Ayotte C, Goudreault D, Charlebois A. Testing for natural and synthetic anabolic agents in human urine. J Chromatogr B 1996;687:3–25.

145. Kicman AT, Fallon JK, Cowan DA, Walker C, Easmon S, Mackintosh D. *Candida albicans* in urine can produce testosterone: impact on the testosterone/epitestosterone sports drug test. Clin Chem 2002;48:1799–1801.

146. Catlin DH, Cowan DA. Detecting testosterone administration. Clin Chem 1992;38:1685–1686.

147. Catlin DH, Hatton CK, Starcevic SH. Issues in detecting abuse of xenobiotic anabolic steroids and testosterone by analysis of athletes' urine. Clin Chem 1997;43: 1280–1288.

148. Raynaud E, Audran M, Pages JCh, Fedou C, Brun JF, Chanal JL, Orseti A. Determination of urinary testosterone and epitestosterone during pubertal development: a cross-sectional study in 141 normal male subjects. Clin Endocrinol 1993;38:353–359.

149. de Boer D, de Jong EG, van Rossum JM, Maes RA. Doping control of testosterone and human chorionic gonadotrophin: a case study. Int J Sports Med 1991;12: 46–51.

150. Perry PJ, MacIndoe JH, Yates WR, Scott SD, Holman TL. Detection of anabolic steroid administration: ratio of urinary testosterone to epitestosterone vs the ratio of urinary testosterone to luteinizing hormone. Clin Chem 1997;43:731–735.

151. de la Torre X, Gonzalez JC, Pichini S, Pascual JA, Segura J. $^{13}C/^{12}C$ isotope ratio MS analysis of testosterone, in chemicals and pharmaceutical preparations. J Pharm Biomed Anal 2001;24:645–650.

152. Aguilera R, Chapman TE, Starcevic B, Hatton CK, Catlin DH. Performance characteristics of a carbon isotope ratio method for detecting doping with testosterone based on urine diols: controls and athletes with elevated testosterone/epitestosterone ratios. Clin Chem 2001;47: 292–300.

153. Coutts SB, Kicman AT, Hurst DT, Cowan DA. Intramuscular administration of 5 alpha-dihydrotestosterone heptanoate: changes in urinary hormone profile. Clin Chem 1997;43:2091–2098.

154. Debruyckere G, de Sagher R, Van Peteghem C. Clostebol-positive urine after consumption of contaminated meat. Clin Chem 1992;38:1869–1873.

155. Reznik Y, Dehennin L, Coffin C, Mahoudeau J, Leymarie P. Urinary nandrolone metabolites of endogenous origin in man: a confirmation by output regulation under human chorionic gonadotropin stimulation. J Clin Endocrinol Metab 2001;86:146–150.

156. Dehennin L, Bonnaire Y, Plou P. Urinary excretion of 19-norandrosterone of endogenous origin in man: quantitative analysis by gas chromatography-mass spectrometry. J Chromatogr B Biomed Sci Appl 1999;721: 301–307.

157. Lenders JW, Demacker PN, Vos JA, Jansen PL, Hoitsma AJ, van't Laar A, Thien T. Deleterious effects of anabolic steroids on serum lipoproteins, blood pressure, and liver function in amateur body builders. Int J Sports Med 1988;9:19–23.

158. Malmendier CL, van den Bergen CJ, Emplit G, Delcroix C. A long-term study of the efficacy of oxandrolone in hyperlipoproteinemias. J Clin Pharmacol 1978;18:42–53.

159. Hartgens F, Kuipers H. Effects of androgenic-anabolic steroids in athletes. Sports Med 2004;34:513–554.

160. Thompson PD, Cullinane EM, Sady SP, Chenevert C, Saritelli AL, Sady MA, Herbert PN. Contrasting effects of testosterone and stanozolol on serum lipoprotein levels. JAMA 1989;261:1165–1168.

161. Brown JT. Anabolic steroids: what should the emergency physician know? Emerg Med Clin North Am 2005;23:815–826.

162. Di Luigi L, Romanelli F, Lenzi A. Androgenic-anabolic steroids abuse in males. J Endocrinol Invest 2005;28(3 Suppl):S81–S84.

Chapter 16

CLENBUTEROL and SALBUTAMOL (ALBUTEROL)

JAMES RHEE, MD
TIMOTHY ERICKSON, MD

CLENBUTEROL

HISTORY

In 1972, the International Olympic Committee (IOC) banned the use of β_2-agonists including clenbuterol because of concern about the stimulant properties of this class of drug. During the next several decades, the approach of the IOC to the use of inhaled β_2-agonists by athletes changed in response to information on the prevalence of asthma in elite athletes and the lack of anabolic effects of inhaled β_2-agonists.[1] The prevalence of the use of inhaled β_2 agonists by athletes is similar to nonathletes from the *same* country, but β_2-agonist use by athletes varies by sport with the use being high in endurance athletes. During the Sydney and Athens Olympic games, the use of inhaled β_2-agonists by cyclists, triathletes, and swimmers exceeded 11% compared with <2% for weightlifters, boxers, and wrestlers.[2] Similarly, the prevalence of β_2 agonists by cross-country skiers and speed skaters at the Salt City Winer Olympic games exceeded 10.5% compared with <3% for alpine skiing and bobsledding athletes.[3]

Clenbuterol was marketed primarily as a veterinary drug to treat asthma in large animals and to improve muscle mass and muscle/fat ratios in meat-producing animals.[4] During the 1980s, bodybuilders and power athletes began using clenbuterol as an anabolic agent. In addition, the illicit use of clenbuterol to increase muscle mass and reduce fat in food-producing and show animals occurred during the late 1980s.[5] During the early 1990s, several outbreaks of clenbuterol toxicity occurred as a result of the consumption of veal and beef contaminated as a result of the illicit feeding of animals with clenbuterol.[6] The increased use of clenbuterol by bodybuilders and power athletes resulted in the addition of this β_2-agonist to the World Anti-Doping Agency (WADA) list of prohibited drugs. Two athletes competing in field events were disqualified for clenbuterol use during the Barcelona Olympic Games in 1992.[1] This drug is also prohibited by the Association of Official Racing Chemists.

IDENTIFYING CHARACTERISTICS

Clenbuterol (CAS RN: 37148-27-9) or 4-amino-α-[(tert-butylamino)methyl]-3,5-dichlorobenzyl alcohol has a structure similar to other phenethanolamine β-adrenergic agonists as displayed in Figure 16.1. This colorless microcrystalline powder is soluble in water, ethanol, and methanol; the molecular weight is 313.65 g/mol. Beta$_2$-adrenergic agonists contain various substitutions to prevent degradation and to increase selectivity for the β_2-adrenergic receptor. Clenbuterol is structurally similar to albuterol, differing from the latter by the presence of 2 chlorine atoms and an amine group on the benzene ring. These changes purportedly enhance oral absorption, beta$_2$-adrenergic receptor activity, and resistance to inactivation by catechol-*O*-methyl transferase.[7]
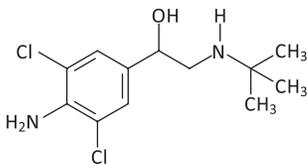
FIGURE 16.1. Chemical structure of clenbuterol.

Clenbuterol is not available as a pharmaceutic preparation in the United States for human use. In some European and South American countries, clenbuterol is available in tablets (10 μg, 20 μg) and as a powder for formulating solutions of varying clenbuterol concentrations. Commercial parenteral preparations are also available.

EXPOSURE

Epidemiology

The prevalence of abuse of clenbuterol or other beta-agonists is not well-documented. According to the WADA in 2004, β_2-adrenergic agonists accounted for 11.5% of all the "adverse analytic findings," defined as "a report from a laboratory or approved testing entity that identifies in a specimen the presence of a prohibited substance or its metabolites or markers (including elevated quantities of endogenous substances) or evidence of the use of a prohibited method.[8]

Sources

Clenbuterol is not approved by the US Food and Drug Administration (FDA), but this drug is approved for the treatment of asthma in some Southern European and South American countries. This drug is approved as oral syrup for the treatment of bronchoconstriction in horses, and illicit sources of clenbuterol in the United States include diversion of veterinary medicine and illegitimate importation. Clenbuterol is the only β-agonist approved in the European Union (EU) for the treatment of nonfood animals including chronic obstructive pulmonary disease in horses and the control of parturition in cattle and sheep.[9] The use of clenbuterol for improvement of the muscle/fat ratio (repartition agent) or muscle mass (anabolic agent) is not approved in the EU.[10] Some dietary supplements marketed through the Internet may contain clenbuterol.[11]

Sources of poisoning with clenbuterol include food poisoning and the contamination of heroin. Several case series report the occurrence of clenbuterol toxicity following the ingestion food contaminated with clenbuterol.[12] Typically, these outbreaks of food poisoning

involve veal, bovine liver, or beef with clenbuterol concentrations exceeding 0.1 μg/kg.[13,14]

Methods of Abuse

The usual route for the abuse of clenbuterol is ingestion as a result of the high oral bioavailability of clenbuterol. Consequently, the administration of clenbuterol parenterally offers no significant increase in bioavailability. Additional drugs used by athletes to enhance the effects of clenbuterol and reduce side effects include niacin, pseudoephedrine, ephedrine, and phenylpropanolamine.[15]

DOSE EFFECT

The recommended adult human dose of clenbuterol in the treatment of asthma is 20–40 μg orally twice daily or 20 μg by inhalation given at 8-hour intervals.[16] There are limited published data on the toxic effects of supratherapeutic doses of clenbuterol. In a controlled clinical study of 5 patients with depression, the ingestion of 100–150 μg clenbuterol daily for 3 weeks was associated with tremor, restlessness, and agitation in all 5 patients.[17] The range of a typical "anabolic" dose of clenbuterol for athletes is about 60–100 μg daily for 6–12 weeks.[18] A case report documented the use of much larger doses of clenbuterol (e.g., 1,575 μg daily for 1½ months) in combination with anabolic steroids.[19] This bodybuilder developed myocardial fibrosis, ventricular hypertrophy, and reduced left ventricular ejection fraction (i.e., 40%).

TOXICOKINETICS

Kinetics

In a study of 9 healthy male volunteers, peak plasma clenbuterol concentrations (i.e., about 0.3 ng/mL) occurred 2–3 hours after the ingestion of a single dose of 20 μg clenbuterol.[20] The bioavailability of a single dose is relatively high (i.e., about 70–80%). The systemic absorption of clenbuterol via inhalation is substantially less than the oral route. *In vitro* studies indicate that clenbuterol is highly protein bound (50–98%);[21] the volume of distribution is also relatively large.

There is evidence of multiple metabolites following hepatic metabolism of clenbuterol, but the structures of these me olites have not been elucidated. The main metabolite is probably a 4-mono-nitro compound and a secondary metabolite formed by dechlorination.[22]

In volunteer studies, renal excretion of unchanged clenbuterol accounts for about 30% of the absorbed dose of clenbuterol.[20] Biliary excretion and renal excretion of conjugated metabolites (i.e., mostly sulfates) account for the remainder of clenbuterol elimination.

The plasma elimination half-life of clenbuterol is approximately 25–39 hours.[14]

Tolerance

Adrenergic receptor desensitization occurs within approximately 2 weeks in rodents following the administration of clenbuterol.[23] Tolerance to β_2-agonists results from the down-regulation of β_2-receptors following daily exposure,[24,25] but this tolerance is rapidly reversible within about 3 days following cessation of use.[26]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Beta-adrenergic agonists activate β-adrenergic receptors located throughout the body. The β_1-adrenergic receptor subtype occurs in the myocardium, and stimulation of these receptors cause inotropic and chronotropic effects. Beta$_2$-adrenergic receptors throughout the body are located in smooth muscle (lung, gastrointestinal tract, uterus, vascular system), skeletal muscle, hepatocytes, and the myocardium. Selective β_2- adrenergic agonists stimulate the β_2-adrenergic receptors by the following mechanisms: 1) directly activating the β_2-receptor (albuterol), 2) transport into a membrane depot (formoterol), and 3) interacting with a receptor-specific auxiliary binding site (salmeterol).[27]

Stimulation of the β_2-adrenergic receptor results in activation of intracellular adenylyl cyclase and the increased conversion of adenosine triphosphate (ATP) to cyclic $3'5'$-adenosine monophosphate (cAMP). cAMP is a major second messenger that activates intracellular protein kinases and ultimately produces biologic effects. These effects include bronchodilation, skeletal muscle arteriolar dilation, splanchnic venodilation, insulin secretion, decreased gut motility, hepatic gluconeogenesis, skeletal muscle glycogenolysis, skeletal muscle potassium uptake, and mast cell silization.

In animal models, the chronic administration of clenbuterol decreases fat deposition, reduces protein breakdown, and stimulates hypertrophy of skeletal muscles, particularly type II fibers.[28,29] The injection of rats with clenbuterol at a dose of 1 mg/kg daily for 15 days reduced epididymal fat pad mass (mean, 39%) while increasing body weight (mean, 9%), protein content (mean, 8%) and water content (mean, 7%) compared with controls.[30] These changes persisted at least 9 days after termination of treatment. However, the doses used in these animal studies are much higher than the doses recommended for humans, and the use of these high doses would be expected to produce adverse effects associated with excessive β_2-adrenergic stimulation.

Although not well studied in humans, existing data do not support improvements in muscle mass or athletic performance in healthy adults using clenbuterol.[31] In animal studies, the administration of clenbuterol increases the rate of muscle protein deposition and independently promotes lipolysis. Skeletal and cardiac muscle hypertrophy, but not hyperplasia occur in animal-feed clenbuterol.[32] The increase in protein synthesis results primarily from inhibition of protein degradation rather than increases in protein synthesis.[23] These animal studies do not demonstrate any alteration of plasma testosterone, insulin, or growth hormone concentrations; furthermore, the anabolic effects may diminish with prolonged treatment.

Mechanism of Toxicity

Toxicity from β_2-adrergic agonists results from excessive β_2 stimulation along with loss of β_2-selectivity following a supratherapeutic dose. Stimulation of β_2-adrenergic receptors promotes smooth muscle relaxation, activate glycogenolysis in the liver, and increases potassium uptake in skeletal muscle. Tachycardia is a common manifestation of toxicity secondary to β_2-adrergic agonists. Mechanisms for the development of tachycardia during an overdose include the following: 1) reflex tachycardia from β_2-adrenergic mediated peripheral vasodilation, 2) direct stimulation of the β_1-adrenergic receptors, and 3) activation of β_2-adrenergic receptors in the right atrium and left ventricle. Beta$_2$-adrenergic receptors stimulate the sodium-potassium ATPase pump and cause an intracellular shift of potassium into the cells, resulting in a relative hypokalemia. Hyperglycemia results from increased glycogenolysis, primarily in the liver. Intracellular sequestration of magnesium and phosphorus also occurs, resulting in hypomagnesemia and hypophosphatemia.[33]

Long-term animal studies suggest the possibility that the chronic administration of β_2-adrenergic agonists alter the structure of the heart. The administration 2 mg clenbuterol/kg daily to rats for 16–18 weeks caused cardiac hypertrophy when compared with controls (no clenbuterol).[34] The structural changes included an increased absolute heart mass of approximately 19% and an increased heart mass/body mass ratio of about 20%.

CLINICAL RESPONSE

Illicit Use

Clinical data on the acute and chronic toxicity of clenbuterol use by athletes is limited. Adverse effects of

β_2-agonists include headache, muscle tremor, muscle cramps during intense exercise, and palpitations.[35] Similarly, toxicity associated with clenbuterol-adulterated heroin includes palpitations, tachycardia, hypotension, tremor, anxiety, diaphoresis, nausea, vomiting, hypokalemia, hypophosphatemia, and hyperglycemia.[36] Rare case reports associate anabolic regimens including clenbuterol use with serious cardiac events. An acute myocardial infarction (peak creatine kinase 1,060 IU/L) occurred in an otherwise healthy 26-year-old bodybuilder using both anabolic steroids (testosterone propionate, cypionate, enanthate, methandrostenolone, stanozolol) and clenbuterol.[37] He stopped using anabolic steroids and started using clenbuterol about 4 weeks before his myocardial infarction. His coronary angiogram was normal. Another case report associated the development of a non-Q wave myocardial infarction with the use of clenbuterol by a 17-year-old male bodybuilder.[38] He reported the use of clenbuterol 40 mg daily for 2 weeks; then a 2-day hiatus prior to the onset of retrosternal chest pain. He denied the use of anabolic steroids. Echocardiography demonstrated hypokinesis of the apex along with mild left ventricular hypertrophy, and an angiogram 5 days after admission was normal. There are few data on the effect of the chronic administration of β_2-adrenergic drugs in humans at doses illicitly used by athletes; therefore, the clinical significance of these animal studies is unclear.

Overdose

The clinical features associated with overdoses involving clenbuterol are characteristic of β_2-adrenergic agonists. Clenbuterol is a long-acting drug that produces prolonged β_2-stimulation during an overdose including headache, dizziness, lightheadedness, tinnitus, muscle weakness, tremor, diaphoresis, nausea, vomiting, anxiety, agitation, chest pain, palpitations, sinus tachycardia (135–160 bpm), hypotension, hypokalemia, hypophosphatemia, metabolic acidosis, and hyperglycemia.[39,40] The most common manifestation of clenbuterol toxicity are tachycardia, agitation, and hypokalemia. Sinus tachycardia is the most common dysrhythmia associated with clenbuterol intoxication, and signs of intoxication can persist up to about 24 hours.[33] A 31-year-old man developed palpitations, dyspnea, and a supraventricular tachycardia (ventricular rate = 254 bpm) after the ingestion of clenbuterol hydrochloride.[41] About 16 hours after ingestion, an electrocardiogram revealed atrial fibrillation with a ventricular rate of 125–147 bpm; later, he was cardioverted to a normal sinus rhythm. Serious ventricular dysrhythmias do not usually occur during clenbuterol intoxication. Mydriasis is an incon-

sistent sign of β_2-adrenergic toxicity.[42] Clenbuterol does not typically cross the blood-brain barrier; therefore seizures do not usually occur during an clenbuterol overdose. This drug is an adulterant of heroin and cocaine, and the insufflation of a white powder containing clenbuterol has been associated with the acute onset of chest pain, palpitations, tremors, headache, nausea, vomiting, and subsequently pulmonary edema with respiratory failure.[43]

Food Poisoning

Outbreaks of clenbuterol toxicity have occurred in Europe (Spain, France, Italy, Portugal) and China following the consumption of clenbuterol-contaminated food, primarily liver from ruminants. In a study of 4 cases of food poisoning involving clenbuterol-contaminated bovine liver, bovine muscle, and lamb meat in Portugal, the most common symptoms were tachycardia, tremors, nausea, and stomach pains as demonstrated in Table 16.1.[44] Random samples of contaminated meat contained approximately 1 mg clenbuterol/kg.

In a series of 15 patients with clinical features of food poisoning after consuming clenbuterol-contaminated veal, the onset of symptoms (palpitations, headache, tachypnea, tremors) occurred between 0.5–3 hours after eating.[12] Symptoms resolved within 3–5 days.

DIAGNOSTIC TESTING

The diagnosis of β_2-adrenergic agonist toxicity in the acute setting is based on the presence of the clinical features of excessive β-adrenergic stimulation. Analytic methods confirm the presence of excessive amounts of β-adrenergic agonist, usually in urine samples.

TABLE 16.1. Incidence of Toxic Effects Associated with the Consumption of Clenbuterol-Contaminated Meat by 50 Patients.[44]

Clinical Effect	Number	% of Total
Tachycardia	41	82
Distal tremors	38	76
Nausea	37	74
Stomach ache	34	68
Diarrhea	25	50
Temperature >37.5°C (99.5°F)	18	36
Headache/dizziness	18	36
Myalgias/weakness	11	22
Hypertension	9	18
Vomiting	9	18
Dry mouth/voice change	5	10

Analytic Methods

Immunoassays (e.g., enzyme-linked immunosorbent assay [ELISA]) are available to screen for the presence of clenbuterol, but cross-reactivity with salbutamol and mabuterol limits the specificity of these tests.[45] For confirmation of the presence of clenbuterol, analytic methods include capillary electrophoresis,[46] high performance liquid chromatography with ultraviolet detection,[47] liquid chromatography/electrospray ionization/mass spectrometry,[48,49] gas chromatography/electrospray ionization/tandem mass spectrometry,[50] and gas chromatography/mass spectrometry.[51] Gas chromatography/mass spectrometry is a sensitive method for the analysis of clenbuterol in urine samples with a detection limit in the range of 0.02 ng/mL.[52] For capillary electrophoresis with laser-induced fluorescence, the limit of detection (LOD) for clenbuterol in biologic samples is about 0.7 ng/mL.[53] The LOD for clenbuterol and salbutamol with liquid chromatography/electrospray tandem mass spectrometry is near 0.1–0.5 ng/mL.[54,55] Using liquid chromatography/tandem mass spectrometry with ion trap technology, the LOD and LLOQ of clenbuterol in liver samples were 0.11 μg/kg and 0.21 μg/kg, respectively.[56]

Biomarkers

Following the ingestion of 80 μg clenbuterol by healthy volunteers, peak plasma concentration of clenbuterol occurred about 2.5 hours after ingestion.[21] In this study, the peak plasma clenbuterol concentration was about 5 ng/mL compared with a steady-state plasma clenbuterol concentration of 0.6 ng/mL following the ingestion of 40 μg clenbuterol twice daily for 4 days. A 28-year-old woman developed tremulousness, vomiting, palpitations, sinus tachycardia, hyperglycemia, hypokalemia, and hypomagnesemia after ingesting a powder containing clenbuterol.[33] Three hours after her ingestion, her serum clenbuterol concentration was 2.93 ng/mL as measured by liquid chromatography/mass spectrometry.

Abnormalities

Laboratory abnormalities associated with β_2-adrenergic overdose include hypokalemia, hypophosphatemia, hypomagnesemia, hyperglycemia, and metabolic acidosis. The severity of hypokalemia correlates to serum clenbuterol concentration. The differential diagnosis of the triad of hypokalemia, hyperglycemia, and hyperlactatemia includes theophylline and caffeine toxicity as well as β-adrenergic agonist overdose.

TREATMENT

The treatment of clenbuterol toxicity is similar to the treatment of other β_2-adrenergic agonists with management focused on symptomatic and supportive care. The principal difference between clenbuterol and other β_2-adrenergic agonists is potency and duration of action. The clinical features of clenbuterol toxicity persist much longer (e.g., half-life 25–39 hours) than salbutamol (half-life 3–6 hours). Patients with signs of clenbuterol toxicity should be monitored closely for dysrhythmias and hypotension secondary to vasodilation with an electrocardiogram, cardiac monitoring, and frequent vital signs. These patients should also be evaluated for the presence of metabolic and electrolyte abnormalities.

Gut Decontamination

The administration of activated charcoal to alert patients who ingest clenbuterol within 1 hour prior to presentation is a therapeutic option,[57] but there are no clinical data to confirm the clinical efficacy of activated charcoal on the outcome of clenbuterol intoxication.

Antidotes

Case reports suggest that α- and β-adrenergic antagonists are *potential* antidotes for β-adrenergic agonist toxicity, particularly following the development of a clinically significant tachydysrhythmia or hypotension unresponsive to intravenous (IV) fluid therapy.[58] However, there are no specific IV β_2-blockers. Typically, noninvasive cardiac monitoring in patients with tachycardia, lactic acidosis, and hypotension demonstrate low systemic vascular resistance with high cardiac output. Nonselective β-adrenergic antagonists should not be administered prematurely or indiscriminately because excessive use of these drugs in the setting of a clenbuterol overdose may cause a serious deterioration in the vital signs as a result of unopposed α-adrenergic stimulation. Although case reports suggest that β-adrenergic antagonist can be administered to asthmatic patients judiciously,[59] these drugs must be administered cautiously to patients with a history of bronchospasm or chronic obstructive pulmonary disease. Theoretically, the initial use of a short-acting β-adrenergic antagonist is advantageous [e.g., esmolol drip, IV metoprolol (5 mg initial IV bolus to adults)] to quickly reverse any adverse effects from the use of these drugs. If the patient responds to initial doses of a short-acting β-adrenergic antagonist and no adverse effects occur, a longer-acting β-adrenergic antagonist (e.g., propranolol 0.5–1 mg intravenously) can be administered to control clinically

significant symptoms of intoxication. Metabolic abnormalities (e.g., hypokalemia, hyperglycemia, hypomagnesemia) resolve with improvement in clinical symptoms; therefore, only supportive care is necessary for these metabolic abnormalities.

Supplemental Care

Most patients with tachycardia and hypotension secondary to β-adrenergic agonist intoxication respond to supportive care with IV saline and vasopressors usually are not required. Hypokalemia typically results from an intracellular shift of potassium rather than a loss of extracellular potassium. Consequently, the total body burden of potassium is not reduced during β-adrenergic agonist poisoning; therefore, potassium supplements should be administered cautiously as small potassium infusions only in the setting of severe or symptomatic hypokalemia. Aggressive treatment of this relative hypokalemia potentially can cause hyperkalemia when the β-adrenergic agonist activity of these drugs resolve. The hypomagnesemia, hypophosphatemia, and hyperglycemia do not usually require therapy, unless severe abnormalities occur. Seizures are uncommon during β-adrenergic agonist toxicity, and IV benzodiazepines are the anticonvulsants of choice. If seizures occur, other sources of seizures should be considered including toxicity by other sympathomimetic agents or theophylline.

SALBUTAMOL (ALBUTEROL)

HISTORY

In 1972, the International Olympic Committee (IOC) banned the use of β₂-agonists because of concern about the stimulant properties of this class of drug. During the next several decades, the approach of the IOC to the use of inhaled β₂-agonists by athletes changed in response to information on the prevalence of asthma in elite athletes and the lack of anabolic effects of inhaled β₂-agonists.[1] In 1993, the Medical Commission of the International Olympic Committee re-introduced the notification requirement for inhaled β₂-agonist and limited the use of inhaled β₂-agonists to salbutamol and terbutaline. Inhaled formoterol was added to this list in 2001. During that year, the IOC established the Independent Asthma Panel to oversee the required submission of evidence of asthma or exercise-induced
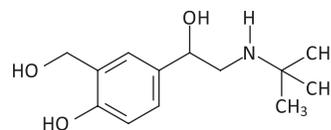
FIGURE   16.2. Chemical   structure   of   salbutamol (albuterol).

asthma by athletes using β₂-agonists. Accepted evidence includes positive bronchoconstrictor response, positive methacholine   challenge,   positive   bronchodilator response, and confirmatory medical records.

IDENTIFYING CHARACTERISTICS

Albuterol (CAS RN: 18559-94-9) is the official generic name in the United States, whereas salbutamol (CAS RN: 35763-26-9) is the name recommended by the World Health Organization. Figure 16.2 displays the chemical structure of salbutamol (albuterol). Salbutamol is a racemic mixture of 2 enantiomers: $S(+)$- and $R(-)$-salbutamol. The $R(-)$ has most of the pharmacological activity, whereas the $S(+)$ enantiomer is inactive.[60]

EXPOSURE

The IOC permits the use of salbutamol in athletes with documented asthma or exercise-induced asthma after the approval of a therapeutic use exemption by an independent medical panel. This β₂-agonists is the only one approved by WADA with the following limitation: 1) the use of only the inhalant is permitted as salbutamol is both a stimulant and an anabolic agent when administered   orally, 2)   the   maximum   permitted   use   is 1,600 μg/24 hours, and 3) the presence of urine salbutamol concentrations exceeding 1,000 ng/mL is presumed evidence of salbutamol abuse.[61] The IOC requires documented evidence of bronchoconstriction prior to the use of these β₂-agonists. All oral β₂-agonists are prohibited. Although salbutamol is available in inhaled form, the lack of anabolic effects by this route limits the abuse of this β₂-adrenergic agonist.

DOSE EFFECT

Although inhaled dose of salbutamol typically do not cause ergogenic or anabolic effects, the oral administration   of   salbutamol   to   non-asthmatic   individuals can improve muscle strength, anaerobic power and endurance. However, the typical oral dose used for ergogenic effects is 10–20-fold higher than the maximal therapeutic dose of inhaled salbutamol (800–1,600 μg daily).[62]

TOXICOKINETICS

Absorption/Distribution

In contrast to the high bioavailability of clenbuterol, the average absorption of salbutamol is about 50% of an oral dose. In a study of 10 healthy volunteers ingesting 4 salbutamol doses of 4 mg divided over 24 hours, the mean systemic bioavailability was 50% ± 4%.[63] The mean bioavailability of salbutamol in 10 healthy volunteers following inhalation of 400 µg and the ingestion of 4 mg was 57.4% ± 14.9% and 63.3% ± 10.9%, respectively.[64] In 10 healthy volunteers receiving IV salbutamol, the mean V_d was 156 L ± 38 L.[63]

Biotransformation

Salbutamol metabolism occurs in the intestine and liver almost exclusively by conjugation with sulfate at the phenolic group. The active $R(-)$-enantiomer undergoes a higher rate of conjugation with sulfur than the inactive $S(+)$-enantiomer; therefore, the kidneys excrete a relatively larger portion of free $S(+)$ in the urine after ingestion than after inhalation. The free $S(+)/R(-)$ ratio is close to unity during the first hour after inhalation of salbutamol because of the lack of salbutamol biotransformation in the lung.[65] Following oral administration the free $S(+)/R(-)$ ratio typically exceeds 2.5 as a result of the higher conjugation of the active $R(-)$-enantiomer. Because of the variable ingestion of inhaled salbutamol, the free $S(+)/R(-)$ ratio >2.5 alone is not sufficient evidence of the ingestion of salbutamol.[66] There are few data on the potential saturation of the sulfate pathway following the ingestion of high doses of salbutamol.

Elimination

The plasma half-life of salbutamol (albuterol) is substantially less than clenbuterol with a mean half-life of about 3–6 hours.[67] The kidneys excrete salbutamol as a mixture of unchanged drug and conjugated metabolites, primarily as sulfates. Experimental studies in healthy volunteers indicate that urine salbutamol concentrations depend on the route of exposure with oral or subcutaneous administration of salbutamol producing higher and more prolonged urine salbutamol concentrations than inhalation.[68] The ratio of free/conjugated drug depends on the route of administration. Following the oral administration of 16 mg salbutamol divided over 24 hours, the mean urinary excretion of unchanged salbutamol and the sulfate conjugate was 31.8% ± 1.9% and 48.2% ± 7.3%, respectively.[63] Following IV administration of salbutamol, the mean urinary excretion of unchanged salbutamol and the sulfate conjugate was

64.2% ± 7.1% and 12.0% ± 3.1%, respectively. The values resulting from the administration of salbutamol by these 2 routes were significantly different ($P < .001$). In a study of asthmatic patients ingesting ^{3}H-salbutamol, approximately 76% (range, 65–84%) of the dose was excreted over 3 days in the urine with a majority of the excretion occurring in the first 24 hours as measured by total radioactivity.[69] During this period, the feces contained about 4% of the absorbed dose (range, 1–7%). In a study of 10 healthy volunteers, the mean urinary elimination half-lives of salbutamol following ingestion (4 mg) and inhalation (400 µg) were 5.7 hours ± 1.4 hours and 6.1 hours ± 2.1 hours, respectively.[64]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

There are limited data on anabolic effects and physical performance parameters following the doses of β_2 agonists typically used to treat asthma. In a study of 12 healthy volunteers receiving 16 mg sustained-release, *oral* salbutamol daily for 3 weeks, quadriceps strength increased 12% ± 3% compared with placebo.[70] However, the effect was variable as there was no change in grip strength in either hand during the trial. Most double-blind, controlled clinical trials do not demonstrate a statistically significant improvement in physical performance of atopic or non-atopic athletes treated with *therapeutic* doses of *inhaled* β_2-agonists including salbutamol and terbutaline, even at supratherapeutic doses up to 1200 µg daily.[62] A double-blind, placebo-controlled study of 7 nonasthmatic, elite cyclists demonstrated no ergogenic benefit of a therapeutic dose of inhaled salbutamol.[71] Measures of physical performance included maximum heart rate, endurance sprint time, peak power, and total work.

In contrast to the lack of ergogenic effects following the therapeutic use of *inhaled* salbutamol, the administration of *oral* doses of salbutamol has been associated with improved strength and endurance during intense submaximal exercise. A randomized, placebo-controlled, double-blind study of 8 recreational, nonasthmatic athletes demonstrated increased endurance during intense submaximal exercise (cycling) following the administration of oral salbutamol 12 mg daily for 3 weeks.[72] The cycling time was significantly increased ($P < .05$) after 3 weeks of salbutamol administration (30.5 min ± 3.1 min) compared with placebo (23.7 min ± 1.6 min). In a double-blind randomized cross-over study of 16 non-asthmatic men, the mean endurance times on a cycle ergometer for the placebo and treatment (i.e., 4 mg salbutamol) groups were 3,039 ± 1,031 seconds and 3,439 ± 1,287, respectively. The difference was not statistically significant ($P = .19$).[73] However, following the

exclusion of 4 athletes with adverse effects, the mean endurance of the salbutamol group was statistically different ($P < .05$) than the placebo group.

CLINICAL RESPONSE

Similar to clenbuterol, the clinical features associated with overdoses involving salbutamol are characteristic of β_2-adrenergic agonists including headache, diaphoresis, tremor, anxiety, lightheadedness, tinnitus, nausea, vomiting, chest pain, palpitations, tachycardia, hypotension, hyperglycemia, metabolic acidosis, hypokalemia, and hypophosphatemia. Serious ventricular dysrhythmias do not usually occur during salbutamol intoxication despite the presence of hypokalemia.[74] Rare case reports temporally associate the therapeutic use of inhaled or IV salbutamol with the development of myocardial injury in patients without evidence of coronary artery obstruction.[75]

DIAGNOSTIC TESTING

Analytic Methods

Immunoassays (e.g., ELISA) are available to screen for the presence of salbutamol, but this technique demonstrates cross-reactivity with other β_2-adrenergic agonists including clenbuterol.[76] Analytic methods to quantify salbutamol in biologic samples in addition to liquid chromatography/thermospray/tandem mass spectrometry include high performance liquid chromatography with fluorometric detection,[77] liquid chromatography/ electrospray ionization/mass spectrometry in selected ion monitoring with nadolol as the internal standard,[78] capillary electrophoresis with ultraviolet detection (205 nm),[79] and gas chromatography/mass spectrometry.[80] The latter method requires extraction (liquid-liquid, solid-phase) and derivatization. Analysis of urine with ultraperformance liquid chromatography/tandem mass spectrometry and electrospray ionization in positive ion mode, the lower limit of quantitation (LLOQ) for salbutamol is about 0.2 ng/mL with intraassay precision and accuracies <8.4%.[81] In comparison, the LLOQ following analysis by liquid chromatography/electrospray ionization/mass spectrometry is 10 ng/mL with an accuracy of ± 2.6%. Using solid-phase extraction and derivatization with N-methyl-trimethylsilytrifluoroaceta mide, the LOD and LLOQ for salbutamol using gas chromatography/mass spectrometry were 30 ng/mL and 111 ng/mL, respectively.[82] In this study, the stability of free salbutamol in urine samples was relatively stable over 60 days in frozen storage ($-18°C/-0.4°F$) and in refrigeration ($4°C/39.2°F$). The estimated loss of free salbutamol over this period was 2–4% and 8–12%, respectively. Rare case reports associate the use of high doses of salbutamol (albuterol) with elevated serum concentrations of creatine kinase, primarily following ingestion and less often with inhaled doses.[83]

Biomarkers

PLASMA. Peak plasma concentrations of salbutamol following the ingestion of therapeutic doses (4–8 mg) by healthy volunteers range from 10–18 ng/mL.[63] Following the ingestion of an estimated 320 mg salbutamol, a 16-year-old asthmatic developed tremor, hypokalemia, and sinus tachycardia.[74] Her plasma salbutamol concentration about 2 hours after ingestion was 449 ng/mL. She recovered without sequelae. The whole blood/plasma ratio of salbutamol is approximately 1. Following the IV administration of salbutamol to healthy volunteers, the mean whole blood/plasma ratio was 0.96 ± 0.13.[63]

URINE. Both the total and free salbutamol concentrations and the ratio of $S(+)$- and $R(-)$-enantiomers are useful biomarkers for distinguishing oral and therapeutic (inhaled) use of salbutamol. The World Anti-Doping Agency established urine total (free plus glucuronides) salbutamol concentration exceeding 1,000 ng/mL in urine samples (uncorrected for urine specific gravity) as an indication of the potential oral use of salbutamol. In experimental studies of healthy volunteers receiving 200–800 µg salbutamol via inhalation, peak urine nonsulfonated salbutamol concentrations up to about 900 ng/mL occurred approximately 1 hour after administration.[84] Several factors besides individual variation in biotransformation of salbutamol can cause falsepositive results using the WADA criteria including the administration of high doses (e.g., 800–1,000 µg over 3 hours prior to competition) and dehydration.[85,86] Immunoassays (e.g., ELISA) measure total salbutamol concentrations in urine samples, whereas gas chromatography/mass spectrometry detects nonsulfated (unconjugated) salbutamol.

The conjugation rate of the $S(+)$-enantiomer is higher than the $R(-)$-enantiomer following oral salbutamol administration when compared with inhalation of salbutamol. The $S(+)/R(-)$ ratio usually exceeds 2.5 after oral use of salbutamol compared with <2.5 following inhalation of salbutamol.[87] However, some false-positive results occur, probably as a consequence of swallowing some of the dose of inhaled salbutamol.

Abnormalities

Laboratory abnormalities associated with β_2-adrenergic overdose include hypokalemia, hypophosphatemia, hypomagnesemia, hyperglycemia, and metabolic acido-

sis.[88,89] The severity of hypokalemia correlates to serum salbutamol concentration.[33]

TREATMENT

The treatment of clenbuterol and salbutamol toxicity is similar. The clinical features of clenbuterol toxicity persist much longer (e.g., half-life 25–39 hours) than salbutamol (half-life 3–6 hours).

References

1. Fitch KD. β2-Agonists at the Olympic Games. Clin Rev Allergy Immunol 2006;31:259–268.

2. Anderson SD, Sue-Chu M, Perry CP, Gratziou C, Kippelen P, McKenzie DC, et al: Bronchial challenges in athletes applying to inhale a beta₂-agonist at the 2004 Summer Olympics. J Allergy Clin Immunol 2006;117:767–773.

3. Anderson SD, Fitch K, Perry CP, Sue-Chu M, Crapo R, McKenzie D, Magnussen H. Responses to bronchial challenge submitted for approval to use inhaled beta₂-agonists before an event at the 2002 Winter Olympics. J Allergy Clin Immunol 2003;111:45–50.

4. Yang YT, McElligott MA. Multiple actions of beta-adrenergic agonists on skeletal muscle and adipose tissue. Biochem J 1989;261:1–10.

5. Mitchell GA, Dunnavfan G. Illegal use of β-adrenergic agonists in the United States. J Anim Sci 1998;76: 208–211.

6. Pulce C, Lamaison D, Keck G, Bostvironnois C, Nicolas J, Descotes J. Collective human food poisonings by clenbuterol residues in veal liver. Vet Hum Toxicol 1991;33: 480–481.

7. Grassi V, Daniotti S, Schiassi M, Dottorini M, Tantucci C. Oral beta₂-selective adrenergic bronchodilators. Int J Clin Pharmacol Res 1986; 6, 93–103.

8. World Anti-Doping Agency, LABSTATS 2004, http://www.wada-ama.org/rtecontent/document/LABSTATS_2004.pdf, accessed 2/10/2011.

9. Prezelj A, Obreza A, Pecar S. Abuse of clenbuterol and its detection. Curr Med Chem 2003;10:281–290.

10. Kuiper HA, Noordam MY, van Dooren-Flipsen MM, Schilt R, Roos AH. Illegal use of β-adrenergic agonists: European community. J Anim Sci 1998;75:195–207.

11. Parr MK, Koehler K, Geyer H, Guddat S, Schanzer W. Clenbuterol marketed as dietary supplement. Biomed Chromatogr 2008;22:298–300.

12. Brambilla G, Cenci T, Franconi F, Galarini R, Macri A, Rondoni F, et al. Clinical and pharmacological profile in a clenbuterol epidemic poisoning of contaminated beef meat in Italy. Toxicol Lett 2000;114:47–53.

13. Salleras L, Dominguez A, Mata E, Taberner JL, Moro I, Salva P. Epidemiologic study of an outbreak of clenbuterol poisoning in Catalonia, Spain. Public Health Rep 1995; 110:338–342.

14. Sporano V, Grasso L, Esposito M, Oliviero G, Brambilla G, Loizzo A. Clenbuterol residues in non-liver containing meat as a cause of collective food poisoning. Vet Hum Toxicol 1998;40:141–143.

15. Prather ID, Brown DE, North P, Wilson JR. Clenbuterol: a substitute for anabolic steroids? Med Sci Sports Exerc 1995;27:1118–1121.

16. Keelan P, Gray P, Cox GA. A multiple-dose response study of clenbuterol in bronchial asthma. Ir Med J 1980;73: 382–384.

17. Wiegand M, Schreiber W, Lauer C, Berger M. The action of clenbuterol on sleep and symptomatology in depressives. Pharmacopsychiatry 1991;24:89–92.

18. Kennedy MC. Newer drugs used to enhance sporting performance. Med J Aust 2000;173:314–317.

19. Nieminen MS, Ramo MP, Viitasalo M, Heikkila P, Karjalainen J, Mantysaari M, Heikkila J. Serious cardiovascular side effects of large doses of anabolic steroids in weight lifters. Eur Heart J 1996;17:1576–1583.

20. Couet W, Girault J, Reigner BG, Ingrand I, Bizouard J, Acerbi D, et al. Steady-state bioavailability and pharmacokinetics of ambroxol and clenbuterol administered alone and combined in a new oral formulation. Int J Clin Pharmacol Ther Toxicol 1989;27:467–472.

21. Yamamoto I, Iwata K, Nakashima M. Pharmacokinetics of plasma and urine clenbuterol in man, rat, and rabbit. J Pharmacobiodyn 1985;8:385–391.

22. Brambilla G, di Bez S, Pietraforte D, Minetti M, Campanella L, Loizzo A. *Ex vivo* formulation of gastric metabolites of clenbuterol preliminary characteristics of their chemical structure. Anal Chim Acta 2007;586:426–431.

23. Reeds PJ, Hay SM, Dorwood PM, Palmer RM. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. Br J Nutr 1986;56:249–258.

24. Rothwell NJ, Stock MJ, Sudera DK. Changes in tissue blood flow and beta-receptor density of skeletal muscle in rats treated with the beta₂-adrenoceptor agonist clenbuterol. Br J Pharmacol 1987;90:601–607.

25. Sillence MN, Matthews ML, Spiers WG, Pegg GG, Lindsay DB. Effects of clenbuterol, ICI118551 and sotalol on the growth of cardiac and skeletal muscle and on beta₂-adrenoceptor density in female rats. Naunyn Schmiedebergs Arch Pharmacol 1991;344:449–453.

26. Haney S, Hancox RJ. Rapid onset of tolerance to beta-agonist bronchodilation. Respir Med 2005;99:566–571.

27. Johnson M. The beta adrenergic receptor. Am J Respir Crit Care Med 1998;58:S146–S153.

28. Kim YS, Sainz RD. Beta-adrenergic agonists and hypertrophy of skeletal muscles. Life Sci 1992;50:397–407.

29. Chow JJ, Horan MA, Little RA. Anabolic effects of clenbuterol on skeletal muscle mediated by beta-2 adrenoreceptor activation. Am J Physiol 1992; 263:E50–E56.

30. Cartana J, Segues T, Yebras M, Rothwell NJ, Stock MJ. Anabolic effects of clenbuterol after long-term treatment and withdrawal in the rat. Metabolism 1994;43: 1086–1092.

31. Spann C, Winter ME. Effect of clenbuterol on athletic performance. Ann Pharmacother 1995; 29:75–77.

32. Emery PW, Rothwell NJ, Stock MJ, Winter PD. Chronic effects of beta $_2$-adrenergic agonists on body composition and protein synthesis in the rat. Biosci Rep 1984;4:83–91.

33. Hoffman RJ, Hoffman RS, Freyberg CL, Poppenga RH, Nelson LS. Clenbuterol ingestion causing prolonged tachycardia, hypokalemia, and hypophosphatemia with confirmation by quantitative levels. J Toxicol Clin Toxicol 2001;39:339–344.

34. Duncan ND, Williams DA, Lynch GS. Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats. Clin Sci (Lond) 2000; 98: 339–347.

35. Whitsett TL, Manion CV, Wilson MF. Cardiac, pulmonary and neuromuscular effects of clenbuterol and terbutaline compared with placebo. Br J Clin Pharmacol 1981;12: 195–200.

36. Werder G, Arora G, Frisch A, Aslam S, Imani F, Missri J. Clenbuterol-contaminated heroin: cardiovascular and metabolic effects. Conn Med 2006;70:5–11.

37. Goldstein DR, Dobbs T, Krull B, Plumb VJ. Clenbuterol and anabolic steroids: a previously unreported cause of myocardial infarction with normal coronary arteriograms. South Med J 1998;91:780–784.

38. Kierzkowska B, Stanczyk J, Kasprzak JD. Myocardial infarction in a 17-year-old body builder using clenbuterol. Circ J 2005;69:1144–1146.

39. Chodorowski Z, Sein Anand J. Acute poisoning with clenbuterol–a case report. Przegl Lek 1997;54:763–764.

40. Ramoska EA, Henretig F, Joffe M, Spiller HA. Propranolol treatment of albuterol poisoning in two asthmatic patients. Ann Emerg Med 1993; 22, 1474–1476.

41. Daubert GP, Mabasa VH, Leung VW, Aaron C. Acute clenbuterol overdose resulting in supraventricular tachycardia and atrial fibrillation. J Med Toxicol 2007;3:56–60.

42. Jarvie DR, Thompson AM, Dyson EH. Laboratory and clinical features of self-poisoning with salbutamol and terbutaline. Clin Chim Acta 1987;168:313–322.

43. Schechter E, Hoffman RS, Stajic M, Tarabar A. Pulmonary edema and respiratory failure associated with clenbuterol exposure. Am J Emerg Med 2007;25:735.e1–735.e3.

44. Barbosa J, Cruz C, Martins J, Silva JM, Neves C, Alves C, et al. Food poisoning by clenbuterol in Portugal. Food Addit Contam 2005;22:563–566.

45. Xu T, Wang BM, Sheng W, Li QX, Shao XL, Li J. Application of an enzyme-linked immunosorbent assay for the detection of clenbuterol residues in swine urine and feeds. J Environ Sci Health B 2007;42:173–177.

46. Toussaint B, Palmer M, Chiap P, Hubert P, Crommen J. On-line coupling of partial filling-capillary zone electrophoresis with mass spectrometry for the separation of clenbuterol enantiomers. Electrophoresis 2001;22: 1363–1372.

47. Blomgren A, Berggren C, Holmberg A, Larsson F, Sellergren B, Ensing K. Extraction of clenbuterol from calf urine using a molecularly imprinted polymer followed by quantitation by high-performance liquid chromatography with UV detection. J Chromatogr A 2002;975:157–164.

48. Lin Z, Zhang S, Zhao M, Yang C, Chen D, Zhang X. Rapid screening of clenbuterol in urine samples by desorption electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 2008;22:1882–1888.

49. Crescenzi C, Bayoudh S, Cormack PA, Klein T, Ensing K. Determination of clenbuterol in bovine liver by combining matrix solid-phase dispersion and molecular imprinted solid-phase extraction followed by liquid chromatography/ electrospray ion trap multiple-stage mass spectrometry. Anal Chem 2001;73:2171–2177.

50. Thevis M, Opfermann G, Schanzer W. Liquid chromatography/electrospray ionization tandem mass spectrometric screening and confirmation methods for beta$_2$ agonists in human or equine urine. J Mass Spectrom 2003;38:1197–1206.

51. Ayotte C, Goudreault D, Charlebois A. Testing for natural and synthetic anabolic agents in human urine. J Chromatogr B Biomed Appl 1996;687:3–25.

52. Keskin S, Ozer D, Temizer A. Gas chromatography-mass spectrometric analysis of clenbuterol from urine. J Pharm Biomed Anal 1998;18:639–644.

53. Zhou J, Xu X, Wang Y. Competitive immunoassay for clenbuterol using capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr B 2007:848: 226–231.

54. Hogendoorn EA, van Zoonen P, Polettini A, Marrubini Bouland G, Montagna M. The potential of restricted access media columns as applied in coupled-column LC/LC-TSP/ MS/MS for the high-speed determination of target compounds in serum. Application to the direct trace analysis of salbutamol and clenbuterol. Anal Chem 1998;70: 1362–1368.

55. van Rhijn JA, O'Keeffe M, Heskamp HH, Collins S. Rapid analysis of beta-agonists in urine by thermospray tandem mass spectrometry. J Chromatogr A 1995;712:67–73.

56. De Wasch K, De Brabander H, Courtheyn D. LC-MS-MS to detect and identify four beta-agonists and quantify clenbuterol in liver. Analyst 1998;123:2701–2705.

57. Leikin JB, Linowieciki KA, Soglin DF. Hypokalemia after pediatric albuterol overdose: A case series. Am J Emerg Med 1994;12:64–66.

58. Maistro S, Chiesa E, Angeletti R, Brambilla G. Beta blockers to prevent clenbuterol poisoning. Lancet 1995;346 (8968):180.

59. Ramoska EA, Henretig F, Joffe M, Spiller HA. Propranolol treatment of albuterol poisoning in two asthmatic patients. Ann Emerg Med 1993;22:1474–1476.

60. Brittain RT, Farmer JB, Marshall RJ. Some observations on the β-adrenoceptor agonist properties of the isomers of salbutamol. Br J Pharmacol 1973;48:144–147.

61. World Anti-Doping Agency. The 2010 prohibited list. International standards. http://www.wada-ama.org/ Documents/World_Anti-Doping_Program/WADP-

Prohibited-list/WADA_Prohibited_List_2010_EN.pdf. Accessed 2/10/2011.

62. Kindermann W. Do inhaled β2-agonists have an ergogenic potential in non-asthmatic competitive athletes? Sports Med 2007;37:95–102.

63. Morgan DJ, Paull JD, Richmond BH, Wilson-Evered E, Ziccone SP. Pharmacokinetics of intravenous and oral salbutamol and its sulphate conjugate. Br J Clin Pharmacol 1986;22:587–593.

64. Hindle M, Chrystyn H. Determination of the relative bio-availability of salbutamol to the lung following inhalation. Br J Clin Pharmacol 1992;34:311–315.

65. Ward JK, Dow J, Dallow N, Eynott P, Milleri S, Ventresca GP. Enantiomeric disposition of inhaled, intravenous and oral racemic-salbutamol in man–no evidence of enantioselective lung metabolism. Br J Clin Pharmacol 2000;49: 15–22.

66. Berges R, Segura J, Ventura R, Fitch KD, Morton AR, Farre M, et al. Discrimination of prohibited oral use of salbutamol from authorized inhaled asthma treatment. Clin Chem 2000;46:1365–1375.

67. Boner AL, Vallone G, Brighenti C, Schiassi M, Miglioranzi P, Richelli C. Comparison of the protective effect and duration of action of orally administered clenbuterol and salbutamol on exercised-induced asthma in childhood. Pediatr Pulmonol 1988;4:197–200.

68. Pichon A, Venisse N, Krupka E, PeraultPochat MC, Denjean A. Urinary and blood concentrations of β2-agonists in trained subjects: comparison between routes of use. Int J Sports Med 2006;27:187–192.

69. Evans ME, Walker SR, Brittain RT, Paterson JW. The metabolism of salbutamol in man. Xenobiotica 1973;3: 113–120.

70. Martineau L, Horan MA, Rothwell NJ, Little RA. Salbutamol, a beta 2-adrenoceptor agonist, increases skeletal muscle strength in young men. Clin Sci (Lond) 1992;83: 615–621.

71. Meeuwisse WH, McKenzie DC, Hopkins SR, Road JD. The effect of salbutamol on performance in elite nonasthmatic athletes. Med Sci Sports Exerc 1992;24:1161–1166.

72. Collomp K, Candau R, Lasne F, Labsy Z, Prefaut C, De Ceaurriz J. Effects of short-term oral salbutamol administration on exercise endurance and metabolism. J Appl Physiol 2000;89:430–436.

73. van Baak MA, Mayer LH, Kempinski RE, Hartgens F. Effect of salbutamol on muscle strength and endurance performance in nonasthmatic men. Med Sci Sports Exerc 2000;32:1300–1306.

74. Lewis LD, Essex E, Volans GN, Cochrane GM. A study of self poisoning with oral salbutamol–laboratory and clinical features. Hum Exp Toxicol 1993;12:397–401.

75. Fisher AA, Davis MW, McGill DA. Acute myocardial infarction associated with albuterol. Ann Pharmacother 2004;38:2045–2049.

76. Hahnau S, Julicher B. Evaluation of commercially available ELISA test kits for the detection of clenbuterol and other beta2-agonists. Food Addit Contam 1996;13: 259–274.

77. Berges R, Segura J, de la Torre X, Ventura R. Analytical methodology for enantiomers of salbutamol in human urine for application in doping control. J Chromatogr B Biomed Sci Appl 1999;723:173–184.

78. Zhang J, Xu Y, Di X, Wu M. Quantitation of salbutamol in human urine by liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr B 2006;831: 328–332.

79. Sirichai S, Khanatharana P. Rapid analysis of clenbuterol, salbutamol, procaterol, and fenoterol in pharmaceuticals and human urine by capillary electrophoresis. Talanta 2008;76:1194–1198.

80. Eenoo PV, Delbeke FT. Detection of inhaled salbutamol in equine urine by ELISA and GC/MS2. Biomed Chromatogr 2002;16:513–516.

81. Ventura R, Ramirez R, Monfort N, Segura J. Ultraperformance liquid chromatography tandem mass spectrometric method for direct quantification of salbutamol in urine samples in doping control. J Pharmaceut Biomed Anal 2009;50:886–890.

82. Forsdahl G, Gmeiner G. Quantification and stability of salbutamol in human urine. J Sep Sci 2004;27:110–114.

83. Craig TJ, Smits W, Soontornniyomkiu V. Elevation of creatine kinase from skeletal muscle associated with inhaled albuterol. Ann Allergy Asthma Immunol 1996;77: 488–490.

84. Sporer BC, Sheel AW, Taunton J, Rupert JL, McKenzie DC. Inhaled salbutamol and doping control: effects of dose on urine concentrations. Clin J Sport Med 2008;18: 282–285.

85. Jacobson GA, Peterson GM, McLean S. Investigation of urinary levels of salbutamol in asthmatic patients receiving inhaled therapy. J Clin Pharm Ther 1997;22:119–126.

86. Schweizer C, Saugy M, Kamber M. Doping test reveals high concentrations of salbutamol in a Swiss track and field athlete. Clin J Sport Med 2004;14:312–315.

87. Ventura R, Segura J, Berges R, Fitch KD, Morton AR, Berruezo S, Jimenez C. Distinction of inhaled and oral salbutamol by urine analysis using conventional screening procedures for doping control. Ther Drug Monit 2000;22: 277–282.

88. Connell JM, Cook GM, McInnes GT. Metabolic consequences of salbutamol poisoning reversed by propranolol. Br Med J (Clin Res Ed) 1982;285:779.

89. King WD, Holloway M, Palmisano PA. Albuterol overdose: a case report and differential diagnosis. Pediatr Emerg Care 1992;8:268–271.

Chapter 17

ERYTHROPOIETIN STIMULATION and OTHER BLOOD DOPING METHODS

JAMES RHEE, MD
TIMOTHY ERICKSON, MD

HISTORY

Erythropoietin stimulation is one of several methods of blood doping. Broadly defined, blood doping refers to the practice of increasing the oxygen-carrying capacity of blood to enhance oxygen delivery to working muscles and tissues with the expectation of improving endurance and athletic performance. Originally, the term referred to the use of autologous or allogenic blood transfusions by athletes; however, the term currently is applied to several other methods that increase the red blood cell count or oxygen-carrying capacity including the following: 1) use of blood substitutes (modified hemoglobin solutions, perfluorochemicals, allosteric effectors of hemoglobin), and 2) artificial stimulation of erythropoiesis (recombinant erythropoietin, gene therapy, erythropoietin mimetics).[1] Additional methods used by some athletes to increase red cell mass include stimulation of endogenous erythropoietin (living and training at high altitude, sleeping in altitude tents).[2] Blood doping is common in endurance athletes (e.g., cycling, cross-country skiing, distance running, rowing, triathlon). The use of blood products as means to enhance strength and resistance to disease dates back at least to the work of Fuster in 1865 as well as later research during the 19th century conducted by Deschiens, Catillon, Adrian, and Richet.[3] At the beginning of the 20th century, several pharmaceutical companies developed blood products derived from animal blood for use in humans. However, interest in these products faded after the Second World War with the discovery of antibiotic therapy.

Since the 1960s and 1970s, blood transfusions have been a popular method to enhance endurance by increasing hemoglobin, aerobic capacity or maximal oxygen uptake ($VO_{2\,max}$), and time to exhaustion at 95% $VO_{2\,max}$. After the 1984 Summer Olympic Games, the US Olympic Committee disclosed the use of blood transfusions by 7 of 24 members of the US Olympic cycling team during training for the Summer Olympics.[4] However, at that time there was no Olympic ban on the use of these blood products. Shortly thereafter, the International Olympic Committee banned all forms of blood doping defined as the administration of blood, red blood cells (RBCs), artificial oxygen carriers, or related blood products to an athlete.[5]

In 1906, Paul Carnot, Professor of Medicine at the Sorbonne in Paris and his research assistant research, Mlle. C. Deflandre postulated the existence of a humoral factor (hémopoïétine) that controlled erythropoiesis.[6,7] They discovered an increase in erythrocytes in rabbits after several days of injecting serum from anemic rabbits; however, lack of confirming studies dampened interest in this area of research for 5 decades. In 1948, Bonsdorff and Jalavisto proposed the replacement of the term hémopoïétine by erythropoietin to limit this term to red cell production.[8] Research in the 1950s confirmed the production of this humoral factor in response to anemia and hypoxia.[9,10] Isolation of native human erythropoietin did not occur until 1977. In 1983, Fu-Kuen Lin and a research team at Amgen, Inc. (Thousand

Oaks, CA) cloned the gene for human erythropoietin, the endogenous hormone responsible for stimulating RBC production. Subsequently, recombinant human erythropoietin (rHuEPO) was developed for the treatment of certain types of anemia and released in Europe in 1987. The US Food and Drug Administration (FDA) approved the use of Epogen® for the treatment of anemia in patients with end-stage renal disease in 1989 as a means to improve survival, cognitive function, and the quality of life for renal patients.[11]

During the first year of the release of rHuEPO in Europe, 5 Dutch cyclists died of unknown causes.[12] This cluster of deaths raised concerns about the use of ergogenic aids including rHuEPO. The International Olympic Committee (IOC) banned the use of rHuEPO as an ergogenic aid in 1990,[13] but deaths of cyclist from causes (e.g., pulmonary embolism, myocardial infarction, stroke) associated with rHuEPO use continued during the 1990s.[5] During this period, the popularity of blood transfusion among endurance athletes decreased dramatically. The abuse of rHuEPO to improve athletic performance continued into the 2000s, particularly in elite and nonprofessional endurance athletes (e.g., cycling, marathon running, cross-country skiing).[14] Four athletes lost their medals at the Salt Lake City 2002 Winter Olympics after testing positive for the hyper-glycosylated erythropoiesis-stimulation protein, darbepoetin alpha, which has a prolonged terminal half-life that allows less frequent dosing than rHuEPO.

Following implementation of reliable tests to screen for EPO-stimulating substances, the use of blood transfusions by endurance athletes has increased substantially.[15] Other potential methods used by athletes to increase oxygen transport and endurance capacity include intermittent transient hypoxia, other erythropoiesis-stimulating substances (continuous erythropoiesis receptor activator, erythropoiesis-mimetic peptides such as hematide), EPO gene therapy, or mimetics (hypoxia-inducible transcription factors pathway), allosteric effectors of hemoglobin (RSR-13, RSR-4), and blood substitutes (e.g., modified hemoglobin-based oxygen carriers, perfluorochemicals).[16]

IDENTIFYING CHARACTERISTICS

Structure

Endogenous human erythropoietin (EPO, CAS RN:11096-26-7) is a 165-amino acid, 30.4-kDa glycoprotein derived from the cleavage of a 27-amino acid leader sequence from a 193-amino acid transcript of chromosome 7 (7q11-22). Subsequently, glycosylation of 4 amino acids (Asn24, Asn38, Asn83, Ser126) and the loss of the carboxyl-terminal arginine from the 166-amino

acid residue produces the 165-amino acid hormone.[17] Two disulfide bonds in the human erythropoietin molecule join cysteine residues at positions 29 and 33 and at 7 and 161. The presence of N-linked carbohydrate chains with terminal sialic acids limits the hepatic clearance of EPO. The structure of EPO is similar to growth hormone, prolactin, granulocyte colony-stimulating factor, and interleukin 6.[18]

The term "epoetin" refers to recombinant human erythropoietin (rHuEPO, CAS RN:113427-24-0) that has an amino acid sequence homologous to endogenous erythropoietin. Although the amino acid sequences of rHuEPO and EPO are identical, rHuEPO has microheterogeneities and minor differences in the glycosylation pattern compared with EPO.[19,20] The addition of Greek letters to epoetin delineates differences in the carbohydrate moieties between different epoetin molecules (e.g., alpha, beta, delta, omega). As a result of these differences in composition, the beta isoform is slightly more basic than the alpha form.

Darbepoetin alfa is a hyper-glycosylated erythropoietin analogue that is biochemically distinct from recombinant human erythropoietin (rHuEPO). In contrast to the 3 N-linked carbohydrate chains in rHuEPO, darbepoetin alfa contains 5 N-linked carbohydrate chains with a resultant carbohydrate content of 51% compared with 40% for rHuEPO.[21] Darbepoetin alfa has a molecular weight of 37.1 kDa and contains up to 22 sialic acid molecules compared with a maximum of 14 sialic acid residues in rHuEPO. Newer EPO analogues include protein conjugates (synthetic erythropoiesis protein) that contain precision-length, monodisperse, negatively charged polymers instead of oligosaccharides.[22] Continuous erythropoietin receptor activator (CERA) incorporates a long polymer chain that substantially extends the erythropoietic activity of this molecule.[23] Oral HIF-1α stabilizers (e.g., FG-2216) are currently under investigation as substitutes for rHuEPO.[24]

Form

Blood doping involves a variety of techniques that increase oxygen transport in the blood. Table 17.1 lists various techniques and substances used to alter oxygen-carrying capacity.

Recombinant human erythropoietin (rHuEPO) mimics endogenous erythropoietin (EPO) and stimulates the production of erythrocytes similar to EPO. Both endogenous EPO and rHuEPO are compendiums of different isoforms. Endogenous erythropoietin has a net negative charge based on the presence of 8–14 sialic acid residues. The net negative charge of rHuEPO differs from EPO based on the number of sialic acid residues. Darbepoetin alfa (new erythropoiesis-stimulating

TABLE 17.1. Blood Doping Techniques and Substances.

Technique	Substances	Forms
Blood transfusion	Autologous blood, allogeneic blood	
Blood substitutes	Perfluorocarbons	Perflubron emulsion
	Hemoglobin-based oxygen carriers	PolyHeme®, HemoLink™, Hemopure®
Recombinant human EPO (rHuEPO)	Epoetin alfa	Epogen®, Eprex®, Epoxitin®, Epypo®, Erypo®, Espo®, Globuren®, Procrit®
	Epoetin beta	Epogin®, Marogen®, NeoRecormon®, Recormon®
	Epoetin delta	Dynepo™
	Epoetin gamma	
	Epoetin omega	Epomax®, Hemax®
Other erythropoiesis-stimulating substances	Darbepoetin alpha	Aranesp®
	Continuous erythropoiesis receptor activator (CERA)	
	Erythropoietin-mimetic peptide	Hematide™
Allosteric hemoglobin modulators	RSR-13	Efaproxiral
	RSR-4	
Supplementation	Iron, cobalt chloride	
Alteration of inspired oxygen	Hypoxic gas mixtures	
	Supplemental oxygen	
	Artificial altitude facilities	
Gene regulation	Hypoxia inducible factor (HIF) pathway	
Gene doping	Human erythropoietin gene transfection	

protein [NESP]) is an analogue of EPO that also stimulates erythropoiesis. Erythropoietin mimetics are peptide and nonpeptide molecules that simulate the action of EPO on dimerizing EPO receptors. Epoetin alfa is available as a solution in the following concentrations: 2,000 units/mL, 4000 units/mL, 10,000 units/mL, and 20,000 units/mL, whereas darbepoetin alpha is available in 0.025 mg/mL, 0.04 mg/mL, 0.06 mg/mL, and 0.2 mg/mL solutions.

EXPOSURE

Epidemiology

The World Anti-Doping Agency (WADA) bans blood doping including blood transfusions, methods to enhance or transport oxygen, and erythropoiesis-stimulating agents (erythropoietin, darbepoetin alpha, hematide).[25] DNA-recombinant human erythropoietin has been administered to patients with chronic renal failure and anemia since the late 1980s. By the middle of the 1990s, rHuEPO was the drug of choice for endurance athletes seeking to improve performance, including cyclists in the Tour de France.[14] The advantages of rHuEPO include ease of use, efficacy, and the absence of the risks associated with blood transfusions. In addition, detection of rHuEPO is technically difficult. The estimated prevalence of rHuEPO by elite endurance athletes ranges from about 3–7%.[14] In a study of 1,015 Italian athletes, the prevalence of blood doping was 7% based on interviews.[26] The type of blood doping was not specified in the report. The use of rHuEPO is probably much less common among young noncompetitive athletes compared with other ergogenic aids (anabolic-androgenic steroids). In a study of 571 Danish general practitioners, none of these physicians reported the use of rHuEPO among their patients; almost all of the reported use of ergogenic aids in this population involved androgenic-anabolic steroids.[27]

Sources

Renal interstitial cells adjacent to the proximal tubules secrete most (i.e., 90%) of endogenously produced EPO in response to hypoxia or reduced erythrocyte concentrations.[28] The liver accounts for most of the remaining production of EPO with small amounts of EPO synthesized in the brain.[29] Recombinant human erythropoietin is produced from mammalian cell lines after insertion of the human erythropoietin gene. The nomenclature for epoetin uses epoetin with a Greek letter to differentiate the same amino acid sequence with varying glycosylation patterns. Different cell lines produce these different isoforms that also vary in pharmacokinetics.[30] The sources of epoetin alfa and epoetin beta are recombinant DNA from Chinese hamster ovary cells after the

insertion of the human erythropoietin gene. Epoetin delta is derived from genetically engineered human fibrosarcoma cell lines, whereas the source of epoetin omega is baby hamster kidney cells. Murine C127 cells are the source for epoetin gamma. Darbepoetin alfa is a glycosylated analog of rHuEPO produced by DNA technology in Chinese hamster ovary cells that binds to the erythropoietin receptor similar to endogenous EPO, resulting in a longer elimination half-life than endogenous EPO. Due to the illicit demand and the high cost of these drugs, there is an underground market for stolen or counterfeit EPO products.[31] Additionally, human and animal blood has also been falsely marketed as EPO to unsuspecting athletes carrying an additional risk of viral infection (e.g., hepatitis C, human immunodeficiency virus [HIV]).[32,33]

Methods of Abuse

Routes of illicit rHuEPO use include intravenous (IV), intramuscular (IM), and subcutaneous (SC) administration. The poor oral bioavailability of rHuEPO limits the use of this route of administration based on animal studies.[34] The athlete using blood doping seeks a competitive advantage to improve athletic performance. The use of rHuEPO increases oxygen-carrying capacity of the blood, improves endurance, and enhances self-esteem, particularly in endurance athletes attributing their self-worth to their athletic performance. Psychologic studies of endurance athletes suggest that the administration of rHuEPO produces a significant increase in their physical condition and their commitment to training.[35] The perception of improved physical condition causes a hedonic link to endurance training and the use of rHuEPO.

The use of transfusions involves either the infusion of autologous blood (i.e., recipient and donor the same) after recovery of the recipient/donor from the effects of previously collected blood or the allogeneic transfusion of blood collected from a cross-matched donor to the recipient.[36] Although allogeneic transfusion can be detected in the recipient by erythrocyte genotyping, autologous transfusions are much more difficult to detect.[37] The typical autologous blood transfusion involves the withdrawal of 1–4 units of blood (450–1800 mL) several weeks before competition.[38] The plasma is reinfused immediately, and the erythrocytes are either refrigerated or frozen. Subsequently, the erythrocytes are infused 1–7 days before endurance events. The average increase in hemoglobin and time to exhaustion at 95% $VO_{2\ max}$ using typical blood doping is approximately 7% and 34%, respectively.[12] The decreased responsiveness of Hb-based oxygen carriers and perfluorocarbons to 2,3-DPG limits the effective-

ness and the abuse of these substances when compared with packed erythrocytes.[39] Additionally, these artificial substances are easily detected by routine laboratory tests and visual changes of the blood.

DOSE EFFECT

Medical Use

The FDA approved the use of rHuEPO for the treatment of anemia associated with chronic renal failure, zidovudine-treated HIV, cancer patients on chemotherapy, and the reduction of allogenic blood transfusions in anemic patients undergoing elective surgery with a high risk for perioperative blood loss. Anemia of chronic renal failure and chemotherapy-induced anemia associated with nonmyeloid malignancies are also an indication for darbepoetin alpha. Other uses of erythropoiesis-stimulating agents include treatment of critical care patients exhibiting severe anemia, blood loss, reduced red cell life span, impaired iron availability, and inhibition of erythropoiesis by inflammatory cytokines.[40] There are no FDA-approved uses of erythropoiesis-stimulating agents in healthy individuals or athletes.

The dose of erythropoiesis-stimulating agent depends on the indication and age beginning with an initial dose and titrating to desired effect (e.g., Hg = 10–12 g/dL, avoidance of transfusions). A typical adult dose of epoetin alfa is 50–300 U/kg 3 times a week by IV or SC routes; however, higher doses may be required. The response to epoetin alfa varies with underlying disease, dose, and dosing regimen. The administration of 500 μg/kg epoetin alfa 3 times weekly to 5 anemic renal dialysis patients resulted in a mean increase of 10 percentage points in the hematocrit after 3 weeks of therapy.[41] The baseline mean hematocrit of this group was 19 ± 3%. A dose-dependent increase in hemoglobin occurs within 2–6 weeks of administration of EPO drugs. In a study of healthy volunteers given single SC doses of rHuEPO (epoetin alfa) ranging from 300–2,400 IU/kg, the mean percentage of reticulocytes began increasing 3–4 days after administration and the maximum response occurred 8–11 days after administration.[42] Saturation of the response to rHuEPO occurred at doses above 1,800 IU/kg. The reticulocyte count returned to baseline 22 days after administration. The response was linear up to 1,800 IU/kg, but above that dose the dose-response curve flattens. Multiple, smaller rHuEPO doses (e.g., 150 IU/kg 3 times weekly) produced more modest (1–2% reticulocytes), sustained responses. Overall, stimulation of a reticulocyte response was more effective following repeated SC administration of rHuEPO than the equivalent dose as a single injection. Following

the SC administration of 10–500 IU/kg epoetin beta in monthly doses to 16 healthy volunteers, the maximum reticulocyte count occurred about 3–4 days after administration.[43]

The initial adult dose of darbepoetin alfa is 0.45–2.25 µg/kg IV or SC once weekly, depending on the underlying cause of the anemia, followed by smaller weekly doses unless higher doses are necessary to produce the desired response. Less frequent doses (e.g., every 2–3 weeks) may be sufficient depending on the indication. Doses of erythropoiesis-stimulating agents are modified at monthly intervals to maintain an acceptable hemoglobin level without hemoglobin concentrations exceeding 12 g/dL or increasing >1 g/dL in 2 weeks.[44] The recommended target hemoglobin concentration for therapeutic indications should not exceed 12 g/dL. Volunteer studies indicate that the use rHuEPO is not associated with elevated blood pressure in anemic chronic renal failure patients with the target hemoglobin of <12 g/dL.[45] In clinical trials, an increase in hemoglobin greater than approximately 1g/dL in 2 weeks was associated with a higher frequency of cardiac arrest, strokes, seizure, worsening hypertension, fluid overload, congestive heart failure, and acute myocardial infarction.[46]

Experimental Studies

Limited data from the study of healthy students and endurance athletes suggest that the administration of moderate doses of rHuEPO can modestly increase oxygen-carrying capacity.[47,48] A review of these studies indicated the SC administration of rHuEPO in doses of 60–232 U/kg weekly for 4–6 weeks produces the following increases: 1) hemoglobin, 9–14%; 2) hematocrit, 11–19%; and 3) VO_{2max} (aerobic capacity, maximal oxygen uptake), 7–9%; and enhanced endurance performance (time to exhaustions on a standard work load), 9–17%.[14] An estimated 1 g/dL increase in hemoglobin in an athlete with an exercise cardiac output of 25 L/min would increase oxygen transport about 335 mL/min (i.e., about 8% increase above normal VO_{2max} of 4,000 mL O_2/min).[49] The response of the hematocrit to rHuEPO was more modest in other studies. In a study of 11 healthy male students (mean age, 26 ± 6 year) receiving 2,000 U rHuEPO/thrice weekly for 6 weeks, the mean hematocrit increased ($P < .05$) from an initial value of 43 ± 2% to 45 ± 2% at the end of the study.[50]

Toxicity

There are limited data on toxic doses of erythropoiesis-stimulating agents associated with the clandestine use of these agents by athletes; potentially, therapeutic doses used in anemic patients may cause adverse effects (e.g., stroke, myocardial infarction, congestive heart failure, hypertension, embolism) following abuse of these agents by athletes or other individuals. The injection of a single dose of rHuEPO probably does not produce toxicity with the exception of short-term elevation of serum hepatic aminotransferases following very high doses. In a clinical study, the inadvertent administration of 318,000 U erythropoietin on the second day of a 3-day course of 33,000 U daily was associated with an approximate 10-fold increase in the serum alanine aminotransferase and aspartate aminotransferase that resolved over 3 months.[51] The hemoglobin increased about 1 g/dL over the admission value; the hemoglobin return to preadmission level within 3 months. Healthy volunteers have tolerated 2,400 U/kg as a single dose without complications.[42]

The effects of erythropoiesis-stimulating drugs require multiple injections over a prolonged period. The risks of adverse effects increases with Hb > 12–13 g/dL and/or with a high rate of hemoglobin increase (i.e., >1 g/dL per 2-week period). The maximum dosage of these agents has not been determined. In patients with end-stage renal disease, no direct toxicity was detected with larger doses of 1,500 U/kg 3 times weekly for 3–4 weeks based on pharmaceutical company data.[52] Doses of up to 8 µg/kg weekly and 15 µg/kg every 3 weeks have been administered to cancer patients for up to 12–16 weeks without complications.[46] A 42-year-old man with acquired immunodeficiency syndrome (AIDS) inadvertently injected himself with 10,000 U rHuEPO daily for several weeks.[53] Subsequently, he developed polycythemia (hematocrit 77%) along with confusion, abdominal pain, and foot pain. He recovered after erythropheresis with mild skin loss over his toes. The inadvertent administration of 384,000 U rHuEPO over 3 days to 2 patients with acute myocardial infarctions was associated with modest elevation of serum hepatic aminotransferase concentrations that resolved over 3 months.[51] The hemoglobin concentrations increased approximately 1 g/dL, and then returned to baseline in 3 months.

TOXICOKINETICS

Absorption

The participants in most pharmacokinetic studies on rHuEPO are patients with chronic renal disease, and there are limited data on the pharmacokinetics of this substance in healthy volunteers or in elite athletes. The average bioavailability of SC epoetin alfa ranges from approximately 14–40%.[54,55] The mean bioavailability of

rHuEPO after the SC administration of 50 U/kg to 6 healthy volunteers was 36% ± 23%.[56] In a single-dose pharmacokinetic study of rHuEPO in pediatric patients with chronic renal disease, the bioavailability of rHuEPO following SC administration of 40 U rHuEPO/kg was approximately 40%.[57] The time to peak concentration for epoetin alfa administered SC ranges from about 5–24 hours. Following the administration of single doses of rHuEPO (300 IU/kg to 2,400 IU/kg) to healthy volunteers, the time to peak rHuEPO concentration ranged from 15.6 ± 5.8 hours to 28.8 ± 7.8 hours.[42] The peak EPO concentration ranged from 429 ± 86 mIU/mL (300 IU/kg dose) to 6,819 ± 764 mIU/mL (2,400 IU/kg dose).

Following SC administration, the mean bioavailability of darbepoetin alfa and rHuEPO in adults are similar. In a study of 11 peritoneal dialysis patients, the mean bioavailability of darbepoetin alfa after the SC administration of about 100 U/kg was approximately 37%.[58] In a study of pediatric patients with chronic kidney disease, the mean bioavailability of darbepoetin alfa was slightly higher (i.e., 54%) compared with the adult patients and patients receiving rHuEPO.[59] Subcutaneous darbepoetin alpha reaches peak concentration within 34–54 hours after administration.[60]

Distribution

rHuEPO and darbepoetin alfa are rapidly distributed with similar volumes of distribution (V_d). Following the IV administration of 100 U/kg to 11 peritoneal dialysis patients, the mean V_d of rHuEPO and darbepoetin alfa were 48.7 ± 2.1 mL/kg and 52.4 ± 2.0 mL/kg, respectively.[58] The volume of distribution of these 2 compounds approximates the plasma volume.[61]

Biotransformation

Epoetin and darbepoetin alpha undergo extensive liver metabolism, desialation and clearance via the hepatic galactose pathway.[54] The galactose binding proteins in hepatic cells rapidly clear erythropoietin from the circulation. Erythropoietin contains *N*-glycans and *N*-acetyllactosamine repeats. The sialic acid of recombinant erythropoietin is probably necessary for this glycoprotein hormone to circulate without degradation, and the desialation of erythropoietin allows the recognition of these galactose-terminated glycoproteins by receptors on the cell surface of hepatocytes.[62,63] Subsequent endocytosis and lysosomal digestion of the asialo-erythropoietin result in the clearance of erythropoietin from the blood.

Elimination

The elimination of rHuEPO probably follows nonlinear saturation kinetics (i.e., Michaelis-Menten).[64] The elimination of rHuEPO primarily results from biotransformation with renal excretion of unchanged rHuEPO accounting for a small portion (<5%) of the total elimination. Over the first 24 hours after administration of 3 different doses of rHuEPO (10 U/kg, 100 U/kg, 500 U/kg) to 10 healthy volunteers, the overall mean percentage of rHuEPO excreted unchanged in the urine was 1.74% ± 1.04%.[65] In this study, the clearance of rHuEPO was nonlinear in doses ranging between 10 U/kg and 500 U/kg intravenously, and Michaelis-Menten elimination kinetics described best the elimination kinetics of rHuEPO. The serum half-life of rHuEPO directly correlates to sialic acid-containing carbohydrate content of the molecule as well as the dose and route of administration.[66] In a study of healthy volunteers receiving 50 U rHuEPO/kg, the mean half-life was 4.5 ± 0.9 hours following IV administration compared with 25 ± 12 hours after SC administration.[56] Similarly, the mean plasma half-life of EPO following IV doses of < 100 IU/kg was approximately 5 hours.[54] Studies in healthy volunteers suggest that the terminal half-life of epoetin beta is slightly longer (i.e., about 20%) than epoetin alfa when administered in equivalent doses.[67] The renal clearance of rHuEPO uncorrected for bioavailability averages about 48–50 mL/h/kg.[64] Assuming a bioavailability of 35% the corrected renal clearance of rHuEPO is about 17 mL/h/kg.

The addition of 2 sialic acid-containing carbohydrate chains to darbepoetin alpha compared with rHuEPO prolongs the serum half-life of darbepoetin alfa in patients with chronic kidney disease or cancer.[68] Following the IV administration of a 100 U/kg-dose to adult patients with end-stage renal disease, the mean serum half-lives of rHuEPO and darbepoetin alpha were 8.5 ± 2.4 hours and 25.3 ± 2.2 hours, respectively.[58] The SC administration of darbepoetin alfa to these patients increased the mean serum half-life to 48.8 ± 5.2 hours. After SC administration of rHuEPO to athletes, the serum erythropoietin concentration returns to baseline values about 7 days after administration compared with 4 days in the urine.[69]

Maternal and Fetal Kinetics

Hematopoiesis occurs independently in maternal and fetal circulations in response to endogenous erythropoietin. The human placenta forms a barrier to endogenous and recombinant erythropoietin, thus allowing the use of recombinant erythropoietin (rHuEPO) in the

treatment of maternal pregnancy and postpartum anemia.[70] There are few data on the excretion of epoetin alfa or darbepoetin alfa into breast milk.

Tolerance

There are few data on the specific factors that limit the hematologic response of some renal dialysis patients to the chronic administration of rHuEPO.[11] Although the therapeutic effects of erythropoietin are dose-dependent at low dose, increased biologic response in humans is not typically observed following the administration of doses exceeding 300 U/kg 3 times weekly.[71]

Drug Interactions

Preliminary data in patients with cancer suggest that concurrent chemotherapy may influence the pharmacokinetics of epoetin and darbepoetin alfa.[21] Patients on iron preparations for iron-deficiency anemia require monitoring while on EPO therapy. A functional iron deficiency developed in 13 of 18 patients with chronic EPO therapy. The decreased heme synthesis exceeded the ability of the reticulocyte to release iron to transferrin, and a relative iron deficiency develops as a result of the drop in the reticulocyte count and transferrin saturation.[41]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Oxygen stores in the body are relatively low compared with the oxygen requirements of the body. The basal oxygen consumption rate is 4 mL/kg/min compared with total body oxygen stores of 20 mL/kg (i.e., 5 minutes of basal oxygen needs).[72] The ability of the tissues to meet oxygen demands depends in part on the number of erythrocytes (red cell mass). The kidneys and to a lesser extent, the liver and brain secrete the glycoprotein, erythropoietin (EPO), which increases the number of circulating erythrocytes (red cell mass) and thus oxygen delivery to muscle tissue.[73] Endogenous human erythropoietin is the primary regulator of red blood cell formation. The red cell mass is constant in a specific individual; however, this mass varies up to about 10% between healthy individuals of the same age and gender. Endogenous human erythropoietin couples long-term oxygen requirements to erythrocyte and hemoglobin production by acting on the early erythroid progenitor cells (erythroid burst-forming units, erythroid colony-forming units, pronormoblasts) in the bone marrow to stimulate growth and prevent apoptosis. This hormone is the paradigm of oxygen-related genes that are controlled by the main regulator of oxygen homeostasis, hypoxia inducible factors (e.g., HIF-1, HIF-2, HIF-3). These heterodimeric proteins are transcription factors that modulate a variety of genes in response to relative low-oxygen conditions. HIF-1 is the primary factor, consisting of 2 subunits, HIF-1α and HIF-β. Both HIF-1α and HIF-β are necessary to generate an active transcription complex in conjunctions with coactivators (e.g., p300/CREB).[74] HIF-1α resides in the cytoplasm where oxygen-dependent degradation occurs as a result of the hydroxylation by prolyl 4-hydroxylases. HIF-1β resides in the nucleus away for the action of these enzymes. Under hypoxic conditions, HIF-1α survives to penetrate the nucleus and forms a heterodimer with HIF-1β.

Endogenous human erythropoietin binds to the homodimeric erythropoietin receptor on the surface of colony-forming unit-erythroid cells (CFU-E), proerythroblasts, and basophilic erythroblasts in the red bone marrow, initiating cellular transcription and differentiating these cells into mature red blood cells.[54] Activation of erythropoietin receptors on the earliest committed erythroid progenitor cells (i.e., erythroid burst-forming units) initially increases proliferation of these cells along with the ability of the progenitor cells to survive and reach the reticulocyte stage. Conformational changes in specific domains on the EPO receptor also cause autophosphorylation and activation of the Janus kinase 2 pathway with subsequent signal transduction. Hematide is a small molecule analog of EPO that also binds to this receptor, but the structure of this dimeric erythropoietin-mimetic peptide is unrelated to the sequence of endogenous EPO. Animal studies suggest that the increase in red cell mass is associated with a reduction in the contribution of anaerobic metabolism to overall energy production, as a result of increased muscle glycogen and free fatty acids along with a decrease in lactate.[75]

Erythropoietin modulates a spectrum of cellular processes in addition to progenitor stem cell development, including cellular integrity and angiogenesis. Downregulation of EPO synthesis results from a variety of mechanisms including hyperoxia, increased catabolism by an expanded erythroid progenitor cell pool, blood hyperviscosity, renal disease, transfusion-dependent anemia, and the cytokines synthesis secondary to inflammatory, infectious, and neoplastic disorders.

Mechanism of Toxicity

Most research on the pathophysiology of the adverse effects of rHuEPO involve medical use rather than the illegitimate use during blood doping with very limited research on normal volunteers. Furthermore, differences in cardiovascular dynamic between renal failure

patient and athletes limits extrapolation of data from anemic chronic renal failure patients to healthy adults. The administration of rHuEPO enhances endothelial activation and platelet reactivity and increases systolic blood pressure during submaximal exercise in healthy adults.[76] These effects potentially increase the risk for thromboembolic events (myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis), ischemia, hypertension and hypertensive encephalopathy, congestive heart failure, and hyperviscosity syndromes. Potentially, mechanisms for the adverse cardiovascular effects of chronic rHuEPO misuse include increased blood viscosity, hypertension, and the vasoconstrictive properties of erythropoietin.[77] Additionally, the increased hematocrit and dehydration associated with intense exercise may reveal previously undetected cardiovascular abnormalities in some athletes.

BLOOD VISCOSITY

Oxygen transport capacity does not necessarily increase as the hematocrit increases. In normal individuals as the hematocrit increases above 40%, the relative oxygen transport capacity of the blood decreases due to increasing blood viscosity. Figure 17.1 displays the relationship between hematocrit, blood viscosity, and oxygen trans-
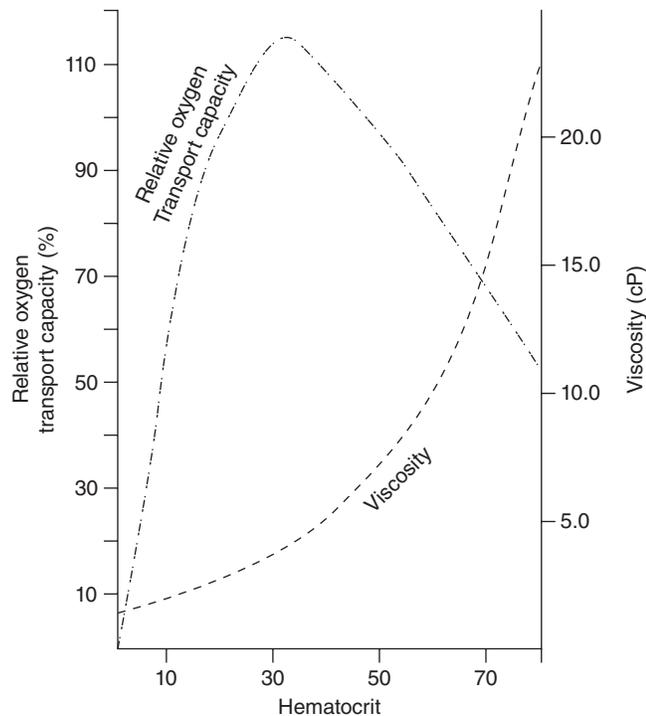
FIGURE 17.1. Correlation of oxygen transport in the brain to hematocrit and blood viscosity. (Reprinted from the American Journal of Emergency Medicine, Vol. 11, KR Brown, W Carter Jr., GE Lombardi, Recombinant erythropoietin overdose, p. 620, Copyright 2003, with permission from Elsevier.)

port in the brain. Adverse side effects of chronic rHuEPO use potentially result from this increased viscosity and the impairment of oxygen delivery.[78] In controlled clinical studies of chronic renal failure patients with anemia, comparison of traditional hemoglobin targets (10-12 g/dL) to increased rHuEPO (epoetin alfa) doses resulting in hemoglobin >13 g/dL suggested that higher doses caused increased cardiovascular events and significant increases in hypertension.[79] However, the risk of death and cardiovascular events (congestive heart failure, myocardial infarction, stroke) may vary with comorbidity among these patients. A reanalysis of this (CHOIR) study using Cox regression indicated that there was an increased risk of cardiovascular events following high compared with low-dose in subgroups without heart failure or diabetes mellitus, but not in the subgroup with congestive heart failure or diabetes mellitus.[80] Consequently, factors in addition to blood viscosity affect the risk of adverse cardiovascular events. Furthermore, the relative importance of blood viscosity and these other factors may vary between healthy individuals, patients with renal disease, and athletes using erythropoiesis-stimulating agents.

HYPERTENSION

Elevation of arterial blood pressure is a suspected cause of the excess cardiovascular events associated with high doses of erythropoiesis-stimulating agents. In normal individuals, the use of erythropoiesis-stimulating agents is associated with an increase in the average mean arterial pressure at rest (e.g., 6 mm Hg following 5,000 U for 15 weeks to maintain hematocrit near 50%)[81] and an increase mean systolic blood pressure (i.e., 14 mm Hg) during submaximal exercise at 200 W after 6 weeks of 20–40 U rHuEPO/kg thrice weekly to maintain the hematocrit near 50%.[82] The effect of rHuEPO on blood pressure in normal individuals is independent of red blood cell volume. Neither rHuEPO therapy or blood transfusions increase total blood volume because the associated increase in red blood cell volume is accompanied by quantitatively similar decrease in plasma volume.[83] In healthy adults, the use of rHuEPO produces blood pressure elevation, whereas transfusions to the same hematocrit produces no increase in blood pressure over baseline (i.e., pretreatment).[84] Consequently, the hypertension associated with rHuEPO therapy is independent of blood viscosity.

THROMBOSIS

In general, partial correction of anemia to subnormal levels (e.g., 36%) in uremic patients does not cause adverse effects with the exception of hypertension

during the first 8 weeks of therapy.[50] However, the risk of thrombotic events increases as the hematocrit exceeds normal levels. Potential mechanisms of toxicity include enhanced thrombogenesis secondary to endothelial activation and increased platelet reactivity.[38] An elevation of the red cell mass causes a reduction in cerebral blood flow. Changes in cerebral blood flow potentially increase the risk of thrombosis, transient ischemic attacks, cerebral vascular accidents, cerebral ischemia, and altered mental status.[79]

VASCULAR-DEPENDENT MECHANISMS

Animal and *in vitro* data suggest that rHuEPO may cause arterial vasoconstriction by both endothelium-dependent and endothelium-independent impairment of arterial vasodilation.[83] Potential mechanisms include changes in nitrous oxide production and the production of vascular endothelin and endothelial prostanoids.[85,86] Neither of these mechanisms account for all of the vasoconstriction associated with the use of rHuEPO.

IMMUNE REACTIONS

Rarely, case reports associated chronic rHuEPO therapy with a severe, progressive, normocytic, normochromic anemia of sudden onset with almost complete absence of erythroid precursor cells in the bone marrow.[87] This pure red cell aplasia is associated with the development of antibodies to this product. Although most of the original cases involved the use of epoetin alpha outside the United States, subsequent case reports associated pure red cell aplasia with all commercial formulations of EPO.[88] The clinical features of red cell aplasia also occur in malnutrition, other drug reactions, and a variety of other infectious, malignant, and autoimmune diseases. In all cases of red cell aplasia associated with erythropoiesis-stimulating agents, neutralizing antierythropoietin antibodies are present. Although pure red cell aplasia usually occurs after the production of neutralizing antierythropoietin antibodies, not all patients with these antibodies will develop anemia.[89] Thus, the exact mechanism of the formation of these antibodies is unconfirmed. Subsequently, these antibodies cross-react and inactive endogenous EPO.

Postmortem Examination

Pathologic findings associated with erythropoietin primarily involve the effects of increased viscosity including cerebral ischemia and thrombosis, myocardial thrombosis, cardiac muscle damage, and deep venous thrombosis.

CLINICAL RESPONSE

Medical Use

Identification of complications associated with the medical use of rHuEPO is complicated by the administration of these drugs to patients with severe chronic diseases. In early dose-finding clinical studies primarily in patients with chronic renal failure, adverse effects associated with the long-term use of rHuEPO included arterial hypertension, iron deficiency, thromboembolism (e.g., renal thrombosis),[44] mesenteric ischemia,[53] hypertensive encephalopathy with seizures (i.e., often with history of seizure disorders),[90] flu-like syndrome varicose veins, myalgias, arthralgias, headache, and hyperkalemia.[91,92] Hypertension and seizures do not usually occur in patients on short-term therapy with erythropoiesis-stimulating agents (e.g., autologous blood transfusions). Cardiovascular complications (e.g., ischemic chest pain, unstable angina, and myocardial infarctions) associated with rHuEPO therapy also occur primarily in patients with end stage renal disease.[93,94] Adverse dermatologic effects in these patients include pruritic papules,[95] angioedema/anaphylaxis (i.e., secondary to gelatin),[96] and local pain at the injection site.[97] Rarely, case reports of patients with chronic renal disease associated the use of rHuEPO with the formation of neutralizing anti-EPO antibodies and the development of red cell aplasia.[98]

Several malignant cell lines express EPO and EPO receptor mRNA that respond to hypoxic stimuli by increasing the secretion of EPO. *In vitro* studies suggest that EPO may stimulate the proliferation of neoplastic cells and protect these cells from apoptosis;[99] however, there are currently no data to indicate that there is an increased risk of cancer in patients treated with rHuEPO. There are no specific adverse effects uniquely associated with rHuEPO therapy. Extrapolation of the frequency and type of adverse effects in these clinical studies of rHuEPO to healthy adults and elite athletes is limited by differences in predisposing factors (e.g., blood viscosity, vascularity, risk factors for thromboembolism) and the lack of data on healthy individuals.

CARDIOVASCULAR

Approximately 20–30% of chronic renal failure patients treated with epoetin alfa develop hypertension or worsening of existing hypertension.[100] The change in blood pressure is usually associated with an excessive (i.e., hemoglobin > 12–13 g/dL) or a rapid rise in hematocrit. In a case series of anemic renal dialysis patients, 1 patient developed hypertensive encephalopathy with

peak blood pressure of 220/140 mmHg during rHuEPO therapy.[101] The patient recovered uneventfully after reduction of hemoglobin concentration and antihypertensive therapy. In a study of 1,598 end-stage renal failure patients on chronic darbepoetin alfa therapy, the percentage of adverse effects were as follows: hypertension, 23%, hypotension, 22%; peripheral edema, 11%; cardiac dysrhythmias, 10%; vascular access thrombosis, 8%; angina, 8%; and congestive heart failure, 6%.[102]

CENTRAL NERVOUS SYSTEM

Adverse neurologic effects associated with the medical use of rHuEPO include headache, dizziness, confusion, visual disturbances, cerebral ischemia, transient ischemic attacks, cerebral vascular accidents, and seizures.[103] A case report associated the onset of venous thrombosis of the sagittal and transverse sinuses with the therapeutic use of rHuEPO by a 37-year-old end-stage renal disease patient.[104] The patient complained of headache, and he recovered uneventfully following treatment with heparin and analgesics.

Illicit Use

There are few clinical data on the medical complication of EPO abuse; expected complications are similar to the complications associated with high-dose, therapeutic use, particularly cardiovascular and thrombotic events including hypertension. Potentially, life-threatening cerebral and cardiac thrombotic events may occur during the dehydration and plasma contraction associated with heavy exercise in elite athletes using rHuEPO. A 62-year-old man developed polycythemia (hematocrit, 70.4%) after the surreptitious injection of rHuEPO.[105] Subsequently, he developed worsening hypertension, exacerbation of chronic lung disease, and new-onset angina. His clinical abnormalities included labored breathing, cyanosis, diaphoresis, hypotension, tachycardia, and acidosis. Respiratory failure required endotracheal intubation with mechanical ventilation, IV hydration, and serial phlebotomies. He refused to divulge the amount of rHuEPO injected.

Overdose

Based on limited clinical data, healthy individuals tolerate large, single doses of rHuEPO without complications. In a clinical study of rHuEPO, 2 patients with myocardial infarctions inadvertently received about 10 times the intended dose of 33,000 U on the second of 3 days of therapy; the patients remained asymptomatic.[51] Repeated, prolonged supratherapeutic doses of rHuEPO potentially cause serious morbidity related to hyperviscosity or immune reactions (red cell aplasia, allergic reactions). Case reports associated the excessive use of rHuEPO with polycythemia, altered mental status, mesenteric ischemia, myalgias, and arthralgias. Following the inadvertent administration of high doses of rHuEPO, a 42-year-old man with AIDS developed confusion, abdominal pain, and pain in his feet.[53] The hemoglobin on presentation was 23.2 g/dL with a hematocrit of 77.1%. His symptoms resolved after treatment including IV fluids, phlebotomy, and 2 sessions of erythropheresis. The only sequelae involved minor skin loss over his toes.

Fatalities

Blood doping is a suspected cause of sudden death in young athletes, particularly in cyclists. The chronic abuse of rHuEPO by elite cyclists is an alleged cause of several deaths from cardiovascular complications.[106,107] Between 1987 and 1990, 19 Belgian and Dutch cyclists died of uncertain causes suspected to be related to the use of rHuEPO.[108] However, there are few data on the clinical features or pathologic abnormalities present in these athletes during the period leading up to their deaths.

Abstinence Syndrome

There is no abstinence syndrome associated with chronic rHuEPO therapy or abuse. However, animal studies suggest that after cessation of chronic rHuEPO therapy, inhibition of endogenous erythropoietic activity may persist, leading to the development of rebound anemia several weeks after treatment ends. Recovery of erythropoietic activity typically follows the occurrence of rebound anemia by 2 months after cessation of treatment.[109] Some factors favoring thrombosis also remain elevated after cessation of rHuEPO therapy. In a study of renal dialysis patients treated with rHuEPO, factor VIII von Willebrand factor antigen and fibrinogen remain elevated after the cessation of rHuEPO therapy, and a significant increase in the concentration of D-dimer occurred.[110]

Reproductive Abnormalities

The FDA lists epoetin and darbepoetin alfa in Pregnancy Category C.[22] The administration of epoetin alfa at 5 times the typical human dose (500 U/kg) to pregnant rats was associated with an increased incidence of decreased body weight, delayed eye opening, and decreased caudal vertebrae in offspring based on pharmaceutical company data.[52] Darbepoetin alpha studies

demonstrated decreased weight in animal models, but no evidence of teratogenetic effects were reported in the pharmaceutical company data.[111] Case reports suggest that rHuEPO is an effective therapy for pregnant patients with severe anemia and renal failure.[112] Complications typically associated with rHuEPO therapy were not reported in the neonates or mothers.[112,113] Treatment with rHuEPO and IV iron increases the mother's capacity to donate blood for autologous and intrauterine transfusions.[114]

DIAGNOSTIC TESTING

Analytic Methods

The effects of rHuEPO continue after the elimination of rHuEPO; however, there is no single, long-term biomarker of rHuEPO use. Direct testing for rHuEPO during competition is impractical because of the complex biotransformation and rapid elimination half-life (i.e., 3–4 days) of rHuEPO.[115] The strategy approved by the International Olympic Committee (IOC) for the detection of rHuEPO use in athletes involves both indirect testing for the effects of rHuEPO and direct testing for the presence of rHuEPO by isoelectric focusing and double immunoblotting.[116] These methods separate rHuEPO from endogenous EPO based on differences in the pattern and extent of glycosylation. There are no direct screening methods for the detection of oxygen carriers, and the best screening method for the detection of hemoglobin-based oxygen carriers is direct visualization of plasma discoloration. The detection limit for this technique is <1% hemoglobin-based oxygen carrier in plasma.[117] The presence of elevated plasma hemoglobin concentrations alone are not sufficient proof of blood doping because 1) hemolysis of erythrocytes during extreme exercise, and 2) storage increases the plasma hemoglobin concentration. The standard cyanmethemoglobin method does not distinguish between endogenous hemoglobin and hemoglobin-based oxygen carriers. Confirmatory methods for the detection of hemoglobin-based oxygen carriers include electrophoresis, size-exclusion high performance liquid chromatography, and liquid chromatography/tandem mass spectrometry.[118] The detection of perfluorocarbons requires specific, expensive analyzers and flow-measuring devices.[119] Methods to detect the presence of the plasma volume expander, dextran in urine samples include liquid chromatography/tandem mass spectrometry.[120] The assay limit of detection (LOD) was 3.8 μg/mL and the lower limit of quantitation (LLOQ) was 12.5 μg/mL. Conventional laboratory techniques do not typically detect gene doping because the detection of gene doping requires molecular techniques with the ability to recognize EPO transgenes and gene transfer vectors.

INDIRECT METHODS

These methods disclose abnormal stimulation of erythropoietic activity by comparing various hematologic parameters in peripheral blood with baseline or population norms. Indirect methods for the detection of rHuEPO use include macrocytic hypochromic erythrocytes, serum soluble transferrin receptor concentration (sTfr), and a combination of 5 biomarkers of enhanced erythropoiesis (hematocrit, reticulocyte percentage, macrocytic erythrocyte percentage, serum EPO, sTfr). Often, the combination of indirect measures of EPO use is called a blood passport; both the International Ski Federation and the International Cycling Union use blood passports to detect blood doping. Changes in these parameters may persist up to 4 weeks after cessation of rHuEPO use based on rHuEPO injections 3 times weekly for up to 8 weeks.[121] However, use of less frequent injections of rHuEPO (e.g., 60–65 IU/kg body weight once weekly) decreases the sensitivity of these indirect methods to detect EPO use.[122]

INDIVIDUAL TESTS.   The use of macrocytic erythrocyte percentage as a marker of rHuEPO administration is a specific, but insensitive technique. In a study of 20 amateur athletes receiving 30 U rHuEPO/kg every other day for 45 days, the use of 0.6% macrocytic erythrocytes as the cutoff detected about 50% of the individuals using rHuEPO.[123] The percentage of macrocytic erythrocytes in blood samples from the 240 elite athletes in the control group did not exceed this cutoff value (i.e., no false-positives). Analytic methods for the detection of serum soluble transferrin receptor (sTfr) include enzyme-linked immunosorbent assay (ELISA), immunoturbidimetry, and a nephelometric technique.[124] This substance is released primarily from erythropoietic progenitors; thus, this test is an indirect marker of erythropoiesis.

ON/OFF MODEL.   The Australian Institute of Sport and the Australian Sports Drug Testing Laboratory developed 2 mathematical models for the detection of rHuEPO use based on Fischer's discriminant analysis of changes in indirect makers of hematopoiesis during the administration of rHuEPO to recreational athletes.[125] The ON model is a screening test to determine the need to collect urine samples for the detection of rHuEPO use. This model utilizes 5 erythrocyte and serum parameters (hematocrit, reticulocyte count, percentage macrocytic erythrocytes, serum EPO, serum sTfr) to screen for the current use of rHuEPO. This model is highly

sensitive during the last 2 weeks of rHuEPO administration and a few days after cessation; however, the sensitivity drops dramatically a few days after stopping the injection of low to moderate doses of rHuEPO.[126] False-positives occur rarely. The OFF model uses 3 indirect markers (hematocrit, percentage macrocytic erythrocytes, serum EPO) to detect the recently (i.e., last 14 days) discontinuation of rHuEPO use; this model is more sensitive than the ON model during the later stages of rHuEPO use up to the last few days prior to cessation.

The OFF model is the only model to detect rHuEPO use >1 week after cessation of use. Figure 17.2 displays the sensitivity of the ON and OFF models in relation to the time of rHuEPO use. The OFF model did not completely separate rHuEPO use from controls; therefore, a confirmatory test (e.g., isoelectric patterning) is required to confirm recent rHuEPO use. The sensitivity of the OFF model up to 3 weeks after cessation of use was 67–72% with no false-positive tests.[127] Some adjustment of the ON- and OFF-model scores for training modalities is necessary because studies of male endurance athletes training at high altitudes demonstrate moderately higher ON-model scores compared with training at sea level.[128] However, physical fitness, training workloads, and sport do not substantially alter the indirect serum biomarkers for the detection of rHuEPO use.[129]
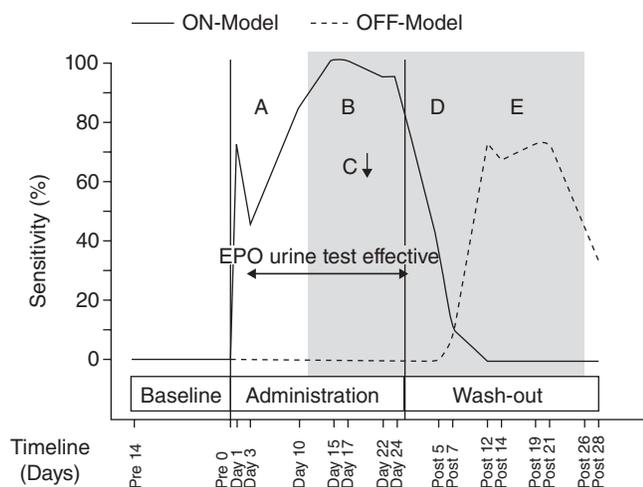
FIGURE 17.2. Sensitivity of ON and OFF models for the detection of rHuEPO use. The shaded area represents the period of enhanced performance.[162] Scenario A: period of rapid increase in rHuEPO concentration before performance enhancement; Scenario B: steady-state rHuEPO use-high dose; Scenario C: steady-state rHuEPO use-low dose; Scenario D: period of increasing OFF model scores while ON model scores decrease; Scenario E: postadministration period with elevated OFF model scores. (Reprinted with permission from R Kazlauskas, C Howe, G Trout, Strategies for rhEPO detection in sport, Clinical Journal of Sport Medicine, Vol. 12, Issue 4, p. 234, copyright 2002.)

Second-generation models (ON-he, ON-hes, OFF-he, OFF-hes) based on standard discriminant analysis are more sensitive than original models, particularly for the detection of the cessation of rHuEPO use and low rHuEPO doses during maintenance regimens.[130] These second-generation models use hemoglobin, reticulocyte percentage, serum erythropoietin, and serum transferrin receptor concentrations rather than volume-dependent parameters (i.e., macrocytic erythrocytes). The incorporation of volume-independent blood parameters allows increased storage and transport times between sample collection and analysis. Longitudinal monitoring of athletes (e.g., hemoglobin, reticulocyte percentage) substantially improves the detection of rHuEPO use weeks after injections cease, particularly when compared with historical values.[131] Blood samples refrigerated for up to 24 hour at 4°C are suitable for the determination of indirect hematologic parameters associated with rHuEPO use.[132] Third-generation models use the athlete's baseline values (hemoglobin, hematocrit, reticulocyte count, third-generation OFF score) rather than population-derived thresholds to reduce within-subject variability.[121]

DIRECT METHODS

IMMUNOASSAYS.   Most standard immunoassays do not distinguish endogenous EPO from recombinant forms of erythropoietin. Immunoassays for the analysis of serum EPO include chemiluminescent immunoassay (CHEM) and ELISA. The LOD and LLOQ are lower for CHEM than ELISA. In an interlaboratory validation study of these 2 immunoassays, the LOD for CHEM was 0.2 mIU/mL compared with 0.6 mIU/mL for ELISA.[133] The LLOQ for CHEM and ELISA were 0.5 mIU/mL and 2 mIU/mL, respectively. The erythropoiesis-mimetic peptide, hematide does not cross reaction with EPO antibodies; thus, conventional tests for rHuEPO do not detect the misuse of this drug.[134]

ELECTROPHORESIS.   The International Olympic Committee Medical Commission (WADA technical document TD2007EPO) approved the use of isoelectric focusing with double immunoblotting and chemiluminescence detection as the confirmation of rHuEPO in urine samples from participating athletes. The second blotting step ("double-blotting") is necessary to avoid nonspecific binding of the secondary antibody and keep interfering urinary proteins on the first membrane.[135] Isoelectric focusing in a horizontal slab-gel containing carrier ampholytes in a pH ranging from 2–6 separates discrete isoforms of endogenous and recombinant EPO based on mass and charge differences in the *N*- and

O-glycans of the isoforms. rHuEPO is less anionic than endogenous EPO;[136] the slightly greater number of sialic acid residues in the glycosylated side chains of the rHuEPO molecule compared with endogenous EPO also allows the separation of these 2 molecules using the isoelectric patterning method in a pH gradient electrical field.[137] The use of rHuEPO by athletes suppresses the production of endogenous EPO, resulting in a continuous shift to the recombinant IEF-profile depending on the serum half-life and the dose of rHuEPO. Some atypical isoelectric focusing patterns result from the partial desialylation, which potentially causes false-negative (but not false-positive) results.[138] To reduce false-positive results, a positive test requires detection of at least 3 consecutive bands with 2-fold greater intensity than those in the acidic region.[39] The use of electrophoresis to detect rHuEPO in urine samples is limited by expense and the short detection interval (i.e., <24–48 hours after the last injection).[139] The unique gene activation technology used to produce epoetin delta limits the effectiveness of current analytic techniques used to detect rHuEPO use.[38] The use of isoelectric focusing techniques requires precautionary measures to prevent EPO degradation by endogenous proteases in urine.[137] Alternate electrophoretic methods for the detection of rHuEPO in urine samples include sodium dodecyl sulfate-polyacrylamide gel electrophoresis and 2-dimensional (2D) electrophoresis. The former method separates proteins based on their apparent molecular mass; an upstream immunoaffinity purification step allows the detection of EPO isoforms.[140] The 2D electrophoretic method combines separation by charge (isoelectric focusing) and by molecular mass (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), resulting in greater resolution than either method alone.[141]

MASS SPECTROMETRY. Mass spectrometric methods for the detection of rHuEPO and darbepoetin alpha in urine samples include liquid chromatography/mass spectrometry,[142] electrospray ionization mass spectrometry (ESI/MS) with separation by either high performance liquid chromatography or capillary electrophoresis,[143] and matrix-assisted laser desorption/ionization mass spectrometry applying a high-resolution time-of-flight mass analyser in the linear mode.[144] The use of enzymes (e.g., trypsin) to cleave the intact glycoproteins allows more detailed structural characterization of EPO isoforms, when analyzing the subsequent peptides/glycopeptides or glycans with a high-resolution time-of-flight mass analyser in the linear mode or electrospray ionization mass spectrometry.[73] The latter method can be operated either on-line or off-line, whereas a high-resolution time-of-flight mass analyser

in the linear mode is primarily an off-line method. Dynepo™ (erythropoietin-delta; Shire Pharmaceuticals, Dublin, Ireland) has no distinct glycosylation pattern that distinguishes this substance from endogenous EPO by direct EPO testing. Although the addition of proteases to urine samples dramatically reduces the sensitivity of these assays, direct tests for protease activity are available to detect sample manipulation.

Biomarkers

BLOOD

In a given individual, the plasma EPO concentration is relatively constant; however, there is about a 2-fold variation among individuals independent of gender, age, and red cell mass.[145] The effects of rHuEPO continue after the elimination of rHuEPO; therefore, analysis of blood primarily involves the detection of indirect biomarkers that suggest rather than confirm rHuEPO use. Increases in the hemoglobin and hematocrit begin about 2–6 weeks after the administration of rHuEPO. Elevation of serum EPO suggests the use of rHuEPO, but standard analytic techniques do not discriminate between endogenous EPO and rHuEPO. Both short-term aerobic exercise and endurance training do not significantly alter the serum erythropoietin (EPO) concentration.[146] In a study of 46 long-distance runners during a training period, the mean serum EPO concentrations of the endurance runners (19.7 ± 5.5 U/L) and sedentary controls (19.7 ± 9.2 U/L) were not statistically different, as measured by radioimmunoassay.[147] There was no statistically significant difference in the serum EPO concentration of a subset (17) of these endurance runners, when comparing the mean serum EPO before and after a 6-hour race. The mean serum EPO concentration was significantly lower ($P < .05$) in a group 15 male triathletes than in a group of 45 male long-distance runners.[148] However, there were no significant differences in the mean serum EPO concentration in female and male athletes in various sports when compared with sedentary controls. Data on the effect of circadian rhythms on the serum EPO concentration are conflicting with some studies demonstrating no effect[149] and other studies indicating lower serum EPO concentration in the morning and higher concentrations in the evening.[150]

The serum sTfr concentration depends on the erythroid proliferation rate and cellular iron demands. Suggested threshold for detection of rHuEPO use based on a 200 U/kg dosing regimen was a serum sTfr concentration exceeding 10 μg/mL.[151] The ratio of sTfr/serum ferritin depends on the amount of rHuEPO administered. In the same study using the 200 U/kg dosing

regimen, statistical analysis indicated a threshold value for the sTfr/serum ferritin ratio of 403 compared with 153 for a 50 U/kg dosing regimen.[48] In contrast to the serum sTfr, the sTfr/serum ferritin ratio is independent of the hematocrit. The use of the sTfr/serum protein ratio has the advantage of being unaffected by the administration of iron supplements that are commonly used by endurance athletes on rHuEPO. The serum sTfr remains elevated for about 1 week after cessation of rHuEPO use compared with about 2 weeks for the sTfr/serum protein ratio.[152] However, these values return to baseline before the ergogenic effect of rHuEPO ceases. Therefore, the use of sTfr or ratios involving sTfr is most useful for out-of-competition testing.

Urine

The typical concentration of EPO in the urine of non-athletes is about 1 IU/L (10 pg/L), whereas the upper limits of elite male and female athletes is 13.5 IU/L and 7.7 IU/L, respectively.[137] Isoelectric focusing differentiates between rHuEPO, endogenous EPO, and darbepoetin alpha, but not Dynepo™ (erythropoietin-delta).[153] The sensitivity of this method decreases dramatically more than 3 days after use of rHuEPO, whereas the longer half-life of darbepoetin alpha allows detection up to about 1 week.[14] In a study of volunteers receiving epoetin alfa 3 times weekly at a dose of 50 IU/kg for 3 weeks, the sensitivity of isoelectric focusing was 100% and about 50% for urine samples collected 3 days and 7 days, respectively, after cessation of the injections.[154] Case reports suggest that the use of microdoses of rHuEPO (i.e., <10% of initial doses) as a maintenance dose reduces the detection interval for rHuEPO in the urine.[155] The detection interval for darbepoetin alfa in urine samples is substantially longer than most forms of rHuEPO. In a study of 3 healthy men, darbepoetin alfa was detectable in urine samples for up to 7 days after the injection of 40 μg (60 IU), as measured by isoelectric focusing.[156] Case reports suggest that false-positive results for epoetin beta occur rarely under conditions of extreme exercise following the analysis of postexercise, highly concentrated, protein-rich urine using isoelectric focusing.[157] Additional tests to identify false-positive results include indirect markers of altered erythropoiesis or 2D electrophoresis.[158]

Abnormalities

Abuse of rHuEPO can decrease endogenous EPO production, depress reticulocytosis, and cause anemia.[159] Blood abnormalities associated with chronic rHuEPO use include thrombosis (increased D-dimer), refractory anemia (decreases reticulocyte count), and hyperkale-mia. The inadvertent administration of 384,000 U rHuEPO over 3 days was associated with modest elevation of serum hepatic aminotransferase concentrations, but no liver dysfunction developed.[51]

TREATMENT

Stabilization

The treatment for excessive use of erythropoiesis-stimulating agents is supportive including withdrawal from these agents and close monitoring for the development of ischemic (angina, myocardial infarction, congestive heart failure, mesenteric ischemia) and thrombotic (cerebral embolism/infarction, deep vein thrombosis, pulmonary embolism) complications. There are no known antidotes for overdoses of erythropoiesis-stimulating agents.

Polycythemia

Life-threatening ischemic and thrombotic complications frequently occur when the hematocrit exceeds 60%. Patients presenting with a hematocrit above this level require emergent phlebotomy along with fluid replacement or in very emergent situations, erythropheresis (i.e., selective removal of erythrocytes with reinfusion of the remaining portion of the blood) in sufficient amounts to decrease the hematocrit to levels near 55%. The ultimate hematocrit target over the first week of therapy is <45% while maintaining intravascular volume.[53] In a patient with a hematocrit of 72% following an inadvertent overdose of rHuEPO, phlebotomy and 2 sessions of erythropheresis removed 898 mL and 640 mL red blood cells, respectively.[53] Reducing his hematocrit below 45% resolved his symptoms of abdominal pain, confusion, and peripheral ischemia. Repeat phlebotomy or erythropheresis may be necessary in the first 4–5 days after cessation of erythropoiesis-stimulating agents because of the continuing presence of erythropoiesis-stimulating agents during this period. In less urgent, asymptomatic patients, phlebotomy (500 mL with fluid replacement) may suffice every other week.

Hypertension

Hypertension is a common adverse effect associated with erythropoiesis-stimulating agents that is multifactorial (e.g., preexisting hypertension, rapid increase in hematocrit, low baseline hematocrit, genetic predisposition, high-dose therapy). The elevation in blood pressure usually responds to conventional therapy including reduction and/or discontinuation of

erythropoiesis-stimulating agents, antiplatelet therapy, standard antihypertensive agents (beta-blockers, vaso-dilators), and fluid removal (diuretics or dialysis in patients with end-stage kidney disease).[160,161] ACE-inhibitors and calcium antagonists are options to beta-blockers and vasodilators as choices for an antihypertensive agents. Rarely, hypertensive encepha-lopathy complicates the use of rHuEPO in patients with end-stage renal disease. Other than the reduction of elevated hemoglobin concentrations as outlined in the Stabilization section, the treatment of hypertensive encephalopathy in these patients is similar to standard therapy for hypertensive encephalopathy including the IV use of nitroprusside (initial infusion rate 0.1 μg/kg/min titrated to desired end point with typical average infusion 0.5–8 μg/kg/min) or, in cases with coronary ischemia, nitroglycerin (10–20 μg/min increased by 5–10 μg/min every 5–10 minutes until desired response).

Gut Decontamination

Decontamination measures are unnecessary because of the poor oral bioavailability of erythropoiesis-stimulating agents.

Elimination Enhancement

The large molecular weight of erythropoiesis-stimulating agents limits the efficacy of extracorporeal methods of removal. Hemodialysis for complications following the use of erythropoiesis-stimulating agents primarily involves the correction of fluid and electrolyte balance in patients with chronic renal disease. Plasmapheresis theoretically could reduce the circulating erythropoiesis-stimulating agents, but there are no clinical data to support the routine use of plasmapheresis.

Supplemental Care

The presence of severe vomiting or diarrhea indicates the need to monitor fluid/electrolyte balance and evalu-ate the patient for mesenteric ischemia. Asymptomatic patients with elevated hematocrit from chronic use of erythropoiesis-stimulating agents require monitoring of hematocrit and blood pressure for at least 1 week after cessation of therapy. Patients with pure red cell aplasia usually require regular packed red blood cell transfu-sions until the anemia resolves and the antierythropoi-etin antibodies disappear. In cases of pure red blood cell aplasia related to drugs other than erythropoiesis-stimulating agents, the anemia typically remits within 1–2 weeks; however, there are inadequate data to deter-mine the course of pure red blood cell aplasia in indi-viduals abusing erythropoiesis-stimulating agents. In the

absence of kidney transplantation, the treatment of pure red blood cell aplasia usually involves prednisone (1 mg/kg/d starting dose).[88]

References

1. Gaudard A, Varlet-Marie E, Bressolle F, Audran M. Drugs for increasing oxygen and their potential use in doping: a review. Sports Med 2003;33:187-212.

2. Wehrlin JP, Zuest P, Hallen J, Marti B. Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. J Appl Physiol 2006;100:1938–1945.

3. Bonnemain B. When blood and meat were drugs. Rev Hist Pharm (Paris) 2003;51:611–624. [French]

4. Klein HG. Blood transfusion and athletics. Games people play. N Engl J Med 1985;312:854–856.

5. Mottram DR. Banned drugs in sport. Does the International Olympic Committee (IOC) list need updat-ing? Sports Med 1999;27:1–10.

6. Jelkmann W. Erythropoietin after a century of research: younger than ever. Eur J Haematol 2007;78:183–205.

7. Lappin TR, Rich IN. Erythropoietin – the first 90 years. Clin Lab Haematol 1996;18:137–145.

8. Bonsdorff I, Jalavisto E. A humoral mechanism in anoxic erythrocytosis. Acta Physiol Scand 1948;16:150–170.

9. Ribatti D. Erythropoietin, the first century. Leuk Res 2008;32:1169–1172.

10. Jacobson LO, Goldwasser E, Fried W, Plzak L. Role of the kidney in erythropoiesis. Nature 1957;179:633–634.

11. Tong EM, Nissenson AR. Erythropoietin and anemia. Semin Nephrol 2001;21:190–203.

12. Tokish JM, Kocher MS, Hawkins RJ. Ergogenic aids: a review of basic science, performance, side effects, and status in sports. Am J Sports Med 2004;32:1543–1553.

13. Scott J, Phillips GC. Erythropoietin in sports: a new look at an old problem. Curr Sports Med Rep 2005;4:422-426.

14. Wilber RL. Detection of DNA-recombinant human epoetin-alfa as a pharmacological ergogenic aid. Sports Med 2002,32:125–142.

15. Robinson N, Giraud S, Saudan C, Baume N, Avois L, Mangin P, Saugy M. Erythropoietin and blood doping. Br J Sports Med 2006;40(suppl 1):i30–i34.

16. Borrione P, Mastrone A, Salvo RA, Spaccamiglio A, Grasso L, Angeli A. Oxygen delivery enhancers: past, present, and future. J Endocrinol Invest 2008;31:185–192.

17. Romanowski RR, Sytkowski AJ. The molecular structure of human erythropoietin. Hematol Oncol Clin N America 1994;8:885–894.

18. Bazan JF. A novel family of growth factor receptors: a common binding domain in the growth hormone, prolac-tin, erythropoietin and IL-6 receptors, and the p75 IL-2

receptor beta-chain. Biochem Biophys Res Commun 1989;164:788–95.

19. Lah J, Prislan I, Krzan B, Salobir M, Francky A, Vesnaver G. Erythropoietin unfolding: thermodynamics and its correlation with structural features Biochemistry 2005;44:13883–13892.

20. Storring PL, Tiplady RJ, Gaines Das RE, Stenning BE, Lamikanra A, Rafferty B, Lee J. Epoetin alfa and beta differ in their erythropoietin isoform compositions and biological properties. Br J Haematol 1998;100:79–89.

21. Zamboni WC, Stewart CE. An overview of the pharmacokinetic disposition of darbepoetin alfa. Pharmacotherapy 2002;22:133S–140S.

22. Pascual JA, Belalcazar V, de Bolos C, Gutierrez R, Llop E, Segura J. Recombinant erythropoietin and analogues: a challenge for doping control. Ther Drug Monit 2004;26: 175–179.

23. Macdougall IC. CERA (Continuous Erythropoietin Receptor Activator): a new erythropoiesis-stimulating agent for the treatment of anemia. Curr Hematol Rep 2005;4:436–440.

24. Hsieh MM, Linde NS, Wynter A, Metzger M, Wong C, Langsetmo I, et al. HIF prolyl hydroxylase inhibition results in endogenous erythropoietin induction, erythrocytosis, and modest fetal hemoglobin expression in rhesus macaques. Blood 2007;110:2140–2147.

25. World Anti-Doping Agency. Available at http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf. Accessed 7/10/2011.

26. Scarpino V, Arrigo A, Benzi G, Garattini S, La Vecchia C, Bernardi LR, et al. Evaluation of prevalence of "doping" among Italian athletes. Lancet 1990;336(8722): 1048–1050.

27. Keld DB, Hahn T. [Use of anabolic androgenic steroids, growth hormone and erythropoietin by patients in general practice] Ugeskr Laeger 2006;168:3121–3124. [Danish]

28. Porter DL, Goldberg MA. Physiology of erythropoietin production. Semin Hematol 1994;31:112–121.

29. Lacombe C, Mayeux P. The molecular biology of erythropoietin. Nephrol Dial Transplant 1999;14(Suppl 2): S22–S28.

30. Deicher R, Horl WH. Differentiating factors between erythropoiesis-stimulating agents: a guide to selection for anaemia of chronic kidney disease. Drugs 2004;64: 499–509.

31. Spalding BJ. Black marketing biotechnology: Athletes abuse EPO and HGH. Biotechnology (NY) 1991; 9: 1050–1053.

32. Schumacher YO, Schmid A, Dinklemann S, Berg A, Northoff H. Artificial oxygen carriers-the new doping threat in endurance sports. Int J Sports Med 2001;22: 566–571.

33. Schumacher YO, Ashenden M. Doping with artificial oxygen carriers—an update. Sports Med 2004;34: 141–150.

34. Maitani Y, Hazama M, Tojo Y, Shimoda N, Nagai T. Oral administration of recombinant human erythropoietin in liposomes in rats. Influence of lipid composition and size of liposomes on bioavailability. J Pharm Sci 1996;85: 440–445.

35. Ninot G, Connes P, Caillaud C. Effects of recombinant human erythropoietin injections on physical self in endurance athletes. J Sports Sci 2006;24:383–391.

36. Cristani A, Boldrini E, Amateis E, Arioli D. [Blood Doping 2.] Recenti Prog Med 2005;96:1–3. [Italian]

37. Lippi G, Banfi G. Blood transfusions in athletes. Old dogmas, new tricks. Clin Chem Lab Med 2006; 44: 1395–1402.

38. Lippi G, Franchini M, Salvagno GL, Guidi GC. Biochemistry, physiology, and complications of blood doping: facts and speculation. Crit Rev Clin Lab Sci 2006; 43:349–391.

39. Elliott S. Erythropoiesis-stimulating agents and other methods to enhance oxygen transport. Br J Pharmacol 2008;154:529–541.

40. Baginski S, Korner R, Frei U, Eckardt KU. [Anemia and erythropoietin in critically ill patients.] Zentralbl Chir 2003;128:487–492. [German]

41. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of anemia of end stage renal disease with recombinant human erythropoietin: results of a phase I and phase II clinical trial. N Eng J Med 1987; 316:73–78.

42. Cheung WK, Goon BL, Guilfoyle MC, Wacholtz MC. Pharmacokinetics and pharmacodynamics of recombinant human erythropoietin after single and multiple subcutaneous doses to healthy subjects. Clin Pharmacol Ther 1998;64:412–423.

43. Flaharty KK, Caro J, Erslev A, Whalen JJ, Morris EM, Bjornsson TD, Vlasses PH. Pharmacokinetics and erythropoietic response to human recombinant erythropoietin in healthy men. Clin Pharmacol Ther 1990;47:557–564.

44. National Kidney Foundation. DOQI clinical practice guidelines for anemia of chronic disease 2000 Am J Kidney Dis 2001;37 (suppl):S182–S238.

45. Verbeelen D, Bossuft A, Smitz J. Hemodynamics of patients with recombinant human erythropoietin. Clin Nephrol 1989;31:6–11.

46. Markham A, Bryson HM. Epoetin alfa: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in nonrenal applications. Drugs 1995; 49:232–254.

47. Balsom PD, Ekblom B, Sjodin B. Enhanced oxygen availability during high intensity intermittent exercise decreases anaerobic metabolite concentrations in blood. Acta Physiol Scand 1994;150:455–456.

48. Audran M, Gareau R, Matecki S, Durand F, Chenard C, Sicart MT, et al. Effects of erythropoietin administration in training athletes and possible indirect detection in doping control. Med Sci Sports Exerc 1999;31:639–645.

49. Williams MH, Branch JD. Ergogenic aids for improved performance. In: Garrett WE, Kirkendall DT, eds. Exercise and sport science. Philadelphia, PA: Lippincott, Williams and Wilkins; 2000:373–384.

50. Clyne N, Berglund B, Egberg N. Treatment with recombinant human erythropoietin induces a moderate rise in hematocrit and thrombin antithrombin in healthy subjects. Thromb Res 1995;79:125–129.

51. Shin D-H, Kwon Y-I, Choi S-I, Park U-S, Lee J, Shin J-H, et al. Accidental ten times overdose administration of recombinant human erythropoietin (rh-EPO) up to 318,000 units a day in acute myocardial infarction: report of two cases. Basic Clin Pharmacol Toxicol 2006;98: 222–228.

52. Product Information. Epogen, epoetin alfa. Thousand Oaks, CA: Amgen; 1999.

53. Hoffman RS, Cobrin G, Nelson LS, Howland MA. Erythropoietin overdose treated with emergent erythropheresis Vet Hum Toxicol 2002;44:157–159.

54. Hughes RT, Cotes PM, Oliver DO, Pippard MJ, Royston P, Stevens JM, et al. Correction of the anaemia of chronic renal failure with erythropoietin: pharmacokinetic studies in patients on haemodialysis and CAPD. Contrib Nephrol 1989;76:122–130.

55. Macdougall IC. An overview of the efficacy and safety of novel erythropoiesis stimulating protein. Nephrol Dial Transplant 2001;16:14–21.

56. Salmonson T, Danielson BG, Wikstrom B. The pharmacokinetics of recombinant human erythropoietin after iv and sc administration to healthy subjects. B J Clin Pharmacol 1990;29:709–713.

57. Evans JH, Brocklebank JT, Bowmer CJ, Ng PC. Pharmacokinetics of recombinant human erythropoietin in children with renal failure. Nephrol Dial Transplant 1991;6:709–714.

58. Macdougall IC, Gray SJ, Elston O, Breen C, Jenkins B, Browne J, Egrie J. Pharmacokinetics of novel erythropoiesis stimulating protein compared with epoetin alfa in dialysis patients. J Am Soc Nephrol 1999;10:2392–2395.

59. Lerner G, Kale AS, Warady BA, Jabs K, Bunchman TE, Heatherington A, et al. Pharmacokinetics of darbepoetin alfa in pediatric patients with chronic kidney disease. Pediatr Nephrol 2002;17:933–937.

60. Overbay DK, Manley HJ. Darbepoetin-alpha: a review of the literature. Pharmacology 2002,22:889–897.

61. Heatherington AC, Dittrich C, Sullivan JT, Rossi G, Schueller J. Pharmacokinetics of darbepoetin alfa after intravenous or subcutaneous administration in patients with non-myeloid malignancies undergoing chemotherapy. Clin Pharmacokinet 2006;45:199–211.

62. Fukuda MN, Sasaki H, Lopez L, Fukuda M. Survival of recombinant erythropoietin in the circulation: The role of carbohydrates. Blood 1989;73:84–89.

63. Briggs DW, Fisher JW, George WJ. Hepatic clearance of intact and desialylated erythropoietin. Am J Physiol 1974;227:1385–1388.

64. Varlet-Marie E, Gaudard A, Audran M, Bressolle R. Pharmacokinetics/pharmacodynamics of recombinant human erythropoietins in doping control. Sports Med 2003;33:301–315.

65. Veng-Pedersen P, Widness JA, Pereira LM, Peters C, Schmidt RL, Lowe LS. Kinetic evaluation of nonlinear drug elimination by a disposition decomposition analysis. Application to the analysis of the nonlinear elimination kinetics of erythropoietin in adult humans. J Pharm Sci 1995;84:760–767.

66. Egrie JC, Dwyer E, Browne JK, Hitz A, Lykos MA. Darbepoetin alfa has a longer circulating half-life and greater *in vivo* potency than recombinant human erythropoietin. Exp Hematol 2003;31:290–299.

67. Halstenson CE, Macres M, Katz SA, Schnieders JR, Watanabe M, Sobota JT, Abraham PA. Comparative pharmacokinetics and pharmacodynamics of epoetin alfa and epoetin beta. Clin Pharmacol Ther 1991;50: 702–712.

68. Marsden JT. Erythropoietin-measurement and clinical applications. Ann Clin Biochem 2006; 43:97–104.

69. Souillard A, Audran M, Bressolle F, Gareau R, Duvallet A, Chanal JL. Pharmacokinetics and pharmacodynamics of recombinant human erythropoietin in athletes. Blood sampling and doping control. Br J Clin Pharmacol 1996;42:355–364.

70. Huch R, Huch A. Erythropoietin in obstetrics. Hematol Oncol Clin North Am 1994;8:1021–1040.

71. Eschbach JW, Kelly MR, Haley NR, Abels RI, Adamson JW. Treatment of the anemia of progressive renal failure with recombinant erythropoietin. N Eng J Med 1989; 321:158–163.

72. Spivak JL. Erythropoietin use and abuse: when physiology and pharmacology collide. Adv Exp Med Biol 2001; 502:207–224.

73. Reichel C, Gmeiner G. Erythropoietin and analogs. Handb Exp Pharmacol 2010;195:251–294.

74. Jelkmann W. Molecular biology of erythropoietin. Intern Med 2004;43:649–659.

75. Lavoie C, Diguet A, Milot M, Gareau R. Erythropoietin (rHuEPO) doping: effects of exercise on anaerobic metabolism in rats. Int J Sports Med 1998;19:281–286.

76. Stohlawetz PJ, Dzirlo L, Hergovich N, Lackner E, Mensik C, Eichler HG, et al. Effects of EPO on platelet activity and thrombopoiesis in humans. Blood 2000;95: 2983–2989.

77. Gauthier J. [Cardiovascular effects of doping]. Ann Cardiol Angeiol (Paris). 2001;50:293–298. [French]

78. Corrigan B. Beyond EPO. Clin J Sport Med. 2002; 12:242–244.

79. Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, Reddan D, CHOIR Investigators. Correction of anemia with epoetin alpha in chronic kidney disease. N Eng J Med 2006;355:2085–2098.

80. Szczech LA, Barnhart HX, Sapp S, Felker GM, Hernandez A, Reddan D, et al. A secondary analysis of the CHOIR

trial shows that comorbid conditions differentially affect outcomes during anemia treatment. Kidney Int 2010;77: 239–46.

81. Lundby C, Thomsen JJ, Boushel R, Koskolou M, Warberg J, Calbet JA, Robach P. Erythropoietin treatment elevates haemoglobin concentration by increasing red cell volume and depressing plasma volume. J Physiol 2007; 578:309–314.

82. Berglund B, Ekblom B. Effect of recombinant human erythropoietin treatment on blood pressure and some haemotological parameters in healthy men. J Intern Med 1991; 229:125–130.

83. Krapf R, Hulter HN. Arterial hypertension induced by erythropoietin and erythropoiesis-stimulating agents (ESA). Clin J Am Soc Nephrol 2009;4:470–480.

84. Celsing F, Svedenhag J, Pihlstedt P, Ekblom B. Effects of anaemia and stepwise-induced polycythaemia on maximal aerobic power in individuals with high and low haemoglobin concentrations. Acta Physiol Scand 1987; 129:47–54.

85. Wilcox CS, Deng X, Doll AH, Snellen H, Welch WJ. Nitric oxide mediates renal vasodilation during erythropoietin-induced polycythemia. Kidney Int 1993;44:430–435.

86. Carlini RG, Dusso AS, Obialo CI, Alvarez UM, Rothstein M. Recombinant human erythropoietin (rHuEPO) increases endothelin-1 release by endothelial cells. Kidney Int 1993;43:1010–1014

87. Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian JJ, Martin-Dupont P, et al. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med 2002; 346:469–475.

88. Pollock C, Johnson DW, Horl WH, Rossert J, Casadevall N, Schellekens H, et al. Pure red cell aplasia induced by erythropoiesis-stimulating agents. Clin J Am Soc Nephrol 2008;3:193–198.

89. Kharagjitsingh AV, Korevaar JC, Vandenbroucke JP, Boeschoten EW, Krediet RT, Daha MR, Dekker FW; NECOSAD Study Group. Incidence of recombinant erythropoietin (EPO) hyporesponse, EPO-associated antibodies, and pure red cell aplasia in dialysis patients. Kidney Int 2005;68:1215–1222.

90. Edmunds ME, Walls J, Tucker B, Baker LR, Tomson CR, Ward M, et al. Seizures in haemodialysis patients treated with recombinant human erythropoietin. Nephrol Dial Transplant 1989;4:1065–1069.

91. Singbartl G. Adverse events of erythropoietin in long-term and in acute/short-term treatment. Clin Investig 1994;72(Suppl 6):S36–S43.

92. Novak BL, Force RW, Mumford BT, Solbrig RM. Erythropoietin-induced hypertensive urgency in a patient with chronic renal insufficiency. Case report and review of the literature. Pharmacotherapy 2003; 23: 265–269.

93. Scheen AJ. [Pharma-clinics. Doping with erythropoietin or the misuse of therapeutic advances.] Rev Med Liege 1998;53:499–502. [French]

94. Dhar R, Stout CW, Link MS, Homoud MK, Weinstock J, Estes NA 3rd. Cardiovascular toxicities of performance-enhancing substances in sports. Mayo Clin Proc 2005; 80:1307–1315.

95. Harwick N, King CM. Generalized eczematous reaction to EPO. Dermatitis 1993;28:123.

96. Schroder-Kolb B. [Cutaneous reactions to treatment with recombinant human erythropoietin.] Derm Beruf Umwelt 1990;38:12–13. [German]

97. Minuto F, Barreca A, Melioli GJ. Indirect evidence of hormone abuse. Proof of doping? Endocrinol Invest 2003;26:919–923.

98. Bennett CL, Cournoyer D, Carson KR, Rossert J, Luminari S, Evens AM, et al. Long-term outcome of individuals with pure red cell aplasia and anti-erythropoietin antibodies in patients treated with recombinant epoetin: a follow-up report from the Research on Adverse Drug Events and Reports (RADAR) Project. Blood 2005;106:3343–3347.

99. Yasuda Y, Fujita Y, Matsuo T, Koinuma S, Hara S, Tazaki A, et al. Erythropoietin regulates tumour growth of human malignancies. Carcinogenesis 2003;24:1021–1029.

100. Maschio G. Erythropoietin and systemic hypertension. Nephrol Dial Transplant 1995;10(Suppl 2):S74–S79.

101. Winearls CG, Oliver DO, Pippard MJ, Reid C, Downing MR, Cotes PM. Effect of human erythropoietin derived from recombinant DNA on the anemia of patients maintained by chronic haemodialysis. Lancet 1986;2(8517): 1175–1178.

102. Lindberg J. Darbepoetin alfa: a new therapy for the management of anaemia associated with chronic kidney disease. Expert Opin Biol Ther 2002;2:977–984.

103. Beccari M. Seizures in dialysis patients treated with recombinant erythropoietin. Review of the literature and guidelines for prevention. Int J Artif Organs 1994;17: 5–13.

104. Finelli PF, Carley MD. Cerebral venous thrombosis associated with epoetin therapy. Arch Neurol 2000; 57: 260–262.

105. Brown KR, Carter W Jr, Lombardi GE. Recombinant erythropoietin overdose. Am J Emerg Med 1993; 11:619-621.

106. Scheen AJ. [Pharma-clinics: Doping with erythropoietin or the misuse of therapeutic advances]. Rev Med Liege 1998;53:499–502. [French]

107. Noakes TD. Tainted glory–doping and athletic performance. N Engl J Med 2004;351:847–849.

108. Eicher ER. Better dead than second. J Lab Clin Med 1992;120:359–360.

109. Piron M, Loo M, Gothot A, Tassin F, Fillet G, Beguin Y. Cessation of intensive treatment with recombinant human erythropoietin is followed by secondary anemia. Blood 2001;97:442–448.

110. Taylor JE, Belch JJ, McLaren M, Henderson IS, Stewart WK. Effect of erythropoietin therapy and withdrawal on

blood coagulation and fibrinolysis in hemodialysis patients. Kidney Int 1993;44:182–190.

111. Product Information. Aranesp, darbepoetin alfa. Thousand Oaks, CA: Amgen; 2001.

112. Scott LL, Ramin SM, Richey M, Hanson J, Gilstrap LC 3rd. Erythropoietin use in pregnancy: two cases and a review of the literature. Am J Perinatol 1995;12:22–24.

113. Braga J, Marques R, Branco A, Goncalves J, Lobato L, Pimentel JP, et al. Maternal and perinatal implications of the use of human recombinant erythropoietin. Acta Obstet Gynecol Scand 1996;75:449–453.

114. Lydaki E, Nikoloudi I, Kaminopetros P, Bolonaki I, Sifakis S, Kikidi K, et al. Transfusion. Serial blood donations for intrauterine transfusions of severe hemolytic disease of the newborn with the use of recombinant erythropoietin in a pregnant woman alloimmunized with anti-Ku. Transfusion 2005;45:1791–1795.

115. Kazlauskas R, Howe C, Trout G. Strategies for rhEPO detection in sport. Clin J Sport Med 2002;12:229–235.

116. Diamanti-Kandarakis E, Konstantinopoulos PA, Papailiou J, Kandarakis SA, Andreopoulos A, Sykiotis GP. Erythropoietin abuse and erythropoietin gene doping: detection strategies in the genomic era. Sports Med 2005;35:831–840.

117. Goebel C, Alma C, Howe C, Kazlauskas R, Trout G. Methodologies for detection of hemoglobin-based oxygen carriers. J Chromatogr Sci 2005;43:39–46.

118. Varlet-Marie E, Ashenden M, Lasne F, Sicart MT, Marion B, de Ceaurriz J, Audran M. Detection of hemoglobin-based oxygen carriers in human serum for doping analysis: confirmation by size-exclusion HPLC. Clin Chem 2004;50:723–731.

119. Audran M, Connes P, Varlet-Marie E. [Oxygen blood transport and doping]. Bull Acad Natl Med 2003;187:1669–1679. [French]

120. Guddat S, Thevis M, Schanzer W. Identification and quantification of the plasma volume expander dextran in human urine by liquid chromatography-tandem mass spectrometry of enzymatically derived Isomaltose. Biomed Chromatogr 2005;19:743–750.

121. Sharpe K, Ashenden MJ, Schumacher YO. A third generation approach to detect erythropoietin abuse in athletes. Haematologica 2006;91:356–363.

122. Borno A, Aachmann-Andersen NJ, Munch-Andersen T, Hulston CJ, Lundby C. Screening for recombinant human erythropoietin using [Hb], reticulocytes, the OFFhr score, Off z score and Hbz score: status of the blood passport. Eur J Appl Physiol 2010;109:537–543.

123. Casoni I, Ricci G, Ballarin E, Borsetto C, Grazzi G, Guglielmini C, et al. Hematological indices of erythropoietin administration in athletes. Int J Sports Med 1993;14:307–311.

124. Abellan R, Ventura R, Pichini S, Sarda MP, Remacha AF, Pascual JA, et al. Evaluation of immunoassays for the measurement of soluble transferrin receptor as an indirect biomarker of recombinant human erythropoietin misuse in sport. J Immunol Methods 2004;295:89–99.

125. Parisotto R, Wu M, Ashenden MJ, Emslie KR, Gore CJ, Howe C, et al. Detection of recombinant human erythropoietin abuse in athletes utilizing markers of altered erythropoiesis. Haematologica 2001;86:128–137.

126. Delanghe JR, Bollen M, Beullens M. Testing for recombinant erythropoietin. Am J Hematol 2008;83:237–241.

127. Parisotto R, Gore CJ, Emslie KR, Ashenden MJ, Brugnara C, Howe C, et al. A novel method utilizing markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. Haematologica 2000;85:564–572.

128. Sharpe K, Hopkins W, Emslie KR, Howe C, Trout GJ, Kazlauskas R, et al. Development of reference ranges in elite athletes for markers of altered erythropoiesis. Haematologica 2002;87:1248–1257.

129. Abellan R, Ventura R, Pichini S, Palmi L, Bellver M, Olive R, et al. Effect of physical fitness and endurance exercise on indirect biomarkers of recombinant erythropoietin misuse. Int J Sports Med 2007;28:9–15.

130. Gore CJ, Parisotto R, Ashenden MJ, Stray-Gundersen J, Sharpe K, Hopkins W, et al. Second-generation blood tests to detect erythropoietin abuse by athletes. Haematologica 2003;88:333–344.

131. Sharpe K, Ashenden MJ, Schumacher YO. A third generation approach to detect erythropoietin abuse in athletes. Haematologica 2006;91:356–363.

132. Lippi G, Salvagno GL, Solero GP, Franchini M, Guidi GC. Stability of blood cell counts, hematologic parameters and reticulocytes indexes on the Advia A120 hematologic analyzer. J Lab Clin Med 2005;146:333–340.

133. Abellan R, Ventura R, Pichini S, Remacha AF, Pascual JA, Pacifici R, et al. Evaluation of immunoassays for the measurement of erythropoietin (EPO) as an indirect biomarker of recombinant human EPO misuse in sport. J Pharm Biomed Anal 2004;35:1169–1177.

134. Thevis M, Thomas A, Kohler M, Beuck S, Schanzer W. Emerging drugs: mechanism of action, mass spectrometry and doping control analysis. J Mass Spectrom 2009; 44:442–460.

135. Lasne F. Double-blotting: a solution to the problem of nonspecific binding of secondary antibodies in immunoblotting procedures. Methods Mol Biol 2009;536:213–219.

136. Wide L, Bengtsson C, Berglund B, Ekblom B. Detection in blood and urine of recombinant erythropoietin administered to healthy men. Med Sci Sports Exerc 1995; 27:1569–1576.

137. Lasne F, Martin L, Crepin N, de Ceaurriz J. Detection of isoelectric profiles of erythropoietin in urine: differentiation of natural and administered recombinant hormones. Anal Biochem 2002;311:119–126.

138. Belalcazar V, Gutierrez Gallego R, Llop E, Segura J, Pascual JA. Assessing the instability of the isoelectric focusing patterns of erythropoietin in urine. Electrophoresis 2006;27:4387–4395.

139. De Frutos M, Cifuentes A, Diez-Masa JC. Differences in capillary electrophoresis profiles of urinary and recombinant erythropoietin. Electrophoresis 2003;24:678–680.

140. Kohler M, Ayotte C, Desharnais P, Flenker U, Lüdke S, Thevis M, et al. Discrimination of recombinant and endogenous urinary erythropoietin by calculating relative mobility values from SDS gels. Int J Sports Med 2008;29:1–6.

141. Khan A, Grinyer J, Truong ST, Breen EJ, Packer NH. New urinary EPO drug testing method using two-dimensional gel electrophoresis. Clin Chim Acta 2005; 358:119–130.

142. Breymann C. Erythropoietin test methods. Baillieres Best Pract Res Clin Endocrinol Metab 2000;14:135–145.

143. Neusüss C, Demelbauer U, Pelzing M. Glycoform characterization of intact erythropoietin by capillary electrophoresis-electrospray-time of flight-mass spectrometry. Electrophoresis 2005;26:1442–1450.

144. Stubiger G, Marchetti M, Nagano M, Reichel C, Gmeiner G, Allmaier G. Characterisation of intact recombinant human erythropoietins applied in doping by means of planar gel electrophoretic techniques and matrix-assisted laser desorption/ionization linear time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2005; 19:728–742.

145. Spivak JL. The clinical physiology of erythropoietin. Semin Hematol 1993;30:2–11.

146. Robinson N, Saugy M, Mangin P. Effects of exercise on the secondary blood markers commonly used to suspect erythropoietin doping. Clin Lab 2003;49:57–62.

147. Remacha AF, Ordonez J, Barcelo MJ, Garcia-Die F, Arza B, Estruch A. Evaluation of erythropoietin in endurance runners. Haematologica 1994;79:350–352.

148. Weight LM, Alexander D, Elliot T, Jacobs P. Erythropoietic adaptations to endurance training. Eur J Appl Physiol Occup Physiol 1992;64:444–448.

149. Roberts D, Smith DJ. Erythropoietin does not demonstrate circadian rhythm in healthy men. J Appl Physiol 1996;80:847–851.

150. Wide L, Bengtsson C, Birgegard G. Circadian rhythm of erythropoietin in human serum. Br J Haematol 1989; 72:85–90.

151. Bressolle F, Audran M, Gareau R, Baynes R, Guidicelli C, Gomeni R. Population pharmacodynamics for monitoring epoetin in athletes. Clin Drug Invest 1997; 14: 233–242.

152. Birkeland KI, Stray-Gundersen J, Hemmersbach P, Hallen J, Haug E, Bahr R. Effect of rhEPO administration on serum levels of sTfR and cycling performance. Med Sci Sports Exerc 2000;32:1238-1243.

153. Lopez-Soto-Yarritu P, Diez-Masa JC, Cifuentes AJ, de Frutos M. Improved capillary isoelectric focusing method for recombinant erythropoietin analysis. J Chromatogr A 2002;968:221–228.

154. Breidbach A, Catlin DH, Green GA, Tregub I, Truong H, Gorzek J. Detection of recombinant human erythropoietin in urine by isoelectric focusing. Clin Chem 2003; 49:901–907.

155. Ashenden M, Varlet-Marie E, Lasne F, Audran M. The effects of microdose recombinant human erythropoietin regimens in athletes. Haematologica 2006;91:1143–1144.

156. Lamon S, Robinson N, Mangin P, Saugy M. Detection window of darbepoetin-alpha following one single subcutaneous injection. Clin Chem Acta 2007;379:145–149.

157. Beullens M, Delanghe JR, Bollen M. False-positive detection of recombinant human erythropoietin in urine following strenuous physical exercise. Blood 2006; 107:4711–4713.

158. Khan A, Grinyer J, Truong ST, Breen EJ, Packer NH. New urinary EPO drug testing method using two-dimensional gel electrophoresis. Clin Chim Acta 2005; 358:119–130.

159. Cazzola M. A global strategy for prevention and detection of blood doping with erythropoietin and related drugs. Haematologica 2000; 85:561–563.

160. Sunder-Plassmann G, Horl WH. Effect of erythropoietin on cardiovascular diseases. Am J Kidney Dis 2001;38 (Suppl 1):S20–S25.

161. Luft FC. Erythropoietin and arterial hypertension. Clin Nephrol 2000;53(Suppl 1):S61–S64.

162. Kazlauskas R, Howe C, Trout G. Strategies for rhEPO detection in sport. Clin J Sport Med 2002;12:229–235.

Chapter 18

HUMAN CHORIONIC GONADOTROPIN

HISTORY

In the late 1920s, Zondek and Ascheim discovered a hormone in the urine of pregnant women that simulated some of the effects of pregnancy in immature female mice.[1] Various modifications of their assay based on biologic activity were developed as a means to diagnose pregnancy until the introduction of immunoassays in the 1970s.[2] Screening for the abuse of human chorionic gonadotropin (hCG) in athletic events began in the early 1980s, when urine screens from some elite athletes demonstrated abnormally large concentrations of hCG. The International Olympic Committee (IOC) banned the use of hCG in 1987. The Medical Commission of the IOC introduced testing for peptide hormones and analogues in 1989, including hCG and related releasing factors (adrenocorticotropic hormone, human growth hormone, erythropoietin). This testing involved immunoassay screens rather than confirmatory methods of analysis (i.e., gas chromatography/mass spectrometry). Only men are tested for hCG because of the lack of effect of this compound on muscle mass and athletic performance in women. In 1999, an initiative by the IOC resulted in the Lausanne Declaration on Doping in Sport and the formation of the World Anti-Doping Agency (WADA) to provide scientific research, education, and monitoring of the use of doping agents in sports; WADA now supervises the implementation of the World Anti-Doping Code including the use of hCG and other doping agents by elite athletes. This private Swiss law agency is funded both by the IOC and world governments.

IDENTIFYING CHARACTERISTICS

Structure

Human chorionic gonadotropin (hCG, CAS RN:9002-61-3) is a highly glycosylated, endogenous hormone that belongs to a family of dimeric, structurally similar glycoprotein hormones (thyroid stimulating hormone, follicle stimulating hormone, leuteinizing hormone). Human chorionic gonadotropin consists of a α-subunit of hCG (hCGα, MW: 14 kDa and a β-subunit (hCGβ, 23.5 kDa). HCGα is part of leuteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone in addition to hCG; therefore, the biologic specificity of these hormones resides in hCGβ. Luteinizing hormone (LH) and hCG have similar affinities for the same target cell receptors in the Leydig cells and similar structure with the exception that hCG has an additional 29-amino acid residues at the C-terminal region containing 4 antigenically distinct, *O*-linked sialic acid capped glycosylation sites. The highly glycosylated β-carboxy terminus on hCG facilitates detection by specific immunoassays or mass spectrometry. Various forms of aberrant glycosylation are common in tumor-derived hCG.

The blood and urine contain a variety of isoforms, fragments, and degradation products of hCG in addition

TABLE 18.1. Nomenclature for Human Chorionic Gonadotropin (hCG) and hCG-Related Molecules.[27]

Molecule	Symbol	Description
Intact hCG	hCG	Purified hCG without nicked forms or free subunits
Nicked hCG	hCGn	Partially degraded hCG with missing peptide bonds in hCGβ40–hCGβ50 region
α-Subunit of hCG	hCGα	Purified hCGα-subunits without intact dimeric hCG or other related molecules
β-Subunit of hCG	hCGβ	Purified hCGβ-subunits without intact dimeric hCG or other related molecules
Nicked β-Subunit of hCG	hCGβn	Partially degraded hCGβ with peptide bonds in hCGβ40–hCGβ50 region
β-Core fragment of hCG	hCGβcf	Residues hCGβ-6 to hCGβ-40 joined by disulfide bonds to hCGβ-55 to hCGβ-90

Abbreviations: hCG = Human chorionic gonadotropin.

to the pure hormone. The heterogeneity of hCG forms in biologic fluids (intact hCG, free subunits, fragments or nicked forms) result from production and metabolic processes as well as degradation during storage (e.g., nicked forms of hCG). Although hCGβ has some growth-promoting activity in *in vitro* studies of cancer cells,[3] the function of free hCG subunits including hCGβ remains unclear. Table 18.1 lists the descriptions of intact hCG and related molecules. Most free subunits of hCG lack biologic activity under normal physiological conditions. The free β-subunit (hCGβ) is a 22-kDa polypeptide consisting of 145 amino acid residues with 4 *O*-linked and 2 *N*-linked carbohydrate chains. The core fragment (hCGβcf) of hCGβ is a 10-kDa 2-chained polypeptide linked by disulfide bonds. This core fragment lacks several sequences present in whole hCGβ; production of hCGβcf probably results from the proteolytic degradation of hCG and hCGβ by the kidney. About one-half of the hCG immunoreactivity in urine results from hCGβcf; however, most commercial hCG assays do not detect this core fragment.[4] The free α-subunit (hCGα) is a glycosylated 14-kDa polypeptide consisting of 92 amino acid residues and 2 *N*-linked carbohydrate chains. The serum concentration of hCGα is substantially higher than hCGβ with the latter compound accounting for about 1% of the total hCG.[5] Partially degraded forms of hCG (hCGn) and hCGβ (hCGβn) also occur in the serum and urine as a result of the spontaneous cleavage of amino acids at various positions between amino acids 44–48. The hCG produced in early pregnancy and by trophoblastic cancers is highly glycosylated, but the hyper-glycosylated hCG

(hCGh) decreases to a few percent after 10 weeks of pregnancy.[6] The various forms of hCG produce differences in antigenicity. The substantial variation of hCG forms in sialic acid (charge), carbohydrate, and peptide structure dramatically affect the recognition of hCG in urine by commercial hCG assays based on the sandwich principle.[7]

Form

Human chorionic gonadotropin is available for exogenous administration in various prescription formulations as either purified hCG derived from the urine of pregnant women or by production through recombinant DNA technology.

RECOMBINANT

Ovidrel® (choriogonadotropin alfa; Serono Laboratories, Inc., Randolph, MA) is a subcutaneous (SC) preparation of recombinant hCG (rhCG) that consists of noncovalently linked α- and β-subunits similar to endogenous hCG. The primary amino structures of the α-subunit of rhCG and endogenous hCG are identical, whereas the glycoform pattern differs slightly between recombinant and endogenous forms in the extent of branching and sialylation of oligosaccharides. The glycosylation patterns of the β-subunits of rhCG and endogenous hCG are also very similar, but not identical. Thus, the antigenicity of rhCG is not identical to the forms of hCG derived from the urine of pregnant women. Furthermore, the core fragment, hCGβcf accounts for much of the immunoreactivity in urine of pregnant women with the exception of the first 5–7 weeks of pregnancy when hCG is the predominant form.

PURIFIED HCG

Pregnyl® (Organon International, Roseland, NJ) is an injectable, highly purified preparation of hCG obtained from the urine of pregnant women. This preparation contains equal amounts of hCG isoforms and the core fragment (hCGβcf) that is available in sterile, lyophilized 10 mL-vials for intramuscular administration. Other pharmaceutic sources of hCG derived from pregnant women include Profasti® (Serono Laboratories, Inc., Randolph, MA) and Novarel® (Ferring Pharmaceuticals Inc., Suffern, NY). Each vial contains either 5,000 IU or 10,000 IU, and administration of the lyophilized powder requires reconstitution with a diluent. The urine from athletes using commercial hCG preparations derived from the urine of pregnant women contains little hyperglycosylated hCG (hCGh) that normal appears primarily in early pregnancy.

EXPOSURE

Epidemiology

Human chorionic gonadotropin is listed as a prohibited drug by WADA.[8] Abuse of hCG in male power athletes usually results from the use of this hormone to stimulate testosterone production before competition or to prevent testicular atrophy/shutdown after prolonged courses of testosterone and anabolic androgenic steroids.[9] Human chorionic gonadotropin is an attractive alternative to LH because the latter hormone is supplied in small doses and has a much shorter plasma half-life compared with hCG. There are inadequate data on the prevalence of hCG use among amateur or elite athletes to determine the extent of hCG use among these athletes. The main medical indication for the administration of hCG is the restoration of endogenous testosterone production and normalization of blood testosterone concentrations in gonadotropin-deficient men (e.g., delayed male puberty).

Sources

The primary natural sources of hCG are the syncytiotrophoblastic cells of the growing placenta and the developing embryo. Other sources in addition to the normal placenta include trophoblastic disease (hydatiform mole, choriocarcinoma), ectopic pregnancy, gonadal cancers (ovarian, testicular, extragonadal teratoma), and nontrophoblastic tumors. Medical uses for hCG include prepubertal cryptorchidism, selected cases of hypogonadotropic hypogonadism in men, and the induction of ovulation and pregnancy in the anovulatory, infertile woman with secondary ovarian failure. There are numerous Internet sources for pharmaceutic preparations of hCG; however, there are inadequate data to determine the source of hCG as an ergogenic aid.

Methods of Abuse

Illicit use of hCG by male power athletes usually involves the use of hCG to stimulate testosterone production, to reduce testicular atrophy during anabolic androgenic steroid use, and/or to normalize the testosterone/epitestosterone (T/E) ratio after the administration of anabolic androgenic steroids.[10,11] The latter 2 effects are the most common reasons for the abuse of hCG because hCG is more expensive and less efficient than testosterone and anabolic androgenic steroids for the enhancement of muscle strength. Some male athletes use pharmaceutic preparations of hCG to stimulate testosterone production before competition to improve performance. The administration of hCG often occurs with the mistaken belief that the use of hCG will help avoid the detection of anabolic steroid use by reducing the testosterone/epitestosterone ratio. The administration of hCG stimulates the endogenous production of both testosterone and epitestosterone without increasing the urinary T/E ratio above normal values. This hormone is also administered with cycles of anabolic steroids in an attempt to prevent reduced spermiogenesis and testicular atrophy. The abuse of hCG by female athletes is unlikely because hCG has negligible, if any, stimulation of blood testosterone concentrations in healthy young women; therefore, the use of hCG by women does not improve athletic performance.

DOSE EFFECT

Medical Use

Therapeutic use of the gonadotropins, LH and chorionic gonadotropin, is uncommon and primarily involves the stimulation of steroid production by the gonads. Recommended doses of purified hCG for prepubertal cryptorchidism include 5,000 USP Units (IU) every second day for 4 injections or 500 USP Units 3 times weekly for 4–6 weeks with an increase to 1,000 USP Units 3 times weekly for 4–6 weeks if the response is inadequate. Dosage regimens for hypogonadotropic hypogonadism include 500–1,000 USP Units 3 times a week for 3 weeks, followed by the same dose twice a week for 3 weeks or 4,000 USP Units 3 times weekly for 6–9 months. The latter dose is reduced to 2,000 USP Units 3 times weekly for an additional 3 months, if needed. For many years, purified urinary hCG (uhCG) has been administered to induce the final maturation of follicles and to support the luteal phase of the cycle in women. The dose of uhCG for the induction of ovulation and pregnancy in anovulatory, infertile woman without primary ovarian failure is 5,000–10,000 USP Units 1 day following the last dose of menotropins. The dose of 250 μg rhCG (Ovidrel®) has similar efficacy for infertile women as 5,000–10,000 USP Units uhCG. There are no common off-label uses of hCG in routine clinical medical practice; the use of hCG to treat obesity lacks validation by clinical data.

Illicit Use

Anecdotal data suggests that steroid abusers often use once monthly injection of 2,500-5,000 IU of hCG during an anabolic steroid cycle.[12] During the last 2 weeks of the anabolic steroid cycle, the dose of hCG typically increases to 10,000–20,000 IU divided into 2–4 injections. Other athletes inject 500–1,000 IU daily during a

cycle. There are few data on hCG doses associated with acute or chronic toxicity, in part because of the lack of reporting of illicit hCG use.

TOXICOKINETICS

Absorption

Based on volunteers studies, the bioavailability of hCG is slightly greater after intramuscular (IM) injection than SC injection and greater in nonobese than obese women. Typically, the absolute bioavailability via IM and SC routes ranges from approximately 40–50%. In a study of 6 men and 6 women, the mean absolute bioavailability of 125 μg rhCG IM was 0.47 ± 0.11 compared with 0.39 ± 0.11 for subjects receiving the same dose subcutaneously.[13] A study of 12 nonobese and 12 obese women (body mass index ≥ 28 kg/m^2) compared the area under the plasma concentration-time curve (AUC) from zero to infinity after IM and SC injection of the 2 groups.[14] In nonobese women and obese women, the mean AUC was approximately 24% and 58% higher, respectively, following IM than SC injection. Figure 18.1 demonstrates the plasma hCG concentrations after IM and SC injection of 10,000 IU hCG into obese and nonobese women. Following IM injection of 10,000 IU of equal amounts of hCG and hCGβcf (Pregnyl®) into nonpregnant women, peak serum concentrations of approximately 200–300 IU hCG/L occurred about 1 day after administration.[15] In a study of 404 women receiving 10,000 IU hCG IM, the mean serum concentration was 204.2 IU/L (range, 51–644 IU/L) 12 hours after administration.[16] Peak serum concentrations of LH following IM or SC injections of 5,000 IU are approximately 20 IU/L 1 day after administration.[4]

Distribution

In a study of 6 women and 6 healthy men receiving 250 μg r-hCG IV, the mean steady-state volume of distribution of rhCG was 6.7 ± 1.2 L.[13] During the same study, the steady-state volume of distribution in 12 subjects receiving 5,000 IU of hCG (urine-derived) intravenously was 6.6 ± 2.6 L.

Biotransformation/Elimination

There are limited data on the biotransformation and elimination of hCG; the kinetics of hCG is complex, depending on the formulation, analytic techniques, and phase of pregnancy. Human chorionic gonadotropin undergoes proteolytic degradation both in the liver and the kidney. Hepatic metabolism accounts for about 80% of the elimination of endogenous hCG, whereas the kidney excretes about 20% of the hCG dose, primarily as subunits (e.g., hCGβcf) and nicked forms.[17] The elimination half-lives of endogenous hCG, purified uhCG, rhCG, and hCG subunits vary substantially. The serum half-life of intravenous (IV) purified hCG is biphasic with an average rapid phase of about 5–6 hours and a slow phase of approximately 24–36 hours as measured by radioimmunoassay.[18,19] As measured by immunofluorometric assays, the clearance of hCG in serum samples from 6 women 3 weeks postpartum followed a 3-component model with reported median half-times of about 3½ hours, 18 hours, and 53 hours. The clearances of hCG subunits (hCGα, hCGβ) are more rapid than purified hCG following IV administration with hCGα cleared more rapidly than hCGβ. The serum elimination half-lives in the former study were about ¼ hour and 1¼ hours for hCGα, and approximately ¾ hour and 4 hours for hCGβ. In the study using immunofluorometric assays to detect hCG in postpartum women, the half-lives of hCGα were substantially shorter than hCGβ and purified hCG. The median reported half-times of hCGα were about ½ hour, 6 hours, and 22 hours compared with approximately 1 hour, 24 hours, and 194 hours for hCGβ. The mean serum elimination half-life of hCG was 1.56 ± 0.12 days after the IM injection of 10,000 IU of equal amounts of hCG and hCGβcf (Pregnyl®) to nonpregnant women, as measured by radioimmunoassay.[15] In a study of 404 women receiving 10,000 IU hCG IM, the mean serum half-life of hCG

hCG (IU/L)

FIGURE 18.1. Plasma human chorionic gonadotropin concentrations following intramuscular or subcutaneous injection in obese and nonobese women. (Reprinted from CCW Chan, EHY Ng, MMY Chan, OS Tang, EYL Lau, WSB Yeung, P Ho, Bioavailability of hCG after intramuscular or subcutaneous injection in obese and non-obese women, Human Reproduction, 2003, Vol. 18, p. 2295, by permission by Oxford University Press.)

was approximately 48 hours.[16] The terminal serum half-life of hCG and rhCG is similar. For healthy subjects receiving 125 µg rhCG IM and SC, the mean serum elimination half-life was 26.6 ± 6.5 hours and 28.3 ± 5.8 hours, respectively, as measured by immunoradiometric assay (MAIAclone™, Serono Biodata, Italy).[13]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Both purified urinary hCG and rhCG are potent, long-acting analogs of LH that produce sustained and dose-dependent increases in blood testosterone concentrations by stimulating the secretion of testosterone from Leydig cells. The biologic action of hCG and LH on the surface receptors of the Leydig cells in the testes are similar, but the plasma half-life of hCG is substantially longer than LH. In men, the administration of hCG produces a consistent, sustained increase in plasma testosterone concentrations. Stimulation of testosterone secretion following the injection of hCG is rapid with a 50% increase in plasma testosterone concentration occurring within 2 hours of the IM administration of 6,000 IU hCG.[20] Maximum increases in plasma testosterone concentrations following single IM injections of hCG occur about 2–4 days after the administration of hCG, and the elevation of testosterone concentrations persists up to 6 days.[21] A single IV dose of 125 µg rhCG or an IM dose of 6,000 U uhCG approximately doubles the serum testosterone concentration within about 2–4 days after administration.[13,22]

Large doses of hCG increase the production of aromatase activity in the Leydig cells and subsequently increase the secretion of estradiol by the aromatization of testosterone. Rising estradiol concentrations partially suppress CYP17 activity (17-α-hydroxylase/17,20-lyase) and the hCG-induced increase in testosterone synthesis by a negative feedback mechanism.[23] There is no direct correlation between concentrations of hCG and testosterone in the blood; the testosterone concentration has a biphasic pattern after hCG administration.[10] A study of 40 men over the age of 60 years with partial androgen deficiencies (i.e., blood testosterone <15 nmol/L) demonstrated that twice weekly SC injections of 5,000 IU rhCG increased body weight and lean body mass approximately 1 kg and 2 kg, respectively, over 3 months.[24] These changes were not associated with increased shoulder/knee strength, enhanced physical activity, or improvements in coordination or gait.

In young, healthy women, limited data suggest that hCG causes no clinically significant change in the blood testosterone concentration.[11] The ovaries, adrenal gland, and some extraglandular sources produce small amounts of testosterone in women, but there is no regulation of testosterone by estradiol as occurs in men by a homeostatic negative feedback mechanism. In addition to the very low endogenous blood testosterone concentrations in women, hCG does not stimulate testosterone production as both hCG and LH increase the secretion of testosterone by the Leydig cells in men. Consequently, hCG in women causes no significant androgenic response to high concentrations of hCG including the massive concentrations of hCG produced by the placenta during pregnancy.

CLINICAL RESPONSE

There are few data on the ergogenic effect of the supraphysiologic doses of hCG on muscle mass or strength in women or men. Furthermore, there is no clear evidence in the published literature that the use of hCG increases strength or improves endurance in athletes. Although hCG does initially increase the serum testosterone concentration, there are inadequate data to determine if the increased testosterone concentrations persist and result in improved strength and/or athletic performance. The expected side effects associated with the misuse of hCG and anabolic steroids are similar with the exception of more gynecomastia following hCG use. The administration of hCG to men increases spermatogenesis despite anabolic steroid-induced suppression of gonadotropin secretion in the presence of continued high serum estradiol concentrations. The administration of exogenous testosterone to normal men markedly increases blood testosterone and estradiol concentrations while suppressing gonadotropin, testicular testosterone, and sperm production. Case reports suggest that in the setting of profound symptomatic hypogonadotrophic hypogonadism secondary to anabolic steroid abuse, the administration of hCG stimulates the production of testosterone by the testes.[25] In a study of 5 healthy men receiving 200 mg testosterone weekly, the IM administration of 5,000 IU hCG 3 times weekly normalized the sperm count as assessed by sperm concentrations, motility, and morphology.[26] Although the use of hCG attenuated some of the reduction in spermatogenesis, some studies suggest that this regimen transiently impairs semen quality. In a study of 18 healthy male power athletes using massive doses of anabolic androgenic steroids, there was a significant positive correlation between the use of hCG during the steroid cycle and the presence of morphologically abnormal spermatozoa.[12] Additionally, there was a concomitant decrease in sperm mobility. The overall effect of hCG on male reproduction is unclear. There are no data on hCG overdoses in the medical literature.

DIAGNOSTIC TESTING

Conversion Factors

Standardization of assays for a heterogeneous analyte (e.g., hCG) is difficult because hCG is a family of closely related molecules (e.g., intact hCG, hCGβ, hCGα, hCGn, hCGβn, hCGβcf) rather than a single species.[27] Table 18.1 lists the nomenclature and description of the family of hCG molecules. Equating micrograms of rhCG to levels of biologic activity of uhCG is complicated by the issues related to mass units, molar conversions, purity of the substance, and standardization of biologic activity, particularly the end point used to determine bioactivity. The standard dose of urinary hCG is 5,000 IU of bioactivity. As measured by *in vitro* testosterone production by the Leydig cell method, the median biopotency of uhCG was 14,100 IU/mg (95% CI: 12,500–16,000 IU/mg).[28] Using an *in vivo* seminal vesicle weight method in male rats, the median biopotency for uhCG was 15,400 IU/mg (95% CI: 14,200–16,800 IU/mg) in 1 study, and 10,300 IU/mg (95% CI: 8,360–12,600 IU/mg) in another study.[28] The estimated potency of 250 μg rhCG is approximately 6,500 IU uhCG.[29]

Analytic Methods

Radioimmunoassay (RIA) was the original assay for hCG, but cross-reaction with LH limits the use of conventional RIA methods.[30] Concentrations of hCG in serum and urine samples are highly dependent on the specific assay as a result of differences in assay design and specificity for the different forms of hCG and associated fragments. Degradation of LH during storage increases the cross-reactivity of this compound with monoclonal antibodies for hCG.[31] The use of soft ionization techniques allows the quantitation of large biologic molecules (e.g., hCG) including matrix-assisted laser desorption and ionization time of flight and electrospray ionization. The concentrations (1 μmol/L) of urinary proteins are about 1,000-fold lower than plasma, whereas the hCG concentration (<10 pmol/L) in urine samples from healthy men is >100,000-fold lower than plasma proteins. Thus, analysis of hCG in urine requires extraction by immunopurification followed by reduction, alkylation, and digestion with trypsin.[4] Quantitation results from the detection of nonglycosylated forms by mass spectrometry. Gonadotropins are much less stable in urine than in serum, and hCG is somewhat more stable than LH. Both hCG and LH are stable in storage at 4°C (39.2°F) with 0.02% azide.[32] Storage of urine samples at room temperature substantially reduces hCG immunoreactivity. An *in vitro* study indicated that urinary hCG reactivity decreased over 10% after 5 days of storage at room temperature and 70% after 90 days of storage at room temperature.[33] Freezing at –20°C (–4°F) causes rapid loss of immunoreactivity.

Biomarkers

BLOOD

Serum samples from men and nonpregnant women contain small amounts of hCG: men <60 years have <1.3 IU/L; men >60 years have <2.3 IU/L; premenopausal women have <1.0 IU/L); and postmenopausal women have <4.8 IU/L.[31] In nonpregnant women, the upper reference limit increases during menopause from 3 IU/L (8.6 pmol/L) to around 5 IU/L (15.5 pmol/L) with values in healthy women ranging up to 8–10 IU/L.[15] The upper reference ranges for men below and above 50 years of age are approximately 0.7 IU/L (2.1 pmol/L) and 2 IU/L (6.1 pmol/L), respectively, with occasional concentrations ranging up 3–4 IU/L. Following IM injection of 10,000 IU of equal amounts of hCG and hCGβcf (Pregnyl®) to nonpregnant women, the serum hCG concentration remained above the upper reference range for 7–11 days. The serum hCG concentration increases in several conditions besides pregnancy including gestational trophoblastic disease, testicular cancer, and other tumors (e.g., biliary, pancreatic). In men and nonpregnant women, the administration of estrogen and progestogen lowers the serum hCG by approximately 50%, whereas the administration of gonadotropin-releasing hormone causes about a 3-fold increase in serum hCG.

URINE

The World Anti-Doping Agency requires a limit of detection of 5 IU/L for hCG in urine samples from athletes; published recommended limits for the cutoff of positive samples range from 10–25 IU/L.[34,35] However, WADA has not set recommendations regarding assay specificity other than to require that the hCG assay be positive in 2 different assays. Substantial variation occurs in results of hCG concentrations between various immunoassays as a result of a difference in reactivity between these assays and various isoforms/degradation products of hCG. Although current urine hCG immunoassays are sensitive within WADA guidelines, quantitative data from different immunoassays of hCG are not directly comparable.[33] Most commercial hCG assays measure hCG and hCGβ together, but the results are expressed in IU/L based on hCG units. These immunoassays reflect molar concentrations of protein rather than bioactivity. There is

substantially more heterogeneity in hCG forms in urine than in the serum.[36] After the IM administration of 10,000 IU of equal amounts of hCG and hCGβcf, the urine concentrations of hCGβcf dropped below the upper reference limit 2–3 days earlier than hCG.[15] In a study of urine samples from 1,400 athletes, the median concentration of hCG was 0.8 IU/L with a range of 0–24 IU/L.[37] As measured by Serono MAIAclone IRMA (Serono Diagnostics, Fleet, UK), only 3 of the data points exceeded 5.0 IU/L. The median hCG concentration in urine samples from 120 healthy adults was 0.4 IU/L with a range of nondetectable to 4.6 IU/L. In a study of 5,663 male athletes, the mean hCG concentration in spot urine samples was 0.56 IU/L with a range of nondetectable to 43.03 IU/L.[38] The limit of detection as measured by microparticle enzyme immunoassay (Abbot IM_x® system, Abbott Park, IL) was 0.21 IU/L. Statistical evaluation of the data indicated a far outside value of 2.28 IU/L with a suggested upper limit of 5 IU/L. Recommended reference ranges for total hCG in urine for menstruating women and men <50 years of age are 13.6 pmol/L and 8.0 pmol/L, respectively.[4]

Although hCG and LH are biologically similar, cross-reactivity of highly specific monoclonal antibodies typically is <1%.[39] The administration of hCG produces up to 2- to 3-fold increases in plasma testosterone concentrations, but only minor changes in the urinary testosterone/epitestosterone ratio because hCG stimulates the production of both testosterone and epitestosterone.[40] Using immunoassays and a urine hCG threshold of 5 IU/L, experimental studies in healthy young men suggest that the urine remains positive for hCG about 1 week following a single injection of a 750 µg rhCG dose, whereas the false-negative rate on postinjection day 8 for a 250 µg rhCG dose was 5 out of 12 (42%) injections.[41] The use of nandrolone decanoate by the subjects did not alter these results. The use of hCG substantially alters the testosterone/leuteinizing hormone ratio in spot urine samples because of the fall in LH secretion secondary to the negative feedback from increasing testosterone concentrations. Potentially, use of the testosterone/leuteinizing hormone ratio is a method for the detection of illicit hCG use in sports.[41]

TREATMENT

There are no reported unique treatments for complications resulting from the chronic use of hCG. Treatment is supportive after cessation of hCG use.

References

1. Editorial. The Zondek-Ascheim test for pregnancy. Can Med Assoc J 1930;22:251–253.

2. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gynecol 1972;113:751–758.

3. Butler SA, Staite EM, Iles RK. Reduction of bladder cancer cell growth in response to hCGβ CTP37 vaccinated mouse serum. Oncol Res 2003;14:93-100.

4. Stenman U-H, Hotakainen K, Alfthan H. Gonadotropins in doping: pharmacological basis and detection of illicit use. Br J Pharmacol 2008;154:569–583.

5. Hoermann R, Spoettl G, Moncayo R, Mann K. Evidence for the presence of human chorionic gonadotropin (hCG) and free beta-subunit of hCG in the human pituitary. J Clin Endocrinol Metab 1990;71:179–186.

6. Kovalevskaya G, Kakuma T, Schlatterer J, O'Connor JF. Hyperglycosylated HCG expression in pregnancy: cellular origin and clinical applications. Mol Cell Endocrinol 2007; 260–262:237–243.

7. Cole LA, Sutton JM, Higgins TN, Cembrowski GS. Between-method variation in human chorionic gonadotropin test results. Clin Chem 2004;50:874–882.

8. World Anti-Doping Agency. Available at http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf. Accessed 2011 February 18.

9. Jarow JP, Lipshultz LI. Anabolic steroid-induced hypogonadotropic hypogonadism. Am J Sports Med 1990; 18:429–431.

10. Kicman AT, Brooks RV, Cowan DA. Human chorionic gonadotropin and sport. Br J Sports Med 1991; 25:73–80.

11. Handelsman DJ. Clinical review: the rationale for banning human chorionic gonadotropin and estrogen blockers in sport. J Clin Endocrinol Metab 2006;91:1646–1653.

12. Karila T, Hovatta O, Seppala T. Concomitant abuse of anabolic androgenic steroids and human chorionic gonadotrophin impairs spermatogenesis in power athletes. Int J Sports Med 2004;25:257–263.

13. Trinchard-Lugan I, Khan A, Porchet HC, Munato A. Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotrophin in healthy male and female volunteers. Reprod Biomed Online 2002;4: 106–115.

14. Chan CC, Ng EH, Chan MM, Tang OS, Lau EY, Yeung WS, Ho P-C. Bioavailability of hCG after intramuscular or subcutaneous injection in obese and non-obese women. Human Reprod 2003;18:2294–2297.

15. Stenman U-H, Unkila-Kallio L, Korhonen J, Alfthan H. Immunoprocedures for detecting human chorionic gonadotropin: clinical aspects and doping control. Clin Chem 1997;43:1293–1298.

16. Al-Hassan S, Fishel S, Fleming S, Thornton S. Low plasma levels of hCG after 10,000-IU hCG injection do not reduce the number or maturation of oocytes recovered in patients undergoing assisted reproduction. J Assist Reprod Genet 1998;15:583–586.

17. Nisula BC, Blithe DL, Akar A, Lefort G, Wehmann RE. Metabolic fate of human choriogonadotropin. J Steroid Biochem 1989;33:733–737.

18. Rizkallah T, Gurpide E, Vande Wiele RL. Metabolism of HCG in man. J Clin Endocrinol Metab 1969;29:92-100.

19. Wehmann RE, Nisula BC. Metabolic and renal clearance rates of purified human chorionic gonadotropin. J Clin Invest 1981;68:184–194.

20. Saez JM, Forest MG. Kinetics of human chorionic gonadotropin-induced steroidogenic response of the human testis. I. Plasma testosterone: implications for human chorionic gonadotropin stimulation test. J Clin Endocrinol Metab 1979;49:278–283.

21. Padron RS, Wischusen J, Hudson B, Burger HG, de Kretser DM. Prolonged biphasic response of plasma testosterone to single intramuscular injections of human chorionic gonadotropin. J Clin Endocrinol Metab 1980;50: 1100–1104.

22. Padrón RS, Wischusen J, Hudson B, Burger HG, de Kretser DM. Prolonged biphasic response of plasma testosterone to single intramuscular injections of human chorionic gonadotropin. J Clin Endocrinol Metab 1980;50: 1100–1104.

23. Miller WL. Molecular biology of steroid hormone synthesis. Endocr Rev 1988;9:295-318.

24. Liu PY, Wishart SM, Handelsman DJ. A double-blind, placebo-controlled, randomized clinical trial of recombinant human chorionic gonadotropin on muscle strength and physical function and activity in older men with partial age-related androgen deficiency. J Clin Endocrinol Metab 2002;87:3125–3135.

25. Gill GV. Anabolic steroid induced hypogonadism treated with human chorionic gonadotropin. Postgrad Med J 1998;74:45–46.

26. Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WJ. Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors, and sperm production despite high estradiol levels. J Clin Endocrinol Metab 1983;56:720–728.

27. Bristow A, Berger P, Bidart JM, Birken S, Norman R, Stenman UH, Sturgeon C, and the IFCC Working Group on hCG. Establishment, value assignment, and characterization of new WHO reference reagents for six molecular forms of human chorionic gonadotropin. Clin Chem 2005; 51:177–182.

28. Birken S, Berger P, Bidart J-M, Weber M, Bristow A, Norman R, et al. Preparation and characterization of new WHO reference reagents for human chorionic gonadotropin and metabolites. Clin Chem 2003;29:144–154.

29. Ludwig M, Doody KJ, Doody KM. Use of recombinant human chorionic gonadotropin in ovulation induction. Fertil Steril 2003;79:1051–1059.

30. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gynecol 1972;113:751–758.

31. Stenman UH, Alfthan H, Ranta T, Vartiainen E, Jalkanen J, Seppala M. Serum levels of human chorionic gonadotropin in nonpregnant women and men are modulated by gonadotropin-releasing hormone and sex steroids. J Clin Endocrinol Metab 1987;64:730–736.

32. Stenman UH, Tiitinen A, Alfthan H, Valmu L. The classification, functions and clinical use of different isoforms of HCG. Hum Reprod Update 2006;12:769–784.

33. Robinson N, Sottas P-E, Saugy M. Evaluation of two immunoassays for the measurement of human chorionic gonadotropin in urine for anti-doping purposes. Clin Lab 2010;56:197–206.

34. Laidler P, Cowan DA, Hider RC, Kicman AT. New decision limits and quality-control material for detecting human chorionic gonadotropin misuse in sports. Clin Chem 1994;40:1306–1311.

35. Gam LH, Tham SY, Latiff A. Immunoaffinity extraction and tandem mass spectrometric analysis of human chorionic gonadotropin in doping analysis. J Chromatogr B Analyt Technol Biomed Life Sci 2003;792:187–196.

36. Alfthan H, Haglund C, Dabek J, Stenman UH. Concentrations of human choriogonadotropin, its beta-subunit, and the core fragment of the beta-subunit in serum and urine of men and nonpregnant women. Clin Chem 1992;38:1981–1987.

37. Laidler P, Cowan DA, Hider RC, Kicman AT. New decision limits and quality-control material for detecting human chorionic gonadotropin misuse in sports. Clin Chem 1994;40:1306–1311.

38. Delbeke FT, van Eenoo P, de Backer P. Detection of human chorionic gonadotrophin misuse in sports. Int J Sports Med 1998;19:287–290.

39. Saller B, Clara R, Spottl G, Siddle K, Mann K. Testicular cancer secretes intact human choriogonadotropin (hCG) and its free beta-subunit: evidence that hCG (+hCG-beta) assays are the most reliable in diagnosis and follow-up. Clin Chem 1990;36:234–239.

40. Cowan DA, Kicman AT, Walker CJ, Wheeler MJ. Effect of administration of human chorionic gonadotrophin on criteria used to assess testosterone administration in athletes. J Endocrinol 1991;131:147–154.

41. Handelsman DJ, Goebelt C, Idan A, Jimenez M, Troutt G, Kazlauskast R. Effects of recombinant human LH and hCG on serum and urine LH and androgens in men. Clin Endocrinol 2009;71:417–428.

Chapter 19

HUMAN GROWTH HORMONE and INSULIN-LIKE GROWTH FACTOR

HUMAN GROWTH HORMONE

HISTORY

The presence of a substance (growth hormone) secreted by the anterior pituitary was discovered in the 1920s, when experimental zoologists demonstrated a growth-maintaining compound in the anterior lobe of amphibian brains.[1] In 1929, Putnam et al produced the disease associated with excessive growth hormone secretion (acromegaly) in an English bulldog following the daily injection of a sterile aqueous extract of bovine anterior pituitary glands for 14 months.[2] Pituitary dwarfism is associated with inadequate production of the peptide hormone, hGH that is secreted by the somatotroph cells located in the anterior pituitary gland. In the 1930s, scientists began searching for an exogenous source of growth hormone to treat human dwarfism;[3] however, Li and Evans did not isolate growth hormone from bovine pituitary glands until 1944.[4] By the early 1950s, sufficient amounts of bovine and ovine pituitary extract were available to conduct clinical studies on the use of nonprimate pituitary extracts for the treatment of pituitary dwarfism. Ultimately, these clinical trials demonstrated that nonprimate pituitary extracts are inactive in humans.[5] Virilization and premature epiphyseal closure complicated the subsequent use of testosterone and chorionic gonadotropin to treatment of pituitary dwarfism in children. Li and Papkoff first purified hGH in 1956.[6] During the early 1960s, clinical trials by the Pituitary Hormone Committee of the UK Medical Research Council began using injections of extracts from acetone-preserved human cadaveric pituitary glands for the treatment of children with short stature from growth hormone deficiencies. The National Hormone and Pituitary Program was the main source of hGH in the United States beginning in 1963, resulting in the administration of pituitary-derived hGH to thousands of US children. Substantial numbers of these children grew to near normal adult height, particularly when treated early with adequate doses of the extract.[7] However, these trials were halted in 1985 because of the discovery of the transmission of a rare, fatal disease characterized by the rapid progression of dementia (Creutzfeldt-Jacob disease) in several US and British patients following injection of infectious prions present in cadaveric pituitary glands administered during the 1960s and 1970s. Contaminated cadaveric hGH was the most common iatrogenic cause of Creutzfeldt-Jacob disease with 139 cases reported by 2000.[8] A long-term cohort study of 1,908 UK patients treated with pituitary growth hormone between 1959 and 1985 revealed that 6 patients died of Creutzfeldt-Jacob disease.[9]

During the early 1980s, the development of recombinant growth hormone (rhGH) using recombinant technology resulted in the replacement of human pituitary extracts with rhGH for those children with growth

hormone deficiencies. The US Food and Drug Administration (FDA) approved the clinical use of rhGH (Protropin®, Genentech, South San Francisco, CA) for the treatment of growth hormone deficiency in 1985. In 1987, Leung et al elucidated the amino acid sequence of the growth hormone receptor, demonstrating a new class of transmembrane receptors.[10] Later that year, Abdel-Meguid et al determined the 3-dimensional crystalline structure of porcine GH, which is similar to hGH.[11] In 1996, the FDA approved the use of rhHG for the treatment of wasting or cachexia in patients with acquired immunodeficiency syndrome (AIDS).

The use of hGH in athletics for the enhancement of size, athletic performance, and muscle strength began in the 1980s, particularly among swimmers, cyclists, and competitive bodybuilders. Although this substance was initially promoted for strength disciplines (e.g., football, baseball), the lipolytic actions of hGH was attractive to endurance athletes as a means to reduce fat mass. This hormone was touted as an expensive, fashionable new athletic drug by the steroid guru, Dan Duchaine.[12] Growth hormone was promoted as an anabolic agent that had synergistic effects with anabolic steroids. Ben Johnson, the former Canadian sprinter and world record holder in the 100 meter dash, admitted using a regimen including hGH after his gold medal was revoked following a positive steroid test at the 1988 Seoul Olympic Games. These regimens gained popularity because of the difficulty detecting the use of hGH. In 1996, the International Olympic Committee launched the Human Growth Hormone 2000 project with the goal of developing reliable screening tests for the use of hGH,[13] but the search for sensitive tests for hGH remains complicated. Recombinant human growth hormone (rhGH) was confiscated at a variety of elite athletic events including the 1998 World Swimming Championships and the Tour de France.[14] The 1994 World Aquatic Championship breast stroke silver medalist, Yuan Yuan was sent home from the 1998 World Aquatic Championships in Perth, Australia when she was caught trying to smuggle 13 vials of hCG into Australia. As a result of the difficulty detecting rhGH use more than a day after cessation of rhGH use, the first athlete disciplined for rhGH use occurred in 2010. A UK rugby player, Terry Newton tested positive for hGH in a blood sample, and he received a 2-year ban for using rhGH ostensibly to increase muscle mass.[15] Since the early 1990s, growth hormone has also been promoted as an antiaging drug. During this time, human studies suggested that growth hormone-deficiencies in elderly patients accounted for reduction in lean body mass, increases in fat mass, and thinning of the skin.[16] However, currently there is no clear evidence that the use of hGH improves longevity or the quality of life.

IDENTIFYING CHARACTERISTICS

Structure

About 70–75% of circulating hGH (Somatropin, CAS RN:12629-01-5) is a 191-residue, 22 kDa peptide, whereas about 5–10% occurs as a 20 kDa isoform as a result of alternative mRNA splicing. Dimers and oligomers of hGH also occur in the plasma along with acidic, acylated, deaminated, and fragmented forms. Recombinant hGH (rhGH) occurs only as a 22 kDa peptide. The binding of hGH to the 2 different growth hormone-binding proteins increases the heterogeneity of hGH isoforms in plasma.[17] The practical consequence of the wide variation in hGH isoforms is the substantial variation in the affinity of different immunoassays for the natural isoforms and fragments of hGH. Recombinant human growth hormone occurs only in the 22 kDa-form that allows differentiation of exogenous hGH use by examining the ratio of 22 kDa- and non-22 kDa-forms.

Form

Human growth hormone is available in injectable forms derived from recombinant technology including Genotropin® (Pfizer, New York, NY), Norditropin® (Novo Nordisk, Inc., Princeton, NJ), Nutropin® (Genentech, South San Francisco, CA), Humatrope® (Eli Lily, Indianapolis, Indiana), Serostim® (Serono, Inc., Rockland, MA), and Saizen® (Serono, Inc., Rockland, MA). These commercial recombinant forms of hGH have the following 2 origins: 1) modified *Escherichia coli* strain (Genotropin®, Norditropin®, Nutropin®, Humatrope®), and 2) mammalian cell line (mouse C127) (Serostim®, Saizen®). The molecular weight of the rhGH is 22.125 kDa, identical to the 22 kDa-form of endogenous hGH. As a result of the popularity of hGH as an antiaging agent, all-natural supplements have been formulated as a low-cost method to stimulate the endogenous secretion of hGH. These naturopathic preparations contain a variety of amino acids (L-arginine, L-isoleucine, L-glutamine, L-glycine, L-lysine, L-tyrosine, L-valine) that are available as capsules, pills, powders, and nasal sprays. Somatrem (*N*-methionyl-human growth hormone) was the first biosynthetic form of growth hormone that contained an extra methionyl amino acid on the *N*-terminus (i.e., 192 amino acid residues). The commercial form of somatrem was Protropin® (Genentech), but manufacture of this form by Genentech ceased in 2004. Sustained release preparations of rhGH are now available for the treatment of growth hormone deficiencies that allow injections every 1–2 weeks instead of daily injections.[18,19]

EXPOSURE

Epidemiology

Although doping with hGH is a significant issue in elite sports based on seizures and anecdotal reports, determination of the prevalence of hGH abuse is complicated by the difficulty developing reliable commercial assays and detecting hGH use more than a day after cessation of hGH. Additionally, the physiologic increases in bone and collagen turnover during normal puberty limit studies to determine the prevalence of hGH use in adolescent athletes as detected by the marker method.[20] Despite the fact that the ergogenic effects of systemic administration of rhGH by athletes and bodybuilders remain unproven, the lack of accurate detection methods contributes to the prevalence of rhGH abuse by sports personnel.[21] Frequently, the use of rhGH is part of a multiple drug regimen that includes anabolic steroids. In an anonymous self-administered questionnaire of 100 athletes attending 4 gymnasiums for weight training, 12% of the athletes admitted the use of hGH, usually in addition to anabolic steroids and other ergogenic drugs.[22] The use of hGH extends to young adolescents. Based on an anonymous questionnaire of 432 adolescents in 2 suburban Chicago high schools, 11 males (5%) and 1 female admitted to the past or present use of hGH.[23] This study indicated that adolescent hGH users often also abuse anabolic steroids. Although many press articles during the 2000 Olympic Games suggested that rhGH was a popular performance-enhancing drugs among athletes, the lack of confirmatory laboratory data limits conclusions regarding the prevalence of rhGH use among elite athletes.[24]

Sources

Endogenous hGH is an anabolic peptide hormone secreted by the anterior pituitary gland. The original products available for hGH replacement were derived from the pituitary glands of human cadaver; however, the human use of cadaver-derived hGH was halted during the 1980s after documentation of the transmission of the prion-medicated spongiform encephalopathy (Creutzfeldt-Jakob disease) with this type of hGH preparation. Pituitary-derived hGH (cadaveric growth hormone) is available on the Internet and may be sold illicitly as rhGH with a risk of developing Creutzfeldt-Jakob disease.[25,26] Cadaveric growth hormone contains the full range of growth hormone isoforms; therefore, cadaveric growth hormone is indistinguishable from endogenously derived growth hormone. Somatrem and rhGH are polypeptide hormones produce by recombinant DNA technology. Pharmaceutic preparations of rhGH are derived from recombinant technology; inser-

tion of the hGH gene into the genome of mammalian or *E. coli* cell lines produces large quantities of 22 kDa-hGH in cell cultures. Subsequently, rhGH is isolated and purified for parenteral administration. These parenteral preparations contain preservatives including benzyl alcohol, *m*-cresol, and phenol.[27]

Methods of Abuse

The administration of hGH usually involves the subcutaneous (SC) or intramuscular (IM) injection of pharmaceutic preparations diverted from legal sources. Typically, the use of hGH is part of a regimen that includes anabolic steroids.

DOSE EFFECT

The mean daily secretion of hGH in adults is about 0.5 mg.

Medical Use

Indications for hGH primarily involves children with growth hormone deficiency and growth retardation. These conditions include pituitary dwarfism, short bowel syndrome, chronic renal insufficiency, Ullrich-Turner syndrome, and Prader-Willi syndrome. Approved US indications for the treatment of adults include HIV-associated wasting or cachexia, short bowel syndrome, and certain types of hGH deficiencies.[28] Over 90% of adults with growth hormone deficiency have pituitary disease, primarily from a pituitary adenoma or the treatment of a tumor.[29]

The recommended dose of hGH depends on the medical condition, gender, and age, titrated to response (e.g., normalization of plasma IFI-I concentrations in adults) and the presence of side effects.[30] In children with growth hormone deficiency, the usual daily doses range from 5–25 µg/kg body weight.[31] In general, the daily dose of rhGH in GH-deficient adults is smaller than doses in children, typically beginning as 150–300 µg and titrated up to 1 mg (3 IU). High-dose regimens in growth hormone-deficient children use up to 80 µg/kg. Although the use of SC rhGH is approved for use in adult cachexia, there is no clear benefit from the use of rhGH in these patients.[32] Off-label uses of rhGH include the treatment of obesity, osteoporosis (70 µg/kg/week divided in 3 subcutaneous injections for 12 months), muscular dystrophy, and infertility, but there are inadequate clinical data to support of efficacy and safety of rhGH for these uses.[31]

Illicit Use

Athletes anecdotally use hGH in doses substantially higher (i.e., up to 5–15 times) higher than the medical

doses used to supplement children with short stature or adults with wasting diseases. These supratherapeutic doses of hGH typically range from 2 mg 3 times weekly to over 5 mg daily.[33,34] Cycles typically continue 6–12 weeks, usually in combination with anabolic steroids in power sports and erythropoietin in endurance sports. A common dose of hGH for body building is 16 mg/week divided in 3 doses subcutaneously.

Toxicity

There are few data reported on the toxicity of hGH following administration of specific doses of hGH. A minimum toxic dose for hGH has not been established; side effects occur with therapeutic doses. The IM LD$_{50}$ in a mouse and rat model exceeds 40 IU/kg (~15 mg/ kg). The administration of porcine growth hormone in doses up to 1 IU/kg/day for 14 weeks to dogs was associated with the development of diffuse glomerulosclerosis (thickening of the glomerular basal lamina, diffuse increase of the mesangial matrix) morphologically similar to diabetic nephropathy.[35]

TOXICOKINETICS

Absorption

Absorption of hGH depends on several factors including the formulation, site of injection, and delivery method (SC, IM); prolonged absorption may result from depots of hGH in SC or IM tissue. Following SC administration of rhGH, peak serum hGH concentrations occur within approximately 3–6 hours. In a study of 6 healthy adults, the SC administration of hGH (bio-hGH 0.2 U/kg) produced a mean hGH concentration of 106 ± 10 mIU/L at 3.3 ± 0.5 hours after administration.[36] In a study of 6 subjects receiving 1.2 IU rhGH (Genotropin®, Pfizer, New York, NY) subcutaneously, mean maximum serum hGH concentrations of 5.56 ± 0.91 mIU/L (95% CI: 3.79–7.33 mIU/L) occurred about 4.75 hours (95% CI: 2.25–5.75 hours) after administration.[37] The SC injection of 0.3 IU rhGH (Norditropin, Novo Nordisk Pharma, Tokyo, Japan)/kg to 12 healthy adults resulted in a mean peak hGH concentration of approximately 180 µg/L about 4 hours (range 3–5 hours) after administration.[38] Figure 19.1 displays the changes in serum growth hormone within 24 hours after SC administration of single rhGH doses of 0.075 IU/kg, 0.15 IU/kg, and 0.3 IU/kg as well as after the 7th daily dose of 0.3 IU/kg. The mean bioavailability (AUC$_{sc}$/ AUC$_{iv}$) of rhGH in this study was approximately 63% (95% CI: 55–71%) as a result of degradation at the site of injection. Typically, the bioavailability of subcutaneously injected hGH in high therapeutic doses ranges
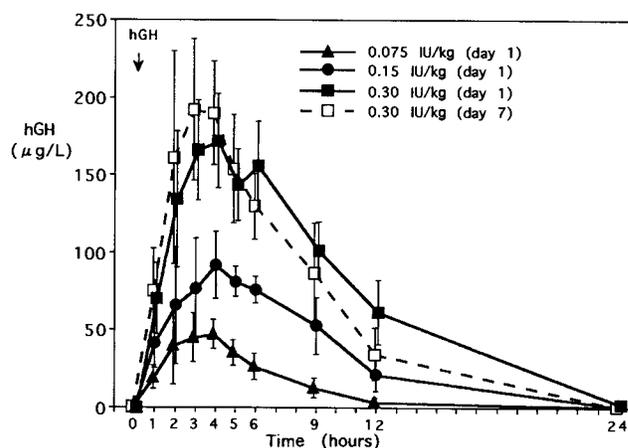
FIGURE 19.1. Serum growth hormone concentrations after single subcutaneous rhGH doses of 0.075 IU/kg, 0.15 IU/kg, and 0.3 IU/kg, as well as after the 7th daily dose of 0.3 IU/kg. (Reprinted with permission from T Tanaka, Y Seino, K Fujieda, Y Igarashi, S Yokoya, K Tachibana, Y Ogawa, Pharmacokinetics and metabolic effects of high-dose growth hormone administration in healthy adult men, Japan Endocrine Journal, Vol. 46, p. 607, copyright 1999.)

from approximately 50–70%; there are inadequate pharmacokinetic data to determine the bioavailability of supratherapeutic hGH doses used in sports doping. Intranasal absorption of physiologic amounts of hGH requires the presence of a permeability enhancer (e.g., didecanoyl-L-alpha-phosphatidylcholine, sodium tauro-24,25-dihydrofusidate) in the product formulation. In a study of 16 healthy young adults without known allergies, the mean peak serum hGH concentration was 28.22 ± 20.85 ng/mL following intranasal application of 6 IU (2 mg) hGH (8% didecanoyl-L-alpha-phosphatidylcholine) with a mean t$_{max}$ of 54.5 ± 30.5 minutes.[39]

Distribution

The estimated volume of distribution for hGH in healthy adults receiving therapeutic doses of hGH is < 1 L/kg; as a peptide, hGH probably distributes into extracellular water. In an Australian study of 6 healthy young adults receiving 0.2 IU bio-hGH/kg/day subcutaneously for 5 days, the mean volume of distribution was reported as 77.7 ± 22.8 L with mean body weights of 81.1 ± 4.3 kg.[36] The mean volume of distribution of the central compartment in a study of 14 healthy men receiving 8.64 IU rhGH (Saizen®) as a single SC injection was 0.35 L/kg (95% CI: 0.28–0.42 L/kg).[40] Animal studies indicate that hGH can penetrate the blood–brain barrier and enter the central nervous system despite the absence of a specific transport system for hGH.[41]

Biotransformation/Elimination

Biotransformation of hGH occurs primarily by metabolism in the liver, kidney, and peripheral tissues with only small amounts (i.e., <0.01%) of hGH excreted unchanged by the kidneys based on rodent studies.[42] The renal tubules reabsorb most of the hGH filtered by the glomeruli. The clearance of hGH depends on several variables including hGH concentrations, body composition, insulin, growth hormone-binding protein, and IGF-binding protein-1. The effect of age on hGH clearance is minimal.[43] The mean clearance of SC somatropin in growth-hormone-deficient adult patients is approximately 0.3 L/h/kg. The plasma elimination half-life of rhGH following IV infusion is very short (i.e., about 15 minutes).[44] The estimated mean plasma half-life of hGH in 12 healthy adults was 17 ± 1.7 minutes.[45] Although the plasma half-life of hGH and rhGH is similar following IV administration, the terminal serum elimination half-life of rhGH following SC administration is 2–4 hours following single SC injections as a result of prolonged absorption and distribution.[40] Serum hGH concentrations are not usually detectable within 20–24 hours after SC injection of therapeutic doses (e.g., up to 0.3 IU/kg) of rhGH.[38,46] Recombinant hGH probably does not degrade into the 20-kDa isoform.

Maternal and Fetal Kinetics

There are few data on maternal and fetal kinetics of rhGH including excretion into breast milk.

Drug Interactions

An *in vivo* study of healthy elderly men indicated that the administration of rhGH induces CYP1A2 and, to a lesser extent, inhibits CYP2C19.[47] There was no effect on CYP2D6 or CYP3A4. Based on this study, drug interactions between rhGH and drugs requiring CYP1A2 are possible, but there are no clinical data to determine the clinical relevance of these potential interactions. Interactions with other CYP450 isoenzyme are unlikely. *In vitro* studies indicate that the administration of rhGH does not alter the efficacy of antiretroviral drugs.[48]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Growth hormone (GH) is a peptide hormone secreted by the somatotrope cells in the anterior pituitary in a pulsatile fashion, producing anabolic and anticatabolic effects by affecting the function of a wide range of tissues throughout the body primarily mediated by GH-induced secretion of a another peptide hormone, insulin-like growth factor (IGF). Growth hormone is the most abundant pituitary hormone. The human pituitary gland secretes hGH in multiple, short (i.e., 20–30 minute) secretory bursts following a circadian rhythm (e.g., daily GH peak occurs at night within the first hour of sleep) as a result of alternating secretion of growth hormone-releasing hormone and growth hormone. Secretory hGH rates fall asymptotically to zero between the pulsatile release of hGH. There is marked individual variation in the secretion of endogenous growth hormone. In a study of 12 healthy men, the mean endogenous production of hGH during a 24-hour period was 0.25 ± 0.033 mg/m^2.[45] During this 24-hour period, production of 67% of the hGH occurred within a 4.4-hour period and 95% of the hGH production occurred within an 8.8-hour period. Amino acids (e.g., ornithine, L-arginine, tryptophan, L-lysine) increase hGH through an unknown mechanism; naturopathic products containing these amino acids are available to promote the secretion of growth hormone.[49] However, there are inadequate data to support the efficacy of these natural products.

PHYSIOLOGY

The somatotrope cells of the anterior pituitary secrete hGH (somatotropin) in response to a variety of factors including growth hormone-releasing factor (somatorelin, CAS RN:83930-13-6), somatostatin (inhibitory), and ghrelin. The source of the latter is the stomach and to a lesser extent, hypothalamus. Other factors affecting the pulsatile release of hGH from the pituitary include exercise, stress, sleep, low calorie diet, estrogen, and androgen. Somatorelin is a peptide amide that causes the release of growth hormone from the anterior pituitary gland, whereas somatostatin is a hormone found in the pancreas and hypothalamus that inhibits the release of hGH. Hypoglycemia, high temperature, deep sleep, and stress increase hGH secretion; carbohydrate-rich diets, obesity, and β-adrenergic agonists decrease hGH secretion. The release of hGH during exercise is dose-related with more intense exercise causing larger releases of hGH, particularly anaerobic exercise.[50] Exercise stimulates the secretion of hGH up to 5- to 10-fold, whereas starvation reduces hGH secretion. Ageing is associated with declining hGH secretion. In a study of 21 healthy subjects aged 21–71 years, the production of hGH decreased by a mean of 14% per decade.[51]

The effects of hGH binding to specific growth hormone receptors found on every cell of the body are primarily anabolic and lipolytic including enhancement of amino acid uptake, promotion of renal and hepatic gluconeogen-
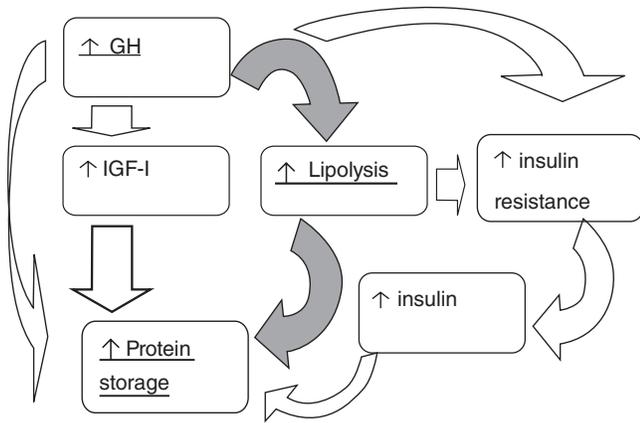
FIGURE 19.2. Schematic representation of the metabolic actions of growth hormone (GH), which directly stimulated lipolysis. Protein storage results from the breakdown of protein, stimulation of protein synthesis, and inhibition of hepatic amino acid degradation. (Reprinted from Clinical Nutrition, Vol. 28, N Moller, MH Vendelbo, U Kampmann, B Christensen, M Madsen, H Norrelund, JO Jorgensen, Growth hormone and protein metabolism, p. 599, Copyright 2009, with permission from Elsevier.)

esis, stimulation of protein synthesis, and increased fat resorption. The stimulation of lipolysis and lipid oxidation results in the increased formation of free fatty acids and ketone bodies; the indirect anabolic effects on protein storage are less dramatic.[52] Figure 19.2 displays a schematic representation of the metabolic actions of growth hormone. Binding to the GH receptors activates the tyrosine kinase, Janus kinase 2, and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway, resulting in cellular proliferation, differentiation, and migration, reduced apoptosis, regulation of metabolic pathways, and cytoskeletal reorganization.[53] These effects are primarily mediated by insulin-like growth factor I (IGF-1). Other effects of hGH include enhancement of bone and collagen turnover and the regulation of selective aspects of metabolic function (e.g., lipolysis, body composition). The anabolic effects of hGH are independent of the actions of testosterone. Growth hormone receptors are glycosylated transmembrane glycoproteins that occur in many tissues (e.g., liver). Human growth hormone contains 2 binding sites that form a dimer when attached to the GH receptor. Binding of hGH to hepatic receptors causes the secretion of anabolic somatomedins (e.g., IGF-1).

Adolescents and Adults

Peak GH secretion occurs during the prepubertal growth spurt and after the mid-20s with GH secretion decreasing about 14% every decade.[54] Detrimental changes in body composition, muscle strength, and somatotropic function occur during the normal aging process. Supplementation of elderly patients with rhGH does not increase body weight, but hGH reduces fat mass and increases lean body mass.[55] Although older adults increase muscle strength as a result of resistance exercise training, these strength gains stabilize after several months, and subsequent improvement in muscle strength is modest despite continued training. Although hGH induces protein anabolic effects in healthy adults, there is little evidence that supraphysiologic hGH doses produce protein anabolic effects in addition to the effects of exercise in healthy adults with normal hGH concentrations.[56] Supplementation with rhGH does not augment the response to strength training in elderly men.[57] In acromegaly, muscle mass increases, but these muscles are relatively weak compared with normal musculature. The use of rhGH by GH-deficient patients improves aerobic exercise capacity (i.e., maximal oxygen consumption while exercising at maximum capacity, VO_{2max}), but hGH does not increase muscle mass or strength beyond the expected norms for healthy adults of the same age and gender.[58]

Athletes

There is substantial evidence that hGH supplementation increases lean body mass in athletes, particularly in men co-administering testosterone. In a meta-analysis of 9 clinical studies (mean duration, 4 weeks), the mean increases in muscle mass in the hGH-treated group was 2.1 kg (95% CI: 1.3–2.9 kg) compared with controls (no treatment).[59] However, the increase in lean body mass probably results from extracellular fluid accumulation rather than increased muscle mass. In a study of 30 healthy, young adults receiving 0.1 or 0.2 IU rhGH/kg, the mean increases in the pooled treatment group compared with placebo were as follows: body weight, 2.7%; fat free mass, 5.3%; total body water, 6.5%; and extracellular water (ECW), 9.6%.[60] The mean intracellular water of the treatment and placebo groups was not statistically different. As intracellular water reflects muscle mass, the results suggest that extracellular water and fluid retention accounts for the increase in lean body mass. Consequently, hGH has little anabolic effect. Short-term use of hGH does not increase the incorporation of amino acids into skeletal muscle protein or reduce the catabolism of whole body protein in experienced weightlifters.[61]

There are no well-controlled studies demonstrating convincing evidence that hGH doses used in clinical studies improve aerobic exercise capacity or increase muscle strength in power athletes with highly lean body mass.[62,63,64] Evaluation of anecdotal reports of

improvement in strength or endurance following use of hGH are difficult to evaluate because hGH is usually part of a regimen that includes other ergogenic agents. Although the use of hGH increases IGF-1, maximum strength during concentric contraction of biceps and quadriceps muscles does not increase in these athletes following hGH use.[65] In a study of 96 recreational athletes receiving 2 mg rhGH daily for 8 weeks, muscle strength as measured by dead lift dynamometer and muscle power as assessed by jump height were not significantly different between the treatment and placebo groups.[66] The addition of rhGH to a group receiving testosterone (250 mg IM weekly) in this study did not improve these muscle parameters over the effects of testosterone alone. Similarly, there was no significant difference between placebo and treatment groups on aerobic exercise capacity as measured by VO_{2max}. Anaerobic capacity assesses the ability to generate relatively high power output over a brief duration from anaerobic energy sources (phosphocreatine degradation, glycogenolysis). The Wingate test (30-second all-out sprint capacity) is a measure of anaerobic capacity. In the above study of recreational athletes, the 8-week course of rhGH increased anaerobic capacity (Wingate test) an average of about 6% (95% CI: 0.8–10.5%) in men with the addition of testosterone increasing the benefit to an average of approximately 8% (95% CI: 3.0–13.6%) compared with the placebo group. Some limitations of these studies include the small number of study subjects, the use of doses below the supratherapeutic doses of hGH covertly used by many athletes, and the lack of power of these studies to detect the small differences (i.e., 0.5–1%) in physical performance that separate Olympic champions from their competitors. Additional limitations include the extrapolation of randomized clinical trials with the power to detect differences of 20–30% to elite competition where 1% difference in performance separates gold medal winners from other top performers.[67]

Mechanism of Toxicity

Human growth hormone is the main stimulus for the release of IGF-1. The effects of hGH are primarily mediated by the action of IGF-1; consequently, the adverse effects of hGH and IFG-I are expected to be similar to diseases associated with growth hormone excess (i.e., acromegaly with fluid retention, hypertension, cardiomyopathy, and diabetes mellitus).[68] Normal hGH levels are an important factor in the development and function of the cardiovascular system.[69] Although hGH improves muscle and cardiac function in hGH-deficient patients, there are few data on the effects of chronic hGH use by healthy subjects on the risk of increased morbidity and/or mortality from cardiovascular disease. Chronic hGH decreases high-density lipoprotein (HDL), which is a risk factor for coronary heart disease.[70] Chronic growth hormone *deficiency* is associated with impaired cardiac performance, reduced left ventricular systolic function, dilated cardiomyopathy, and congestive heart failure.[71] Growth hormone excess (acromegaly) is associated with an increased risk of hypertension, cardiomyopathy, cardiovascular disease, diabetes mellitus, osteoporosis, and impotence.

CLINICAL RESPONSE

Medical Use

Adverse effects of hGH during medical use include insulin resistance, pre- and postpubertal gynecomastia, arthralgias, edema, headache, erectile dysfunction, low-grade fevers, and carpal tunnel syndrome.[72,73] In clinical trials using hCG to treat GH-deficient adult patients, the most common side effects are edema, arthralgias, and myalgias. The large muscle mass associated with acromegaly is associated with myopathy and relative weakness.[74] Case reports associate sudden death with the early treatment phase of Prader-Willi syndrome with growth hormone,[75,76] but the presence of other potential complicating factors (e.g., pneumonia, sleep apnea, pulmonary hypertension) limit conclusions regarding the causal role of hGH. Complications of treating familial short stature with rhGH include non-ketotic hyperglycemia,[77] hypothyroidism, benign intracranial hypertension,[78] and acute pancreatitis.[79] In patients with GH deficiency, replacement therapy with rhGH improves mood and sense of well-being. Clinical studies indicate that withdrawal of rhGH treatment from adults with severe GH deficiency produces detrimental psychologic effects manifest by fatigue, pain, irritability, and depression.[80] Rarely, case reports associated the development of hypersensitivity reactions (pruritus, urticaria) with rhGH therapy, particularly with the use of somatrem.[81,82]

Illicit Use

The abuse of hGH in athletes typically involves the use of supraphysiologic doses of this compound in cycles with other ergogenic aids. In addition, determining the long-term safety of hGH use is difficult as a result of problems associated with collecting clinical data on off-label and illicit uses of hGH. Many adverse effects from supraphysiologic doses of hGH result from fluid retention. Case reports associate the use of high doses of

rhGH with the development of entrapment syndromes (e.g., carpel tunnel syndrome) manifest by paresthesias and dysesthesias of the hands and feet.[83,84] Other complaints associated with the use of rhGH include myalgias, arthralgias, myositis, peripheral edema, diaphoresis, fatigue, and lightheadedness. Chronic use of supraphysiologic doses of hGH are associated with the clinical features of acromegaly, an insidious, chronic debilitating disease associated with soft tissue and bony overgrowth as a result of growth hormone excess. Complications of chronic hGH use involve slipped capital femoral epiphysis, premature physial closure, coarse facial features, voice change, hypercalcemia and renal stones, enlargement of facial bones, increased skull circumference, macroglossia, myopathy, congestive heart failure, cardiomegaly, goiter, impotence, insulin resistance, and diabetes mellitus. Dermatologic changes during chronic rhGH use include increased skin viscosity, oily skin, enhancement of melanocytic (nonmalignant) nevi, and local complications of parenteral drug use.[85] Case-control studies suggest an increased risk of prostate cancer in subjects with elevated serum IGF-1 concentrations,[86] but there is no direct evidence that the doses of rhGH used by athletes increase the risk of prostate cancer.

Overdose

There are inadequate clinical data to evaluate the effects of a massive overdose of hGH.

Abstinence Syndrome

An abstinence syndrome associated with cessation of the chronic use of rhGH in healthy subjects is not well defined. Physiologically, the administration of exogenous hGH suppresses the secretion of endogenous hGH, but there are few data on the clinical and pathologic effects of chronic suppression of hGH secretion. In patients with severe growth hormone deficiencies, withdrawal of rhGH therapy results in fatigue, pain, irritability, and depression. However, the effects of hormone deficiency are difficult to separate from a true withdrawal syndrome.

Reproductive Abnormalities

There are no well-controlled studies of reproductive abnormalities associated with the use of rhGH in healthy subjects. The FDA classifies rhGH in pregnancy category B (fetal harm possible, but unlikely) and somatrem in pregnancy category C (lack of animal and human studies).

DIAGNOSTIC TESTING

Conversion Factors

$$1 \text{ IU} = 0.37 \text{ mg}$$
$$1 \text{ mg} = 2.7027 \text{ IU}$$

Analytic Methods

The IOC classifies hGH in prohibited class E; therefore, the use of hGH by athletes is doping. However, detection of hGH use is difficult as a result of wide individual variation in hGH secretion, analytic problems separating exogenous and endogenous hGH, and the relatively short half-life of rhGH; most infractions result from the confiscation of ampules.

Screening

The detection of exogenous hGH is complicated by the presence of endogenous hGH. Radioimmunoassay (RIA) and immunoradiometric assays (IRMA) detect whole hGH isoforms, whereas immunofunctional assay methods (IFA) detect only biologically active hGH isoforms. Consequently, the hGH concentrations by IFA are about 27–30% lower than determinations by RIA or IRMA because of the interference by GH-binding protein and the inclusion of several different molecular forms of hGH by the latter methods.[87] IFA is an ELISA method that incorporates 2 binding sites of the transmembrane growth hormone receptor. Nonfunctional isoforms do not bind to the growth hormone-binding protein; therefore, these isoforms of hGH are not detected by this method. However, the presence of isoform fragments, which contain binding sites for antibodies, can cause overestimation of the serum hGH concentration when high concentrations of rhGH are present.[88]

Confirmatory

Practical analytic methods to separate exogenous rhGH use from endogenous hGH based on serum or urine hGH concentrations are not well studied. Analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis with tandem mass spectrometry allows the quantitation of hGH isoforms including the 20 and 22 kDa isoforms, 9 and 12 kDa fragments, and glycosylated 23 kDa GH variants.[89] The direct measurement of 22 kDa-/20 kDa-hGH isoforms is a potential biomarker of hGH abuse as this ratio increases following the

TABLE 19.1. Characteristics of Growth Hormone-Dependent Markers.[97]

Marker	Source	Physiologic Role	Disappearance Half-Time (h)	Effect Acute Exercise
Insulin-like growth factor I (1GF-1)	Liver	Mitogenic protein	89.5	+20%
Insulin-like growth factor binding protein II (IGFBP-2)	Liver	Regulates lGF-1 bioavailability and action		No change
Insulin-like growth factor binding protein III (IGFBP-3)	Liver	Regulates lGF-1 bioavailability and action	176	+18%*,†
Acid labile subunit (ALS)	Liver	Regulates lGF-1 bioavailability and action	119	+21%*,†
Type III procollagen (P-III-P)	Soft Tissue	Marker of soft tissue formation	693	+5%
Osteocalcin (OC)	Bone	Marker of bone formation	770	No change
C-terminal propeptide of type I procollagen (PICP)	Bone	Marker of bone formation	433	+14%†
Type I collagen telopeptide (ICTP)	Bone	Marker of bone resorption	248	+10%

*Affected by ethnicity.
†Affected by gender.

administration of 22 kDa isoforms (rhGH).[90] Other methods to detect hGH misuse require analysis of specific biomarkers from the GH/IGF-1 axis and bone markers because these specific variables are sensitive to exogenous rhGH rather than changes in endogenous hGH related to exercise. The detection of hGH abuse with only 1 parameter is difficult, and analysis of several different serum marker may be necessary (e.g., IGF-1, IGFBP-3, ALS, N-terminal propeptide of pro-collagen III [PIIINP], C-terminal cross-linked telopeptide of type I collagen [ICTP], osteocalcin, leptin).[91] ICTP and PIIINP are GH-dependent peripheral markers of bone and collagen turnover, respectively. Reference ranges for each marker in each gender are available, but these reference ranges have not been formally accepted by governing bodies for the detection of hGH abuse in sports.[92,93]

Biomarkers

BLOOD

Blood sampling is necessary to detect hGH abuse because of the low concentration of hGH and related peptides in urine samples. Direct measurement of hGH as a means of detecting hGH abuse is complicated by the diurnal variation in hGH secretion and the increase in serum hGH in response to exercise. In a study of 17 physically fit men exercised to exhaustion, the mean serum total growth hormone increased from a baseline value of 5.2 ± 2.2 mIU/L to 72.8 ± 10.9 mIU/L at the termination of exercise.[14] The administration of rhGH increased the pre-exercise serum total growth hormone and markedly attenuated the response of hGH to exer-

cise. The 2 main investigative methods for detecting hGH abuse are 1) the isoform method, the detection of altered endogenous pituitary hGH isoforms, and 2) the marker method, the measurement of hGH-dependent proteins (i.e., GH-dependent biomarkers including IGF, IGF-binding proteins, and collagen peptides).[94] The latter peptides include osteocalcin, the marker of bone resorption (*C*-terminal telopeptide of type I collagen, ICTP), and the marker of connective tissue synthesis (*N*-terminal propeptide of type III procollagen, PIIIP). These tests are an alternative to the direct analysis of hGH, but the cut-off concentrations of these tests for hGH abuse are not well defined.[95,96] Table 19.1 lists the characteristics of the biomarkers chosen to detect hGH abuse by the GH-2000 Project. In general, these biomarkers are unaffected by injury and do not display diurnal variation.[97]

ISOFORM METHOD.   Recombinant growth hormone consists only of the 22-kDa isoform, whereas endogenous hGH contains several different isoforms. The circulating isoforms of endogenous hGH are the 191 amino acid 22-kDa and 20-kDa variants with several other minor monomeric size variants. The former isoform accounts for about 50–70% of the circulating hGH compared with 5–15% for the latter isoform. The 20-kDa isoform lacks 15 amino acids (residues 32–46) of the 191 amino acid sequence of the 22-kDa isoform. Administration of rhGH suppresses the secretion of endogenous hGH by the pituitary as a result of a negative feedback system. Consequently, abnormally high serum concentrations of the 22-kDa isoform suggest the use of rhGH.[98] In studies of normal children and children with hGH deficiency,

the administration of rhGH (22-kDa variant) increases the 22-kDa/20-kDa hGH ratio.[95] This ratio increases because the serum concentration of 22 kDa increases, whereas the serum concentration of the 20-kDa variant remains low to undetectable. In adults, the usefulness of this ratio as a screening test is limit by the short time (i.e., about 3 hours after injection) available for analysis of this ratio, unless depot hGH is administered. A confirmation test is required of all positive samples because of the variable affinity of different immunoassays for the variety of hGH isoforms. The isoform method does not detect the use of cadaveric, pituitary-derived growth hormone.[99] Exercise increases the serum concentration of the 22-kDa isoform;[100] therefore, post-race testing may reduce the sensitivity of this screening test. Based on studies in children with short stature receiving rhGH, endogenous hGH secretions returns to baseline within 48 hours after administration.[101]

Marker Method.   The anabolic effects of hGH produces several proteins, and alterations in the serum concentration or ratios of these proteins suggests the use of endogenous hGH. These proteins include IGF-1, IGF binding proteins, and markers of bone and collagen turnover.

The potential advantage of these biomarkers (e.g., IGF-1, PIIIP) is the extended window of detection, which reaches several weeks compared with a 1–2 days for the ratio of hGH isoforms.[87] During the medical use of rhGH, IGF-1 is the most common biochemical marker of hGH use. Potential bone and soft tissue biomarkers, which change with exogenous hGH administration, include procollagen III terminal peptide (PIIIP), osteocalcin, and bone alkaline phosphatase.[102] Although endurance exercise transiently activated bone and collagen turnover, the administration of rhGH produces a substantially greater increase than endurance exercise alone.[103] Currently, no 1 biomarker provides sufficient sensitivity and specificity to allow the use of a single test; the normal gender- and age-specific reference limits of these compounds in athletes currently are under study.[104,105] Immunoassays of the blood from athletes indicate that some variation in IGF-binding proteins and ICTP occurs between elite athletes, recreational athletes, and sedentary individuals as well as some differences between the type of elite athlete (e.g., swimming, gymnastics, weightlifting).[106]

Urine

The very short elimination half-life of hGH and the low concentrations (i.e., <0.1%) of this compound in urine samples limits the practicality of using urine samples as a means of detecting hGH abuse. Although hGH is officially banned by the IOC, there is no official limit for hGH in the urine.

Abnormalities

Case reports associate both hyperglycemia and hypoglycemia with the use of rhGH as part of a regimen for body building.[107,108] In patients with hypopituitarism, supplementation with 6–25 µg/kg/day up to a maximum of 1.48 mg for 6 months increased fasting serum glucose, total serum cholesterol, and the peripheral conversion of T4 to T3.[109] The short-term administration of 0.2 IU/kg daily for 5 consecutive days to 6 healthy adults produced statistically significant increases in fast serum glucose, free fatty acids, and serum T_3.[36] During this experiment, a marked reduction in urinary sodium exaction occurred. Potential blood abnormalities associated with chronic hGH use include hypernatremia, hyperphosphatemia, and elevated alkaline phosphatase as well as abnormalities suggestive of hypothyroidism. Abnormal lipid profiles may occur during chronic hGH use including elevated serum cholesterol and triglyceride concentrations, but these changes are not specific to hGH use. In a volunteer study of 12 healthy, young adults, the SC injection of 0.3 IU rhGH/kg daily for 1 week was associated with an increase in the serum triglyceride concentration when compared with baseline.[38] Case reports associate hematuria with the therapeutic use of rhGH.[18]

TREATMENT

There are inadequate clinical data on the treatment of toxicity associated with the chronic use of rhGH to determine the efficacy of specific aspects of clinical management other than cessation of illicit hGH use. The treatment of adverse effects associated with chronic rhGH use is supportive. Primary attention should be directed at identifying and correcting any serum electrolyte, lipid, or glucose abnormalities. Hypothyroidism is a rare complication of the therapeutic use of rhGH, and thyroid function should be evaluated when suggested by the clinical presentation. Human growth hormone use typically occurs in combination with other ergogenic aids (anabolic steroids, erythropoietin); therefore, these patients should be evaluated for complications of anabolic steroid and erythropoietin use including a complete blood count (CBC). There are no antidotes for rhGH, and the rapid clearance of this compound limits the usefulness of any extracorporeal methods for removal. Referral to an endocrinologist may be necessary for complicated cases of chronic hGH use.

INSULIN-LIKE GROWTH FACTOR

IDENTIFYING CHARACTERISTICS

Somatomedin C (CAS RN:67763-96-6) is a synonym for the 70-amino acid, 7.65 kDa peptide, insulin-like growth factor I (IGF-1). This single chain polypeptide with 3 cross-linking disulfide bridges is structurally similar to proinsulin.[110] Insulin-like growth factor II (IGF-2, CAS RN:67763-97-7) is another somatomedin compound. IGF-1 and IGF-2 are straight chain polypeptides consisting of 70 and 67 amino acid residues, respectively. IGF-2 is an important fetal growth factor, whereas IGF-1 is primarily an adult HG factor that mediates many of the anabolic effects of growth hormone. IGF-1 circulates in the plasma as a 150 kDa complex formed by the IGF peptide, IGF binding protein III (IGFBP-3), and the acid-labile subunit (ALS). Minor amounts of IGF-1 are bound as 45 kDa-binary complexes containing IGFBP-1, IGFBP-2, IGFBP-3, or IGFBP-5.

EXPOSURE

Unlike growth hormone, there is no readily available natural source of IGF-1; therefore, the source of IGF-1 is recombinant human DNA technology (rhIGF-1). The following 2 pharmaceutic preparations of IGF-1 are available for the treatment of growth disorders in children: 1) Increlex® (recombinant human IGF-1; Tercica, Brisbane, CA); and 2) Iplex® (mecasermin rinfabate; INSMED Incorporated, Richmond, VA).[111] The latter product is recombinant human IGF-1 in equal molar concentration with the major binding protein, IGFBP-3 that together form a ternary complex in blood with the acid-labile subunit. Increlex® contains rhIGF-1 alone. Potential medical uses for rhIGF-1 include the treatment of growth disorders, severe insulin resistance (Rabson-Mendenhall syndrome), osteopenia, and burns.

The prevalence of doping with IGF-1 is difficult to determine, in part because of the complicated analytic processes required to detect IGF-1 use. The abuse of IGF-1 is probably much less common than the abuse of hGH, but abuse of rhIGF-1 is expected to increase as improvements in the detection of hGH use occur.[112] Suspected use of rhIGF-1 by athletes involves use as an anabolic and lipolytic agent either alone or in combination with hGH and anabolic androgenic steroids. Purported benefits of rhIGF-1 use include improvements in muscle growth, tissue and ligament repair, skin rejuvenation, sexual function, energy, endurance, fat reduction, mood, mental acuity, bone density, immune balance, and aging.[113] None of these claims are supported by clinical studies. IGF-1 appears on the World Anti-Doping Agency list of prohibited substances.[114]

TOXICOKINETICS

IGF-1 circulates in the blood bound to a family of highly specific IGF-binding proteins (IGFBP-1 through IGFBP-6), with about 80% of the circulating IGF-1 bound to a ternary complex consisting of IGF-1, IGFBP-3, and an 85 kDa glycoprotein acid-labile subunit (ALS).[115] The free fraction of IGF-1 is negligible (<1–5%). In the serum, GH-dependent IGFBP-3 binds approximately 90% of IGF-1 even though IGF-2 occupies most of the binding sites on IGFBP-3.[116] The binding of free IGF-1 markedly prolongs the serum IGF-1 half-life. The serum elimination half-lives of bound IGF-1 (binary, ternary complexes) and IGFBP-3 are about 16–20 hours, whereas the half-life of free IGF-1 is only a few minutes based on tracer studies in healthy volunteers.[117] Peak serum IGF-1 concentrations after the SC administration of 40 μg rhIGF-1/kg to healthy volunteers occurs about 7 hours after administration with the trough serum IGF-1 concentration was increased by a mean of 277 ± 50 ng/mL from baseline.[118] The volume of distribution of IGF-1 is relatively low (0.20–0.36 L/kg).

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Human growth hormone stimulates the release of IGF-1, primarily from the liver. As growth hormone secretion falls with aging, serum IGF-1 concentrations also decline along with decreases in muscle mass and increases in fat mass.[119] IGF-1 mediates the effect of hGH including activation of tyrosine kinase, Janus kinase 2, the Janus kinase/signal transducers, and activators of transcription (JAK/STAT) pathway, resulting in cellular proliferation, differentiation and migration, reduced apoptosis, regulation of metabolic pathways, and cytoskeletal reorganization.[120] Insulin and nutritional status also modulate IGF-1 synthesis. The anabolic effects of both growth hormone and IGF-1 are probably mediated through the induction of amino acid transporters in the cell membrane.

The actions of IGF-1 and insulin on carbohydrate metabolism, lipid formation, and protein synthesis are similar, but not identical. Insulin, IGF-1, and growth hormone act synergistically in regulating protein synthesis as displayed in Figure 19.3. Together these hormones efficiently store nutrients during the postprandial period while minimizing protein breakdown. IGF-1
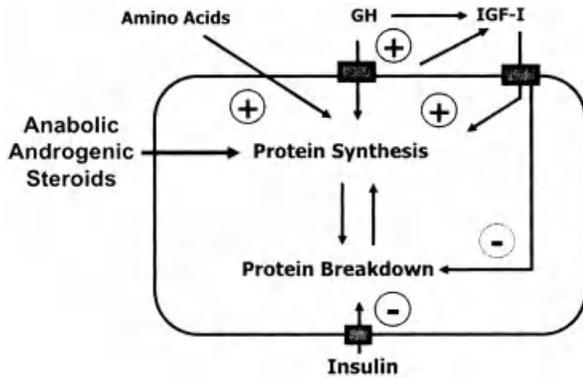
FIGURE 19.3. Synergistic interactions of insulin-like growth factor I (IGF-1), growth hormone, and insulin on protein regulation. (Reprinted from Endocrinology and Metabolism Clinics of North America, Vol. 39, I Erotokritou-Mulligan, RIG Holt, Insulin-like growth factor I and insulin and their abuse in sport, p. 36, Copyright 2010, with permission from Elsevier.)

mediates the side effects of hGH; the adverse effects of IFG-I and hGH are similar (e.g., acromegaly).[68] Production of IGF-2 also occurs in the liver, kidney, and peripheral target tissues in response to the secretion of hGH. Although IGF-1 has potential effects on competitive athletes similar to rhGH (e.g., reduced glycogen breakdown during endurance activities, reduced muscle breakdown during power or sprinting events), there are inadequate clinical data to determine if these effects result in improved athletic performance.

CLINICAL RESPONSE

There are few data on the adverse effects associated with the use of rhIGF-1 by athletes. Adverse effects reported in studies of rhIGF-1 for insulin-resistant diabetes mellitus and growth hormone deficiency in children include headache, myalgias, fluid retention, jaw pain, tonsillar hypertrophy, alteration of facial features, and hypoglycemia.[121,122]

DIAGNOSTIC TESTING

IGF-1 is the most common biochemical marker of the medical use of rhGH; the methodology for detecting rhIGF-1 abuse is based on the analytic methods developed for the detection of exogenous rhGH use. These methods are based on GH-sensitive biomarkers rather than the measurement of pituitary GH isoforms because the ratio of endogenous GH isoforms is unaffected by IGF-1 administrations and IGF-1 exists as a single isoform in humans. The kidney excretes very small amounts of IGF-1 and methods for analyzing urinary IGF-1 are complex and lengthy; therefore, blood is the preferred sample for analyzing IGF-1 and related biomarkers (e.g., PIIIP). The serum concentration of IGF-1 is relatively stable during the day with little change during exercise,[123] and this marker increases several fold after administration of exogenous hGH. Almost all (99%) IGF-1 in the blood is bound to binding proteins (IGFBP-1 to IGFBP-6), and total IGF-1 is typically measured because free IGF-1 is less responsive to exogenous hGH administration. Serum concentrations of IGF-1 are relatively high compared with other peptide hormones, reaching up to about 800 ng/mL. The major determinants of variability in serum IGF-1 concentrations are age and gender with ethnicity and sport type having relatively minor influences. Based on cross-sectional studies of elite athletes, age accounts for a majority (i.e., about 80%) of the variability in serum IGF-1 concentrations with a significant negative correlation with age for both IGF-1 and collagen markers.[124] The contribution of gender was smaller than age. The influence of ethnicity on serum IGF-1 concentrations is relatively minor; a study of 1,085 elite athletes of a variety of different ethnicities demonstrated statistically significant differences only between Afro-Caribbeans (i.e., about 20% lower) and white Europeans.[125]

Methods for the quantitation of IGF-1 in biologic media include high performance liquid chromatography/electrospray ion trap/mass spectrometry,[126] ultra high performance liquid chromatography/tandem mass spectrometry in selected reaction monitoring,[127] and liquid chromatography/tandem mass spectrometry with immunoaffinity purification.[128] rhIGF-1 has an identical amino acid sequence as endogenous IGF-1; thus, separation techniques based on electrical charge differences (e.g., electrophoresis) do not distinguish between rhIGF-1 and endogenous IGF-1. The use of sodium dodecyl sulfate or strong acids with ethanol allows the determination of total IGF-1 by cleaving the noncovalent bond between IGF-1 and the IGF-1 binding proteins 3 and 5. Smaller IGF-1 binding proteins (e.g., 1 and 4) do not bind as well as other IGF-1 binding proteins; consequently, some of IGF-1 bound to these smaller binding proteins are not extracted during this process.[129] Potential abnormalities following the chronic use of IGF-1 include hypoglycemia and elevated serum hepatic aminotransferases based on clinical studies.

TREATMENT

Treatment is supportive.

References

1. Tatersall R. A history of growth hormone. Horm Res 1996;46:236–247.

2. Putnam TJ, Benedict EB, Teel HM. Studies in acromegaly: experimental canine acromegaly produced by injection of anterior lob pituitary extract. Arch Surg 1929;18:1708–1736.

3. Engelbach W, Schaeffer RL, Brosius WL. Endocrine growth deficiencies: diagnosis and treatment. Endocrinology 1933;17:250.

4. Strobl JS, Thomas MJ. Human growth hormone. Pharmacol Rev 1994;46:1–34.

5. Li CH, Papkoff H, Jordan CW Jr. Difference in biological behavior between primate and beef or whale pituitary growth hormones. Proc Soc Exp Biol Med 1959;100: 44–45.

6. Li CH, Papkoff H. Preparation and properties of growth hormone from human and monkey pituitary glands. Science 1956;124:1293–1294.

7. Ranke MB, Bierich JR. Treatment of growth hormone deficiency. Clin Endocrinol Metab 1986;15:495-510.

8. Brown P, Preece M, Brandel JP, Sato T, McShane L, Zerr I, et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. Neurology 2000;55:1075–1081.

9. Buchanan CR, Preece MA, Milner RD. Mortality, neoplasia, and Creutzfeldt-Jakob disease in patients treated with human pituitary growth hormone in the United Kingdom. BMJ 1991;302(6780):824–828.

10. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, et al. Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 1987;330:537–543.

11. Abdel-Meguid SS, Shieh HS, Smith WW, Dayringer HE, Violand BN, Bentle LA. Three-dimensional structure of a genetically engineered variant of porcine growth hormone. Proc Natl Acad Sci U S A 1987;84:6434–6437.

12. Duchaine D. (Ed.). Underground steroid handbook. 1st ed. Venice, CA: HLR Technical Books; 1983. p 84.

13. Sonksen P. The international Olympic Committee (IOC) and GH-2000. Growth Horm IGF Res 2009;19:341–345.

14. Wallace JD, Cuneo RC, Baxter R, Orskov H, Keay N, Pentecost C, et al. Responses of the growth hormone and insulin-like growth factor axis to exercise, GH administration, and withdrawal in trained adult athletes: a potential test for GH abuse in sport. J Clin Endocrinol Metab 1999; 84:3591–3601.

15. Travis J. Growth hormone test finally nabs first doper. Science 2010;327:1185.

16. Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, et al. Effects of human growth hormone in men over 60 years old. N Engl J Med 1990;323:1–6.

17. Baumann G. Growth hormone heterogeneity in human pituitary and plasma. Horm Res 1999; 51(Suppl 1):2–6.

18. Bidlingmaier M, Kim J, Savoy C, Kim MJ, Ebrecht N, de la Motte S, Strasburger CJ. Comparative pharmacokinetics and pharmacodynamics of a new sustained-release growth hormone (GH), LB03002, versus daily GH in adults with GH deficiency. J Clin Endocrinol Metab 2006; 91:2926–2930.

19. Jostel A, Mukherjee A, Alenfall J, Smethurst L, Shalet SM. A new sustained-release preparation of human growth hormone and its pharmacokinetic, pharmacodynamic and safety profile. Clin Endocrinol (Oxf) 2005; 62:623–627.

20. Rogol AD. Growth hormone and the adolescent athlete: what are the data for its safety and efficacy as an ergogenic agent? Growth Horm IGF Res 2009;19:294–299.

21. Jenkins PJ. Growth hormone and exercise: physiology, use and abuse. Growth Horm IGF Res 2001;11(Suppl A):S71–S77.

22. Evans NA. Gym and tonic: a profile of 100 male steroid users. Br J Sports Med 1997;31:54–58.

23. Rickert VI, Pawlak-Morello C, Sheppard V. Human growth hormone: a new drug of abuse among adolescents? Clin Pediatr 1992;31:723–726.

24. Bidlingmaier M, Wu Z, Strasburger CJ. Doping with growth hormone. J Pediatr Endocrinol Metab 2001; 14:1077–1083.

25. Deyssig R, Frisch H. Self administration of cadaveric GH in power athletes. Lancet 1993;341:768–769.

26. Jacquemot C, Cuche C, Dormont D, Lazarini F. High incidence of scrapie induced by repeated injections of subinfectious prion doses. J Virol 2005;79:8904–8908.

27. Kappelgaard AM, Bojesen A, Skydsgaard K, Sjogren I, Laursen T. Liquid growth hormone: preservatives and buffers. Horm Res. 2004;62(Suppl 3):98–103.

28. Bae RJ, Orgill DP, DeBiasse MA, Demling R. Management of a patient with advanced AIDS and toxic epidermal necrolysis using human growth hormone and G-CSF. AIDS Patient Care STDS 1997;11:125–129.

29. Attanasio AF, Lamberts SW, Matranga AM, Birkett MA, Bates PC, Valk NK, et al. Adult growth hormone (GH)-deficient patients demonstrate heterogeneity between childhood onset and adult onset before and during human GH treatment. Adult Growth Hormone Deficiency Study Group. J Clin Endocrinol Metab 1997; 82:82–88.

30. Krysiak R, Gdula-Dymek A, Bednarska-Czerwińska A, Okopień B. Growth hormone therapy in children and adults. Pharmacol Rep 2007;59:500–516.

31. Vance ML, Mauras N. Growth hormone therapy in adults and children. N Eng J Med 1999;341:1206–1216.

32. Takala J, Ruokonen E, Webster NR, Nielsen MS, Zandstra DF, Vundelinckx G, Hinds CJ. Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med 1999;341:785–792.

33. Saugy M, Robinson N, Saudan C, Baume N, Avois L, Mangin P. Human growth hormone doping in sport. Br J Sports Med 2006;40(Suppl 1):i35–i39.

34. Stacy JJ, Terrell TR, Armsey TD. Ergogenic aids: human growth hormone. Curr Sports Med Rep 2004;3:229–233.

35. Molon-Noblot S, Laroque P, Prahalada S, Stabinski LG, Peter CP, Duprat P, van Zwieten MJ. Morphological changes in the kidney of dogs chronically exposed to exogenous growth hormone. Toxicol Pathol 2000;28: 510–517.

36. Ho KY, Weissberger AJ, Stuart MC, Day RO, Lazarus L. The pharmacokinetics, safety and endocrine effects of authentic biosynthetic human growth hormone in normal subjects. Clin Endocrinol (Oxf) 1989;30:335–345.

37. Janssen YJ, Frolich M, Roelfsema R. The absorption profile and availability of a physiological subcutaneously administered dose of recombinant human growth hormone (GH) in adults with GH deficiency. Br J Clin Pharmacol 1999;47:273–278.

38. Tanaka T, Seino Y, Fujieda K, Igarashi Y, Yokoya S, Tachibana K, Ogawa Y. Pharmacokinetics and metabolic effects of high-dose growth hormone administration in healthy adult men. Endocrine J 1999;46:605–612.

39. Laursen T, Ovesen P, Grandjean B, Jensen S, Jorgensen JO, Illum P, Christiansen JS. Nasal absorption of growth hormone in normal subjects: studies with four different formulations. Ann Pharmacother 1994;28:845–848.

40. de la Motte S, Klinger J, Kefer G, King T, Harrison F. Pharmacokinetics of human growth hormone administered subcutaneously with two different injection systems. Arzneim Forsch Drug Res 2001;51:613–617.

41. Pan W, Yu Y, Cain CM, Nyberg F, Couraud PO, Kastin AJ. Permeation of growth hormone across the blood-brain barrier. Endocrinology 2005;146:4898–4904.

42. Johnson V, Maack T. Renal extraction, filtration, absorption, and catabolism of growth hormone. Am J Physiol 1977;233:F185–F196

43. Hanasen TK, Jorgensen JO, Christiansen JS. Body composition and circulating levels of insulin, insulin-like growth factor-binding protein-1 and growth hormone (GH)-binding protein affect the pharmacokinetics of GH in adults independently of age. J Clin Endocrinol Metab 2002;87:2185–2193.

44. Sohmiya M, Kato Y. Renal clearance, metabolic clearance rate, and half-life of human growth hormone in young and aged subjects. J Clin Endocrinol Metab 1992;75:1487–1490.

45. Hartman ML, Faria AC, Vance ML, Johnson ML, Thorner MO, Veldhuis JD. Temporal structure of *in vivo* growth hormone secretory events in humans. Am J Physiol 1991;260:E101–E110.

46. Jorgensen JO, Flyvbjerg A, Dinesen J, Lund H, Alberti KG, Orskov H, Christiansen JS. Serum profiles and short-term metabolic effect of pituitary and authentic biosynthetic human growth hormone in man. A double-blind cross-over study. Acta Endocrinol (Copenh) 1987;116: 381–386.

47. Jurgens G, Lange KH, Reuther LO, Rasmussen BB, Brosen K, Christensen HR. Effect of growth hormone on hepatic cytochrome P450 activity in healthy elderly men. Clin Pharmacol Ther 2002;71:162–168.

48. Wainberg MA, Brenner BG, Daar E, Gertner JM, Olivier C, Kenley S. Lack of effect of recombinant human growth hormone on the *in vitro* activities of antiretroviral drugs against human immunodeficiency virus type 1. Antimicrob Agents Chemother 2004;48:2337–2340.

49. Sturmi JE, Diorio DJ. Anabolic agents. Clin Sports Med 1998;17:261–282.

50. Godfrey RJ, Madgwick Z, Whyte GP. The exercise-induced growth hormone response in athletes. Sports Med. 2003;33:599–613.

51. Iranmanesh A, Lizarralde G, Veldhuis JD. Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. J Clin Endocrinol Metab 1991;73: 1081–1088.

52. Moller N, Vendelbo MH, Kampmann U, Christensen B, Madsen M, Norrelund H, Jorgensen JO. Growth hormone and protein metabolism. Clin Nutr 2009;28:597–603.

53. Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. Rev Endocr Metab Disord 2006;7: 225–235.

54. Toogood AA. Growth hormone (GH) status and body composition in normal ageing and in elderly adults with GH deficiency. Horm Res 2003;60(Suppl 1):105–111.

55. Taaffe DR, Jin IH, Vu TH, Hoffman AR, Marcus R. Lack of effect of recombinant human growth hormone (GH) on muscle morphology and GH-insulin-like growth factor expression in resistance-trained elderly men. J Clin Endocrinol Metab 1996;81:421–425.

56. Birzniece V, Nelson AE, Ho KK. Growth hormone administration: is it safe and effective for athletic performance? Endocrinol Metab Clin North Am 2010;39: 11–23.

57. Taaffe DR, Pruitt L, Reim J, Hintz RL, Butterfield G, Hoffman AR, Marcus R. Effect of recombinant human growth hormone on the muscle strength response to resistance exercise in elderly men. J Clin Endocrinol Metab 1994;79:1361–1366.

58. Cuneo RC, Salomon F, Wiles CM, Hesp R, Sönksen PH. Growth hormone treatment in growth hormone-deficient adults. II. Effects on exercise performance. J Appl Physiol 1991;70:695-700.

59. Liu H, Bravata DM, Olkin I, Friedlander A, Liu V, Roberts B, et al. Systematic review: the effects of growth hormone on athletic performance. Ann Intern Med 2008; 148:747–758.

60. Ehrnborg C, Ellegård L, Bosaeus I, Bengtsson BA, Rosén T. Supraphysiological growth hormone: less fat, more extracellular fluid but uncertain effects on muscles in healthy, active young adults. Clin Endocrinol (Oxf) 2005;62:449–457.

61. Yarasheski KE, Zachweija JJ, Angelopoulos TJ, Bier DM. Short-term growth hormone treatment does not increase muscle protein synthesis in experienced weight lifters. J Appl Physiol 1993;74:3073–3076.

62. Tokish JM, Kocher MS, Hawkins RJ. Ergogenic acids: a review of basic science, performance, side effects, and status in sports. Am J Sports Med 2004;32:1543–1553.

63. van der Lely AJ. Hormone use and abuse: what is the difference between hormones as fountain of youth and doping in sports? J Endocrinol Invest 2003;26:932–936.

64. Dean H. Does exogenous growth hormone improve athletic performance? Clin J Sport Med 2002;12:250–253.

65. Deyssig R, Frisch H, Blum WF, Waldhor T. Effect of growth hormone treatment on hormonal parameters, body composition and strength in athletes. Acta Endocrinol (Copenh) 1993;128:313–318.

66. Meinhardt U, Nelson AE, Hansen JL, Birzniece V, Clifford D, Leung KC, et al. The effects of growth hormone on body composition and physical performance in recreational athletes: a randomized trial. Ann Intern Med 2010;152:568–577.

67. Holt RI. Is human growth hormone an ergogenic aid? Drug Test Analysis 2009;1:412–418.

68. Russell-Jones DL, Umpleby M. Protein anabolic action of insulin, growth hormone and insulin-like growth factor I. Eur J Endocrinol 1996;135:631–642.

69. Isgaard J. Cardiovascular disease and risk factors: the role of growth hormone Horm Res 2004;62(Suppl 4): 31–38.

70. Zuliani U, Bernardini B, Catapano A, Campana M, Cerioli G, Spattini M. Effects of anabolic steroids, testosterone, and HGH on blood lipids and electrocardiographic parameters in body builders. Int J Sports Med 1989; 10:62–66.

71. Svensson J, Tivesten A, Isgaard J. Growth hormone and the cardiovascular function. Minerva Endocrinol 2005;30: 1–13.

72. Malozowski S, Stadel BV. Prepubertal gynecomastia during growth hormone therapy. J Pediatr 1995;126: 659–661.

73. Cohn L, Feller AG, Draper MW, Rudman IW, Rudman D. Carpal tunnel syndrome and gynaecomastia during growth hormone treatment of elderly men with low circulating IGF-1 concentrations. Clin Endocrinol (Oxf) 1993;39:417–425.

74. Mastaglia FL, Barwich DD, Hall R. Myopathy in acromegaly. Lancet 1970;2(7679):907–909.

75. Riedl S, Blumel P, Zwiauer K, Frisch H. Death in two female Prader-Willi syndrome patients during the early phase of growth hormone treatment. Acta Paediatr 2005; 94:974–977.

76. Van Vliet G, Deal CL, Crock PA, Robitaille Y, Oligny LL. Sudden death in growth hormone-treated children with Prader-Willi syndrome. J Pediatr 2004;44:129–131.

77. Botero D, Danon M, Brown RS. Symptomatic non-insulin-dependent diabetes mellitus during therapy with recombinant human growth hormone. J Pediatr 1993; 123:590–592.

78. Malozowski S, Tanner LA, Wysowski DK, Fleming GA, Stadel BV. Benign intracranial hypertension in children

with growth hormone deficiency treated with growth hormone. J Pediatr 1995;126:996–999.

79. Malozowski S, Hung W, Scott DC. Acute pancreatitis associated with growth hormone therapy for short stature. N Eng J Med 1995;332:401–402.

80. McMillan CV, Bradley C, Gibney J, Healy ML, Russell-Jones DL, Sonksen PH. Psychological effects of withdrawal of growth hormone therapy from adults with growth hormone deficiency. Clin Endocrinol (Oxf) 2003; 59:467–475.

81. Junprasert J, Javier FC 3rd, Rodriguez JA, Moore C, Sorensen RU. Successful intravenous desensitization of growth hormone hypersensitivity. J Pediatr Endocrinol Metab 1997;10:223–226.

82. Walker SB, Weiss ME, Tattoni DS. Systemic reaction to GH with acute desensitization. Pediatrics 1992; 90: 108–109.

83. Caliandro P, Padua L, Aprile I, Conti V, Pazzaglia C, Pavone A, Tonali P. Adverse effects of GH self administration on peripheral nerve. A case report. J Sports Med Phys Fitness 2004;44:441–443.

84. Dickerman RD, Douglas JA, East JW. Bilateral medial neuropathy and growth hormone use: a case report. Arch Phys Med Rehabil 2000;81:1594–1595.

85. Pierard-Franchimont C, Henry F, Crielaard JM, Pierard GE. Mechanical properties of skin in recombinant human growth factor abusers among adult bodybuilders. Dermatology 1996;192:389–392.

86. Stattin P, Bylund A, Rinaldi S, Biessy C, Dechaud H, Stenman UH, et al. Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. J Natl Cancer Inst 2000; 92:1910–1917.

87. McHugh CM, Park RT, Sonksen PH, Holt RI. Challenges in detecting the abuse of growth hormone in sport. Clin Chem. 2005;51:1587–1593.

88. Wallace JD, Cuneo RC, Bidlingmaier M, Lundberg PA, Carlsson L, Boguszewski CL, et al. Changes in non-22-kilodalton (kDa) isoforms of growth hormone (GH) after administration of 22-kDa recombinant human GH in trained adult males. J Clin Endocrinol Metab 2001; 86:1731–1737.

89. Kohler M, Thomas A, Puschel K, Schanzer W, Thevis M. Identification of human pituitary growth hormone variants by mass spectrometry. J Proteome Res 2009;8: 1071–1076.

90. Irie M, Ueki M, Kishikawa Y, Nishii M, Kawahara T. 20K-GH and its use in detecting GH abuse. Growth Horm IGF Res 2009;19:352–356.

91. Kniess A, Ziegler E, Kratzsch J, Thieme D, Muller RK. Potential parameters for the detection of hGH doping. Anal Bioanal Chem. 2003;376:696–700.

92. Sartorio A, Marazzi N, Agosti F, Faglia G, Corradini C, De Palo E, et al. Elite volunteer athletes of different sport disciplines may have elevated baseline GH levels divorced from unaltered levels of both IGF-1 and GH-

dependent bone and collagen markers: a study on-the-field. J Endocrinol Invest 2004;27:410–415.

93. Ehrnborg C, Lange KH, Dall R. The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test. J Clin Endocrinol Metab 2003;88:394–401.

94. Holt RI, Sonksen PH. Growth hormone, IGF-1 and insulin and their abuse in sport. Br J Pharmacol 2008; 154:542–556.

95. Radetti G, Buzi F, Tonini G, Bellone J, Pagani S, Bozzola M. Growth hormone (GH) isoforms following acute 22-kDa GH injection: is it useful to detect GH abuse? Int J Sports Med 2004;25:205–208.

96. Rigamonti AE, Cella SG, Marazzi N, Di Luigi L, Sartorio A, Muller EE. Growth hormone abuse: methods of detection. Trends Endocrinol Metab 2005;16:160–166.

97. Holt RI. Detecting growth hormone abuse in athletes. Drug Test Anal 2009;1:426–433.

98. Nelson AE, Ho KK. A robust test for growth hormone doping – present status and future prospects. Asian J Androl 2008;10:416–425.

99. Bidlingmaier M, Strasburger CJ. Growth hormone. Handb Exp Pharmacol 2010;195:187–200.

100. Banfi G, Marinelli M, Roi GS, Colombini A, Pontillo M, Giacometti M, Wade S. Growth hormone and insulin-like growth factor I in athletes performing a marathon at 4000 m of altitude. Growth Regul 1994;4:82–86.

101. Wu RH, St Louis Y, DiMartino-Nardi J, Wesoly S, Sobel EH, Sherman B, Saenger P. Preservation of physiological growth hormone (GH) secretion in idiopathic short stature after recombinant GH therapy. J Clin Endocrinol Metab 1990;70:1612–1615.

102. Longobardi S, Keay N, Ehrnborg C, Cittadini A, Rosen T, Dall R, et al. Growth hormone (GH) effects on bone and collagen turnover in healthy adults and its potential as a marker of GH abuse in sports: a double blind, placebo-controlled study. The GH-2000 Study Group. J Clin Endocrinol Metab 2000;85:1505–1512.

103. Wallace JD, Cuneo RC, Lundberg PA, Rosén T, Jørgensen JO, Longobardi S, et al. Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. J Clin Endocrinol Metab 2000;85:124–133.

104. Sartorio A, Jubeau M, Agosti F, Marazzi N, Rigamonti A, Muller EE, Maffiuletti NA. A follow-up of GH-dependent biomarkers during a 6-month period of the sporting season of male and female athletes. J Endocrinol Invest 2006;29:237–243.

105. Healy ML, Dall R, Gibney J, Bassett E, Ehrnborg C, Pentecost C, et al. Toward the development of a test for growth hormone (GH) abuse: a study of extreme physiological ranges of GH-dependent markers in 813 elite athletes in the postcompetition setting. J Clin Endocrinol Metab 2005;90:641–649.

106. Abellan R, Ventura R, Palmi I, di Carlo S, Bascosi A, Bellver M, et al. Immunoassays for the measurement of

IGF-2, IGFP-2, and -3, and ICTP as indirect biomarkers of recombinant human growth hormone misuse in sport values in selected populations of athletes. J Pharmaceut Biomed Anal 2008;48:844–852.

107. Davies JS, Morgan CL, Currie CJ, Green JT. Growth hormone use by body builders. Br J Sports Med 1997; 31:352–353.

108. Sein Anand J, Chodorowski Z, Wisniewski M. Multifactorial hypoglycemic coma in female bodybuilder. Przegl Lek 2005;62:520–521.

109. Binnerts A, Swart GR, Wilson JH, Hoogerbrugge N, Pols HA, Birkenhager JC, Lamberts WJ. The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol 1992;37:79–87.

110. Rinderknecht E, Humbel RE. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J Biol Chem 1978;253: 2769–2776.

111. Kemp SF. Insulin-like growth factor-I deficiency in children with growth hormone insensitivity: current and future treatment options. Bio Drugs 2009;23:155–163.

112. Guha N, Sonksen PH, Holt RI. IGF-I abuse in sport: current knowledge and future prospects for detection. Growth Horm IGF Res 2009;19:408–411.

113. Erotokritou-Mullian I, Holt RI. Insulin-like growth factor 1 and insulin and their abuse in sport. Endocrinol Metab Clin North Am 2010;39:33–43.

114. World Anti-Doping Agency. The 2010 prohibited list: international standard, 2010. Available at http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf. Accessed 2011 June 17.

115. Guha N, Dashwood A, Thomas NJ, Skingle AJ, Sonksen PH, Holt RI. IGF-1 abuse in sport. Curr Drug Abuse Rev 2009;2:263–272.

116. Blum WF, Ranke MB, Kietzmann K, Gauggel E, Zeisel HJ, Bierich JR. A specific radioimmunoassay for the growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. J Clin Endocrinol Metab 1990;70:1292–1298.

117. Guler HP, Zapf J, Schmid C, Froesch ER. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. Acta Endocrinol (Copenh) 1989;121:753–758.

118. Grahnén A, Kastrup K, Heinrich U, Gourmelen M, Preece MA, Vaccarello MA, et al. Pharmacokinetics of recombinant human insulin-like growth factor I given subcutaneously to healthy volunteers and to patients with growth hormone receptor deficiency. Acta Paediatr Suppl 1993;82(Suppl 391):9–14.

119. Toogood AA. Growth hormone (GH) status and body composition in normal ageing and in elderly adults with GH deficiency. Horm Res 2003;60(Suppl 1):105–111.

120. Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. Rev Endocr Metab Disord 2006;7: 225–235.

121. Cheetham TD, Holly JM, Clayton K, Cwyfan-Hughes S, Dunger DB. The effects of repeated daily recombinant human insulin-like growth factor I administration in adolescents with type 1 diabetes. Diabet Med 1995;12: 885–892.

122. Chernausek SD, Backeljauw PF, Frane J, Kuntze J, Underwood LE; GH Insensitivity Syndrome Collaborative Group. Long-term treatment with recombinant insulin-like growth factor (IGF)-I in children with severe IGF-I deficiency due to growth hormone insensitivity. J Clin Endocrinol Metab 2007;92:902–910.

123. Ehrnborg C, Lange KH, Dall R, Christiansen JS, Lundberg PA, Baxter RC, et al. The growth hormone/insulin-like growth factor-I axis hormones and bone markers in elite athletes in response to a maximum exercise test. J Clin Endocrinol Metab 2003;88:394–401.

124. Nelson AE, Ho KK. Demographic factors influencing the GH system: implications for the detection of GH doping in sport. Growth Horm IGF Res 2009;19:327–332.

125. Erotokritou-Mulligan I, Bassett EE, Cowan DA, Bartlett C, McHugh C, Sönksen PH, et al. Influence of ethnicity on IGF-I and procollagen III peptide (P-III-P) in elite athletes and its effect on the ability to detect GH abuse. Clin Endocrinol (Oxf) 2009;70:161–168.

126. Popot MA, Woolfitt AR, Garcia P, Tabet JC. Determination of IGF-1 in horse plasma by LC electrospray ionisation mass spectrometry. Anal Bioanal Chem 2008;390: 1843–1852.

127. Kay RG, Barton C, Velloso CP, Brown PR, Bartlett C, Blazevich AJ, et al. High-throughput ultra-high-performance liquid chromatography/tandem mass spectrometry quantitation of insulin-like growth factor-I and leucine-rich alpha-2-glycoprotein in serum as biomarkers of recombinant human growth hormone administration. Rapid Commun Mass Spectrom 2009;23:3173–3182.

128. Thevis M, Bredehoft M, Kohler M, Schanzer. Mass spectrometry-based analysis of IGF-1 and hGH. Handb Exp Pharmacol 2010;195:201–207.

129. Mohan S, Libanati C, Dony C, Lang K, Srinivasan N, Baylink DJ. Development, validation, and application of a radioimmunoassay for insulin-like growth factor binding protein-5 in human serum and other biological fluids. J Clin Endocrinol Metab 1995;80:2638–2645.

Chapter 20

NUTRITIONAL SUPPLEMENTS

Ergogenic aids are techniques or regimens that increase performance capacity, improve the efficiency to perform work, or enhance the ability to recover from exercise. Diet has been part of Olympic training at least since Roman and Greek times, and diets used for ergogenic purposes included various combinations of dried figs, moist cheese, wheat, meat, white bread sprinkled with poppy seeds, fish and pork, along with the limited use of wine.[1] The US Food and Drug Administration (FDA) defines a dietary supplement as follows: 1) a product (other than tobacco) intended to supplement the diet that bears or contains 1 or more of the following dietary ingredients: a vitamin, mineral, amino acid, herb or other botanical; 2) a dietary substance for use to supplement the diet by increasing the total dietary intake; or 3) a concentrate, metabolite, constituent, extract, or combination of any ingredient described previously. In general, nutritional supplements are not produced under the same strict quality control measures as pharmaceutic drugs. The FDA monitors structure/function claims under the Dietary Supplement Health Education Act of 1994, but the FDA does not routinely monitor the quality of these supplements with the same scrutiny as pharmaceutic drugs.

The potency and composition of nutritional supplements may vary substantially between different sources. Additionally, nutritional supplements may contain adulterants not listed on the label. The World Anti-Doping Agency (WADA) does not ban dietary supplements or caffeine.[2] However, nutritional supplements may contain prohibited stimulants (e.g., ephedrine, sibutramine) or anabolic androgenic steroids (androstenedione, dehydroepiandrosterone) that are not listed on the labels.

The latter compounds in these intentionally faked products include both classic anabolic steroids (boldenone, dehydrochloromethyl-testosterone, methandienone, oxandrolone, stanozolol) and newer designer steroids (e.g., androstatrienedione, methasterone, prostanozol).[3] Androstenedione (CAS RN: 63-05-8) and dehydroepiandrosterone (DHEA, CAS RN: 2283-82-1) are popular endogenous steroid precursors that are weak androgenic compounds; the body converts DHEA to androstenedione, which subsequently is converted to testosterone or estrone.[4]

Currently, there are a large number (>250) of dietary supplements being used as ergogenic aids, but there are few data on the ergogenic properties of most of these compounds. In a meta-analysis of dietary supplements, 6 of these supplements had more than 2 studies that met the criteria for inclusion in the meta-analysis.[5] Of these 6 supplements, only creatine and, to a lesser extent, β-hydroxy-β-methylbutyrate demonstrated statistically significant increases in net lean mass and strength.

CREATINE

HISTORY

Chevreul originally discovered creatine in the late 1830s, and Liebeg confirmed the existence of creatine in 1847.[6] However, creatine was not isolated until the early 1900s.[7] In 1926, Chanutin suggested that creatine was associated with skeletal muscle anabolism.[8] However, the use

of creatine as an ergogenic aid did not begin until the 1970s, when athletes from the Soviet Union and the Eastern bloc countries used creatine supplementation to enhance performance. The use of creatine by athletes in the United States and United Kingdom did not begin until the early 1990s.[9] During the 1992 Barcelona Olympics, high profile athletes associated improved performance in power and sprint events with the use of creatine supplements. Creatine is not listed on WADA's list of banned substances.

IDENTIFYING CHARACTERISTICS

Physiochemical Properties

Creatine (α-methyl guanidino acetic acid, CAS RN: 57-00-1) is a nitrogenous organic acid composed of 3 amino acids (arginine, glycine, methionine) that is formed endogenously by methylation of guanidinoacetic acid found primarily in muscle. Figure 20.1 displays the chemical structure of creatine ($C_4H_9N_3O_2$). Creatine salts (e.g., glycinate, citrate, malate, ethyl ester, pyruvate) are relatively water soluble and easily dissolved in sports drinks or gels. In contrast, creatine monohydrate must be consumed soon after dissolved in solution to avoid precipitation. Table 20.1 lists some of the physiochemical properties of creatine.

Form

Creatine supplements appear commercially in several forms as a result of the relatively poor solubility of creatine monohydrate compared with some creatine salts. Creatine monohydrate is the most common form of creatine supplements, available as a powder from a
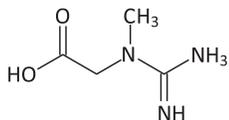
FIGURE 20.1. Chemical structure of creatine.

TABLE 20.1. Some Physical Properties of Creatine.

Physical Property	Value
Melting Point	303°C (577.4°F)
log P (Octanol-Water)	−3.720
Water Solubility	1.33E+04 mg/L (18°C/64.4°F)
Vapor Pressure	7.90E-04 mm Hg (25°C/77°F)

variety of sources including health food stores, grocery stores, and pharmacies. Creatine is a component of many protein supplements, sport drinks, and energy bars that is also available as capsules and tablets. Each gram of creatine monohydrate provides 0.879 g creatine.

EXPOSURE

The liver, kidneys, and pancreas produce endogenous creatine by the methylation of guanidinoacetic acid from dietary sources of arginine and glycine.[6] The diet is the exogenous source of creatine (3–7 g/kg), particularly meat, fowl, and fish. The typical US diet provides about 1 g creatine daily; endogenous creatine production and the diet are about equal sources of creatine for the human body. Clinical trials on the ergogenic effects of creatine supplementation use doses about 3- to 12-fold higher than the daily dietary intake of creatine.[10] Exogenous and endogenous sources account for approximately equal amounts of creatine in the body.

Creatine is a nonessential, legal food supplement that is regulated in the United States as a food supplement rather than a drug. Although creatine is a natural constituent of fish and meat, most creatine in dietary supplements is produced synthetically by reacting sarcosine (CAS RN: 107-97-1) with cyanamide (CAS RN:420-04-2). Potential contaminants of the manufacturing process include creatinine, dicyandiamide, dihydrotriazine, and trace metals (e.g., arsenic).[11] Creatine monophosphate is a very common nutritional supplement used primarily as a method to increase strength and power; this substance is the most widely marketed nonsteroidal, nonstimulant ergogenic aid.[12] In a survey of 674 high school athletes from Georgia and Tennessee, 16% of these athletes used creatine to enhance athletic performance based on responses to questionnaires distributed by their athletic trainers or coaches.[13] In this study, creatine use increased with age and was more prevalent among male than female athletes (23% vs. 2%, respectively). Frequently, adolescents used creatine supplements at dosages exceeding standard recommendations. In a study of the use of complementary medicine and dietary supplements by the US adolescent population, 4.7% of the participants reported they ever used creatine supplements on the survey.[14] The use of creatine by college athletes probably is probably at least several times higher than adolescent athletes.[15]

DOSE EFFECT

The common dose of creatine is an initial oral loading dose of 15–20 g daily for 5–7 days followed by a maintenance dose of 3–5 g daily for up to 6 months.[16]

Anecdotally, higher daily maintenance doses (e.g., 10 g) are used, but there are few medical data on the dose effect of these regimens. Because of concern about reduced response to creatine and a slow decrease in muscle creatine during maintenance dosing, creatine regimens frequently include a 4-week wash-out period following 8–10 weeks of maintenance dosing.[17] The observed safe level of creatine for chronic supplementation in healthy adults is approximately 5 g/day based on risk assessment.[10]

TOXICOKINETICS

Absorption

The absorption of oral doses of creatine probably involves active transport (i.e., with sodium- and chloride-dependent transporters) similar to the uptake of amino acids and peptides by transporters.[18] There are inadequate pharmacokinetic data to determine the absolute bioavailability of creatine. The dissolution rate and solubility may limit the bioavailability of the capsules and chewable tablets formulations of creatine. The bioavailability of creatine is probably <100% as a result of the potential for saturation of the active transport process and degradation of creatine in the gut.[18] The absorption of creatine may be dose-dependent. Creatine is not subject to first-pass metabolism. Peak plasma concentrations of creatine usually occur within 2–3 hours after ingestion. In a study of 6 healthy volunteers, the mean peak plasma creatine after the ingestion of 71 mg creatine monohydrate/kg was 102.1 ± 11.2 mg/L at 1.9 ± 0.88 hours after administration.[19] The steady-state plasma concentration of creatine after administration of 71 mg/kg 4 times daily for 6 days was 97.4 ± 13.0 mg/L.

Distribution

Although uptake of creatine occurs in a variety of cells (erythrocytes, nervous tissue, spermatozoa, retina), the main site of creatine absorption is skeletal muscle.[20] Therefore, skeletal muscle contains most of the creatine in the body; in muscle, creatine exists as either the free or phosphorylated form in a ratio of approximately 2:3 depending on muscle fiber type. The average pool of creatine is about 2 g/kg, and the storage capacity of skeletal muscle is finite.[21] About 95% of creatine is distributed to skeletal muscle; the remaining occurs in the heart, brain, and testes. Creatine enters the muscle cell in the free form against a concentration gradient as a result of the action of the sodium-dependent transporter, creatine transporter-1. The creatine transporter is specific for creatine, but the chemical structure of the creatine transporter is similar to the transporters for

dopamine and γ-aminobutyric acid (GABA). Several factors increase creatine uptake into muscle including exercise, insulin, catecholamines, insulin-like growth factor 1 (IGF-1), and simultaneous ingestion with carbohydrates, probably as a result of insulin-induced enhancement of creatine transport into muscle.[22,23] The volume of distribution is probably near total body water (i.e., about 45 L).[24] Protein binding of creatine in plasma is negligible (<10%) as a result of the hydrophilic properties of creatine.[25]

Biotransformation

Animal studies indicate that creatine undergoes spontaneous (i.e., nonenzymatic) degradation in the blood almost exclusively to creatinine.[26,27] Increasing the stores of creatine in muscle elevates serum creatinine concentrations.[18] Metabolism of creatine to methylamine is a minor pathway.[28] Formaldehyde is a potential metabolite of methylamine as a result of the enzymatic action of semicarbazide-sensitive amine oxidase; however, the extent of formation is probably small.[29] Figure 20.2 displays the synthesis, metabolism, and phosphorylation of creatine.

Elimination

Clearance of creatine involves biotransformation to creatinine, renal filtration, and irreversible uptake into skeletal muscle.[18] In a study of 6 healthy volunteers, the mean terminal plasma elimination half-life of creatine after a single dose (71 mg/kg) and 4 doses daily for 6 days was 2.0 ± 0.68 hours and 2.7 ± 0.63 hours, respectively.[19] Urinary excretion accounts for the loss of about 2 grams of creatine daily, primarily as creatinine. The elimination rate of creatine in the urine is not well defined following creatine supplementation, but the renal clearance of free creatine in patients without supplementation is probably near the glomerular filtration rate. With increasing supplementation, the amount of creatine in the urine increases. Although the percentage of clearance by metabolism to creatinine, renal elimination of creatine, and skeletal muscle uptake; the latter mechanism probably accounts for most of the clearance of creatine during creatine supplementation.[18] Total creatine concentrations return to baseline values within 1 month after cessation of creatine supplementation.[30] During this period, urinary creatinine concentrations are elevated.

Maternal and Fetal Kinetics

There are few data on the maternal fetal kinetics of creatine.
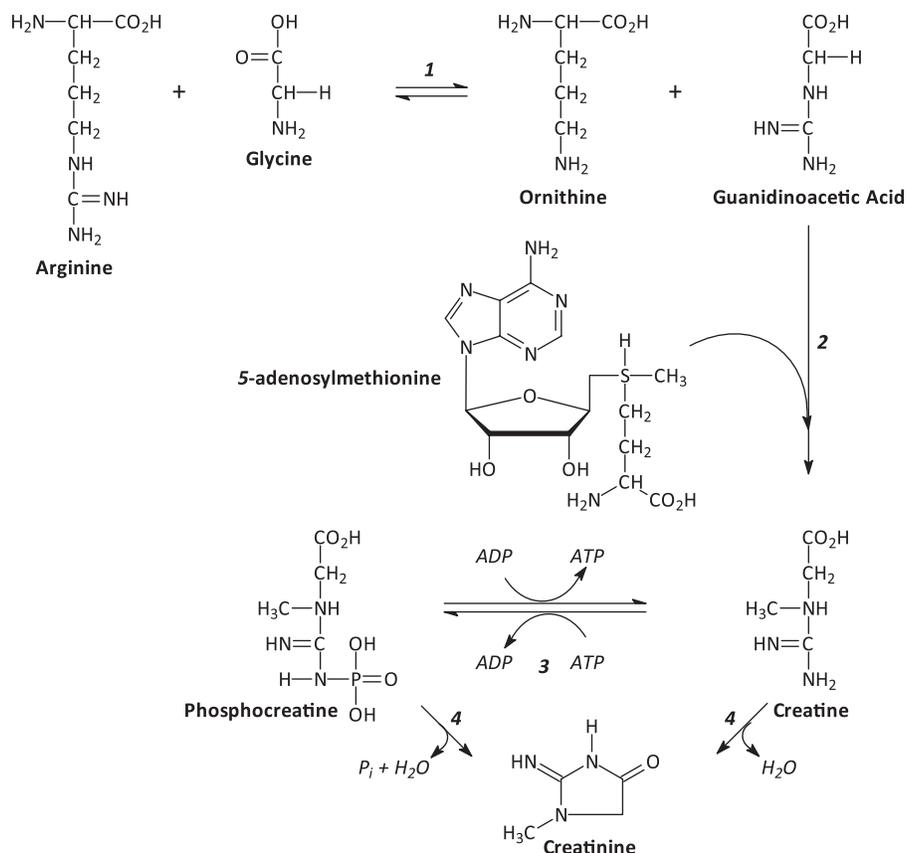
FIGURE 20.2. Synthesis, metabolism, and phosphorylation of creatine. Enzymes for the chemical reactions include the following: 1) arginine:glycine amidino-transferase, 2) guanidinoacetate *N*-methyltransferase, 3) creatine kinase, and 4) none—spontaneous conversion.[28]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

The skeletal muscles contain over 95% of the total creatine in the human body. The primary ergogenic effect of creatine supplementation involves improvement in repeated short bouts (i.e., <1 minute) of high-intensity, repetitive physical activity rather than endurance events.[31] During these activities (e.g., sprinting, jumping, cycling), the amount of phosphocreatine stored in the muscle determines the amount of energy available based on the rephosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). Beside repeated short bouts of high-intensity physical exercise, creatine supplementation increases maximal force or strength of both dynamic and isotonic contractions. There is little evidence to support the use of creatine to improve isometric muscular performance or endurance activities.[16] Creatine, in the phosphorylated form (phosphocreatine) helps buffers hydrogen ions during

maximal exercise in muscle cells along with maintaining the adenosine triphosphate/adenosine diphosphate ratio in both skeletal muscle and brain tissue that is critical for normal cellular function. During physical activity, phosphocreatine maintains adenosine triphosphate (ATP) concentrations by donating a phosphate to adenosine diphosphate (ADP). Short-term creatine supplementation (20 g daily for 5–7 days) increases total creatine content in the muscles of healthy young adults by about 10–30% and phosphocreatine stores in muscle by approximately 10–40%.[32] Creatine supplementation does not increase muscle glycogen content at rest or after exhaustive exercise.[33] In the brain, similar creatine supplementation protocols increase creatine and phosphocreatine concentrations about 9% and 4%, respectively, as measured by nuclear magnetic resonance spectroscopy.[34,35]

The use of creatine probably increases the energy available for maximal force of dynamic or isotonic contractions, particularly in less-trained individuals.[36] The benefit of creatine depends on the study group with the greatest gains in nonelite athletes, and volunteer studies

are inconsistent regarding the positive effects of creatine on athletic performance.[37] In the positive studies, short-term creatine supplementation improves single-effort sprint performance about 1–5% and maximal power (peak power, total work)/strength (maximal force or strength) about 5–15%.[32] Up to approximately 30% of individuals do not respond to creatine supplementation.[38] In a study of National Collegiate Athletic Association division I players with at least 1 year of college football experience, creatine supplementation did not increase strength gains compared with a control group (placebo).[39] However, a study of red-shirt freshmen indicated that creatine supplementation produces a statistically significant increase in strength, peak torque (300° knee flexion only), and anaerobic capacity as assessed by the Wingate protocol, when compared with placebo (sodium phosphate monohydrate) and control (no supplementation).[40] All participants underwent 4 weeks of resistance training. Body fat, global muscle strength, and extracellular fluid did not demonstrate statistically significant differences between these groups. In a study of healthy young adults, postexercise supplementation with protein-carbohydrate (10 g casein, 75 g glucose) produced similar increases in strength after a resistance exercise training program, when compared with creatine-carbohydrate (10 g creatine, 75 g glucose) supplementation.[41] However, mean total body mass increased more ($P < .05$) for creatine-carbohydrate group (+4.3 kg, 5.4%) compared with protein-carbohydrate group (+1.9 kg, 2.4%). The increase in body mass results primarily from increased intracellular water in muscle cells rather than an increase in protein synthesis or decrease in muscle proteolysis. The results of creatine supplementation on muscle mass and muscle performance in older men (>60 years) is equivocal.[42]

Creatine does not prevent myalgias or muscle breakdown that follows intense exercise, and there is little evidence that creatine supplementation increases isometric muscular exercise.[43] Additionally, there is little evidence that creatine loading improves the adverse effects of neuromuscular fatigue on power output. Five days of creatine loading (20 g dicreatine citrate/day) had minimal effect on the electromyographic fatigue threshold as measured in the vastus lateralis of the right thigh of 16 college-age men performing a discontinuous cycle-ergometer exercise.[44]

The mechanism of action of creatine is not well defined. Possible mechanisms of action include increased phosphocreatine-enhanced rephosphorylation of adenosine diphosphate, buffering of pH changes, increased diffusion of high-energy phosphates into myosin heads, and increased muscle hypertrophy.[45,46] There is equivocal evidence that creatine supplementation improves

muscle strength in disease states (e.g., atherosclerosis, congestive heart failure, neuromuscular diseases).[47,48] Although some studies suggest that creatine supplementation improves performance of older adults and sleep-deprived young adults on some cognitive and psychomotor tests,[49] 6 weeks of creatine supplementation (0.03 g/kg/d) did not improve cognitive function in healthy, rested young adults.[50] Neuropsychologic testing included simple reaction time, code substation, logical reasoning symbolic, running memory, Sternberg memory recall, and mathematical processing.

Mechanism of Toxicity

There are limited data on the potential of creatine supplementation to cause chronic toxicity. Most reported data on toxicity is derived from clinical studies on healthy adults or patients with muscle wasting. There are inadequate data to determine the mechanism of toxicity of permanent adverse effects, if any, associated with the use of creatine supplementation at standard doses.

CLINICAL RESPONSE

In most open label studies and randomized clinical trials to date, creatine supplements were associated with infrequent and relatively minor side effects, when reported.[10] However, not all studies specifically report measuring adverse effects or other safety outcomes. A few case reports document more severe effects (e.g., renal dysfunction, atrial fibrillation, rhabdomyolysis), but the precise role of creatine in the causation of these abnormalities is unclear. Side effects most commonly associated with the use of creatine supplements based on anecdotal reports include gastrointestinal (GI) distress, muscle cramps, and renal dysfunction.[51] Currently, there are no clinical data to indicate that creatine supplementation causes liver dysfunction.

Gastrointestinal Distress

The most common adverse effect associated with creatine supplementation in clinical trials evaluating the effect of creatine supplementation on athletic performance is GI distress (nausea, vomiting, diarrhea), particularly if ingested during exercise.[52,53] In a placebo-controlled trial of creatine monohydrate in patients with amyotrophic lateral sclerosis, 3 of 175 patients in the treatment group ceased using creatine because of severe GI complaints (diarrhea, vomiting) compared with none in the placebo group.[54] Most clinical studies on creatine supplementation do not report a significant incidence of adverse effects as compared with baseline.[55] In a 28-day study of elite college football players

receiving phosphorus and creatine (15.75 g) supplementation daily, there were no complaints of GI symptoms.[56]

Muscle

Based on responses to questionnaires, the most common adverse effects associated with creatine supplementation in high school athletes were muscle cramps, increased thirst, and stomach cramps.[13] However, there was no control group to determine the effect of physical exercise alone on the development of these symptoms. Follow-up studies up to 5 years suggest that supplementation with common doses of creatine up to 80 g daily does not alter hepatic or renal function.[57,58] In the above 28-day study on elite athletes, there were no complaints of excess muscle cramps.[56] Volunteer studies suggest that creatine supplementation can increase anterior compartment pressures of the lower leg during the period immediately after exercise.[59] However, the clinical significance of this increase remains unclear. Rhabdomyolysis and an acute quadriceps compartment syndrome developed in a weight lifter on long-term, high-dose creatine supplementation (25 g daily for 1 year).[60] The chest x-ray demonstrated cardiomegaly, pulmonary venous congestion and bilateral effusions, attributed to pulmonary edema secondary to iatrogenic fluid overload. There were no laboratory tests for other ergogenic aids.

Renal Dysfunction

Clinical studies indicate that creatine supplementation does not alter glomerular filtration rate, glomerular permeability, or renal tubular reabsorption, when taken in standard doses (20 g/d for 5 days, <5 g thereafter) by healthy young adults.[51,61] Several case reports suggest that creatine supplementation may decrease renal function in patients with preexisting renal disease. A 25-year-old man had normal renal function while receiving cyclosporin for focal segmental glomerular sclerosis; 7 weeks of creatine supplementation at 2 g/day was associated with a decrease in creatinine clearance from 93 mL/min prior to supplementation to 54 mL/min after supplementation.[62] The creatinine clearance returned to baseline values after cessation of creatine supplementation. Another case report associated creatine supplementation (10 g daily 6 weeks prior to surgery) with the development of nonoliguric renal dysfunction and rhabdomyolysis immediately after arthroscopic knee surgery.[63] These 2 patients responded to medical therapy and they did not develop any permanent sequelae. A case report associated the development of lactic acidosis and acute renal failure with the use of creatine for 2

months by a 42-year-old bodybuilder treated with metformin for his diabetes mellitus.[64]

Cardiovascular

Cardiovascular effects are not usually associated with the use of creatine supplementation. A case report of a 30-year-old man associated the development of atrial fibrillation with the loading phase of creatine supplementation.[65] Although there was no other obvious cause of this dysrhythmia, the causal link between atrial fibrillation and creatine supplementation is unclear.

Carcinogenesis

Creatine is not considered a carcinogen. Nitrosation of creatine to the animal carcinogen, *N*-nitrososarcosine, probably does not occur to any clinical significant extent based on pharmacology studies.[66] Although creatine supplementation potentially stimulates the production and renal excretion of methylamine and formaldehyde, there are inadequate data to determine if this enhanced production increases the risk of cancer.

DIAGNOSTIC TESTING

Analytic Methods

Creatine supplementation interferes with serum and urine creatinine measurements as a result both of increased daily excretion of creatinine from the degradation of creatine and analytic interference of creatine with some creatinine assays. In addition to creatine and bilirubin, the presence of ascorbic acid in biological samples interferes with enzymatic methods for determining creatinine concentrations.[67] The effect of creatine on creatinine determination depends on the specific method, and the presence of creatine can either falsely increase or decrease the serum creatinine concentration. Inulin clearance is an alternative to the use of creatinine to evaluate kidney function, but there are no human data on the use of inulin in the setting of creatine supplementation. A liquid chromatography method with ultraviolet detection is available for the determination of creatine in commercial products.[68] Methods to determine creatine in biologic samples include isocratic high performance liquid chromatography.[25] The lower limit of quantitation for creatine with this assay is about 5 mg/L.

Abnormalities

Clinical studies on the use of creatine supplements indicate that creatine supplementation causes no significant

long-term changes in measures of renal function in young healthy adults.[61] Although creatine supplementation initially increases serum and urine creatinine concentrations during use, follow-up studies of athletes using creatine supplementation do not indicate that the use of these supplements permanently alters serum measurements of liver, kidney, or muscle function. Both serum and urine creatinine concentrations increase during creatine supplementation, resulting in no significant change in urine creatinine clearance. In a study of healthy young participants receiving a loading dose of creatine (20 g daily for 5 days) followed by a maintenance dose (3 g daily for 9 weeks), there were no clinical significant changes in serum markers of hepatorenal function or muscle damage during the study.[69] These serum markers included sodium, potassium, urea, creatinine alkaline phosphatase, alanine aminotransferase, bilirubin, creatine kinase, albumin, and γ-glutamyl transferase. A study of 116 National Collegiate Athletic Association Division 1A college football players did not detect clinically significant difference in a number of urinary and blood markers of liver, kidney, and muscle function when comparing athletes with and without creatine supplementation (daily maintenance creatine doses of 5–10 g for up to 21 months).[70] The biomarkers included serum creatine kinase, serum electrolytes, hepatic aminotransferases, serum alkaline phosphatase, hemoglobin, and serum uric acid. Creatine supplementation was associated with a statistically significant increase in the magnetic resonance pool of creatine in the deep frontal cerebral white matter in some,[71] but not all studies.[72] The reason for the differences (e.g., dosage, duration of treatment, analytical method, study group) are unclear.

TREATMENT

There are few data on the treatment of toxicity associated with creatine use. Any adverse effects associated with the use of creatine typically resolve with cessation of use and respond to supportive care.

β-HYDROXY-β-METHYLBUTYRATE (HMB)

IDENTIFYING CHARACTERISTICS

β-Hydroxy-β-methylbutyrate (β-hydroxyisovaleric acid, CAS RN:625-08-1) is a metabolic product of leucine
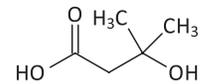
FIGURE 20.3. Chemical Structure of β-hydroxy-β-methylbutyrate (HMB).

TABLE 20.2. Some Physical Properties of β-Hydroxy-β-Methylbutyrate.

Physical Property	Value
Melting Point	−80°C (−112°F)
log P (Octanol-Water)	−0.020

that is a precursor of cholesterol. Leucine is an important amino acid and precursor for protein synthesis and energy metabolism in the muscle. Figure 20.3 displays the chemical structure of HMB. Table 20.2 displays some physical properties of β-hydroxy-β-methylbutyrate.

EXPOSURE

β-Hydroxy-β-methylbutyrate is a popular "anticatabolic," ergogenic agent that is usually part of a training supplementation program designed to improve strength and increase lean muscle mass.[73] Products containing HMB include fat burners, muscle builders, and recovery enhancers. This compound is a natural constituent of citrus, catfish, and breast milk. Both nonprimate animals and humans produce HMB endogenously. The FDA lists this compound as a nutritional supplement rather than a drug. HMB is not listed on WADA's list of banned substances or any other sporting organizations. HMB is an investigative supplement for the improvement of nitrogen balance in both hospitalized and nonhospitalized elderly patients. In a double-blind controlled study of 77 elderly participants receiving a supplement containing HMB, L-arginine, and L-lysine for 1 year, the lean mass in the treated group increased 1.2% ($P = .05$) compared with controls receiving an iso-nitrogenous supplement.[74] However, there was no decrease in the reduction of handgrip and leg strength between the 2 groups over the 1-year study.

DOSE EFFECT

The typical dose of HMB is 3–5 g daily alone or as part of a regimen of other supplements and/or exercise program at bedtime or before training. In a rodent study, the no-observed-adverse-effect level (NOAEL) was 5% calcium HMB (3.49 g/kg body weight males, 4.16 g/kg body weight females) in the diet.[75] There was
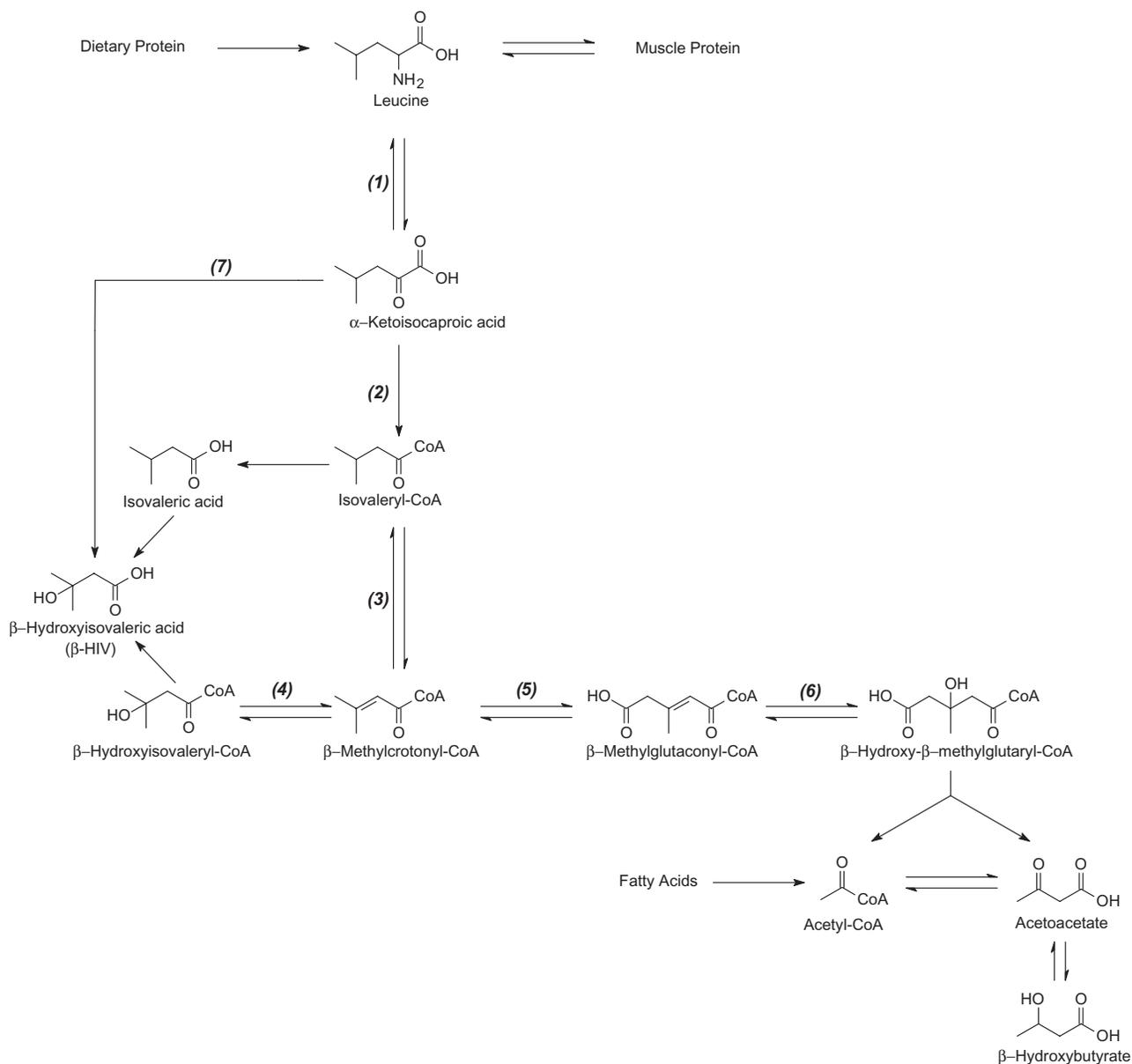
FIGURE 20.4. Formation of β-hydroxy β-methylbutyrate (β-hydroxyisovaleric acid). 1 = Branched chain amino acid transaminase; 2 = branched chain ketoacid dehydrogenase; 3 = isovaleryl-CoA dehydrogenase; 4 = enoyl-CoA hydratase; 5 = β-methylcrotonyl-CoA carboxylase; 6 = β-methylglutaconyl-CoA hydratase; 7 = cytosolic α-ketoisocaproate oxygenase.[95]

a statistically significant increase in inorganic phosphate in male animals in the 5% feeding group, but the effect was not considered adverse.

TOXICOKINETICS

Transamination of leucine produces α-ketoisocaproate. The action of the cytosolic enzyme, α-ketoisocaproate dioxygenase, on this latter compound forms β-hydroxy-β-methylbutyrate (HMB). This reaction accounts for about 5% of the oxidation of leucine in animal studies.[76]

Most α-ketoisocaproate undergoes metabolism in the mitochondria by α-ketoisocaproate dehydrogenase. Additionally, production of HMB results from the hydration of methylcrotonyl-CoA by crotonase, particularly in patients with 3-methylcrotonyl-CoA carboxylase or biotin deficiencies. Figure 20.4 displays the formation of HMB from leucine and from methylcrotonyl-CoA. The major metabolic pathway of HMB is probably conversion to β-hydroxy-β-methylglutaryl-CoA and then to mevalonate or acetoacetyl-CoA.[77] There are no data on the maternal-fetal kinetics of HMB.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The mechanism of action of HMB is not well defined. Clinical studies suggest that this mechanism is different from creatine as a result of the additive effect of HMB when added to regimens including creatine and resistance training.[78] Potential mechanisms of action include the following: 1) promoting muscle growth and cell integrity by enhanced production of membrane precursors (i.e., cholesterol) and 2) improving recovery time by decreasing muscle catabolism and excretion of 3-methylhistidine. Cholesterol enhances membrane fluidity and reduces stretch rupture in the muscle fibers. In the cytosol, β-hydroxy-β-methylglutaryl-coenzyme A forms from HMB, and subsequently cholesterol is produced from fatty acid β-oxidation and/or glycolysis via β-hydroxy-β-methylglutaryl-coenzyme A.

Most clinical studies on HMB supplementation are limited by small sample size, specific populations (e.g., auto immunodeficiency syndrome [AIDS] patients), and unique settings. The existing studies suggest the *potential* for modest enhancement of strength in resistance trained athletes, primarily in lower extremities.[79] The increase in strength measures is variable with some studies demonstrating no clear improvement in strength following HMB supplementation. A meta-analysis of 9 studies of HMB supplementation during resistance training estimated the average net weekly increase in strength gain over about 5 weeks was 1.4% (95% CI: 0.41–2.39%).[5] In a study of 34 resistance-trained men receiving 3 g HMB daily for 9 weeks, 1-repetition maximum leg extension strength increased 9.1% (90% CI: ± 7.5%) between baseline and the end of the study.[80] The effect of HMB supplementation on upper body strength (bench press, biceps preacher curl) was insignificant. Another meta-analysis of 9 studies of both trained and untrained participants demonstrated little benefit of daily HMB supplementation over an average of 5 ± 2 weeks for trained athletes.[81] For untrained participants, there were small benefits for average lower body strength (mean ± 90% CI = 9.9 ± 5.9%) with negligible changes in upper body strength (2.1 ± 5.5%).

Improvements in lean body mass occur following the use of HMB in certain participants including the elderly[82] and patients with muscle wasting diseases (i.e., in combination with L-glutamine and L-arginine).[83] However, the benefits of HMB supplementation in athletes is less clear. In the above meta-analysis of 9 studies, HMB supplementation during resistance training increased the average net weekly gain in lean mass over about 5 weeks ~0.28% (95% CI: 0.13–0.42%).[5] Benefits of HMB supplementation depends on the fitness level of the athlete with untrained athletes probably benefiting more than conditioned athletes.[73] In a 4-week study of athletes training 3 times weekly, the upper body strength increased more ($P = .008$) in the HMB supplementation group (7.5 ± 0.6 kg) compared with the placebo group (5.2 ± 0.6 kg).[84] However, in a double-blind, placebo-controlled study of 35 college football players, there were no statistically significant differences in muscle strength (power cleans, squats, bench press) or lean body mass between the HMB supplementation group (3 g daily for 4 weeks) and the placebo group.[85] Most studies do not demonstrate significant difference in lean body mass before and after HMB supplementation.[81,86]

Intense exercise often causes delayed-onset muscle membrane damage. Short-term supplementation with HMB probably does not reduce the symptoms associated with strenuous exercise. Although some clinical studies suggest that HMB supplementation for 6 weeks decreases muscle damage associated with prolonged exercise as assessed by serum biomarkers (creatine kinase, lactate dehydrogenase), other studies do not demonstrate improvement in measures of exercise-induced muscle damage including delayed-onset muscle soreness, knee extensor range of motion, serum creatine kinase activity, and isometric muscle function.[87] A study of nonresistance-trained individuals demonstrated no benefit from short-term HMB supplementation (40 mg/kg daily for 6 days prior to exercise) on symptoms associated with eccentric muscle damage secondary to a bout of 24 maximal isokinetic eccentric contractions of the elbow flexors, when compared with placebo.[88] Assessment measures included muscle soreness, upper arm girth, and torque measures. Similar negative results occurred in a study of college football players receiving 10 days of HMB supplementation (3 g daily) during preseason training.[89]

CLINICAL RESPONSE

To date, limited data from short-term studies (i.e., up to 8 weeks) have not demonstrated serious adverse effects following the use of HMB. The incidence of side effects associated with HMB supplementation in clinical studies is low. In short-term studies, the administration of 3 g HMB daily for up to 8 weeks does not adversely affect surrogate markers of tissue health and function.[90] The long-term effects of HMB supplementation are unknown.

DIAGNOSTIC TESTING

Analytic Methods

Techniques to detect HMB include high performance liquid chromatography,[91] gas chromatography/mass

spectrometry,[92] and gas chromatography/ammonia chemical ionization/mass spectrometry of the trimethylsilyl derivatives of HMB.[93]

Biomarkers

Normal plasma concentrations of HMB range from about 1–4 μmol/L, but the HMB concentration increases up to 10-fold following leucine supplementation. The kidneys normally excrete small amounts of HMB. Increased urinary concentrations of HMB occur in rare inborn errors of leucine metabolism and in patients with ketoacidosis (e.g., diabetic ketoacidosis).[94,95]

Abnormalities

A study of elite rugby players did not demonstrate any clinical significant abnormalities of health indices in these well-trained athletes when comparing 6 weeks of HMB supplementation and 6 weeks of HMB plus creatine to baseline values.[96] Assessment methods included full blood count, plasma testosterone and cortisol, serum electrolytes, lipids, urea and glucose, sperm count and motility, and evaluation of psychological state. Analysis of clinical studies of HMB supplementation suggests a modest decrease in total cholesterol (about 5%) and LDL cholesterol (about 7%) during use.[90] Short-term supplementation with HMB does not alter the urinary testosterone/epitestosterone ratio. In a study of 6 healthy men, supplementation with 3 g HMB daily for 3 weeks did not cause significant changes in the testosterone/epitestosterone ratio.[97] The baseline ratio was 1.02 ± 0.68 compared with 0.92 ± 0.62 after 2 weeks of daily supplementation with HMB.

TREATMENT

There are few data on the treatment of toxicity associated with HMB use. Adverse effects associated with the use of HMB typically resolve with cessation of use and respond to supportive care.

OTHER SUPPLEMENTS

There are few medical data on the effect of dietary supplements on athletic performance. In a meta-analysis of dietary supplements, only 6 supplements had more than 2 studies that met the criteria for inclusion in the meta-analysis.[5] Most of these supplements (protein, chromium, dehydroepiandrosterone or DHEA, androstenedione) did not significantly increase lean mass or

improve strength. Although chromium has an essential role in maintaining carbohydrate and lipid metabolism (e.g., insulin signalling),[98] most studies on chromium do not demonstrate that chromium picolinate is an effective ergogenic aid including a lack of improvement in body composition,[99,100] lean muscle mass,[101] muscle strength,[102] and exercise capacity.[103] There are few adverse effects associated with the administration of chromium picolinate doses up to 1 mg daily in randomized clinical trials; the use of chromium picolinate at these doses does not alter glucose metabolism in healthy participants.[104,105] However, case reports associate excessive use of chromium picolinate with systemic contact dermatitis (several weeks of diet supplements containing chromium picolinate),[106] rhabdomyolysis (1.2 mg over 2 days for bodybuilding),[107] and hepatorenal dysfunction (1.2–2.4 mg daily for 4–5 months to enhance weight loss).[108] Other trace minerals (e.g., vanadyl sulfate) lack efficacy as ergogenic aids for improving body composition or athletic performance.[109]

The addition of sodium bicarbonate to the body buffers the acidosis associated with anaerobic glycolysis. Theoretically, sodium bicarbonate enhances performance when the exercise is sufficient to cause significant acidosis in the muscles along with adenine nucleotide loss. Although some studies suggest that the ingestion of sodium bicarbonate or citrate (300 mg/kg) up to 1–3 hours before short-term, high-intensity exercise improves performance (e.g., power output),[110,111] there is no clear or consistent pattern of improvement between studies.[112] Adverse GI effects (vomiting, diarrhea) and the need for large volumes to buffer the lactic acidosis limit the usefulness of this supplement for improving athletic performance.[113]

References

1. Grivetti LE, Applegate EA. From Olympia to Atlanta: a cultural-historical perspective on diet and athletic training. J Nutr 1997;127(Suppl 5):860S–868S.
2. World Anti-Doping Agency. Available at http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibited-list/WADA_Prohibited_List_2010_EN.pdf. Accessed 2011 February 20.
3. Geyer H, Parr MK, Koehler K, Mareck U, Schanzer W, Thevis M. Nutritional supplements cross-contaminated and faked with doping substances. J Mass Spectrom 2008;43:892–902.
4. Calfee R, Fadale P. Popular ergogenic drugs and supplements in young athletes. Pediatrics 2006;117:e577–e589.
5. Nissen SL, Sharp RL. Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. J Appl Physiol 2003;94:651–659.

6. Demant TW, Rhodes EC. Effects of creatine supplementation on exercise performance. Sports Med 1999;28:49–60.

7. Myers VC, Fine MS. The metabolism of creatine and creatinine. VII. The fate of creatine when administered to man. J Biol Chem 1915;21:377–383.

8. Chanutin A. The fate of creatine when administered to man. J Biol Chem 1926;67:29–41.

9. Greenhaff PL, Casey A, Short AH, Harris R, Soderlund K, Hultman E. Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. Clin Sci (Lond) 1993;84:565–571.

10. Shao A, Hathcock JN. Risk assessment for creatine monohydrate. Regul Toxicol Pharmacol 2006;45:242–251.

11. Benzi G, Ceci A. Creatine as nutritional supplementation and medicinal product. J Sports Med Phys Fitness 2001;41:1-10.

12. Metzl JD, Small E, Levine SR, Gershel JC. Creatine use among young athletes. Pediatrics 2001;108:421–425.

13. Ray TR, Eck JC, Covington LA, Murphy RB, Williams R, Knudtson J. Use of oral creatine as an ergogenic aid for increased sports performance: perceptions of adolescent athletes. South Med J 2001;94:608–612.

14. Wilson KM, Klein JD, Sesselberg TS, Yussman SM, Markow DB, Green AE, et al. Use of complementary medicine and dietary supplements among U.S. adolescents. J Adolesc Health 2006;38:385–394.

15. Lattavo A, Kopperud A, Rogers PD. Creatine and other supplements. Pediatr Clin N Am 2007;54:735–760.

16. Bemben MG, Lamont HS. Creatine supplementation and exercise performance. Sports Med 2005;35:107–125.

17. Hespel P, Maughan RJ, Greenhaff PL. Dietary supplements for football. J Sports Sci 2006;24:749–761.

18. Persky AM, Brazeau GA, Hochhaus G. Pharmacokinetics of the dietary supplement creatine. Clin Pharmacokinet 2003;42:567–574.

19. Persky AM, Muller M, Derendorf H, Grant M, Brazeau GA, Hochhaus G. Single- and multiple-dose pharmacokinetics of oral creatine. J Clin Pharmacol 2003;43:29–37.

20. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. Physiol Rev 2000;80:1107–1213.

21. Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin Sci (Lond) 1992;83:367–374.

22. Green AL, Simpson EJ, Littlewood JJ, Macdonald IA, Greenhaff PL. Carbohydrate ingestion augments creatine retention during creatine feeding in humans. Acta Physiol Scand 1996;158:195–202.

23. Robinson TM, Sewell DA, Hultman E, Greenhaff PL. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. J Appl Physiol 1999;87:598–604.

24. Brault JJ, Abraham KA, Terjung RL. Muscle creatine uptake and creatine transporter expression in response to creatine supplementation and depletion. J Appl Physiol 2003;94:2173–2180.

25. Persky AM, Hochhaus G, Brazeau GA. Validation of a simple liquid chromatography assay for creatine suitable for pharmacokinetic applications, determination of plasma protein binding and verification of percent labeled claim of various creatine products. J Chromatogr B Analyt Technol Biomed Life Sci 2003;794:157–165.

26. Fitch CD, Shields RP. Creatine metabolism in skeletal muscle. I. Creatine movement across muscle membranes. J Biol Chem 1966;241:3611–3614.

27. Fitch CD, Shields RP, Payne WF, Dacus JM. Creatine metabolism in skeletal muscle. 3. Specificity of the creatine entry process. J Biol Chem 1968;243:2024–2027.

28. Persky AM, Brazeau GA. Clinical Pharmacology of the dietary supplement creatine monohydrate. Pharmacol Rev 2001;53:161–176.

29. Yu PH, Deng Y. Potential cytotoxic effect of chronic administration of creatine, a nutrition supplement to augment athletic performance. Med Hypotheses 2000;54:726–728.

30. Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in men. J Appl Physiol 1996;81:232–237.

31. McNaughton LR, Dalton B, Tarr J. The effects of creatine supplementation on high-intensity exercise performance in elite performers. Eur J Appl Physiol 1998;78:236–240.

32. Kreider RB. Effects of creatine supplementation on performance and training adaptations. Mol Cell Biochem 2003;244:89–94.

33. Sewell DA, Robinson TM, Greenhaff PL. Creatine supplementation does not affect human skeletal muscle glycogen content in the absence of prior exercise. J Appl Physiol 2008;104:508–512.

34. Pan JW, Takahashi K. Cerebral energetic effects of creatine supplementation in humans. Am J Physiol Regul Integr Comp Physiol 2007;292:R1745–R1750.

35. Lyoo IK, Kong SW, Sung SM, Hirashima F, Parow A, Hennen J, et al. Multinuclear magnetic resonance spectroscopy of high-energy phosphate metabolites in human brain following oral supplementation of creatine-monohydrate. Psychiatry Res 2003;123:87–100.

36. Kilduff LP, Pitsiladis YP, Tasker L, Attwood J, Hyslop P, Dailly A, et al. Effects of creatine on body composition and strength gains after 4 weeks of resistance training in previously nonresistance-trained humans. Int J Sport Nutr Exerc Metab 2003;13:504–520.

37. Ahmun RP, Tong RJ, Grimshaw PN. The effects of acute creatine supplementation on multiple sprint cycling and running performance in rugby players. J Strength Condition Res 2005;19:92–97.

38. Jacobs I. Dietary creatine monohydrate supplementation. Can J Appl Physiol 1999;24:503–514.

39. Wilder N, Gilders R, Hagerman F, Deivert RG. The effects of a 10-week, periodized, off-season

resistance-training program and creatine supplementation among collegiate football players. J Strength Cond Res 2002;16:343–352.

40. Bemben MG, Bemben DA, Loftiss DD, Knehans AW. Creatine supplementation during resistance training in college football athletes. Med Sci Sports Exerc 2001;33:1667–1673.

41. Tarnopolsky MA, Parise G, Yardley NJ, Ballantyne CS, Olatinji S, Phillips SM. Creatine-dextrose and protein-dextrose induce similar strength gains during training. Med Sci Sports Exerc 2001;33:2044–2052.

42. Candow DG, Chilibeck PD, Chad KE, Chrusch MJ, Davison KS, Burke DG. Effect of ceasing creatine supplementation while maintaining resistance training in older men. J Aging Physical Activity 2004;12:219–231.

43. Stevenson SW, Dudley GA. Dietary creatine supplementation and muscular adaptation to resistive overload. Med Sci Sports Exerc 2001;33:1304–1310.

44. Walter AA, Smith AE, Herda TJ, Ryan ED, Moon JR, Cramer JT, Stout JR. Effects of creatine loading on electromyographic fatigue threshold in cycle ergometry in college-age men. Int J Sport Nutr Exerc Metab 2008;18:142–151.

45. Tsintzas K, Williams C, Constantin-Teodosiu D, Hultman E, Boobis L, Clarys P, Greenhaff P. Phosphocreatine degradation in type I and type II muscle fibres during submaximal exercise in man: effect of carbohydrate ingestion. J Physiol 2001;537:305–311.

46. Hespel P, Eijnde BO, Derave W, Richter EA. Creatine supplementation: exploring the role of the creatine kinase/phosphocreatine system in human muscle. Can J Appl Physiol 2001;26(Suppl):S79–S102.

47. Escolar DM, Buyse G, Henricson E, Leshner R, Florence J, Mayhew J, et al. CINRG randomized controlled trial of creatine and glutamine in Duchenne muscular dystrophy. Ann Neurol 2005;58:151–155.

48. Tarnopolsky MA, Mahoney DJ, Vajsar J, Rodriguez C, Doherty TJ, Roy BD, Biggar D. Creatine monohydrate enhances strength and body composition in Duchenne muscular dystrophy. Neurology 2004;62:1771–1777.

49. McMorris T, Harris RC, Swain J, Corbett J, Collard K, Dyson RJ, et al. Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. Psychopharmacology (Berl) 2006;185:93–103.

50. Rawson Es, Lieberman HR, Walsh TM, Zuber SM, Harhart JM, Matthews TX. Creatine supplementation does not improve cognitive function in young adults. Physiol Behav 2008;95:130–134.

51. Francaux M, Poortmans JR. Side effects of creatine supplementation in athletes. Int J Sports Physiol Perform 2006;1:311–323.

52. Juhn MS, O'Kane JW, Vinci DM. Oral creatine supplementation in male collegiate athletes: a survey of dosing habits and side effects. J Am Diet Assoc 1999;99:593–595.

53. Vandebuerie F, Vanden Eynde B, Vandenberghe K, Hespel P. Effect of creatine loading on endurance capacity and sprint power in cyclists. Int J Sports Med 1998;19:490–495.

54. Groeneveld GJ, Beijer C, Veldink H, Kalmin S, Wokke JH, van den Berg LH. Few adverse effects of long-term creatine supplementation in a placebo-controlled trial. Int J Sports Med 2005;26:307–313.

55. Volek JS, Ratamess NA, Rubin MR, Gomez AL, French DN, McGuigan MM, et al. The effects of creatine supplementation on muscular performance and body composition responses to short-term resistance training overreaching. Eur J Appl Physiol 2004;91:628–637.

56. Kreider RB, Ferreira M, Wilson M, Grindstaff P, Plisk S, Reinardy J, et al. Effects of creatine supplementation on body composition, strength, and sprint performance. Med Sci Sports Exerc 1998;30:73–82.

57. Poortmans JR, Francaux M. Long-term oral creatine supplementation does not impair renal function in healthy athletes. Med Sci Sports Exerc 1999;31:1108–1110.

58. Mayhew DL, Mayhew JL, Ware JS. Effects of long-term creatine supplementation on liver and kidney functions in American college football players. Int J Sport Nutr Exerc Metab 2002;12:453–460.

59. Schroeder C, Potteiger J, Randall J, Jacobsen D, Magee L, Benedict S, Hulver M. The effects of creatine dietary supplementation on anterior compartment pressure in the lower leg during rest and following exercise. Clin J Sport Med 2001;11:87–95.

60. Robinson SJ. Acute quadriceps compartment syndrome and rhabdomyolysis in a weight lifter using high-dose creatine supplementation. J Am Board Fam Pract 2000;13:134–137.

61. Pline KA, Smith CL. The effect of creatine intake on renal function. Ann Pharmacother 2005;39:1093–1096.

62. Pritchard NR, Kalra PA. Renal dysfunction accompanying oral creatine supplements. Lancet 1998;351(9111):1252–1253.

63. Sheth NP, Sennett B, Berns JS. Rhabdomyolysis and acute renal failure following arthroscopic knee surgery in a college football player taking creatine supplements. Clin Nephrol 2006;65:134–137.

64. Saidi H, Mani M. Severe metabolic acidosis secondary to coadminstration of creatine and metformin, a case report. Am J Emerg Med 2010;28:388.e5–388.e6.

65. Kammer RT. Lone atrial fibrillation associated with creatine monohydrate supplementation. Pharmacotherapy 2005;25:762–764.

66. Derave W, van den Eede E, Hespel P, Carmella SG, Hecht SS. Oral creatine supplementation in humans does not elevate urinary excretion of the carcinogen *N*-nitrososarcosine. Nutrition 2006;22:332-333.

67. Weber JA, van Zanten AP. Interferences in current methods for measurements of creatinine. Clin Chem 1991;37:695–700.

68. Dash AK, Sawhney A. A simple LC method with UV detection for the analysis of creatine and creatinine and its application to several creatine formulations. J Pharm Biomed Anal 2002;29:939–945.

69. Robinson TM, Sewell DA, Casey A, Steenge G, Greenhaff PL. Dietary creatine supplementation does not affect some hematological indices, or indices of muscle damage and hepatic and renal function. Br J Sports Med 2000;34:284–288.

70. Kreider RB, Melton C, Rasmussen CJ, Greenwood M, Lancaster S, Cantler EC, et al. Long-term creatine supplementation does not significantly affect clinical markers of health in athletes. Mol Cell Biochem 2003;244:95–104.

71. Dechent P, Pouwels PJ, Wilken B, Hanefeld F, Frahm J. Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. Am J Physiol 1999;277:R698–R704.

72. Wilkinson ID, Mitchel N, Breivik S, Greenwood P, Griffiths PD, Winter EM, van Beek EJ. Effects of creatine supplementation on cerebral white matter in competitive sportsmen. Clin J Sport Med 2006;16:63–67.

73. Palisin T, Stacy JJ. β-Hydroxy-β-methylbutyrate and its use in athletics. Curr Sports Med Rep 2005;4:220–223.

74. Baier S, Johannsen D, Abumrad N, Rathmacher JA, Nissen S, Flakoll P. Year-long changes in protein metabolism in elderly men and women supplemented with a nutrition cocktail of beta-hydroxy-beta-methylbutyrate (HMB), L-arginine, and L-lysine. JPEN J Parenter Enteral Nutr 2009;33:71–82.

75. Baxter JH, Carlos JL, Thurmond J, Rehani RN, Bultman J, Frost D. Dietary toxicity of calcium β-hydroxy-β-methyl butyrate (CaHMB). Food Chem Toxicol 2005;43:1731–1741.

76. Van Koevering M, Nissen S. Oxidation of leucine and alpha-ketoisocaproate to beta-hydroxy-beta-methylbutyrate in vivo. Am J Physiol 1992;262:E27–E31.

77. Nissen SL, Abumrad NN. Nutritional role of the leucine metabolite β-hydroxy-β-methylbutyrate (HMB). Nutr Biochem 1997;8:300–311.

78. Jowko E, Ostaszewski P, Jank M, Sacharuk J, Zieniewicz A, Wilczak J, Nissen S. Creatine and beta-hydroxy-beta-methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program. Nutrition 2001;17:558–566.

79. Nissen S, Sharp R, Ray M, Rathmacher JA, Rice D, Fuller JC Jr, et al. Effect of leucine metabolite β-hydroxy-β-methylbutyrate on muscle metabolism during resistance-exercise training. J Appl Physiol 1996;81:2095–2104.

80. Thomson JS, Watson PE, Rowlands DS. Effects of nine weeks of β-hydroxy-β-methylbutyrate supplementation on strength and body composition in resistance trained men. J Strength Cond Res 2009;23:827–835.

81. Rowlands DS, Thomson JS. Effects of β-hydroxy-β-methylbutyrate supplementation during resistance training on strength, body composition, and muscle damage

in trained and untrained young men: a meta-analysis. J Strength Cond Res 2009;23:836–846.

82. Vukovich MD, Stubbs NB, Bohlken RM. Body composition in 70-year-old adults responds to dietary beta-hydroxy-beta-methylbutyrate similarly to that of young adults. J Nutr 2001;131:2049–2052.

83. Clark RH, Feleke G, Din M, Yasmin T, Singh G, Khan FA, Rathmacher JA. Nutritional treatment for acquired immunodeficiency virus-associated wasting using beta-hydroxy beta-methylbutyrate, glutamine, and arginine: a randomized, double-blind, placebo-controlled study. JPEN J Parenter Enteral Nutr 2000;24:133–139.

84. Panton LB, Rathmacher JA, Baier S, Nissen S. Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) during resistance training. Nutrition 2000;16:734–739.

85. Ransone J, Neighbors K, Lefavi R, Chromiak J. The effect of beta-hydroxy beta-methylbutyrate on muscular strength and body composition in collegiate football players. J Strength Cond Res 2003;17:34–39.

86. Knitter AE, Panton L, Rathmacher JA, Petersen A, Sharp R. Effects of beta-hydroxy-beta-methylbutyrate on muscle damage after a prolonged run. J Appl Physiol 2000;89:1340–1344.

87. Nunan D, Howatson G, van Someren KA. Exercise-induced muscle damage is not attenuated by β-hydroxy-β-methylbutyrate and α-ketoisocaproic acid supplementation. J Strength Cond Res 2010;24:531–537.

88. Paddon-Jones D, Keech A, Jenkins D. Short-term beta-hydroxy-beta-methylbutyrate supplementation does not reduce symptoms of eccentric muscle damage. Int J Sport Nutr Exerc Metab 2001;11:442–450.

89. Hoffman JR, Cooper J, Wendell M, Im J, Kang J. Effects of beta-hydroxy beta-methylbutyrate on power performance and indices of muscle damage and stress during high-intensity training. J Strength Cond Res 2004;18:747–752.

90. Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC Jr. β-hydroxy-β-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. J Nutr 2000;130:1937–1945.

91. Watanabe T, Oguchi K-I, Ebara S, Fukui T. Measurement of 3-hydroxyisovaleric acid in urine of biotin-deficient infants and mice by HPLC. J Nutr 2005;135:615–618.

92. Nissen S, Van Koevering M, Webb D. Analysis of beta-hydroxy-beta-methyl butyrate in plasma by gas chromatography and mass spectrometry. Anal Biochem 1990;188:17–19.

93. Jakobs C, Sweetman L, Nyhan WL, Packman S. Stable isotope dilution analysis of 3-hydroxyisovaleric acid in amniotic fluid: contribution to the prenatal diagnosis of inherited disorders of leucine catabolism. J Inherit Metab Dis 1984;7:15–20.

94. Landaas S. Increased urinary excretion of 3-hydroxyisovaleric acid in patients with ketoacidosis. Clin Chim Acta 1974;54:39–46.

95. Yu W, Kuhara T, Inoue Y, Masumoto I, Iwasaki R, Morimoto S. increased urinary excretion of β-hydroxyisovaleric acid in ketotic and nonketotic type II diabetes mellitus. Clin Chim Acta 1990;188:161–168.

96. Crowe MJ, O'Connor DM, Lukins JE. The effects of beta-hydroxy-beta-methylbutyrate (HMB) and HMB/creatine supplementation on indices of health in highly trained athletes. Int J Sport Nutr Exerc Metab 2003;13:184–197.

97. Slater GJ, Logan PA, Boston T, Gore CJ, Stenhouse A, Hahn AG. β-Hydroxy β-methylbutyrate (HMB) supplementation does not influence the urinary testosterone: epitestosterone ratio in healthy males. J Sci Med Sport 2000;3:79–83.

98. Vincent JB. The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. Sports Med 2003;33:213–230.

99. Walker LS, Bemben MG, Bemben DA, Knehans AW. Chromium picolinate effects on body composition and muscular performance in wrestlers. Med Sci Sports Exerc 1998;30:1730–1737.

100. Campbell WW, Joseph LJ, Anderson RA, Davey SL, Hinton J, Evans WJ. Effects of resistive training and chromium picolinate on body composition and skeletal muscle size in older women. Int J Sport Nutr Exerc Metab 2002;12:125–135.

101. Hallmark MA, Reynolds TH, DeSouza CA, Dotson CO, Anderson RA, Rogers MA. Effects of chromium and resistive training on muscle strength and body composition. Med Sci Sports Exerc 1996;36:139–144.

102. Livolsi JM, Adams GM, Laguna PL. The effect of chromium picolinate on muscular strength and body composition in women athletes. J Strength Condition Res 2001;15:161–166.

103. Davis JM, Welsh RS, Alderson NA. Effects of carbohydrate and chromium ingestion during intermittent high-intensity exercise to fatigue. Int J Sport Nutr Exerc Metab 2000;10:476–485.

104. Joseph LJ, Farrell PA, Davey SL, Evans WJ, Campbell WW. Effect of resistance training with and without chromium picolinate supplementation on glucose metabolism in older men and women. Metab 1999;48:546–553.

105. Anderson RA, Cheng N, Bryden NA, Polansky MM, Cheng N, Chi J, Feng J. Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. Diabetes 1997;46:1786–1791.

106. Fowler JF Jr. Systemic contact dermatitis caused by oral chromium picolinate. Cutis 2000;65:116.

107. Martin WR, Fuller RE. Suspected chromium picolinate-induced rhabdomyolysis. Pharmacotherapy 1998;18:860–862.

108. Cerulli J, Grabe DW, Gauthier I, Malone M, McGoldrick MD. Chromium picolinate toxicity. Ann Pharmacother 1998;32:428–431.

109. Fawcett JP, Farquhar SJ, Walker RJ, Thou T, Lowe G, Goulding A. The effect of oral vanadyl sulfate on body composition and performance in weight-training athletes. Int J Sport Nutr 1996;6:382–390.

110. Douroudos II, Fatouros IG, Gourgoulis V, Jamurtas AZ, Tsitsios T, Hatzinikolaou A, et al. Dose-related effects of prolonged NaHCO3 ingestion during high-intensity exercise. Med Sci Sports Exerc 2006;38:1746–1753.

111. Bishop D, Edge J, Davis C, Goodman C. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. Med Sci Sports Exerc 2004;36:807–13.

112. Santalla A, Perez M, Montilla M, Vicente L, Davison R, Earnest C, Lucia A. Sodium bicarbonate ingestion does not alter the slow component of oxygen uptake kinetics in professional cyclists. J Sports Sci 2003;21:39–47.

113. Maughan RJ, King DS, Lea T. Dietary supplements. J Sports Sci 2004;22:95–113.

V Ethanol

Chapter 21

ETHANOL

HISTORY

Wine has been part of the human diet and culture for over 6,000 years. According to the Bible (Genesis Chapter IX, Verse 20), Noah established the first vineyard soon after the Ark rested safely on dry land. The oldest chemical evidence for the use of wine is the presence of the calcium salt of tartaric acid on a pottery jar recovered from a mud-brick building in Iran that dates back to 5,400–5,000 BC.[1] Large quantities of this chemical were present only in grapes and in terebinth tree resin used as a bacteriostatic agent for ancient wines. The domestication of the wine grape (*Vitis vinifera* L.) probably occurred in southern Caucasus, and winemaking spread southward to Palestine, Syria, Egypt, and Mesopotamia.[2] By the third millennium BC, wine was used for sacramental purposes in Egypt, but the consumption of wine by the Egyptian population did not occur for about 2,000 more years. The Old Testament contains many references to the physical, psychological, and social consequences of alcohol abuse including descriptions of Noah becoming intoxicated.[3]

IDENTIFYING CHARACTERISTSICS

Ethanol or ethyl alcohol is a relatively simple aliphatic alcohol (i.e., saturated hydroxylated carbon) with the structure of CH_3-CH_2-OH. Ethyl alcohol (C_2H_5OH, CAS RN 64-17-5) is a clear, colorless liquid with a sweet odor having a molecular weight of 46 g/mol and a boiling point of 78.5°C (173.3°F) at atmospheric pressure. The density of ethanol at room temperature (15°C/59°F) is 0.789;

therefore, a 10% v/v solution is equivalent to a 7.89% w/v solution. Ethanol is slightly less polar than water; consequently, ethanol easily crosses cell membranes and diffuses into tissues. The odor threshold of pure ethanol is about 80 ppm in air with the minimum identifiable odor threshold of about 350 ppm.[4]

EXPOSURE

Epidemiology

The medical consequences of acute and chronic ethanol consumption are a ubiquitous part of modern culture as a result of the relationship between ethanol intoxication and a variety of adverse health effects.[5] A study of European mortality rates suggests a positive association between the blood alcohol concentration (BAC) in drivers and fatal traffic accidents in central and southern Europe as well as between ethanol consumption, work-related accidents, and other nontraffic-related accidents in northern Europe.[6] Every year about 40,000 persons in the United States die as a result of motor vehicle collisions. Approximately 17% of the drivers involved in fatal crashes had a BAC exceeding 100 mg/dL, whereas about 6% of these drivers had a BAC between 10–99 mg/dL.[7] The relative risk of involvement in a car crash by a driver under the age of 21 with a positive BAC is greater than predicted from the additive risk of the BAC and age.[8] Overall, the percentage of alcohol-related fatal traffic accidents decreased from 57% in 1982 to 42% in 2000 as defined by anyone involved in the accident having a BAC exceeding 10 mg/dL. Hence,

an alcohol-related accident does not necessarily indicate a causal relationship between ethanol use and the accident. The French population has the highest per capita ethanol consumption in the world. The relative risk of total mortality adjusted for age, smoking habits, and body mass index for men consuming >5 alcohol drinks/day was 1.9 (95% CI: 1.1–3.5) compared with the control group (i.e., men consuming <2 alcohol drinks/day).[9] There was no significant increased risk of premature death in Frenchmen consuming <5 alcohol drinks/day when compared with the control group.

Alcohol dependence is one of the most common psychologic disorders in the United States, affecting about 20% of men and about 8% of women during some part of their lives.[10] In the United States, an estimated 620,000 emergency department visits occur yearly as a result alcohol abuse or dependence.[11] Overall, the mortality rate of chronic alcohol-dependent patients is at least several times higher than the general population.[12] The use of ethanol increases the risk of suicide both in alcohol-dependent and nondependent individuals, particularly with alcohol dependence and completed suicide.[13] The overall lifetime risk of suicide in alcohol-dependent patients is about 2–3%, and the likelihood of suicide is approximately 60–120 times higher in an alcohol-dependent patient than in a patient without psychiatrically definable illness.[14] Thus, alcohol abuse and dependence are contributing factors in about 25% of suicides. Furthermore, acute ethanol use is more common in emergency department patients attempting suicide than in control groups of emergency department patients presenting for illness or injuries (e.g., workplace accidents, domestic animal bites, recreational accidents) traditionally not associated with ethanol use.[15]

Sources

Commercial products containing ethyl alcohol include after-shaves lotions, beverages, colognes, medicinal liquids (antitussives, liniments, rubbing alcohol), mouthwashes, perfumes, and solvents.[16] The production of ethanol beverages involves fermentation when certain yeasts convert carbohydrates and water into ethanol and carbon dioxide. Different types of alcoholic beverages result from different types of fermenting ingredients. Fruits with high sugar contents (e.g., grapes, berries, apricots) are the fermenting ingredients for wine. The production of beer uses starch from cereal grains, whereas winemakers use sugar from grapes. When the ethanol concentration reaches 15% (v/v), most yeast die; therefore, the production of hard liquor (bourbon, gin, vodka, whiskey) requires a distillation process after the fermentation is completed.[17]

PRODUCTS

Ethylene from the cracking of petroleum hydrocarbons is the major source of ethanol for use in commercial products other than alcoholic beverages. The alcoholic beverage industry does not usually use synthetic ethanol for the production of alcoholic beverages as a result of the impurities present in the production of ethanol from ethylene. Commercial uses of ethanol include solvents for resins, fats and oils, synthesis of denatured alcohol, constituent of pharmaceuticals and cosmetics, chemical intermediate, and fuel additives. Ethanol is a constituent of many over-the-counter (OTC) medications and household products including hand sanitizers.[18]

BEVERAGES

Drinking patterns vary between countries as defined by a single beverage type accounting for >50% of the per capita alcohol consumption. Beer-drinking countries include New Zealand, Ireland, Australia, Scotland, England, Canada, Belgium, Denmark, and Czechoslovakia. Prominent wine-drinking countries are Bulgaria, Switzerland, Italy, Spain, France, Portugal, and Greece. Distilled spirits traditionally have been the major source of ethanol consumption in Norway, Sweden, Finland, Poland, Romania, Yugoslavia, and the United States.[19] Overall, the per capita (>15 years of age) alcohol consumption decreased in the United States from 2.76 gallons in 1981 to 2.21 gallons in 1994.[20] However, the decline did not affect ethnic groups equally with the decline being greater in the white population than in African Americans or Hispanics.[21] The natural fermentation of carbohydrates with yeast produces the ethanol present in alcoholic beverages with maximum concentrations of about 14–15%, whereas the production of ethanol concentrations exceeding 14–15% by volume requires distillation. This process involves the evaporation of ethanol from the solution by heating and then collecting the ethanol contained in the distillate by cooling the steam produced.

Alcoholic beverages contain volatile and nonvolatile flavor compounds. Volatile compounds include aliphatic carbonyl compounds, alcohols, monocarboxylic acids and related esters, heterocyclic and aromatic compounds, hydrocarbons, terpenic compounds, and nitrogen- and sulfur-based compounds.[22] The nonvolatile component of alcoholic beverages contains tannic and polyphenolic substances, inorganic salts, dibasic and tribasic carboxylic acids, and unfermented sugars. Most alcoholic beverages contain byproducts of the fermentation or distillation process called congeners (e.g., n-propyl, n-butyl, isoamyl alcohols). Collectively, these

congeners are called fusel oils. Additives and contaminants of alcoholic beverages that are present in minor amounts include sulfur dioxide, cadmium, nitrosamines, urethane, and mycotoxins (e.g., aflatoxins, ochratoxin A, zearalenone).[22] The concentration of ethanol in beverages is usually defined by ethanol volume in volume of solution (i.e., % v/v). Aqueous ethanol is approximately 21% lighter than water (i.e., specific gravity = 0.79) as defined in Equation 21.1. Therefore, an ethanol concentration defined by % v/v must be multiplied by 0.79 (i.e., reduced 21%) to determine an equivalent weight per volume (% w/v). Alternately, the proof of an ethanol concentration is divided by 2 and then multiplied by 0.79 to determine the % w/v.

$$\begin{aligned} \text{Ethanol (w/v)} &= \text{Ethanol (v/v)} \times (0.79) \\ &= \text{Ethanol (Proof/2)} \times (0.79) \end{aligned}$$

(Equation 21.1)

Although the amount of ethanol in a drink varies with glass size and configuration as well as ethanol content, standard US drink equivalents typically are defined as follows: 1) 12-oz beer, 5% v/v; 2) 5-oz glass of wine, 12% v/v; and 3) mixed or straight drink containing 1.5 oz, 40% ethanol (80 proof).[23] Consequently, the standard (US) drink contains about 14 g ethanol.

Beer.   Types of beers include lagers (European pilsner, Oktoberfest, bocks, Australian lagers, American lagers) and ales (amber ale, bitter, mild, pale ale, brown ale, hefeweizen, stout, porter, barley wine). The production of ales involves the action of top fermenting yeast on malted barley, hops, water and yeast at temperatures between 16–21°C (60.8–69.8°F). During the production of lagers, the bottom fermenting yeast produce beer by initial fermentation at 4–16°C (39.2–60.8°F) and then a secondary fermentation at temperatures <4°C (<39.2°F). The exception is steam beer, which is brewed at higher temperatures. Ice beers use the same fermentation process, but the chilling of the beer to near freezing temperatures and the removal of ice crystals produces higher ethanol content. American lagers are typically highly carbonated and are brewed with barley malt and rice, corn, or wheat. Light beers contain fewer calories and slightly lower (i.e., about 15% lower) ethanol content than standard lager beers. The production processes for regular and nonalcoholic beers are similar with the exception that vacuum evaporation at the end of the brewing process removes most of the ethanol in nonalcoholic beer. By legislation, nonalcoholic beers contain <0.5% v/v ethanol; therefore, consumption of unrealistically large quantities of light beer are neces-sary to produce intoxication from the ingestion of nonalcoholic beers. The major flavoring compounds in beer typically include carbonyl compounds (i.e., primarily acetaldehyde), aliphatic ketones (e.g., acetone), alcohols (e.g., glycerol), volatile acids (e.g., acetic acid), lactic acid, esters (e.g., ethyl acetate), amines, phenols (e.g., cresol, 4-vinylphenol), and aromatic acids (e.g., benzoic acid). Formaldehyde, methanol, and oxalic acid are minor constituents of beer.

The ethanol content of beer varies both by type and by brand with the average beer containing about 4–5% ethanol by volume. Analysis of about 50 North American and European beers demonstrate the following median ethanol content for specific types of beer: 1) low-alcohol, 2.3% v/v, 2) light beers, 4.2% v/v, 3) regular beers, 4.7% v/v, and 4) malt liquors/ales, 6.15% v/v.[24] In a study of 113 lagers, the median ethanol concentration was 5.00 % v/v (range, 4.02–15.66% v/v).[25] The median ethanol concentration of 256 ales was 5.25% v/v (range, 2.29–12.69% v/v). Table 21.1 lists the measured ethanol content of samples from American and other beers.[26]

Wine.   Wine contains over 500 compounds in addition to ethanol and water with about one-third of the compounds being esters.[2] The primary ingredients responsible for the taste of wine are aromatic compounds including fusel alcohols, volatile acids, and fatty acid esters. Acetal (1,1-diethoxyethane) is a major constituent of the volatile compounds in wine, whereas beer contains small amounts of this volatile acid.[22] Glycerol and 2,3-butanediol are the principal alcohols in wine. Acetic acid and acetaldehyde are the primary acidic and aldehyde components of the volatile portion of wine, respectively. The principal ester of aliphatic monocarboxylic acids is ethyl acetate with concentrations higher in red wine than in white wine. Phenolic compounds (flavonoids) including phenol and m-cresol are minor constituents of white wine, but these compounds contribute to the taste and quality of red wines. The ethanol content of wines varies from about 8–14% with an average ranging from approximately 10–13%, whereas the ethanol content of fortified wines is somewhat higher (about 15–25%). Japanese sake (rice wine) contains about 10–20% ethanol content with an average of approximately 15% depending on the variety.

Distilled Spirits.   The distillation process alters the concentration of volatile compounds by reducing the concentration of low-boiling and high-boiling compounds. Like wine, acetaldehyde is the major carbonyl compound in distilled spirits. Acrolein is the only unsaturated aldehyde found in unaged whisky distillates. Some spirits (e.g., vodka) contain few flavoring agents

TABLE 21.1. Measured Ethanol Content in American and Other Beers. (Adapted from Reference 26.)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Abbaye De Saint Landelin Blond Abbey Ale	NA	5.98	Ale	Les Brasseurs de Gayant, France
Adelscott Smoked Malt Liquor	8.45	6.27	Malt Liquor	Brasserie et Maltere Du Pecheur, France
Admiralty Ale Eagles Roost Pale Ale	12	5.65	Ale	Aviator Ales, Washington
Admiralty Ale Seafarer Amber Ale	12	5.00	Ale	Aviator Ales, Washington
Admiralty Ale Summer Sojourn Ale	12	5.25	Ale	Aviator Ales, Washington
Alaskan Amber Alt Style Beer	12	5.44	Ale	Alaskan Brewing & Bottling Co., Arkansas
Alaskan Frontier American Amber Ale	12	5.32	Ale	Alaskan Brewing & Bottling Co., Arkansas
Alaskan Pale Golden Ale	12	5.32	Ale	Alaskan Brewing & Bottling Co., Arkansas
Amstel Light Beer	12	3.61	Light	Amstel Brouwerij B. V., Holland
Anchor Liberty Ale	12	5.70	Ale	Anchor Brewing Co., California
Anchor Our Special Ale Christmas 1997	12	5.28	Ale—Seasonal	Anchor Brewing Co., California
Anchor Steam Beer	12	5.09	Lager	Anchor Brewing Co., California
Asahi Draft Beer	12	4.91	Lager	Asahi Breweries Ltd, Japan
Augsburger Golden Beer	12	5.09	Lager	Augsburger Brewing Co., Minnesota
Aviator Harvest Pale Ale	12	4.68	Ale	Aviator Ales, Washington
Aviator India Pale Ale	12	5.12	Bitter	Aviator Ales, Washington
Bacardi Bahama Mama	12	3.20	Malt Beverage	Bacardi & Co. Ltd., California
Bacardi Calypso Berry	12	3.20	Malt Beverage	Bacardi & Co. Ltd., California
Bacardi Key Lime	12	3.04	Malt Beverage	Bacardi & Co. Ltd., California
Bacardi Pina Colada	12	3.04	Malt Beverage	Bacardi & Co. Ltd., California
Bacardi Strawberry Daiquiri	12	3.10	Malt Beverage	Bacardi & Co. Ltd., California
Bachelor Bitter	12	5.06	Bitter	Deschutes Brewery Co., Oregon
Bass Pale Ale	12	5.38	Ale	Bass Brewers Ltd., England
Bateman's Salem Porter	16.9	5.57	Porter	George Bateman and Son, England
Bavarian Schweizerhof-Brau		5.28	Lager	Privatbrauerei Franz Joseph Sailer, Germany
Beartooth Raspberry Lager	12	4.37	Fruit	Beartooth Brewing Co., Minnesota, USA
Beck's	12	5.22	Lager	Braueri Beck & Co., Germany
Beck's Dark	12	5.22	Lager	Braueri Beck & Co., Germany
Belhaven Scottish Ale	16.9	3.70	Ale	Belhaven Brewing Co., Scotland
Belhaven St. Andrews Ale "Scotland"	16.9	4.33	Ale	Belhaven Brewing Co., Scotland
Belle-Vue Framboise	12.67	5.25	Lambic	Brewery Belle-Vue, Belgium
Belle-Vue Gauze	12.67	5.32	Lambic	Brewery Belle-Vue, Belgium
Bell-Vue Kriek	12.67	4.91	Lambic	Brewery Belle-Vue, Belgium
Bert Grant's Amber Ale	12	4.72	Ale	Yakima Brewing & Malting Co., Washington
Bert Grant's Apple Honey Ale	12	4.18	Fruit	Yakima Brewing & Malting Co., Washington
Bert Grant's Celtic Ale	12	2.92	Ale	Yakima Brewing & Malting Co., Washington
Bert Grant's Hefeweizen	12	4.62	Hefeweizen	Yakima Brewing & Malting Co., Washington
Bert Grant's Imperial Stout	12	6.68	Stout	Yakima Brewing & Malting Co., Washington

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Bert Grant's India Pale Ale	12	5.03	Bitter	Yakima Brewing & Malting Co., Washington
Bert Grant's Perfect Porter	12	3.64	Porter	Yakima Brewing & Malting Co., Washington
Bert Grant's Scottish Ale	12	4.68	Ale	Yakima Brewing & Malting Co., Washington
Bert Grant's Winter Ale	12	6.36	Ale—Seasonal	Yakima Brewing & Malting Co., Washington
Big Fog Amber Lager	12	6.84	Lager	The Great Northern Brewing Co., Montana
Big Rock Black Amber Ale	11.5	7.25	Ale	Big Rock Brewery, Canada
Big Rock Buzzard Breath Ale	11.5	5.32	Ale	Big Rock Brewery, Canada
Big Rock Grasshopper Wheat Ale	12	4.94	Hefeweizen	Big Rock Brewery, Canada
Big Rock Magpie ale brewed with rye	11.5	5.51	Ale	Big Rock Brewery, Canada
Big Rock McNally's Ale	11.5	7.79	Ale	Big Rock Brewery, Canada
Big Rock Warthog Ale	11.5	4.81	Ale	Big Rock Brewery, Canada
Bird Creek Anchorage Ale	12	5.19	Ale	Bird Creek Brewery, Arkansas
Bird Creek Denali Style Ale	12	5.25	Ale	Bird Creek Brewery, Arkansas
Bird Creek Iliamna Raspberry Style Wheat	12	4.59	Fruit	Bird Creek Brewery, Arkansas
Bird Creek Old 55	12	4.91	Ale	Bird Creek Brewery, Arkansas
Black Label Carling's	12	4.68	Lager	G. Heileman Brewing Co., Michigan
Blue Moon Abbey Ale	12	5.35	Ale	Blue Moon Brewing Co., Ohio
Blue Moon Belgian White	12	5.32	Ale	Blue Moon Brewing Co., Ohio
Blue Moon Nut Brown Ale	12	5.00	Ale	Blue Moon Brewing Co., Ohio
Blue Moon Raspberry Cream	12	5.63	Fruit	Blue Moon Brewing Co., Ohio
Boddingtons Pub Ale Draught	14.9	5.19	Ale	Strangeways Brewery, England
Bohemia	12	4.68	Lager	Cerveceria Cuauhtemoc Moctezuma, Mexico
Bokrijks Kruikenhier	29.4	7.59	Beer	Br. Sterken's Meer, Belgium
Bos Keun Special Paasbier	12	6.04	Lager—Seasonal	DDB 8160, Belgium
Boulder Amber Ale	12	4.74	Ale	Rockies Brewing Co., Colorado
Boulder Brown Ale	12	4.52	Ale	Rockies Brewing Co., Colorado
Boulder Cliffhanger Ale	12	4.62	Ale	Rockies Brewing Co., Colorado
Boulder ESB extra special bitter	12	5.13	Bitter	Rockies Brewing Co., Colorado
Boulder Extra Pale Ale	12	5.16	Ale	Rockies Brewing Co., Colorado
Boulder Porter	12	5.00	Porter	Rockies Brewing Co., Colorado
Boulder Stout	12	5.92	Stout	Rockies Brewing Co., Colorado
Brasal	11.5	5.63	Lager	Brasserie Brasal Brewing, Canada
Brasal Bock	11.5	6.90	Bock	Brasserie Brasal Brewing, Canada
Bridgeport Black Strap Stout	12	5.60	Stout	Bridgeport Brewing Co., Oregon
Bridgeport Blue Heron Amber Ale	12	5.06	Ale	Bridgeport Brewing Co., Oregon
Bridgeport ESB Extra Special Bitter	12	5.63	Bitter	Bridgeport Brewing Co., Oregon
Bridgeport India Pale Ale	12	5.13	Bitter	Bridgeport Brewing Co., Oregon
Bridgeport Porter	12	5.35	Porter	Bridgeport Brewing Co., Oregon
Budweiser	12	4.76	Lager	Anheuser-Busch Brewing Co., Missouri
Budweiser Ice	12	5.30	Ice	Anheuser-Busch Brewing Co., Missouri

(*Continued*)

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Budweiser Ice Light	12	5.41	Light	Anheuser-Busch Brewing Co., Missouri
Budweiser Light	12	4.15	light	Anheuser-Busch Brewing Co., Missouri
Bull Ice	16	8.16	Ice	The Stroh Brewery Co., Michigan
Burton Ale Double Diamond	12	5.44	Ale	Burton Brewery, England
Busch Beer	12	5.38	Lager	Anheuser-Busch Brewing Co., Missouri
Busch Ice	12	5.82	Ice	Anheuser-Busch Brewing Co., Missouri
Captains City Coupe's Success Cream Ale	22	4.49	Ale	Captains City Brewery Inc., Washington
Carlsberg Elephant	12	6.68	Lager	Carlsberg Bryggerierne, Denmark
Cascade Golden Ale	12	4.05	Ale	Deschutes Brewery, Oregon
Chapeau Exotic Lambic	12.7	3.42	Lambic	Chapeau, Belgium
Chapeau Fraises Lambic	12.7	2.97	Lambic	Chapeau, Belgium
Chapeau Kriek Lambic	12.7	3.29	Lambic	Chapeau, Belgium
Chapeau Peche Iambic	12.7	3.96	Lambic	Chapeau, Belgium
Chung Hua Beer	12	5.28	Lager	Chung Hua Beer Co. LTD, China
Coors Banquet Beer	12	4.87	Lager	Coors Brewing Co., Colorado
Coors Light	12	4.11	Light	Coors Brewing Co., Colorado
Corona Extra Beer	12	4.46	Lager	Cerveceria Modelo, Mexico
Crested Butte Red Lady Ale	12	4.72	Ale	Crested Butte Brewery & Pub, Colorado
Crested Butte White Buffalo Peace Ale	12	4.91	Ale	Crested Butte Brewery & Pub, Colorado
Cutter Non-Alcohol Brew	12	0.47	Nonalcoholic	Coors Brewing Co., Colorado
Deschutes Black Butte Porter	12	5.47	Porter	Deschutes Brewing Co., Oregon
Dos Equis XX Beer	12	4.34	Lager	Cerveceria Moctezuma, Mexico
Dragon Stout	12	7.15	Stout	Desnoes-Goeddes, Jamaica
Dubuque Star Big Muddy Red	12	5.47	Ale	Dubuque Star Brewing Co., Iowa
Dubuque Star River Town Brown	12	5.06	Lager	Dubuque Star Brewing Co., Iowa
Duvel Belgian Ale (Green)	25.4	7.34	Ale	Moortgat Breweries, Belgium
Duvel Belgian Ale (Red)	11.2	8.04	Ale	Moortgat Breweries, Belgium
Ename Dubbel Abbey Ale	11.2	7.31	Ale	Abdij Sint-Salvator Ename Annd, Belgium
Ename Tripel Abbey Ale	11.2	8.48	Ale	Abdij Sint-Salvator Ename Annd, Belgium
Fischer Hoopla Bitter	22	5.03	Bitter	Brasserie et Malterie Du Pecheur, France
Fischer La Belle Strasbourgeoise	22	4.97	Porter	Brasserie et Malterie Du Pecheur, France
Fish Brewing Co. India Pale Ale	12	5.06	Bitter	Fish Brewing Co., Washington
Fish Brewing Co. Mudshark Porter	12	6.08	Porter	Fish Brewing Co., Washington
Foster's Lager	12	4.94	Lager	Carlton and United Breweries, Australia
Foster's Special Bitter	25.4	5.63.	Bitter	Carlton and United Breweries, Australia
Full Sail Amber Ale	12	6.02	Ale	Full Sail Brewing Co., Oregon
Full Sail Equinox ESB extra special bitter	12	5.82	Bitter	Full Sail Brewing Co., Oregon
Full Sail India Pale Ale	12	6.93	Bitter	Full Sail Brewing Co., Oregon

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Full Sail Nut Brown Ale	12	5.50	Ale	Full Sail Brewing Co., Oregon
Full Sail Oktoberfest	12	5.28	Lager—Seasonal	Full Sail Brewing Co., Oregon
Full Sail Very Special Pale Golden Ale	12	5.79	Ale	Full Sail Brewing Co., Oregon
Full Sail Wassail Winter Ale	12	6.99	Ale	Full Sail Brewing Co., Oregon
Fuller's London Pride Imported Pale Ale	16.9	4.52	Ale	Griffin Brewery Fuller's, England
George Killian's Irish Red Ale	12	5.03	Ale	Coors Brewing Co., Colorado
George Killian's Wilde Honey	12	5.57	Fruit	Unived Ltd, Colorado
Golden Gate Original Ale	12	5.25	Ale	Golden Pacific Brewing Co., California
Golden Promise Organically Produced Ale	16.7	5.00	Ale	Caledonian Brewing Co., Scotland
Gordon Biersch Marzen Smooth Auburn Lager	12	5.41	Lager	Gordon Biersch Brewing Co., California
Gordon Biersch Pilsner Crisp Golden Lager	12	5.28	Lager	Gordon Biersch Brewing Co., California
Grimbergen Double Belgian Special Dark Abbey Ale	12	6.08	Ale	N.V. Br Alken-Maes Kontidr, Belgium
Grimbergen Triple Belgian Amber Abbey Ale	12	8.89	Ale	N.V. Br Alken-Maes Kontidr, Belgium
Grolsch Amber Beer	12	4.91	Ale	Grolsch Bierbrouweri, Holland
Grolsch Lager Beer	16	5.13	Lager	Grolsch Bierbrouweri, Holland
Guiness EX Stout	12	6.08	Stout	St. James Gate Brewery, Ireland
Guiness Pub Draught	14.9	4.18	Stout	St. James Gate Brewery, Ireland
Hair of the Dog Adam	12	10.28	Ale	Hair of the Dog Brewing Co., Oregon
Hair of the Dog Golden Rose	12	9.21	Ale	Hair of the Dog Brewing Co., Oregon
Hales Moss Bay Extra	12	3.99	Ale	Hale's Ale Brewing Co., Washington
Hales Pale Ale	12	4.49	Ale	Hale's Ale Brewing Co., Washington
Hamm's	12	4.75	Lager	Pabst Brewing Co., Wisconsin
Harp Imported Lager Beer	12	5.00	Lager	Harp Brewery, Ireland
Haystack Black	12	4.74	Ale	Portland Brewing Co., Oregon
Heineken Lager Beer	12	5.06	Lager	Heineken Brouwerijen, Holland
Henry Weinhard's Amber Ale	12	5.28	Ale	Blitz-Weinhard Brewing Co., Oregon
Henry Weinhard's Porter	12	5.13	Porter	Blitz-Weinhard Brewing Co., Oregon
Henry Weinhard's Private Reserve Beer	12	4.78	Lager	Blitz-Weinhard Brewing Co., Oregon
Henry Weinhard's Private Reserve Dark Beer	12	5.09	Lager	Blitz-Weinhard Brewing Co., Oregon
Henry Weinhard's Private Reserve Light	12	3.92	Light	Blitz-Weinhard Brewing Co., Oregon
Hite	2	4.19	Lager	Chosun Brewery, Korea
Hoegaarden Original White Ale	12	4.94	Ale	Brouwerij De Kluis, Belgium
Humbolt Red Nectar Ale	12	4.87	Ale	Humbolt Brewing Co., California
Hurricane Malt Liquor	40	5.63	Malt Liquor	Anheuser-Busch Brewing Co., Missouri
Icehouse	12	5.25	Ice	Miller & Plank Road Brewing Co., Wisconsin
Icicle Creek Winter Ale	12	5.47	Ale—Seasonal	Portland Brewing Co., Oregon

(*Continued*)

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Jubelale	12	6.46	Ale—Seasonal	Deschutes Brewery, Oregon
Kokanee	11.5	4.91	Lager	Columbia Brewing Co. Labatt, Canada
Labatt Blue	11.5	4.97	Lager	Labatt Breweries, Canada
Lagunitas Dog Town Pale Ale	12	5.28	Ale	Laqunitas Brewing Co., California
LaRossa Moretti Birra	12	6.52	Lager	Birra Moretti, Italy
Latrobe American Pale Ale	12	5.06	Ale	Latrobe Brewing Co., Pennsylvania
Latrobe Bavarian Black	12	4.62	Lager	Latrobe Brewing Co., Pennsylvania
Latrobe Bohemian Pilsner	12	4.68	Lager	Latrobe Brewing Co., Pennsylvania
Liefmans Frambozenbier	12.7	4.65	Fruit	Brouwerij Liefmans, Belgium
Lowenbrau Dark Special Beer	12	4.68	Lager	Miller Brewing Co., Wisconsin
Lowenbrau Special Beer	12	4.75	Lager	Miller Brewing Co., Wisconsin
Lucky	12	4.53	Lager	General Brewing Co., Washington
MacGillivrays Australian Original Two Dogs Lemon Brew	12	4.34	Malt Beverage	Kirin Brewery Co., Japan
Mackeson XXX Stout	12	4.43	Stout	Whitbread PLC, England
MacTarnahan's Scottish Style Amber Ale	12	5.19	Ale	Portland Brewing Co., Oregon
Mad River Jamaica Red Ale	12	6.68	Ale	Mad River Brewing Co., California
Mad River John Barleycorn 7.75–9.25	12	10.41	Barley Wine	Mad River Brewing Co., California
Mad River Steelhead Extra Pale Ale	12	6.67	Ale	Mad River Brewing Co., California
Mad River Steelhead Extra Stout	12	6.65	Stout	Mad River Brewing Co., California
Maes Pils	12	4.87	Lager	Mae Waarloos, Belgium
Maredsous Blonde Abbey Ale	11.2	6.61	Ale	Moortgat Breweries, Belgium
Maritime Pacific Flagship Red Ale	12	5.95	Ale	Maritime Pacific Brewing Co., Washington
Maritime Pacific Islander Pale Ale	12	6.20	Ale	Maritime Pacific Brewing Co., Washington
Maritime Pacific Jolly Roger Christmas Ale 96	NA	8.58	Ale—Seasonal	Maritime Pacific Brewing Co., Washington
Maritime Pacific Jolly Roger Christmas Ale 97	12	9.62	Ale—Seasonal	Maritime Pacific Brewing Co., Washington
Maritime Pacific Nightwatch Dark Ale	12	5.63	Ale	Maritime Pacific Brewing Co., Washington
McMullen's Anniversary Porter	NA	5.00	Porter	Hertfordshire Family Brewers, England
McMullen's Oatmeal Ale	NA	5.03	Ale	Hertfordshire Family Brewers, England
McMullen's Original Bitter	16.7	4.97	Bitter	Hertfordshire Family Brewers, England
Mendocino Black Hawk Stout	12	5.25	Stout	Mendocino Brewing Co., California
Mendocino Red Tail Ale	12	6.17	Ale	Mendocino Brewing Co., California
Michelob	12	5.25	Lager	Anheuser-Busch Inc., Missouri
Michelob Amber Bock	12	4.87	Bock	Anheuser-Busch Inc., Missouri
Michelob Golden Pilsner All Malt Beer	12	4.49	Lager	Anheuser-Busch Inc., Missouri
Michelob Hefeweizen Wheat Beer Premium Brewed	12	4.59	Hefeweizen	Anheuser-Busch Inc., Missouri
Michelob Honey Lager	12	4.24	Lager	Anheuser-Busch Inc., Missouri
Michelob Light Beer	12	4.34	Light	Anheuser-Busch Inc., Missouri
Michelob Pale Ale Dry Hopped	12	4.78	Ale	Anheuser-Busch Inc., Missouri

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Michelob Porter All Malt	12	4.49	Porter	Anheuser-Busch Inc., Missouri
Michelob Winter Brew Spiced Ale	12	5.79	Ale—Seasonal	Anheuser-Busch Inc., Missouri
Mickey's Malt Liquor	12	5.66	Malt Liquor	G. Heileman Brewing Co., Michigan
Miller Genuine Draft Beer	12	4.62	Lager	Miller Brewing Co., Wisconsin
Miller Genuine Draft Light Beer	12	4.08	Light	Miller Brewing Co., Wisconsin
Miller High Life	12	4.59	Lager	Miller Brewing Co., Wisconsin
Miller Lite	12	3.99	Light	Miller Brewing Co., Wisconsin
Milwaukee's Best Beer	12	4.43	Lager	Miller Brewing Co., Wisconsin
Milwaukee's Best Ice	12	5.76	Ice	Miller Brewing Co., Wisconsin
Milwaukee's Best Light Beer	12	4.05	Light	Miller Brewing Co., Wisconsin
Mirror Pond Pale Ale	12	4.87	Ale	Deschutes Brewing Co., Oregon
Mississippi Mud	32	5.16	Porter	Memphis Brewing Co., New York
Molson Canadian Beer	12	5.09	Lager	Molson Breweries of Canada, Canada
Molson Golden Ale	12	5.06	Ale	Molson Breweries of Canada, Canada
Molson Ice	12	5.69	Ice	Molson Breweries of Canada, Canada
Moosehead Canadian Lager Beer	12	4.62	Lager	Moosehead Brewery, Canada
Moretti Birra Friulana	12	4.84	Fruit	Birra Moretti; S.P.A., Italy
Mrs. Pucker's Lemon Malt Beverage	12	4.87	Malt Beverage	Mrs. Puckers, Minnesota
Murphy Irish Stout Pub Draught	16	3.73	Stout	Murphy's Brewing Co., Ireland
Natural Ice Beer	12	5.60	Ice	Anheuser-Busch Brewing Co., Missouri
Natural Light	12	4.15	Light	Anheuser-Busch Brewing Co., Missouri
Negra Modelo	12	5.79	Ale	Cerveceria Modelo S.A., Mexico
Newcastle Brown Ale	12	4.49	Ale	Newcastle Breweries Ltd., England
North Coast Blue Star Wheat Beer	12	4.59	Hefeweizen	North Coast Brewing Co., California
North Coast Old No. 38 Stout	12	5.22	Stout	North Coast Brewing Co., California
North Coast Old Rasputin Russian Imperial Stout	12	8.89	Stout	North Coast Brewing Co., California
North Coast Red Seal Ale	12	5.47	Ale	North Coast Brewing Co., California
North Coast Scrimshaw Pilsner Style Beer	12	4.02	Lager	North Coast Brewing Co., California
North Coast Traditional Bock	22	6.77	Bock	North Coast Brewing Co., California
Northstone Amber Ale	12	4.81	Ale	Miller & Plank Road Brewery, Wisconsin
Norwester Hefeweizen	12	5.12	Hefeweizen	Nor'Wester Brewing Co., Oregon
Obsidian Stout	12	6.30	Stout	Deschutes Brewing Co., Oregon
O'Doul's Non-Alcohol Brew	12	0.20	Nonalcoholic	Anheuser-Busch Brewing Co., Missouri
Old Knucklehead	12	9.02	Barley Wine	Bridgeport Brewing Co., Oregon
Old Milwaukee	12	4.62	Lager	Stroh's Brewing Co., Michigan
Old Peculier	12	5.82	Ale	T&R Theakston Ltd., England

(*Continued*)

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Olde English 800 Ice	12	7.97	Ice	Pabst Brewing Co., Wisconsin
Olde English 800 Malt Liquor	12	7.69	Malt Liquor	Pabst Brewing Co., Wisconsin
Olde English 800 Special Reserve Mixed Fruit	22	6.11	Fruit	Pabst Brewing Co., Wisconsin
Olympia Beer	12	4.65	Lager	Pabst Brewing Co., Wisconsin
One-Eyed Jack Alcohol Lemon Brew	12	5.16	Malt Beverage	The Thickhead Beverage Co., New Jersey
One-Eyed Jack Raspberry Brew	12	5.06	Malt Beverage	The Thickhead Beverage Co., New Jersey
Oregon Honey Beer	12	4.81	Fruit	Portland Brewing Co., Oregon
Oregon Original Hop Harvest Ale	12	5.95	Ale	Oregon Ale and Beer Co., Oregon
Oregon Original India Pale Ale	12	5.89	Bitter	Oregon Ale and Beer Co., Oregon
Oregon Original Nut Brown Ale	12	5.41	Ale	Oregon Ale and Beer Co., Oregon
Oregon Original Raspberry Wheat	12	5.47	Fruit	Oregon Ale and Beer Co., Oregon
Paulaner Hefe-Weizen	16.9	5.54	Hefeweizen	Paulaner Brewing, Germany
Paulaner Oktoberfest Marzen amber	16.9	5.80	Ale	Paulaner Brewing, Germany
Paulaner Premium Pils extra dry	12	4.90	Lager	Paulaner Brewing, Germany
Pete's Wicked Ale	12	4.94	Ale	Pete's Brewing Co., California
Pete's Wicked Amber Ale	12	5.06	Ale	Pete's Brewing Co., California
Pete's Wicked Honey Wheat	12	4.97	Hefeweizen	Pete's Brewing Co., California
Pete's Wicked Mardi Gras	12	5.06	Ale—Seasonal	Pete's Brewing Co., California
Pete's Wicked Summer Brew	12	4.56	Ale—Seasonal	Pete's Brewing Co., California
Pete's Wicked Winter Brew	12	5.32	Ale—Seasonal	Pete's Brewing Co., California
Pike Pale Ale	12	5.38	Ale	The Pike Brewing Co., Washington
Pike Street Stout	12	5.73	Stout	The Pike Brewing Co., Washington
Pilsner Urquell	12	4.56	Lager	Pilsner Urquell, Czechoslovakia
Poorter	25.4	6.61	Porter	Sterkens, Belgium
Portland Summer Pale Ale	12	4.87	Ale—Seasonal	Portland Brewing Co., Oregon
Pyramid Apricot Ale	12	4.91	Fruit	Pyramid Hart Brewing Co., Washington
Pyramid Best Brown	12	5.16	Ale	Pyramid Hart Brewing Co., Washington
Pyramid Hefeweizen	12	5.00	Hefeweizen	Pyramid Hart Brewing Co., Washington
Pyramid Pale Ale	12	4.94	Ale	Pyramid Hart Brewing Co., Washington
Pyramid Porter	12	5.85	Porter	Pyramid Hart Brewing Co.,
Pyramid Scotch Ale	12	6.90	Ale	Pyramid Hart Brewing Co., Washington
Pyramid Snow Cap Ale	12	7.50	Ale—Seasonal	Pyramid Hart Brewing Co., Washington
Pyramid Wheaten Ale	12	4.80	Ale	Pyramid Hart Brewing Co., Washington
Rainier	12	4.91	Lager	Rainier Brewing Co., Washington
Rainier Ale	12	7.47	Ale	Rainier Brewing Co., Washington
Rainier Ice	12	5.30	Ice	Rainier Brewing Co., Washington
Rainier Light	12	4.18	Light	Rainier Brewing Co., Washington
Red Dog	12	4.81	Lager	Miller Brewing Co.

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Red Hook Ballard Bitter IPA	12	7.02	Bitter	Red Hook Ale Brewery, Washington
Red Hook Black Hook Porter	12	5.28	Porter	Red Hook Ale Brewery, Washington
Red Hook Blond Ale	12	5.28	Ale	Red Hook Ale Brewery, Washington
Red Hook Double Black Stout	12	7.34	Stout	Red Hook Ale Brewery, Washington
Red Hook Extra Special Bitter	12	5.60	Bitter	Red Hook Ale Brewery, Washington
Red Hook Winterhook	12	5.70	Ale	Red Hook Ale Brewery, Washington
Red River Valley Honey Brown Ale	12	4.97	Ale	Northern Plains Brewing Co., Minnesota
Red Wolf Red Lager Beer	12	5.28	Lager	Anheuser-Busch Brewing Co., Missouri
Rogue Ale	12	4.24	Ale	Oregon Brewing Co., Oregon
Rogue American Amber Ale	12	4.91	Ale	Oregon Brewing Co., Oregon
Rogue Dead Guy Ale	22	6.65	Ale	Oregon Brewing Co., Oregon
Rogue Golden Ale	22	4.94	Ale	Oregon Brewing Co., Oregon
Rogue Imperial Stout	7	10.22	Stout	Oregon Brewing Co., Oregon
Rogue McRogue Scotch Ale	7	7.09	Ale	Oregon Brewing Co., Oregon
Rogue Oregon Golden Ale	12	4.65	Ale	Oregon Brewing Co., Oregon
Rogue Shakespeare Stout	22	5.09	Stout	Oregon Brewing Co., Oregon
Rogue Younger's Special Bitter	12	4.15	Bitter	Oregon Brewing Co., Oregon
Rogue-XS Old Crustacean	7	10.51	Barley Wine	Oregon Brewing Co.. Oregon
Rogue-XS Smoke Ale	7	5.79	Ale	Oregon Brewing Co., Oregon
Rolling Rock Extra Pale Premium Beer	12	4.65	Ale	Latrobe Brewery, Pennsylvania
Royal Oak Pale Ale	11.5	4.59	Ale	Eldridge Pope & Co. Plc
Samuel Adams Boston Ale	12	4.56	Ale	Boston Beer Brewing Co., Massachusetts
Samuel Adams Boston Lager	12	5.13	Lager	Boston Beer Brewing Co., Massachusetts
Samuel Adams Cranberry Lambic	12	5.35	Lambic	Boston Beer Brewing Co., Massachusetts
Samuel Adams Cream Stout	12	4.78	Stout	Boston Beer Brewing Co., Massachusetts
Samuel Adams Golden Pilsner	12	5.19	Lager	Boston Beer Brewing Co., Massachusetts
Samuel Adams Honey Porter	12	5.32	Porter	Boston Beer Brewing Co., Massachusetts
Samuel Adams Octoberfest	12	5.98	Lager— Seasonal	Boston Beer Brewing Co., Massachusetts
Samuel Adams Scotch Ale	12	5.82	Ale	Boston Beer Brewing Co., Massachusetts
Samuel Adams Triple Bock	12	15.66	Bock	Boston Beer Brewing Co., Massachusetts
Samuel Adams White Ale	12	5.32	Ale	Boston Beer Brewing Co., Massachusetts
Samuel Adams Winter Lager	12	6.77	Seasonal	Boston Beer Brewing Co., Massachusetts
Samuel Smith's India Pale Ale	18.7	5.09	Bitter	The Old Brewery Tadcaster, England
Samuel Smith's Nut Brown Ale	18.7	5.20	Ale	The Old Brewery Tadcaster, England
Samuel Smith's Oatmeal Stout	18.7	5.06	Stout	The Old Brewery Tadcaster, England

(*Continued*)

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Samuel Smith's Winter Ale Welcome	18.7	6.20	Ale—Seasonal	The Old Brewery Tadcaster, England
Sapporo Black Stout Draft	22	5.44	Stout	Sapporo Beer, Japan
Saxer's Jackfrost Winter Dobbelbock	12	7.56	Bock	Saxer Brewing Co., Oregon
Saxer's Lemon Lager	12	3.96	Lager— Seasonal	Saxer Brewing Co., Oregon
Schell Doppel Bock Beer	12	6.36	Bock	August Schell Brewing Co., Minnesota
Schell German Style Pale Ale	12	5.16	Ale	August Schell Brewing Co., Minnesota
Schell Schmaltz's Alt Dark Ale	12	5.47	Ale	August Schell Brewing Co., Minnesota
Schmidt's Ice	12	5.92	Ice	G. Heilman Brewing Co., Michigan
Schmidt's Premium Beer	12	4.56	Lager	G. Heilman Brewing Co., Michigan
Schmidt's Premium Beer Light	12	4.05	Light	G. Heilman Brewing Co., Michigan
Seagrams Fuzzy Navel Flavored Malt Beverage	12	3.80	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Margarita Flavored Malt Cooler	12	3.86	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Peach Daiquiri Flavored Malt Beverage	12	3.80	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Pineapple Pina Colada Flavored Malt Beverage	12	4.30	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Ruby Red Seabreeze flavored malt beverage	12	3.96	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Strawberry Daiquiri flavored malt beverage	12	4.08	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Berries Flavored Cooler	12	3.83	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Black Cherry Flavored Cooler	12	3.92	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Kiwi Strawberry Flavored Cooler	12	4.02	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Mango Flavored Cooler	12	3.80	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Orange Strawberry Passion Flavored Cooler	12	3.83	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Tropical Fruits Flavored Cooler	12	3.64	Malt Beverage	Seagrams Beverage Co., Indiana
Seagrams Wild Watermelon Flavored Cooler	12	3.61	Malt Beverage	Seagrams Beverage Co., Indiana
Sharp's Non-Alcohol Brew	12	0.37	Nonalcoholic	Miller Brewing Co., Wisconsin
Sheaf Stout	25.6	5.79	Stout	Carleton & United, Australia
Sierra Nevada Celebration Ale	12	7.15	Ale	Sierra Nevada Brewing Co., California
Sierra Nevada Pale Ale	12	5.57	Ale	Sierra Nevada Brewing Co., California
Sierra Nevada Porter	12	5.66	Porter	Sierra Nevada Brewing Co., California
Snake Eyes Alcohol Black Cherry Drink	22	4.46	Malt Beverage	Schoeling Brewing Co., Ohio

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Snake Eyes Alcohol Lemon Drink	22	4.37	Malt Beverage	Schoeling Brewing Co., Ohio
Snake Eyes Alcohol Passion Punch Drink	22	4.52	Malt Beverage	Schoeling Brewing Co., Ohio
Spanish Peaks Black Dog Ale	12	5.32	Ale	Black Dog Brewing Co., Wisconsin
Spanish Peaks Black Dog Sweetwater Wheat Ale	12	3.92	Ale	Black Dog Brewing Co., Wisconsin
Spanish Peaks Black Dog Yellowstone Pale Ale	12	5.60	Ale	Black Dog Brewing Co., Wisconsin
Spanish Peaks Honey Raspberry Ale	12	4.59	Fruit	Black Dog Brewing Co., Wisconsin
Spanish Peaks Peaches and Cream	12	4.13	Fruit	Black Dog Brewing Co., Wisconsin
Spanish Peaks Porter	12	4.65	Porter	Black Dog Brewing Co., Wisconsin
St Ides Special Brew Berry	20	6.01	Malt Beverage	St. Ides Brewing Co., Michigan
St. Ides Premium Malt Liquor	20	7.40	Malt Liquor	St. Ides Brewing Co., Michigan
St. Ides Special Brew Mixed Fruit malt Beverage	20	5.76	Malt Beverage	St. Ides Brewing Co., Michigan
3t. Ides Special Brew Passion	20	5.66	Malt Beverage	St. Ides Brewing Co., Michigan
St. Ides Special Brew Pineapple-Coconut Malt Beverage	20	5.89	Malt Beverage	St. Ides Brewing Co., Michigan
St. Pauli Girl Beer	12	5.03	Lager	St. Pauli, Germany
Steel Reserve	40	8.42	Malt Liquor	Steel Brewing Co., Minnesota
Steinlager	11.2	4.91	Lager	Unibev Ltd, New Zealand
Storm	17	9.56	Malt Liquor	Lancaster Brewing Co., England
Stroh's Beer	12	4.81	Lager	Stroh's Brewing Co., Michigan
Sun Devil Alcohol Lemon Drink	12	4.91	Malt Beverage	Sun Devil
Tecate	12	4.52	Lager	Cerveceria Cuahtemoc, Mexico
Thames Festive Ale	17	7.72	Ale—Seasonal	Thames America Trading Co., England
Thames Welsh Ale	17	5.25	Ale	Thames America Trading Co., England
Thames Welsh Bitter Ale	17	4.18	Bitter	Thames America Trading Co., England
Thomas Brau Non-Alcoholic	12	0.47	Nonalcoholic	Paulander Brewing, Germany
Thomas Hardy's Ale	11.2	12.69	Ale	Eldridge Pope & Co., England
Thomas Kemper Amber Lager	12	4.62	Lager	Thomas Kemper Brewing Co., Washington
Thomas Kemper Bohemian Dunkel Lager	12	5.32	Lager	Thomas Kemper Brewing Co., Washington
Thomas Kemper Hefeweizen	12	4.94	Hefeweizen	Thomas Kemper Brewing Co., Washington
Thomas Kemper Honey Weizen	12	4.97	Ale	Thomas Kemper Brewing Co., Washington
Thomas Kemper Weizenberry	12	4.97	Fruit	Thomas Kemper Brewing Co., Washington
Thomas Kemper Winterbrau	12	6.30	Ale—Seasonal	Thomas Kemper Brewing Co., Washington
Three Finger Jack Roasted Red	12	4.97	Lager	Saxer Brewing Co., Oregon
Three Finger Jack Stout	12	4.97	Stout	Saxer Brewing Co., Oregon
Three Finger Jack Summer	12	5.44	Lager—Seasonal	Saxer Brewing Co., Oregon

(*Continued*)

TABLE 21.1. (*Continued*)

Brand	Fluid Ounces	Measured Alcohol (% v/v)*	Classification	Manufacturer
Tri-Motor Amber Ale	12	4.97	Ale	Lang Creek Brewery, Montana
Tsingtao Beer	12	4.62	Lager	Tsingtao Beer, China
Watney's Cream Stout	12	4.34	Stout	Watney Truman LTD, England
Weinhard's Blue Boar Hefeweizen	12	4.91	Hefeweizen	Blitz-Weinhard's Brewery, Oregon
Weinhard's Blue Boar Ice Ale	12	6.01	Ice	Blitz-Weinhard's Brewery, Oregon
Weinhard's Blue Boar Pale Ale	12	4.68	Ale	Blitz-Weinhard's Brewery, Oregon
Weinhard's Blue Boar Red Lager	12	4.97	Lager	Blitz-Weinhard's Brewery, Oregon
Whistler Black Tusk Ale	12	5.57	Ale	Whistler Brewing Co., Canada
Whistler Mother's Pale Ale	12	5.26	Ale	Whistler Brewing Co., Canada
Whistler Premium Lager	12	5.16	Lager	Whistler Brewing Co., Canada
Widmer Amberbier	12	5.70	Ale	Widmer Brother's Brewing Co., Oregon
Widmer Blackbier	12	6.55	Ale	Widmer Brother's Brewing Co., Oregon
Widmer Czech Pilsner	12	5.00	Lager	Widmer Brother's Brewing Co., Oregon
Widmer Hefeweizen	12	4.88	Hefeweizen	Widmer Brother's Brewing Co., Oregon
Widmer Vienna	12	5.19	Ale	Widmer Brother's Brewing Co., Oregon
Widmer Widberry Black Raspberry	12	4.68	Fruit	Widmer Brother's Brewing Co., Oregon
Widmer Winternacht	12	6,58	Ale— Seasonal	Widmer Brother's Brewing Co., Oregon
Yakima Honey Wheat	12	4.94	Hefeweizen	Rainier Brewing Co., Washington
Yakima Red	12	5.47	Ale	Rainier Brewing Co., Washington
Younger's Tartan Special	12	4.37	Ale	Scottish & Newcastle Breweries Plc, Scotland
Young's Oatmeal Stout	18.7	4.87	Stout	The Ram Brewery, England
Young's Old Nick Ale	18.7	6.77	Ale	The Ram Brewery, England
Young's Ram Rod Pale Ale	18.7	4.84	Ale	The Ram Brewery, England
Young's Special London Ale	12	5.76	Ale	The Ram Brewery, England
Zig Zag	12	4.91	Lager	Portland Brewing Co., Oregon
Zima	12	4.97	Malt Beverage	Zima Beverage Co., Tennessee

Abbreviation: NA = not available.

*Approximate ethanol concentration. Actual ethanol concentration may change over time.

and these spirits consist principally of ethanol and water. In contrast, whisky, cognac, brandy, and rum usually contain many volatile flavoring compounds. Methanol is a minor constituent of commercial alcoholic beverages with the exception of some brandies produced from stone fruits (e.g., cherries, plums, apricot) that contain methanol concentrations ranging up to 10 g/L.[22] Small quantities of volatile compounds including acetone, isopropanol, acetaldehyde, *n*-propanol, ethanol, and methanol appear in the blood.[27] Acetic acid, and to a lesser extent decanoic acid, are the major constituents of the acid component in distilled beverages. Distilled spirits typically contain ethanol volumes of 40–50% (80–100 proof).

DOSE EFFECT

Acute

Although the reported lethal ethanol dose is 5–8 g/kg for adults and 3 g/kg for children, acute toxicity varies because of the occurrence of hypoglycemia, drug interactions, and tolerance. The American Academy of Pediatrics arbitrarily established a serum ethanol con-

centration of 25 mg/dL as the maximum safe concentration for a single dose of alcohol-containing medication,[16] but most central nervous system (CNS) effects (e.g., muscular incoordination, behavioral changes, reaction times) occur at serum ethanol concentrations above 100 mg/dL. For example, the ingestion of 40 mL of a 10% ethanol solution produces a serum ethanol concentration of approximately 25 mg/dL in an average-sized 6-year-old child. Chronic alcohol abusers develop a marked tolerance to ethanol, even at blood ethanol concentrations considered potentially fatal to nontolerant individuals (i.e., 500 mg/dL).[28] Ambient ethanol concentrations in air exceeding 20 mg/L are highly irritating with easily recognizable odors that limit exposure to ambient air concentrations of ethanol capable of producing CNS effects.[29]

Chronic

The Royal College of Physicians (UK) recommends a weekly limit of 21 units (168 g) of ethanol for men and 14 units (112 g) for women.[30] One unit equals 8 g ethanol according to the British system. A 360 mL-bottle of beer contains about 1.25 units (10 g), a 750 mL-bottle of wine contains about 7–10 units (55–80 g), and a bottle of hard liquor contains about 30 units (240 g). Consumption of >21 units ethanol weekly does not necessarily produce medical effects, but the ingestion >50 units weekly in men and >35 units in women significantly increases the risk of harm. Social drinkers typically drink <2–3 units of ethanol daily, and these individuals do not become intoxicated. Heavy drinkers regularly drink >6 units ethanol daily without obvious harm. Problem drinkers develop physical, social, family, financial, and occupational problems as a result of their drinking behavior, but evidence of ethanol dependence may be absent. Ethanol dependence occurs in individuals who compulsively drink daily, develop tolerance, and exhibit signs of withdrawal following the cessation of drinking. These individuals continue to drink despite clear evidence of harm.

TOXICOKINETICS

There is substantial variation in the absorption, distribution, and metabolism of ethanol as a result of genetic and environmental factors.[31] Absorption of ethanol following oral ethanol doses occurs primarily in the small intestine, where the rate of absorption depends on a variety of factors including stomach contents, rate of ingestion, composition of the food, rate of gastric emptying, and the timing of food consumption in relation to ethanol ingestion. Ethanol is a small, polar molecule that distributes evenly throughout the body depending on the water content of individual tissues; the volume of distribution is equivalent to total body water. Factors that affect total body water also affect the distribution of ethanol. Elimination of ethanol occurs almost exclusively by metabolism with only about 2–10% of the ethanol dose excreted in the breath, sweat, and urine. Because elimination depends on the hepatic oxidation of ethanol by enzymes (alcohol dehydrogenase, aldehyde dehydrogenase), elimination rates vary considerably depending on the allelic variants of the genes encoding these enzymes.

Absorption

The gastrointestinal (GI) tract is the major route of absorption with about 20% of the absorbed ethanol dose entering the systemic circulation by passive diffusion through the stomach and the remainder through the duodenum and other parts of the small intestine. The potential for ethanol toxicity via the pulmonary and dermal routes is limited by the high water solubility and low lipid solubility of ethanol. Experimental studies indicate that the estimated fraction of ethanol removed from ambient air containing 10–15 mg ethanol/L via inhalation is approximately 55–65%.[29,32] Because of the rapid rate of ethanol metabolism (~7–8 g/h) and the sensory irritation caused by high ambient air concentrations of ethanol, the pulmonary route of ethanol exposure usually produces very low blood ethanol concentrations.[29,33] The low oil-water partition coefficient of ethanol significantly limits the diffusion of ethanol through the keratin layer of intact skin; therefore, dermal absorption is not a significant route of exposure to ethanol.[34]

Physiology of Gastrointestinal Absorption

There is no active transport system for ethanol; consequently, the GI tract absorbs ethanol by passive diffusion along a concentration gradient according to Fick's law. A variety of physiologic factors affect the rate of ethanol absorption from the GI tract including the following: 1) the concentration of ethanol in contact with mucosal surfaces, 2) the time the ethanol remains in contact with the mucous membranes, 3) the amount of blood flow to the area of contact, and 4) the surface area available for ethanol absorption. The absorption of ethanol is slow from the stomach compared with the duodenum. During normal conditions, the stomach absorbs <10–20% of an ethanol dose with a majority of the ethanol passing into the intestines.[29] The small intestine extracts about 80% of an oral ethanol dose because of its large surface area and efficient exchange mechanisms provided by the microvilli. Although the colon is

capable of absorbing ethanol, little absorption occurs in the colon because of the small amount of ethanol remaining in the intestinal tract during transit through the colon.

First-Pass Metabolism

The ingestion of ethanol produces lower blood ethanol concentrations and smaller areas under the concentration-time curves compared with the intravenous (IV) infusion of an equivalent dose of ethanol. The difference between these 2 routes of exposure is the result of first-pass metabolism. Depending on hepatic blood flow, the blood ethanol concentration, and the absorption rate of ethanol, the average amount of ethanol removed during first-pass metabolism is approximately 20%.[35] At low doses (0.15 g/kg) of ethanol, first-pass metabolism occurs primarily in the liver with metabolism in the gastric mucosa accounting for about 2% of the ethanol dose.[36] Delayed gastric emptying increases the first-pass metabolism of ethanol in the stomach, whereas the slower absorption of ethanol into the small intestine enhances hepatic first-pass metabolism.[37] First-pass metabolism is greater in fed individuals with low ethanol doses, intact gastric mucosa, and after chronic ethanol consumption.[38] Fasting and gastrectomy significantly decrease the first-pass metabolism of ethanol. Furthermore, the extent of first-pass metabolism in women is variable and concentration-dependent. In a small study of 6 men and 6 women given a 60-minute IV infusion of 0.3 g ethanol/kg *and* an equimolar dose of deuterium-labeled ethanol/kg orally or intraduodenally, there was limited (i.e., <10%) first-pass metabolism and minor gender-related differences for dilute solutions of ethanol (i.e., 5%).[39] The ethanol content of this solution and beer is similar. However, the ingestion of drinks containing higher ethanol concentrations reduced first-pass metabolism more in women than in men. Baraona et al also demonstrated that first-pass metabolism of oral ethanol was significantly lower in women than in men (35.6±7.4 mg/kg and 72.9±8.9 mg/kg, respectively), when administered as a concentrated ethanol solution (i.e., 40%) similar to whisky.[40] There were no statistically significant gender differences in first-pass metabolism when ethanol was administered as a dilute ethanol solution (i.e., 5%). The smaller amount of first-pass metabolism in the stomach (i.e., reduced gastric alcohol dehydrogenase activity) increases the bioavailability of ethanol in women compared with men.[41]

Determinants of Absorption

Studies of ethanol kinetics in twins indicate that short-term environmental factors account for a large portion of the variance in ethanol kinetics, particularly during the absorptive phase.[42] The rate of increase in the blood ethanol concentration during the absorptive phase depends on the drinking pattern, the type of beverage (beer, wine, hard liquor), gastric contents (fasting vs. fed), type (liquid, solid) and composition (fat, carbohydrate, protein) of food, and changes in GI motility. Factors that delay and reduce peak ethanol concentrations relative to the fasting state include high volumes, high protein diet, trauma, gastric surgery, and reduced bowel motility. Such factors may delay absorption to such an extent that peak blood ethanol concentrations occur several hours after consumption. Additionally, there is substantial variation in peak BAC and the time to reach peak BAC between individuals and in the same individual at different times. In a study of healthy volunteers given low doses (0.3 g/kg) of ethanol, the coefficient of variation (i.e., standard deviation as a percentage of the mean) for intravolunteer and intervolunteer peak BAC during the fed state was 38% and 34%, respectively.[43] The corresponding values of the coefficient of variation for the time to peak BAC were 70% and 52%, respectively. Laboratory studies suggest that heavy smoking may reduce peak ethanol concentrations by delaying gastric emptying. In a study of 8 current cigarette smokers ingesting a 400 mL-radiolabeled nutrient test meal containing 0.5 g ethanol/kg, the mean peak ethanol concentration decreased approximately 20% when comparing smoking (4 cigarettes for first hour) with baseline (no smoking for 1 week).[44] The smoking cessation was associated with an average 50% decrease in gastric emptying.

Food. The presence of food in the stomach before or shortly after ethanol ingestion is the single most important determinate of ethanol absorption.[45] The ingestion of food with ethanol reduces and delays the peak ethanol concentration by prolonging the retention of ethanol in the stomach, where ethanol absorption is much slower than in the duodenum.[46] The consumption of ethanol with a meal reduced the mean blood ethanol concentration about 25–35% compared with the fasting state as well as delaying the mean time to reach the peak BAC by approximately 1.5–2 hours after drinking ceased.[47,48] In 1 study, the peak BAC was approximately 50% higher following a high fat diet than after a high protein diet.[48] High carbohydrate and high sucrose diets produced intermediate BAC. Consumption of food prolongs ethanol absorption past the peak BAC, which defines the time at which the rate of absorption equals the rate of distribution/elimination.[49] Eating during drinking may delay complete absorption for 4–6 hours.

BEVERAGE. Both the dose of ethanol and the type of beverage (e.g., ethanol concentration) impact the shape of the blood ethanol curve. There is substantial variation in blood ethanol concentrations between individuals drinking the same beverage as well as among individuals drinking the same amount of ethanol from different alcoholic beverages. In a study of 6 volunteers fasted at least 2 hours, the consumption of equivalent doses of ethanol as beer and wine produced mean peak blood ethanol concentrations about 20% below peak blood ethanol concentration from an equivalent amount of ethanol as hard liquor.[50] Alcoholic beverages are hyperosmolar; therefore, these beverages delay gastric emptying until the osmolarity of the gastric content approaches isotonicity. Consequently, the time to peak ethanol concentration for the same concentration of ethanol is longer for larger volumes compared with smaller volumes of ethanol. In a study of fasting, healthy adult men, the mean time to peak ethanol concentrations following the ingestion of 0.5 g/kg and 1.0 g/kg was 29.3 minutes (range, 9–114 minutes) and 52.0 minutes (range, 12–166 minutes).[51] In a study of 83 healthy, fasted volunteers given 0.68 g ethanol/kg as neat whiskey, 77% and 97% of the volunteers had peak blood ethanol concentration about 45 minutes and 75 minutes, respectively, after the end of a 30-minute drinking episode.[52] The peak ethanol concentrations ranged from 52–136 mg/dL. Maximum rates of ethanol absorption occurred following the ingestion of beverages containing 10–30% ethanol (v/v). The large volume associated with dilute ethanol solutions limits the contact of ethanol with the intestinal mucosa, and higher concentrations of ethanol probably delay gastric emptying by irritating the gastric mucosa.[53] The consumption of dilute beer (i.e., <1%) does not usually produce blood ethanol concentrations that cause impairment as a result of first-pass metabolism of dilute ethanol solutions and the very large volumes needed to reach high blood ethanol concentrations.[54]

In the fasted state, the ingestion of low doses of concentrated beverages (e.g., whiskey) produces equal or higher blood ethanol concentrations than more diluted ethanol beverages (e.g., beer), whereas the reverse occurs following the consumption of these beverages within 1 hour of a standard meal. In a study of 10 men given 0.3 g ethanol/kg as whiskey or beer 1 hour after a standard meal, the mean peak BAC was approximately 36% higher for the beer-drinking group compared with the whiskey-drinking group (i.e., approximately 31 mg/dL and 23 mg/dL, respectively).[55] The consumption of wine and sherry during the postprandial period yielded intermediate results between beer and whiskey when consumed 1 hour after a standard meal.[56] In a study of 11 fasted Mexican Americans and 11 fasted, non-Hispanic Caucasians, the ingestions of 450 mL of beer delayed gastric emptying more in the Mexican American group than in non-Hispanic Caucasians.[57] However, there was no statistically significant difference in the blood ethanol concentrations. Because of the wide variation of BAC between participants, the use of nomograms based on *mean* kinetic data, gender, and body weight may seriously underestimate or overestimate the BAC.[58]

RATE OF ABSORPTION

The rate of ethanol absorption in the GI tract is a first-order process that depends on the speed of gastric emptying.[59] Factors that delay gastric emptying decrease the rate of ethanol absorption. In healthy, fasting adults receiving single ethanol doses in pharmaceutic studies, about 80–90% of ethanol absorption occurs within 30–60 minutes. However, there is substantial variation between individual participants even when comparing fasting participants.[60] Consequently, accurately estimating the exact time of reaching the peak blood ethanol concentrations at the end of the absorptive phase is difficult because of wide interindividual and intraindividual variation in gastric emptying, portal blood flow, and intestinal transit time. In a study of 24 healthy volunteers administered ethanol in the fed and fasting state, the interindividual variability as measured by the coefficient of variation for the area under the concentration-time curve (AUC) of ethanol was 44% and 21%, respectively.[43] Intraindividual variability for the fed and fasted state as measured by the coefficient of variation for AUC of ethanol was 35% and 22%, respectively.

Ethanol consumption in social settings typically involves divided-dose drinking, and the blood ethanol concentrations during this type of drinking produces slower and stepwise increases in blood ethanol concentrations compared with the ingestion of a single bolus dose in experimental settings. In most social situations without substantial food consumption, the peak blood ethanol concentrations occur within approximately 1 hour after the cessation of drinking,[61] and volunteer studies suggest that the time to peak effect of ethanol occurs earlier than peak BAC. In a study of 40 fasted volunteers, the mean time to peak BAC was 24 minutes earlier than the self-reported peak ethanol effect as measured by a psychological scale on ethanol effects.[62] The menstrual cycle has a minor effect on ethanol pharmacokinetics, including variations in body composition and gastric emptying.[63]

Distribution

Because ethanol is highly water soluble, ethanol distributes into tissues based on the water content. The

ingestion of equivalent doses of ethanol produces higher ethanol concentrations in obese than in lean individuals of the same weight because adipose tissue contains only 10–30% water.[64] The administration of ethanol according to the total body water rather than body weight limits the effect of age and sex on peak blood ethanol concentrations. The highest initial concentrations of ethanol occur in tissues with a large ratio of blood flow to tissue mass (e.g., brain, lungs, kidney, liver). During the absorption and distribution phase, substantial differences (up to 50–100%) occur in ethanol concentrations between the arterial and venous blood.[65] During the absorption phase, the ethanol concentration typically is slightly higher (i.e., about 10–20 mg/dL) in the arterial blood than in venous blood, and the magnitude of the arteriovenous difference depends partly on the rate of ethanol absorption and muscular activity. Rapid absorption exaggerates the difference, whereas slow absorption minimizes the arteriovenous difference. During the postabsorptive phase, the venous BAC slightly exceeds (i.e., 2–10 mg/dL) arterial and capillary BAC, particularly following the ingestion of moderate doses of ethanol.[66] The ethanol concentration in capillary blood more closely resembles the ethanol concentration in arterial blood rather than venous blood.[67]

In a study of fasted volunteers ingesting 0.8 g ethanol/kg, the mean capillary BAC exceeds the venous BAC during the absorptive phase, but the capillary-venous difference decreased when ethanol absorption slowed during the later part of the absorptive phase.[68] The capillary-venous difference 30 minutes and 60 minutes after the start of drinking was 13.6 ± 7.8 mg/dL and 3.3 ± 6.9 mg/dL, respectively. Between 90–360 minutes after drinking started (i.e., postabsorptive phase), the venous BAC exceeded the capillary BAC by a mean of 5.8 ± 3.4 mg/dL. During the IV infusion of 0.4 g ethanol/kg to 13 healthy men over 30 minutes, arterial ethanol concentrations exceeded the venous ethanol concentration by about 10 mg/dL with mean values of 94.8 ± 2.06 mg/dL and 84.7 ± 1.54 mg/dL, respectively.[69] The blood ethanol concentrations decreased rapidly after the infusion ceased with a mean apparent distribution half-life of 7–8 minutes. The arteriovenous ethanol difference was small (1–2 mg/dL) by 5–10 minutes after the infusion stopped.

Volume of Distribution

Ethanol distributes into tissue in direct proportion to the water content, and thus the V_D of ethanol reflects total body water. The V_D of ethanol varies between individuals based on obesity, sex, and age.[70] Adipose tissue contains less water than lean body tissue, and therefore individuals with higher fat content contain relatively less total body water than lean individuals. For example, women have a slightly smaller V_D because they have less water and more fat than men with of an equivalent body weight. The consumption of 45 g of ethanol daily for 3 weeks increased the V_D in men from 0.73 to 0.88 L/kg, but this pattern of ethanol consumption did not affect the mean V_D (0.7 L/kg) in women.[71] In a study of 6 men and 4 women, the average V_D for the women and men was 0.63 (range, 0.54–0.71) and 0.69 (range, 0.63–0.76), respectively.[72] Consequently, women usually attain higher BAC compared with men given the same dose of ethanol and the same body weight, although the time to peak BAC is similar for both sexes.[73] Experimental studies indicate that the anthropometric equation for calculating total body water and the body mass index are more reliable methods for calculating peak BAC than body weight alone.[74,75] In a study of ethanol kinetics in men and women, the mean body water as a percentage of body weight was $65 \pm 2\%$ and $51 \pm 2\%$, respectively.[76] Higher peak BAC concentrations occur in the elderly compared with younger individuals given the same dose of ethanol because of the lower lean body mass and the decreased volume of distribution that accompanies the aging process.[77] The volume of distribution for ethanol is smaller if the concentration-time profile is based on the plasma or serum ethanol concentration rather than whole blood.[78]

Protein Binding

Ethanol does not bind to plasma proteins or tissue, and as a result, the volume of distribution correlates closely to the total amount of water in the body.[78] Ethanol also does not interfere with the binding of other compounds to plasma proteins.

Biotransformation

The main metabolic pathways for ethanol in humans involve alcohol dehydrogenase and the microsomal-ethanol oxidizing system. Although early studies suggested that ethanol oxidation occurs with catalase, the lack of hydrogen peroxide necessary for this reaction limits the use of this pathway for ethanol oxidation *in vivo*. The most important pathway in the biotransformation of ethanol is catalyzed by alcohol dehydrogenase. The end products of ethanol oxidation by this pathway are water and carbon dioxide. In the human brain and other tissues, fatty acid ethyl esters are nonoxidative metabolites of ethanol.[79]

ALCOHOL DEHYDROGENASE (ADH)

The main route of ethanol elimination is hepatic oxidation by alcohol dehydrogenase (ADH) with the cofactor nicotinamide adenine dinucleotide (NAD^+) acting as a hydrogen acceptor during the oxidation of ethanol to acetaldehyde. ADH resides in the cytosol (soluble cell fraction) of hepatocytes, and this pathway becomes saturated at relatively low concentrations of ethanol. There is significant polymorphism of ethanol-metabolizing enzymes including ADH, aldehyde dehydrogenase (ALDH), and CYP2E1. ADH is a dimeric molecule controlled by at least 7 human ADH genes and subunits as defined in Table 21.2. This variability produces gene products with different K_m values. For example, the K_m of ADH1 and ADH2 are 30 µM and 3 µM, respectively.[80] The Michaelis constant (K_m) is the concentration at which the rate of the enzyme reaction is half the enzyme's maximum rate (V_{max}); K_m is an approximation of the affinity of the enzyme for the substrate. Pyrazole, 4-methylpyrazole, other pyrazole derivatives, and monosubstituted formamide compounds inhibit ADH1 and ADH2 isoenzymes.[81,82] The latter selective inhibitors include N-benzylformamide (ADH1B*1), N-1-methylheptylformamide (ADH1C*2), and N-heptylformamide (ADH1B*1, ADH4). There are also several molecular forms of aldehyde dehydrogenase (ALDH) involved with the oxidation of acetaldehyde. ALDH1 is the cytosolic enzyme with limited catalytic efficiency that is distributed throughout the body including the brain. ALDH2 is the mitochondrial enzyme present in the liver and stomach. This enzyme possesses high catalytic efficiency (low K_m) for acetaldehyde oxidation, and ALDH2 is the primary enzyme responsible for the *in vivo* oxidation of acetaldehyde. Asian populations with a deficient ALDH2 phenotype demonstrate characteristic ethanol sensitivity reactions (elevated heart rate, intense facial flushing, erythema, diaphoresis) as a result of acetaldehyde accumulation.[83]

Conversion of ethanol to acetaldehyde by alcohol dehydrogenase involves the re-oxidation of NADH to NAD^+; this reaction is the rate-limiting step of ethanol metabolism. Both alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) require NAD^+, which reduces the hepatic NAD/NADH ratio during ethanol oxidation. The shift in this redox state of the liver to a more reduced potential causes profound metabolic abnormalities in heavy drinkers and alcohol abusers. The final step of this pathway is conversion of acetate to acetyl CoA and then to CO_2 and H_2O via the Krebs cycle. Inhibitors of ALDH include prunetin (5,4′-dihydroxy-7-methoxyisoflavone, CAS RN: 552-59-0) and daidzin (CAS RN: 552-66-9). The latter compound is the major active principle in extracts of *radix puerariae*, a traditional Chinese medication that suppresses the ethanol intake of Syrian golden hamsters and selectively inhibits ALDH2.[84]

MICROSOMAL ETHANOL-OXIDIZING SYSTEM (MEOS)

The microsomal ethanol-oxidizing system (MEOS) is located in the smooth endoplasmic reticulum, and this pathway requires oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The major cytochrome P450 enzyme involved in this reaction is CYP2E1, which has a K_m of 12–16 mM (55–75 mg/dL).[80] This microsomal enzyme is an important part of the metabolism of ethanol when large doses (>100 g daily) are ingested over long periods.[85] During the oxidation of ethanol via this inducible pathway, a significant loss of energy occurs along with an increased formation of acetaldehyde. Chronic ethanol consumption induces the

TABLE 21.2. Nomenclature for Alcohol Dehydrogenase Genes and Subunits.[31]

ADH Class	Nomenclature	Former Name	Subunit Name	Efficiency[†]
I	ADH1A	ADH1	α	Low
	ADH1B*1	ADH2*1	β_1	High
	ADH1B*2	ADH2*2	β_2	High
	ADH1B*3	ADH2*3	β_3	Low
	ADH1C*1	ADH3*1	γ_1	High
	ADH1C*2	ADH3*2	γ_2	High
II	ADH2	ADH4	π	Low
III	ADH3	ADH5	χ	Very Low
IV	ADH4	ADH7	σ	High
V	ADH5	ADH6	NA	NA

Abbreviation: NA = not applicable.
†Ethanol catalytic efficiency.

hepatic cytochrome CYP2E1 up to 4–10 times depending on the ethanol dose and duration of drinking.[86] Immunoblot analysis of human liver microsomes indicate that the CYP2E1 content is approximately 4-fold higher in currently-drinking alcohol abusers than in nondrinkers.[87] The induction of CYP2E1 occurs relatively quickly in moderate drinkers. In a volunteer study using the chlorzoxazone test to measure CYP2E1 induction during the daily ingestion of 40 g ethanol (i.e., about 3 standard drinks), CYP2E1 activity increased within 1 week and continued to increase over the 4-week study.[88] After cessation of drinking, CYP2E activity decreased within 3 days and returned to baseline value within approximately 1 week. MEOS includes other cytochrome P450 enzymes, primarily CYP1A2 ($K_m = 24$ mM) and CYP3A4.[89] The MEOS pathway becomes more important as the blood ethanol concentration rises[90] because the velocity constant (K_m) is 4–5 times higher for the MEOS than for alcohol dehydrogenase. Inhibitors of CYP2E1 include pyrazole compounds, diallyl sulfide, and chlormethiazole.

CATALASE

In the peroxisomes, catalase oxidizes ethanol in the presence of a hydrogen peroxide-generating system.[91] However, the peroxidase-catalase system is a minor pathway under physiologic conditions *in vivo* because of the lack of availability of hydrogen peroxide.[92]

Elimination

Metabolic pathways located primarily in the liver account for about 90–95% of the overall elimination of ethanol. Most of the remainder of an ethanol dose is excreted unchanged into the urine, breath, saliva, and sweat. The urinary excretion of unchanged ethanol is relatively small compared with biotransformation, and the portion of unchanged ethanol in the urine accounts for about 0.5–2.5% of the absorbed ethanol dose.[93] The kidney excretes a small fraction (i.e., 0.02%) of the ethanol dose as ethyl β-glucuronide as a result of the conjugation of ethanol with uridine 5′-diphospho-glucuronic acid in the presence of uridine 5′-diphospho-glucuronosyltransferase.[94] Formation of minor amounts of ethyl sulfate also occurs after ingestion of ethanol as a result of the activity of sulfotransferases. Substantial interindividual variations in the formation of these 2 compounds result from considerable polymorphisms in the genes coding uridine 5′-diphospho-glucuronosyl-transferases (UGT1A1, UGT2B7) and sulfotransfer-ases.[95] In a study of 13 healthy volunteers receiving moderate doses of ethanol (mean, 0.51 ± 0.17 g/kg), the mean times from ingestion to peak ethyl glucuronide

and ethyl sulfate concentrations in urine samples were 2.3 ± 0.9 hours and 1.2 ± 0.5 hours, respectively.[96] Eleven hours after the start of drinking, 11 of 13 volunteers still had detectable quantities of ethyl glucuronide (lower limit of quantitation [LLOQ], 0.45 µmol/L) in their urine compared with 2 of 13 volunteers for ethyl sulfate (LLOQ, 0.79 µmol/L).

WIDMARK EQUATION

In 1932, Widmark developed a mathematical equation for the elimination of ethanol from the bloodstream based on Lord Mellanby's observation that the rate of ethanol oxidation was constant during ethanol metabolism.[97] Figure 21.1 displays the graphical representation of the Widmark curve as defined by the Widmark equation. The basis of this equation is a 1-compartment model with zero-order kinetics characterized by a constant rate of ethanol elimination per unit time. Equation 21.2 describes the Widmark equation

$$A = pr(C_t + \beta_t) \qquad \text{(Equation 21.2)}$$

where A = absorbed dose of ethanol (g); p = body weight (kg); r = Widmark factor that represents the apparent volume of distribution as a fraction of body weight (kg/kg); C_t = concentration of ethanol in blood at time t (mg/g blood); β = slope of apparent linear decline of the concentration-time profile of ethanol [mg ethanol/(g blood × minutes)]; and t = time after oral dosing (minutes).
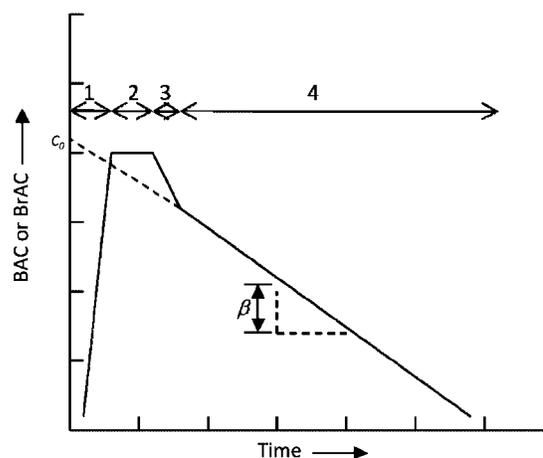
FIGURE 21.1. Theoretical blood ethanol curve based on Widmark equation. BAC = blood ethanol concentrations; BrAC = breath ethanol concentration; 1 = absorption phase; 2 = plateau; 3 = diffusion-equilibration; 4 = elimination (post-absorptive) phase with slope of β.

Widmark calculated the β value from the relatively linear slope of the blood ethanol decay curve between the end of the absorption/distribution phase and the curvilinear portion near the end of the blood ethanol curve. The factor β is the rate of decline of blood ethanol concentration during the postabsorptive phase of the blood ethanol curve that occurs about 1–3 hours after ingestion. The average value for β was 16 mg/dL/h with considerable variation between individuals and a slightly increased slope for women.[98] Widmark defined the *r* value as the ratio of total body ethanol and the blood ethanol concentration. Because of the close relationship between ethanol concentration and the water content in tissues throughout the body, the Widmark factor *r* corresponds to the ratio of total body water and the water content of blood. To calculate the extrapolated, theoretical maximum concentration of alcohol in blood C_0. Equation 21.3 is rearranged as follows:

$$C_0 = \text{Absorbed dose of ethanol (g)}/$$
$$\text{reduced body mass (kg)}$$
$$= A/(p \times r)$$

$$\text{(Equation 21.3)}$$

This equation assumes instantaneous and complete absorption (e.g., during rapid infusion) and distribution without elimination.[99] The use of this equation often overestimates the maximum blood ethanol concentration because of the reduction in bioavailability of ethanol after ingestion by several factors (e.g., ingestion of food, low ethanol-containing beverages). In addition, the use of the traditional average *r* values of 0.7 for men and 0.6 for women tends to overestimate C_0 if calculated according to Equation 21.3. In German-speaking countries, a 10–30% minimum resorption deficit is used to adjust C_0 for this apparent loss of ethanol.[100] The use of total body water calculations improves the accuracy of the Widmark equation when applied to a specific individual because the blood water content is relatively constant except in certain conditions (e.g., anemia, polycythemia, pregnancy).

To calculate the *r* value, Widmark estimated the blood ethanol concentration at time zero (C_0) by using the β slope to draw a straight line through C_t to give C_0, the intercept on the y-axis at time zero. Equation 21.4 defines the calculation of the distribution volume of ethanol corresponding to the Widmark *r* factor.

$$r = A/pC_0 \qquad \text{(Equation 21.4)}$$

The Widmark (distribution) factor was a dimensionless ratio used to correct the body weight for total body water based on drinking experiments with 20 fasting male and 10 fasting female volunteers, who ingested cognac brady over a short drinking time.[97] There was considerable variation in the *r* factor between individuals with a mean value of 0.68 (range, 0.51–0.85) and 0.55 (range, 0.47–0.64) for men and women, respectively. In an experimental study using direct measurements of total body water, the mean distribution factor *r* was 0.75 ± 0.05 (range, 0.60–0.82) for men and 0.66 ± 0.05 (range, 0.52–0.72) for women.[101] These fasting participants did not consume food for at least 2 hours prior to the consumption of beer or wine over a 2.5-hour drinking period. The uncertainty in the calculation of total body water is about ±10–20%, when using a V_D of 0.7 and 0.6 for men and women, respectively. Equation 21.5 estimates the blood ethanol concentration at time *t* during the postabsorptive phase, assuming complete absorption and distribution of the ethanol dose,

$$C_t = C_0 - \beta_t \qquad \text{(Equation 21.5)}$$

where C_0 = extrapolated, theoretical maximum blood ethanol concentration at the start of drinking, Ct = blood ethanol concentration at time *t* during the postabsorptive phase, β = elimination rate, and *t* = time during postabsorptive phase.

POSTABSORPTIVE ELIMINATION

MODEL. The hepatic elimination of ethanol from the body occurs primarily by capacity-limited (i.e., concentration independent) metabolic processes except at very low or very high concentrations in blood where the elimination rate is more concentration-dependent.[102] A zero-order elimination operates during a large portion of the blood-concentration time profile as a result of the saturation of ADH after the absorption of a few drinks. Overall, elimination of ethanol resembles a 1-compartment; the Michaelis-Menten kinetic model describes the concentration-dependent kinetics at very high and very low ethanol concentrations in blood, whereas concentration-independent (zero-order) kinetics best describe the BAC over the middle of the blood ethanol curve. Suggested values for the Michaelis-Menten parameters include an average K_m (Michaelis constant) of 9–11 mg/dL and a V_m (maximal ethanol rate velocity) of 22 mg/dL serum water/hour at ethanol levels <300 mg/dL.[103] Equation 21.6 defines Michaelis-Menten kinetics where C equals the concentration of blood ethanol.

$$-dC/dt = V_m C/(K_m + C) \qquad \text{(Equation 21.6)}$$

Consequently, when the blood ethanol concentration is large compared with K_m (11 mg/dL), the rate of ethanol

elimination is equal to V_m. During the postabsorptive phase of the BAC curve, ethanol elimination resembles zero-order kinetics; however, the elimination rate is not entirely linear, particularly during the immediate postabsorptive phase.[104] Assuming K_m = 9.5 mg/dL and V_m = 22.8 mg/kL/h, the estimated ethanol elimination rate at BAC of 50 mg/dL and 300 mg/dL is about 19.1 mg/dL/h and 22.1 mg/dL/h, respectively. The estimated ethanol elimination rate is substantially less at lower BAC (e.g., 11.7 mg/dL/h at BAC = 10 mg/dL). Alternate routes of elimination of ethanol (e.g., breath, urine) contribute relatively more to overall ethanol elimination when the BAC is very high (>300 mg/dL).

RATE.   Ethanol elimination occurs almost exclusively by hepatic metabolism with unchanged parent drug excreted in the urine, breath, and sweat accounting for about 1–10% of the ethanol dose.[35,77,105] The rate-limiting step during ethanol oxidation is the conversion of ethanol to acetaldehyde by alcohol dehydrogenase in the cytosol. There is substantial polymorphism of this enzyme, leading to racial and ethnic variations in ethanol pharmacokinetics. Although individual variation has the greatest impact on ethanol elimination rate, other factors (chronic ethanol consumption,[106] initial ethanol concentration and point on blood-alcohol curve,[107] gender, day-to-day variation[108]) also alter the rate of ethanol disappearance from the blood.[17,69] The ingestion of ethanol immediately after a meal increases the mean ethanol elimination rate slightly, but the rate of increase is somewhat variable. Volunteer studies using oral doses of ethanol suggest that the effect of food on the ethanol elimination rate is small (1–3 mg/dL/h), but these oral studies do not separate the effect of food on absorption and elimination.[80] The increase in the ethanol elimination rate following food consumption is slightly greater (i.e., up to about 1–4 mg/dL/h) in breath alcohol clamping studies than in oral studies.[109] Age alone does not alter the elimination rate of ethanol significantly.[77] Neither does the administration of IV fluids increase the rate of ethanol elimination. A study of 10 volunteers administered 0.81 g ethanol/kg did not detect a statistically significant difference in elimination rates with and without the rapid infusion of 1 L normal saline (0.9% NaCL).[110] During experimental studies producing peak BAC of 120 mg/dL in blood, the rate of ethanol clearance rate was approximately 10% faster in plasma compared with whole blood.[111] The blood flow to the liver represents about 20–25% of the cardiac output, and factors (e.g., trauma) that reduce hepatic blood flow can theoretically reduce the clearance rate of ethanol from the bloodstream.

Dose Response.   There are limited data on the dose-response curve for ethanol metabolism following large doses of ethanol. However, the ethanol elimination rate usually increases when the blood ethanol concentration exceeds 200–300 mg/dL. In a study of 14 nonrandomly selected alcoholic patients presenting with ethanol intoxication (C_{max} = 336 ± 114 mg/dL), the mean elimination rate was 21.9 ± 7.2 mg/dL/h with a range of 6–36 mg/dL/h.[112] The chronic ethanol use by these patients was not reported; therefore, this elimination rate may not reflect the ethanol elimination rate for nontolerant individuals. Two days later, these 14 patients ingested a test dose of 0.5 g ethanol/kg that produced a C_{max} = 42 ± 11 mg/dL. The mean elimination rate was 33% lower (14.6 ± 4.8 mg/dL/h) than the ethanol elimination rate during the acute intoxication phase. The mean serum elimination rate of 103 patients presenting to the emergency department with acute ethanol intoxication (mean serum ethanol concentration, 299 mg/dL) was 20.43 ± 6.86 mg/dL/h with a range of approximately 10–34 mg/dL/h.[113] Similarly, the chronic ethanol use of these patients was not reported. Case reports document an ethanol half-life of approximately 4 hours at blood ethanol concentrations exceeding 700 mg/dL, which suggest first-order kinetics at such high blood concentrations.[114,115] The BAC declines at a near-constant rate near the lower end of the blood ethanol curve until the BAC reaches about 3 mM (10–15 mg/dL); thereafter, first-order kinetics is a better description of the concentration-time profile.[116]

General Population.   The average rate of ethanol elimination following ingestion of typical doses of ethanol by moderate drinkers is approximately 15–16 mg/dL/h with a range of 8–25 mg/dL/h.[117,118] Blood ethanol concentrations decrease more rapidly (e.g., 23–43 mg/dL/h) at concentrations above 200–300 mg/dL compared with the elimination rate at blood concentrations below this concentration, perhaps because of induction of the microsomal ethanol-oxidizing system.[119,120] Children have higher metabolic rates than adults as a result of larger liver weight/kg body weight and potentially faster rates of ethanol elimination. Although there is a lack of controlled clinical trials in children for ethical reasons, case series document ethanol elimination rates during ethanol intoxication ranging up to 28 mg/dL/h.[121] Several studies suggest that the ethanol elimination rate is slightly faster in women compared with men. In studies involving apprehended drunken drivers and volunteers, the mean ethanol elimination rate in women averaged about 12–18% higher than the elimination rate in men for BAC in the range of 100–200 mg/dL.[122,123] Experimental studies indicate that the time during the

menstrual cycle does not affect the ethanol elimination rate in women.[124]

Chronic Heavy Ethanol Use. Chronic ethanol consumption generally increases the elimination rate of ethanol from blood compared with the general population, primarily as a result of the induction of CYP2E1 from the microsomal ethanol oxidizing system. The change in ethanol elimination rate for alcohol abusers typically involves an increase of <60–100%, but the increase is variable from individual to individual with the ethanol elimination rate exceeding 30–40 mg/dL/h in a few individuals. In a series of 81 chronic alcohol abusers arrested while driving with a BAC >400 mg/dL, the mean clearance rate of ethanol from whole blood was 23 ± 0.01 mg/dL/h with a range of 13–61 mg/dL/h.[125] Three consecutive hourly blood samples were drawn from a convenience sample of 21 drivers arrested with very high blood ethanol concentrations (mean, 405 mg/dL; range, 271–518 mg/dL).[126] The mean blood ethanol elimination rate for this group was 33 mg/dL/h (range, 20–62 mg/dL/h). The mean ethanol elimination rate was about 23 mg/dL/h (range, approximately 20–29 mg/dL/h) in a case series of 8 alcohol abusers with serum ethanol concentrations ranging from 200–400 mg/dL.[85] In the detoxification ward, the ethanol elimination rate was remeasured at serum ethanol concentrations of approximately 50 mg/dL. At this concentration, the mean elimination rate decreased on average by 49% to give a mean of 16 mg/dL/h. Substantial increases in the ethanol elimination rate occur following chronic ethanol use over more than several weeks. After the administration of 45 g ethanol daily for 3 weeks to volunteers without a history of alcohol abuse, the plasma ethanol elimination rate did not change significantly compared with control values before the beginning of the study.[71]

Maternal and Fetal Kinetics

Because ethanol distributes into total body water, ethanol diffuses into the placenta and breast milk.[127] The average water content of breast milk and blood are 87.5% and 85%, respectively. Although ethanol rapidly distributes into breast milk, the concentration in breast milk is close to the mother's BAC; further dilution into the total body water of the neonate results in relative low ethanol concentrations compared with the mother. The concentration of ethanol in a breastfeeding child depends on the mother's BAC, the volume of breast milk consumed, and the drinking time. Only a small fraction of the maternal blood ethanol concentration can be measured in the blood of the neonate; however, the rate of ethanol elimination in the neonate is sub-

stantially less than later in life.[128] Nomograms are available to determine the time between consumption of alcohol and nondetectable ethanol concentrations in breast milk.[129] The risks associated with drinking ethanol while breastfeeding are not well defined. Potential adverse effects include altered sleep patterns, decreased milk intake, hypoglycemia, and slowed motor development. Limited clinical data are conflicting with some studies demonstrating impaired motor development in 1-year-old infants breastfeed by drinking, lactating mothers,[130] while other studies of 18-month infants failed to duplicate these findings.[131]

Tolerance

ACUTE

Acute (within-session) tolerance refers to the greater effect of a specific blood ethanol concentration on the ascending portion of the blood ethanol curve compared with the effect of the same ethanol concentration on the descending portion of the curve. Mellanby first described acute tolerance in 1919,[132] and the Mellanby effect is associated with the phenomena of acute tolerance. Acute tolerance is a complex phenomenon that depends on a variety of factors including the dose of ethanol, speed of drinking, rate of absorption, and prior experience with ethanol as well as the measure of outcome.[133] The presence of chronic tolerance interferes with the expression of acute alcohol tolerance. Generally, the occurrence of acute tolerance is greater in light drinkers than in moderate or heavy drinkers,[134] especially for more complex cognitive tasks (e.g., learning, recall, coding) rather than simpler motor performance (e.g., pursuit rotor).[135] Although the results of human studies on acute tolerance are inconsistent,[136,137,138] impairment of information processing tasks displays less variation between the ascending and descending BAC curve than pure motor skills (e.g., pursuit rotor task), when tested at moderate BAC (≤100 mg/dL).[139] In a study of social drinkers, peak self-reported intoxication levels were significantly lower and occurred earlier for heavy drinkers than for light or moderate social drinkers.[140] Self-reported responses to questions using the alcohol scale of the Addiction Research Centre Inventory were less on the descending limb than on the ascending limb of the blood-alcohol curve for all types of social drinkers.

CHRONIC

Chronic (between-session) tolerance refers to the reduced effect of a specific blood ethanol concentration following repeated use compared with the effect of the

same concentration in alcohol-naive individuals. Chronic tolerance involves both metabolic (pharmacokinetic) and functional (pharmacodynamic) processes. Functional tolerance implies resistance to ethanol effects at the cellular level, whereas metabolic tolerance refers to changes in duration of ethanol effects that result from alterations of absorption, distribution, and elimination.[141] Functional tolerance to the CNS effects of ethanol may occur without proportional increases in the elimination rate of ethanol from the body.[142] The clinical effects of high BAC may not be obvious in patients, who chronically consume large amounts of ethanol. In a study of alcohol abusers presenting voluntarily to a detoxification center, 24% of the patients with serum ethanol concentrations exceeding 200 mg/dL did not demonstrate clinical signs of ethanol intoxication.[28] Although all patients with serum ethanol concentrations exceeding 350 mg/dL demonstrated some signs of ethanol intoxication, some of these individuals also had normal speech, stable gait, or adequate verbal comprehension. The development of chronic tolerance is dose-dependent with high ethanol doses producing more rapid appearance of chronic tolerance.[143] The dose-response curve for the development of functional tolerance to the effects of ethanol is not well defined. Experimental studies suggest that small changes in the performance of psychomotor tasks begin about 1 week after chronic administration of ethanol, depending on the ethanol dose.[144] Following heavy continuous ethanol use, experimental studies of alcohol abusers with a history of withdrawal suggest that impairment of some psychomotor skills in this group begins at higher BAC compared with the general population.[145]

Drug Interactions

The effect of chronic ethanol consumption is difficult to predict because of the multitude of pharmacokinetic and pharmacodynamic effects of ethanol use. Prolonged ethanol intake induces CYP2E1, whereas acute ethanol use inhibits the metabolism of drugs (warfarin, barbiturates, phenytoin, rifampin) that require the CYP2E1 isoenzyme.[146] Although chronic ethanol consumption decreases the metabolism of carbamazepine, fewer side effects occur because of the increased tolerance to the CNS side effects of carbamazepine.[147] There is no clear evidence that OTC medication (e.g., H$_2$-receptor antagonists, acetaminophen, aspirin) alter ethanol metabolism or cause higher ethanol-blood concentrations.[148]

Disulfiram-like Reactions

Disulfiram blocks the metabolism of acetaldehyde to acetate, leading to an accumulation of acetaldehyde in the blood and adverse effects (e.g., nausea, vomiting). Case reports suggest that certain other drugs produce a disulfiram-like reaction, possibly by inhibiting the enzyme aldehyde dehydrogenase. These include metronidazole, sulfonamides, cefamandole, cefoperazone, griseofulvin, hypoglycemic agents, the mushroom *Coprinus atramentarius* (Bull.) Fr., and some industrial chemicals (amides, oximes, carbamates, dithiocarbamate compounds, thiram derivatives).[149,150]

Gastric Motility and ADH Secretion

The daily administration of 1,200 mg cimetidine and 300 mg ranitidine did not alter the ethanol elimination rate in volunteers given 45 g ethanol daily for 3 weeks.[71] Many drugs that decrease hydrochloric acid and pepsin secretion (e.g., cimetidine, cisapride, erythromycin) accentuate the delay in gastric emptying produced by food, but the overall effect of BAC depends on the individual drug.[151] For example, ranitidine affects intrinsic cholinergic mechanisms in addition to H$_2$-blocking effects; therefore, the overall effect of ranitidine on BAC is minor. Consequently, the weight of the evidence suggests that the interaction of H$_2$-blocking agents with ethanol is unlikely to affect BAC at concentrations associated with impairment of psychomotor skills.[152] Proton pump inhibitors (e.g., omeprazole) probably do not have an effect on ethanol pharmacokinetics.[153] In a study of 12 healthy men ingesting 0.80 g ethanol/kg body weight on an empty stomach, pretreatment for 7 days with ranitidine, cimetidine, or omeprazole did not alter the pharmacokinetics of ethanol compared with controls.[154]

Pharmacodynamic Effects

Common direct interactions involve sedation following the concomitant ingestion of CNS depressants and ethanol. The pharmacokinetic interaction between acute ethanol ingestion and single doses of benzodiazepines are complex, depending on the dose, timing of ingestion, and chronic use of ethanol and/or other drugs. Most fatalities resulting from the ingestion of ethanol and benzodiazepines involve triazolam.[155] Impairment of psychomotor performance may occur following the ingestion of ethanol with older, sedating antihistamines, tricyclic antidepressants, and benzodiazepines.[152] The specific interaction varies depending on the pharmacodynamic properties of the particular drug involved.

Caffeine

The interaction of caffeine and ethanol is complex, and this interaction depends on a number of variables

including type of task, BAC, portion of the BAC curve, the dose of caffeine, and the timing of the ingestion of these 2 compounds.[156,157] Although caffeine increases alertness and reduces reaction time when ingested alone, the ingestion of caffeine and ethanol does not completely antagonize impairment of psychomotor skills, even at low BAC.[158] In a study of 15 healthy, young adults, the mean brake latency during testing in a driving simulator for the ethanol limb (75–85 mg/dL) and the caffeine (400 mg) and ethanol limb increased 17% and 11% compared with controls (no drugs).[159] Additionally, caffeine does not reverse the subjective effects of ethanol.[160] Most of these studies do not simulate the situation where an intoxicated individual drinks coffee and then drives shortly thereafter. However, existing data suggest that the ingestion of caffeine will not ameliorate ethanol-induced impairment of driving skills.[161]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

There are no specific ethanol receptors in the CNS. However, ethanol affects endogenous opiates and several CNS receptors including γ-aminobutyric acid (GABA), dopamine, and glutamate.[162] Ethanol alters the function of several ligand-activated ion channels including N-methyl-D-aspartate (NMDA), serotonin (5-HT$_3$), glycine, and γ-aminobutyric acid type A (GABA$_A$) receptors.[163] Ethanol activates the inhibitory chloride influx mediated by the GABA$_A$ receptors; this property contributes to the anxiolytic and sedative properties as well as to the impairment of coordination associated with ethanol use. *In vitro* studies indicate that action of ethanol on GABA$_A$ receptors is more complex than benzodiazepines with individual receptor subtype sensitivity to ethanol varying substantially between different neurons in the brain (e.g., in hippocampus); these studies suggest that modulation of the GABA$_A$ receptors does not account for all of the effect of ethanol on the brain.[164] Furthermore, ethanol increases GABAergic neurotransmission through indirect effects including the presynaptic release of GABA, elevation of endogenous GABAergic neuroactive steroids, and dephosphorylation of GABA$_A$ receptors promoting increases in GABA sensitivity.[165] Ethanol inhibits the function of the excitatory NMDA type of glutamate receptor as well as interacting with serotonin and dopamine receptors to increase dopamine in the reward centers of the brain.

Mechanism of Toxicity

ACUTE

Ethanol is a CNS depressant that selectively depresses the reticular activating system. The mechanism of action probably involves interference with ion transport (i.e., sodium flux) at the cell membrane rather than at synapses, similar to the action of general anesthetic agents. The frontal lobes are sensitive to low ethanol concentrations, resulting in alteration of thought and mood before changes in vision (occipital lobe) and coordination (cerebellum). Preferential suppression of inhibitory (GABA) neurons probably causes the excitation seen at low ethanol concentrations. Ethanol is a vasodilator that produces decreased preload, afterload, and systemic vascular resistance in healthy adults after acute ingestion.[166] Chronic ethanol consumption is associated with dilated cardiomyopathy (alcoholic cardiomyopathy) characterized by cardiomegaly, reduced myocardial contractility, myocardial fibrosis, and disruption in the myofibrillary architecture. Potential toxins (ethanol, acetaldehyde) may directly contribute to this cardiomyopathy by enhancing catecholamine concentrations and reactive oxygen species; however, other potential mechanisms (oxidative stress, protein-acetaldehyde adduct formation, accumulation of fatty acid ethyl esters, modification of lipoprotein and apolipoprotein particles) may also contribute to the development of alcohol cardiomyopathy.[167]

CHRONIC

Chronic alcohol abuse produces profound metabolic changes as a result of alterations in the cellular redox state resulting from a decreased NAD+/NADH ratio. These changes include 1) shift from pyruvate to lactate that results in lactic acidosis, 2) elevation of serum uric acid from reduced clearance, 3) alteration of lipid metabolism with accumulation of hepatic triglycerides, 4) reduced gluconeogenesis, 5) depressed protein synthesis, and 6) reduced active bone resorption and formation resulting from inhibition of bone remodeling by a mechanism independent of calciotropic hormones.[168]

ADDICTION.   Although the specific gene associated with alcohol dependence has not been identified, epidemiologic studies on alcohol dependence (twin, adoption) suggest that genetic factors account for up to about one-half of the risk of alcohol dependence.[169] First-degree relatives of alcohol abusers have an approximate 3- to 4-fold increased risk of alcohol dependence and the risk of alcohol dependence increases another 2-fold for identical twins.[170] Alcohol sensitivity is related to

pharmacogenetic variation in the ability to rapidly remove acetaldehyde during ethanol metabolism through the enzymatic action of aldehyde hydrogenase, primarily ALDH2. Individuals with the biologic inactive form of ALDH2 (ALDH2*2) develop increased heart rate, facial flushing, and nausea after ethanol ingestion as a result of the decreased ability to reduce aldehyde concentrations. Less than 10% of Japanese alcohol abusers are heterozygotes for the inactive form of aldehyde hydrogenase (ALDH2*2), whereas the prevalence of ALDH2*2 heterozygotes in the general Japanese population is approximately 40%.[171]

WITHDRAWAL.   Although the exact mechanism of withdrawal remains poorly defined, tolerant patients develop a compensatory physiologic state that opposes the depressant actions of ethanol. The withdrawal of ethanol leads to unopposed compensatory mechanisms, which are clinically manifested as autonomic hyperexcitability. The chronic ingestion of ethanol inhibits NMDA receptors leading to upregulation, reduces excitatory glutamatergic transmission, and excites GABA$_A$ receptors resulting in downregulation.[172] During abstinence from prolonged ethanol use, enhancement of NMDA receptor function, reduced transmission at GABA receptors, and dysregulation of the dopaminergic system contribute to the clinical features of ethanol withdrawal.[173] Although hypomagnesemia and hypokalemia may also contribute to clinical effects, changes of electrolyte concentrations in plasma or in cerebrospinal fluid do not cause delirium tremens (DTs).[174] Proposed compensatory mechanisms accounting for the hyperadrenergic state include a decreased affinity for the inhibitory transmitter (GABA), false transmitter formation, decreased prostaglandin E synthesis, and increased Na/K-ATPase activity. Ethanol itself does not exhibit proconvulsant properties. Etiologies of seizures during alcohol withdrawal include cerebral trauma (subdural or epidural hematoma), alcohol-related metabolic brain disorder, intracranial hemorrhage or infarct, CNS infection, and precipitation of idiopathic or posttraumatic epilepsy as well as alcoholic withdrawal seizures. Alterations in the number and function of NMDA (e.g., NR1, NR2B subtypes) and GABA$_A$ receptors probably contribute to the occurrence of seizures during ethanol withdrawal.

WERNICKE ENCEPHALOPATHY AND KORSAKOFF PSYCHOSIS.
Wernicke encephalopathy is an acute neuropsychiatric condition that results from biochemical changes secondary to inadequate stores of intracellular thiamine (vitamin B$_1$). The body stores little thiamine; depletion occurs within 2–3 weeks of reduced thiamine intake. These changes involve impairment of carbohydrate metabolism and nerve cell conduction resulting from inadequate amounts of the active form of thiamine (diphosphate ester of thiamine); initially these changes are reversible. However, the continuing lack of adequate thiamine stores produces the chronic form of the disease (Korsakoff psychosis), which is characterized by loss of short-term memory and difficulty processing new information. The Wernicke-Korsakoff syndrome refers to these 2 entities. Thiamine deficiency disrupts several thiamine-dependent enzyme complexes [i.e., pyruvate dehydrogenase complex, transketolase (pentose phosphate pathway), 2-oxoglutarate or α-ketoglutarate dehydrogenase (Krebs cycle)] resulting in damage to the myelin sheaths, inhibition of glucose and lipid metabolism, decreased branched chain amino acid production, and increased lactic acid production (See Figure 21.2).[175] Thiamine deficiency leads to decreased concentrations of the coenzyme thiamine pyrophosphate, causing alterations in the metabolism and the membrane structure of the nervous system. Ethanol interferes with active GI transport of thiamine and chronic liver disease reduces thiamine activation and storage.[176] Symmetrical pathologic changes occur in anterior cerebellum (vermis), periaqueductal region, floor of the fourth ventricle, mammillary bodies, and the paraventricular portions of the anterior medial nuclei, thalamus, and medial dorsal nuclei.[177] Patients with acute Wernicke encephalopathy have edema in these areas on T2-weighted late-echo sequences during magnetic resonance imaging (MRI),[178] whereas MRI studies demonstrate atrophy of the mammillary bodies and thalamus on sagittal cuts in patients with Korsakoff syndrome as a result of the loss of surrounding gray matter nuclei.[179] Modest volume deficits also occur in the medial septum/diagonal band of Broca as well as in the cerebellar hemisphere, anterior superior vermis, and pons. The lack of the Wernicke-Korsakoff syndrome in most thiamine-deficient alcohol abusers suggests genetic susceptibility to the development of this syndrome.

MARCHIAFAVA-BIGNAMI DISEASE.   This rare disease results from the necrosis and demyelination of the corpus callosum, particularly the medial zone.[180] Patients are usually middle-age male alcohol abusers with poor nutrition and heavy consumption of ethanol over 10–20 years.[181] The course of the illness may be acute or chronic with signs of severe global dementia, seizures, alteration of conscious progressing to coma and death. MRI studies demonstrate T1 hypointensities and T2 hyperintensities in the corpus callosum, centrum semiovale, and other periventricular white matter.[179]

POLYNEUROPATHY.   A polyneuropathy commonly occurs in alcohol abusers, particularly in association with nutritional deficiencies (i.e., thiamine). However, the clinico-
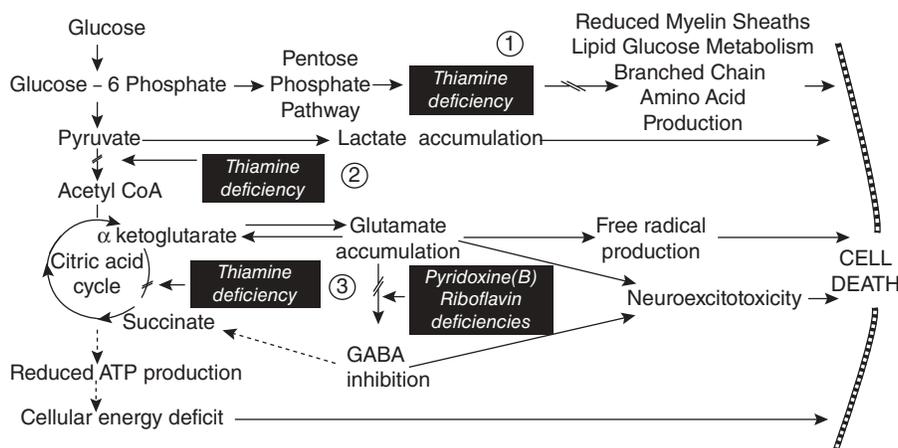
FIGURE 21.2. Pathophysiology of brain damage from Wernicke encephalopathy. (1) Thiamine-dependent enzymes, (2) pyruvate dehydrogenase complex, and (3) α-ketoglutarate dehydrogenase complex. (Reprinted from AD Thomson, CCH Cook, R Touquet, JA Henry, The Royal College of Physicians report on alcohol: guidelines for managing Wernicke's encephalopathy in the accident and emergency department, 2002, Vol. 37 Issue 6, p. 516, by permission of Oxford University Press.)

pathologic features of these 2 neuropathies are distinct.[182] Alcoholic neuropathy is a painful, slowing progressive, sensory-dominant polyneuropathy affecting primarily superficial sensation. Sural nerve biopsies demonstrate predominately small-fiber loss of axons with segmental demyelination and remyelination resulting from widening of consecutive nodes of Ranvier. In contrast, thiamine-associated polyneuropathy (i.e., beriberi neuropathy) manifests an acute, motor-dominant polyneuropathy affecting both superficial and deep sensation. These clinical effects are associated with large-fiber axonal loss and subperineurial edema. The combination of chronic alcohol abuse and thiamine deficiency obscures the clinicopathologic differences between alcoholic neuropathy and thiamine-deficiency neuropathy.

METABOLIC DISTURBANCES. The oxidation of ethanol by the alcohol dehydrogenase pathway causes the loss of hydrogen and the concomitant reduction of NAD^+ to NADH. The formation of large amounts of reducing equivalents disrupts redox homeostasis and produces metabolic alterations including hyperlactacidemia and hypoglycemia, particularly during reduced carbohydrate intake by alcohol abusers. During glycolysis, glucose is converted to pyruvate. Under normal aerobic metabolism, acetyl CoA is formed from pyruvate, and acetyl CoA enters the Krebs cycle where the end products are ATP, water, and carbon dioxide. The accumulation of NADH during ethanol metabolism favors the conversion of pyruvate to lactate by the enzymatic action of lactate dehydrogenase. Elimination of lactate involves the regeneration of glucose in the liver via gluconeogenesis, which involves the cofactors biotin and thiamine. Ethanol increases serum lactate concen-

tration by increasing the formation of NADH and shifting the redox potential to favor lactate formation. Additionally, chronic ethanol consumption and malnutrition impairs the elimination of lactate by limiting the availability of cofactors necessary for gluconeogenesis. The inducible microsomal ethanol-oxidizing system (MEOS) produces acetaldehyde, which can enhance the formation of oxygen radicals and toxic metabolites from other xenobiotics.[183]

Alcohol-induced ketosis reduces the capacity of the kidney to excrete uric acid, producing a secondary hyperuricemia. Increasing amounts of NADH restricts lipid oxidation and promotes fatty acid synthesis and accumulation, resulting in increased formation of keto-acids, β-hydroxybutyrate, and acetoacetate.[184] The hypoglycemia associated with ethanol intoxication in young children appears related primarily to the inhibition of hepatic gluconeogenesis.[185] In the presence of significant depletion of hepatic glycogen stores (i.e., secondary to starvation or fasting), alcohol impairs hepatic gluconeogenesis, probably by reducing the intracellular NAD^+/NADH ratio. Consequently, life-threatening hypoglycemia may occur in alcohol abusers.

Postmortem Examination

ALCOHOL-RELATED LIVER INJURY. The earliest and most common hepatic response to ethanol abuse is the asymptomatic accumulation of fat in the liver cells with minimal elevation of serum hepatic transaminases.[186] Occasionally, the severe form of fatty liver disease mimics extrahepatic obstructive jaundice with dark urine, acholic stools, and mild to moderate elevation of serum hepatic transaminases. Long-term ethanol use

transforms stellate cells into collagen-producing myofibroblast-like cells that cause fibrosis around central veins and venules (i.e., terminal hepatic venules).[187] Even after decades of ethanol abuse, only a fraction of heavy ethanol users develop alcoholic hepatitis characterized as inflammatory necrosis of the liver. Histologic changes of alcoholic hepatitis include ballooning and disarray of the hepatocytes in the perivenular area, lymphocytic and polymorphonuclear infiltration of the parenchyma and portal areas, steatosis, cholestasis, necrosis, and fibrosis.[186] Mallory bodies (i.e., irregular hyaline cytoplasmic inclusions) are diagnostic features of alcoholic hepatitis, but these inclusion bodies are not present in all cases of alcoholic hepatitis. Cirrhosis distorts the normal architecture of the liver by the formation of fibrous bands between the central and portal zones that may develop in the absence of overt alcoholic hepatitis.[188] As cirrhosis advances, irregular nodules and perivenular fibrosis impair blood flow from the sinusoids, producing postsinusoidal portal hypertension. Eventually, portal hypertension causes splenomegaly, increasing hepatomegaly, esophageal and gastric varices, and GI hemorrhage.

ALCOHOL-RELATED SUDDEN DEATH. Sudden death occurs in chronic alcohol abusers, particularly in those alcohol abusers that drink heavily over several days without adequate dietary intake. Although postmortem examination demonstrates diseases (e.g., GI hemorrhage, cardiovascular disease, trauma, hepatorenal failure) that account for death in a majority of these cases, a cause of death is not apparent in some cases.[189] The most common histologic abnormality in these cases is the presence of extensive microvesicular fatty droplets in hepatocytes along with varying amounts of macrovesicular fatty change.[190] Metabolic disturbances (e.g., metabolic acidosis, ketoacidosis, hypoglycemia, hyperammonemia), renal dysfunction, hypothermia, and cardiac arrhythmias probably contribute to the occurrence of alcohol-related sudden death.

CLINICAL RESPONSE

Use

Ethanol is a selective CNS depressant in low doses, whereas high ethanol doses cause global depression of consciousness. The degree of neurologic impairment depends on a number of variables, including genetic factors, absolute amount ingested, tolerance, rate, and direction of blood ethanol elevation, simultaneous ingestion of other drugs and alcohol products, trauma, underlying nutritional status, and complications of chronic alcohol abuse.

INTOXICATION

ACUTE.   The most common symptoms in children and young adolescents under medical supervision for ethanol intoxication were coma and vomiting.[119] The rapid ingestion of ethanol by adolescents increases the risk of the rapid onset of coma and hypothermia in cold environments. Although ethanol intoxication causes vomiting and diuresis by inhibiting antidiuretic hormone, dehydration is uncommon in patients without other causes. Initially, ethanol produces exhilaration that progresses to loss of restraint, behavioral abnormalities, loquaciousness, slurred speech, ataxia, gait disturbances, irritability, drowsiness, stupor, coma, and respiratory depression as the ethanol dose increases. Other clinical features of ethanol intoxication include flushed face, dilated pupils, excessive sweating, nausea, vomiting, and rarely, alcohol-induced urticaria.[191] The ingestion of ethanol can produce dysrhythmias (e.g., atrial fibrillation) in nontolerant binge drinkers as well as in chronic alcohol abusers, but dysrhythmias are not a common complication of ethanol intoxication. Seizures during acute ethanol intoxication are rare without the presence of underlying intracranial pathology.[192] Common complicating factors associated with acute ethanol intoxication include concurrent trauma (e.g., subdural hematoma) and underlying medical diseases (liver, kidney, heart) as well as the use of other drugs and toxic alcohol substitutes (i.e., methanol, ethylene glycol). There is no direct evidence that acute ethanol intoxication predisposes or causes sleepwalking or related disorders.[193]

VISIBLE OR OBVIOUS.   Visible or obvious intoxication refers to a series of observable actions and behaviors that are consistent with gross impairment of cognitive and motor abilities following the ingestion of ethanol or other drugs. This impairment involves 1) diminished inhibitions (inappropriate response to social and/or environmental cues), 2) psychomotor impairment (slurred or slow speech, poor coordination, unsteady gait), and 3) cognitive impairment (poor concentration/memory, forgetfulness, difficulty performing simple tasks, inability to follow directions).[194] Observational instruments are available to assess the presence and level of alcohol intoxication; however, the reliability of observation alone in detecting moderately elevated BAC (100–140 mg/dL, 0.1–0.14 % w/v) is relatively low. Items in these observational instruments include the smell of alcohol, fine motor control (dropping or difficulty handling objects), gross motor control (stumbling, difficulty walking, falling), slurred speech, change in volume or pace of speech, sleepiness or decreased alertness, conjunctival erythema, sweating and change in

respiratory pattern.[195] None of these items is uniquely associated with visible intoxication; therefore, the combination of items must be interpreted together with the circumstances surrounding the actions. Some behaviors associated with visible intoxication also occur in sober individuals; consequently, observational instruments do not usually include behavioral changes (e.g., boisterous, argumentative, confrontational, obnoxious) in the evaluation of individuals for visible intoxication.

The ability of observers to reliably determine visible intoxication in an individual is limited until the BAC is high (>150 mg/dL), regardless of the experience of the evaluator.[196] In a study of videos containing moderate drinkers seated in a chair, experienced police officers were unable to reliably determine if individuals were too intoxicated to be served another drink or to drive until the BAC exceeded 150 mg/dL.[197] This study was an observational study that did not allow the police officers to smell the odor of ethanol or to conduct a field sobriety test. Other studies using college counselors and college students also indicate that the determination of alcohol impairment is difficult without specific testing (e.g., BAC, field sobriety test) unless the BAC is relatively high (>150 mg/dL).

Although the likelihood of visible intoxication increases with the BAC, not all individuals with the same BAC are visibly intoxicated. Factors affecting the clinical appearance of ethanol intoxication at a given BAC include chronic tolerance, the phase on the BAC curve (acute tolerance), fatigue, hangover, drinking habits, the environment, culturable norms on acceptable behavior, personality, age, ingestion of other drugs, underlying disease states, and general health.[198,199,200] A review of experimental data dating back to the 1930s concluded that visible signs of intoxication are present in a majority of individuals with BAC exceeding 150 mg/dL, and almost all individuals with BAC exceeding 200 mg/dL are visibly intoxicated, regardless of tolerance.[194] However, a few exceptional individuals do not display visible signs of intoxication despite a BAC well above 200 mg/dL as a result of chronic ethanol abuse.[201] Therefore, the determination of visible intoxication is not based on the BAC alone, but requires observation of the individual for visible signs of impaired motor and cognitive abilities.

The definition of what specifically (i.e., number and type of observable signs and symptoms) constitutes visible intoxication is not well defined, even for court proceedings. In a study of patients seeking admission for alcohol dependence, the criteria for visible signs of intoxication were gross gait abnormalities (staggering, swaying, reeling) plus 2 of the following signs: speech abnormality, flushed face, dilated pupils, or alcoholic odor on breath.[202] About 50% of the individuals averag-

ing 150 mg/dL (range, 125–175 mg/dL) met this criteria compared with 84% in individuals averaging 200 mg/dL (range, 175–225 mg/dL), as measured by the analysis of oxalated blood samples using the potassium dichromate method. With the same criteria for occasional drinkers and nondrinkers, 4 of 8 (50%) individuals with BAC averaging 100 mg/dL (range, 75–125 mg/dL) were considered intoxicated compared with 4 of 7 (57%) in the range of 125–175 mg/L and all 5 individuals in the 175–225 mg/dL range.[203]

Hangover. Although a hangover shares some of the adverse effects associated with acute alcohol withdrawal, a hangover is probably a separate entity that has a different, albeit poorly understood etiology.[204] Although relatively uncommon in alcohol abusers, an alcoholic hangover occurs frequently in mild to moderate ethanol users as a result of binge drinking. Common symptoms include a variety of affective, biologic, and physiologic effects including general misery, malaise, headache, anorexia, tremulousness, dizziness, dry mouth/thirst, fatigue, nausea, diarrhea, poor concentration, decreased attention, irritability, anxiety, and decreased sleep (rapid-eye-movement [REM] and total sleep time).[205,206] There is substantial interindividual and intraindividual variability in the severity of a hangover. Symptoms develop as the BAC declines substantially, reaching maximum effects when the BAC falls to zero; resolution of symptoms occurs within 12–24 hours after ethanol consumption ends. The consumption of alcoholic beverages (e.g., wine, brandy, whiskey, tequila, dark liquors) that contain relatively high concentrations of congeners (e.g., fusel oil, acetaldehyde, ethyl acetate) increases the severity of a hangover compared with alcoholic beverages with no congeners (e.g., vodka); however, ethanol consumption is a stronger determinant of hangover than congener content.[207,208] Medical and social factors that increase the severity of a hangover include sleep deprivation, poor food consumption, dehydration, increased physical activity during acute ethanol intoxication, and poor physical conditioning.[209] The etiology of a hangover remains unclear;[210] sleep deprivation and dehydration do not account for all the effects of a hangover.[211] The evidence for impairment of psychomotor skills, memory, and attention during an alcohol hangover in nonalcohol abusers is conflicting as a result of methodologic differences between studies (e.g., differences in timing of testing between controls and drinking study participants, documentation of zero blood ethanol concentrations, matching baseline skills, sleep deprivation, and personality differences between study participants and controls).[212,213] For crew members of civilian aircraft, the US Federal Aviation Administration prohibits the use of ethanol within 8

hours of flying or a BAC of ≥0.4% w/v during flight. There is no clear evidence that currently popular interventions for the prevention or treatment of a hangover are efficacious including the use of propranolol, glucose, fructose, ethanol, borage (*Borago officinalis* L.), and prickly pear [*Opuntia ficus-indica* (L.) P. Mill.].[214]

BEHAVIORAL ABNORMALITIES

The existence of alcohol idiosyncratic intoxication (i.e., pathologic intoxication) is highly controversial.[215] The *Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition* (*DSM-III*) listed a syndrome (291.40) involving marked behavioral changes (usually aggressive) following ethanol doses that are usually insufficient to produce intoxication.[216] The behavioral changes frequently involve combative, irrational behavior that results in both property damage and personal injury followed by amnesia. The definition of this syndrome did not include aggressive behavior associated with marked intoxication.[217] Because of lack of medical data to support the existence of this syndrome, pathologic intoxication was no longer included as a separate diagnosis in *DSM-IV*.[218] The *DSM-IV-TR* includes clinically significant maladaptive behavioral and psychologic changes (e.g., inappropriate sexual or aggressive behavior, mood lability, impaired judgment, impaired social or occupational functioning) with other signs of acute alcohol intoxication (e.g., slurred speech, incoordination, unsteady gait, nystagmus, impairment of cognition, memory, and consciousness).[219] There is no category for this type of behavior without the usual signs of acute ethanol intoxication.

MENTAL DISORDERS

ALCOHOL ABUSE AND DEPENDENCE. Alcohol abuse and dependence is a chronic, often progressive and fatal disorder characterized by impaired control over ethanol use and preoccupation with drinking despite serious adverse social and physical consequences.[220] The *DSM-IV-TR* defines alcohol abuse as a maladaptive pattern of ethanol use during the last year that is manifest by clinically significant impairment in at least one of the following areas:[219]

1) Recurrent ethanol use resulting in a failure to fulfill major obligations at work, school, or home (e.g., absenteeism, poor work performance, suspension, neglect of children or household duties).

2) Recurrent ethanol use that causes physically hazardous behavior (e.g., driving while intoxicated, operating machinery while impaired).

3) Recurrent legal problems associated with ethanol use (e.g., disorderly conduct, driving while intoxicated).

4) Continued ethanol abuse despite recurrent legal, social, or medical problems.

The presence of symptoms of tolerance, withdrawal, and compulsive use of ethanol suggests the diagnosis of ethanol dependence rather than ethanol abuse. However, some patients with alcohol dependence do not develop clinically significant signs of alcohol withdrawal, and only about 5% of alcohol-dependent patients develop severe complications (grand mal seizures, DTs). Patients with alcohol dependence devote substantial time to obtaining and consuming alcoholic beverages; this compulsive behavior persists despite serious adverse psychologic and physical effects (e.g., blackouts, liver disease, depressions).[219]

Early detection of alcohol abuse and dependence requires a careful medical and psychosocial evaluation to prevent the serious health effects associated with this disease. To promote early detection, the National Council on Alcoholism assembled data in 1972 that divided criteria for alcohol dependence into the following 2 areas with major and minor categories:[221] Track I: Physiological and Clinical, and Track II: Behavioral, Psychological, and Attitudinal. Each criterion was weighted to grade the degree of implication for the presence of alcohol dependence that the specific sign or symptom suggested. Depending on the overall evaluation, there were 3 diagnostic categories:

1) *Diagnostic Level 1 (Definite).* A person who fits this criterion must be diagnosed as an alcohol dependent (e.g., withdrawal seizures, delirium tremens, alcoholic hepatitis, serious disruption of interpersonal relationships).

2) *Diagnostic Level 2 (Indicative).* In a person who satisfies this criterion, the diagnosis of alcohol dependence should be strongly suspected.

3) *Diagnostic Level 3 (Possible).* These manifestations may arouse the suspicion of alcohol dependence, but other evidence is needed to confirm the diagnosis of alcohol dependence.

The diagnosis of alcohol dependence requires 1 or more major criteria or the presence of several minor criteria in both Tracks I and II. However, the use of this set of criteria and diagnostic levels is relatively complex compared with the *DSM-IV-TR* definitions, and use of the National Council on Alcoholism criteria results in some overlap between various diagnostic levels and major/minor criteria.[220]

WERNICKE ENCEPHALOPATHY AND KORSAKOFF PSYCHOSIS. The Wernicke-Korsakoff syndrome refers to an acute or subacute neurologic disorder (i.e., Wernicke encephalopathy) that progresses to a chronic memory disorder called Korsakoff psychosis (amnestic-confabulatory psychosis with relative preservation of other intellectual function), when the underlying thiamine deficiency is not treated promptly. The classical signs of Wernicke encephalopathy are confusion, ataxia, and ophthalmoplegia, but the classic triad occurs only in a small portion of patients with Wernicke encephalopathy.[222] Typically, Wernicke encephalopathy occurs along with signs of alcohol withdrawal (e.g., tremor, hypotension, hypothermia, obtundation), but some alcohol abusers may develop this illness without obvious withdrawal symptoms.[223] The most reversible signs of the Wernicke encephalopathy are ocular abnormalities, including diplopia, blurred vision, nystagmus (bidirectional), and lateral gaze palsy. The latter sign is the most common; the palsy may be asymmetrical. Gait ataxia results from a polyneuropathy (decreased deep tendon reflexes and sensation) as well as from CNS dysfunction.

The term alcoholic cerebellar degeneration refers to the gait disturbances when these neurologic abnormalities occur with relatively little dysfunction of speech, upper motor movement, or ocular motility. The pathologic gait disturbances of alcoholic cerebellar degeneration and Wernicke encephalopathy are clinically indistinguishable. Other abnormalities associated with thiamine-deficient states include cardiac failure and GI distress (nausea, vomiting, abdominal pain). Thiamine deficiency produces Wernicke encephalopathy, the common underlying neuropathologic cause of most cognitive impairment in chronic alcohol abusers. Wernicke encephalopathy is also associated with other medical conditions causing nutritional deprivation (malignancy, hyperemesis gravidarum, intestinal obstruction). Changes in mental status occur in >90% of patients with Wernicke encephalopathy, and the cognitive abnormalities of Korsakoff syndrome are the least responsive to treatment. Approximately 80% of surviving patients with Wernicke encephalopathy develop Korsakoff syndrome characterized by retrograde (inability to recall information) and anterograde (inability to assimilate new information) amnesia. Memory is usually divided into working memory (information held seconds while allocating resources) and secondary memory (information held on a semipermanent or permanent basis). Episodic memory involves recalling memories from the past; this type of secondary memory is severely affected in these patients, often the amnesia extends 20–30 years.[224] Semantic memory involves the learning and recall of facts, concepts, and language; the effect of Korsakoff syndrome on this type of memory is variable. Implicit memory (i.e., response to priming and perceptuomotor memory) usually remains intact. Confabulation and decreased initiative also occur, but otherwise impairment of sensorium is minimal. Although some improvement in memory and learning occurs over years in 75% of abstinent Korsakoff patients, the remainder does not improve and may require long-term institutional care.[223] The improvement occurs much more slowly than resolution of the acute clinical features of Wernicke encephalopathy.[224]

Alcoholic pellagra encephalopathy is a neurologic condition associated with niacin deficiency and chronic ethanol abuse. Clinical features of this disease include confusion, oppositional hypertonus and myoclonus, hallucinations, insomnia, tremor ataxia, incontinence, peripheral neuropathy, seizures, anxiety, and depression.[225]

MEDICAL COMPLICATIONS

The complications of ethanol abuse are protean as outlined in Table 21.3. Polydrug use and alcohol substitutes (methanol, ethylene glycol) may complicate the diagnosis of ethanol abuse/dependence. Nutritional deficiencies (e.g., B_1, B_6, B_{12}, Zn) produce a variety of diseases including alcoholic beriberi and Wernicke encephalopathy. A substantial portion of abstinent alcohol abusers exhibit cognitive dysfunction after detoxification, particularly in visuospatial abilities, perceptual-motor integration, abstract reasoning, short-term memory, and mental flexibility.[226] Although most of the improvement in cognitive function occurs in the first several months of abstinence, some of these cognitive deficits may improve over a 2-year period.[227]

CARDIOMYOPATHY. An idiopathic (nonischemic), dilated cardiomyopathy occurs in chronic alcohol abusers.[228] The onset of alcoholic cardiomyopathy begins after consuming at least 7–8 standard drinks daily for over 5 years. The average duration of heavy drinking for these patients is approximately 15 years.[229] Clinical features of alcoholic cardiomyopathy include dilated left ventricle, fatty infiltration in the perivascular region, the intraventricular septum and the liver, atrophy of the myocytes, normal or reduced left ventricular wall thickness, and increased left ventricular muscle mass. Symptoms range from none to the typical symptoms associated with severe congestive heart failure (New York Heart Association Class IV).[230] There are no specific criteria or laboratory abnormalities that separate alcoholic cardiomyopathy from other causes of cardiomyopathy. Consequently, the diagnosis of alcoholic cardiomyopathy is a diagnosis of exclusion based on a history of

TABLE 21.3. Diseases Associated with Excessive Ethanol Consumption.

Organ System	Disease/Disorder
Skin/Musculoskeletal	Pellagra, nutritional stomatitis, cheilosis, scabies, alopecia, myopathy
Trauma	Automobile accidents, rib fractures, pneumothorax, chronic subdural hematoma, suicide
Blood	Hemolysis, anemia, Zieve syndrome (jaundice, hyperlipidemia, hemolytic anemia)
Neurologic System	Acute intoxication, dementia, seizures, Wernicke encephalopathy, Korsakoff psychosis, stroke, subarachnoid hemorrhage, amblyopia, alcoholic withdrawal/delirium tremens, central pontine myelinolysis, cerebral atrophy, cerebellar degeneration, polyneuropathy, Marchiafava-Bignami disease, peripheral motor/sensory neuropathy
Respiratory	Tuberculosis, pneumonia, atelectasis, chronic obstructive pulmonary disease from heavy long-term smoking
Cardiovascular	Cardiomyopathy, hypertension, congestive heart failure, arrhythmias, beriberi
Reproduction	*Male*: hypogonadism, male impotence, reduced testosterone concentrations, erectile dysfunction, loss of libido, impaired spermatic function, small testes, scant pubic hair
	Females: infertility, sexual dysfunction, loss of secondary sexual characteristics, menstrual irregularities, fetal alcohol syndrome
Metabolic/Endocrine	Malnutrition, vitamin B deficiencies, diabetes mellitus, hypoglycemia, hyperglycemia, excessive lactate, hyperuricemia, alcoholic ketoacidosis, hypophosphatemia, electrolyte disorders, hypomagnesemia, hypercholesterolemia, hypertriglyceridemia
Gastrointestinal	Esophagitis, diffuse esophageal spasm, Mallory-Weiss tear, esophageal perforation with mediastinitis, gastrointestinal cancer, erosive gastritis, peptic ulcer, diarrhea, upper gastrointestinal bleeding, pancreatitis, pancreatic pseudocyst
Liver	Fatty infiltration, alcoholic hepatitis, cirrhosis, liver failure

heavy alcohol abuse. Sudden death may occur in chronic alcohol abusers with cardiomyopathy despite the absence of histologic evidence of coronary artery thrombosis or a recent myocardial infarction.[231,232] Heavy beer drinkers also may develop alcoholic cardiac beriberi.

DYSRHYTHMIAS. Ischemic heart disease is the most common natural cause of out-of-hospital death in alcohol dependent men, and sudden cardiac death contributes significantly to the excess mortality in chronic alcohol abusers even several years after detoxification.[233] Dysrhythmias are particularly common during and immediately after alcohol withdrawal.[234] Heavy drinking of ethanol can induce arrhythmias (e.g., atrial fibrillation, ventricular dysrhythmias) in chronic alcohol abusers without underlying cardiovascular disease or electrolyte imbalance, particular during withdrawal.[235,236] In a prospective study of sudden cardiac death in England, Wales, and Scotland, the unadjusted risk of sudden death was increased several fold in heavy drinking (>6 drinks daily) men without preexisting ischemic heart disease compared with occasional or light drinkers without preexisting ischemic heart disease.[237] Adjustment for age, social class, smoking, and systolic hypertension reduced the mean relative risk of sudden death in heavy drinking men to about 1.7, but the difference between the 2 groups was not statistically sig-

nificant. In chronic alcohol abusers with preexisting heart disease and a history of dysrhythmias, the acute ingestion of moderate ethanol doses (e.g., 90 mL of 80-proof whiskey) may produce atrial or ventricular dysrhythmias including ventricular tachycardia and atrial flutter/fibrillation.[238] Heavy drinking is associated with hypertension, particularly during withdrawal.[239] The elevation of blood pressure is relatively mild following the daily consumption of 2–3 drinks per day.[240]

Abstinence Syndrome

This hyperadrenergic syndrome of physical dependence ranges from mild tremor and anxiety to hallucinations and seizures, particularly in regular heavy drinkers after a 5- to 10-year latency period. These individuals frequently demonstrate high tolerance to the acute effects of ethanol and to other drugs. The severity of the syndrome depends on individual susceptibility (i.e., genetic variability) as well as ethanol dose during the period immediately before the cessation of drinking.[241,242] Consequently, substantial differences in withdrawal symptoms occur in alcohol abusers consuming similar amounts of ethanol. A period of binge drinking for several days to a week by a heavy drinker frequently precedes the onset of withdrawal symptoms. In a study of heroin addicts confined to a closed ward, mild with-

drawal symptoms (tremulousness, nausea, diaphoresis, insomnia) occurred in 4 volunteers after discontinuance of alcohol after about 2–4 weeks of daily intoxication.[243] Of the 6 volunteers who drank heavily for 1½–3 months, withdrawal symptoms were more severe. Two of these inpatients had seizures, 2 had hallucinations, and 3 patients had delirium. One of the latter patients required treatment for DTs. In a study of 10 alcohol abusers with a prior history of withdrawal, the administration of progressive increasing daily doses of 86-proof whiskey (maximum 40 ounces) over 24 days produced withdrawal symptoms in 8 of 10 volunteers following cessation of drinking.[244]

Structured, withdrawal severity assessment scales (Clinical Institute Withdrawal Assessment Scale-Revised, Alcohol Withdrawal Scale)[245,246] are available to objectively quantify the severity of withdrawal. Table 21.4 outlines the scoring used for the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) Scale. A CIWA-Ar score below 15 indicates mild withdrawal, whereas scores between 16 and 20 indicate moderate withdrawal symptoms.[247] Severe withdrawal is consistent with scores above 20. High total scores on the Alcohol Use Disorders Identification Test (AUDIT) suggest an increased risk of the development of withdrawal symptoms. A total score of ≥27 was sensitive (0.92), but not specific (0.24) for the necessity of medication for the treatment of withdrawal symptoms during the hospitalization of 55 patients at an inpatient addiction treatment unit.[248]

MINOR ABSTINENCE SYNDROME

The earliest and most common manifestations of alcohol withdrawal are generalized tremulousness, irritability, and GI distress (nausea, vomiting, anorexia) that develop within about 6–8 hours after the cessation of drinking. Other clinical features of the abstinence syndrome include diaphoresis, tachycardia, agitation, hyperexcitability, preoccupation with personal misery, muscular weakness, headache, insomnia, flushed facies, and conjunctival injection. Although most patients are asymptomatic within 72 hours, some symptoms persist for a week.

ALCOHOL DEPENDENT HALLUCINOSIS

Disordered perception occurs in about one fourth of tremulous alcohol abusers typically within 24–36 hours after cessation of drinking. This disorder of perception ranges from disturbing dreams to illusions and hallucinations (visual, auditory). The patient usually remains fully oriented.

SEIZURES (RUM FITS)

Seizures associated with ethanol usually occur during periods of abstinence preceded by binge drinking; the etiology of these seizures is multifaceted and complex.[249] Less than 10% of alcohol abusers develop seizures during withdrawal. The occurrence of seizures during the withdrawal phase is an indication of serious alcohol addiction with the potential for the development of DTs. Although seizures rarely develop after hallucinations, about one third of alcohol abusers with withdrawal seizures develop DTs. Most of these seizures are single, generalized, tonic-clonic seizures that occur with a peak incidence of 24 hours after the cessation of drinking and a range of about 6–48 hours.[250] The occurrence of focal seizures suggests the presence of concurrent lesions (e.g., hemorrhage, infection, metabolic abnormalities, illicit drug use, trauma, neoplastic lesions, cerebrovascular diseases). Multiple seizures may occur within several hours, but only about 3% of hospitalized alcohol abusers develop status epilepticus.[177]

DELIRIUM TREMENS

Symptoms of DTs typically develop 3–5 days after cessation of ethanol and rarely symptoms occur during the first 2 weeks of abstinence. Prodromal symptoms include sleep disturbances, restlessness, anxiety, and GI distress. Seizures occur in approximately one third of patients with DTs, and seizures almost always precede the development of DTs.[177] Prominent features of DTs include agitation, confusion, disorientation, delusions, hallucinations (visual, tactile), and autonomic hyperactivity (fever, sweating, tachycardia, dilated pupils, hypertension, and piloerection). The average duration of the acute psychosis is about 3 days.[251] Delirium tremens represents a true medical emergency with a 5–10% mortality rate. Alcohol dependent patients with a previous history of DTs, concomitant medical illness (infection, metabolic disease, trauma), and chronic alcohol-related illness are high-risk candidates for the development of DTs.

Reproductive Abnormalities

Heavy abuse of alcohol during the prenatal period produces a wide range of facial abnormalities, cognitive dysfunction, and neurodevelopmental delay.[252] In 1973, Jones and Smith described the fetal alcohol syndrome as the pattern of anomalies associated with children born to alcohol dependent mothers.[253] The fetal alcohol syndrome is a combination of intrauterine or postnatal growth retardation, CNS dysfunction (reduced head circumference, developmental delay, mental retardation),

TABLE 21.4. Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) Scale.[245,246]

Clinical Effect	Question/ Observation	Scoring	Score
Nausea and Vomiting	Ask "Do you feel sick to your stomach? Have you vomited?"	0 No nausea and no vomiting 1 Mild nausea with no vomiting 4 Intermittent nausea with dry heaves 7 Constant nausea, frequent dry heaves, and vomiting	
Tremor	Arms extended and fingers spread apart.	0 No tremor 1 Not visible, but can be felt fingertip to fingertip 4 Moderate, with patient's arms extended 7 Severe, even with arms not extended	
Paroxysmal Sweats		0 No sweat visible 1 Barely perceptible sweating, palms moist 4 Beads of sweat obvious on forehead 7 Drenching sweats	
Anxiety	"Do you feel nervous?"	0 No anxiety, at ease 1 Mildly anxious 4 Moderately anxious, or guarded, so anxiety is inferred 7 Equivalent to acute panic states as seen in severe delirium or acute schizophrenic reactions	
Agitation		0 Normal activity 1 Somewhat more than normal activity 4 Moderately fidgety and restless 7 Paces back and forth during most of the interview, or constantly thrashes about	
Tactile Disturbances	Have you any itching, pins and needles sensations, burning sensations, numbness or do you feel bugs crawling on or under your skin?"	0 None 1 Very mild itching, pins and needles, burning or numbness 2 Mild itching, pins and needles, burning or numbness 3 Moderate itching, pins and needles, burning or numbness 4 Moderately severe hallucinations 5 Severe hallucinations 6 Extremely severe hallucinations 7 Continuous hallucinations	
Auditory Disturbances	Are you more aware of sounds around you? Are they harsh? Do they frighten you? Are you hearing anything that is disturbing to you? Are you hearing things you know are not there?"	0 Not present 1 Very mild harshness or ability to frighten 2 Mild harshness or ability to frighten 3 Moderate harshness or ability to frighten 4 Moderately severe hallucinations 5 Severe hallucinations 6 Extremely severe hallucinations 7 Continuous hallucinations	
Visual Disturbances	Does the light appear to be too bright? Is its color different? Does it hurt your eyes? Are you seeing anything that is disturbing to you? Are you seeing things you know are not there?"	0 Not present 1 Very mild sensitivity 2 Mild sensitivity 3 Moderate sensitivity 4 Moderately severe hallucinations 5 Severe hallucinations 6 Extremely severe hallucinations 7 Continuous hallucinations	
Headache, Fullness in Head	"Does your head feel different? Does it feel as if there is a band around your head?" Do not rate for dizziness or lightheadedness. Otherwise, rate severity.	0 Not present 1 Very mild 2 Mild 3 Moderate 4 Moderately severe 5 Severe 6 Very severe 7 Extremely severe	

TABLE 21.4.   (*Continued*)

Clinical Effect	Question/ Observation	Scoring	Score
Orientation and Clouding of Sensorium	"What day is this? Where are you? Who am I?"	0 Oriented and can do serial additions 1 Cannot do serial additions or is uncertain about date 2 Disoriented for date by no more than 2 calendar days 3 Disoriented for date by more than 2 calendar days 4 Disoriented for place and/or person Total CI WA-Ar score*:	

*Maximum possible score 67.

TABLE 21.5.   Abnormalities Associated with Fetal Alcohol Syndrome.*

Category	Abnormalities
Facial Anomalies	Short palpebral fissures, small eye opening, premaxillary zone changes (flat, thin upper lip, flat midface, flattened philtrum, low nasal bridge, short nose)
Growth Retardation	Low birth weight, poor weight gain, disproportional height-to-weight ratio
Neurodevelopmental	CNS anomalies (small head size, structural brain abnormalities), performance deficits (fine and gross motor movement, visual-spatial tasks, tandem gait, eye-hand coordination, intelligence)

*The partial fetal alcohol syndrome label applies to children born to alcohol dependent mothers when these children manifest only some components of facial anomalies along with complex cognitive and behavioral abnormalities (learning deficits, behavioral problems, memory and attention difficulties, poor school performance).

and facial dysmorphology (short palpebral fissures, abnormal philtrum, thin upper lip) in children with known exposure *in utero* to alcohol.[254,255] Table 21.5 lists the birth abnormalities associated with the fetal alcohol syndrome. Functional abnormalities associated with facial hypoplasia include dental malocclusions and malalignments as well as eustachian tube dysfunction. The degree of craniofacial abnormalities correlates to the persistence of cognitive dysfunction and growth retardation.[254] The alcohol-related neurodevelopmental disorder is applied to individuals born to mothers using moderate to heavy doses of ethanol who do not have the characteristic facial abnormalities associated with fetal alcohol syndrome. These individuals have more subtle neurobehavioral deficits than individuals with the fetal alcohol syndrome, particularly in the sequential manipulation of information linking environmental input with information retrieved from memory (i.e., working memory).[256] Sustained attention, focusing of attention in the presence of distractions and execution of appropriate responses are less affected.

There is no single mechanism responsible for the wide array of fetal abnormalities associated with maternal alcohol abuse. Risk factors include maternal age, socioeconomic status, ethnicity, genetic/metabolic factors, smoking, diet, and multiple-drug dependence.[257] The risk of the recurrence of the fetal alcohol syndrome in alcohol dependent mothers is high when the mother continues to drink, but not all children born to these mothers develop fetal alcohol syndrome. The incidence of fetal alcohol syndrome is about 4% among heavy drinkers.[258] The safe level of ethanol use during pregnancy is not clearly established, in part because of the variable definition of heavy drinking and the averaging of drinking behavior among groups in most epidemiologic studies on fetal alcohol syndrome. However, most of the serious teratologic effects of ethanol result from alcohol abuse rather than casual ethanol consumption. There is no conclusive evidence that the ingestion of ≤83 g ethanol/weekly (low-moderate ethanol consumption) during pregnancy causes adverse fetal effects (e.g., miscarriage, stillbirth, intrauterine growth restriction, prematurity, reduced birth weight, birth defects).[259]

Fetal alcohol syndrome is a diagnosis of exclusion. There are 2 sets of diagnostic criteria for the evaluation of children with suspected fetal alcohol syndrome including the 1996 Institute of Medicine (IOM) criteria and the highly-detailed Washington criteria[260] as well as a combination of the IOM criteria and the 4-Digit Diagnostic Code.[261] The IOM criteria developed the following 5 categories: fetal alcohol syndrome with confirmed maternal alcohol exposure, fetal alcohol syndrome without confirmed maternal alcohol exposure, partial fetal alcohol syndrome with confirmed maternal alcohol exposure, alcohol-related birth defects, and alcohol-related neurodevelopmental disorder. Although no specific parameters were originally established for each diagnostic category, recommended revi-

399

sions provide more specific details.[262] The presence of short palpebral fissures (≤3%), smooth or flattened groove between lip and nose called the philtrum (4 or 5 on the 5-point Likert scale of the lip-philtrum guide) and thin vermillion border of the upper lip (4 or 5 on the 5-point Likert scale) is highly suggestive of fetal alcohol syndrome.[261]

Carcinogenesis

The International Agency for Research on Cancer lists ethanol as a known human carcinogen (Group 1) as a result of epidemiologic studies causally relating alcohol drinking to increased incidences of cancers of the oral cavity and pharynx (2- to 5-fold), larynx (2- to 5-fold), esophagus (2- to 4-fold), and liver (2- to 3-fold).[263] Both chronic alcohol consumption and heavy cigarette smoking are strong risk factors for the development of upper aerodigestive tract cancers including the oropharynx, hypopharynx, larynx, and esophagus.[264] The epidemiologic data for a causal relationship between alcohol consumption and breast, colon, or stomach cancer is inconclusive. There is no direct relationship between lung cancer and ethanol consumption. Most of the statistically significant associations between ethanol and cancer involve patients with high alcohol consumption (>35 drinks/week).[265] These associations persist following adjustment for smoking, and there is no indication that the causal effect is dependent on type of beverage. Although there are insufficient data on the carcinogenicity of ethanol in animals, a metabolite (acetaldehyde) of ethanol is a known animal carcinogen.

DIAGNOSTIC TESTING

Conversion Factors

SI Units

$$1 \, mg/dL = 0.217 \, mmol/L$$

$$100 \, mg/dL = 21.7 \, mmol/L$$

$$1 \, mmol/L = 4.606 \, mg/dL$$

Mass/Volume

$$0.1\% \, w/v = 100 \, mg/dL$$

[100 mg/dL (whole blood) = 94.8 mg/100 g = 0.948 g/kg, assuming the relative density of 1 mL whole blood = 1.055 g. Typically, the relative whole blood density of 1.055 g/L is based on a blood water fraction of 80% (w/w), and the plasma density of 1.027 g/L is based on a plasma water content of 92% (w/w).]

$$1 \, ‰ = 1 \, mg/mL = 100 \, mg/dL$$

[Promile (‰) means part per thousand; this measure is commonly used in Germany, Poland, Netherlands, Lithuania, and Latvia.]

Volume/Volume

$$80 \, proof = 40\% \, v/v$$

Mass/Mass

1 mg ethanol/g whole blood =
   1 mg ethanol/g whole blood×1.055 g whole blood/mL
   = 1.055 mg/mL

Air

$$1 \, ppm = 1.88 \, mg/m^3$$

$$1 \, mg/L = 531 \, ppm$$

Analytic Methods

Techniques

Currently, gas chromatography (GC) with a flame ionization detector using either a direct injection technique or headspace sampling is the reference method of choice for ethanol determinations, particularly for legal purposes.[266] Headspace analysis measures the blood ethanol concentration in the equilibrated vapor phase above the liquid sample within a closed container maintained at constant temperature (50°C/122°F or 60°C/140°F). This procedure has relatively good specificity for ethanol; the presence of other alcohols (e.g., methanol, isopropanol) or acetone does not usually interfere with the quantitative determination of ethanol using this method.[267] The precision of this method is high with interlaboratory coefficients of variation ranging from about 3–5% and within laboratory <1%.[268] Total uncertainty (random plus systemic errors) using this method of alcohol determination ranges from ~4–6%.[269] Typically, BAC below 10 mg/dL is reported as negative because ethanol concentrations below this value are near the limit of quantitation for head space GC.[270]

Although GC is the reference method of choice for ethanol analysis, enzymatic ethanol assays are less costly and useful as more rapid screening methods. These enzymatic assays use the oxidation of ethanol to acetaldehyde by alcohol dehydrogenase with the concurrent

reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. The generation of NADH increases the absorbance at 340 nm that correlates to the ethanol concentration in the sample, whereas oxidized NAD^+ does not absorb UV light at 340 nm. Other substances (e.g., methanol, isopropanol, formaldehyde) that use NAD as a coenzyme to generate NADH potentially interfere with the enzymatic assays for ethanol. However, the ADH enzyme from yeast is more specific; additionally, the very slow oxidation of methanol at room temperature limits the interference of methanol in forensic and clinical practice. Elevated serum lactate and lactate dehydrogenase (LDH) also produce falsely elevated serum ethanol concentrations when analyzed by enzymatic methods. Although standard blood collection techniques use nonalcohol containing skin antiseptics, experimental studies indicate that the type of antiseptic does not significantly alter the results from blood ethanol determinations when using current enzymatic methods. In a study of 50 emergency department patients presenting with suspected ethanol intoxication, there was no statistically significant difference in the serum ethanol concentration between paired samples using 70% isopropanol or povidone-iodine impregnated cotton swabs as measured with the DuPont automatic clinical analyzer (ACA).[271] An experimental study using 100% ethanol as a skin preparation and the same clinical analyzer did not demonstrate an increased serum ethanol concentration unless the ethanol-containing sponge directly contaminated the needle during withdrawal.[272] The cross-reactivity of isopropanol with ethanol using the ACA was 3.9%. Consequently, the type of skin-cleansing agent used during collection of blood specimens should not affect the results of ethanol measurements determined with the ACA, when the correct phlebotomy technique is used.

Techniques for the qualitative determination of ethanol in urine specimens include adding volumes 10% potassium dichromate in 50% sulfuric acid to an equal volume of urine.[266] In a strongly acidic potassium dichromate solution, reduction of the yellow-orange chromic ion to the chromous ion changes the solution to blue-green; a green color indicates a positive test for alcohol. The Widmark method and modified Widmark methods (Cavett, Southgate and Carter) are based on the colorimetric changes associated with acidifying potassium dichromate solutions.[273] Older methods for the determination of ethanol concentrations include the use of the Conway cell where ethanol diffuses from the biologic fluid in the outer chamber to an acid dichromate solution in the inner compartment.[274] The change in the color of the reduced solution is analyzed spectrophotometrically. The presence of methanol, formaldehyde, and paraldehyde, but not acetone, interfere with this reaction leading to false high ethanol results. The dichromate solution remains bright orange in the absence of ethanol and these other compounds.

Enzyme immunoassays (DRI® EtG immunoassay, Thermo Fisher Scientific, Waltham, MA) are available for the detection of ethyl glucuronide in urine samples with lower limits of quantitation <0.1 mg/L.[275] Cutoffs typically are set at 0.5 mg/L to increase specificity. Case reports suggest that cross-reaction of this assay with chloral hydrate or metabolites (trichloroethyl glucuronide) may occur.[276] Detection of ethyl glucuronide in hair requires high sensitivity (pg/mg range), such as achieved by liquid chromatography/tandem mass spectrometry or gas chromatography/mass spectrometry (GC/MS). The limit of detection (LOD) for ethyl glucuronide in a 100-mg hair sample using liquid chromatography/tandem mass spectrometry after solid-phase extraction was 51 pg/mg.[277] Analysis of the same sample size by gas chromatography/electron impact/mass spectrometry after derivatization with pentafluoropropionic anhydride produces a LOD of 25 pg/mg with interassay precision <7%.[278] Using gas chromatography/negative chemical ionization/tandem mass spectrometry following solid-phase extraction and derivatization with perfluoropentanoic anhydride, the LOD and lower limit of quantitation (LLOQ) for ethyl glucuronide in a 30-mg hair sample are 3 pg/mg and 8.4 pg/mg, respectively. The bias (trueness) is <15% and <20% at the LLOQ.

SERUM/WHOLE BLOOD RATIO

ETHANOL.   Plasma and serum ethanol concentrations are essentially identical; therefore, these values are interchangeable.[279] However, the ethanol concentrations in serum and plasma averages at least 11–14% higher than the corresponding concentrations in whole blood samples from healthy individuals.[280] In a study of 235 healthy volunteers administered ethanol orally, the mean ratio of serum ethanol/whole blood ethanol was 1.14 ± 0.04 with a range of 1.04–1.26 as measured by head-space GC.[281] Consequently, blood samples from healthy individuals with a plasma or serum ethanol concentration >100 mg/dL reliably predicts a whole blood ethanol concentration exceeding 80 mg/dL.

The ethanol content of whole blood is a weighted average of the ethanol concentrations in plasma as well as various blood elements including erythrocytes, leukocytes, and platelets. Medical conditions (e.g., hypovolemic shock, dialysis, anemia, pregnancy, hyperlipidemia, dehydration) alter intracellular fluid balance and these conditions lower the ratio of plasma or serum ethanol to whole blood ethanol.[280] In a study of duplicate blood samples from 211 emergency department patients containing serum ethanol concentrations up to 622 mg/dL,

the mean serum/whole blood ethanol ratio was 1.16 (95% CI: 1.14–1.17).[282] There were several outliers resulting in a range of serum/whole blood ethanol ratios from 0.88–1.59. In a study of 17 hospitalized patients receiving IV ethanol, the mean plasma/whole blood ratio was 1.10 with a range of 1.03–1.24.[280] Consequently, a single serum ethanol concentration can correspond to a range of whole blood concentrations, and the conversion of a single serum ethanol concentration to a corresponding whole blood ethanol concentration requires the use of a range (e.g., 1.10–1.18) covering individuals with variable distribution of water between blood components.

ETHYL GLUCURONIDE/SULFATE. Analysis of whole blood measures the total amount of a substance in blood, whereas serum excludes the erythrocytes and leukocytes. In contrast to serum, plasma does not contain fibrinogen; however, fibrinogen is not known to bind drugs and therefore plasma and serum concentrations for ethyl glucuronide and ethyl sulfate are almost identical as noted above for ethanol. In a study of 13 serum and whole blood samples from patients admitted for ethanol detoxification, the median serum/whole blood ratio of ethyl glucuronide was 1.69 (range, 1.33–1.90), whereas the median ratio for ethyl sulfate was 1.30 (range, 1.08–1.47).[283]

STORAGE

ETHANOL.   Optimum storage conditions for the determination of postmortem ethanol concentrations include the collection of blood in an air-tight container containing 1–2% w/v sodium or potassium fluoride, particularly if analysis occurs more than a few days after autopsy. Significant variation in BAC from living individuals does not usually develop in blood specimens collected within 14 days of analysis, regardless of the storage conditions.[284] In a study of whole blood, plasma, and serum stored with and without preservatives at room temperature and under refrigeration (4°C/39.2°F), there was no statistically significant difference between type of collection tube or the storage condition during the 10-day study.[285] The blood ethanol concentration ranged from 60–90 mg/dL. The loss of ethanol from preserved, well-sealed samples stored at ambient temperatures is also small during the first month of storage.[286] However, loss of ethanol may occur during long-term storage of blood samples, particularly when multiple aliquots are removed from the container. Mechanisms of ethanol loss from stored samples include diffusion from the air (headspace) inside improperly sealed containers, ethanol metabolism by microorganisms, and temperature-dependent ethanol oxidation reactions.[287] Specimen

containers should contain small air-space to minimize evaporation of the ethanol. Temperature, fluoride concentration, tightness of the seal, and length of storage are the most important variables in loss of ethanol during storage.[288,289] The loss of ethanol from whole blood samples is minimal when stored at 4°C (39.2°F) in sealed containers with 1% sodium fluoride. The average monthly loss of ethanol from unopened whole blood samples stored at 4°C (39.2°F) with 1% sodium fluoride averaged about 0.2–0.3% per month over 1 year.[290] The ethanol loss was independent of initial BAC in the samples. Even at ambient temperature, the loss of ethanol from preserved samples over the course of 1 year at ambient temperatures is usually small (<10%).[291] In unopened blood specimens containing 0.36% w/v sodium fluoride and stored at room temperature, the mean loss of ethanol over 3.0 and 6.75 years was 19 mg/dL and 33 mg/dL, respectively.[292]

One study compared the change in ethanol concentrations of 32 paired, refrigerated samples of postmortem whole blood and vitreous humor.[293] The former was stored in 50-mL polypropylene tubes containing sodium fluoride as a preservative and potassium oxalate as an anticoagulant, whereas the vitreous humor was stored in 10-mL gray-top Vacutainer tubes containing 20 mg potassium oxalate and 25 mg sodium fluoride. Over 5–6 years the average reduction in ethanol concentration in the postmortem blood samples was 48% (absolute range, 10–290 mg/dL) compared with an average decline in ethanol concentration in vitreous humor samples of 9% (absolute range 0–40 mg/dL).

Frequent opening of the container increases the loss of ethanol. Following the inoculation of whole blood samples with 150 mg/dL ethanol, the loss of ethanol averaged about 10–20% during the month of storage at ambient temperatures despite the presence of 1% sodium fluoride.[294] The containers were opened 16 times during the 35 days of the study. The serum concentrations of ethanol were not significantly different over the course of the study. Microbial action in a blood specimen usually reduces the ethanol concentration in the sample. Strains of *Serratia marcescens* and *Pseudomonas* species are capable of metabolizing ethanol in blood samples containing 1% sodium fluoride stored at room temperature.

The production of ethanol in well-preserved blood samples is unlikely. In a study of BAC in blood samples preserved with sodium fluoride/potassium oxalate and stored at 4°C (39.2°F) for 1 year, significant increases in BAC did not occur in any of the samples.[295] Production of ethanol in nonpreserved, antemortem blood specimens may occur as a result of the fermentation by microbes, particularly in diabetic individuals with high blood glucose concentrations. Fermentation (i.e., the

metabolic conversion of glucose to ethanol) by most microbes is inhibited by 1% sodium fluoride with the exception of *Candida albicans*.[296] Experimental studies suggest that fermentation processes following direct inoculation or contamination with *C. albicans* depend on ambient storage temperatures. Under refrigerated conditions, no ethanol was formed from fermentation during 6 months of storage in blood samples preserved with 1% sodium fluoride and inoculated with *C. albicans*.[297] Experimental studies indicate that the storage of urine in 1% sodium fluoride at 0°C (32°F) prevents the formation and loss of ethanol from the urine sample.[298,299]

ETHYL GLUCURONIDE. Ethyl glucuronide remains fairly stable in urine samples in air-tight containers, when stored at 4°C (39.2°F) for 5 weeks; however, storage at room temperature in ventilated vials during this period resulted in some loss (mean, 37.5%; range, 30–80%) of ethyl glucuronide from the samples.[300] At the end of the study period, ethanol was no longer detectable in samples stored at room temperature in ventilated vials. There was no ethyl glucuronide formation in postmortem samples of blood or liver spiked with 100 mg/dL ethanol. False-negative results may occur in the analysis of ethyl glucuronide in heavily putrefied samples, particularly when stored at room temperature without preservatives.[301] The use of preservatives (chlorhexidine, potassium fluoride) improves the stability of ethyl glucuronide in samples stored at room temperature for 1 week.[302] Although ethyl sulfate is relatively stable in storage, experimental studies suggest that extreme conditions (e.g., putrefaction) for 1 week may cause some bacterial degradation of ethyl sulfate.[303]

Biomarkers

Ethanol diffuses into a variety of body fluids including the aqueous portion of the blood, bile, urine, saliva, cerebrospinal fluid, vitreous humor, and bone marrow.[304] Saliva is an alternative sample for the determination of ethanol, but studies on the correlation of saliva to blood are conflicting. In a study of volunteers ingesting 0.72 g ethanol/kg body weight, the mean saliva/blood ethanol ratio *during* the postabsorptive phase was 1.077 (95% CI: 1.065–1.088).[305] Experimental studies suggest that the salivary ethanol concentration correlates better to capillary blood than to venous blood.[306] Analysis of vitreous humor samples helps confirm the validity of blood ethanol samples because postmortem vitreous humor is relatively resistant to putrefaction processes.[307] Analyses of these specimens also provide information about the status of ethanol absorption (i.e., absorptive vs. elimina-

tion phase). Variation in the ratio of liver to blood ethanol concentrations is relatively large. Postmortem analysis of 71 cases with a blood ethanol concentration ≥40 mg/dL demonstrated a mean liver/heart blood ratio (± SD) of 0.56 ± 0.30 (range, 0–1.40).[308]

BREATH

The ethanol in expired air occurs almost exclusively as a result of the diffusion of ethanol from the bronchial circulation to conducting airways rather than from the alveoli.[309] Portable methods of breath ethanol detection provide rapid, simple, and noninvasive ways of estimating the arterial ethanol concentrations based on the ethanol concentration in expired air and a blood/breath ratio ranging from 2,000–2,300. The standard conversion value in the United States for forensic purposes is 2,100:1. The estimation of the blood/breath ethanol ratio depends on the extrapolation of Henry's law, which determines partition of a chemical between the liquid and gas phases at equilibrium based on constant pressure and temperature in a closed system. However, Henry's law provides only an estimation of the concentration of ethanol in blood from the vapor phase because the lungs are not a closed system. Consequently, the actual ratio varies substantially between individuals and within individuals as a result of a variety of physiologic factors (e.g., temperature, humidity, breathing pattern, etc.) that affect the equilibrium of ethanol between end-tidal gas and the blood in the bronchial circulation.[310] Additionally, the blood/breath ratio is lower during the absorption phase compared with the postabsorption phase. In the absorptive phase, the breath/blood ethanol concentration is a better measure of arterial ethanol concentrations and exposure of the brain to ethanol than venous ethanol concentrations. To minimize the effects of physiologic variables, sampling techniques require the use of breath samples obtained from deep expiration after at least 15 minutes of breathing ambient air of normal temperature.

PHYSIOLOGIC VARIABLES. In volunteer studies, hyperventilation decreases the BrAC, whereas breath holding increases the BrAC.[311] Increasing body temperature distorts the BrAC curve upward compared with the BAC. During an experimental study, the core body temperature of 9 male volunteers increased a mean of about 2.5°C.[312] The average increase of the BrAC above the BAC was approximately 8.6%/°C increase in core body temperature. Similar volunteer experiments on reducing the core body temperature indicated that the BrAC curve was displaced downward an average of 6.8%/°C.[313] Thus, alterations of core temperature change the likelihood of detecting ethanol concentrations, which exceed

legal limits. At these core temperatures (i.e., ± 2.5°C), the ethanol clearance did not change significantly.

Variables impacting on the accuracy of a BrAC measurement include tests performed with 15 min of the use of alcohol or alcohol-containing medications or products.[314,315,316] Other factors include arteriovenous differences in ethanol concentrations during the absorption phase, recent belching or vomiting, inadequate end expiratory specimen (i.e., poor cooperation), presence of obstructive pulmonary disease (FEV$_1$< 2L, FVC <2.6 L),[317] and poor technique or maintenance.[318] Experimental studies suggest that the presence of gastrointestinal reflux disease (GERD) does not significantly alter the BrAC curve during the postabsorptive phase.[319] Swallowing ethanol-containing products including beverages is unlikely to produce falsely high BrAC values when >15 minutes elapses between the ingestion and the testing. In a study of volunteers swallowing 10 mL of diluted gin (20% v/v ethanol), about 7% of the breath samples were positive as measured by the Intoxilyzer 5000™ (CMI, Inc., Owensboro, KY) in tests performed 10 minutes after ingestion.[320] The range of positive samples was 0.007–0.024 g% (7–24 mg ethanol/dL). Potential interfering substances include volatile compounds with enough vapor pressure to diffuse across the alveolar membrane and absorb infrared light at the same wavelength regions as ethanol; these substances include acetone, ether, isopropanol, methyl ethyl ketones, toluene, and methanol. Newer machines in the Intoxilyzer™ series (e.g., 5000EN) display an "interferent" message that appears with aborting of the test when the interferent concentration exceeds 0.01 g/210 L. However, case reports indicate that some interference may occur without detection. A 47-year-old intoxicated, suicidal man was found in a public park with a breath ethanol concentration of 0.288 g/210 L breath as analyzed by an Intoxilyzer 5000™ EN.[321] Analysis of his blood in the emergency department demonstrated a methanol concentration of 589 mg/dL and no detectable ethanol. The presence of ether in expired air may interfere with the analysis of BrAC, when analyzed by machines using a single wavelength of 9.5 μm to determine the presence of ethanol.[322]

Blood/Breath Ratio.   The mean blood/breath ratio of ethanol is approximately 2,400–2,450. In a study of 799 drivers apprehended for driving under the influence, the mean of the time-adjusted blood/breath ratio was 2,407 ± 213 (95% CI: 1,981–2,833) as measured by the Intoxilyzer 5000S™.[323] In a similar study using the Intoxilyzer 5000S™ in 18 healthy volunteers administered 0.4–0.65 g ethanol/kg over 20–30 minutes, the mean blood/breath ratio was 2,448 ± 540 (95% CI: 1,836–4,082).[324] Although the mean elimination rates in venous blood and breath samples were statistically different, the absolute differences were small (i.e., 0.157 ± 0.021 mg/g/h and 0.161 ± 0.021 mg/g/h, respectively). There was no statistical difference in the blood/breath ethanol ratio between men and women. The dynamics of ethanol exchange in the conducting airways produce a positively sloped plateau in the breath ethanol concentration that contributes to the dependence of breath ethanol concentrations on breathing pattern. At a constant flow rate, the breath ethanol concentration increases as an individual exhales into the breath analyzer. Consequently, ethanol breath measurements are approximations of the actual BAC.

The coefficient of variation for the blood/breath ratio ranges from approximately 9–19% as a result of both analytic and biologic factors.[325] The variability of the blood/breath ratio is substantially greater during the absorptive phase compared with the postabsorptive phase,[326] and the interindividual variation in BrAC is too large to accurately determine ethanol elimination rates between successive samples.[327] The breath ethanol measurement depends on instrumental, biologic, and procedural factors, and the variability increases as the blood ethanol concentration increases.[328] Interfering substances identified in the breath of drinking drivers with Intoxilyzer 5000S™ and Intoxilyzer 4011AS-A™ usually involve high concentrations of toluene, acetone, isopropanol, or methyl ethyl ketone.[329,330] Typically, the Intoxilyzer 5000S™ aborts the evidential breath test when these substances appear in the breath samples above a certain threshold value. Modern infrared instruments can distinguish ethanol from acetone and if necessary correct the result or abort the test.[331] Variability in the blood/breath ratio indicates that breath ethanol concentrations should be reported as such, and not as blood ethanol concentrations. Studies of populations of drivers suspected of driving under the influence suggest that the Intoxilyzer 5000S™ tends to underestimate the actual venous BAC. In a study of drivers arrested for operating a motor vehicle while intoxicated (mean BAC, 180 mg/dL, range, 0–338 mg/dL), the breath ethanol concentration was higher by >0.01 g/210 L than the corresponding BAC (<1 hour sampling difference) in approximately 67% of the samples.[332] Only 2% of the samples contained a breath ethanol concentration >0.01 g/210 L lower than the corresponding BAC. There are few data on the correlation of breath ethanol concentrations to BAC in populations with BAC <80–100 mg/dL. In many social situations, peak BrAC occurs within 0.45–1 hour of the cessation of drinking.[333]

ANTEMORTEM BLOOD

Although yeasts (e.g., *Candida albicans*) readily produce ethanol *in vitro*, the endogenous production of clinically significant quantities of ethanol in healthy adults is unlikely. In healthy adults and adults with diabetes, hepatitis, and cirrhosis, the typical range of ethanol in venous blood from endogenous ethanol production is about 0–0.08 mg/dL.[334] Consequently, the endogenous ethanol concentration in these conditions does not cause impairment. Rare case reports document the presence of BAC >80 mg/dL in Japanese individuals with serious yeast infections.[335] These individuals probably have genetic defects (e.g., aldehyde dehydrogenase-2) that cause the formation of higher concentrations of ethanol and acetaldehyde in the gut. Significant concentrations of endogenous ethanol can occur in children with short bowel syndrome. The formation of clinically significant concentrations of ethanol during rectal or vaginal candidiasis in healthy adults is highly unlikely.

ACUTE CLINICAL EFFECTS.   Ethanol consumption produces a dose-related depression of consciousness, and the BAC correlates generally to clinical signs, depending on several factors including the presence of tolerance, trauma, hypoglycemia, drug interactions, and preexisting diseases.[336,337] Table 21.6 correlates clinical symptoms with the BAC (whole blood) in a population without a history of substantial ethanol use. Signs of ethanol intoxication are less prominent in chronic drinkers compared with nondrinkers or occasional drinkers.[203] The clinical presentation of a tolerant patient at a given BAC is less predictable, and serum ethanol concentra-

tions of 400–500 mg/dL in these patients may not cause serious CNS depression or vital sign abnormalities.[338] In a series of 204 consecutive patients presenting to an emergency department with serum ethanol concentrations >400 mg/dL (mean, 467 mg/dL, range, 400–719 mg/dL), 88% of the patients were oriented to person, place, and time.[339] Although none of the 4 patients with serum ethanol concentration >600 mg/dL were alert and oriented, neither did any of these patients require measures of advanced cardiac life support (intubation, vasopressors).

Gross intoxication involves obvious impairment of perception, psychomotor tasks, motor coordination, reaction time, cognition, and judgment. These impairments usually produce easily observable signs of intoxication including stumbling, falls, breaking of objects, poor execution of motor tasks, increased laughter, agitation, and quarreling.[340] Typically, individuals displaying these signs have serum ethanol concentrations >150–200 mg/dL. However, many chronic drinkers with a serum ethanol concentration within this range can adequately perform simple and even moderately complex tasks, particularly if the tasks are not prolonged. Estimation of the serum ethanol concentration is difficult both for the drinking individual and the observer. In a study of 161 volunteers with BAC ranging from 0–295 mg/dL as measured by gas chromatography with flame ionization detector, only 31% of the participants estimated their actual BAC within 20 mg/dL.[341] There was a tendency for individuals with low BAC to overestimate their actual BAC and for individuals with a high BAC to underestimate their BAC. Experimental studies suggest that observers tend to underestimate the

TABLE 21.6. Correlation of Blood Ethanol Concentration with Acute Clinical Effects in Individuals without History of Heavy Ethanol Use.[266]

BAC*	Clinical Stage	Signs and Symptoms
10–50	Sobriety	Behavior normal with observable effects. Minimal changes potentially present by special testing.
30–120	Euphoria	Mild euphoria with increased sociability, loquaciousness, self-confidence. Some decreased inhibitions and diminution of attention and judgment. Reduced performance on complex psychomotor tests.
90–250	Excitement	Emotional instability with decreased inhibitions, loss of critical judgment, impairment of memory and comprehension. Some loss of muscular coordination, increased reaction time, and decreased sensory response.
150–300	Confusion	Disorientation, mental confusion, dizziness, exaggerated emotional states (fear, anger, grief), disturbance of vision (diplopia, distortion of forms and motion), reduced pain sensation, impaired balance and coordination, slurred and/or incoherent speech.
270–400	Stupor	Apathy, marked decreased responsiveness, marked lack of muscular coordination with inability to stand, incontinence, impaired consciousness (lethargy, stupor).
350–500	Coma	Unconsciousness, depressed reflexes, respiratory depression, aspiration.
>450–500	Death	Respiratory insufficiency.

*Whole blood ethanol concentration in mg/dL.

BAC, and that there is substantial variation between observers.[342]

Chronic ethanol users often do not demonstrate signs of gross ethanol intoxication even when the serum ethanol concentration is 200–400 mg/dL. In particular, recognizing chronic alcohol abusers with a serum ethanol concentrations >150–200 mg/dL is frequently difficult for trained health professionals (physicians, alcohol counselors) despite the use of observation instruments (e.g., alcohol symptoms checklist).[343] In a study of 110 consecutive admissions to a detoxification unit, patients were evaluated for intoxication based on measures of vision, pupil size, speech, verbal comprehension, coordination, and the ability to dress.[28] There was no clinical evidence of intoxication in 24% of 54 patients with a serum ethanol concentration >200 mg/dL. Although none of the 26 patients with serum ethanol concentrations >300 mg/dL exhibited normal responses to all field sobriety tests, 11 patients had normal speech and 6 patients had normal gait. In a study of 76 emergency department patients that admitted to recent ethanol ingestion and were released after minor trauma, the mean blood ethanol concentration was 268 mg/dL with a range of 120–540 mg/dL as measured by gas chromatography.[344] The criteria for release was whether the patient was ambulatory, oriented, able to calculate change for money, had no obvious neurologic abnormalities, and was capable of assuming his or her own care.

A serum ethanol concentration exceeding 450–500 mg/dL is usually considered a potentially fatal concentration in individuals without a history of chronic ethanol use, depending on the presence of supportive care. A 30-month-old child with an initial blood ethanol concentration of 455 mg/dL after ingestion up to 16 oz of wine was comatose when admitted to the emergency department and evaluated by the clinical laboratory (method not reported).[345] The victim survived without intubation and recovered without sequelae. Two case reports document the presence of high serum ethanol concentrations (1,121 mg/dL, 1,510 mg/dL) in patients with slight confusion.[346] Both these patients were alert and able to respond to questions. Several patients were deeply comatose with serum ethanol concentrations ranging from 700–1,500 mg/dL; these patients survived without sequelae.[114,115,347] These chronic ethanol abusers survived without sequelae.

Death occurs from respiratory depression at ethanol concentrations in blood samples exceeding 500 mg/dL. Alcohol dependence is common in fatalities associated with BAC exceeding 300 mg/dL.[348] Chronic alcohol abusers may exhibit few clinical signs of intoxication at ethanol concentrations in blood from 300–450 mg/dL, and these patients may survive ethanol concentrations exceeding 500 mg/dL if they do not aspirate.[338]

ESTIMATING BLOOD ETHANOL CONCENTRATION. Calculation of the serum ethanol concentration based on the serum osmolality or the presence of an osmolal gap *approximates* the blood ethanol concentration.[349] However, the presence of other, potentially unmeasured solutes limits the accuracy of these determinations, particularly in trauma patients.[350] Accurate estimation of blood ethanol concentrations based on antegrade or retrograde extrapolation requires the acceptance of several assumptions about elimination rates, status of absorption/distribution, and an accurate measurement of the blood ethanol concentration.

Antegrade (Forward Estimation). The accuracy of forward estimations of BAC depends on the presence of the following factors: 1) the forward estimate of the BAC at time "t" occurs during the postabsorptive phase, 2) known clearance rate or range of clearance rates that probably includes the true clearance rate, 3) there is 100% availability of the dose of ethanol, 4) the metabolic rate is constant from the beginning of drinking, and 5) the true volume of distribution is known. Under these conditions Equation 21.7 defines the BAC at time t,

$$BAC_t = BAC_0 - (\beta_{60} \times t) \qquad \text{(Equation 21.7)}$$

where BAC_t = blood alcohol concentration (mg/dL) at time t since drinking started; BAC_0 = theoretical BAC (mg/dL) at time 0 if total dose of ethanol is distributed instantaneously throughout total body water; β_{60} = blood alcohol clearance rate (mg/dL/h); t = time between start of drinking and time in question (h).

The theoretical BAC_0 is equivalent to the C_0 defined by Widmark and illustrated in Figure 21.1. This value represents the theoretical BAC if the entire dose was absorbed and distribution in total body water, which is the volume of distribution (V_D) into which the dose of ethanol diffuses. Consequently, the value of BAC_0 depends on the ethanol dose, the amount of first-pass metabolism, age, gender, total body water, and the fraction of water in the blood as defined by Equation 21.8,

$$BAC_0 = D/TBW \times F_w \times 100 \qquad \text{(Equation 21.8)}$$

where D = dose of ethanol (g); TBW = total body water volume (L), F_w = fraction of water in the blood (v/v).

The ethanol elimination rate in whole blood samples from healthy, nontolerant adults typically ranges from about 8–25 mg/dL/h with a mean of approximately 15–16 mg/dL. The actual rate may vary during the descending portion of the blood alcohol curve as a result of environmental factors and prevailing ethanol concentration. Sources of error in the forward and backward

estimation of the BAC involve inaccurate elimination rates, variability in the onset of the postabsorptive phase, and inappropriate values of V_D. Determination of the onset of the postabsorption phase depends on a number of variables including the type of beverage, the drinking history, and the consumption of food. When the drinking pattern involves the ingestion of a few drinks at the end of a drinking binge over a period of hours, the delayed absorption of the last few drinks probably does not alter the estimation of the already high BAC.[351] The V_D depends on total body water, which varies with body habitus, sex, and age.[352] Compared with other tissues, ethanol is relatively excluded from fat with a fat-water partition coefficient of 0.018. The volume of distribution (i.e., total body water) decreases with age and obesity, resulting in relatively higher blood ethanol concentrations for the same dose at a given body weight compared with younger individuals with ideal body weights.

In general, 1 ounce of 80-proof liquor (e.g., 1 glass of wine, 1 shot of whiskey, 1 can of beer) raises the blood ethanol concentration about 20–25 mg/dL for 100% bioavailability of the ethanol dose. Factors affecting the extrapolation of the blood ethanol concentration after a specific amount of ethanol include an accurate time of consumption, time for complete absorption, variability of test method (i.e., typically ± 5%), time between incident and withdrawal of the blood sample, storage and collection methods, variability in volume of distribution, and position on the blood alcohol curve (i.e., greater on ascending than descending portion).[353] There are few clinical data on the effect of trauma and blood loss on the blood alcohol curve. The rapid alteration of fluids in the intravascular space during hemorrhagic shock theoretically increases the whole blood ethanol concentration as the intravascular water content increases relative to the erythrocyte content.[354] However, serum ethanol concentrations do not vary substantially. Although trauma potentially can reduce ethanol metabolism by reducing hepatic blood flow, studies so far have not detected major changes in the blood alcohol curve as a result of infusion of fluids during the resuscitation of multiple trauma victims except during periods of severe hypotension or poor hepatic perfusion.[355] In a volunteer study of 10 individuals ingesting ethanol to produce BAC of 105–210 mg/dL, the removal of 200–500 mL blood (4.5–8.7% total blood volume) did not alter the blood alcohol curve when compared with the same individuals under control conditions.[356]

Retrograde Extrapolation (Back Estimation). Estimating blood ethanol concentrations at a time prior to analysis requires careful evaluation of a number of factors that affect the blood ethanol curve. These factors include the phase of the blood alcohol curve during the time in question, the elimination rate during the time in question, the time of the last drink, the type of sample (serum, whole blood, breath), interindividual variation in the elimination rate, the time of reaching the peak BAC, and any short-term fluctuations in the elimination rate.[65] The accurate estimation of the BAC at a time prior to the collection of a blood sample requires the presence of several of the following conditions: 1) the time of the estimated BAC and the blood sample must occur during the postabsorptive phase of the blood alcohol curve, 2) the ethanol elimination rate must be known or estimated as a range of probable values, and 3) the time of the sample and the estimated BAC must occur during the linear declining phase of the elimination curve (i.e., BAC = 20–200 mg/dL). In general, studies of healthy, fasted volunteers indicated that the blood alcohol curve is linear over the period 2–7 hours after rapid consumption of ethanol.[357] However, the onset of the postabsorptive phase varies with a variety of factors including food, medications, drinking pattern, and physiologic abnormalities (see Toxicokinetics-Absorption). Attempted back extrapolation of the BAC during a period of continuing ethanol absorption (i.e., the absorption and distribution phases) causes serious errors in the estimation of the BAC.[358]

Timing of Postabsorptive Phase. Most absorption of ethanol in *fasted* individuals occurs within 60 minutes of starting to drink; therefore, the mean peak ethanol usually occurs within 1 hour after the cessation of drinking, particularly after a period of heavy drinking. However, some absorption may occur after reaching the peak ethanol concentrations. Drinking scenarios during experimental settings frequently differ from those encountered during real-world social situations. Factors that affect the timing of the postabsorptive phase include the dose and concentration of ethanol, the rate of consumption, the timing and type of food consumed near the time of drinking, age, weight, sex, and race. In an experimental study of 54 beer-drinking participants, who drank variable amounts of beer as fast as possible (range, 20–103 minutes) beginning about 3 hours after a light meal, the mean peak BAC was about 132 mg/dL (range, 52–254 mg/dL), and the mean time between the cessation of drinking and the peak BAC was approximately 1 hour with a range of about 0.3–2 hours.[359,360] Consequently, the postabsorptive phase usually begins about 1–2 hours after drinking ceases in individuals, who have not consumed a meal within several hours before drinking starts. The peak blood ethanol concentration may also occur within approximately 1 hour of cessation of drinking of relatively small quantities of beer. The ingestion of 660 mL of light beer (about 3%

w/v ethanol) by 9 volunteers produced a mean time to peak capillary ethanol concentration of approximately 32 minutes (range 30–50 minutes).[361] Because ethanol absorption continues after peak BAC, retrograde extrapolation of the BAC to the period within 2 hours after drinking stops produces considerable variation with the true BAC, even in fasted individuals.[104] Therefore, retrograde extrapolation should be limited to the portion of the blood alcohol curve 2 hours after the cessation of drinking. Cross-sectional studies suggest that a majority of drivers with BAC exceeding 100 mg/dL are in the postabsorptive phase at the time of the accident. In a study of 129 fatalities, about 67% of the drivers were in the postabsorptive phase at the time of the accident based on a urine/blood ethanol ratio exceeding 1.2.[362]

Elimination Rate.   The accuracy and precision of retrograde and antegrade extrapolations depend on the assumed rate of ethanol elimination from blood. In a study of healthy fasted volunteers, the use of the actual mean plasma elimination rate (18.6 mg/dL/h) to extrapolate plasma ethanol concentrations during the postabsorptive phase produced small mean errors, but there was substantial interindividual variation in the difference between estimated and actual plasma ethanol concentrations (-28 mg/dL to +21 mg/dL).[357] The use of bracketing plasma elimination rates (15 mg/dL/h, 23.8 mg/dL/h) produced large mean errors between estimated and actual plasma ethanol concentrations as well as large interindividual variations in the retrograde extrapolations. Consequently, calculations involving retrograde extrapolation should include a sufficiently broad range of elimination rates (e.g., 10–25 mg/dL) that likely includes the individual's elimination rate as well as a mean rate of elimination (13–17 mg/dL/h). Although there are theoretical reasons for assuming that the ethanol elimination rate decreases during trauma and shock, there are limited clinical data to support this notion. A German study did not detect substantial changes in the ethanol elimination rate between patients with traumatic intracranial lesions and patients with torso injuries.[363] However, this study did not separate patients by the severity of shock.

Ethanol elimination rates of selected populations (e.g., suspected drunk drivers) and the general population may differ because of the relative overrepresentation of alcohol abuse in the former group. In a study of drivers apprehended in the Netherlands for driving under the influence, the mean elimination rate (whole blood) for drivers with BAC <250 mg/dL was approximately 21 mg/dL/h with a range of 11–57 mg/dL/h.[364] For the subset of 197 drivers with an initial BAC ranging from 50–99 mg/dL, the mean elimination rate was

20 mg/dL (range, 10–36 mg/dL). At least 2% of the drivers were not in the postabsorptive phase of the blood alcohol curve based on rising BAC between the 2 blood samples. Compared with this study, the mean ethanol elimination rates in whole blood from healthy, fasted volunteers during experimental studies using BAC near 100 mg/dL are lower (11–17 mg/dL/h) with substantially lower upper ranges (i.e., <30 mg/dL/h).[111,117,365] Low elimination rates calculated from 2 blood samples suggests that the sampling occurred near the peak of the blood-alcohol curve, which may be on a plateau as a result of delayed gastric emptying (e.g., after food). The elimination rate of ethanol is nonlinear at very low BAC (i.e., <10–20 mg/dL).[116]

ALCOHOL DEPENDENCE

Recall bias produces substantial underreporting of ethanol consumption. Despite careful sampling and assessment of ethanol intake, surveys based on self-reported history account for only about 40–60% of the known ethanol consumption of a country or region.[366] No laboratory test is reliable enough to support the diagnosis of alcohol dependence without confirmation from other sources.[367] Questionnaires depend on the awareness and willingness of the patient to acknowledge the negative effects of drinking, and the questionnaire and serum biomarkers of excessive ethanol consumption probably detect ethanol abuse in overlapping populations.[368] Consequently, questionnaires used in combination with biomarkers (MCV/GGT, CDT/GGT) may be the most sensitive tests for the detection of excessive drinking.

Serum ethanol concentrations are not sensitive tests for the detection of alcohol abuse.[369] Popular questionnaires on the detection of alcohol dependence include CAGE, MAST (Michigan Alcoholism Screening Test), and AUDIT (Alcohol Use Disorders Identification Test—World Health Organization). Common biochemical markers of ethanol abuse include gamma glutamyl-transferase (GGT), aspartate aminotransferase (AST), and erythrocyte mean cell volume (MCV). Newer biomarkers of excessive ethanol use include serum mitochondrial AST, carbohydrate-deficient transferrin (CDT), serum 5-hydroxytryptophol, acetaldehyde adducts, and β-hexosaminidase. Of these newer markers, only the serum CDT offers some limited advantages over older biomarkers because of much higher specificity.

QUESTIONNAIRES. Both the CAGE questionnaire (Table 21.7) and the Brief Michigan Alcoholism Screening Test (Table 21.8) are useful screening tests for covert alcohol abusers, particularly in young, male

patients in a general hospital psychiatric ward. However, these questionnaires are less-sensitive tests for the detection of alcohol dependence in the general community or in populations containing large numbers of female or elderly patients.[370] Both the CAGE and MAST questionnaires involve abnormal past drinking behavior (morning drinking, alcohol-related problems), but these 2 questionnaires probably cover different aspects of unsafe drinking as reflected in the fact that persons testing positive on one questionnaire frequently do not test positive on the other.[371] In addition to abnormal drinking behavior, the AUDIT questionnaire (Table 21.9) includes the level and frequency of recent (i.e., past year) ethanol consumption as well as adverse consequences of drinking. The AUDIT questionnaire was designed to identify heavy drinkers, and this questionnaire specifically seeks information about alcohol consumption, alcohol-related problems, adverse psychologic reactions, and symptoms of dependence over the past year.[372] Any positive response to the 4 questions on the CAGE questionnaire indicates the need for a more detailed clinical assessment for alcohol dependence. The major criticism of the CAGE questionnaire is lack of

specificity for individuals highly concerned about the health and religious implications of ethanol use as well as the failure to detect recent heavy drinking, particularly in women and in the elderly.[373,374] The sensitivity and specificity of the CAGE questionnaire for the detection of excessive ethanol consumption ranges from

TABLE 21.7. CAGE Questionnaire.*

1. Have you ever felt you should <u>C</u>ut down on your drinking?
2. Have people <u>A</u>nnoyed you by criticizing your drinking?
3. Have you ever felt bad or <u>G</u>uilty about your drinking?
4. Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover (<u>E</u>ye-opener)?

*One positive answer indicates the need for follow-up, whereas 2 or more affirmatives indicates alcohol dependence.

TABLE 21.9. Alcohol Use Disorders Identification Test (AUDIT) Questionnaire.*

1. How often did you have a drink containing alcohol in the past year?
2. How many drinks containing alcohol did you have on a typical day when you were drinking in the past year?
3. How often did you have 6 or more drinks on one occasion in the past year?
4. How often during the last year have you found that you were not able to stop drinking once you had started?
5. How often during the last year have you failed to do what was normally expected from you because of drinking?
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
7. How often during the last year have you had a feeling of guilt or remorse after drinking?
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
9. Have you or someone else been injured as a result of your drinking?
10. Has a relative or friend or doctor or other health care worker been concerned about your drinking or suggested you cut down?

*Responses are rated 0 to 4: 0 = never, 1 = less than monthly, 2 = monthly, 3 = weekly, and 4 = daily or almost daily. Almost all alcohol abusers score 10 or above. The maximum score is 40.

TABLE 21.8. Brief Michigan Alcoholism Screening Test (MAST) Questionnaire.*

Question	Yes -Points	No -Points
Do you feel you are a normal drinker?	0	2
Do friends or relatives think you are a normal drinker?	0	2
Have you ever attended a meeting of Alcoholics Anonymous?	5	0
Have you ever lost friends or girlfriends/boyfriends because of drinking?	2	0
Have you ever gotten into trouble at work because of drinking?	2	0
Have you ever neglected your obligations, your family, or your work for 2 or more days in a row because you were drinking?	2	0
Have you ever had delirium tremens (DTs) or severe shaking, or heard voices or seen things that weren't there after heavy drinking?	2	0
Have you ever gone to anyone for help about your drinking?	5	0
Have you ever been in a hospital because of drinking?	5	0
Have you ever been arrested for drunk driving or driving after drinking?	2	0

*Score ≥6 suggests the diagnosis of alcohol-use disorder.

about 60–95% and 40–95%, respectively, depending on the particular population assessed.[367,375,376] For example, the CAGE questionnaire is relatively insensitive in primary care populations containing large numbers of Caucasian women.[377] The original MAST questionnaire contained 25 questions, and there are several modifications including the Brief MAST (Table 21.8). The sensitivity of the original MAST questionnaire for the detection of alcohol dependence ranges up to 85%,[378] but the length of the questionnaire limits the clinical usefulness of this test for the general practitioner. The sensitivity and specificity of the AUDIT questionnaire ranges from about 80–95%.[379]

BLOOD TESTS.   Laboratory markers of excessive ethanol consumption potentially help identify alcohol dependent patients. However, no laboratory marker is reliable enough alone to diagnosis alcohol dependence, and the diagnosis of alcohol dependence requires the combination of physician interview, questionnaire, and biomarkers.[380] Typically, these biomarkers indicate prolonged heavy ethanol consumption (≥5 standard drinks/day for 5 days or more/week); these markers usually return to normal values within several weeks of abstinence. The usefulness of biomarkers (GGT, CDT, MCV) remains controversial because of the low correlation of biomarkers to alcohol consumption and the wide range of normal values in the nondrinking population.[381] Both the CDT and GGT are sensitive markers for the excessive use of alcohol, although GGT is less specific than CDT.[382] The BAC alone is not a sensitive screening test for alcohol dependence or problem drinking. In a cross-sectional study of 86 drinking drivers, a BAC >150 mg/dL identified only approximately one-third of problem drinkers in the sample.[383]

Gamma-Glutamyl Transferase (GGT). Gamma-glutamyl transferase is a membrane-bound enzyme that catalyzes the transfer of the γ-glutamyl moiety of glutathione to peptide acceptors. GGT is a sensitive marker of ethanol-induced liver damage in the absence of biliary stasis, but specificity of this biomarker is limited by elevation of GGT in some patients with pancreatitis, prostate disease, diabetes mellitus, obesity, other liver disease, or use of some drugs (anticoagulants, antiepileptics).[384] Although the serum GGT does discriminate between alcohol-induced liver disease and other hepatic disease, about 75% of established alcohol abusers have elevated serum GGT concentrations with a range of 60–90% depending on the comparison population.[385] The initial elevation of serum GGT in chronic ethanol abusers results from enzyme induction rather than hepatic damage; therefore, the serum GGT may be the only hepatic enzyme elevated during the early stages of

alcohol dependence.[386] The sensitivity of the serum GGT as a marker for alcohol dependence drops substantially (<25–50%) when screening patients in the primary care setting where the population contains large numbers of women and healthy young (<30 years of age) male, episodic drinkers.[387,388] The serum GGT rarely increases in persons under the age of 30 years despite their heavy consumption of ethanol. The presence of drug interactions, obesity, medications (barbiturates, anticonvulsants, anticoagulants), and hepatobiliary disease limits the specificity of the serum GGT.[389] The serum half-life of the GGT is about 2–4 weeks, and the serum GGT returns to normal in alcohol abusers during the first 2 months of abstinence.[387] Potential confounders of detecting alcohol dependence by elevated concentrations of GGT include obesity and smoking.[390]

Serum Carbohydrate-deficient Transferrin (CDT). Carbohydrate-deficient transferrin (CDT) is a component of serum transferrin with an abnormally high isoelectric point that results from changes in the carbohydrate composition of these chains in the presence of chronic ethanol consumption. In general, this biomarker has similar sensitivities as other serum biomarkers of chronic ethanol consumption, but CDT is somewhat more specific than GGT.[391] The serum CDT has a high sensitivity (80%) and specificity (80–95%) for detecting alcohol dependence in men, but the sensitivity of this test for detecting heavy drinking (>60 g/day) in the general population and in women is relatively low (12–45%).[392] The specificity of serum CDT for alcohol abuse is relatively high with some rare hepatic diseases (hepatocellular carcinoma, chronic hepatitis C, chronic active hepatitis, primary biliary cirrhosis) and the genetic D-variant of transferrin-limiting specificity.[393] In general, the use of CDT for the detection of alcohol dependence in the general population does not offer an advantage over the use of GGT, particularly in women. The main advantage of using the serum CDT over serum GGT is the monitoring of patients for increased ethanol consumption during abstinence or in the detection of alcohol dependence in patients with liver disease, particularly changes of the baseline GGT in men.[367] Elevation of the CDT requires at least the average daily intake of >4–6 drinks over the preceding 1–2 weeks.[394] In large, multicenter, community-based populations, the CDT is probably not significantly better than the GGT in detecting high- or intermediate-risk alcohol consumption, particularly in women.[395]

Serum Aminotransferases (AST, ALT).   Although elevation of the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) often occurs in alcohol abusers, the sensitivity of these tests for the

detection of heavy ethanol consumption is relatively low (25–60% and 15–40%, respectively).[367,396] The amount of AST and ALT elevation depends on the extent of hepatic damage and the timing of ethanol intake. Acute ethanol consumption (3–4 g/kg body weight) may increase the concentration of these 2 markers within 1–2 days after ingestion by healthy individuals. The AST/ALT ratio >1.5–2.0 is highly suggestive of alcoholic hepatitis, particularly if these enzymes do not exceed 400 IU/L.[397]

Mean Corpuscular Volume (MCV). The increases in the MCV correlates to the amount and frequency of ethanol consumption, but generally the ingestion of >60 g ethanol daily for >1 month is necessary to increase the MCV above the reference range.[398] The MCV is less sensitive (i.e., 40–50%) than GGT in alcohol abusers, particularly in the primary care setting.[399] However, a variety of diseases increase the MCV including hemolysis, vitamin deficiencies (B_{12}, folic acid), hypothyroidism, nonalcoholic liver disease, smoking, and medications (anticonvulsants, azathioprine, zidovudine). The presence of both elevated concentrations of GGT and MCV increases the likelihood of alcohol dependence. Some individuals may ingest large doses of ethanol (>60 g/day) for a prolonged period without developing alteration of the MCV or hepatic aminotransferases.[400]

HAIR. Fatty acid ethyl esters (FAEE) are minor, non-oxidative metabolites of ethanol biotransformation. These compounds are formed by the etherification of fatty acids or fatty acyl-CoA by fatty acid ethyl ester synthase or acyl CoA/ethanol *O*-acyltransferase, respectively; almost all tissue contains FAEE synthase activity. The dominant fatty acid ethyl ester is ethyl oleate followed by ethyl palmitate with substantially lower concentrations of ethyl myristate and ethyl stearate. These compounds are eliminated from blood within 24 hours.[401] Several studies suggest that the concentration of fatty acid ethyl esters in scalp hair is a biomarker of chronic ethanol consumption. In studies of chronic alcohol abusers and teetotalers, the total concentrations of these 4 FAEE in scalp hair above 1 ng/mg indicated chronic excessive ethanol consumption, whereas ≤0.4 ng/mg indicates abstinence.[402,403] However, there is large inter-individual variation in the deposition of FAEE in hair, and drinking histories do not correlate well to the concentrations of these compounds in hair samples. Furthermore, the use of cosmetic products (deodorants, hair spray, hair lotion) with very high concentrations of ethanol may produce false-positive tests (i.e., FAEE > 1 ng/mg hair).[404]

Ethyl glucuronide is detectable by high performance liquid chromatography/tandem mass spectrometry with LLOQ in the range of 0.05 ng/mg (LOD, 0.025 ng/mg).[405] In general, studies of alcohol abusers and teetotalers suggest that the mean ethyl glucuronide concentration in hair correlates to the daily intake of ethanol; however, the range of ethyl glucuronide concentrations is relatively large.[406,407] Although external contamination and differences in melanin content are unlikely to affect ethyl glucuronide concentrations in hair, some differences occur in the deposition of ethyl glucuronide in hair at different sites on the body. Analysis of paired hair samples suggest that higher ethyl glucuronide concentrations may occur in pubic hair and sites other than scalp hair when compared with scalp hair samples.[408,409]

POSTMORTEM SAMPLES

Ethanol distributes into tissues antemortem based on water content. Therefore, postmortem changes in ethanol concentrations may result from changes in blood water content. Under certain conditions during decomposition, postmortem production of ethanol may also occur. A number of factors affect the postmortem redistribution of ethanol including the postmortem interval between death and sampling (>10 hours), ambient temperature, the presence of microorganisms in the body, chest and abdominal trauma, location and quality of the sample collection, diffusion of ethanol from the stomach to pericardial fluid, and diffusion of aspirated ethanol into cardiac blood.[410] Several of these factors increase the concentration of ethanol in chest fluid; therefore, the ethanol concentration in fluid drawn from the chest cavity does not reliably reflect the BAC at the time of death.[411]

BLOOD. Case series of postmortem examination of fatalities in alcohol abusers indicate that alcohol and drugs are common causes of death in addition to natural causes (coronary artery disease, pneumonia, intracranial lesions, trauma).[412] Based on postmortem examination and toxicologic analysis of 73 alcohol abusers, death was attributed to the following causes: nonethanol-related drugs, 19%; alcohol/drug combination, 15%; acute ethanol intoxication, 8%; and alcoholic ketoacidosis, 7%.[413] The causes of death for the other alcohol abusers in these data were not attributed to drugs or ethanol. The presence of postmortem ethanol does not necessarily imply the chronic use or abuse of ethanol.[414] Postmortem ethanol concentrations potentially result from several sources including ethanol administration during the period immediately before death, diffusion from the stomach or aspirated vomitus after death, or from the postmortem formation of ethanol. The concentration of ethanol necessary to cause death depends on several factors including

tolerance, genetic susceptibility, and the presence of other CNS depressants. In postmortem blood samples from patients dying of acute ethanol intoxication in the absence of the detection of other drugs or causes of death, the majority of the blood ethanol concentrations range from approximately 300–450 mg/dL.[415,416] The determination of acute ethanol intoxication as the cause of death requires careful evaluation of the events surrounding death including evidence of asphyxiation, aspiration, and hypothermia. Average postmortem blood ethanol concentrations of alcohol abusers dying of chronic ethanol abuse are substantially lower (i.e., median, ~150 mg/dL) than the blood ethanol concentration from fatal, acute ethanol intoxication (360 mg/dL). Deaths in chronic alcohol abusers are usually the result of the presence of serious underlying medical diseases (e.g., cardiomyopathy, hepatitis, cirrhosis, liver failure, pancreatitis).[417]

Factors to consider when evaluating postmortem ethanol concentrations include the site and method of sample collection, time after death and condition of body during sample collection, storage conditions and preservatives (e.g., sodium fluoride), condition of the sample (desiccation, evidence of putrefaction including smell, color, fluidity, and consistency), and analytic procedures (method, time interval between collection and analysis).[418] Both water content and ethanol concentrations in blood samples tend to decrease after death, particularly with long postmortem intervals.[419] In living humans, the average water content of fresh whole blood is about 80% w/w (84% w/v assuming a density of 1.05 g/mL for whole blood) with the average for men being slightly less than women because of the lower female hematocrit.[270] Ethanol is hydrophilic; therefore, this substance distributes easily into total body water with relatively small differences between sampling sites after distribution is complete. However, some differences in postmortem ethanol concentrations between different sampling sites will occur as a result of differences in water distribution from postmortem redistribution (clotting, collapsed vessels). Because ethanol distributes to a greater extent into serum than into red blood cells, the ethanol concentration in blood samples will vary depending on the hematocrit. Hemorrhagic shock increases the blood water content and therefore the blood ethanol concentration as a result of the influx of extracellular fluid and rapid equilibrium of alcohol between liquid and solid parts.[410] However, the correction factor is relatively small (i.e., 80% × normal body water/existing water content). Trauma also potentially complicates the interpretation of intrathoracic ethanol specimens, particularly extensive GI trauma (e.g., aviation accidents). The ethanol concentrations in a majority of transthoracic samples *approximate* the ethanol

concentration in heart blood.[420] However, postmortem diffusion of gastric ethanol during decomposition or direct contamination from a ruptured stomach can falsely elevate the ethanol concentration in transthoracic fluid and heart blood, particular in deaths occurring shortly after a period of heavy ethanol consumption.[421] In a retrospective study of 6,000 postmortem cases, 8 of the 19 cases with BAC >500 mg/dL in heart blood resulted from truncal trauma, aspiration, or postmortem contamination.[422]

Ethanol Production. Fermentation of glucose by certain fungi as well as decomposition of protein by specific bacteria leads to production of ethanol, particularly in anatomic sites with high glucose content (e.g., liver, blood, muscle). Many postmortem blood samples contain large amounts of bacteria or yeast as contaminants, whereas microbial contamination is less common in the intact vitreous humor.[423] Many yeast (*Candida albicans, Saccharomyces cerevisiae*), gram-positive bacteria (*Bacillus* spp., *Staphylococcus aureus*, *Streptococcus faecalis*, α- and β-hemolytic *Streptococcus*, *Clostridium perfringens*) and gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris, Pseudomonas* spp., *Enterobacter* spp., *Klebsiella pneumoniae*) are capable of fermenting carbohydrates (primarily glucose), lactate, glycerol, and amino acids in human blood to form ethanol.[424] Other carbohydrate substrates for postmortem ethanol formation include galactose, maltose, sucrose, and lactose. The combination of abundant bacteria, high glucose concentrations, insufficient preservatives, and prolonged postmortem interval are a factor that favors postmortem formation of ethanol. In postmortem blood samples from a 40-year-old diabetic (vitreous glucose, 996 mg/dL) stored at 4°C (39.2°F) in 0.21% potassium fluoride, the blood ethanol concentration increased from 40 mg/dL on storage day 2 to 350 mg/dL on day 10.[425]

Little ethanol production occurs in corpses stored at low temperature, but the rise in ethanol concentration of putrefying blood reaches a peak within 5–15 days of storage without preservatives at room temperature in association with a rapid decrease in blood glucose concentration.[426] Blood ethanol produced by postmortem decomposition does not usually exceed 50–70 mg/dL unless the body is in advanced stages of putrefaction and/or high glucose concentrations are present along with ethanol-producing microorganisms.[427,428] Analysis of samples from 130 bodies in various stages of putrefaction demonstrated endogenous production of ethanol in 23 bodies.[429] The blood ethanol concentration ranged up to 220 mg/dL with 83% of the samples containing ≤70 mg/dL. In another study of 286 bodies in various stages of putrefaction, the highest ethanol concentra-

tion attributable only to putrefactive production of ethanol was 70 mg/dL, but the ethanol concentration from the combination of endogenous production and exogenous ethanol ranged up to 160 mg/dL.[430] A study of 27 decomposed bodies (9 slight, 9 moderate, 9 severe), the endogenous ethanol concentration in postmortem samples from the chest cavity averaged 31 mg/dL with a range of 8–76 mg/dL.[431]

Abnormal distribution of ethanol between blood sampling sites suggests the possibility of postmortem ethanol formation, whereas relative similarity of ethanol concentrations between various postmortem sampling sites supports the absence of significant amounts of postmortem ethanol formation. The presence or absence of volatiles (e.g., *n*-propanol, isopropanol, *n*-butanol, isobutanol, acetone, acetaldehyde) alone does not provide sufficient information to determine the origin of ethanol in postmortem samples or to exclude endogenous formation of ethanol.[432] In a study of 62 human bodies with water content ranging from 62–86% (mean, 72%), the postmortem blood ethanol concentration increased 0.01 g/kg for each 1 mg/kg increase in the postmortem concentration of *n*-propanol.[410] Most embalming fluids contain methanol rather than ethanol, but the use of ethanol by the embalmer can contaminate postmortem fluids (e.g., vitreous humor).[433] Methanol is not usually formed postmortem by microbial synthesis.[270] Postmortem blood samples from individuals with poorly controlled diabetes should be interpreted cautiously (i.e., with vitreous humor samples) because of the potential of microorganisms to transform high concentrations of blood glucose into ethanol. Postmortem fermentation of glucose to ethanol is highly variable, in part because many microorganisms also consume ethanol. The presence of ethyl glucuronide in the liver helps confirm the antemortem consumption of ethanol.

Fluoride ion, mercuric ion, and cold storage inhibit the formation of ethanol in tissue by microorganisms. The addition of 1% sodium fluoride to tissues from fatal airplane accidents prevented the formation of ethanol in tissue stored at 4°C (39.2°F) and 25°C (77°F) for 96 hours.[434] Within 24 hours of death, little ethanol is formed even at room temperature. No blood specimen from bodies refrigerated within 4 hours of death and stored up to 28 hours contained ethanol concentrations exceeding 10 mg/dL.[435] Criteria suggestive for endogenous ethanol production include the following: 1) presence of ethanol only in blood or chest fluid samples, 2) atypical distribution of ethanol among several body fluids, 3) reliable historical information, and 4) the presence of high concentrations of other low-molecular-weight alcohols (*n*-butanol, *n*-propanol).[430] Fatty acid ethyl esters are esterification products of ethanol and

fatty acids, and these compounds are present in the blood for up to approximately 24 hours after the ingestion of ethanol. The presence of >10,000 pmol fatty acid ethyl esters/g liver helps confirm the antemortem ingestion of ethanol.[436]

Ethanol Diffusion. In general, case series of postmortem ethanol specimens indicate that the mean ethanol concentration is similar between different sites in the body (e.g., femoral, heart blood, central veins, aorta).[437,438] However, on an individual basis there can be substantial variation between different sampling sites, particularly if ethanol is present in the stomach at the time of death.[439] Large variations (>50%) between postmortem ethanol concentrations in femoral and central (e.g., heart, aorta, chest) samples develop most often when the stomach contains an ethanol concentration >500–800 mg/dL or aspiration of stomach contents has occurred.[440] These conditions usually result from the ingestion of ethanol within 1 hour of death. Potential contamination of heart blood with ethanol results from inadvertent puncture of the stomach depending on the presence of high concentrations of ethanol in the stomach and diffusion of decomposing chest fluid from sources near the stomach. In a study of multiple sampling sites in 61 cadavers, there was >25% variation in the blood ethanol concentration between different sampling sites in 33% of the cadavers, particularly when the ethanol concentration in the stomach exceeded 500 mg/dL.[441] Pleural or pericardial samples may contain ethanol concentrations up to 190 mg/dL higher than those of the corresponding femoral blood samples because ethanol can diffuse from the stomach into the pericardium.[442] The presence of ethanol concentrations in blood below 50 mg/dL suggests that the gastric diffusion of ethanol is relatively small, particularly in peripheral samples.[443] Postmortem blood circulation patterns may alter the ethanol concentration both in peripheral and central sampling sites as a result of changes in water content.[441] Additionally, collection techniques may account for some of the differences between collection sites. The collection of low volume-blood samples in fluoride-containing containers usually produces lower blood ethanol concentrations when compared with companion specimens of larger volume specimens unless special techniques are employed (e.g., saturation of the blood sample with excess sodium chloride) when measured by headspace gas chromatography.[438] Fatalities from drowning in sea water can exacerbate differences in postmortem ethanol concentrations between right heart or femoral blood and left heart blood as a result of changes in tonicity of blood in the lungs and GI tract, particularly after antemortem and postmortem aspiration.[444]

VITREOUS HUMOR.   The use of vitreous humor for post-mortem ethanol analysis helps distinguish endogenous from exogenous ethanol sources because microbial infiltration of the vitreous humor occurs late in the time course of the putrefaction process, provided that the globe remains intact.[429] Anatomically, the vitreous humor is relatively protected from trauma. This clear, serous fluid demonstrates good chemical stability because of the protected position of the vitreous humor and the relative resistance to postmortem ethanol formation.

Ethanol.   Typically, the BAC is less than the concentration of ethanol in vitreous humor unless death occurred before steady-state equilibrium of ethanol, postmortem ethanol formation developed in blood, or the sample was contaminated internally by trauma or externally by ethanol.[445] The absence of ethanol in vitreous humor specimens and the presence of ethanol in postmortem blood samples suggest the postmortem formation of ethanol. In a retrospective review of nonpreserved specimens from 347 cases, almost all (99%) of blood samples with BAC >50 mg/dL had detectable quantities of ethanol in vitreous humor samples.[446] The highest BAC with a nondetectable concentration of ethanol in vitreous humor was 120 mg/dL. The presence of other putrefactive products (e.g., *n*-propanol, *n*-butanol) in the vitreous helps confirm the endogenous postmortem production of ethanol.[447] Factors other than endogenous ethanol formation that account for substantial differences (postmortem blood > vitreous ethanol concentrations) include contamination of the blood sample prior to collection, diffusion of ethanol from the stomach into surrounding tissue, and the ingestion of large doses of ethanol immediately prior to death.[448]

Blood/Vitreous Ethanol Ratio.   Based on the ratio of water content between blood and vitreous, the theoretical ethanol blood/vitreous ratio is approximately 0.79, assuming full distribution of ethanol at the time of death and the absence of postmortem ethanol formation or diffusion.[419] Early forensic literature suggested a conversion factor for the blood/vitreous humor ethanol ratio (± SD) ranging from 0.72 ± 0.09[449] to 1.01 ± 0.04.[450] Correlation of the blood ethanol concentration to the vitreous humor ethanol concentration decreases as the blood ethanol concentration exceeds 200 mg/dL even during the postabsorptive phase.[451] Although analysis of vitreous humor samples helps confirm the presence of exogenous sources of ethanol in the blood, the variation of the vitreous/blood ratio is too large to accurately predict blood ethanol concentrations from vitreous humor samples alone.[452] In a study of 349 cases involving the collection of postmortem vitreous humor and blood samples, regression analysis suggested that a vitreous humor ethanol concentration >150 mg/dL was necessary to predict a blood ethanol concentration of 80 mg/dL with 95% certainty.[453] In a study of 706 forensic necropsies, the median femoral blood/vitreous humor ratio was 0.847 (95% CI: 0.52–1.63).[452]

The blood/vitreous humor ratio depends on the phase of the blood ethanol curve at the time of death. During the early absorption phase (urine/blood ethanol ratio <1.20), the blood/vitreous ratios demonstrate large variation as the ethanol content of vitreous fluid lags behind the ethanol concentration in blood. A urine/blood ethanol ratio of <1.2 suggests that death occurred during the early absorption phase, whereas a urine/blood ethanol ratio >1.33 indicates that death occurred during the elimination phase.[454] Using the urine/blood ethanol ratio of <1.2 as indicating the early absorptive phase, the blood/vitreous humor ethanol ratio during this period in 200 postmortem specimens averaged 1.21 ± 0.45 (range, 0.84–3.07).[455] During the late absorption and elimination phase (urine/blood ethanol ratio >1.20), the mean blood/vitreous ethanol ratio was 0.84 ± 0.15 (range, 0.43–1.21). A blood/vitreous ethanol ratio exceeding unity suggests that death occurred during the absorptive phase.[454] However, both the standard deviation (0.57) and the range (0.71–3.7) were large for the blood/vitreous humor ethanol ratio, and this range overlapped the range (0.32–1.28) for the late absorptive and elimination phases. Consequently, the blood/vitreous humor ethanol ratio helps confirm the phase of ethanol distribution, but this ratio alone does not prove when death occurred in relation to the blood alcohol curve.

Ethyl Glucuronide.   In general, the concentration of ethyl glucuronide in vitreous is lower than urine and blood.[456] Because vitreous is relatively protected from bacterial contamination, the presence of ethyl glucuronide in vitreous is an aid to determine the occurrence of antemortem drinking, depending on the time and amount of drinking prior to death. However, both false-positive and false-negatives may occur,[301,457] and the vitreous ethyl glucuronide concentration must be interpreted along with the BAC, ethyl sulfate, the medical history, and the circumstances surrounding the death.

URINE.
Urine/Blood Ethanol Ratio.   Compared with ethanol biotransformation, the urinary excretion of unchanged ethanol is relatively small with renal excretion of unchanged ethanol accounting for about 0.5–10% of the ethanol dose. The ingestion of ethanol produces a temporary increase in urine flow, and volunteer studies indi-

cate the maximum diuresis in healthy adults occurs during the absorptive phase (i.e., <1–2 hours after drinking ceases).[93] Based on comparative water content, the urine/blood ethanol ratio is about 1.23–1.33.[458,459] The actual ratio depends on the average BAC during the time of the collection of the urine in the bladder.

Fresh–Voided Specimens. The accuracy of estimating the BAC from the UAC in antemortem samples is controversial. For the estimation of BAC, the first voided specimen is discarded and a second urine sample obtained 30–60 minutes after emptying the bladder. The ethanol concentration in this second void is used to estimate the BAC using a UAC/BAC ratio of 1.3.[460] Generally, during the postabsorptive phase, the urine ethanol concentration exceeds the BAC. Therefore, a urine/blood ethanol ratio ≥1.3 in a fresh-voided specimen indicates that drinking occurred at least 1–2 hours prior to the collection of the fresh-voided sample.[461]

The validity of this estimate depends, in part, on the phase of ethanol absorption. Although wide variation exists between individuals, volunteer studies in healthy adults suggest, but do not prove, that a UAC/BAC ratio <1.0–1.2 indicates continuing ethanol absorption.[462] A UAC/BAC >1.3 suggests the sample was obtained during the postabsorptive phase. The combination of UAC/BAC >1.3 and decreasing UAC in 2 successive urine samples indicates that the ingestion of ethanol within 1–2 hours of the sample is unlikely. The postsampling formation of ethanol in urine complicates the interpretation of the UAC/BAC ratio in diabetics and in patients with urinary tract infections.[463,464] Incomplete emptying of the bladder also complicates the interpretation of the UAC/BAC ratio, depending on the amount of urinary retention. Correction of the urine sample for dilution using the urine creatinine or specific gravity is not necessary because experimental studies indicate that the urine creatinine does not correlate to the UAC or to the UAC/BAC ratio.[458]

Postmortem Specimens. Postmortem urine samples represent pooled bladder specimens; therefore, the postmortem urine ethanol concentrations (UAC) do not necessarily correlate to the BAC at the time of death. Although the average urine/blood ratio of ethanol in autopsy cases frequently average about 1.3:1, the range is usually large; therefore, correlations between postmortem BAC and urine ethanol concentrations is usually low.[465] Analysis of UAC and BAC obtained simultaneously from postmortem specimens demonstrated a mean UAC/BAC ratio of 1.28 (95 % CI: 0.36–2.20).[466] Using a urine/heart blood conversion factor of 1.28, only 44 % of the 148 autopsy cases had <20 mg/dL

difference between the calculated and actual BAC. Consequently, the main value of postmortem UAC is the determination of the likelihood of postmortem ethanol changes in blood. Postmortem fermentation occurs much later in the bladder than in the blood as long as the urine does not contain high concentrations of glucose (i.e., as in diabetes). A large discrepancy between the BAC and UAC suggests postmortem ethanol formation, particularly if death occurred during the postabsorptive phase (i.e., low ethanol concentrations in gastric contents).[467]

Ethyl Glucuronide. The rapid elimination of ethanol results in the disappearance of ethanol within 12 hours in the plasma and slightly longer in the urine. Ethyl glucuronide is a potential biomarker of recent ethanol ingestion that remains measurable in urine samples longer than ethanol.[468,469] However, back-extrapolation of urine ethyl glucuronide concentrations to blood ethanol concentrations or levels of impairment is inaccurate. Hence, urine ethyl glucuronide concentrations indicate prior ethanol exposure rather than the ethanol dose or impairment. This minor, nonoxidative metabolite of ethanol is a nonvolatile, acidic, water-soluble substance that forms during phase II reactions by uridine diphosphate-glucuronosyltransferase (UGT). *In vitro* studies indicate that multiple UGT isoforms catalyze the addition of glucuronic acid to ethanol with UGT1A1 and UGT2B7 being the 2 most prevalent isoforms;[470] however, the involvement of multiple UGT isoforms limits the forensic consequence of polymorphism with UGT1A1.[471] In a study of 10 male volunteers receiving 0.5 g ethanol/kg, the median excretion of ethyl glucuronide over about 2 days was 0.017% of the administered dose.[472] Pharmacokinetic studies indicate that ethyl glucuronide does not accumulate in urine following repeated dermal or oral exposures. In a study of 9 female volunteers receiving 0.4 g ethanol/kg twice daily for 8 days, no significant accumulation of ethyl glucuronide occurred in urine samples.[473]

Detection Times. Urinary ethyl glucuronide concentrations are detectable within 1 hour after ethanol ingestion and for at least 6–10 hours and longer after blood ethanol concentrations are undetectable depending on the ethanol dose, urine specific gravity, individual variation, timing of previous drinking, and the sensitivity of the test (i.e., cutoff concentrations).[474] Volunteer studies indicate that detection of ethyl glucuronide in oral fluid is only a few hours longer than ethanol; therefore, analysis of ethyl glucuronide in urine is superior to oral fluid for detection of ethanol use.[475] In a volunteer study, the peak urine concentration of ethyl glucuronide occurred about 2–3.5 hours after the peak ethanol

concentration.[476] The presence of ≥100 ng ethyl glucuronide/mL indicates ethanol exposure within the previous 3–4 days.

Nineteen volunteers were divided into low dose (1.4 standard drinks), medium dose (2.9 standard drinks), and high dose (4.3 standard drinks) groups.[477] At 24-hours postingestion, all urine samples were positive for ethyl glucuronide in the high-dose group, whereas ethyl glucuronide was not detectable in the urine samples from the low-dose group as measured by liquid chromatography/tandem mass spectrometry (cutoff, 100 ng/mL). All urine samples obtained 48 hours after ethanol ingestion contained no detectable amounts of ethyl glucuronide. In a study of volunteer students ingesting an average of about 9 standard drinks based on questionnaire data, 100% were positive for ethyl glucuronide in urine samples 40 hours after the last drink, 77% of the urine samples were positive for ethyl glucuronide at 54 hours, and 18% at 78 hours after the last drink as measured by liquid chromatography/tandem mass spectrometry (cutoff, 100 ng/mL).[478] The students ingested ethanol in various drinks (wine, beer, spirits) over a 6-hour meeting; ethanol was nondetectable in all blood samples 15 hours after the last reported drink. Ethyl glucuronide was not detectable in all urine samples at 103.5 hours postingestion. In general, the elimination half-lives of ethyl glucuronide in social and heavy drinkers are similar, depending on renal function. In a study of 16 alcohol abusers admitted to an alcohol withdrawal clinic, the median elimination half-life of ethyl glucuronide in their blood samples was 3.3 hours (range, 2.6–4.3 hours).[479] In a study of 32 alcohol dependent patients undergoing detoxification with initial breath ethanol concentrations from 100–340 mg/dL, the median time for urinary ethyl glucuronide concentrations to drop below the cutoff (<0.5 mg/L) was 78 hours (range, ~40–130 hours).[480] The median urinary detection time for ethyl glucuronide after the estimated zero ethanol concentration was 66 hours (range, ~30–110 hours) compared with 56 hours (range, ~30–70 hours) for the urine concentration adjusting for relative dilution. The cutoffs were 0.5 mg/L and 0.5 mg/g creatinine, respectively.

Sensitivity/Specificity.   The presence of ethyl glucuronide in the urine is not a unique biomarker of recent ethanol ingestion as a result of the high sensitivity of this biomarker to trivial quantities of ethanol. Small quantities of ethyl glucuronide (i.e., <120 ng/mL) may appear in the urine as a result of unrecognized ethanol exposure (e.g., mouthwash, nonalcoholic wine, cough medication, cosmetics, etc.) or endogenous ethanol metabolism. The presence of ethyl glucuronide must be interpreted cautiously because of the occurrence of false-positive as well as false-negative reactions.[481,482] The former occurs following exposure to ethanol-containing products or after bacterial or yeast contamination that produces ethyl glucuronide, particular when the sample contains *E. coli* and glucose.[483] Case reports indicate that false-positive results may result from the absorption of ethanol from mouthwash or ethanol-containing hand sanitizer.[484] In a study of 9 volunteers, gargling 4 ounces of 12% ethanol-containing mouthwash over 15 minutes, 39 urine samples were collected during the first 24 hours after gargling.[485] Twenty of these urine samples had ethyl glucuronide concentrations over 50 ng/mL and 12 samples contained ethyl glucuronide concentrations exceeding 100 ng/mL. In a drinking study of 12 volunteers ingesting 1 g or 3 g ethanol, the maximum ethyl glucuronide concentration in urine normalized to creatine was 320 ng/mL and 1,530 ng/mL, respectively.[486] The estimated ingestion of ethanol in food and nonalcoholic beverages is <1 g compared with 1–2 g for ingesting 10–20 mL of mouthwash containing 12% ethanol. There was substantial interindividual variation in the urinary excretion of ethyl glucuronide during this study. Following repetitive daily dermal exposure to hand sanitizer (60% ethanol) by 9 adults, the ethyl glucuronide concentrations in 88 first-morning void urine samples ranged from <10–114 ng/mL.[487] False-negative results may occur as a result of the hydrolysis of ethyl glucuronide by strains of β-glucuronidase-containing *E. coli* that contaminate urine samples. Additionally, the sensitivity of this biomarker decreases significantly in dilute urine when substantial amounts of water are ingested within 1 hour of sampling.[488] False-negative results may occur during analysis of dilute urine samples (e.g., urine creatinine <25 mg/dL); normalization of the urine ethyl glucuronide to urine creatinine (e.g., 100 mg/dL) eliminates the effect of urine dilution on the sensitivity of the urine ethyl glucuronide analysis.[489]

Postmortem.   The presence of ethyl glucuronide in postmortem urine suggests recent ethanol consumption, but does not necessarily prove that a person died with a high blood ethanol concentration. Ethyl glucuronide is stable in urine samples stored at room temperature at least 4 days. Although *in vitro* formation of ethanol occurs as result of the fermentation of glucose, particularly in those with diabetes, formation of ethyl glucuronide does not result from glucose fermentation.[490] This biomarker along with increased ratio of 5-hydroxytryptophol/5-hydroxyindole-3-acetic acid can help to distinguish recent ethanol ingestion from postmortem ethanol artifacts.[491,492] Case reports suggest that ethyl glucuronide (and ethyl sulfate) does not form during the putrefaction process; these compounds may

persist for years in mummified corpses (i.e., without preservation) despite some postmortem hydrolysis during the putrefaction process.[493]

Serum Ethanol/ Ethyl Glucuronide Ratio.   In a study of 10 male volunteers consuming 0.5 g ethanol/kg body weight in a fasted state, the median peak serum ethanol ethyl glucuronide concentrations occurred about 4 hours (range, 3.5–5.0 hours) after ingestion, whereas the median peak ethanol in blood was 1 hour (range, 0.5–2.0 hours).[472] The blood ethanol/ethyl glucuronide ratio as measured in g/L and mg/L, respectively, was >1 for the first 3.5 hours after drinking (range 2.5–3.5). Thus, a ratio above 1 suggests drinking < 3.5 hours before sampling, whereas a ratio less than 1 suggests drinking > 2.5 hours before sampling. The ingestion of multiple doses of ethanol complicates the interpretation of this ratio.

Ethyl Sulfate.   Ethyl sulfate is another minor, water-soluble metabolite of ethanol that is a potential biomarker of ethanol ingestion; this metabolite is formed during phase II reactions by sulfotransferase (SULT). The median times of occurrence and ranges for ethyl sulfate and ethyl glucuronide concentrations in urine following ethanol consumption are similar.[480] Ethyl sulfate is less susceptible to postsampling formation than ethyl glucuronide but there is less kinetic data for ethyl sulfate compared with ethyl glucuronide.[494]

Serotonin Metabolites.   Ethanol alters serotonin metabolism by 1) decreasing the reduction of the intermediate serotonin metabolite, 5-hydroxyindole-3-acetaldehyde (5-HIAL) to 5-hydroxyindole-3-acetic acid (5-HTOL) and 2) increasing the oxidation of 5-HIAL to 5-hydroxytryptophol (5-HIAA).[495] The ratio, 5-HTOL/5-HIAA, remains elevated 5–15 hours after the elimination of ethanol from the body depending on the ethanol dose.[496] Consequently, a 5-HTOL/5-HIAA ratio exceeding 15 pmol/nmol is not definitive proof of the presence of ethanol at the time of death. A 5-HTOL/5-HIAA ratio below 15 pmol/nmol indicates that the consumption of ethanol is unlikely within about 24 hours prior to the collection of the urine sample.[497] This change in the 5-HTOL/5-HIAA ratio is probably not affected by the use of selective serotonin reuptake inhibitors or the consumption of serotonin rich foods.[498]

LIVER.   At equilibrium, the ethanol concentration is lower in liver tissue than in blood as a result of the relatively lower water content of the liver. In a case series of 10 deaths attributed to acute ethanol intoxication, the liver/blood ratio was 0.60 ± 0.056 (range, 0.54–0.72).[502] The average liver/heart blood ratio in 71 postmortem samples that contained heart blood concentrations exceeding

40 mg/dL was 0.56 ± 0.30 (range, 0–1.40).[308] The presence of ethanol in the liver in the absence of detectable concentrations of ethanol in the vitreous humor and the urine suggests postmortem ethanol formation.

BILE.   The gallbladder in relatively well protected from trauma and this organ usually remains free of contamination, even in violent deaths. In general, the postmortem blood and bile concentrations are similar.[499] Typically, mean postmortem blood/bile ratio is about 0.92–1.10. In a study of autopsied cases without thoracic or GI trauma, 19 paired heart blood and bile samples yielded a mean blood/bile ratio of 1.09 (range, 0.56–1.54).[422] A study of ethanol concentrations in 33 cadavers demonstrated a mean heart or aorta blood/bile ratio of 1.01 ± 0.19 (range, 0.44–1.40).[304] In a study of specimens from 189 cadavers, the mean heart or aorta blood/bile ratio was 1.03 (range, 0.32–2.91).[500] The wide variation in the blood/bile ratio for ethanol concentration limits the reliability of the extrapolation of bile ethanol concentrations to blood ethanol concentrations. The correlation of ethanol concentrations in simultaneous blood and bile samples improves when the blood ethanol concentration exceeds 100 mg/dL.[501] Bile ethanol concentrations exceeding 175 mg/dL suggest that blood ethanol concentrations exceed 150 mg/dL, whereas bile ethanol concentrations below 50 mg/dL usually correlate to blood ethanol concentration below 100 mg/dL. Extrapolation of blood/bile ratios for determining blood ethanol concentrations improves substantially when death occurs immediately (i.e., 30 minutes) after an incident and no drinking occurred shortly (i.e., 30 minutes) before the incident. The presence of high ethanol concentrations in bile compared with other sampling sites (e.g., blood) suggests that death occurred rapidly after ethanol ingestion.[502,503] The presence of a substantially lower postmortem ethanol concentration in blood than in bile also suggests the possibility of metabolism of blood ethanol shortly before death. However, there are inadequate data to determine the ethanol time-concentration curve for bile after ethanol ingestion.

Abnormalities

ACUTE INGESTION

The acute ingestion of large doses of ethanol produces an increase in serum osmolality along with a fall in the concentration of plasma vasopressin and an increase in free water clearance. In a volunteer study of 6 men ingesting 3.3 L of beer over 3 hours, the changes in water balance included a small decrease in the plasma concentrations of urea and creatinine as well as a mild rise in plasma potassium.[504] Under these experimental

conditions, there was no hypoglycemia or significant increase in serum transaminase, creatine kinase, or γ-glutamyl transferase. Acute ethanol ingestion by itself does not usually produce dehydration or electrolyte imbalance, and the presence of laboratory abnormalities consistent with dehydration indicates the presence of a condition (e.g., reduced fluid intake, vomiting, pancreatitis) other than acute ethanol intoxication.[505]

Serum Osmolality.   Osmolality (osmoles per kilogram solvent) and osmolarity (osmoles per liter of solution) are the number of particles dissolved in solution, and the osmolal gap is a rapid approximation of the unmeasured, osmotically active constituents.[506] Equation 21.9 approximates the serum osmolarity (O_C) based on the concentrations of sodium, glucose, and blood urea nitrogen (BUN) in traditional units including correction of the serum water fraction (i.e., assumed to be 93% in healthy individuals). The correction factor for traditional units is the BUN concentration in mg/dL divided by 2.8 and the glucose concentration in mg/dL divided by 18,

$$O_C = 2[\text{Na}^+ \text{ in meq/L}] + 0.39[\text{BUN in mg/dL}] + 0.059 \text{ [glucose in mg/dL]}$$

(Equation 21.9)

where O_C = Calculated osmolarity.

The measured osmolality (O_M) is normally about 270–290 mOsm/kg H_2O, and the difference between the measured osmolality and the calculated osmolality is the osmolal gap. Freezing point depression is the method of choice for the determination of osmolality because the vapor pressure method underestimates the contribution of volatile alcohols.[507,508] Equation 21.10 calculates the osmolal gap (O_G), which usually ranges from 10–15 mOsm/kg H_2O depending on the variability of the reference ranges and coefficients of variation for the specific laboratory instruments.[509]

$$O_G = O_M - O_C$$ (Equation 21.10)

The presence of an elevated osmolal gap suggests the presence of significant concentrations of osmotically active compounds (ethylene glycol, propylene glycol, methanol, ethanol, isopropanol, acetone). The ingestion of ethanol causes an increase in the osmolal gap. For each milligram of ethanol per deciliter, the osmolal gap rises by about 0.22 mOsm. An ethanol concentration of 100 mg/dL raises the osmolal gap approximately 22 mOsm/kg H_2O, whereas serum ethanol concentrations of 300 mg/dL result in an osmolal gap of about 66 mOsm/kg H_2O. Serum osmolal gaps are screening measures for the presence of selected osmotically active constituents rather than estimates of serum ethanol concentrations. A discrepancy between serum osmolality and serum ethanol concentration suggests, but does not confirm, the presence of other osmotically active solvents. In the absence of an increased osmolal gap, ethanol is an unlikely cause of coma and other causes of coma should be considered (e.g., subdural hematoma, stroke, another CNS depressant drug). Nonethanol-related causes for elevated osmolal gaps include the presence of fructose, glycerin, IVP dye (diatrizoate), mannitol, propylene glycol, sorbitol, and alcoholic ketoacidosis.[510] Occasionally, alcoholic ketoacidosis may cause a high anion gap metabolic acidosis and markedly increased serum osmolal gap in the absence of detectable concentrations of ethanol.[511] This metabolic pattern may be misdiagnosed as ethylene glycol or methanol poisoning in chronic alcohol abusers as a result of the formation of high concentrations of acetone and other ketone bodies (acetoacetate, β-hydroxybutyrate) during alcoholic ketoacidosis.

Hypoglycemia.   Ethanol-induced hypoglycemia occurs during ethanol intoxication, particularly in children under the age of 5 years,[512] but this laboratory abnormality is a relatively uncommon occurrence.[513,514] In a study of 143 children under the age of 14 years admitted for acute alcohol intoxication, 12% of the children developed hypoglycemia.[515] The vast majority of these children were under 5 years of age. The severity of the hypoglycemia is not necessarily related to the quantity of alcohol ingested. Clinically significant hypoglycemia is rare during ethanol intoxication in older children, and the biochemical disturbances associated with ethanol intoxication in children aged 11–16 years old and adults are similar. In a series of 268 children (8–16 years) hospitalized for ethanol intoxication, there were no cases of severe hypoglycemia.[516] Other laboratory abnormalities associated with ethanol intoxication in adolescents include mild hypokalemia and mild respiratory acidosis.[517]

Chronic Ethanol Abuse

Alcohol dependence and alcohol dependent liver disease produce a variety of laboratory abnormalities including hypoalbuminemia, mild to moderate serum hepatic aminotransferase elevation, hyperbilirubinemia (primarily unconjugated), low BUN, and a prolonged prothrombin time depending on the severity of hepatic impairment.[186] Hypoalbuminemia results from protein malnutrition, malabsorption, albuminuria, loss into the peritoneal space, and impaired hepatic production. Typically, serum electrophoresis demonstrates a decrease in the albumin and prealbumin along with a

broad elevation of the β- and γ-globulins and occasionally elevation of IgA, IgG, and IgM. Failure of the prothrombin time to respond to vitamin K indicates serious hepatic damage.[518] Even during alcoholic hepatitis, the elevation of hepatic aminotransferase concentrations is usually mild to moderate (<10 times normal), and a >10-fold increase in these enzymes suggests another cause (viral, drug, toxin).[519] The serum AST is typically elevated to a greater degree than the ALT with a ratio of approximately 1.5–2.0 in alcoholic liver disease. The presence of an AST/ALT ratio <1 suggests, but does not necessarily indicate another cause of hepatocellular disease.[520] Common electrolyte disorders, particularly in alcoholic cirrhosis, include hyponatremia, hypokalemia, hypophosphatemia, and hypomagnesemia. Hyperuricemia occurs following acute ethanol intoxication as well as long-term ethanol abuse.[521]

Metabolic acidosis in an alcohol abuser may also be associated with uremia, diabetic ketoacidosis, lactic acidosis, alcoholic ketoacidosis, or fructose therapy. Alcoholic ketosis (acetoacetate, β-hydroxybutyrate) typically occurs in a alcohol dependent patients, who stops drinking after a heavy binge. β-Hydroxybutyrate is usually elevated disproportionally to acetoacetate, and the nitroprusside reaction may not be strongly positive despite large amounts of ketones because this reaction is relatively insensitive to β-hydroxybutyrate. The ketosis is not usually severe, and a metabolic acidosis may not be present. Patients with alcoholic ketoacidosis usually have low or nondetectable concentrations of ethanol, normal or low blood glucose, urinary ketones, and normal or moderately elevated urea and creatinine, whereas alcohol dependent patients with lactic acidosis typically have elevated serum ethanol concentrations along with an increased risk of sepsis, seizures, thiamine deficiency, or severe impaired liver function.[522] The metabolic acidosis with ethylene glycol, methanol, or paraldehyde occurs after the elimination of ethanol from the blood and the restoration of alcohol dehydrogenase function. A hypochloremic metabolic alkalosis may result from volume depletion and loss of hydrochloric acid from vomiting. Anemia occurs commonly in alcohol dependent patients, and mild macrocytosis (mean corpuscular volume 100–110) develops in about one-half of alcohol dependent patients after several months of heavy drinking.[523] Causes of anemia in alcohol abusers include anemia of chronic disease, folate deficiency, sideroblastic anemia, and GI bleeding.

WITHDRAWAL

Risk factors for alcohol-related seizures include hypoglycemia, electrolyte disturbances, intracranial hemorrhage, CNS infection, illicit drug use, isoniazid toxicity, prior withdrawal seizures, multiple previous detoxifications, and high total ethanol consumption. The electroencephalogram helps separate alcohol withdrawal seizures from epilepsy. The typical pattern in the former is a normal low-amplitude recording, whereas generalized spike and wave points occur with epilepsy.[524] However, the predictive value of a normal electroencephalogram in this setting is limited.

Driving

Driving involves a variety of behavioral and psychomotor tasks including perception (visual acuity, depth perception, glare recovery), divided attention, information processing, sensory and motor skills (coordination, reaction time, steering, tracking, maintenance of speed, and braking), and judgment. Driving is a monotonous task interrupted by unpredictable demands on decision-making and reactions. The threshold of impairment, as defined by changes from baseline measurements of psychomotor testing, depends on the type of testing and the specific body function tested as well as the BAC. Because driving is a complex task, no single psychomotor test provides valid information to determine the causal role of ethanol in driving accidents. All behavioral skills are not equally impaired by the same dose of ethanol, and there are gender differences in some psychomotor skills (choice reaction time, tracking).[525] In general, tests of simple sensory and motor tasks (e.g., visual acuity, brightness thresholds, tracking, braking) demonstrate little impairment at BAC <80–100 mg/dL, particularly in experienced drinkers with good psychomotor skills.[58,526] Psychomotor testing indicates impairment at BAC <100 mg/dL on complex tasks (e.g., processing and acquisition of information, performance on divided-attention tasks, judgment). However, biologic differences between individuals cause some overlap in performance measures at different ethanol concentrations.[527] The magnitude of the decrements in performance on psychomotor tests depends on a variety of factors, including the type of test, the specific BAC, and the phase of the BAC curve. In general, impairment is greater on the ascending than the descending BAC curve (i.e., Mellanby effect), but the differences are usually relatively small (<20%) and not all cognitive tasks are similarly affected.[528,529] Above a BAC of 100 mg/dL impairment of almost all driving skills occurs, and the relative risk of an accident increases dramatically when the BAC reaches or exceeds this concentration.[530]

SENSORY, MOTOR, AND COGNITIVE TASKS

Driving involves a variety of behavioral and psychomotor skills that are sensitive to the effects of ethanol, but

there are limited data on which impairments (e.g., reaction times, tracking errors, psychomotor skills, vigilance, perceptual speed) cause an increase in the risk of vehicular accidents. Typically, the ethanol-related accidents involve a single vehicle leaving the road. Intoxicated drivers are also frequently involved in rear-end and head-on crashes. These types of crashes suggest the presence of reduced alertness, decreased attentiveness, and impaired tracking abilities. Driving is primarily a monotonous visuomotor task with visual input accounting for about 90% of information received by the driver.[531] Correlating a BAC to a specific level of impairment is complicated by the wide range of BAC and individual variation in most studies; however, there is a progressive impairment with increasing BAC. Modest impairment in some psychomotor tasks does correlate selectively to BAC <100 mg/dL; above this range, the number of tasks and degree of impairment progressively increases.

VISION.   Ethanol intoxication has negative effects on visual perception and eye movements at BAC concentrations up to 100 mg/dL, primarily involving central information processing rather than sensory receptors.[532] Not all visual functions are equally sensitive to the effects of ethanol in the range of 80–100 mg/dL. In general, ethanol concentrations near this range decrease contrast sensitivity modestly (10–20%), but visual acuity, color vision, accommodation (near vision), and perception of motion are relatively unaffected.[533] Studies on the effect of ethanol in this range on stereoacuity (i.e., smallest detectable difference in images presented to both eyes) and binocular vision (i.e., ability to use both eyes to fuse slightly dissimilar objects) is inconsistent. A variety of environmental factors add to the visual impairment caused by ethanol including weather (fog, rain, snow, dust, fog), illumination during twilight or night, local obstructions (foliage, buildings, cars, curves in the road), objects in the car (dirty windshield), and speed. The latter effects result from the reduction of peripheral visual input as the driver looks progressively ahead as the speed of the car increases.[534] As the BAC increases above 100 mg/dL, the number of impaired visual functions and the degree of impairment increases. There are limited data on the effect of BAC in the range of 125 mg/dL and above because of ethical constraints. Existing data indicates that most visual functions are impaired significantly in this range including visual acuity, eye movements, depth perception and distance judgment, glare recovery, and peripheral vision; however, there is some interindividual variation.[535] The baseline visual acuity also affects the visual field and the individual's ability to detect objects ahead of the vehicle. An individual with 20/20 vision driving at 60 mph requires

3.9 seconds to read a highway sign with 6-inch letters; whereas the time to read the sign decreases to 1.95 seconds and 0.78 seconds when the baseline visual acuity is 20/40 and 20/100, respectively.[536] Substantially more illumination is necessary at night to read these signs as 20/40 vision during the day is equivalent to 20/100 at night; the effect of ethanol on visual acuity is relatively greater under twilight conditions compared with daytime lighting conditions.[537]

Visual Acuity.   Visual acuity is the ability to resolve fine detail. Although visual acuity depends on a variety of variables (e.g., luminance, pupil size, eye movements, accommodation, chromatic aberrations, contrast, motivation/attention), experimental studies typically measure visual acuity in high-contrast environments. Dynamic visual acuity task determines the number of correct responses to the detection of the direction of a broken circle on a moving screen. Within 1 second of the target sweep, the participant must indicate the orientation of the missing arch from the circle. The visual near point is the distance at which an individual begins to lose focus on a visual cue (e.g., card). In general, low to moderate doses of ethanol (BAC <100 mg/dL) do not substantially impair optometric visual function (e.g., visual acuity, color vision, dark adaption, perception of motion, accommodation, peripheral vision).[538] In experimental studies, minor decrements in visual acuity occurred in individuals with BAC 100–125 mg/dL.[539] Some loss of near vision (accommodation, convergence) occurs as the BAC increases above 100–125 mg/dL.[532] Experimental studies suggest that low doses of ethanol (<70 mg/dL) do not significantly alter the perception of velocity.[540] At BAC concentrations in the range of 80–100 mg/dL, lighting conditions do not affect driving skills associated with simulated driving tests.[541] Motion parallax is the ability to recover depth from retinal motion generated by observer translation. The visual perception of depth depends on motion parallax to determine the relative positions and direction of obstacles in the visual field. In a repeat measures within-participants study of 15 paid volunteers, the average observer threshold for motion parallax was significantly higher ($P = .004$) in the intoxicated state compared with the sober state.[542] The mean BrAC at the beginning of testing was 0.085% (range, 0.07–0.14%). However, there was wide interparticipant variability with no difference in the threshold for motion parallax for the intoxicated and sober states in 5 participants. Depth perception as measured by thresholds for binocular stereopsis was unaffected in this study, whereas perception of depth from motion parallax that relies on slow eye movements was affected.

The critical fusion frequency (CFF) test determines the threshold (i.e., highest frequency) at which an individual ceases to perceive the flickering of a flashing light and sees the flashing light as a continuous source; the critical flicker frequency test requires judgment about whether the frequency of a flickering light is increasing or steady. Reduction in critical fusion frequency (CFF) suggests impairment of visual temporal processing. CFF is the temporal analogue of spacial resolution acuity. Visual acuity and CFF provide information about the detection of fine detail and rapidly changing targets. The critical flicker fusion test is relatively insensitive to BAC <100 mg/dL.

Contrast/Glare Recovery.   Contrast sensitivity involves distinguishing spatially distinct differences in luminance over a range of different spatial frequencies; in contrast to visual acuity, contrast sensitivity measures visual process in low-contrast situations. The testing of contrast sensitivity involves the use of a series of light and dark bars (i.e., grating pattern) with modulating luminance profiles. Contrast sensitivity is the reciprocal of the lowest detectable level of contrast; higher values mean greater contrast sensitivity. Experimental studies suggest that the consumption of ethanol delays light adaptation and reduces contrast sensitivity after intense light exposure,[543,544] but the dose response of this effect is not well defined.[545] In general, changes in contrast sensitivity are relatively small to nondetectable at BAC below 80–100 mg/dL, depending on testing of static or dynamic conditions.[546] In a crossover, controlled study of 20 individuals studied with and without ethanol, the mean contrast sensitivity for stationary sine wave grating decreased about 8% under white light conditions.[547] The projected ethanol concentration during testing was 80 mg/dL, but no confirmation of ethanol concentrations was reported.

In a crossover study of 12 healthy volunteers, the reduction in contrast sensitivity in the intoxicated state was about 2½ times greater during the testing of moving gratings of high spatial frequency (-0.29 log-unit) than for stationary gratings (-0.11 log-unit); for the moving gratings, the reduction was <10–15% of baseline.[548] The estimated BAC was 100 mg/dL based on the ingestion of 1.4 mL 95% grain ethanol/kg body weight, but the actual BAC was not measured. The estimated mean BAC was 88 mg/dL; the estimated BAC correlated to the dynamic contrast sensitivity, but not the static contrast sensitivity. Pearson and Timney reported larger decrements in stationary contrast sensitivity.[549] In their crossover study of 6 paid graduate students, the mean reduction in stationary contrast sensitivity over all spatial frequencies was 43% . The measured BrAC for this group was 60 mg/dL. There was substantial interin-

dividual variation (e.g., 215% change in 1 participant) with the reduction generally higher at higher spatial frequencies. The effect of ethanol on glare recovery is variable with some studies demonstrating decrements while other studies indicated some enhancement of glare recovery scores.

Depth Perception/Binocular Vision.   Gain is the ratio of eye velocity to target velocity; maintaining fixation on a moving target requires a gain near 1. Inhibition of slow eye movements results in the necessity of recruiting fast eye movements to maintain fixation. Ethanol reduces the gain of slow eye movements as well as the slowing of the initiation and the velocity of saccadic eye movements.[550] By requiring the visual system to recruit fast eye movements to generate a "catch-up saccade," jerky eye movements develop during ethanol intoxication, as exhibited by horizontal gaze nystagmus. Saccadic eye movements are quick jumps of the eye (20–700°/sec) in response to a visual target stimulus jumping 3–25°. Measured variables include saccade latency, maximum saccade velocity, saccade amplitude, and saccade overshoot. Smooth pursuit eye movements measure the ability of an individual to match velocity of the eye with the velocity of a visual target stimulus that moves at a rate of 1–30°/s as measured by the ratio of eye velocity to target velocity. BAC near 100 mg/dL decreases smooth-pursuit eye movement and increases the number of catch-up saccades.[551] Individuals still recognize exciting and relevant areas of visual scenes, but there is a reduction of visual exploration. Distance judgment and depth perception depends on sensory feedback from ocular muscles that control binocular convergence. In general, moderate ethanol doses (80–100 mg/dL) cause some esophoria (i.e., tendency for inward deviation of eyes) for far viewing and somewhat less exophoria (i.e., tendency for outward deviation of the eyes) for near viewing.[552,553] The effect of these changes on driving skills has not been established.

Fusion/Diplopia/Accommodation.   Under normal circumstances, adjustment (i.e., vergence) of ocular muscles focuses the sensory input from both eyes on the respective retinas in a similar pattern that allows fusion of the 2 images. Failure of this fusion causes diplopia. The near point and far point of fusion are the minimum and maximum viewing distances, respectively, at which fusion occurs. Fusion latency is the time required to attain fusion. Laboratory studies of healthy volunteers suggest that moderate doses of alcohol that produce BAC in the range of 80–100 mg/dL reduce the near and far point of fusion as well as increase the fusion latency, particularly at maximum viewing distances.[554]

421

Accommodation typically is not affected by BAC <100 mg/dL.

HEARING.   There are limited data on the effects of ethanol consumption on hearing. Existing data are conflicting, probably as a result of differences in ethanol dose, experimental design, and/or duration of ethanol exposure. One volunteer study indicated that ethanol reduces the auditory threshold at all frequencies tested (250–8,000 Hz) beginning 30 minutes after the ingestion of 250 mL beer (estimated peak BAC 25 mg/dL).[555] However, the effect of ethanol at higher frequencies was smaller and the duration shorter than lower frequencies. All effects of ethanol on auditory threshold resolved within 480 minutes. Another volunteer study demonstrated increased auditory threshold for low frequencies (monaural and binaural 200 Hz, monaural only 400 Hz) following the ingestion of ethanol (estimated peak BAC approximately 60–80 mg/dL).[556] In this study, the effects of ethanol on detection thresholds were greater on lower frequencies (200 Hz, 400 Hz) than higher frequencies (800 Hz, 1,600 Hz, 3,200 Hz). Additionally, monaural stimuli were more affected by ethanol than binaural stimuli. A study of 8 healthy volunteers receiving moderate doses of ethanol sufficient to cause mild clinical intoxication (BAC not reported) did not detect any effect of ethanol consumption on pure-tone auditory thresholds.[557] There was a temporary reduction in distortion product otoacoustic emissions (i.e., sounds emitted in response to 2 simultaneous tones of different frequencies) at high frequencies, but not at low frequencies. These studies suggest that the effect of ethanol on hearing is a central effect rather than a peripheral one.

VIGILANCE AND ATTENTION.   Vigilance is a state of readiness to detect and respond to unpredictable and rare events. Vigilance tasks (e.g., continuous reaction times) require the ability to concentrate attention on a task for a prolonged period. Vigilance is relatively resistant to the effects of ethanol.[558] Volunteer studies indicate that ethanol intoxication impairs cognitive control of attention by limiting the ability to voluntarily allocate and use attention in a variety of tasks.[559] The spatial distribution of visual tasks is probably affected by ethanol consumption as well as the difficulty of the task. In a volunteer study using mean BrAC concentrations equal to 80 ± 10 mg/dL, participants in the alcohol group had difficulty disengaging attention from the center of their visual field; they fixed their attention more diffusely in the center when compared with the control group.[560] This experiment suggests that ethanol concentrations in the range of 80 mg/dL impair the ability to dissociate attention from gaze.

Driving is a divided attention task involving 1) a tracking task that maintains the vehicle in the proper lane and direction, and 2) a search and recognition task that monitors the driving environment for vital information on vehicles, pedestrians, traffic signals, and hazardous events. Negotiating a turn is a classic divided attention task requiring an accurate estimation of the degree of curvature and precise tracking of the curved portion of the road. Because maintaining the vehicle in the correct position is a continuous task, ethanol-impaired drivers tend to focus on steering and miss important cues in the periphery of the driving environment.[561] Divided attention tasks require individuals to perform 2 tasks simultaneously (e.g., central tracking and peripheral visual search). Sustained attention tasks require maintaining attention to a single source of information for an unbroken period. Sustained attention tasks are less sensitive to ethanol than selective attention tasks that involve attention to 1 source of information and the exclusion of another.[562]

SIMPLE MOTOR TASKS.
Reaction Times.   Reaction times vary from individual to individual as well as with the type of task and the level of attention (fatigue) over different sensory areas.[563] For example, alcohol increases reaction time for foot responses (e.g., braking) relatively more than for hand responses (e.g., steering).[564] Theoretically, reaction times are divided into perception time (i.e., time from the presentation of the stimulus until movement) and movement time (i.e., time from start of movement until a defined motor end point). Reaction times slow as the BAC increases above 80–100 mg/dL. In general, increased reactions times in this range are <10%, whereas large increases (>40–50%) occur at BAC above 150 mg/dL.[565] Choice reaction times typically are slower than simple reaction times, depending on the complexity of the task; ethanol diminishes the difference between these 2 tests as the BAC increases above 125–150 mg/dL.

Simple.   Reaction times measure the speed of a response to an auditory or visual stimulus, and these tests involve components of attention and motor coordination. Tests of simple reaction time measure mostly perception because the length of time required for the motor component is minimal compared with the time required for sensory input. To simulate real driving situations, brake time represents the time from the presentation of a stimulus (e.g., auditory, visual) to the time the foot touches the brake pedal. Simple reaction times are an important component of braking; the braking

reaction to an unexpected obstacle in the driving path is an example of simple reaction time.[530] In general, the motor response is more rapid to an auditory stimulus than to a visual stimulus.[566] The average braking time (i.e., time between presentation of visual stimulus and movement of foot to the brake pedal) in simulated driving situation is about 0.6–0.8 seconds with substantial interindividual variation.[567,568] Experimental studies of simple reaction times indicate that decrements in response times are small (<10–15%) in individuals with BAC of 80–100 mg/dL,[539,569,570,571] whereas large decrements occur when the BAC exceeds 150 mg/dL.[530] The consumption of ethanol increases both simple reaction times and perceptual speed.[572] Under laboratory conditions with a fairly high degree of expectancy, the mean brake reaction time ranges from approximately 0.45–0.60 seconds with perception time accounting for most of the total brake reaction time.[573] In a Swedish study of 321 drivers using their own cars, the median brake reaction time to an *anticipated* auditory stimulus on a road was 0.66 seconds with a wide range (i.e., 0.3–2.0 seconds).[573] A road block was established by police on a local road, and police requested that the driver participate in a braking study. The test group represented all 321 drivers stopped by police. There were no measurements of ethanol concentrations.

A randomized, placebo-controlled study of 18 healthy adults using a driving simulator set to approximately 84 ft/s (i.e., 55–60 mph) measured the mean brake latency as represented by the time between the appearance of a road hazard and the time the participant touched the brake pedal.[574] The mean brake latency increased from about 0.6 seconds under placebo conditions to approximately 0.7 seconds with a BrAC of 90 mg/dL. At a speed of 60 mph, this difference in the reaction time means that the car traveled about 10 feet further under the latter condition. The average brake latency commonly cited by the US National Highway Traffic and Safety Administration (NHTSA) is 0.75 seconds.[575]

Choice.   Simple reaction time measures 1 behavioral response to a stimulus, whereas choice reaction time tasks use multiple stimuli and response possibilities. Therefore, performance on choice reaction tasks places a greater information processing load on individuals than simple reaction tasks, depending on the difficulty and the familiarity of the individual with the tasks. Complicated choice reaction tests measure information retrieval time from short-term memory, and choice reaction tests are more sensitive to ethanol than simple reaction tasks.[564] There are minor differences (<10–12%) in choice reaction times between sexes (i.e., men are faster than women) and age categories (i.e., young adults faster than adults near retirement).[568] The interaction between speed and accuracy in these tests reflects rates of information processing. Significant decrements (i.e., >10–15%) in choice reaction times does not usually occur below BAC 80–100 mg/dL unless the task is very difficult.[530] In a study requiring the selection of the appropriate key after a visual display of 5 choices, the IV infusion of ethanol sufficient to produce a BAC of 80 mg/dL caused a statistically significant ($P < .01$) increase in reaction time compared with placebo (no ethanol).[576] The mean choice reaction time increased 0.12 seconds, which corresponds to traveling an additional 13 feet at a speed of 70 mph.

Tracking.   Steering is classically a skill requiring sensorimotor coordination to keep an object on a path, and a tracking task that uses hand–eye–limb coordination necessary to steer. The effect of ethanol on tracking tasks depends on the type of tracking test. Adaptive tracking requires individuals to match the movement of a stimulus with an index. The difficulty level is adjusted to the ability of the individual. Pursuit tracking requires individuals to maintain a control index in a constant position relative to a moving index. Compensatory tracking entails the maintenance of an index on a predetermined position while a forcing function continuously moves the index off target. This test is analogous to driving on a racetrack with a lateral incline. Critical tracking is an unstable compensatory tracking task that becomes increasingly difficult as the forcing function gradually increases in magnitude. Pursuit tracking tasks are more sensitive to the effects of ethanol compared with compensatory tracking. Simple tasks of tracking are relatively resistant to the effects of ethanol <100 mg/dL,[577] whereas tests simulating evasive maneuvers required for emergency situations are much more sensitive to the effects of ethanol.[578]

DRIVING COURSES AND SIMULATORS

Driving simulators rely on sensorimotor skills (e.g., tracking) along with some judgment. The effects of ethanol on performance in driving simulators are variable depending on the task as well as age, health, and baseline psychomotor abilities. Ageing is associated with some loss of visual acuity, reduced peripheral vision, response slowing, increased reaction time, decline in ability to divide attention, and difficulty with problem-solving and perceptual speed.[579] In a study comparing the driving skills of older (mean age 69 ± 5.2 years) and middle-aged (36 ± 5.8 years) individuals on a driving simulator, the older drivers demonstrated greater driving impairments in speed control and braking.[580]

The older drivers drove slower, spent more time nego- tiating left turns, had more inappropriate braking, made fewer appropriate full stops, had more speed variability, and had more crashes. However, the older men were no more sensitive to ethanol intoxication (BAC >80 mg/ dL) than middle-age drivers in regards to driving per- formance, awareness of intoxication, and judgment.

Drivers with BAC in the range of 70–100 mg/dL demonstrate some increase in braking time and steering errors in response to changes in angulation of the simu- lated roadway.[530] Generally, the most consistent changes in drivers using driving simulators with BAC of 70– 100 mg/dL involve information processing, decision- making, and judgment as manifest in speed and braking parameters. Limitation of closed-course driving involves the use of low speeds and the lack of unpredictable road hazards. Tests of driving in closed course conditions usually measure braking time, steering errors, and other control maneuvers, as well as subjective changes in driving behavior. Some adaption to the effects of ethanol on driving skills under closed-course conditions may occur following the chronic use of ethanol. In a study of light, intermediate, and heavy drinkers, the percentage of participants in each group with evidence of impair- ment at a mean BAC of 79 mg/dL was 68%, 47%, and 40%, respectively.[623]

Culpability Studies

Although the consumption of ethanol is a major risk factor for collisions, the consumption of ethanol per se is not a necessary or sufficient cause of automobile acci- dents. Many fatal accidents and most nonfatal accidents are not associated with the use of ethanol by the driver. However, the risk of involvement increases dramatically as the BAC exceeds 80–100 mg/dL, and the relative risk of involvement in a crash exceeds 20 when the BAC increases above 150–200 mg/dL.[58] Sharp increases in the risk of a fatal accident for more experienced drinkers do not occur until the BAC increases above 100 mg/ dL.[581] Although the relative risk of a fatal accident increases substantially in the range of 80–100 mg/dL, many drivers with a BAC >80 mg/dL do not crash.[582] In general, the use of alcohol is relatively more common in single-vehicle accidents.[583]

Human Factors.   Factors that affect the relative risk of driving accidents include personality, mood, stress, fatigue, and marital status. Personality traits that predis- pose an individual to an increased risk of auto accidents include impulsiveness, overt hostility or aggression, low frustration tolerance, feelings of discontent, depression, irritability, emotional instability, and thrill-seeking

motives.[584] The effect of ethanol on these traits and the increased risk of driving accidents are not well defined. In a study of 20 female volunteers administered a risk- taking paradigm as part of a randomized, double-blind, 2-period laboratory study, testing 45 minutes after the ingestion of 0.7 g ethanol/kg did not increase risk taking.[585] The mean blood ethanol concentration was 65 ± 10.5 mg/dL; therefore, almost all of these volun- teers had a BAC below 80 mg/dL. The effect of acute ethanol intoxication on executive function and decision making is not well defined. Experimental studies suggest that doses of ethanol producing mean blood ethanol concentrations near 70 mg/dL (i.e., as estimated by breath analysis) affect executive function by subtly impairing the ability to use emotional signals (gains, losses, probabilities) while making risky decisions.[586] Measuring the effect of acute ethanol intoxication on impulsivity is complicated by disagreement on the defi- nition of impulsivity (e.g., outcome of the decision, the load placed on working memory, other measures).

Motor Vehicle Accidents. The consumption of alcohol is a major factor in both fatal and nonfatal inju- ries from motor vehicle accidents. Studies of alcohol use in patients requiring hospital care after nonfatal acci- dents indicate that the incidence of alcohol use is high in this population, ranging from ~23–66%.[587] Alcohol involvement is also relatively greater in single-vehicle accidents and those crashes occurring on weekends.[588] Frequently, alcohol-related, single-vehicle accidents occur during the negotiation of a curve, which demands attention divided between estimation of the degree of curvature and precise tracking of the curved path.[589] The major difference between alcohol-related and nonalcohol-related single-vehicle crashes on rural Australian roads was road alignment. The percentages of alcohol-related and nonalcohol-related, single-vehicle accidents occurring on a curve were 50% and 36%, respectively.

The Fatal Accident Reporting System (FARS) of the US National Highway Traffic Safety Administration (NHTSA) collects yearly data on all fatal crashes (i.e., death within 30 days) that occur on public roadways and involve a motor vehicle in transport. A fatal crash is considered alcohol-related if *any* of the following 3 con- ditions occur: 1) the investigating officer determines that at least 1 driver was drinking, 2) the BAC is >10 mg/ dL, or 3) the police officer cites an involved driver for driving while intoxicated. When any of these 3 condi- tions occur, the accident is presumed to be alcohol- related without determining actual fault based on accident analysis. The term drinking refers to any sign of alcohol consumption by a driver, including BAC below the legal limit of intoxication for driving. Because

a BAC is not available for many drivers involved in fatal crashes, the NHTSA uses a statistical method to estimate missing BACs.[590]

In 1999, approximately 36,000 vehicle occupants and about 6,000 nonmotorists (pedestrians/pedal cyclists) died within 30 days of a motor vehicle accident.[591] Since 1982, there has been a steady decline in the number of fatal accidents associated with ethanol.[592] Between 1981 and 1999, the percentage of all fatal accidents involving at least 1 drinking driver (i.e., BAC >10 mg/dL) declined from 57% to 38%. During this period, the percentage of fatally injured motor vehicle occupants with no detectable alcohol increased from 43% to 62%, whereas the percentage of dead occupants with BAC 10–99 mg/dL and ≥100 mg/dL decreased from 11% to 8% and 46% to 30%, respectively. According to 1999 FARS data, the percentage of all drivers in fatal accidents with BAC 10–99 mg/dL and ≥100 mg/dL was 23% and 17%, respectively. Drinking drivers were overrepresented during the evening and night hours (6:00 PM–5:59 AM). During the evening hours, the percentage of drivers with BAC 10–99 mg/dL and ≥100 mg/dL was 40% and 31%, respectively, compared with 10% and 6%, respectively, during the daytime. The percentage of drinking drivers (BAC ≥10 mg/dL) involved in fatal accidents peaks from midnight to 3 AM. During this period, the percentage of drinking drivers in single-vehicle and multiple-vehicle accidents was 77% and 70%, respectively, compared with 14% and 8%, respectively, for the period between 9 AM and noon. Analysis of FARS data and the 1996 National Roadside Survey of Drivers indicates that the relative risk of involvement in a fatal, single-vehicle accident decreases with age and female gender.[593] At a BAC of 90 mg/dL, the relative risk of a fatal single-vehicle crash varied between 11.4 (≥35 years old) to 51.9 (male driver, age 16–20 years). With few exceptions, women and older drivers had lower relative risk of involvement in fatal, single-vehicle crashes compared with men and younger drivers. However, the use of alcohol among young female drivers is increasing compared with earlier data.[594] Among fatally injured drivers, heavy drinkers are overrepresented compared with social drinkers. In a study of fatal traffic accidents in Texas, the mean BAC values for drivers judged to be responsible or not responsible for the accident were 190 mg/dL and 150 mg/dL, respectively.[595]

PEDAL CYCLES. The etiology of serious bicycle crashes is multifactorial. Potential factors include environmental causes (night-time riding, rain, time during the day), adverse road conditions (pot holes, traffic congestion, poor visibility, areas of construction), bicycle-related factors (speed, use of helmets, poor decisions regarding traffic), and the sharing of transportation routes with motorists. Severe head injury is the most common cause of death, and falls from bicycles cause more head injuries than limb or spinal trauma.[596] Age is also a variable for fatal bicycle accidents. In a retrospective study, bicyclist error accounted for 79% and 55% of the fatal bicycle accidents in the age groups <10-years-old and 10–19-years-old, respectively, whereas motorist error accounted for 63% of the fatal bicycle accidents among bicyclists 20–44-years-old.[597] In general, the relative risk of a fatal bicycle accident increases with age >44, night-time bicycle riding, and male gender.[598] Night-time fatality rates are approximately triple the day-time fatality rates after adjustment for mileage.[599] Exclusive of alcohol-related accidents, reduced visibility during dark hours contributes to the relative increase in fatalities for pedestrians and pedal cyclists. Analysis of FARS data from 1980–1990 demonstrated that about 12% of the fatal pedestrian/pedal cycle collisions involved a drinking driver, whereas approximately 44% of all other fatal accidents involved a drinking driver.[600]

The FARS reporting system includes only bicyclists who die on a public road after a collision with a motor vehicle. This data system does not include fatal bicycle accidents when the accident occurs on private property or when a motor vehicle is not involved. A review of the FARS data between 1987–1991 demonstrated that of the 1,711 fatal bicycle accidents reported in the 15 and over age group, an estimated 32% of the bicyclists had been drinking and 23% were legally intoxicated.[601] There are few data on the BAC of motor vehicle drivers involved in fatal bicycle accidents. In an unmatched case-control study of injured bicyclists who arrived at a hospital within 6 hours of injury, the risk of injury was approximately 10 times higher for bicyclists with BAC >100 mg/dL when compared with sober controls.[602]

PEDESTRIANS. Pedestrians provide lower contrast and smaller obstacles compared with vehicles. During 1999, approximately 4,400 pedestrians died within 30 days of an accident involving a motor vehicle based on FARS data.[591] Of these fatalities, the percentage with BAC 10–99 mg/dL and ≥100 mg/dL was 7% and 34%, respectively. In a study of injured pedestrians presenting to a trauma center, 30% had detectable BAC and about 22% had a BAC >100 mg/dL.[603]

Standardized Field Sobriety Tests (SFSTs)

Police officers use field sobriety tests to help assess whether or not a driver is impaired and to give probable cause for an arrest. In 1977, Burns and Moskovitz conducted laboratory tests to determine which field sobriety tests were the most sensitive indicators of impairment (i.e., BAC >100 mg/dL).[604] The horizontal gaze nystag-

mus, the walk-and-turn, and the one-leg stand test were the 3 most sensitive tests for the assessment of impairment based on BAC >100 mg/dL. A follow-up of the original work of Burns and Moskovitz by Stuster and Burns concluded that the field sobriety tests were 91–94% accurate in estimating BAC < 80 mg/dL or >80 mg/dL in drivers arrested for suspected driving under the influence.[605] The preferred method of analyzing the BAC during this study was breath testing (i.e., BRAC), but the participants had the choice of testing their ethanol concentration by analysis of their blood or urine samples. The report did not contain a distribution of these 3 methods of testing the BAC during the study. The data from these studies are heavily weighted to very high measured blood alcohol concentrations (MBAC), and reanalysis of the data indicate that the accuracy of the field sobriety tests depend on the MBAC, particularly in the MBAC range of 0.04–0.12%.[606] Table 21.10 demonstrates the traditional statistics of test performance for symmetrical ranges around the MBAC of 0.08% (80 mg/dL). Although the accuracy of determining whether the MBAC is above or below 0.08% is 91% for the whole range of MBAC from 0–0.33%, the accuracy is much less when the sample contains only MBAC below 0.12%.

Although these field sobriety tests are sensitive for determining if the MBAC is ≥80 mg/dL, the results of this and other studies[607,608] indicated that these tests lack specificity below MBAC <100–120 mg/dL.[609] In these 2 studies, about 32–47% of the participants judged impaired by the field sobriety tests had MBAC <100 mg/dL. In addition, there is substantial variation in test-retest and interrater reliability, depending on the scoring criteria and the training of the officers. Scoring systems are available to increase reliability based on a standardized scoring system involving a maximum of 6 points for the horizontal gaze nystagmus (HGN), 5 points for the one-leg stand, and 9 points for the walk-and-turn.[610] The HGN test accounted for most of the sensitivity of the standardized field sobriety test; similar results occurred following the use of HGN test along with A to Z alphabet recital and rapid, alternate hand clapping for detection of elevated BAC during boating.[611]

HORIZONTAL GAZE NYSTAGMUS (HGN).   Of the ocular signs (conjunctival erythema, eyelid lag, slow pupillary light reflex, convergent pupils) associated with ethanol intoxication, HGN correlates best to BAC. The HGN measures the angle of gaze from the sagittal plane to the onset of rapid jerking movements (saccades) of the eye while visually tracking an object horizontally. As the BAC increases, the movement of the eyes becomes jerky as a succession of irregular saccades replaces smooth pursuit movements.[612] The amplitude of the rapid, jerking movements in the direction of gaze increases with increased deviation from the midline. Although HGN correlates roughly to BAC, HGN is *not* a direct measure of impairment because the presence of saccadic eye movements does not impair vision. The main value of the HGN is a screening test to detect high BAC rather than an indicator of impairment. Consequently, the HGN test does not eliminate the necessity of determining the BAC by standard laboratory methods.

At a BAC of 100 mg/dL, the onset of nystagmus usually begins at approximately 40° laterally from the midline.[608] The angle of onset of nystagmus decreases as the BAC increases,[613] and the onset of nystagmus at 45° may occur at BAC <80 mg/dL.[614] Consequently, the presence of a positive HGN test by itself does not necessarily imply a BAC >80 mg/dL. In an experimental setting involving 20 healthy volunteers, BAC in the ranges of 40–60 mg/dL and 80–100 mg/dL were associated with the onset of nystagmus at 45–50°, whereas BAC in the range of 100–120 mg/dL was associated with the onset of nystagmus at 35–40°.[615] The coadministration of the equivalent of 2–4 cups of coffee (i.e., up to 500 mg caffeine) did not alter the angle of onset of nystagmus.

TABLE 21.10. Reanalysis of National Highway Traffic Safety Administration 1998 Report on the Standardized Field Sobriety Test Battery at Various Breath Ethanol Ranges.[606]

MBAC*	Number	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0.07%-0.09%	36	72	96	36	70	83
0.06%-0.10%	65	75	95	44	73	85
0.05%-0.11%	97	79	97	55	75	92
0.04%-0.12%	135	82	95	63	79	90

Abbreviations: MBAC = measured blood alcohol concentration in g%, where 0.1 g% = 100 mg/dL; Accuracy = percentage of cases correctly classified as above or below MBAC 0.08%; PPV = positive predictive value; NPV= negative predictive value.

*The follow-up study (Reference 605) used breath alcohol concentrations preferentially, but measurements of blood and urine also were accepted. The study did not report the distribution of these 3 types of ethanol measurements.

The 6-point scoring system of HGN includes a point for the following signs in each eye: 1) onset of nystagmus before 45° (40° between 12 PM and 5 AM), 2) moderate, rapid, and distinct nystagmus in the direction of gaze when eye moves as far as possible laterally (end-position nystagmus), and 3) eye cannot follow a moving object smoothly.[610] End-position nystagmus occurs in the normal nondrinking population, particularly in a fatigued individual.[616] Nonpeer-reviewed, pilot studies indicated that about 80% of individuals with scores >4 have a BAC exceeding 100 mg/dL,[608] but there are few peer-reviewed data on the predictive value of total test scores from the general population. During ethanol intoxication, the ocular findings are usually similar in both eyes. Visual acuity does not affect HGN; therefore, glasses are usually removed prior to testing.[617] Hard contacts may limit lateral movement of the eye and other field sobriety tests are used for individuals wearing hard contacts. Other conditions associated with nystagmus include high refractive errors, myasthenia gravis, multiple sclerosis, cerebellar atrophy, congenital nystagmus, Ménière disease, cataracts, corneal lesions, encephalitis, poliomyelitis, stroke, sedative-hypnotic medications, and other drugs of abuse (e.g., phencyclidine, opiates, marijuana).[618] There are few data on the effect of various medications (e.g., sedatives, anticonvulsants, analgesics) on the presence of HGN. Under normal conditions, nystagmus occurs at extreme lateral gaze, and the presence of mild, endpoint nystagmus does not indicate ethanol intoxication.[609] Chronic alcohol abusers may not demonstrate HGN at BAC >100 mg/dL.[619] The accuracy of the HGN test is unaltered by the position (i.e., standing, sitting, supine) of the individual during testing.[620]

DIVIDED ATTENTION TASKS.   Other sobriety tests require divided attention during mental and physical tasks. The walk-and-turn test requires the individual to stand on a line in a heel-to-toe position while listening to instructions. The individual then takes 9 steps in a heel-to-toe fashion, pivots, and returns along the same straight line also in a heel-to-toe fashion. The one-leg stand is a balance task that requires individuals to stand with arms at their side while extending 1 foot 6 inches off the ground. The individual must maintain that position while counting for 30 seconds without extending the arms or losing balance.

BODY SWAY.   Along with the finger-to-nose test, body sway is a simple test (Romberg test) of sensorimotor coordination. These tests classically are part of the field sobriety tests administered by police personnel. The Romberg test is relatively sensitive to BAC exceeding 100 mg/dL[621] Compared with other psychomotor tests, tasks involving sensorimotor coordination (e.g., finger-to-nose, Romberg test) demonstrate the greatest degree of resistance to the effects of ethanol consumption by chronic ethanol consumption.[622,623]

ODOR.   Although pure ethanol is relatively odorless, alcoholic beverages (i.e., beer, wine, spirits) contain a variety of high aroma compounds at concentrations as low as 1–10 ng/L; fractionation of aroma extracts of Spanish wine with high pressure liquid chromatography identified over 70 aroma compounds.[624] Detection of the odor of alcohol is highly subjective, depending on a variety of factors in addition to the BAC including the sensitivity of the evaluator's odor perception, the type of beverage, recent ingestion of a meal, and the presence of aromatic compounds in the beverage. Experimental data suggests that the odor threshold of alcoholic beverages is in the range of 60–80 mg/dL; however, the odor of ethanol is not necessarily present in individuals with blood ethanol concentrations exceeding older or newer levels of legal intoxication. In a study of 562 drinking adults evaluated by physicians trained to detect ethanol intoxication, the odor of ethanol was detected in 33% of participants with blood ethanol between 61–80 mg/dL compared with slightly less than 50% of participants with blood ethanol in the range of 140 mg/dL.[625]

The presence of an odor of alcohol is an indication of recent alcohol consumption, but the strength of the odor is not an accurate measure of the BAC or impairment. Although breath odor is frequently cited by police officers in alcohol-related traffic cases, there is little scientific data to support the use of odor as a means of estimating blood alcohol concentration or impairment. In a pilot study of the cues used by officers to detect drivers under the influence of alcohol, 75 volunteers were either controls (no alcohol) or they drank sufficient alcohol over 1.5–2 hours to produce a BAC ranging from 50–150 mg/dL.[626] After a 30-minute waiting period, the participants drove a car over a closed course to a check-point where officers examined the participants for various cues including the odor of alcohol on their breath. For controls, the false-positive rate was 7%; for participants with a BAC 50–100 mg/dL and BAC 100–150 mg/dL, the false-negative rates were 61% and 39%, respectively. Moskowitz et al studied the ability of 20 experienced police officers to detect an alcohol odor on the breath of 14 individuals with blood alcohol concentrations ranging from 0–0.13% (0–130 mg/dL).[627] After a 30-minute waiting period, the individuals were placed behind a screen and asked to blow into a tube next to the officer. For controls, the false-positive rate was 17–23%. When asked to categorize a detected odor into 1 of 3 concentrations (<40 mg/

dL, 40–80 mg/dL, >80 mg/dL), the correct responses ranged from 19–35%. Consequently, the odor strength estimates were poorly correlated to BAC.

HEALTH SURVEILLANCE

Exposure of workers to ambient air concentrations of ethanol does not usually produce blood ethanol concentrations high enough to cause impairment. Exposure of a healthy volunteer to 1,900 mg/m^3 (UK occupational exposure limit) for 3 hours produced a blood ethanol concentration below the LOD (<0.2 mg/dL).[33]

TREATMENT

Stabilization

Acute ethanol intoxication usually responds to supportive care with special attention to prevention of aspiration, monitoring of respirations, and replacement of fluid and electrolytes as necessary. All obtunded patients should be rapidly evaluated for the adequacy of oxygenation (pulse oximetry, arterial blood gases [ABGs]), circulation, and serum glucose (e.g., Accu-Chek®, Roche Diagnostics, Indianapolis, IN). If rapid measures of blood glucose are not available, 50 mL 50% dextrose in water (D$_{50}$W) should be administered intravenously after blood samples are drawn. Other drugs of immediate importance in obtunded, alcohol dependent patients include naloxone (0.4–0.8 mg IV titrated up to 2 mg IV, if no effect) for suspected opioid use and thiamine (100 mg IV). Hypothermia may complicate the course of acute ethanol intoxication; depending on severity, warming measures should include passive rewarming (blanket) and active rewarming (warm humidified oxygen, warmed IV fluids, heating blanket). A severe metabolic acidosis may occur in alcohol dependent patients as a result of alcoholic ketoacidosis and primary lactic acidosis (hypoxia, hypoperfusion).[628] Management includes IV fluid therapy, sodium bicarbonate for pH <7.1, correction of underlying abnormalities, and treatment of associated conditions (trauma, GI bleeding, hepatorenal failure, encephalopathy). Evidence of trauma in the alcohol dependent patient indicates the need for cervical immobilization, cervical x-rays, and a careful search for cervical and cerebral trauma. A computed tomography (CT) scan of the head is usually necessary in alcohol dependent patients with altered consciousness and evidence of head trauma.

Gut Decontamination

The rapid absorption of alcohol limits the effectiveness of decontamination measures, and there are few clinical data to support the use of activated charcoal in cases of ethanol poisoning. In general, decontamination measures do not alter the clinical course of drug ingestions, when initiated >1–2 hours after ingestion. A study of volunteers administered ethanol did not detect a statistically significant difference (P = .08) in peak concentrations or time-to-peak concentrations when comparing volunteers receiving superactivated charcoal prior to ethanol with the same volunteers ingesting only ethanol.[629] However, animal studies indicated that the concomitant administration of charcoal with 95% ethanol reduces ethanol concentrations at 1, 2, and 3 hours to 62%, 73%, and 82% of control values, respectively.[630] There are no data on the efficacy of cathartics following ethanol ingestions. Asymptomatic children who ingest an ethanol-containing product and remain symptom free for 1–2 hours do not require decontamination.

Elimination Enhancement

Although hemodialysis increases ethanol clearance by 3- to 4-fold, supportive care is usually sufficient to prevent serious complications. Potential indications for hemodialysis include blood ethanol concentrations >500 mg/dL with deteriorating vital signs or poor hepatic function with the failure of supportive care. The IV administration of fructose or saline are not clinically effective methods for increasing the elimination of ethanol from the blood.[631]

Antidotes

There are inadequate clinical data to support the use of naloxone during ethanol intoxication, and the efficacy of naloxone for the reversal of an ethanol-induced coma is equivocal. Although rare case reports associate the reversal of ethanol-induced coma by naloxone, there are no well-controlled studies that confirm the usefulness of naloxone in ethanol-induced coma. A volunteer study composed mostly of alcohol abusers did not detect a significant reversal of the clinical features of ethanol intoxication following the administration of naloxone.[632]

Supplemental Care

Supportive care includes close observation of ventilation, replacement of nutritional deficiencies (magnesium, thiamine [vitamin B$_1$], riboflavin [vitamine B$_2$], pyridoxine [vitamin B$_6$], nicotinamide, vitamins K and C), correction of dehydration, electrolyte and acid-base imbalance, and evaluation for underlying illness and withdrawal symptoms. Poorly nourished alcohol abusers

require high, initial doses (i.e., at least 200 mg) of par-enteral thiamine diluted in 100 mL of saline over 30–60 minutes, particularly when these patients receive IV glucose.[633] Oral thiamine does not adequately replace depleted stores of thiamine.[223] Laboratory evaluation of the seriously ill alcohol dependent patients includes the following tests: electrolyte depletion abnormalities (potassium, magnesium, phosphorus, calcium), ECG abnormalities (dysrhythmias, cardiomyopathy, hypertension), arterial blood gases (metabolic acidosis, hypoxemia secondary to aspiration pneumonia), urinalysis (myoglobin, unusual crystals such as calcium oxalate), serum amylase or lipase (pancreatitis), chest x-ray (aspiration pneumonia, pulmonary tuberculosis, congestive heart failure), blood glucose (hypoglycemia/hyperglycemia), complete blood count with red cell indices for anemia, coagulopathies (prothrombin time and platelet count for hepatic dysfunction), and serum creatinine (renal function). Patients with alcoholic keto-acidosis should be hydrated with 5% dextrose (IV saline may paradoxically worsen acidosis) as well as potassium, magnesium, and phosphate supplementation as needed.[522]

Withdrawal Syndromes

Standard supportive care for alcohol withdrawal includes general nursing care in a quiet environment, monitoring of vital signs, reassurance, reality orientation, hydration, and the administration of thiamine. The usual initial dose of thiamine to prevent Wernicke encephalopathy is at least 50–100 mg intravenously. Nutritionally deficient alcohol dependent patients receiving glucose should also receive thiamine, but the urgent administration of glucose for hypoglycemia should not be withheld pending thiamine administration.[634] In otherwise healthy alcohol-dependent patients undergoing withdrawal, the minimum oral dose of thiamine during detoxification is 300 mg/day.[635] For patients with a high risk of developing Wernicke encephalopathy, larger doses of thiamine (i.e., 250 mg thiamine intramuscularly or intravenously daily for 3–5 days) are recommended. The value of the prophylactic use of multivitamins and other B vitamins remains unproven. Patients with CIWA-Ar scores ≤10 and normal mentation (i.e., no hallucination or disorientation) usually require only supportive care unless comorbid illness is present. Treatment for alcohol dependence after acute withdrawal and detoxification involves maintaining the patient in remission and developing a lifestyle compatible with long-term abstinence. Traditional approaches include psychosocial intervention (Alcoholics Anonymous, counseling) and pharmaceutic adjuncts (naltrexone, acamprosate, disulfiram) to reduce

the risk of relapse to drinking. Clinical trials indicate that naltrexone and acamprosate are more effective than placebo in reducing the risk of relapse to heavy drinking,[636] but there are inadequate data to conclude that these drugs substantially enhance complete abstinence even when administered with cognitive–behavioral therapy.[637,638] The efficacy of disulfiram, lithium, and serotonergic agents to maintain abstinence in alcohol abusers is unproved.

COMMON ABSTINENCE SYNDROME

Symptoms of alcohol withdrawal typically begin about 8 hours after substantial reduction in drinking, peak on day 2, and decrease significantly by day 4–5.[639] Although clonidine, carbamazepine, beta blockers, and neuroleptics all reduce the symptoms associated with alcohol withdrawal, benzodiazepines are the drugs of choice for alcohol withdrawal, particular for the treatment of DTs and seizures.[640] All benzodiazepines appear equally efficacious in the amelioration of withdrawal symptoms. Longer-acting benzodiazepines (e.g., diazepam) produce a smoother withdrawal from alcohol and reduce the incidence of seizures, but these agents may cause oversedation. The dosage of benzodiazepines should be individualized depending on the severity of withdrawal symptoms, previous history of serious complications during withdrawal, comorbid illness, and the specific benzodiazepine selected. Mild withdrawal (CIWA-Ar scores <8–10) may be managed with supportive care without pharmacologic intervention, whereas patients with higher scores benefit from benzodiazepine administration.[640]

Therapeutic regimens for alcohol withdrawal include fixed-schedule, front-loading, and symptom-based schedules. Candidates for fixed-schedule therapy include patients who require treatment because of the potential for serious withdrawal symptoms (seizures, DTs) as well as patients with concurrent illness, particularly if the patient is pregnant (see Table 21.11). The front-loading regimen is an alternative to the fixed-schedule regimen that delivers high doses of mediation early in the course of withdrawal rather than over 3 days. A symptom-based regimen is appropriate for symptomatic patients (AIWA-Ar ≥8–10), and this regimen usually delivers less medication over a shorter period compared with the fixed regimens.[641] Short-acting oxazepam (i.e., half-life, ~ 8 h) may be safer than diazepam in patients with severe liver or lung disease (15–60 mg every 6–8 h). Carbamazepine (600–800 mg in divided doses on day 1 tapered over 7 days to 200 mg) is an alternative to lorazepam, particularly for patients with severe underlying liver disease.[642] Gabapentin is also an alternative to lorazepam. In a study of 100 individuals seeking

TABLE 21.11. Benzodiazepine Therapy for Alcohol Withdrawal.

Regimen	Drug	Dose
Fixed-Schedule	Chlordiazepoxide	Day 1: 50–100 mg orally every 6 hours*
		Day 2–3: 25–50 mg orally every 6 hours
Front-Loading	Diazepam	20 mg orally every 2 hours until symptoms resolve[†]
Symptom-Based	Chlordiazepoxide	25–100 mg orally every hour while symptomatic[†]
Delirium Tremens	Diazepam	10 mg intravenously, then 5 mg every 5 minutes until awake and calm

*Additional 25–100 mg every 1–2 hours as needed for severe tremor or autonomic dysfunction. A dose may deleted if the patient is sleeping or resting comfortably.
†CIWA-Ar ≤8.

outpatient treatment of alcohol withdrawal, gabapentin (400 mg 3 times a day [tid] × 3 days, then 400 mg twice daily [bid]) was at least as effective as lorazepam (2 mg tid × 3 days,then 2 mg bid) as measured by the Clinical Institute Withdrawal Assessment for Alcohol—Revised (CIWA-Ar) scale.[643] There was no statistically significance difference between these 2 groups in self-reported side effects. Clonidine and beta-blockers are adjunctive agent for the treatment of mild to moderate withdrawal symptoms (e.g., tachycardia, hypertension, tremor), whereas preliminary data suggests that the glutamate release inhibitor, lamotrigine, and the AMPA/kainate receptor inhibitor, topiramate may improve the dysphoric mood and reduce the severity of withdrawal in combination with benzodiazepines.[644]

ALCOHOL DEPENDENT HALLUCINOSIS

Treatment involves primarily protection from harm and maintenance of a supportive environment. If behavior disturbances threaten the patient, physical restraint combined with benzodiazepines and the possible addition of haloperidol (2–5 mg intramuscularly or 5–10 mg orally) may be necessary.

ALCOHOL DEPENDENT SEIZURES

Most seizures are brief and self-limited, and therapy primarily involves protection from harm and support of the airway. Intravenous diazepam or lorazepam are the anticonvulsants of choice, and prophylactic or long-term phenytoin therapy is not usually indicated.[645] Although hypomagnesemia often occurs in alcohol abusers undergoing withdrawal, routine supplementation of benzodiazepines with magnesium does not reduce the severity or frequency of seizures or delirium.[646] The administration of phenytoin does not prevent the recurrence of seizures during alcohol withdrawal.[647] Recurrent seizures require evaluation for concurrent illness (e.g., intracranial hemorrhage, infection) and electrolyte imbalances (e.g., hypoglycemia, hypokalemia, hypo-

magnesemia). Disposition options for patients with a single withdrawal seizure vary from discharge from the emergency department observation unit after 6–12 hours to hospital admission for at least 24 hours depending on the severity of the withdrawal symptoms and the presence of concurrent illness. The consensus recommendations from a European panel on alcohol-related seizures included hospital admission of all of these patients for at least 24 hours.[648]

DELIRIUM TREMENS

Although initially overhydration may occur during alcoholic withdrawal as a result of the relative increase in the secretion of antidiuretic hormone, serious dehydration may result from the presence of diaphoresis, hyperthermia, tachypnea, and vomiting. Fluid requirements may approach 5 L within 24 hours.[649] Supportive care includes the correction of magnesium and phosphorus deficiencies as required by serum analysis, administration of IV thiamine before the administration of IV glucose (unless hypoglycemic), and a quiet well-lit room with reassurance and reorientation. Parenteral magnesium is not required unless indicated by low serum magnesium concentrations. Patients with impending DTs should be observed in an intensive care setting with frequent monitoring of vital signs and physical restraints as needed. Risk factors for the development of DTs should be evaluated including hypothermia, hypoglycemia, electrolyte imbalance, pancreatitis, thiamine deficiency, alcoholic ketoacidosis, GI bleeding, cerebral trauma, and chronic liver disease. High doses of benzodiazepines are necessary to ameliorate the symptoms associated with DTs. The use of benzodiazepines should be started early to limit the progression of withdrawal. Intravenous diazepam is effective in 5- to 10-mg loading dose followed by 5 mg every 5–10 minutes until the patient is calm and lightly sedated.[650] Large doses of benzodiazepines may be necessary to calm the patient. The administration of large benzodiazepine doses based

on withdrawal symptoms should be used with caution when comorbid illness is present because the symptoms of alcohol withdrawal are nonspecific. Large doses of diazepam can be safely administered in this setting as long as the patient is observed closely for excessive sedation and the development of hepatic encephalopathy. Lorazepam has a relatively short half-life (~5–10 hours) compared with diazepam, and this property limits oversedation in the elderly and patients with severe liver or lung disease. The use of lorazepam 1–4 mg intravenously every 10–15 minutes is an alternative to diazepam. Diazepam 20 mg is equivalent to lorazepam 4 mg. However, there are no clear clinical data to indicate the superiority of any specific benzodiazepine during the treatment of DTs.[651] Although neuroleptics are not routinely recommended for DTs because of the potential to lower seizure threshold, the use of haloperidol (0.5–5 mg IM or IV) in addition to benzodiazepines may be necessary every 1–4 hours to control severe agitation or hallucinations. Intravenous infusion of propofol (10–100 µg/kg/min) with intubation is an option for severe refractory DTs.[652,653]

Wernicke Encephalopathy

The presence of Wernicke encephalopathy requires high parenteral doses of thiamine (e.g., 500 mg tid for 3 days, then 250 mg daily for 3–5 days) as a result of the poor oral absorption of thiamine during this illness.[633] Parenteral solutions of thiamine may contain benzyl alcohol, and the dilution of the thiamine in 100 mL saline followed by infusion over 30 minutes reduces adverse reactions. Resolution of the ophthalmoplegia is the first response to thiamine administration for Wernicke encephalopathy, usually within hours of thiamine administration. Improvement in cognitive impairment requires much longer, and the cognitive abilities of the patient may not return to baseline levels despite adequate treatment. Korsakoff syndrome is the chronic form of Wernicke encephalopathy, characterized by short-term memory loss and confabulation. The relative preservation of other intellectual functions separates Korsakoff syndrome from the global reduction in intellectual function associated with alcohol dependent dementia.

Disulfiram Reaction

Disulfiram (Antabuse®, Teva Pharmaceuticals, Petach Tivka, Israel) is one of several aldehyde dehydrogenase inhibitors that raise plasma acetaldehyde concentrations following the consumption of ethanol. Elevated plasma acetaldehyde concentrations are associated with histamine release that produces cutaneous and systemic vasodilation, facial flushing, hypotension, tachycardia, tachypnea, palpitations, anxiety, headache, nausea, and vomiting.[654,655] Management of the ethanol-disulfiram reaction involves monitoring of blood pressure and cardiac rhythm, support of blood pressure [IV infusion of saline with pressor support (e.g., dopamine) as needed], and antihistamines.[656] 4-Methylpyrazole (Antizol®, Paladin Labs, Dover, DE) is a potentially useful antidote for the ethanol-disulfiram reaction because of the blockade of ethanol metabolism into acetaldehyde.[657] However, there are few clinical data on the efficacy of 4-methylpyrazole during the ethanol-disulfiram reaction.

References

1. McGovern P, Glusker D, Exner L. Neolithic resinated wine. Nature 1996;381:480–481.

2. Soleas GJ, Diamandis EP, Goldberg DM. Wine as a biological fluid: history, production, and role in disease prevention. J Clin Lab Anal 1997;11:287–313.

3. Seller SC. Alcohol abuse in the Old Testament. Alcohol Alcoholism 1985;20:69–76.

4. Scherberger RF, Happ GP, Miller FA, Fassett DW. A dynamic apparatus for preparing air-vapor mixtures of known concentrations. Am Ind Hyg Assoc J 1958;19: 494–498.

5. Brismar B, Bergman B. The significance of alcohol for violence and accidents. Alcoholism Clin Exp Res 1998; 22(suppl):S299–S306.

6. Skog O-J. Alcohol consumption and mortality rates from traffic accidents, accidental falls, and other accidents in 14 European countries. Addiction 2001;96(suppl 1): S49–S58.

7. National Center for Statistics and Analysis, National Highway Traffic Safety Administration. Traffic safety facts 2000: a compilation of motor vehicle crash data from the fatality analysis reporting system and the general estimates system. Washington, DC: NHTSA, National Center for Statistics and Analysis, US Department of Transportation; 2002.

8. Peck C, Gebers MA, Voas RB, Romano E. The relationship between blood alcohol concentration (BAC), age, and crash risk. J Safety Res 2008;39:311–319.

9. Zureik M, Ducimetiere P. High alcohol-related premature mortality in France: concordant estimates from a prospective cohort study and national mortality statistics. Alcohol Clin Exp Res 1996;20:428–433.

10. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshelman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Arch Gen Psychiatry 1994;51:8–19.

11. Pletcher MJ, Maselli J, Gonzales R. Uncomplicated alcohol intoxication in the emergency department: an

analysis of the national hospital ambulatory medical care survey. Am J Med 2004;117:863–867.

12. Lindberg S, Agren G. Mortality among male and female hospitalized alcoholics in Stockholm 1962–1983. Br J Addict 1988;83:1193–1200.

13. Hufford MR. Alcohol and suicidal behavior. Clin Psychol Rev 2001;21:797–811.

14. Murphy GE, Wetzel RD. The lifetime risk of suicide in alcoholism. Arch Gen Psychiatry 1990;47:383–392.

15. Borges G, Rosovsky H. Suicide attempts and alcohol consumption in an emergency room sample. J Stud Alcohol 1996;57:543–548.

16. Committee on Drugs, 1983–1984, American Academy of Pediatrics. Ethanol in liquid preparations intended for children. Pediatrics 1984;73:405–407.

17. Pohorecky LA, Brick J. Pharmacology of ethanol. Pharmacol Ther 1988;36:335–427.

18. Engel JS, Spiller HA. Acute ethanol poisoning in a 4-year-old as a result of ethanol-based hand-sanitizer ingestion. Pediatr Emerg Care 2010;26:508–509.

19. Nanji AA, French SW. Increased susceptibility of women to alcohol. Is beer the reason? N Engl J Med 1985;312:585.

20. Williams GD, Stinson FS, Lane JD, Tunson SL, Dufour MC. Apparent per capita alcohol consumption: national, state and regional trends, 1977–1994. Rockville, MD: Alcohol epidemiologic Data System, National Institute on Alcohol Abuse and Alcoholism; 1996.

21. Caetano R, Clark CL. Trends in alcohol consumption patterns among whites, blacks and Hispanics: 1984 and 1995. J Stud Alcohol 1998;59:659–668.

22. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Chemical composition of alcoholic beverages, additives and contaminants. IACR Monogr Eval Carcinogenic Risks Hum 1988;44:71–99.

23. Brick J. Standardization of alcohol calculations in research. Alcohol Clin Exp Res 2006;30:1276–1287.

24. Martin CS, Nirenberg TD. Alcohol content variation in the assessment of alcohol consumption. Addict Behav 1991;16:555–560.

25. Logan BK, Case GA, Distefano S. Alcohol content of beer and malt beverages: forensic considerations. J Forensic Sci 1999;44:1292–1295.

26. Case GA, Distefano S, Logan BK. Tabulation of alcohol content of beer and malt beverages. J Anal Toxicol 2000;24:202–210.

27. Zuba D, Piekoszewski W, Pach J, Winnik L, Parczewski A. Concentration of ethanol and other volatile compounds in the blood of acutely poisoned alcoholics. Alcohol 2002;26:17–22.

28. Perper JA, Twerski A, Wienand JW. Tolerance at high blood alcohol concentrations: A study of 110 cases and review of the literature. J Forensic Sci 1986;31:212–221.

29. Lester D, Greenberg LA, Smith RF, Carroll RP. The inhalation of ethyl alcohol by man. I. Industrial hygiene and medicolegal aspects. Quar J Stud Alcohol 1951;12:167–178.

30. Royal College of Physicians. A great and growing evil— the medical consequences of alcohol abuse. Report of a working party. London: Tavistock; 1987.

31. Ramchandani VA, Bosron WF, Li TK. Research advances in ethanol metabolism. Pathol Biol 2001;49:676–682.

32. Kruhoffer PW. Handling of inspired vaporized ethanol in the airways and lungs (with comments on forensic aspects). Forensic Sci Int 1983;21:1–17.

33. Campbell L, Wilson HK. Blood alcohol concentrations following the inhalation of ethanol vapor under controlled conditions. J Forensic Sci Soc 1986;26: 129–135.

34. Pendlington RU, Whittle E, Robinson JA, Howes D. Fate of ethanol topically applied to skin. Food Chem Toxicol 2001;39:169–174.

35. Holford NH. Clinical pharmacokinetics of ethanol. Clin Pharmacokinet 1987;13:273–292.

36. Levitt MD, Li R, DeMaster EG, Elson M, Furne J, Levitt DG. Use of measurements of ethanol absorption from stomach and intestine to assess human ethanol metabolism. Am J Physiol 1997;273:G951–G957.

37. Oneta CM, Simanowski UA, Martinez M, Allali-Hassani A, Pares X, Homann N, et al. First pass metabolism of ethanol is strikingly influenced by the speed of gastric emptying. Gut 1998;43:612–619.

38. DiPadova C, Worner TM, Julkunen RJK, Lieber CS. Effects of fasting and chronic alcohol consumption on the first-pass metabolism of ethanol. Gastroenterology 1987;92:1169–1173.

39. Ammon E, Schafer C, Hofmann U, Klotz U. Disposition and first-pass metabolism of ethanol in humans: Is it gastric or hepatic and does it depend on gender? Clin Pharmacol Ther 1996;59:503–513.

40. Baraona E, Abittan CS, Dohmen K, Moretti M, Pozzato G, Chayes ZW, et al. Gender differences in pharmacokinetics of alcohol. Alcoholism Clin Exp Res 2001;25: 502–507.

41. Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS. High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. N Engl J Med 1990;322:95–99.

42. Martin NG, Perl J, Oakeshott JG, Gibson JB, Starmer GA, Wilks AV. A twin study of ethanol metabolism. Behav Genetics 1985;15:93–109.

43. Fraser AG, Rosalki SB, Gamble GD, Pounder RE. Inter-individual and intra-individual variability of ethanol concentration-time profiles: comparison of ethanol ingestion before or after an evening meal. Br J Clin Pharmacol 1995;40:387–392.

44. Johnson RD, Horowitz M, Maddox AF, Wishart JM, Shearman DJ. Cigarette smoking and rate of gastric emptying: effect on alcohol absorption. BMJ 1991;302 (6767):20–23.

45. Greenberg LA. Pharmacology of alcohol and its relationship to drinking and driving. Q J Stud Alcohol 1968;(suppl 4):252–266.

46. Sedman AJ, Wilkinson PK, Sakmar E, Weidler DJ, Wagner JG. Food effects on absorption and metabolism of alcohol. J Stud Alcohol 1976;37:1197–1214.

47. Jones AW, Jonsson KA. Food-induced lowering of blood-ethanol profiles and increased rate of elimination immediately after meal. J Forensic Sci 1994;39:1084–1093.

48. Pikaar NA, Wedel M, Hermus RJ. Influence of several factors on blood alcohol concentrations after drinking alcohol. Alcohol Alcoholism 1988;23:289–297.

49. Gentry RT. Effect of food on the pharmacokinetics of alcohol absorption. Alcoholism Clin Exp Res 2000;24: 403–404.

50. Gustafson R, Kallmen H. The blood alcohol curve as a function of time and type of beverage: methodological considerations. Drug Alcohol Dependence 1988;21: 243–246.

51. Dubowski KM. Human pharmacokinetics of ethanol. Further studies. Clin Chem 1976;22:1199.

52. Jones AW, Jonsson KA, Neri A. Peak blood-ethanol concentrations and the time of its occurrence after rapid drinking on an empty stomach. J Forensic Sci 1991;36: 376–385.

53. Hopkins A. The pattern of gastric emptying: a new view of old results. J Physiol 1966;182:144–149.

54. Neuteboom W, Vis AA. The effects of low alcohol beers on the blood alcohol concentration. Blutakohol 1991;28; 393–396.

55. Roine R. Interaction of prandial state and beverage concentration on alcohol absorption. Alcohol Clin Exp Res 2000;24:411–412.

56. Roine RP, Gentry RT, Lim TR Jr, Helkkonen E, Salaspuro M, Lieber CS. Comparison of blood alcohol concentrations after beer and whiskey. Alcohol Clin Exp Res 1993; 17:709–711.

57. Schwartz JG, Salman UA, McMahan CA, Phillips WT. Gastric emptying of beer in Mexican-Americans compared with non-Hispanic Whites. Metabolism 1996;45: 1174–1178.

58. Chan AW. Factors affecting the drinking driver. Drug Alcohol Depend 1987;19:99–119.

59. Shumate RP, Crowther RF, Zarafshan M. A study of the metabolism rates of alcohol in the human body. J Forensic Med 1967;14:83–100.

60. Hahn RG, Norberg A, Jones AW. "Overshoot" of ethanol in the blood after drinking on an empty stomach. Alcohol Alcohol 1997;2:501–505.

61. Winek CL, Wahba WW, Dowdell JL. Determination of absorption time of ethanol in social drinkers. Forensic Sci Int 1996;77:169–177.

62. Radlow R, Hurst PM. Temporal relations between blood alcohol concentration and alcohol effect: an experiment with human subjects. Psychopharmacology 1985;85: 260–266.

63. Haddad L, Milke P, Zapata L, Ramon de la Fuente J, Vargas-Vorackova F, Lorenzana-Jimenez M, Corte G, et al. Effect of the menstrual cycle in ethanol pharmacokinetics. J Appl Toxicol 1998;18:15–18.

64. Mills KC, Bisgrove EZ. Body sway and divided attention performance under the influence of alcohol: dose-response differences between males and females. Alcohol Clin Exp Res 1983;7:393–397

65. Dubowski KM. Absorption, distribution and elimination of alcohol: highway safety aspects. J Stud Alcohol 1985; 10(suppl):98–108.

66. Jones AW, Lindberg L, Olsson SG. Magnitude and time-course of arteriovenous differences in blood alcohol concentration in healthy men. Clin Pharmacokinet 2004;43: 1157–1166.

67. Wilkinson PK, Rheingold JL. Arterial-venous blood alcohol concentration gradients. J Pharmacokinet Biopharmaceut 1981;9:279–307.

68. Jones AW, Jonsson KA, Jorfeldt L. Differences between capillary and venous blood-alcohol concentrations as a function of time after drinking, with emphasis on sampling variations in left vs right arm. Clin Chem 1989;35: 400–404.

69. Jones AW, Norberg A, Hahn RG. Concentration-time profiles of ethanol in arterial and venous and end-expired breath during and after intravenous infusion. J Forensic Sci 1997;42:1088–1094.

70. Devgun MS, Dunbar JA. Alcohol consumption, blood alcohol level and the relevance of body weight in experimental design and analysis. J Stud Alcohol 1990;51: 24–28.

71. Holtzman JL, Gebhard RL, Eckfeldt JH, Mottonen LR, Finley DK, et al. The effects of several weeks of ethanol consumption on ethanol kinetics in normal men and women. Clin Pharmacol Ther 1985;38:157–163.

72. Cowan JM Jr, Weathermon A, McCutcheon JR, Oliver RD. Determination of the volume of distribution for ethanol in male and female subjects. J Anal Toxicol 1996;20:287–290.

73. van Thiel DH, Gavaler JS, Rosenblum E, Tarter RE. Ethanol, its metabolism and hepatotoxicity as well as its gonadal effects: effects of sex. Pharmacol Ther 1989; 41:27–48.

74. Wang MQ, Nicholson ME, Jones CS, Fitzhugh EC, Westerfield CR. Acute alcohol intoxication, body composition, and pharmacokinetics. Pharmacol Biochem Behav 1992;43;641–643.

75. Goist KC, Sutker PB. Acute alcohol intoxication and body composition in women and men. Pharmacol Biochem Behav 1985;22:811–814.

76. Marshall AW, Kingstone D, Boss M, Morgan MY. Ethanol elimination in males and females relationship to menstrual cycle and body composition. Hepatology 1983; 3:701–706.

77. Vestal RE, McGuire EA, Tobin JD, Andres R, Norris AH, Mezey E. Aging and ethanol metabolism. Clin Pharmacol Ther 1977;21:343–354.

78. Norberg A, Jones AW, Hahn RG, Gabrielsson JL. Role of variability in explaining ethanol pharmacokinetics. Clin Pharmacokinet 2003;42:1–31.

79. Bora PS, Lange LG. Molecular mechanism of ethanol metabolism by human brain to fatty acid ethyl esters. Alcohol Clin Exp Res 1993;17:28–30.

80. Lands WE. A review of alcohol clearance in humans. Alcohol 1998;15:147–160.

81. Schindler JF, Berst KB, Plapp BV. Inhibition of human alcohol dehydrogenases by formamides. J Med Chem 1998;41:1696–1701.

82. Matsumoto H, Fukui Y. Pharmacokinetics of ethanol: a review of the methodology. Addiction Biol 2002;7:5–14.

83. Crabb DW, Edenberg J, Bosron WF, Li TK. Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive ALDH (2) allele is dominant. J Clin Invest 1989;83:314–316.

84. Rooke N, Li DJ, Li J, Keung WM. The mitochondrial monoamine oxidase-aldehyde dehydrogenase pathway: a potential site of action of daidzin. J Med Chem 2000; 43:4169–4179.

85. Keiding S, Christensen NJ, Damgaard SE, Dejgard A, Iversen HL, Jacobsen A, et al. Ethanol metabolism in heavy drinkers after massive and moderate alcohol intake. Biochem Pharmacol 1983;32;3097–3102.

86. Lieber CS. Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)–a review. Alcohol Clin Exp Res 1999;23;991–1007.

87. Tsutsumi M, Lasker JM, Shimizu M, Rosman AS, Lieber CS. The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. Hepatology 1989;10: 437–446.

88. Oneta CM, Lieber CS, Li JJ, Ruttimann R, Schmidt B, Lattmann J, et al. Dynamics of cytochrome P4502E1 activity in man; induction by ethanol and disappearance during withdrawal phase. J Hepatol 2002;36:47–52.

89. Salmela KS, Kessova IG, Tsyrlov IB, Lieber CS. Respective roles of human cytochrome P-4502E1, 1A2, and 3A4 in the hepatic microsomal ethanol oxidizing system. Alcohol Clin Exp Res 1998;22;2125–2132.

90. Lieber CS. Effects of chronic alcohol consumption on the metabolism of ethanol. Prog Clin Biol Res 1987;241: 161–72.

91. Keilin D, Hartree EF. Properties of catalase: catalysis of coupled oxidation of alcohols. Biochem J 1945;39: 293–301.

92. Matsumoto H, Fukui Y. Pharmacokinetics of ethanol: a review of the methodology. Addiction Biol 2002;7: 5–14.

93. Jones AW. Excretion of alcohol in urine and diuresis in healthy men in relation to their age, the dose administered and the time after drinking. Forensic Sci Int 1990;45: 217–224.

94. Dahl H, Stephanson N, Beck O, Helander A. Comparison of urinary excretion characteristics of ethanol and ethyl glucuronide. J Anal Toxicol 2002;26:201–204.

95. Schneider H, Glatt H. Sulpho-conjugation of ethanol in humans *in vivo* and by individual sulphotransferase forms *in vitro*. Biochem J 2004;383:543–549.

96. Halter CC, Dresen S, Auwaerter V, Wurst FM, Weinmann W. Kinetics in serum and urinary excretion of ethyl sulfate and ethyl glucuronide after medium dose of ethanol intake. Int J Legal Med 2008;122:123–128.

97. Widmark EM. Verteilung und unwandltung des athyl alohols in organimus des hundes. Biochem Z 1933;267: 128–134.

98. Andreasson R, Jones AW. Erik MP. Widmark (1889–1945): Swedish pioneer in forensic alcohol toxicology. Forensic Sci Int 1995;72;1–14.

99. Andreasson R, Jones AW. The life and work of Erik M. P. Widmark. Am J Forensic Med Pathol 1996;17; 177–190.

100. Watson PE, Watson ID, Batt RD. Prediction of blood alcohol concentrations in human subjects. J Stud Alcohol 1981;42:547–556.

101. Seidl S, Jensen U, Alt A. The calculation of blood ethanol concentrations in males and females. Int J Legal Med 2000;114:71–77.

102. Wilkinson PK. Pharmacokinetics of ethanol: A review. Alcohol Clin Exp Res 1980;4:6–21.

103. Wagner JG, Wilkinson PK, Ganes DA. Parameters Vm and Km for elimination of alcohol in young male subjects following low doses of alcohol. Alcohol Alcohol 1989;24: 555–564.

104. Lewis MJ. Blood alcohol: the concentration-time curve and retrospective estimation of level. J Forensic Sci Soc 1986;26:95–113.

105. Norberg A, Gabrielsson J, Jones AW, Hahn RG. Within- and between-subject variations in pharmacokinetic parameters of ethanol by analysis of breath, venous blood and urine. Br J Clin Pharmacol 2000;49:399–408.

106. Brennan DF, Betzelos S, Reed R, Falk JL. Ethanol elimination rates in an ED population. Am J Emerg Med 1995; 13;276–280.

107. Wilson JR, Erwin G, McClearn GE. Effects of ethanol: I. Acute metabolic tolerance and ethnic differences. Alcohol Clin Exp Res 1984;226–232.

108. Nagoshi CT, Wilson JR. Long-term repeatability of human alcohol metabolism, sensitivity and acute tolerance. J Stud Alcohol 1989;50:162–169.

109. Ramchandani VA, Kwo PY, Li TK. Effect of food and food composition on alcohol elimination rates in healthy men and women. J Clin Pharmacol 2001;41:1354–1350.

110. Li J, Mills T, Erato R. Intravenous saline has no effect on blood ethanol clearance. J Emerg Med 1999;17:1–5.

111. Jones AW, Hahn RG, Stalberg HP. Pharmacokinetics of ethanol in plasma and whole blood: estimation of total body water by the dilution principle. Eur J Clin Pharmacol 1992;42:445–448.

112. Bogusz M, Pach J, Stasko W. Comparative studies on the rate of ethanol elimination in acute poisoning and in controlled studies. J Forensic Sci 1977;22:446–451.

113. Gershman H, Steeper J. Rate of clearance of ethanol from the blood of intoxicated patients in the emergency department. J Emerg Med 1991;9:307–311.

114. Hammond KB, Rumack BH, Rodgerson DO. Blood ethanol: a report of unusually high levels in a living patient. JAMA 1973;226:63–64.

115. O'Neill S, Tipton KF, Prichard JS, Quinlan A. Survival after high blood alcohol levels. Arch Intern Med 1984; 144:641–642.

116. Levitt MD, Levitt DG. Use of a two-compartment model to assess the pharmacokinetics of human ethanol metabolism. Alcohol Clin Exp Res 1998;22:1680–1688.

117. Jones AW. Evidence-based survey of the elimination rates of ethanol from blood with applications in forensic casework. Forensic Sci Int 2010;200:1–20.

118. Jones AW. Disappearance rate of ethanol from the blood of human subjects: implications in forensic toxicology. J Forensic Sci 1993;38:104–118.

119. Lamminpaa A. Alcohol intoxication in childhood and adolescence. Alcohol Alcohol 1995;30:5–12.

120. Mumenthaler MS, Taylor JL, Yesavage JA. Ethanol pharmacokinetics in white women: nonlinear model fitting versus zero-order elimination analyses. Alcohol Clin Exp Res 2000;24:1353–1362.

121. Ragan FA Jr, Samuels MS, Hite SA. Ethanol ingestion in children: A five year review. JAMA 1979;242: 2787–2788.

122. Jones AW, Andersson L. Influence of age, gender, and blood-alcohol concentration on the disappearance rate of alcohol from blood in drinking drivers. J Forensic Sci 1996;41:922–926.

123. Thomasson H. Alcohol elimination: faster in women? Alcohol Clin Exp Res 2000;24:419–420.

124. Cole-Harding S, Wilson JR. Ethanol metabolism in men and women. J Stud Alcohol 1987;48:380–387.

125. Jones AW. The drunkest drinking driver in Sweden: blood alcohol concentration 0.545% w/v. J Stud Alcohol 1999; 60:400–406.

126. Jones AW. Ultra-rapid rate of ethanol elimination from blood in drunken drivers with extremely high blood-alcohol concentrations. Int J Legal Med 2008; 122:129–134.

127. Mennella JA, Beauchamp GK. The transfer of alcohol to human milk effects on flavor and the infant's behavior. N Engl J Med 1991;325:981–985.

128. Lawton ME. Alcohol in breast milk. Aust N Z J Obstet Gynecol 1985;25;71–73.

129. Ho E, Collantes A, Kapur BM, Moretti M, Koren G. Alcohol and breast feeding: calculation of time to zero level in milk. Biol Neonate 2001;80:219–222.

130. Little RE, Anderson KW, Ervin CH, Worthington-Roberts B, Clarren SK. Maternal alcohol use during breast-feeding and infant mental and motor development at one year. N Engl J Med 1989;321:425–430.

131. Little RE, Northstone K, Golding J, ALSPAC Study Team. Alcohol, breastfeeding, and development at 18 months. Pediatrics 2002;109:e72.

132. Mellanby E. Alcohol: its absorption and disappearance from the blood under different conditions. Medical Research Committee, Special Report Series No. 31, London, Her Majesty's Stationery Office, 1919.

133. Hiltunen AJ, Saxon L, Skagerberg S, Borg S. Acute tolerance during intravenous infusion of alcohol: comparison of performance during ascending and steady state concentrations—a pilot study. Alcohol 2000;22:69–74.

134. Hiltunen AJ. Acute alcohol tolerance in social drinkers: changes in subjective effects dependent on the alcohol dose and prior alcohol experience. Alcohol 1997; 14:373–378.

135. Goddwin DW, Powell B, Stern J. Behavioral tolerance to alcohol in moderate drinkers. Am J Psychiatry 1971; 127:87–89.

136. Kaplan HL, Sellers EM, Hamilton C, Naranjo CA, Dorian P. Is there acute tolerance to alcohol at steady state? J Stud Alcohol 1985;46:253–256.

137. Vogel-Sprott M, Fillmore MT. Impairment and recovery under repeated doses of alcohol: effects of response-outcomes. Pharmacol Biochem Behav 1993;45:59–63.

138. Wang MQ, Taylor-Nicholson ME, Airhihenbuwa CO, Mahoney BS, Fitzhugh EC, Christina R. Psychomotor and visual performance under the time-course effect of alcohol. Percept Mot Skills 1992;75:1095–1106.

139. Fogarty JN, Vogel-Sprott M. Cognitive processes and motor skills differ in sensitivity to alcohol impairment. J Stud Alcohol 2002;63:404–411.

140. Portans I, White JM, Staiger PK. Acute tolerance to alcohol: changes in subjective effects among social drinkers. Psychopharmacology 1989;97:365–369.

141. Tabakoff B, Cornell N, Hoffman PL. Alcohol tolerance. Ann Emerg Med 1986;15:1005–1012.

142. Lindblad B, Olsson R. Unusually high levels of blood alcohol? JAMA 1976;1600–1602.

143. Kalant H, LeBlanc E, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 1971;23:135–191.

144. Bennett RH, Cherek DR, Spiga R. Acute and chronic alcohol tolerance in humans: effects of dose and consecutive days of exposure. Alcohol Clin Exp Res 1993;17:740–745.

145. Talland GA, Mendelson JH, Ryack P. Experimentally induced chronic intoxication and withdrawal in alcoholics. Tests of motor skills. Q J Stud Alcohol 1964;25(Suppl 2):53–73.

146. Song BJ. Ethanol-inducible cytochrome P450 (CYP2E1): biochemistry, molecular biology and clinical relevance: 1996 update. Alcohol Clin Exp Res 1996;20(suppl 8): 138A–146A

147. Sternebring B, Liden A, Andersson K, Melander A. Carbamazepine kinetics and adverse effects during and

after ethanol exposure in alcoholics and in healthy volunteers. Eur J Clin Pharmacol 1992;43:393–397.

148. Monroe ML, Doering PL. Effect of common over-the-counter medications on blood alcohol levels. Ann Pharmacother 2001;35:918–924.

149. Vasiliou V, Malamas M, Marselos M. The mechanism of alcohol intolerance produced by various therapeutic drugs. Acta Pharmacol Toxicol 1986;58:303–310.

150. Hills BW, Venable HL. The interaction of ethyl alcohol and industrial chemicals. Am J Ind Med 1982;3:321–333.

151. Edelbroek MA, Horowitz M, Wishart JM, Akkermans LM. Effects of erythromycin on gastric emptying, alcohol absorption and small intestinal transit in normal subjects. J Nucl Med 1993;34:582–588.

152. Fraser AG. Pharmacokinetic interactions between alcohol and other drugs. Clin Pharmacokinet 1997;33:79–90.

153. Minocha A, Singh Rahal P, Brier ME, Levinson SS. Omeprazole therapy does not affect pharmacokinetics of orally administered ethanol in healthy male subjects. J Clin Gastroenterol 1995;21:107–109.

154. Jonsson KA, Jones AW, Bostrom L, Andersson T. Lack of effect of omeprazole, cimetidine and ranitidine on the pharmacokinetics of ethanol in fasting male volunteers. Eur J Clin Pharmacol 1992;42;209–212

155. Tanaka E. Toxicological interactions between alcohol and benzodiazepines. Clin Toxicol 2002;40:69–75.

156. Hasenfratz M, Bunge A, Dal Pra G, Battig K. Antagonistic effects of caffeine and alcohol on mental performance parameters. Pharmacol Biochem Behav 1993;46:463–465.

157. Nash H. Psychological effects and alcohol antagonizing properties of caffeine. Q J Stud Alcohol 1966;27:727–734.

158. Franks HM, Hagedorn H, Hensley VR, Hensley WJ, Starmer GA. The effect of caffeine on human performance, alone and in combination with ethanol. Psychopharmacologia 1975;45:177–181.

159. Liguori A, Robinson JH. Caffeine antagonism of alcohol-induced driving impairment. Drug Alcohol Depend 2001;63:123–129.

160. Azcona O, Barbanoj MJ, Torrent J, Jane F. Evaluation of the central effects of alcohol and caffeine interaction. Br J Clin Pharmacol 1995;40:393–400.

161. Fudin R, Nicastro R. Can caffeine antagonize alcohol-induced performance decrements in humans? Percept Mot Skills 1988;67:375–391.

162. Gordis E. Alcohol research: at the cutting edge. Arch Gen Psychiatry 1996;53:199–201.

163. Aguayo LG, Peoples RW, Yeh HH, Yevenes GE. GABA(A) receptors as molecular sites of ethanol action. Direct or indirect actions? Curr Top Med Chem 2002;2:869–885.

164. Fleming RL, Manis PB, Morrow AL. The effects of acute and chronic ethanol exposure on presynaptic and post-synaptic gamma-aminobutyric acid (GABA) neurotransmission in cultured cortical and hippocampal neurons. Alcohol 2009;43:603–618.

165. Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL. 1,2 The role of GABAA receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl) 2009;205:529–564.

166. Lang RM, Borow KM, Neumann A, Feldman T. Adverse cardiac effects of acute alcohol ingestion in young adults. Ann Intern Med 1985;102:742–747.

167. Ren J, Wold LE. Mechanisms of alcoholic heart disease. Ther Adv Cardiovasc Dis 2008;2:497–506.

168. Bikle DD, Genant HK, Cann C, Recker RR, Halloran BP, Strewler GJ. Bone disease in alcohol abuse. Ann Intern Med 1985;103:42–48.

169. Schuckit MA. New findings in the genetics of alcoholism. JAMA 1999;281:1875–1876.

170. Prescott CA, Kendler KS. Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. Am J Psychiatry 1999;156:34–40.

171. Murayama M, Matsushita S, Muramatsu T, Higuchi S. Clinical characteristics and disease course of alcoholics with inactive aldehyde dehydrogenase-2. Alcohol Clin Exp Res 1998;22:524–527.

172. Sanna E, Mostallino MC, Busonero F, Talani G, Tranquilli S, Mameli M, et al. Changes in GABA(A) receptor gene expression associated with selective alterations in receptor function and pharmacology after ethanol withdrawal. J Neurosci 2003;23:11711–11724.

173. Heinz A, Schmidt K, Baum SS, Kuhn S, Dufeu P, Schmidt LG, Rommelspacher H. Influence of dopaminergic transmission on severity of withdrawal syndrome in alcoholism. J Stud Alcohol 1996;57:471–474.

174. Hemmingsen R, Kramp P. Delirium tremens and related clinical states: psychopathology, cerebral pathophysiology and psychochemistry: a two-component hypothesis concerning etiology and pathogenesis. Acta Psychiatr Scand 1988;78(suppl 345):94–107.

175. Todd KG, Hazell AS, Butterworth RF. Alcohol-thiamine interactions: an update on the pathogenesis of Wernicke encephalopathy. Addiction Biol 1999;4:261–277.

176. Reuler JB, Girard DE, Cooney TG. Current concepts. Wernicke's encephalopathy. N Engl J Med 1985;312:1035–1039.

177. Victor M, Laureno R. Neurologic complications of alcohol abuse: epidemiologic aspects. Adv Neurol 1978;19:603–617.

178. Sullivan EV, Pfefferbaum A. Neuroimaging of Wernicke–Korsakoff's syndrome. Alcohol Alcohol 2009;44:155–165.

179. Lang CJ. The use of neuroimaging techniques for clinical detection of neurotoxicity: a review. Neurotoxicology 2000;21:847–856.

180. Chang KH, Cha SH, Han MH, Park SH, Nah DL, Hong JH. Marchiafava-Bignami disease: serial changes in

corpus callosum on MRI. Neuroradiology 1992;34: 480–482.

181. Kohler CG, Ances BM, Coleman AR, Ragland JD, Lazarev M, Gur RC. Marchiafava-Bignami disease: literature review and case report. Neuropsychiatry Neuropsychol Behav Neurol 2000;13;67–76.

182. Koike H, Iijima M, Sugiura M, Mori K, Hattori N, Ito H, et al. Alcoholic neuropathy is clinicopathologically distinct from thiamine-deficiency neuropathy. Ann Neurol 2003;54:19–29.

183. Lieber CS. Mechanisms of ethanol-drug-nutrition interactions. Clin Toxicol 1994;32:631–681.

184. Lieber CS, Schmid R. The effect of ethanol on fatty acid metabolism: stimulation of hepatic fatty acid synthesis *in vitro*. J Clin Invest 1961;40:394–399.

185. Wright J. Alcohol induced hypoglycemia. Br J Alcohol Alcohol 1979;14:174–176.

186. Lieber CS. Alcoholic liver injury: pathogenesis and therapy in 2001. Pathol Biol 2001;49:738–752.

187. Mak KM, Lieber CS. Lipocytes and transitional cells in alcoholic liver disease: a morphometric study. Hepatology 1988;8:1027–1033.

188. Rubin E, Popper H. The evolution of human cirrhosis deduced from observations in experimental animals. Medicine (Baltimore) 1967;46:163–183.

189. Yuzuriha T, Nakamura T, Shoji M, Matsushita S, Takagi S, Kono H. Alcohol and sudden death: a survey on alcohol-related deaths at the Tokyo Metropolitan Medical Examiner's Office (1989). Jap J Alcohol Stud Drug Depend 1993;28:95–119.

190. Yuzuriha T, Okudaira M, Tominaga I, Hori S, Suzuki H, Matsuo Y, et al. Alcohol-related sudden death with hepatic fatty metamorphosis: a comprehensive clinico-pathological inquiry into its pathogenesis. Alcohol Alcohol 1997;32:745–752.

191. Elphinstone PE, Kobza Black A, Greaves MW. Alcohol-induced urticaria. J R Soc Med 1985;78:340–341.

192. Chan AW. Alcoholism and epilepsy. Epilepsia 1985;26: 323–333.

193. Pressman MR, Mahowald MW, Schenck CH, Bornemann MC. Alcohol-induced sleepwalking or confusional arousal as a defense to criminal behavior: a review of scientific evidence, methods and forensic considerations. J Sleep Res 2007;16:198–212.

194. Brick J, Erickson CK. Intoxication is not always visible: an unrecognized prevention challenge. Alcohol Clin Exp Res 2009;33:1489–1507.

195. Teplin LA, Lutz GW. Measuring alcohol intoxication: the development, reliability and validity of an observational instrument. J Stud Alcohol 1985;46:459–466.

196. Carroll N, Rosenberg H, Funke S. Recognition of intoxication by alcohol counselors. J Subst Abuse Treat 1988;5:239–246.

197. Brick J, Carpenter JA. The identification of alcohol intoxication by police. Alcohol Clin Exp Res 2001;25: 850–855.

198. Penttila A, Tenhu M. Clinical examination as medicolegal proof of alcohol intoxication. Med Sci Law 1976;16: 95–103.

199. O'Malley SS, Maisto SA. Factors affecting the perception of intoxication: dose, tolerance, and setting. Addict Behav 1984;9:111–120.

200. Smith RC, Parker ES, Noble EP. Alcohol and affect in dyadic social interaction. Psychosom Med 1975;37; 25–40.

201. Roberts JR, Dollard D. Alcohol levels do not accurately predict physical or mental impairment in ethanol-tolerant subjects: relevance to emergency medicine and dram shop laws. J Med Toxicol 2010;6:438–442.

202. Jetter WW. Studies in alcohol. I. Diagnosis of acute alcoholic intoxication by a correlation of clinical and chemical findings. Am J Med Sci 1938;196:475–487.

203. Jetter WW. Studies in alcohol. II. Experimental feeding of alcohol to non-alcoholic individuals. Am J Med Sci 1938;196:487–493.

204. Prat G, Adan A, Sanchez-Turet M. Alcohol hangover: a critical review of explanatory factors. Hum Psychopharmacol Clin Exp 2009;24:259–267.

205. Wiese JG, Shlipak MG, Browner WS. The alcohol hangover. Ann Int Med 2000;132:897–902.

206. Harburg E, Davis D, Cummings KM, Gunn R. Negative affect, alcohol consumption and hangover symptoms among normal drinkers in a small community. J Stud Alcohol 1981;42:998–1012.

207. Rohsenow DJ, The role of beverage congeners in hangover and other residual effects of alcohol intoxication: a review. Curr Drug Abuse Rev 2010;3:76–79.

208. Damrau F, Goldberg AH. Adsorption of whiskey congeners by activated charcoal. Chemical and clinical studies related to hangover. Southwest Med 1971;52:179–182.

209. Tornros J, Laurell H. Acute and hang-over effects of alcohol on simulated driving performance. Blutalkohol 1991;28:24–30.

210. Verster JC, Stephens R, Penning R, Rohsenow D, McGeary J, Levy D, et al. The alcohol hangover research group consensus statement on best practice in alcohol hangover research. Curr Drug Abuse Rev 2010;3: 116–126.

211. Stephens R, Ling J, Heffernan TM, Heather N, Jones K. A review of the literature on the cognitive effects of alcohol hangover. Alcohol Alcohol 2008;43:163–170.

212. Prat G, Adan A, Perez-Pamies M, Sanchez-Turet M. Neurocognitive effects of alcohol hangover. Addict Behav 2008;33:15–23.

213. Baker CT. The alcohol hangover and its potential impact on the UK Armed Forces: a review of the literature on post-alcohol impairment. JR Army Med Corps 2004;150: 168–174.

214. Pittler MH, Verster JC, Ernst E. Interventions for preventing or treating alcohol hangover: systematic review of randomized controlled trials. BMJ 2005;331: 1515–1518.

215. Perr IN. Pathological intoxication and alcohol idiosyncratic intoxication—Part I: Diagnostic and clinical aspects. J Forensic Sci 1986;31:806–811.

216. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 3rd ed. Washington, DC: American Psychiatric Association; 1980.

217. Perr IN. Pathological intoxication and alcohol idiosyncratic intoxication—Part II: Legal aspects. J Forensic Sci 1986;31:812–817.

218. American Psychiatric Association: Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association; 1994.

219. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed., text rev. Washington, DC: American Psychiatric Association; 2000.

220. Morse RM, Flavin DK, and the Joint Committee of the National Council on Alcoholism and Drug Dependence and the American Society of Addiction Medicine to Study the Definition and Criteria for the Diagnosis of Alcoholism. The definition of alcoholism. JAMA 1992;268:1012–1014.

221. Criteria Committee, National Council on Alcoholism. Criteria for the diagnosis of alcoholism. Am J Psychiatry 1972;129:127–135.

222. Donnino MW, Vega J, Miller J, Walsh M. Myths and misconceptions of Wernicke's encephalopathy: what every emergency physician should know. Ann Emerg Med 2007;50:715–721.

223. Cook CCH, Hallwood PM, Thomson AD. B vitamin deficiency and neuropsychiatric syndromes in alcohol misuse. Alcohol Alcohol 1998;33:317–336.

224. Kopelman MD, Thomson AD, Guerrini I, Marshall EJ. The Korsakoff syndrome: clinical aspects, psychology and treatment. Alcohol Alcohol 2009;44:148–154.

225. Serdaru M, Hausser-Hauw C, Laplane D, Buge A, Castaigne P, Goulon M, Lhermitte F, Hauw JJ. The clinical spectrum of alcoholic pellagra encephalopathy. A retrospective analysis of 22 cases studied pathologically. Brain 1988;111:829–842.

226. Loberg T. Alcohol misuse and neuropsychological deficits in men. J Stud Alcohol 1980;41:119–128.

227. Fein G, Bachman L, Fisher S, Davenport L. Cognitive impairments in abstinent alcoholics. West J Med 1990; 152:531–537.

228. Piano MR. Alcoholic cardiomyopathy incidence, clinical characteristics, and pathophysiology. Chest 2002; 121:1638–1650.

229. Gavazzi A, De Maria R, Parolini M, Porcu M. Alcohol abuse and dilated cardiomyopathy in men. Am J Cardiol 2000;85:1114–1118.

230. Fauchier L, Babuty D, Poret P, Casset-Senon D, Autret ML, Cosnay P, Fauchier JP. Comparison of long-term outcome of alcoholic and idiopathic dilated cardiomyopathy. Eur Heart J 2000;21:306–314.

231. Vikhert AM, Tsiplenkova VG, Cherpachenko NM. Alcoholic cardiomyopathy and sudden cardiac death. J Am Coll Cardiol 1986;8:3A–11A.

232. Clark JC. Sudden death in the chronic alcoholic. Forensic Sci Int 1988;36:105–111.

233. Denison H, Berkowicz A, Oden A, Wendestam C. The significance of coronary death for the excess mortality in alcohol-dependent men. Alcohol Alcohol 1997;32: 517–526.

234. Buckingham TA, Kennedy HL, Goenjian AK, Vasilomanolakis EC, Shriver KK, Sprague MK, Lyyski D. Cardiac arrhythmias in a population admitted to an acute alcoholic detoxification center. Am Heart J 1985;110: 961–965.

235. Lowenstein SR, Gabow PA, Cramer J, Oliva PB, Ratner K. The role of alcohol in new onset atrial fibrillation. Arch Intern Med 1983;143:1882–1885.

236. Rich EC, Siebold C, Campion B. Alcohol related acute atrial fibrillation. A case-control study and review of 40 patients. Arch Intern Med 1985;145:830–833.

237. Wannamethee G, Shaper AG. Alcohol and sudden cardiac death. Br Heart J 1992;68:443–448.

238. Greenspon AJ, Schaal SF. The "holiday heart": electrophysiologic studies of alcohol effects in alcoholics. Ann Intern Med 1983;98:135–139.

239. Klatsky AL. Alcohol, coronary disease, and hypertension. Annu Rev Med 1996;47:149–160.

240. MacMahon S. Alcohol consumption and hypertension. Hypertension 1987;9:111–121.

241. Ng SKC, Hauser WA, Brust JCM, Susser M. Alcohol consumption and withdrawal in new-onset seizures. N Engl J Med 1988;319:666–673.

242. Pristach CA, Smith CM, Whitney RB. Alcohol withdrawal syndromes—Prediction from detailed medical and drinking history. Drug Alcohol Depend 1983;11: 177–199.

243. Isbell H, Fraser HF, Wikler A, Belleville RE, Eisenman AJ. An experimental study of the etiology of "rum fits" and delirium tremens. Q J Stud Alcohol 1955;16:1–33.

244. Mendelson JH, La Dou J. Experimentally induced chronic intoxication and withdrawal in alcoholics: Psychophysiological findings. Q J Study Alcohol 1964(suppl 2):14–39.

245. Wetterling T, Kanitz RD, Besters B, Fischer D, Zerfass B, John U, et al. A new rating scale for the assessment of the alcohol-withdrawal syndrome (AWS scale). Alcohol Alcohol 1997;32:753–760.

246. Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM. Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). Br J Addict 1989;84: 1353–1357.

247. Foy A, March S, Drinkwater V. Use of an objective clinical scale in the assessment and management of alcohol withdrawal in a large general hospital. Alcohol Clin Exp Res 1988;12:360–364.

248. Reoux JP, Malte CA, Kivahan DR, Saxon AJ. The alcohol use disorders identification test (AUDIT) predicts alcohol withdrawal symptoms during inpatient detoxication. J Addict Dis 2002;21:81–91.

249. Rathlev NK, Ulrich AS, Delanty N, D'Onofrio G. Alcohol-related seizures. J Emerg Med 2006;31: 157–163.

250. Hillbom M, Pieninkeroinen I, Leone M. Seizures in alcohol-dependent patients: epidemiology, pathophysiology and management. CNS Drugs 2003;17:1013–1030.

251. Kramp P, Hemmingsen R. Delirium tremens some clinical features. Part I. Acta Psychiatr Scand 1979;60: 383–404.

252. Mattson SN, Schoenfeld AM, Riley EP. Teratogenic effects of alcohol on brain and behavior. Alcohol Res Health 2001;25:185–191.

253. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. Lancet 1973;2:999–1001.

254. O'Leary CM. Fetal Alcohol syndrome: diagnosis, epidemiology, and developmental outcomes. J Paediatr Child Health 2004;40:2–7.

255. Miller L, Tolliver R, Druschel C, Fox D, Schoellhorn J, Podvin D, et al. Fetal alcohol syndrome—Alaska, Arizona, Colorado, and New York, 1995–1997. MMWR Morb Mortal Wkly Rep 2002;51:433–435.

256. Burden MJ, Jacobson SW, Sokol RJ, Jacobson JL. Effects of prenatal alcohol exposure on attention and working memory at 7.5 years of age. Alcoholism Clin Exp Res 2005;29:443–452.

257. Warren KR, Foudin LL. Alcohol-related birth defects— the past, present, and future. Alcohol Res Health 2001; 25:153–158.

258. Abel EL. An update on incidence of FAS: FAS is not an equal opportunity birth defect. Neurotoxicol Teratol 1995;17:437-–43.

259. Henderson J, Gray R, Brocklehurst P. Systematic review of effects of lows-moderate prenatal alcohol exposure on pregnancy outcome. Br J Obstet Gynecol 2007;114: 243–252.

260. Astley SJ, Clarren SK. Diagnosing the full spectrum of fetal alcohol-exposed individuals: introducing the 4-digit diagnostic code. Alcohol Alcohol 2000;35:400–410.

261. Chudley AE, Conry J, Cook JL, Loock C, Rosales T, LeBlanc N. Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. CMAJ 2005;172(suppl 5): S1–S21.

262. Hoyme HE, Kalberg WO, Kodituwakku P, Gossage P, Trujillo PM, Buckley DG, et al. A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 Institute of Medicine criteria. Pediatrics 2005;115:39–47.

263. International Agency for Research on Cancer. Alcohol drinking. Monogr Eval Carcinog Risk Chem Hum 1988; 44:35–45.

264. Poschl G, Seitz HK. Alcohol and cancer. Alcohol Alcohol 2004;39:155–165.

265. Franceschi S. Alcohol and cancer. Adv Exp Med Biol 1999;472:43–49.

266. Dubowski KM. Alcohol determination in the clinical laboratory. Am J Clin Pathol 1980;74:747–750.

267. Dubowski KM. Recent developments in alcohol analysis. Alcohol Drugs Driv 1986;2:13–46.

268. Macchia T, Mancinelli R, Gentili S, Lugarei EC, Raponi A, Taggi F. Ethanol in biological fluids: headspace GC measurement. J Anal Toxicol 1995;19;241–246.

269. Fung WK, Chan KL, Mok VK, Lee CW, Choi WM. The statistical variability of blood alcohol concentration measurements in drink-driving cases. Forensic Sci Int 2000; 110:207–214.

270. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. Forensic Sci Int 2007;16:10–29.

271. Goldfinger TM, Schaber D. A comparison of blood alcohol concentration using non-alcohol and alcohol-containing skin antiseptics. Ann Emerg Med 1982; 11:665–667.

272. Ryder KW, Glick MR. The effect of skin cleansing agents on ethanol results measured with the DuPont automatic clinical analyzer. J Forensic Sci 1986;31:574–579.

273. Jain NC, Cravey RH. Analysis of alcohol. I. A review of chemical and infrared methods. J Chromatogr Sci 1972; 10:257–262.

274. Feldstein M, Klendshoj NC. The determination of ethyl alcohol in biological fluids by micro diffusion analysis. Can J Med Technol 1954;16:48–52.

275. Böttcher M, Beck O, Helander A. Evaluation of a new immunoassay for urinary ethyl glucuronide testing. Alcohol Alcohol 2008;43:46–48.

276. Arndt T, Gierten B, Gussregen B, Werle A, Gruner J. False-positive ethyl glucuronide immunoassay screening associated with chloral hydrate medication as confirmed by LC-MS/MS and self-medication. Forensic Sci Int 2009; 184:e27–e29.

277. Janda I, Weinmann W, Kuehnle T, Lahode M, Alt A. Determination of ethyl glucuronide in human hair by SPE and LC-MS/MS. Forensic Sci Int 2002;128:59–65.

278. Jurado C, Soriano T, Giménez MP, Menéndez M. Diagnosis of chronic alcohol consumption. Hair analysis for ethyl-glucuronide. Forensic Sci Int 2004;145: 161–166.

279. Winek CL, Carfagna M. Comparison of plasma, serum and whole blood ethanol concentrations. J Anal Toxicol 1987;11:267–268.

280. Jones AW, Hahn RG, Stalberg HP. Distribution of ethanol and water between plasma and whole blood; inter- and intra-individual variations after administration of ethanol by intravenous infusion. Scand J Clin Lab Invest 1990; 50:775–780.

281. Charlebois RC, Corbett MR, Wigmore JG. Comparison of ethanol concentrations in blood, serum, and blood cells for forensic application. J Anal Toxicol 1996;20; 171–178.

282. Rainey PM. Relation between serum and whole-blood ethanol concentrations. Clin Chem 1993;39:2288–2292.

283. Hoiseth G, Morini L, Polettini A, Christophersen AS, Johnsen L, Karinen R, Morland J. Serum/whole blood concentration ratio for ethyl glucuronide and ethyl sulfate. J Anal Toxicol 2009;33:208–211.

284. Winek CL, Paul LJ. Effect of short-term storage conditions on alcohol concentrations in blood from living human subjects. Clin Chem 1983;29:1959–1960.

285. Penetar DM, McNeil JF, Ryan ET, Lukas SE. comparison among plasma, serum and whole blood ethanol concentrations: impact of storage conditions and collection tubes. J Anal Toxicol 2008;32:505–510.

286. Dick GL, Stone HM. Alcohol loss arising from microbial contamination of drivers' blood specimens. Forensic Sci Int 1987;34:17–27.

287. Smalldon KW, Brown GA. The stability of ethanol in stored blood: Part II. The mechanism of ethanol oxidation. Anal Chim Acta 1973;66:285–290.

288. Brown GA, Neylan D, Reynolds WJ, Smalldon KW. The stability of ethanol in stored blood: Part I. Important variables and interpretation of results. Anal Chim Acta 1973;66:271–283.

289. Pella PA, Diamondstone BI. Stability of aqueous ethanol solutions stored in glass ampules. J Forensic Sci 1975;20:537–538.

290. Dubowski KM, Gadsden RH Sr., Poklis A. The stability of ethanol in human whole blood controls: an inter-laboratory evaluation. J Anal Toxicol 1997;21:486–491.

291. Shajani NK, Image BA, Chu EB. The stability of ethanol in stored forensic blood samples. Can Soc Forensic Sci J 1989;22:335–339.

292. Chang RB, Smith WA, Walkin E, Reynolds PC. The stability of ethyl alcohol in forensic blood specimens. J Anal Toxicol 1984;8:66–67.

293. Olsen T, Hearn WL. Stability of ethanol in postmortem blood and vitreous humor in long-term refrigerated storage. J Anal Toxicol 2003;27:517–519.

294. Winek T, Winek CL, Wahba WW. The effect of storage at various temperatures on blood alcohol concentration. Forensic Sci Int 1996;78:179–185.

295. Jones AW. Are changes in blood-ethanol concentration during storage analytically significant? Importance of method imprecision. Clin Chem Lab Med 2007;45:1299–1304.

296. Blume P, Lakatua DJ. The effect of microbial contamination of the blood sample on the determination of ethanol levels in serum. Am J Clin Pathol 1973;60:700–702.

297. Chang J, Kollman SE. The effect of temperature on the formation of ethanol by *Candida albicans* in blood. J Forensic Sci 1989;34:105–109.

298. Neuteboom W, Zweipfenning PG. The stability of the alcohol concentration in urine specimens. J Anal Toxicol 1989;13:141–143.

299. Sulkowski HA, Wu AHB, McCarter YS. *In-vitro* production of ethanol in urine by fermentation. J Forensic Sci 1995;40:990–993.

300. Schloegl H, Dresen S, Spaczynski K, Stoertzel M, Wurst FM, Weinmann W. Stability of ethyl glucuronide in urine, post-mortem tissue and blood samples. Int J Legal Med 2006;120:83–88.

301. Høiseth G, Karinen R, Johnsen L, Normann PT, Christophersen AS, Mørland J. Disappearance of ethyl glucuronide during heavy putrefaction. Forensic Sci Int 2008;176:147–151.

302. Thierauf A, Serr A, Halter CC, Al-Ahmad A, Rana S, Weinmann W. Influence of preservatives on the stability of ethyl glucuronide and ethyl sulphate in urine. Forensic Sci Int 2008;182:41–45.

303. Halter CC, Laengin A, Al-Ahmad A, Wurst FM, Weinmann W, Kuemmerer K. Assessment of the stability of the ethanol metabolite ethyl sulfate in standardized degradation tests. Forensic Sci Int 2009;186:52–55.

304. Winek CL, Esposito FM. Comparative study of ethanol levels in blood versus bone marrow, vitreous humor, bile and urine. Forensic Sci Int 1981;17:27–36.

305. Jones AW. Inter- and intra-individual variation in the saliva/blood alcohol ratio during ethanol metabolism in man. Clin Chem 1979;25:1394–1398.

306. Haeckel R, Bucklitsch I. The comparability of ethanol concentrations in peripheral blood and saliva (the phenomenon of variation in saliva to blood concentration ratios). J Clin Chem Clin Biochem 1987;25:199–204.

307. Harper DR. A comparative study of the microbiological contamination of postmortem blood and vitreous humour samples taken for ethanol determination. Forensic Sci Int 1989;43:37–44.

308. Jenkins AJ, Levine BS, Smialek JE. Distribution of ethanol in postmortem liver. J Forensic Sci 1995;40:611–613.

309. Hlastala HP. The alcohol breath test—a review. J Appl Physiol 1998;84:401–408.

310. Jones AW. Variability of the blood:breath alcohol ratio *in vivo*. J Stud Alcohol 1978;39:1931–1939.

311. Ohlsson J, Ralph DD, Mandelkorn MA, Babb AL, Hlastala MP. Accurate measurement of blood alcohol concentration with isothermal rebreathing. J Stud Alcohol 1990;51:6–13.

312. Fox GR, Hayward JS. Effect of hyperthermia on breath-alcohol analysis. J Forensic Sci 1989;34:836–841.

313. Fox GR, Hayward JS. Effect of hypothermia on breath-alcohol analysis. J Forensic Sci 1987;32:320–325.

314. Logan BK, Distefano S. Ethanol content of various foods and soft drinks and their potential for interference with a breath-alcohol test. J Anal Toxicol 1998;22:181–183.

315. Gomez HF, Moore L, McKinney P, Phillips S, Guven H, Brent J. Elevation of breath ethanol measurements by metered-dose inhalers. Ann Emerg Med 1995;25:608–611.

316. Modell JG, Taylor JP, Lee JY. Breath alcohol values following mouthwash use. JAMA 1993;270:2955–2956.

317. Morris MJ. Alcohol breath testing in patients with respiratory disease. Thorax 1990;45:717–721

318. Dubowski KM. Quality assurance in breath-alcohol analysis. J Anal Toxicol 1994;18:306–311.

319. Kechagias S, Jonsson K-A, Franzen T, Andersson L, Jones AW. Reliability of breath-alcohol analysis in individuals with gastroesophageal reflux disease. J Forensic Sci 1999; 44:814–818.

320. Wigmore JG, Leslie GM. The effect of swallowing or rinsing alcohol solution on the mouth alcohol effect and slope detection of the Intoxilyzer 5000. J Anal Toxicol 2001;25:112–114.

321. Caravati EM, Anderson KT. Breath alcohol analyzer mistakes methanol poisoning for alcohol intoxication. Ann Emerg Med 2010;55:198–200.

322. De Baere S, Meyer E, Lambert W, De Leenheer A. Testing for driving under the influence of ethanol: misleading results caused by ether. J Anal Toxicol 1998;22: 78–79.

323. Jones AW. Variability of the blood/breath alcohol ratio in drinking drivers. J Forensic Sci 1996;41:916–921.

324. Jones AW, Andersson L. Comparison of ethanol concentrations in venous blood and end-expired breath during a controlled drinking study. Forensic Sci Int 2003;132: 18–25.

325. Jones AW, Andersson L. Variability of the blood/breath alcohol ratio in drinking drivers. J Forensic Sci 1996; 414:916–921.

326. Simpson G. Accuracy and precision of breath alcohol measurements for subjects in the absorptive state. Clin Chem 1987;33:753–757.

327. Gullberg RG. Differences between roadside and subsequent evidential breath alcohol results and their forensic significance. J Stud Alcohol 1991;52:311–317.

328. Gullberg RG. Breath alcohol measurement variability associated with different instrumentation and protocols. Forensic Sci Int 2003;131:30–35.

329. Jones AW, Andersson L, Berglund K. Interfering substances identified in the breath of drinking drivers with Intoxilyzer 5000S. J Anal Toxicol 1996;20:522–527.

330. Cowan JM Jr, McCutcheon JR, Weatherman A. The response of the Intoxilyzer 4011AS-A to a number of possible interfering substances. J Forensic Sci 1990; 35:797–812.

331. Logan BK, Gullberg RG, Elenbaas JK. Isopropanol interference with breath alcohol analysis: a case report. J Forensic Sci 1994;39:1107–1111.

332. Harding PM, Laessig RH, Field PH. Field performance of the Intoxilyzer 5000: a comparison of blood- and breath-alcohol results in Wisconsin drivers. J Forensic Sci 1990;35:1022–1028.

333. Breen MH, Dang QT, Jaing JT, Boyd GN. The effect of a "one for the road" drink of hard liquor, beer or wine on peak breath alcohol concentration in a social drinking environment with food consumption. Med Sci Law 1998; 38:62–69.

334. Logan BK, Jones AW. Endogenous ethanol "auto-brewery syndrome" as a drunk-driving defence challenge. Med Sci Law 2000;40:206–215.

335. Kaji H, Asanumo Y, Shibue H, Hisamura M, Saito N, Kawakami Y, Murao M. Intra-gastrointestinal alcohol fermentation syndrome: report of two cases and review of the literature. J Forensic Sci Soc 1984;24:461–471.

336. Pach J, Marek Z, Bogusz M, Stasko W. The clinical appearance and blood alcohol level in acute poisoning and blood alcohol level in fatal non-treated poisoning. Acta Pharmacol Toxicol (Copenh) 1977;41(suppl 2): 362–368.

337. Reed TE. The myth of "the average alcohol response". Alcohol 1985;2:515–519.

338. Davis AR, Lipson AH. Central nervous system depression and high blood ethanol levels. Lancet 1986;1:566.

339. Minion GE, Slovis CM, Boutiette L. Severe alcohol intoxication: A study of 204 consecutive patients. Clin Toxicol 1989;27:375–384.

340. Paredes A, Hood WR, Seymour H. Sobriety as a symptom of alcohol intoxication: a clinical commentary on intoxication and drunkenness. Br J Addict 1975;70:233–243.

341. Sharman JR, Lindley TN, Abernethy MH. Blood alcohol levels. How accurately can they be guessed? NZ Med J 1978;87:438–440.

342. Langenbucher JW, Nathan PE. Psychology, public policy, and the evidence for alcohol intoxication. Am Psychol 1983;38:1070–1077.

343. Sullivan JB Jr, Hauptman M, Bronstein AC. Lack of observable intoxication in humans with high plasma alcohol concentrations. J Forensic Sci 1987;32: 1660–1667.

344. Urso T, Gavaler JS, van Thiel DH. Blood ethanol levels in sober alcohol users seen in an emergency room. Life Sci 1981;28:1053–1056.

345. Lopez GP, Yealy DM, Krenzelok EP. Survival of a child despite unusually high blood ethanol levels. Am J Emerg Med 1989;7:283–285.

346. Johnson RA, Noll EC, Rodney WM. Survival after a serum ethanol concentration of 1½%. Lancet 1982;2:1394.

347. Watanabe A, Kobayashi M, Hobara N, Nakatsukasa H, Nagashima H, Fujimoto A. A report of unusually high blood ethanol and acetaldehyde levels in two surviving patients. Alcoholism Clin Exp Res 1985;9:14–16.

348. Taylor HL, Hudson RP. Acute ethanol poisoning: A two year study of deaths in North Carolina. J Forensic Sci 1977;22:639–653.

349. Osterloh JD, Kelly TR, Khayam-Bashi H, Romeo R. Discrepancies in osmolal gaps and calculated alcohol concentrations. Arch Pathol Lab Med 1996;120:637–641.

350. Britten JS, Myers RA, Benner C, Carson S, Cowley RA. Blood ethanol and serum osmolality in the trauma patient. Am Surg 1982;48:451–455.

351. Stowell AR, Stowell LI. Estimation of blood alcohol concentrations after social drinking. J Forensic Sci 1998; 43:14–21.

352. O'Neill B, Williams AF, Dubowski KM. Variability in blood alcohol concentrations. Implications for estimating individual results. J Stud Alcohol 1983;4:222–230.

353. Jones BM, Vega A. Cognitive performance measured on the ascending and descending limb of the blood alcohol curve. Psychopharmacologia 1972;23:99–114.

354. Brettel HF, Maske B. Zur alkoholbestimmung bei blutennahme im schockzustand. Blutalkohol 1971;8:360–373.

355. Kleemann WJ, Seibert M, Tempka A, Wolf M, Weller JP, Troger HD. [Arterial and venous alcohol elimination in 10 polytrauma patients]. Blutalkohol 1995;32:162–173. [German]

356. Gumbel B. [Acute blood loss and the blood alcohol curve.] Munch Med Wochenschr 1956;98:337–340. [German]

357. Al-Lanqawi Y, Moreland TA, McEwen J, Halliday F, Durnin CJ, Stevenson IH. Ethanol kinetics: extent of error in back extrapolation procedures. Br J Clin Pharmacol 1992;34:316–321.

358. Jackson PR, Tucker GT, Woods HF. Backtracking booze with Bayes—the retrospective interpretation of blood alcohol data. Br J Clin Pharmacol 1991;31:55–63.

359. Bayly RC, McCallum NE. Some aspects of alcohol in body fluids. Part II. The change in blood alcohol concentration following consumption. Med J Aust 1959;2; 173–176.

360. McCallum NEW, Scroggie JG. Some aspects of alcohol in body fluids. Part 1. Correlation between blood alcohol concentration and alcohol consumption. Med J Aust 1959;2:169–172.

361. Jones AW. Concentration-time profiles of ethanol in capillary blood after ingestion of beer. J Forensic Sci Soc 1991;31:429–439.

362. Levine B, Smialek JE. Status of alcohol absorption in drinking drivers killed in traffic accidents. J Forensic Sci 2000;45:3–6.

363. Raszeja S, Olszewska I. [The influence of traumatic shock on alcohol elimination.] Z Rechtsmed 1981;86:277–280. [German]

364. Neuteboom W, Jones AW. Disappearance rate of alcohol from the blood of drunk drivers calculated from two consecutive samples; what do the results really mean? Forensic Sci Int 1990;45:107–115.

365. Loomis TA. Blood alcohol in automobile drivers: measurement and interpretation for medicolegal purposes. Q J Stud Alcohol 1974;35:458–472.

366. Midanik L. The validity of self-reported alcohol consumption and alcohol problems. A literature review. Br J Addict 1982;77:357–382.

367. Sharpe PC. Biochemical detection and monitoring of alcohol abuse and abstinence. Ann Clin Biochem 2001;38; 652–664.

368. Di Bari M, Silvestrini G, Chiarlone M, De Alfieri W, Patussi V, Timpanelli M, et al. Features of excessive alcohol drinking in older adults distinctively captured by behavioral and biological screening instruments: An epidemiological study. J Clin Epidemiol 2002;55:41–47.

369. Trevett AJ, Currie NM, MacConnell TJ. Alcohol intoxication and alcoholism in acute male medical admissions. Scot Med J 1990;35:134–135.

370. Saunders WM, Kershaw PW. Screening tests for alcoholism. Findings from a community study. Br J Addict 1980;75:37–41.

371. Moore AA, Seeman T, Morgenstern H, Beck JC, Reuben DB. Are there differences between older persons who screen positive on the CAGE questionnaire and the Short Michigan Alcoholism Screening Test—Geriatric version? J Am Geriatr Soc 2002;50;858–862.

372. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption—II. Addiction 1993;88:791–804.

373. Adams WL, Barry KL, Fleming MF. Screening for problem drinking in older primary care patients. JAMA 1996;276:1964–1967.

374. Bradley KA, Bush KR, McDonell MB, Malone T, Fihn SD, and the Ambulatory Care Quality Improvement Project. Screening for problem drinking comparison of CAGE and AUDIT. J Gen Intern Med 1998;13: 379–388.

375. Ewing JA. Detecting alcoholism. The CAGE questionnaire. JAMA 1984;252:1905–1907.

376. Wallace P, Cutler S, Haines A. Randomized controlled trial of general practitioner intervention in patients with excess alcohol consumption. Br Med J 1988;297: 663–668.

377. Bradley KA, Boyd-Wickizer J, Powell SH, Burman ML. Alcohol screening questionnaires in women a critical review. JAMA 1998;280:166–171.

378. Storgaard H, Nielson SD, Gluud C. The validity of the Michigan Alcoholism Screening Test (MAST). Alcohol Alcohol 1994;29;493–502.

379. Allen JP, Litten RZ, Fertig JB, Babor T. A review of research on the Alcohol Use Disorders Identification test (AUDIT). Alcohol Clin Exp Res 1997;21:613–619.

380. Musshoff F, Daldrup T. Determination of biological markers for alcohol abuse. J Chromatogr B 1998;713: 245–264.

381. Alte D, Ludemann J, Piek M, Adam C, Rose H-J, John U. Distribution and dose response of laboratory markers to alcohol consumption in a general population: results of the Study of Health in Pomerania (SHIP). J Stud Alcohol 2003;64:75–82.

382. Scouller K, Conigrave KM, Macaskill P, Irwig L, Whitfield JB. Should we use carbohydrate-deficient transferrin instead of gamma-glutamyltransferase for detecting problem drinkers? A systematic review and metaanalysis. Clin Chem 2000;46:1894–1902.

383. Gijsbers AJ, Raymond A, Whelan G. Doses a blood alcohol level of 0.15 or more identify accurately problem drinkers in a drink-driver population? Med J Aust 1991; 154:448–452.

384. Mancinelli R, Ceccanti M. Biomarkers in alcohol misuse: their role in the prevention and detection of thiamine deficiency. Alcohol Alcohol 2009;44:177–182.

385. Conigrave KM, Saunders JB, Whitfield JB. Diagnostic tests for alcohol consumption. Alcohol Alcohol 1995;30: 13–26.

386. Teschke R, Brand A, Strohmeyer G. Induction of hepatic microsomal gamma-glutamyltransferase activity following chronic alcohol consumption. Biochem Biophys Res Commun 1977;75:718–724.

387. Alatalo P, Koivisto H, Puukka K, Hietala J, Anttila P, Bloigu R, Niemela O. Biomarkers of liver status in heavy drinkers, moderate drinkers and abstainers. Alcohol Alcohol 2009;4:199–203.

388. Whitfield JB, Hensley WJ, Bryden D, Gallagher H. Some laboratory correlates of drinking habits. Ann Clin Biochem 1978;15:297–303.

389. Allen JP, Litten RZ. The role of laboratory tests in alcoholism treatment. J Subst Abuse Treat 2001;20: 81–85.

390. Lee DH, Ha MH, Christiani DC: Body weight, alcohol consumption and liver enzyme activity—a 4-year follow-up study. Int J Epidemiol 2001;30:766–770.

391. Godart B, Mennetrey L, Schellenberg F, Pages J-C, Bacq Y. Carbohydrate-deficient transferrin and gamma-glutamyl transpeptidase in the evaluation of alcohol consumption. Gastroenterol Clin Biol 2005;29:113–116.

392. Vermes I, van den Berg FA. Clinical utility of carbohydrate deficient transferrin to detect alcohol abuse in a general population. Clin Chem 1996;42:2048–2050.

393. Lakshman R, Tsutsumi M, Ghosh P, Takase S, Anni H, Nikolaea O, Israel Y, et al. Alcohol biomarkers: clinical significance and biochemical basis. Alcohol Clin Exp Res 2001;25(suppl)67S–70S.

394. Stibler H. Carbohydrate deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. Clin Chem 1991;37:2029–2037.

395. Conigrave KM, Degenhardt LJ, Whitfield JB, Saunders JB, Helander A, Tabakoff B. CDT, GGT, and AST as markers of alcohol use: the WHO/ISBRA Collaborative Project. Alcohol Clin Exp Res 2002;26:332–339.

396. Bell H, Steensland H. Serum activity of gamma-glutamyl transpeptidase (GGT) in relation to estimated alcohol consumption and questionnaires in alcohol dependence syndrome. Br J Addict 1987;82:1021–1026.

397. Cohen JA, Kaplan MM. The SGOT/SGPT ratio: an indicator of alcoholic liver disease. Dig Dis Sci 1979;24: 835–838.

398. Whitehead TP, Clarke CA, Whitfield AG. Biochemical and hematological markers of alcohol intake. Lancet 1978;1:978–981.

399. Chick J, Kreitman N, Plant M. Mean-cell volume and gamma glutamyl-transpeptidase as markers of drinking in working men. Lancet 1981;1:1249–1251.

400. Saunders JB, Conigrave KM. Early identification of alcohol problems. Can Med Assoc J 1990;143: 1060–1069.

401. Laposata M. Fatty acid ethyl esters: short-term and long-term serum markers of ethanol intake. Clin Chem 1997;43:1527–1534.

402. Hartwig S, Auwarter V, Pragst F. Fatty acid ethyl esters in scalp, pubic axillary, beard and body hair as markers for alcohol misuse. Alcohol Alcohol 2003;38:163–167.

403. Auwarter V, Sporkert F, Hartwig S, Pragst F, Vater H, Diefenbacher A. Fatty acid ethyl esters in hair as markers of alcohol consumption. Segmental hair analysis of alcoholics, social drinkers, and teetotalers. Clin Chem 2001; 47:2114–2123.

404. Hartwig S, Auwarter V, Pragst F. Effect of hair care and hair cosmetics on the concentrations of fatty acid ethyl esters in hair as markers of chronically elevated alcohol consumption. Forensic Sci Int 2003;131:90–97.

405. Concheiro M, Cruz A, Mon M, de Castro A, Quintela O, Lorenzo A, Lopez-Rivadulla M. Ethyl glucuronide determination in urine and hair from alcohol withdrawal patients. J Anal Toxicol 2009;33:155–161.

406. Politi L, Morini L, Leone F, Polettini A. Ethyl glucuronide in hair: is it a reliable marker of chronic high levels of alcohol consumption? Addiction 2006;101:1408–1412.

407. Yegles M, Labarthe A, Auwärter V, Hartwig S, Vater H, Wennig R, Pragst F. Comparison of ethyl glucuronide and fatty acid ethyl ester concentrations in hair of alcoholics, social drinkers and teetotallers. Forensic Sci Int 2004;145:167–173.

408. Kintz P, Villain M, Vallet E, Etter M, Salquebre G, Cirimele V. Ethyl glucuronide: unusual distribution between head hair and pubic hair. Forensic Sci Int 2008; 176:87–90.

409. Kerekes I, Yegles M, Grimm U, Wennig R. Ethyl glucuronide determination: head hair versus non-head hair. Alcohol Alcohol 2009;44:62–66.

410. Felby S, Nielsen E. The postmortem blood alcohol concentration and the water content. Blutalkohol 1994;31: 24–32.

411. Hardin GG. Postmortem blood and vitreous humor ethanol concentrations in a victim of a fatal motor vehicle crash. J Forensic Sci 2002;47:402–403.

412. Thomsen JL. Chronic alcoholism in a forensic material. 2. Causes and manners of death in alcoholics. Med Sci Law 1996;36:209–216.

413. Thomsen JL, Simonsen KW, Felby S, Frohlich B. A prospective toxicology analysis in alcoholics. Forensic Sci Int 1997;90:33–40.

414. Ferrada-Noli M, Ormstad K, Asberg M. Pathoanatomic findings and blood alcohol analysis at autopsy (BAC) in forensic diagnoses of undetermined suicide. A cross-cultural study. Forensic Sci Int 1995;78:157–163.

415. Heatley MK, Crane J. The blood alcohol concentration at postmortem in 175 fatal cases of alcohol intoxication. Med Sci Law 1990;30:101–105.

416. Marek Z, Pach J, Bogusz M. Ethyl alcohol intoxication. Acta Med Leg Soc (Liege) 1980;30:167–171.

417. Jones AW, Holmgren P. Comparison of blood-ethanol concentrations in deaths attributed to acute alcohol poisoning and chronic alcoholism. J Forensic Sci 2003;48:874–879.

418. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. Forensic Sci Int 2007;165:10–29.

419. Felby S, Nielsen E. Postmortem blood alcohol concentration. Blutalkohol 1993;30:244–250.

420. Budd RD. Validity of post mortem chest cavity blood ethanol determination. J Chromatogr 1988;229:337–340.

421. Yarema MC, Becker CE. Key concepts in postmortem drug redistribution. Clin Toxicol 2005;43:235–241.

422. Winek CL Jr, Winek CL, Wahba WW. The role of trauma in postmortem blood alcohol determination. Forensic Sci Int 1995;71:1–8.

423. Harper DR. A comparative study of the microbiological contamination of postmortem blood and vitreous humour samples taken for ethanol determination. Forensic Sci Int 1989;43:37–44.

424. Nanikawa, R, Moriya F, Hashimoto Y. Experimental studies on the mechanism of ethanol formation in corpses. Z Rechtsmed 1988;101:21–26.

425. Hoiseth G, Kristoffersen L, Larssen B, Arnestad M, Hermansen NO, Morland J. *In vitro* formation of ethanol in autopsy samples containing fluoride ions. Int J Legal Med 2008;122:63–66.

426. Bogusz M, Guminska M, Markiewicz J. Studies on the formation of endogenous ethanol blood putrefying *in vitro*. J Forensic Med 1970;17:156–168.

427. Appenzeller BM, Schuman M, Wennig R. Was a child poisoned by ethanol? Discrimination between antemortem consumption and post-mortem formation. Int J Legal Med 2008;122:429–434.

428. Levine B, Smith ML, Smialek JE, Caplan YH. Interpretation of low postmortem concentrations of ethanol. J Forensic Sci 1993;38:663–667.

429. Zumwalt RE, Bost RO, Sunshine I. Evaluation of ethanol concentrations in decomposed bodies. 1982;27:549–554.

430. Gilliland MG, Bost RO. Alcohol in decomposed bodies: postmortem synthesis and distribution. J Forensic Sci 1993;38:1266–1274.

431. de Lima IV, Midio AF. Origin of blood ethanol in decomposed bodies. Forensic Sci Int 1999;106:157–162.

432. Canfield DV, Kupiec T, Huffine E. Postmortem alcohol production in fatal aircraft accidents. J Forensic Sci 1993;38:914–917.

433. Coe JI. Comparative postmortem chemistries of vitreous humor before and after embalming. J Forensic Sci 1976; 21:583–586.

434. Lewis RJ, Johnson RD, Angier MK, Vu NT. Ethanol formation in unadulterated postmortem tissues. Forensic Sci Int 2004;146:17–24.

435. Clark MA, Jones JW. Studies on putrefactive ethanol production: 1. Lack of spontaneous ethanol production in intact human bodies. J Forensic Sci 1982;27:366–371.

436. Refaai MA, Nguyen PN, Steffensen TS, Evans RJ, Cluette-Brown JE, Laposata M. Liver and adipose tissue fatty acid ethyl esters obtained at autopsy are postmortem markers for premortem ethanol intake. Clin Chem 2002;48:77–83.

437. Plueckhahn V. The significance of blood alcohol levels at autopsy. Med J Aust 1967;2:118–124.

438. Prouty R, Anderson W. A comparison of postmortem heart blood and femoral blood ethyl alcohol concentration. J Anal Toxicol 1987;11:191–197.

439. Turkel HW, Gifford H. Erroneous blood alcohol findings at autopsy. Avoidance by proper sampling technique. JAMA 1957;164:1077–1079.

440. Pounder DJ, Smith DR. Postmortem diffusion of alcohol from the stomach. Am J Forensic Med Pathol 1995;16: 89–96.

441. Briglia EJ, Bidanset JH, Dal Cortivo LA. The distribution of ethanol in postmortem blood specimens. J Forensic Sci 1992;37:991–998.

442. Pluekhahn VD. Alcohol levels in autopsy heart blood. J Forensic Med 1968;15:12–21.

443. Backer RC, Pisano RV, Sopher IM. The comparison of alcohol concentrations in postmortem fluids and tissues. 1980;25:327–331.

444. Pelissier-Alicot A-L, Fornaris M, Bartoli C, Piercecchi-Marti M-D, Sanvoisin A, Leonetti G. An unusual case of post-mortem redistribution of ethanol. Forensic Sci Int 2005;150:81–83.

445. Honey D, Caylor C, Luthi R, Kerrigan S. Comparative alcohol concentrations in blood and vitreous fluid with illustrative case studies. J Anal Toxicol 2005;29:365–369.

446. Caplan YH, Levine B. Vitreous humor in the evaluation of postmortem blood ethanol concentrations. J Anal Toxicol 1990;14:305–307.

447. de Lima IV, Midio AF. Origin of blood ethanol in decomposed bodies. Forensic Sci Int 1999;106:157–162.

448. Hardin GG. Postmortem blood and vitreous humor ethanol concentrations in a victim of a fatal motor vehicle crash. J Forensic Sci 2002;47:402–403.

449. Felby S, Olsen J. Comparative studies of postmortem ethyl alcohol in vitreous humor, blood, and muscle. J Forensic Sci 1969;14:93–101.

450. Sturner WQ, Coumbis RJ. The quantitation of ethyl alcohol in vitreous humor and blood by gas chromatography. Am J Clin Pathol 1966;46:349–351.

451. Leahy MS, Farber ER, Meadows TR. Quantitation of ethyl alcohol in the postmortem vitreous humor. J Forensic Sci 1968;13:498–502.

452. Jones AW, Holmgren P. Uncertainty in estimating blood ethanol concentrations by analysis of vitreous humour. J Clin Pathol 2001;54:699–702.

453. Pounder DJ, Kuroda N. Vitreous alcohol is of limited value in predicting blood alcohol. Forensic Sci Int 1994; 65:73–80.

454. Yip DC, Shum BS. A study on the correlation of blood and vitreous humour alcohol levels in the late absorption and elimination phases. Med Sci Law 1990;30;29–33.

455. Chao TC, Lo DS. Relationship between postmortem blood and vitreous humor ethanol levels. Am J Forensic Med Pathol 1993;14:303–308.

456. Keten A, Tumer AR, Balseven-Odabasi A. Measurement of ethyl glucuronide in vitreous humor with liquid chromatography-mass spectrometry. Forensic Sci Int 2009;193:101–105.

457. Baranowski S, Serr A, Thierauf A, Weinmann W, Grosse Perdekamp M, Wurst FM, Halter CC. *In vitro* study of bacterial degradation of ethyl glucuronide and ethyl sulphate. Int J Legal Med 2008;122:389–393.

458. Jones AW. Lack of association between urinary creatinine and ethanol concentrations and urine/blood ratio of ethanol in two successive voids from drinking drivers. J Anal Toxicol 1998;22:184–190.

459. Dunnett N, Kimber KJ. Urine-blood alcohol ratio. J Forensic Sci Soc 1968;8:15–24.

460. Biasotti AA, Valentine TE. Blood alcohol concentration determined from urine samples as a practical equivalent or alternative to blood and breath alcohol tests. J Forensic Sci 1985;30:194–207.

461. Iffland R, Jones AW. Evaluating alleged drinking after driving—the hip-flask defence. Part 1. Double blood samples and urine-to-blood alcohol relationship. Med Sci Law 2002;42;207–224.

462. Jones AW. Ethanol distribution ratios between urine and capillary blood in controlled experiments and in apprehended drinking drivers. J Forensic Sci 1992;37:21–34.

463. Alexander WD, Wills PD, Eldren N. Urinary ethanol and diabetes mellitus. Diabet Med 1988;5:463–464.

464. Saady JJ, Poklis A, Dalton HP. Production of urinary ethanol after sample collection. J Forensic Sci 1993; 38;1467–1471.

465. Heatley MK, Crane J. The relationship between blood and urine alcohol concentration at autopsy. Med Sci Law 1989;29:209–217.

466. Kaye S, Cardona E. Errors of converting a urine value into a blood alcohol level. Am J Clin Pathol 1969;52: 577–584.

467. Iwasaki Y, Yashiki M, Namera A, Miyazaki T, Kojima T. On the influence of postmortem alcohol diffusion from the stomach contents to the heart blood. Forensic Sci Int 1998;94:111–118.

468. Schmitt G, Aderjan R, Keller T, Wu M. Ethyl glucuronide: an unusual ethanol metabolite in humans. Synthesis, ana-

lytical data, and determination in serum and urine. J Anal Toxicol 1995;19:91–94.

469. Helander A, Beck O. Ethyl sulfate: a metabolite of ethanol in humans and a potential biomarker of acute alcohol intake. J Anal Toxicol 2005;29:270–274.

470. Foti RS, Fisher MB. Assessment of UDP-glucurono-syltransferase catalyzed formation of ethyl glucuronide in human liver microsomes and recombinant UGTs. Forensic Sci Int 2005;153:109–116.

471. Palmer RB. A review of the use of ethyl glucuronide as a marker for ethanol consumption in forensic and clinical medicine. Semin Diagn Pathol 2009;26:18–27.

472. Hoiseth G, Bernard JP, Karinen R, Johnsen L, Helander A, Christophersen AS, Morland J. A pharmacokinetic study of ethyl glucuronide in blood and urine: applications to forensic toxicology. Forensic Sci Int 2007;172: 119–124.

473. Sarkola T, Dahl H, Eriksson CJ, Helander A. Urinary ethyl glucuronide and 5-hydroxytryptophol levels during repeated ethanol ingestion in healthy human subjects. Alcohol Alcohol 2003;38:347–351.

474. Wurst FM, Kempter C, Metzger J, Seidl S, Alt A. Ethyl glucuronide: a marker of recent alcohol consumption with clinical and forensic implications. Alcohol 2000;20: 111–116.

475. Hoiseth G, Yttredal B, Karinen R, Gjerde H, Morland J, Christophersen A. Ethyl glucuronide concentrations in oral fluid, blood, and urine after volunteers drank 0.5 and 1.0 g/kg doses of ethanol. J Anal Toxicol 2010;34: 319–324.

476. Schmitt G, Droenner P, Skopp G, Aderjan R. Ethyl glucuronide concentration in serum of human volunteers, teetotalers, and suspected drinking drivers. J Forensic Sci 1997;42:1099–1102.

477. Wojcik MH, Hawthorne JS. Sensitivity of commercial ethyl glucuronide (ETG) testing in screening for alcohol abstinence. Alcohol Alcohol 2007;42:317–320.

478. Borucki K, Schreiner R, Dierkes J, Jachau K, Krause D, Westphal S, et al. Detection of recent ethanol intake with new markers: comparison of fatty acid ethyl esters in serum and of ethyl glucuronide and the ratio of 5-hydroxytryptophol to 5-hydroxyindole acetic acid in urine. Alcohol Clin Exp Res 2005;29:781–787.

479. Hoiseth G, Morini L, Polettini A, Christophersen A, Morland J. Blood kinetics of ethyl glucuronide and ethyl sulphate in heavy drinkers during alcohol detoxification. Forensic Sci Int 2009;188:52–56.

480. Helander A, Bottcher M, Fehr C, Dahmen N, Beck O. Detection times for urinary ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. Alcohol Alcohol 2009;44:55–61.

481. Kissack JC, Bishop J, Roper AL. Ethyl glucuronide as a biomarker for ethanol detection. Pharmacotherapy 2008; 28:769–781.

482. Hoiseth G, Karinen R, Christophersen AS, Olsen L, Normann PT, Morland J. A study of ethyl glucuronide in

post-mortem blood as a marker of ante-mortem ingestion of alcohol. Forensic Sci Int 2007;165:41–45.

483. Helander A, Olsson I, Dahl H. Post-collection synthesis of ethyl glucuronide by bacteria in urine may cause false identification of alcohol consumption. Clin Chem 2007;53:1855–1857.

484. Rohrig TP, Huber C, Goodson L, Ross W. Detection of ethyl glucuronide in urine following the application of Germ-X. J Anal Toxicol 2006;30:703–704.

485. Costantino A, Digregorio EJ, Korn W, Spayd S, Rieders F. The effect of the use of mouthwash on ethylglucuronide concentrations in urine. J Anal Toxicol 2006;30:659–662.

486. Thierauf A, Halter CC, Rana S, Auwaerter V, Wohlfarth A, Wurst FM, Weinmann W. Urine tested positive for ethyl glucuronide after trace amounts of ethanol. Addiction 2009;104:2007–2012.

487. Rosano TG, Lin J. Ethyl glucuronide excretion in humans following oral administration of and dermal exposure to ethanol. J Anal Toxicol 2008;32:594–600.

488. Goll M, Schmitt G, Ganssmann B, Aderjan RE. Excretion profiles of ethyl glucuronide in human urine after internal dilution. J Anal Toxicol 2002;26:262–266.

489. Bergström J, Helander A, Jones AW. Ethyl glucuronide concentrations in two successive urinary voids from drinking drivers: relationship to creatinine content and blood and urine ethanol concentrations. Forensic Sci Int 2003;133:86–94.

490. Skipper GE, Weinmann W, Thierauf A, Schaefer P, Wiesbeck G, Allen JP, et al. Ethyl glucuronide: a biomarker to identify alcohol use by health professionals recovering from substance use disorders. Alcohol Alcohol 2004;39:445–449.

491. Johnson RD, Lewis RJ, Canfield DV, Dubowski KM, Blank CL. Utilizing the urinary 5-HTOL/5-HIAA ration to determine ethanol origin in civil aviation accident victims. J Forensic Sci 2005;50:670–675.

492. Helander A. Biological markers in alcoholism. J Neural Transm 2003;66(suppl):15–32.

493. Politi L, Morinin L, Mari F, Groppi A, Bertol E. Ethyl glucuronide and ethyl sulfate in autopsy samples 27 years after death. Int J Legal Med 2008;122:507–509.

494. Helander A, Dahl H. Urinary tract infection: a risk factor for false-negative urinary ethyl glucuronide but not ethyl sulfate in the detection of recent alcohol consumption. Clin Chem 2005;51:1728–1730.

495. Beck O, Helander A. 5-hydroxytryptophol as a marker for recent alcohol intake. Addiction 2003;98;(suppl 2):63–72.

496. Helander A, Beck O, Jacobsson G, Lowenmo C, Wikstrom T. Time course of ethanol-induced changes in serotonin metabolism. Life Sci 1993;53:847–855.

497. Johnson RD, Lewis RJ, Canfield DV, Dubowski KM, Blank CL. Utilizing the urinary 5-HTOL/5-HIAA ratio to determine ethanol origin in civil aviation accident victims. J Forensic Sci 2005;50:1–6.

498. Helander A, Eriksson CJ, WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence Investigators. Laboratory tests for acute alcohol consumption: results of the WHO/ISBRA Study on State and Trait Markers of Alcohol Use and Dependence. Alcohol Clin Exp Res 2002;26:1070–1077.

499. Jones GR, Pounder DJ. Site dependence of drug concentrations in postmortem blood—a case study. J Anal Toxicol 1987;11:186–190.

500. Winek CL, Henry D, Kirkpatrick L. The influence of physical properties and lipid content of bile on the human blood/bile ethanol ratio. Forensic Sci Int 1983;22:171–178.

501. Stone BE, Rooney PA. A study using body fluids to determine blood alcohol. J Anal Toxicol 1984;8:95–96.

502. Christopoulos G, Kirch ER, Gearien JE. Determination of ethanol in fresh and putrefied post mortem tissues. J Chromatogr 1973;87:455–472.

503. Budd RD. Ethanol levels in postmortem body fluids. J Chromatogr 1982;252:315–318.

504. Gill GV, Baylis PH, Flear CT, Skillen AW, Diggle PH. Acute biochemical responses to moderate beer drinking. Br Med J 1982;285:1770–1773.

505. Pach J, Sancewicz-Pach K, Targosz D, Osuch M, Adamaszek Z. Acute ethanol poisonings during the New Year's Eve party "95/'96 in Krakow. Przegl Lek 1996;53:324–328.

506. Kruse JA, Cadnapaphornchai P. The serum osmole gap. J Crit Care 1994;9:185–197.

507. Bekeris L, Baker C, Fenton J, Kimball D, Bermes E. Propylene glycol as a cause of elevated serum osmolality. Am J Clin Pathol 1979;72:633–636.

508. Eisen TF, Lacouture PG, Woolf A. Serum osmolality in alcohol ingestions: differences in availability among laboratories of teaching hospital, non-teaching hospital, and commercial facilities. Am J Emerg Med 1989;7:256–259.

509. Aabakken L, Johansen KS, Rydningen E-B, Bredesen JE, Ovrebo S, Jacobsen D. Osmolal and anion gaps in patients admitted to an emergency medical department. Hum Exp Toxicol 1994;13:131–134.

510. Almaghamsi AM, Yeung CK. Osmolal gap in alcoholic ketoacidosis. Clin Nephrol 1997;48:52–53.

511. Ku E, Cheung EL, Khan A, Yu AS. Acid-base and electrolyte teaching case anion and osmolal gaps after alcohol intoxication. Am J Kid Dis 2009;54:385–388.

512. Hollstedt C, Olsson O, Rydberg U. The effect of alcohol on the developing organism. Med Biol 1977;55:1–14.

513. Cummins LH. Hypoglycemia and convulsions in children following alcohol ingestion. J Pediatr 1961;58:23–26.

514. Williams HE. Alcoholic hypoglycemia and ketoacidosis. Med Clin North Am 1984;68:33–38.

515. Beattie JO, Hull D, Cockburn F. Children intoxicated by alcohol in Nottingham and Glasgow, 1973–84. Br Med J 1986;292:519–521.

516. Lamminpaa A, Vilska J. Acute alcohol intoxications in children treated in hospital. Acta Paediatr Scand 1990; 79:847–854.

517. Lamminpaa A, Vilska J, Korri UM, Riihimaki V. Alcohol intoxication in hospitalized young teenagers. Acta Paediatr 1993;82:783–788.

518. Chedid A, Mendenhall CL, Gartside P, French SW, Chen T, Rabin L. Prognostic factors in alcoholic liver disease. VA Cooperative Study Group. Am J Gastroenterol 1991; 86:210–216.

519. Magarian GJ, Lucas LM, Kumar KL. Clinical significance in alcoholic patients of commonly encountered laboratory test results. West J Med 1992;156:287–294.

520. Kawachi I, Robinson GM, Stace NH. A combination of raised serum AST:ALT ratio and erythrocyte mean cell volume level detects excessive alcohol consumption. N Z Med J 1990;103:145–148.

521. Drum DE, Goldman PA, Jankowski CB. Elevation of serum uric acid as a clue to alcohol abuse. Arch Intern Med 1981;141:477–479.

522. McGuire LC, Cruickshank AM, Munro PT. Alcoholic ketoacidosis. Emerg Med J 2006;23:417–420.

523. Wu A, Chanarin I, Levi AJ. Macrocytosis of chronic alcoholism. Lancet 1974;1:829–831.

524. Sand T, Bråthen G, Michler R, Brodtkorb E, Helde G, Bovim G. Clinical utility of EEG in alcohol-related seizures. Acta Neurol Scand 2002;105:18–24.

525. Hindmarch I, Bhatti JZ, Starmer GA, Mascord DJ, Kerr JS, Sherwood N. The effects of alcohol on the cognitive function of males and females and on skills relating to car driving. Hum Psychopharmacol 1992;7:105–114.

526. Kearney SA, Guppy A. The effects of alcohol on speed perception in a closed-course driving situation. J Stud Alcohol 1988;49:340–345.

527. Council on Scientific Affairs. Alcohol and the driver. JAMA 1986;255:522–527.

528. Schweizer TA, Vogel-Sprott M, Danckert J, Roy EA, Skakum A, Broderick CE. Neuropsychological profile of acute alcohol intoxication during ascending and descending blood alcohol concentrations. Neuropsychopharmacology 2006;31:1301–1309.

529. Nicholson ME, Wang M, Airhihenbuwa CO, Mahoney BS, Christina R, Maney DW. Variability in behavioral impairment involved in the rising and falling BAC curve. J Stud Alcohol 1992;53:349–356.

530. Mitchell MC. Alcohol-induced impairment of central nervous system function: behavioral skills involved in driving. J Stud Alcohol 1985;46(suppl 10):109–116.

531. Hills BL. Vision, visibility, and perception in driving. Perception 1980;9:183–216.

532. Hill JC, Toffolon G. Effect of alcohol on sensory and sensorimotor visual functions. J Stud Alcohol 1990;51: 108–113.

533. Watten RG, Lie I. Visual functions and acute ingestion of alcohol. Ophthalmic Physiol Opt 1996;16:460–466.

534. Danielson RW. The relationship of fields of vision to safety in driving with a report of 680 drivers examined by various screening methods. Am J Ophthalmol 1957; 44:657–680.

535. Newman H, Fletcher E. The effect of alcohol on vision. Am J Med Sci 1941;202:723–731.

536. Allen MJ. Vision and driving. Traffic Saf 1969;69:8–9, 38–40.

537. Wilhelmi F, Lindner H-J, Audrlicky I. Untersuchugen über das sehen in der dämmerung nach alkoholaufnahme. Blutalkohol 1972;9:473–485.

538. Moskowitz H, Sharma S. Effects of alcohol on peripheral vision as a function of attention. Hum Factors 1974; 16:174–180.

539. Newman H, Fletcher E. The effect of alcohol on driving skills. JAMA 1940;115:1600–1602.

540. McNamee JE, Tong JE, Piggins DJ. Effects of alcohol on velocity perception: I. Stimulus velocity and change in performance over time. Percept Mot Skills 1980;51: 779–785.

541. Vanakoski J, Mattila MJ, Seppala T. Driving under light and dark conditions: effects of alcohol and diazepam in young and older subjects. Eur J Clin Pharmacol 2000;56:453–458.

542. Nawrot M, Nordenstrom B, Olson A. Disruption of eye movements by ethanol intoxication affects perception of depth from motion parallax. Psychol Sci 2004;15: 858–865.

543. Sekuler R, MacArthur RD. Alcohol retards visual recovery from glare by hampering target acquisition. Nature 1977;270:428–429.

544. Adams AJ, Brown B. Alcohol prolongs time course of glare recovery. Nature 1975;257:481–483.

545. Adams AJ, Brown B, Haegerstrom-Portnoy G, Flom MC, Jones RT. Marijuana, alcohol, and combined drug effects on the time course of glare recovery. Psychopharmacology 1978;56:81–86.

546. Harris A, Swartz D, Engen D, Beck D, Evans D, Caldemeyer K, Martin B. Ocular hemodynamic effects of acute ethanol ingestion. Ophthalmic Res 1996;28: 193–200.

547. Zulauf M, Flammer J, Signer C. Short-term influence of alcohol on spatial brightness contrast sensitivity. Ophthalmologica 1988;197:159–165.

548. Andre JT, Tyrrell RA, Leibowitz HW, Nicholson ME, Wang M. Measuring and predicting the effects of alcohol consumption on contrast sensitivity for stationary and moving gratings. Percept Psychophys 1994;56: 261–267.

549. Pearson P, Timney B. Effects of moderate blood alcohol concentrations on spatial and temporal contrast sensitivity. J Stud Alcohol 1998;59:163–173.

550. Guedry FE Jr, Gilson RD, Schroeder DJ, Collins WE. Some effects of alcohol on various aspects of oculomotor control. Aviat Space Environ Med 1975;46:1008–1013.

551. Moser A, Heide W, Kompf D. The effect of oral ethanol consumption on eye movements in healthy volunteers. J Neurol 1998;248:542–550.

552. Miller RJ, Pigion RG, Takahama M. The effects of ingested alcohol on accommodative, fusional, and dark vergence. Percept Psychophys 1986;39:25–31.

553. Hogan RE, Linfield PB. The effects of moderate doses of ethanol on heterophoria and other aspects of binocular vision. Ophthalmic Physiol Opt 1983;3:21–31.

554. Miller RJ. The effect of ingested alcohol on fusion latency at various viewing distances. Percept Psychophys 1991;50: 575–583.

555. Murata K, Kawashima M, Inaba R. Auditory threshold reduction on alcohol ingestion. Psychopharmacology 2001;157:188–192.

556. Pearson P, Dawe LA, Timney B. Frequency selective effects of alcohol on auditory detection and frequency discrimination thresholds. Alcohol Alcohol 1999;34: 741–749.

557. Hwang J-H, Tan C-T, Chiang C-W, Liu T-C. Acute effects of alcohol on auditory thresholds and distortion product otoacoustic emissions in humans. Acta Otolaryngol 2003; 123:936–940.

558. Fagan D, Tiplady B, Scott DB. Effects of ethanol on psychomotor performance. Br J Anaesth 1987;59:961–965.

559. Peterson JB, Rothfleisch J, Zelazo PD, Pihl RO. Acute alcohol intoxication and cognitive functioning. J Stud Alcohol 1990;51:114–122.

560. do Canto-Pereira LH, de PA David I, Machado-Pinheiro W, Ranvaud RD. Effects of acute alcohol intoxication on visuospatial attention. Hum Exp Toxicol 2007;26: 311–319.

561. Buikhuisen W, Jongman RW. Traffic perception under the influence of alcohol. Q J Stud Alcohol 1972;33:800–806.

562. Schulte T, Muller-Oehring EM, Strasburger H, Warzel H, Sabel BA. Acute effects of alcohol on divided and covert attention in men. Psychopharmacology 2001;154:61–69.

563. Teichner WH. Recent studies of simple reaction time. Psychol Bull 1954;51:128–149.

564. Perrine MW. Alcohol and highway crashes. Closing the gap between epidemiology and experimentation. Mod Probl Pharmacopsychiatry 1976;11:22–41.

565. Wallgren H, Barry H III. Actions of alcohol biochemical, physiological and psychological aspects. Vol I. Amsterdam: Elsevier; 1970:287–329.

566. Franks HM, Hensley VR, Hensley WJ, Starmer GA, Teo RK. The relationship between alcohol dosage and performance decrement in humans. J Stud Alcohol 1976;37: 284–297.

567. Summala H. Driver/vehicle steering response latencies. Hum Factors 1981;23:683–692.

568. Konz S, Daccarett J. Controls for automotive brakes. Highway Res Rec 1967;195:75–79.

569. Heacock D, Wikle R. The effect of alcohol and placebo on reaction time and distance judgment. J Gen Psychol 1974;91:265–268.

570. Linnoila M, Erwin CW, Cleveland WP, Logue PE, Gentry WD. Effects of alcohol on psychomotor performance of men and women. J Stud Alcohol 1978;39:745–758.

571. Connors GJ, Maisto SA. Effects of alcohol, instructions and consumption rate on motor performance. J Stud Alcohol 1980;41:509–517.

572. Jones MB, Chronister JL, Kennedy RS. Effects of alcohol on perceptual speed. Percept Mot Skills 1998;87: 1247–1255.

573. Johansson G, Rumar K. Drivers' brake reaction times. Hum Factors 1971;13:23–27.

574. Liguori A, D'Agostino RB Jr, Dworkin SI, Edwards D, Robinson JH. Alcohol effects on mood, equilibrium, and simulated driving. Alcohol Clin Exp Res 1999; 23:815–821.

575. National Highway Traffic Safety Administration. The highway safety desk book. Washington, DC: National Center for Statistics and Analysis; 1996.

576. Grant SA, Millar K, Kenny GN. Blood alcohol concentration and psychomotor effects. Br J Anaesth 2000;85: 401–406.

577. Valeriote C, Tong JE, Druding B. Ethanol, tobacco and laterality effects on simple and complex motor performance. J Stud Alcohol 1979;40:823–830.

578. Linoila M, Erwin CW, Cleveland WP, Logue PE, Gentry WD. Effects of alcohol on psychomotor performance of men and women. J Stud Alcohol 1978;39:745–758.

579. Ball K, Owsley C. Identifying correlates of accident involvement for the older driver. Hum Factors 1991;33: 583–595.

580. Quillian WC, Cox DJ, Kovatchev BP, Phillips C. The effects of age and alcohol intoxication on simulated driving performance, awareness and self-restraint. Age Ageing 1999;28:59–66.

581. Raffle PA. Interrelation between alcohol and accidents. J Roy Soc Med 1989;82:132–136.

582. Simpson HM, Mayhew DR, Warren RA. Epidemiology of road accidents involving young adults: alcohol, drugs and other factors. Drug Alcohol Depend 1982;10:35–63.

583. Haddon W Jr, Bradess VA. Alcohol in the single vehicle fatal accident. JAMA 1959;169:1587–1593.

584. Donovan DM, Marlatt GA, Salzberg PM. Drinking behavior, personality factors and high-risk driving. A review and theoretical formulation. J Stud Alcohol 1983;44;395–428.

585. Farquhar K, Lambert K, Drummond GB, Tiplady B, Wright P. Effect of ethanol on psychomotor performance and on risk taking behaviour. J Psychopharmacol 2002; 16:379–384.

586. George S, Rogers RD, Duka T. The acute effect of alcohol on decision making in social drinkers. Psychopharmacology 2005;182:160–169.

587. Stoduto G, Vingilis E, Kapur BM, Sheu W-J, McLellan BA, Liban CB. Alcohol and drug use among motor vehicle collision victims admitted to a regional trauma

unit: demographic, injury, and crash characteristics. Accid Anal Prev 1993;25:411–420.

588. Holubowycz OT, Kloeden CN, McLean AJ. Age, sex, and blood alcohol concentration of killed and injured drivers, riders, and passengers. Accid Anal Prev 1994;26: 483–492.

589. Johnston IR. The role of alcohol in road crashes. Ergonomics 1982;25;941–946.

590. Klein T. A method for estimating posterior BAC distributions for persons involved in fatal traffic accidents (DOT HS-807-094). Washington, DC: US Department of Transportation; 1986.

591. National Highway Traffic Safety Administration. Traffic safety facts 1999: a compilation of motor vehicle crash data from the fatality analysis reporting system and the general estimates system. Washington, DC: National Center for Statistics and analysis, US Department of Transportation; 2000.

592. Zobeck TS, Grant BF, Stinson FS, Bertolucci D. Alcohol involvement in fatal traffic crashed in the United States: 1979–90. Addiction 1994;89:227–231.

593. Zador PL, Krawchuk SA, Voas RB. Alcohol-related relative risk of driver fatalities and driver involvement in fatal crashes in relation to driver age and gender: an update using 1996 data. J Stud Alcohol 2000;61: 387–395.

594. Popkin CL. Drinking and driving by young females. Accid Anal Prev 1991;23:37–44.

595. Mounce NH, Pendleton OJ. The relationship between blood alcohol concentration and crash responsibility for fatally injured drivers. Accid Anal Prev 1992;24: 201–210.

596. Friede AM, Azzara CV, Gallagher SS, Guyer B. The epidemiology of injuries to bicycle riders. Pediatr Clin North Am 1985;32;141–151.

597. Rowe BH, Rowe AM, Bota GW. Bicyclist and environmental factors associated with fatal bicycle-related trauma in Ontario. Can Med Assoc J 1995;152:45–53.

598. Rodgers GB. Bicyclist deaths and fatality risk patterns. Acid Anal Prev 1995;27:215–223.

599. National Safety Council. Accident facts. Itasca, IL: National Safety Council;1994.

600. Owens DA, Sivak M. Differentiation of visibility and alcohol as contributors to twilight road fatalities. Hum Factors 1996;38;680–689.

601. Li G, Baker SP. Alcohol in fatally injured bicyclists. Accid Anal Prev 1994;26:43–48.

602. Olkkonen S, Honkanen R. The role of alcohol in nonfatal bicycle injuries. Accid Anal Prev 1990;2:89–96.

603. Jehle D, Cottington E. Effect of alcohol consumption on outcome of pedestrian victims. Ann Emerg Med 1988;17; 953–956.

604. Burns M, Moskowitz H. Psychophysical tests for DWI arrest. Final report (DOT-HS-806-676). Washington, DC: National Highway Traffic Safety Administration; 1977.

605. Stuster J, Burns M. Validation of the standardized field sobriety test battery at BACs below 0.10 percent. Santa Barbara, CA: Anacapa Sciences; 1998.

606. Hlastala MP, Polissar NL, Oberman S. Statistical evaluation of standardized field sobriety tests. J Forensic Sci 2005;50:1–8.

607. Cole S, Nowaczyk RH. Field sobriety tests: are they designed for failure? Percept Mot Skills 1994;79:99–104.

608. Tharp V, Burns M, Moskowitz H. Development and field test of psychophysical tests for DWI arrests. Final report (DOT-HS-805-864). Washington, DC: National Highway Traffic Safety Administration; 1981.

609. Goding GS, Dobie RA. Gaze nystagmus and blood alcohol. Laryngoscope 1986;96:713–717.

610. National Highway Traffic Safety Administration. DWI detection and standardized field sobriety testing. Student manual (PB96-780739INT). Springfield, VA: National Technical Information Services; 1995.

611. McKnight AJ, Lange JE, McKnight AS. Development of a standardized boating sobriety test. Accid Anal Prev 1999;31:147–152.

612. Wilkinson IM, Kime R, Purnell M. Alcohol and human eye movement. Brain 1974;97:785–792.

613. Lehti HM. The effect of blood alcohol concentration on the onset of gaze nystagmus. Blutalkohol 1976;13: 411–414.

614. Aschan G. Different types of alcohol nystagmus. Acta Otolaryngol 1958;140(suppl):69–78.

615. Nuotto E, Mattila MJ, Seppala T, Konno K. Coffee and caffeine and alcohol effects on psychomotor function. Clin Pharmacol Ther 1982;31:68–76.

616. Booker JL. End-position nystagmus as an indicator of ethanol intoxication. Sci Justice 2001;41:113–116.

617. Good GW, Augsburger AR. Use of horizontal gaze nystagmus as a part of roadside sobriety testing. Am J Optometry Physiol Opt 1986;63:467–471.

618. Simpson-Crawford T, Slater SW. Eye signs in suspected drinking drivers: clinical examination and relation to blood alcohol. NZ Med J 1971;74:92–96.

619. Howells DE. Nystagmus and other eye signs in acute alcoholism. Br Med J 1952;2:862–864.

620. Citek K, Ball B, Rutledge DA. Nystagmus testing in intoxicated individuals. Optometry 2003;74:695–710.

621. Goldberg L. Quantitative studies on alcohol tolerance in man: the influence of ethyl alcohol on sensory, motor and psychological functions referred to blood alcohol in normal and habituated individuals. Acta Physiol Scand 1943;5(suppl 16)1–128.

622. Carpenter JA. Effects of alcohol on some psychological processes. Q J Stud Alcohol 1962;23:274–314.

623. Coldwell BB, Penner DW, Smith HW, Lucas GH, Rodgers RF, Darroch F. Effect of ingestion of distilled spirits on automobile driving skill. Q J Stud Alcohol 1958; 19:590–616.

624. Ferreira V, Hernandez-Orte P, Escudero A, Lopez R, Cacho J. Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. J Chromatogr A 1999; 864:77–88.

625. Widmark EM. Principles and applications of medicolegal alcohol determination. Davis, CA: Biomedical Publications; 1981.

626. Compton RP. Pilot test of selected DWI detection procedures for use at sobriety checkpoints (Technical Report HS 806-724). Washington, DC: Office of Driver and Pedestrian Research, National Highway Traffic Safety Administration, US Department of Transportation; 1985.

627. Moskowitz H, Burns M, Ferguson S. Police officers' detection of breath odors from alcohol ingestion. Accid Anal Prev 1999;31:175–180.

628. Lien D, Mader TJ. Survival from profound alcohol-related lactic acidosis. J Emerg Med 1999;17:841–846.

629. Minocha A, Herold HA, Barth JA, Gideon DA, Spyker DA. Activated charcoal in oral ethanol absorption: lack of effect in humans. Clin Toxicol 1986;24:225–234.

630. North DS, Thompson JD, Peterson CD. Effect of activated charcoal on ethanol blood levels in dogs. Am J Hosp Pharm 1981;38:864–866.

631. Li J, Mills T, Erato R. Intravenous saline has no effect on blood ethanol clearance. J Emerg Med 1999;17:1–5.

632. Nuotto E, Palva ES, Seppala T. Naloxone-ethanol interaction in experimental and clinical situations. Acta Pharmacol Toxicol (Copenh) 1984;54:278–284.

633. Thomson AD, Cook CCH, Touquet R, Henry JA. The Royal College of Physicians report on alcohol: guidelines for managing Wernicke's encephalopathy in the accident and emergency department. Alcohol Alcohol 2002;37: 513–521.

634. Hack JB, Hoffman RS. Thiamine before glucose to prevent Wernicke encephalopathy: examining the conventional wisdom. JAMA 1998;279:583–584.

635. Lingford-Hughes AR, Welch S, Nutt DJ. Evidence-based guidelines for the pharmacological management of substance misuse, addiction and comorbidity: recommendations from the British Association for Psychopharmacology. J Psychopharmacol 2004;18:293–335.

636. Kiritze-Topor P, Huas D, Rosenzweig C, Comte S, Paille F, Lehert P. A pragmatic trial of acamprosate in the treatment of alcohol dependence in primary care. Alcohol Alcohol 2004;39:520–527.

637. Feeney GF, Connor JP, Young RM, Tucker J, McPherson A. Is acamprosate use in alcohol dependence treatment reflected in improved subjective health status outcomes beyond cognitive behavioural therapy alone? J Addict Dis 2006;25:49–58.

638. Garbutt JC, West SL, Carey TS, Lohr KN, Crews FT. Pharmacological treatment of alcohol dependence a review of the evidence. JAMA 1999;281:1318–1325.

639. Schuckit MA. Alcohol-use disorders. Lancet 2009;373: 492–501.

640. Mayo-Smith MF and the American Society of Addiction Medicine Working Group on Pharmacological Management of Alcohol Withdrawal: pharmacological management of alcohol withdrawal a meta-analysis and evidence-based practice guideline. JAMA 1997;278: 144–151.

641. Saitz R, Mayo-Smith MF, Roberts MS, Redmond HA, Bernard DR, Calkins DR. Individualized treatment for alcohol withdrawal. A randomized double-blind controlled trial. JAMA 1994;272;519–523.

642. Malcolm R, Myrick H, Roberts J, Wang W, Anton RF, Ballenger JC. The effects of carbamazepine and lorazepam on single versus multiple previous alcohol withdrawals in an outpatient randomized trial. J Gen Intern Med 2002;17:349–355.

643. Myrick H, Malcolm R, Randall RK, Boyle E, Anton RF, Becker HC, Randall CL. A double-blind trial of gabapentin versus lorazepam in the treatment of alcohol withdrawal. Alcohol Clin Exp Res 2009;33:1582–1588.

644. Krupitsky EM, Rudenko AA, Burakov AM, Slavina TY, Grinenko AA, Pittman B, et al. Antiglutamatergic strategies for ethanol detoxification: comparison with placebo and diazepam. Alcohol Clin Exp Res 2007;31:604–611.

645. D'Onofrio G, Rathlev NK, Ulrich AS, Fish SS, Freedland ES. Lorazepam for the prevention of recurrent seizures related to alcohol. N Engl J Med 1999;340:915–919.

646. Wilson A, Vulcano B. A double-blind, placebo-controlled trial of magnesium sulfate in the ethanol withdrawal syndrome. Alcohol Clin Exp Res 1984;8:542–545.

647. Chance JF. Emergency department treatment of alcohol withdrawal seizures with phenytoin. Ann Emerg Med 1991;20;520–522.

648. EFNS Task Force on Diagnosis and Treatment of Alcohol-related Seizures. EFNS guideline on the diagnosis and management of alcohol-related seizures: report of an EFNS task force. Eur J Neurol 2005;12:575–581.

649. Turner RC, Lichstein PR, Peden JG Jr, Busher JT, Waivers LE. Alcohol withdrawal syndromes: a review of pathophysiology, clinical presentation, and treatment. J Gen Intern Med 1989;4:432–444.

650. Mayo-Smith MF, Beecher LH, Fischer TL, Gorelick DA, Guillaume JL, Hill A, et al. Management of alcohol withdrawal delirium. An evidence-based practice guideline. Arch Intern Med 2004;164:1405–1412.

651. Saitz R, O'Malley SS. Pharmacotherapies for alcohol abuse withdrawal and treatment. Med Clin North Am 1997;82:881–907.

652. Subramaniam K, Gowda RM, Jani K, Zewedie W, Ute R. Propofol combined with lorazepam for severe polysubstance misuse and withdrawal states in intensive care unit: a case series and review. Emerg Med J 2004;21: 632–634.

653. Coomes TR, Smith SW. Successful use of propofol in refractory delirium tremens. Ann Emerg Med 1997;30: 825–828.

654. Peachey JE, Brien JF, Roach CA, Loomis CW. A comparative review of the pharmacological and toxicological properties of disulfiram and calcium carbimide. J Clin Psychopharmacol 1981;1:21–26.

655. Brien JF, Loomis CW. Aldehyde dehydrogenase inhibitors as alcohol-sensitizing drugs: a pharmacological perspective. Trends Pharm Sci 1985;6:477–480.

656. Petersen EN. The pharmacology and toxicology of disulfiram and its metabolites. Acta Psychiatr Scand 1992;86: 7–13.

657. Lindros KO, Stowell A, Pikkarainen P, Salaspuro M. The disulfiram (Antabuse)-alcohol reaction in male alcoholics: its efficient management by 4-methylpyrazole. Alcohol Clin Exp Res 1981;5:528–530.

Chapter 22

LYSERGIC ACID DIETHYLAMIDE (LSD)

HISTORY

In 1938 at Sandoz Laboratories, Arthur Stoll and Albert Hoffman synthesized LSD from the ergot alkaloid, lysergic acid during their search for an analeptic drug similar to nikethamide.[1] The ergot alkaloid was a hydrolysis product of the fungus, *Claviceps purpurea* (Fr.) Tul., which commonly infects grains. Historically, ergot alkaloids were the active ingredient in the visionary Eleusinian rite of classical times, the epidemic ergotism (St. Anthony's fire) of the Middle Ages, and some New World religious rites (ololiuqui). A group of ergot alkaloids rather than lysergic acid diethylamide (i.e., LSD) probably was the biologic source for these mind-altering effects.[2] Lysergic acid diethylamide is abbreviated *LSD* rather than *LAD* because the German word for acid is *saeure*. LSD had little analeptic or oxytocic activity, and Hoffman abandoned his research on LSD for 5 years. In 1943 after renewing his research on the compound, Hofmann inadvertently discovered its psychotropic properties (intense kaleidoscopic colors, images of extraordinary vividness); he decided to verify the effects of LSD by intentionally ingesting 250 µg LSD.[3] Subsequently, he developed vertigo, agitation, unpleasant visual illusions, and synesthesias. He returned to normal the following day. Subsequent research confirmed that *d*-lysergic acid diethylamide is the only 1 of 4 isomers derived from lysergic acid that has powerful mind-altering properties.

Publication of the experiences of Stoll and Hoffman with LSD initiated the experimental use of LSD on animals, healthy volunteers, and psychotic patients by psychiatrists and other researchers.[4] During the 1950s and 1960s, unpleasant reactions eventually limited the use of LSD in clinical experiments. Later in life, Hoffman admitted that he spent far too much of his research time on LSD.[5] In 1954, Gaddum and Hameed reported that LSD antagonized the effect of serotonin on peripheral tissues in animal bioassays.[6] Of 8 ergot compounds, LSD was the most potent presynaptic serotonin antagonist. Later studies demonstrated that LSD was best described as a mixed $5\text{-HT}_2/5\text{-HT}_1$ receptor partial agonist.

During the 1950s, the self-administration and the administration of LSD to volunteer house staff and psychiatric patients was associated with changes in body ideation, visual illusions, loss of concentration, feelings of irresponsibility, and occasional severe adverse effects (schizophrenia-like behavior, bad trip).[7] In 1957, the Canadian research psychologist, Humphry Osmond introduced the word, psychedelic ("mind-manifesting") as a term to describe hallucinogenic drugs (e.g., LSD, mescaline) that provide clear, euphonious effects uncontaminated by other associations. Illegal use of LSD increased dramatically in the 1960s, and the US Drug Enforcement Administration (DEA) added LSD to the list of schedule I controlled substances in 1966. Medical uses in the 1950s included the treatment of alcoholism, psychoneurosis, sexual abnormalities, autism in children, sociopathy, and analgesia in terminal patients, but controlled clinical trials did not confirm the efficacy of LSD when compared with psychotherapy alone.

IDENTIFYING CHARACTERISTICS

Structure

LSD (CAS RN: 50-37-3) is the semisynthetic diethylamide derivative of the ergot alkaloid, lysergic acid (CAS

RN: 82-58-6), that is structurally similar to nikethamide as displayed in Figure 22.1. LSD does not occur naturally.[8] As lysergic acid has 2 asymmetric carbon atoms (C5, C8), 4 possible stereoisomers exist (*d*- and *l*-lysergic acid and *d*- and *l*-isolysergic acid). Of the 4 isomers, only the *d*-LSD analogs (e.g., *d*-LSD) are active psychedelic drugs. Most LSD derivatives are less potent psychotomimetic agents than LSD. *d*-Lysergic acid amide (CAS RN: 478-94-4, ergine or lysergamide) is a naturally occurring compound that is a structurally similar ergot alkaloid to LSD. This compound has about one tenth the hallucinogenic activity of the semisynthetic *d*-LSD.[9] Only LSD derivatives substituted at the N6 position are nearly as potent as the parent compound.

Physiochemical Properties

LSD is a colorless, tasteless, odorless compound that is water- and alcohol-soluble. The molecular weight of LSD is 323.44 g/mol with a molecular formula of $C_{20}H_{25}N_3O$. *d*-LSD crystallizes from benzene or isopropyl ether in the form of pointed prisms with a melting point of 80–85°C (176–185°F). The tartrate salt of LSD is stable; LSD usually occurs in solution as either the free base or the tartrate salt. Rapid decomposition of LSD occurs following exposure to sunlight. LSD is one of several common drugs of abuse that cause hallucinations (see Table 22.1). Psychedelic is a poorly defined term that commonly refers to drugs (e.g., LSD, mescaline, peyote, dimethyltryptamine, harmaline) that produce thought, mood, and perceptual changes rarely experienced except in dreams, flashbacks, or acute psychoses. Although the perceptual alterations associated with LSD may be similar to hallucinations associated with psychotic disorders, LSD intoxication is not usually associated with the delirium (i.e., alteration in consciousness with cognitive changes) present during a psychotic disorder. In contrast to the psychotic patient, most LSD users retain insight into their altered perceptions, and they understand the drug-induced origin of these altered perceptions of reality.[10]

Terminology

The official US Pharmacopoeia name for LSD is lysergide. Other synonyms include delysid, LSD-25, *N*,*N*-diethyl-*d*-lysergamide, and 9,10-didehydro-*N*,*N*-diethyl-6-methylergoline-8-β-carboxamide. Street names include acid; blotter acid; paper acid; orange or purple wedges; yellow, blue, brown, or green caps; blue, pink, or yellow drops; white lightning; purple haze; California sunshine; sunshine; window panes; beast; microdots; the ghost; or hawk.

Form

LSD is a white, crystalline compound. Previously, a popular form of LSD use involved the use of tiny, round tablets (microdots) that were impregnated with LSD. Now a more popular form of LSD use involves spraying or basting of the drug on colorful sheets of blotting paper that are dried, perforated, and sold as blotter paper, postage stamps, or squares of gelatin (windowpane acid; Figure 22.2). These forms of LSD may be blank or imprinted with characteristic designs (e.g., Bart Simpson, Beavis and Butthead, Felix the Cat, Tasmanian Devil).[11]

FIGURE 22.1. Chemical structure of lysergic acid diethylamide (LSD).

TABLE 22.1. Commonly Abused Drugs Causing Hallucinations.

Class	Common Drugs of Abuse
Indole Alkaloids	Bufotenine, dimethyltryptamine, harmine, ibogaine, LSD, psilocin, psilocybin
Phenylethylamine Compounds	Mescaline, *p*-methoxyamphetamine (PMA), methylenedioxyamphetamine (MDA), 2,5-dimethoxy-4-amphetamine (STP), 3,4-methylenedioxymethamphetamine (MDMA), 2,5-dimethoxy-4-methylamphetamine (DOM)
Cannabinoids*	Marijuana
Piperidines†	Atropine, hyoscine, hyoscyamine, ketamine, phencyclidine, dextromethorphan

*Although hallucinations may occur during cannabis intoxication, the effects of cannabis are substantially different than classic hallucinogens and cannabis is usually discussed separately from hallucinogens.
†Although included in some classifications of hallucinogens, these drugs are more dissociative agents that reduce or block sensory input to the conscious part of the brain.

FIGURE 22.2. Confiscated blotters containing lysergic acid diethylamide (LSD). (Photo courtesy of the US Drug Enforcement Agency).

EXPOSURE

Epidemiology

The popularity of LSD peaked during the mid-1960s; then use declined during the 1970s and 1980s. However, the illicit use of LSD began increasing again during the 1990s, particularly in midwestern and western United States among middle-class Caucasian high school and college students. In general, the highest risk of using psychedelic drugs occurs during the transition from adolescence to adulthood.[12] According to the National Institutes of Drug Abuse (NIDA) 1993 nationwide survey, the percentage of 8th-, 10th-, and 12th-grade students with a history of experimenting with LSD was 3.5%, 6%, and 10%, respectively.[13] Updates of this survey indicate that LSD use among these 3 grades declined sharply from about 2001 to 2005; since that time, annual prevalence rates remained relatively stable ranging from 1.3–2.7% in these 3 grades. LSD is an inexpensive drug that is popular at rave parties along with other psychedelic drugs [e.g., MDMA (Ecstasy; Adam), MDA (Love drug; Eve), methamphetamine (Meth)].[14]

Sources

Lysergic acid and diethylamine are precursors used in the synthesis of *d*-lysergic acid diethylamide. Lysergic acid occurs naturally in grain parasitized by the fungus *Claviceps purpurea*. The seeds from the heavenly blue morning glory (*Ipomoea violacea* L.), ololiuqui or Christmas vine [*Turbina corymbosa* (L.) Raf. or *Rivea corymbosa*] and Hawaiian baby wood rose or elephant creeper (*Argyreia nervosa* (Burm. f.) Bojer] contain *d*-lysergic acid amide (ergine).[15,16] Minor indole alkaloids in these plants include *d*-isolysergic acid amide (iso-ergine), chanoclavine, elymoclavine, lysergol, seto-clavine, and agroclavine. LSD is a semisynthetic drug that is frequently imported into the US illicitly. The Federal Controlled Substances Act classifies LSD as a schedule I drug (high abuse potential, no medical uses in the United States), whereas the LSD precursors lysergic acid and *d*-lysergic acid amide (ergine) are DEA schedule III drugs.

Typically, samples of illicit LSD are relatively pure, and adulteration has been uncommon since the 1970s. Prior to that time, substitutions for LSD included meth-amphetamine, PCP, and caffeine. Of all illicit drugs, LSD is probably the least adulterated with the validity rate for LSD analysis being almost 90% in some studies of street samples.[17] Although there are 4 stereoisomers of LSD as a result of the asymmetric carbon atoms at positions 5 and 8, only the *d*-LSD isomer is an active psychedelic drug.[9] Most illicit samples of LSD are racemic mixtures of all 4 stereoisomers. Diethylamine is a potential contaminant of illicit LSD synthesis. The epimer, *iso*-LSD and the metabolite, *iso-nor*-LSD are inactive. These synthetic compounds are byproducts of the illicit preparation of LSD; consequently, the presence of *iso*-LSD in urine is an indication of LSD use as detected by gas chromatography/mass spectrometry or liquid chromatography/electrospray ionization/tandem mass spectrometry.[18,19]

Methods of Abuse

The preferred route of LSD administration is almost always orally with intravenous (IV), pulmonary, and nasal routes rarely used. Typically, the pattern of LSD use is intermittent rather than intense and sustained, in part reflecting the rapid development of tolerance with chronic use. LSD users are frequently polydrug abusers (e.g., alcohol, amphetamines, marijuana), but the use of sedative-hypnotics and opiates occurs less frequently in LSD users than in other polydrug users. Although the use of LSD may become the focal point of the user's lifestyle, physical withdrawal symptoms do not occur following abrupt cessation of use.

DOSE EFFECT

The administration of LSD produces dose-dependent changes in mentation, perception, emotion, arousal, and self-image. LSD is a potent, perception-altering drug that is approximately 3,000–5,000 times more powerful than mescaline on a weight-for-weight basis. The minimal, clinically perceptible adult oral dose is approximately 25 μg with optimal psychedelic doses ranging from 100–500 μg, depending on tolerance. The usual street dose varies between 50–300 μg and averages about 100–150 μg. Single oral doses of 30 μg/kg produce an intense depersonalization and derealization experience lasting up to 24 hours.[10] Flashbacks and acute anxiety attacks can reoccur in experienced LSD users without the ingestion of additional doses of LSD. Fatal doses of LSD for humans are not well defined on a clinical basis. There is substantial interspecies variability in the susceptibility of animal to LSD with the LD_{50} ranging approximately 500 times between the Asiatic elephant (0.1 mg/kg or 300 mg intramuscular [IM])[20] and mice (46 mg/kg).

TOXICOKINETICS

Absorption

LSD easily crosses the mucosa of the gastrointestinal tract and nose with clinically significant concentrations of the drug appearing in the blood within 15–20 minutes.[21] Following the ingestion of 2 μg LSD/kg, peak plasma concentrations as measured by a spectrofluorometric method occurred in 30–75 minutes with heavy meals reducing and slightly delaying the peak plasma LSD concentration.[22] There are few data on the absorption of LSD by the lungs, but smoking is not a popular form of LSD abuse, probably as a result of the degradation of LSD during heating.

Distribution

After an IV dose of 2 ug LSD/kg to 5 volunteers, the estimated V_d of LSD correlated to extracellular water (i.e., ~0.25 L/kg) as measured by fluorometry.[23] About 90% of LSD in plasma is protein-bound.

Biotransformation

There are limited data on the metabolites of LSD in humans, but *in vitro* studies indicate that *N*-demethylation (*N*-demethyl-LSD), *N*-deethylation (*N*-desethyl-LSD), and hydroxylation (13-OH-LSD, 14-OH-LSD) are the common metabolic pathways in humans. *N*-demethyl-LSD is probably a minor metabolite (i.e., 1–2 %).[24] Potential metabolites of LSD include *N*-demethyl-LSD (*nor*-LSD), 13-OH-LSD, 14-OH-LSD, lysergic acid ethylamide (LAE), *iso*-LAE, 2-oxo-LSD, and 2-oxo-3-hydroxy-LSD.[25,26] Figure 22.3 demonstrates the structure of LSD and major metabolites. *iso*-LSD is a contaminant of illicit LSD synthesis rather than a part of the biotransformation of LSD. Studies in animals suggest that the major metabolic route is hydroxylation of the phenyl ring to form phenols, which are subsequently conjugated as glucuronides and excreted in the bile. De-ethylation and demethylation probably are minor metabolic pathways in rats.[27]

Elimination

The plasma elimination half-life of LSD in humans is about 2–3 hours with substantially shorter half-lives in some experimental animals (mice, monkeys, cats).[28] Intravenous LSD doses of 2 μg/kg to 5 volunteers produced plasma LSD concentrations ranging from about 4–6 ng/mL 1–2 hours after administration to <1 ng/mL 8 hours after administration as measured by fluorometry.[29] The kidney excretes small amounts (i.e., <1% of the absorbed dose) of unchanged LSD in the urine. The urinary elimination half-life of the *N*-demethyl-LSD metabolite is somewhat longer (i.e., 8–10 hours vs. 4–6 hours) compared with unchanged LSD.[24] Renal excretion of hydroxylated metabolites (13-OH-LSD, 14-OH-LSD) occurs after conjugation with glucuronic acid. Preliminary studies indicate that the metabolite, 2-oxo-3-hydroxy-LSD is not conjugated with glucuronide prior to renal excretion.[30] Animals studies suggest that biliary excretion accounts for most of the elimination of LSD from the body.

Tolerance

Tolerance to the behavioral effects of LSD occurs rapidly in both humans and animals.[31] Although a
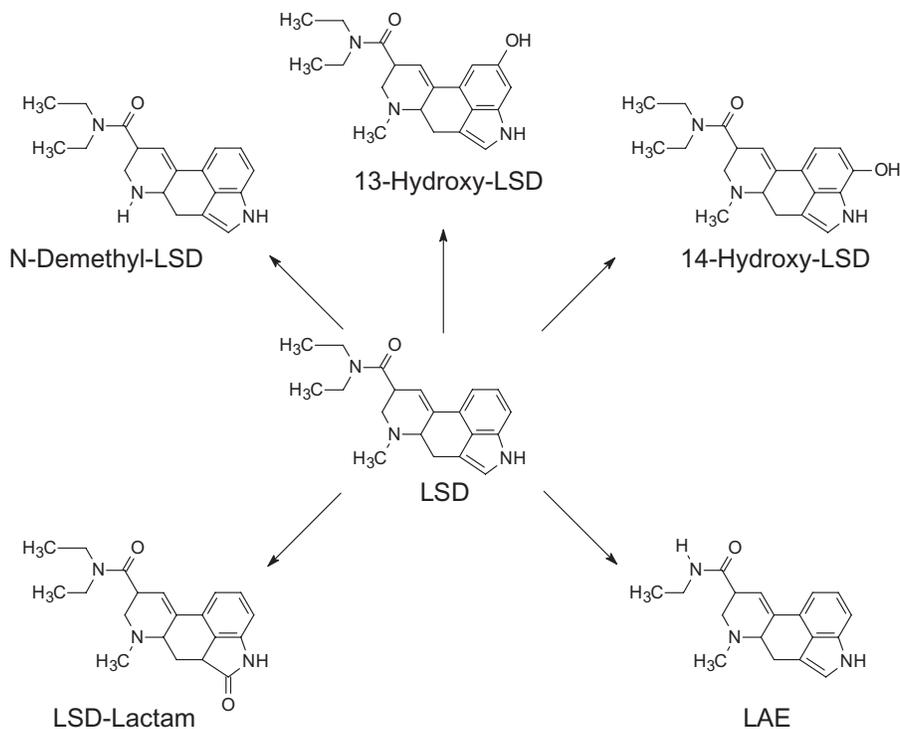
FIGURE 22.3. Lysergic acid diethylamide and major metabolites. LSD = lysergic acid diethylamide; LAE = lysergic acid ethylamide.[132]

300-μg LSD dose produces typical psychotomimetic reactions in adults without recent LSD use, subsequent daily 100-μg doses cause minimal reactions after the second dose.[9] Tolerance typically develops after a few days of daily use, but acute tolerance disappears within 3–4 days of the cessation of LSD use.[32] In animal models, cross-tolerance develops to LSD following the administration of psilocybin and mescaline,[33,34] but not with *d*-amphetamine.[35]

Drug Interactions

In a study of 32 experienced LSD users, structured interviews using a standardized questionnaire indicated a subjective decrease in the response of 88% (28 participants) to LSD following the administration of selective and nonselective serotonin reuptake inhibiting antidepressant drugs (fluoxetine, paroxetine, sertraline, trazodone) for at least 3 weeks.[36] In this study, an additional participant receiving fluoxetine for only 1 week had an increased response to LSD. In contrast to selective serotonin reuptake inhibitors, the chronic administration of tricyclic antidepressants and lithium was associated with an enhanced response of volunteers to LSD, whereas decreases in the subjective response to LSD was reported after the chronic use of monoamine oxidase inhibitors.[37]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Although the exact mechanism of action of LSD has not been identified, animal studies indicate that central serotonin receptors (5HT) mediate the acute discriminative stimulus effects of LSD, particularly the $5HT_{2A}$ receptor.[38,39] LSD is probably best described as a mixed $5HT_2/5HT_1$ receptor partial agonist.[40] This compound binds to $5HT_{1A}$ and to G-protein coupled $5-HT_2$ receptor subtypes in areas of the hippocampus, corpus striatum, cerebral cortex, and, to a much lesser extent, cerebellum.[41] The partial agonist action of LSD on these receptors results from LSD-stimulated phosphoinositide hydrolysis at these receptor sites, particularly at the postsynaptic $5-HT_{2A}$ receptor.[42,43] Although stimulation of the $5-HT_{2A}$ receptor is a necessary prerequisite for the hallucinogenic effects of LSD, the biochemical and signaling pathways responsible for these effects remain undetermined.[44] LSD also interacts as a mixed agonist/antagonist with central dopamine D_1 and D_2 receptors,[45] but the clinical significance of this interaction is unclear. The cause of hallucinogen persisting perception disorder (*flashbacks*) also has not been determined, but the use of benzodiazepines to treat this disorder suggests that the mechanism of toxicity involves receptors (e.g., inhibitory GABA) other than, or in addition to, serotonin receptors.

CLINICAL RESPONSE

Illicit Use

Following the ingestion of 1-2 μg LSD/kg, psychedelic effects typically begin within approximately 30–90 minutes with peak effects in 1.5–2.5 hours, although some sympathomimetic effects (e.g., tachycardia, pilo-erection, tremor, flushing) may occur slightly earlier (i.e., 10–15 minutes).[2] Pupillary dilation is a consistent, very prominent sign associated with LSD use that often measures 8 mm in diameter.[10] Pulse rate, body temperature, and respirations increase slightly during intoxication, but the blood pressure usually remains normal.[46] Peripheral sympathomimetic effects of LSD include uterine contraction, piloerection, and bronchoconstriction (i.e., high LSD doses). Parasympathomimetic effects (e.g., nausea, vomiting, salivation, lacrimation) occur occasionally.

The administration of LSD causes changes in arousal, cognition, and self-image as well as alterations of sensory cues.[10] There is considerable variability in the interindividual and intraindividual response to LSD depending on the set. The main psychologic experience during LSD intoxication involves alterations of perception (sound, color intensity, crossover of sensory modalities) and cognition (decreased attention, unusual associations, less abstract thought), euphoria, subjective slowing of time, distortion of body image, and loss of physical boundaries (i.e., depersonalization). Afterimages become prolonged, with flat surfaces assuming depth and fixed objects undulating. Although visual illusions are common at therapeutic doses, the individual usually retains the ability to discriminate between these altered perceptions and reality. Hyperacusis and amplification of background noise occur occasionally; however, the presence of true auditory hallucinations suggests an underlying psychosis. The blending of sensory inputs (i.e., synesthesias or seeing sounds/hearing colors) is relatively rare.

Alteration of thought processing during LSD intoxication includes the loosening of associations, alteration of body image, feeling of rapid aging, concrete thinking, and depersonalization. The individual usually remains oriented, but the development of paranoia or ideas of persecution can cloud judgment. Weakness and insomnia may develop after resolution of the acute effects of LSD. Psychomimetic effects begin declining about 4 hours after ingestion depending on dose; these symptoms usually disappear within 6–12 hours.

Adverse physiologic reactions following LSD use include nausea, headache, diaphoresis, general weakness, impaired concentration, and exhaustion. Higher incidences of adverse reactions occur in individuals unaware of the administration of LSD;[47] however, adverse reactions may occur despite the frequent use of LSD without complications.[48] The differential diagnosis of a patient with perceptual illusions and mydriatic pupils includes the administration of atropine, scopolamine (Jimson weed), psilocybin mushrooms, phencyclidine (PCP), cocaine, and amphetamine-related psychedelic drugs (MDMA, MDA, DOM). The sympathomimetic effects are generally much more prominent after the use of cocaine or amphetamine than with LSD. In contrast to PCP intoxications, nystagmus is usually absent with pupils typically being dilated during LSD intoxication. The prominent signs of anticholinergic poisoning (atropine, scopolamine) include dry, flushed skin, dry mucous membranes, urinary retention, and reduced bowel sounds (i.e., gastrointestinal hypomotility). These clinical features are typically absent during LSD intoxication.

BEHAVIORAL ABNORMALITIES

Acute anxiety and acute panic attacks are common adverse reactions to LSD intoxication, particularly after the accidental ingestion of LSD by children.[49] The ingestion of 50–150 μg LSD by a 2-year-old child produced tachycardia, tachypnea, diaphoresis, visual illusions, and acute anxiety that resolved within 1 day without residual effects.[50] Similar symptoms (anxiety, hyperreflexia, perceptual distortion) occurred in a middle-aged woman following the accidental ingestion of LSD.[51] A 2-mg LSD ingestion in a 2-year-old child caused mydriasis and ataxia followed by areflexic catatonia, mild hypertension, tachycardia, and tachypnea.[52] Although myalgias and muscle tremor developed later, all symptoms resolved within 48 hours.

Behavioral complications of LSD use typically involve accidental trauma as a result of aggressive behavior during panic attacks, suicide attempts during depressive states,[53] reckless behavior during recurrent flashbacks, and self-inflicted trauma (e.g., solar maculopathy,[54] self-enucleation[55]). Although homicides occur during and after LSD intoxication,[56,57] multiple-drug use, bias, and premorbid personality confound the causal link between LSD use and murder, particularly when goal-directed planning and delayed gratification occur during LSD-induced psychosis.[58] Amnesia does not usually occur during LSD intoxication. Predicting behavior is difficult because of the number of variables (e.g., setting, expectation, underlying personality, emotional stress) that affect behavior in addition to the dose of LSD. Intoxicated LSD users are usually quiet, passive, and withdrawn, but hostility may develop. Sociability

and sexual drives frequently are diminished. Moods are labile, ranging from euphoria to depression.

MENTAL DISORDERS

Psychiatric complications associated with LSD use include psychosis, persistent or recurrent major affective disorders (i.e., depression), deterioration of underlying psychiatric illness, acute panic or paranoid reactions, disruption of normal function (i.e., burnout), and posthallucinogen perceptual disorder (i.e., flashbacks). Depressive and schizophrenic patients may decompensate, and susceptible schizophrenics may develop prolonged hallucinations.[59] Severe depression and intense loneliness may occur within the 24-hour period after the psychomimetic effects of LSD resolve.[14]

ACUTE PSYCHOSIS.   A drug-induced schizophreniform reaction may develop in patients vulnerable to both substance abuse (e.g., LSD) and psychosis.[60] In a study of 52 patients with LSD-associated psychosis and 29 matched first-break schizophrenics, the LSD patients were fundamentally similar to the schizophrenics in genealogy, phenomenology, and clinical features except the prominence of alcohol abuse in their parents.[61] Whether LSD hastens the onset of chronic undifferentiated schizophrenia remains unclear. In a matched, controlled study of 37 chronic schizophrenics with and without LSD use prior to the onset of schizophrenia, retrospective chart review did not detect a difference in the 2 groups in regard to the age of onset of symptoms or the age of first admission to a psychiatric hospital.[62]

FLASHBACKS AND HALLUCINOGEN PERSISTING PERCEPTION DISORDER (HPPD).   Flashbacks are pseudo-hallucinations that typically involve false perception of movement (i.e., stationary objects in motion), color flashes, intensification of colors, halos around objects, comet-like trailing images, moving and shimmering objects, and reappearance of kaleidoscopic, brightly-colored geometric patterns. These visual alterations, time distortions, and changes in body image recur in experienced LSD users during periods of abstinence. Flashbacks are usually benign, spontaneous, recurrent, transient occurrences that do not cause emotional distress. The impaired color perception may persist in chronic LSD users for 2 years after cessation of LSD use.[63] Both physiologic (e.g., fatigue, driving, sepsis, electrolyte disorders, heavy ethanol consumption) and emotional stress can precipitate flashbacks.[64,65]

More frightening flashbacks involve the sudden appearance of hideous apparitions (e.g., Satan) or the distortion of people's body images. In contrast to relatively benign flashbacks, hallucinogen persisting perception disorder (HPPD) is an uncommon, persistent, distressing, spontaneous, pervasive condition that causes an unpleasant dysphoria months rather than days after the cessation of LSD use.[66] The diagnosis of this disorder requires the onset of this perceptual disorder after LSD use and the exclusion of other similar disorders (visual epilepsy, migraine, dementia, schizophrenia, delirium, hypnopompic hallucinations).[67] This phenomenon involves the experience of one or more perceptual symptoms from prior LSD experiences that causes significant distress and/or impairment of social functioning. HPPD often occurs in patients with no prior history of psychiatric disease. These patients are usually aware of these debilitating illusions, and they frequently seek psychiatric care. The clinical course of HPPD is highly variable ranging from subtle perceptual disturbances to severe, debilitating images with associated anxiety and depression. These clinical features may resolve spontaneously or progress to a chronic relapsing syndrome.[68]

MEDICAL COMPLICATIONS

Several case reports suggest that neurologic complications (e.g., seizures,[69] headache with small-artery occlusive changes, [70] occlusion of internal carotid artery[71]) may develop after recreational doses of LSD. However, a causal link between these complications and the use of LSD remains unproven because of the confounding of multiple-drug abuse and biases associated with case reports.

Overdose

Several case reports indicate that hyperthermia may complicate large ingestions of LSD along with rhabdomyolysis, renal failure, and hypotension.[21,72] All LSD overdose patients in these case reports recovered without medical or psychiatric sequelae after supportive care that included ventilatory support. Neuroleptic malignant syndrome (NMS)-like clinical features developed in a 21-year-old man admitted to the hospital with coma, bilateral extrapyramidal rigidity, and rhabdomyolysis.[73] The serum LSD concentration (69.9 ng/mL) drawn 24 hours after admission confirmed the ingestion of massive amounts of LSD. Deaths associated with medical consequences of LSD intoxication are rare, and these case reports are not well-documented.[74] A 25-year-old man died within 24 hours after ingesting LSD secondary to hyperpyrexia (41.6°C/106.9°F), hypotension, renal failure, rhabdomyolysis, and liver necrosis.[75] Analysis of antemortem blood confirmed a high LSD concentration (14.4 ng/mL). The presence of respiratory depression during massive LSD overdose suggests that death may occur following the use of very large LSD doses.[21]

Abstinence Syndrome

The chronic use of LSD is not associated with symptoms or signs of withdrawal; the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV-TR*) does not list a LSD-dependence syndrome.[66] There are few data on the neuropsychologic effects of chronic LSD use, in part because of the frequent use of other drugs of abuse and the difficulty controlling various confounding variables including premorbid cognitive function, personality, and previous drug use.[76] Existing data suggest that any changes are subtle and there are few, if any, long-term neuropsychologic deficits attributable to chronic LSD use.[32] Affective abnormalities following LSD use include exhaustion, profound depression, loss of motivation, and emotional lability.

Reproductive Abnormalities

There is no clear evidence that LSD is teratogenic.[77] Case reports associate the illicit maternal use of LSD during pregnancy with rare congenital abnormalities including anophthalmia (absence of ocular tissue),[78] true limb aplasia (absence of entire limb bud),[79] and multiple cerebral and cerebellar malformations.[80] Uncontrolled studies of women using LSD during pregnancy suggest elevated incidences of spontaneous abortions[81] and major congenital abnormalities[82] compared with national averages. However, the potential presence of a number of confounders (e.g., ingestion of other illicit drugs, infectious diseases, poor maternal nutrition) preclude a definitive correlation between LSD and the occurrence of human birth defects.

At normal recreational doses, there are no unequivocal data to indicate that LSD is a mutagen.[83] *In vitro* human and experimental animal studies are inconsistent, and the data from the 1960s and 1970s indicate that LSD is, at most, a weak mutagen.[84,85] Chromosomal studies in LSD users are also inconsistent. Elevated chromosomal abnormalities occurred in the leukocytes of LSD users, but these changes were not dose-related.[86] Studies of lymphocytes from 50 hospitalized psychiatric patients administered LSD did not demonstrate a statistically significant elevation of chromosomal abnormalities when compared with matched controls.[87,88]

DIAGNOSTIC TESTING

Analytic Methods

SCREENING

Identification of LSD in biologic fluids is a difficult task. The rapid and extensive metabolism of LSD produces very low urine concentrations (i.e., <1 ng/mL) of unchanged parent LSD, limiting the detection of this compound by various screening methods. Fluorometric and thin-layer chromatographic methods of LSD detection are relatively insensitive. Radioimmunoassay is a sensitive method of detection (i.e., limit of detection [LOD], 0.020 ng/mL), but this procedure is time consuming and uses radioactive material. Commercial immunoassays for LSD are rapid, relatively low cost analytic procedures for the detection of LSD, but these assays do not provide the specificity and quantitative capabilities of gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry. Simple, non-isotope immunoassays have been developed on automated analyzers to screen urine samples for LSD including microplate enzyme immunoassay (EIA),[89] EMIT, microparticle-based immunoassay (OnLine®),[90] and cloned enzyme donor immunoassay (CEDIA).[91] These immunoassays cross-react with LSD metabolites depending on the individual assay;[92,93] confirmation techniques are necessary to separate LSD and LSD metabolites.[94,95] The cross-reactivity of EIA with *nor*-LSD was 16–28% depending on the *nor*-LSD concentration.[88] Cross-reactions with ergot alkaloids (e.g., ergonovine, ergotamine, dihydroergocristine, dihydroergotamine) are relatively minor, but some commonly used medications may cause false-positive results with LSD immunoassays as a result of cross-reactivity. Potential medications that may produce false-positive results with LSD immunoassays include amitriptyline, bupropion,[96] fentanyl, haloperidol, metoclopramide, sertraline, and ambroxol.[97,98] Common adulterants (e.g., ascorbic acid, bleach, salt, apple juice, vinegar, liquid soap) do not usually produce false-negative results on immunoassays, but false positive results for LSD may occur.[99] The current US Department of Defense cutoff for positive confirmation of LSD in positive screening assays in urine is 0.020 ng LSD/mL, whereas the cutoff for screening LSD in urine is 0.050 ng/mL. LSD is not often included in urine drug abuse screens.

CONFIRMATORY

Low volatility, instability at high temperatures, and the high adsorptive capacity of LSD for chromatographic columns complicate the analysis of LSD by chromatographic methods. Derivatization of LSD is necessary to prevent sample loss during chromatography as a result of irreversible adhesion of LSD to the column.[100] Analytic methods for the confirmation of LSD in biologic fluids include radioimmunoassay, high performance liquid chromatography coupled with fluorometric detection,[101] high performance liquid chromatography/mass spectrometry, and gas chromatography/mass

spectrometry (GC/MS).[102] These techniques are targeted toward LSD rather than LSD metabolites; therefore, immunoassays, which cross-react with LSD metabolites, may detect LSD use longer than these confirmatory procedures as a result of the inclusion of parent LSD as well as cross-reacting metabolites.[90] Methods that do not require derivatization include liquid chromatography/electrospray ionization/tandem mass spectrometry and immunoaffinity chromatography/high performance liquid chromatography/mass spectrometry.[103] The lower limit of quantitation (LLOQ) for LSD and iso-LSD in whole blood samples was 0.01 ng/mL, as measured by liquid chromatography/tandem mass spectrometry.[104] This method also quantitates the metabolite, 2-oxo-3-hydroxy-LSD in urine samples. Atmospheric pressure matrix-assisted laser desorption/ion trap/mass spectrometry is a relative easy and rapid method that is a good quantitative measure of LSD when interference from high-background ions is eliminated.[105] These analytic procedures are highly sensitive. The LLOQ and LOD for LSD using gas chromatography/tandem mass spectrometry and an internal ion trap detector were 0.080 ng/mL and 0.020 ng/mL, respectively.[106] Established cutoff limits for RIA procedures are usually near 0.50 ng/mL, although actual detection limits may be slightly lower (0.25 ng/mL). Analysis of urine samples for 2-oxo-3-hydroxy-LSD increases the detection window for LSD because this metabolite is usually present in substantially higher urinary concentrations compared with LSD, iso-LSD, and nor-LSD.[107] In this study, the mean ratio of 2-oxo-3-hydroxy-LSD/LSD was approximately 15. LSD possesses a native fluorescent chromophore (fluorophore) and HPLC with fluorescence detection is a sensitive method of detecting LSD. However, this method is not specific because of interference from other fluorescent compounds absorbing at similar wavelengths. The use of ultra performance liquid chromatography/tandem mass spectrometry allows the detection of LSD and LSD metabolites (N-demethyl-LSD, iso-LSD, 2-oxo-3-hydroxy-LSD) in blood with an LOD of 0.005–0.010 ng/mL.[108] The LLOQ in blood or urine for LSD and iso-LSD is 0.020 ng/mL and 0.050 ng/mL, respectively, similar to other LSD metabolites.

STORAGE

LSD is unstable under prolonged exposure to heat, alkaline conditions, and ultraviolet light irradiation. Proper storage of LSD typically involves the use of amber glass or nontransparent polyethylene containers. Frozen samples of LSD are stable for at least 3 months.[109,110] However, LSD is photosensitive, and rapid deterioration of LSD occurs in sunlight. The amount of deterioration under lighted conditions depends on the wavelength, light intensity, and exposure time.[111] The loss of LSD from spiked samples stored at 4°C and -20°C under dark conditions was minimal over 2 weeks, whereas the loss of LSD from samples stored at room temperature under normal fluorescent lights in clear vials was approximately 50% over 2 weeks.[112] Under experimental conditions, loss of LSD from spiked samples stored in polyethylene bottles at room temperature in light and dark conditions was limited to 10%.[109] The LSD metabolite, 2-oxo-3-hydroxy lysergic acid diethylamide is relatively stable up to 2 months under refrigerated and frozen conditions, but some deterioration occurs if the sample is stored at or above room temperature.[113]

Biomarkers

BLOOD

EXPERIMENTAL/ILLICIT USE.   Typically, the LSD concentration in blood following the ingestion of recreational LSD doses is in the low ng/mL range.[114] Peak plasma LSD concentrations following the IV administration of 2 μg/kg are approximately 6–7 ng/mL within about 30 minutes; the LSD concentration decreases to nondetectable concentrations in about 8 hours.[29] In 5 patients presenting to an urban emergency department with bizarre, agitated behavior after ingesting LSD, the serum LSD concentrations 2–10 hours after ingestion ranged from 1.3–1.8 ng/mL as measured by high performance liquid chromatography with fluorescent detection.[115] This method is not specific for LSD because of the interference of other compounds with fluorescent properties. The LSD concentration did not correlate to adverse effects (agitation, flashbacks).

OVERDOSE.   Plasma LSD concentrations ranged from 4–6 ng/mL 1–2 hours after the IV administration of 2 μg/kg, [23] and up to 8.8 ng/mL 2 hours after the ingestion of 160 μg of LSD as measured by a less-specific fluorometric method.[116] A massive LSD overdose occurred following the insufflation of LSD by 8 patients who presented to an emergency department 15 minutes later.[21] On admission to the emergency department, 2 patients were comatose, vomiting, and displaying prominent sympathomimetic signs. Analysis of blood samples demonstrated admission LSD plasma concentrations of 6.6 ng/mL and 11.6 ng/mL. Another patient had vomiting, catatonia, and hyperreflexia with fixed dilated pupils; the admission plasma LSD concentration of the latter patient was 16.0 ng/mL.

POSTMORTEM.   In blood samples from a 25-year-old man, who died 16 hours after hospital admission, the

postmortem LSD concentrations was 4.8 ng/mL as measured by radioimmunoassay.[117] Details of the clinical course were not reported; however, the coroner concluded that the man died from LSD intoxication. The antemortem plasma was 14.8 ng/mL. No other drugs were detected as analyzed by radioimmunoassay, high performance liquid chromatography, and GC/MS.

URINE

Following ingestion, the kidneys excrete small amounts of LSD rapidly in urine; therefore, the concentration of LSD usually decreases below the confirmation cutoff (0.200 ng/mL) within 12–24 hours following the ingestion of a recreational dose (50–100 µg) of the drug.[118] Lowering the detection limit to 0.010 ng/mL with ion trap GC/MS can extend the detection time to approximately 72 hours after ingestion of a 50 µg recreational dose of LSD.[119] Some metabolites of LSD appear in higher concentrations than LSD, and analysis directed toward the detection of these urinary metabolites may extend the detection time to 96 hours. Following the ingestion of 4 µg LSD/kg by volunteers, 2-oxo-3-hydroxy-LSD remained >0.200 ng/mL for 48 hours and above the LOD for 96 hours as measured by gas chromatography/tandem mass spectrometry with solid-phase extraction. Current immunoassays for LSD do not significantly cross-react with 2-oxo-3-hydroxy-LSD.[30] In a series of 49 urine specimens from workplace drug testing programs that were positive for LSD by screening immunoassay, the mean ratio of 2-oxo-3-hydroxy-LSD/LSD was approximately 24 with a range of 0.03–136.[120] Analysis of urine samples by radioimmunoassay demonstrated LSD concentrations ranging from 1.5–55 ng/mL within 24 hours after the ingestion of LSD doses of 200–400 µg.[121]

Abnormalities

Experimental doses of LSD produce laboratory evidence of a nonspecific stress reaction (i.e., leukocytosis, eosinophilia).[10] Rhabdomyolysis may occur, particularly in severely agitated, restrained patients[73] or in unrestrained patients.[122] After LSD administration, the amplitude of the electroencephalogram (EEG) tracings decreases with alpha rhythms disappearing and low-voltage fast or desynchronized discharges predominating, particularly the slower frequencies. The rhythmic after-discharges associated with photo-evoked potentials are suppressed, but the clinical significance of these changes is unclear.[123] There are inadequate clinical data on the chronic effects of LSD use on neuropsychologic testing, in part because of the difficulty controlling for the effects of multiple drug use and the absence of data on premorbid cognitive and personality function. A review of the limited EEG and neuropsychologic testing data on chronic LSD users in the 1960s and 1970s demonstrated few, if any, long-term neuropsychologic deficits attributable to LSD use.[75,124] The cognitive effects of LSD complicate the interpretation of neuropsychologic testing of individuals given LSD acutely.

Driving

There are few data on the effects of LSD on driving skills. Case series of polydrug abusers suggest that prolonged afterimages, visual illusions, and alteration of cognition and judgment may occur while driving under the influence of psychedelic drugs including LSD.[125] However, the prevalence of these effects and the exact role of LSD remain unclear.

TREATMENT

Stabilization

Life-threatening medical reactions to LSD intoxication are extremely rare. All patients should be evaluated for organic disease with vital signs, physical examination, and a thorough history that includes current symptoms, premorbid function, and prior medical and psychiatric conditions. Vital signs should include an accurate rectal temperature that is repeated if the patient deteriorates. The sympathomimetic effects of LSD are usually mild and transient, except in patients with underlying cardiovascular disease or very large overdose. Laboratory investigations in patients with severe LSD intoxication to exclude organic disease include complete blood count, serum electrolytes, hepatic aminotransferases, creatinine and creatine kinase, urinalysis, and urine for drugs of abuse as well as computed tomography of the brain if neurologic disease is suspected. Seizures may occur in patients using LSD, but the causal link between LSD use and seizures is not well delineated. Therefore, these patients should be evaluated for other causes of seizures; benzodiazepines are first-line drugs for the treatment of agitation and persistent seizures.

Gut Decontamination

The usual methods of decontamination (e.g., activated charcoal) are almost always unnecessary because of the rapid absorption of LSD and the relatively small amounts of the ingested drug. Additionally, the use of decontamination measures complicate the treatment of agitated patients.

Elimination Enhancement

As the volume of distribution is large, there are no clinical data to support the use of methods to enhance the elimination of LSD.

Antidotes

There is no specific antidote for LSD intoxication.

Supplemental Care

Occasionally, peripheral vascular ischemia and lower extremity gangrene is associated with LSD use that does not respond to conventional treatment including IV heparin, nicardipine, methylprednisolone, and topical nitroglycerin. A case report documented restoration of normal luminal caliber and prompt reperfusion of LSD-induced extensive lower extremity vasospasm in a previously healthy 19-year-old woman.[126] Acute renal failure secondary to LSD-induced rhabdomyolysis is one of the few indications for hemodialysis after LSD intoxication.

ACUTE PANIC ATTACK

Reassurance that the frightening images are temporary and illusory along with reduction of sensory stimulation are the most important aspects of the management of LSD-related panic attacks.[47] The patient should be placed in a safe, quiet environment, preferably with a familiar person who can provide constant reassurance (i.e., "talkdown"). This person should reorient the patient by interpreting the anxious feelings while attempting to restore a sense of control and well-being to the patient overwhelmed with frightening visions and emotions. Continuous interpretations of sensory misperceptions and pseudo-hallucination are necessary to reduce the anxiety associated with a "bad trip".[11] This "talkdown" period typically lasts 8–12 hours, and during that time the patient may move in the room as long as the patient does not display destructive behavior. Restraints should be avoided if possible, but impulsive and potentially dangerous actions should be controlled as necessary to prevent destructive behavior. Diazepam (5 mg IV every 1–2 hours, adult) or lorazepam (1–2 mg IV every 1–2 hours) are the drugs of choice for sedation, particularly for restrained patients. Haloperidol (2–5 mg IM, adult) may be administered as a second-line drug if the above measures fail to calm the patient and continued agitation represents an substantial medical risk unless the patient has symptoms consistent with neuro-leptic malignant syndrome. There are few published data on the use of droperidol, midazolam, ziprasidone, or newer generation antipsychotic drugs (aripiprazole, olanzapine, quetiapine, risperidone) for the treatment of LSD-induced agitation. Patients sedated for agitation should be observed closely for respiratory depression.

All patients requiring the continued use of restraints should have the first voided urine specimen sent for myoglobin and urinalysis. A positive dipstick test for hematuria in the absence of microscopic evidence of red blood cells suggests myoglobinuria and the need for generous fluid replacement along with sedation as required by the mental status of the patient. If the patient does not respond to reassurance and regain control over the frightening thoughts, 24-hour hospitalization may be necessary. Follow-up to a mental health facility is important to assess the need for further therapy.

ACUTE PSYCHOTIC REACTIONS

Most visual hallucinations are actually illusions rather than true hallucinations, and these patients understand that what they are seeing is drug-induced. Haloperidol has been administered successfully in the setting of LSD-induced psychotic reactions,[127] but there are no clinical data to compare haloperidol with other antipsychotic drugs including the newer antipsychotic drugs.

HALLUCINOGEN PERSISTING PERCEPTION DISORDER (HPPD)

The treatment of benign flashbacks is similar to the treatment of acute panic attacks. Reassurance that the flashbacks diminish with time frequently reduces the anxiety associated the occurrence of flashbacks. Avoidance of stress, antihistamines, fatigue, ethanol, and other drugs of abuse helps reduce the recurrence of flashbacks.[128] The treatment of HPPD often requires drug therapy depending on the severity of the anxiety and social disruption caused by pervasive perceptual disturbances. Treatment modalities include psychotherapy, identification of triggers, benzodiazepines, and other drugs (clonidine, perphenazine, clonazepam).[129] Case series of HPPD patients suggests that high potency benzodiazepines (e.g., clonazepam 2 mg/day for 2 months) are more effective than low potency benzodiazepines (e.g., alprazolam) for the treatment of HPPD.[130] In this case series, the improvement continued after tapering the patients off clonazepam over 1 month. Older medical literature suggests that haloperidol is an option for the treatment of HPPD.[131]

References

1. Stoll A, Hoffman A. [Ergot alkaloids III. Partial synthesis of ergobasine, a natural ergot alkaloid and its optical antipodes.] Z Physiol Chem 1938;251:155–163. [German]

2. Shulgin AT. LSD. J Psychedelic Drugs 1980;12:173–174.

3. Fusar-Poli P, Borgwardt S. Albert Hofmann, the father of LSD (1906-2008). Neuropsychobiology 2008;58:53–54.

4. Stoll WA. [Lysergic acid diethylamide, a hallucinogen from the ergot group.] Schweiz Arch Neurol Psychiatr 1947;60:279–323. [German]

5. Lee MR. The history of ergot of rye (*Claviceps purpurea*) III: 1940–80. J R Coll Physicians Edinb 2010;40:77–80.

6. Gaddum JH, Hameed KA. Drugs which antagonize 5-ydroxytryptamine. Br J Pharmacol 1954;9:240–248.

7. Green AR. Gaddum and LSD: the birth and growth of experimental and clinical neuropharmacology research on 5-HT in the UK. Br J Pharmacol 2008;154: 1583–1599.

8. Schultes RE. The botanical and chemical distribution of hallucinogens. J Psychedelic Drugs 1977;9:247–261.

9. Hoffer A. *d*-Lysergic acid diethylamide (LSD): A review of its present status. Clin Pharmacol Ther 1965;6: 183–255.

10. Cohen S. Psychotomimetic agents. Annu Rev Pharmacol 1967; 7:301–318.

11. Schwartz RH. LSD Its rise, fall, and renewed popularity among high school students. Pediatr Clin N Am 1995; 42:403–413.

12. Chilcoat HD, Schutz CG. Age-specific patterns of hallucinogen use in the US population: an analysis using generalized additive models. Drug Alcohol Depend 1996;43:143–153.

13. Johnson LD, O'Malley PM, Bachman JG. National survey results on drug use from the monitoring of the Future Study, 1975-1993, Volume 1, Secondary Students. Rockville, MD, US Department of Health and Human Services, National Institute on Drug Abuse, 1994.

14. Schwartz RH, Comerci GD, Meeks JE. LSD: patterns of use by chemically dependent adolescents. J Pediatr 1987; 111:936–938.

15. Chao J-M, der Marderosian AH. Ergoline alkaloidal constituents of Hawaiian baby wood rose, *Argyreia nervosa* (Burn. F.) Bojer. J Pharmaceut Sci 1973;62:588–591.

16. Schultes RE. Indole alkaloids in plant hallucinogens. Planta Med 1976;29:330–342.

17. Renfroe CL, Messinger TA. Street drug analysis: an eleven year perspective on illicit drug alteration. Semin Adolesc Med 1985;1:247–257.

18. Clarkson ED, Lesser D, Paul BD. Effective GC-MS procedure for detecting *iso*-LSD in urine after base-catalyzed conversion to LSD. Clin Chem 1998;44:287–292.

19. Favretto D, Frison G, Maietti S, Ferrara SD. LC-ESI-MS/MS on an ion trap for the determination of LSD, *iso*-LSD, *nor*-LSD and 2-oxo-3-hydroxy-LSD in blood, urine and vitreous humor. Int J Legal Med 2007;121:259–265.

20. West LJ, Pierce CM, Thomas WD. Lysergic acid diethylamide: Its effects on a male Asiatic elephant. Science 1962;133: 1100–1103.

21. Klock JC, Boemer U, Becker CE. Coma, hyperthermia and bleeding associated with massive LSD overdose: a report of eight cases. West J Med 1974;120:183–188.

22. Upshall DG, Wailling DG. The determination of LSD in human plasma following oral administration. Clin Chim Acta 1972; 36:67–73.

23. Wagner JG, Aghajanian GK, Bing OH. Correlation of performance test scores with "tissue concentrations" of lysergic acid diethylamide in human subjects. Clin Pharmacol Ther 1968; 9:635–638.

24. Lim HK, Andrenyak D, Francom P, Foltz RL. Quantification of LSD and *N*-demethyl-LSD in urine by gas chromatography/resonance electron capture ionization spectrometry. Anal Chem 1988;60:1420–1425.

25. Klette KL, Anderson CJ, Poch GK, Nimrod AC, ElSohly MA. Metabolism of lysergic acid diethylamide (LSD) to 2-oxo-3-hydroxy LSD (O-H-LSD) in human liver microsomes and cryopreserved human hepatocytes. J Anal Toxicol 2000;24:550–556.

26. Cai J, Henion J. Elucidation of LSD *in vitro* metabolism by liquid chromatography and capillary electrophoresis coupled with tandem mass spectrometry. J Anal Toxicol 1996;20:27–37.

27. Siddik ZH, Barnes RD, Dring LG, Smith RL, Williams RT. The metabolism of lysergic acid di[14C]ethylamide ([14C]LSD) in the isolated perfused rat liver. Biochem Pharmacol 1979;28:3081–3091.

28. Axelrod J, Brody RO, Withop B, Evarts EV. The distribution and metabolism of lysergic acid diethylamide. Ann NY Acad Sci 1957;66:435–444.

29. Aghajanian GK, Bing OH. Persistence of lysergic acid diethylamide in plasma of human subjects. Clin Pharmacol Ther 1964;5:611–614.

30. Verstraete AG, Van de Velde EJ. 2-oxo-3-hydroxy-LSD: an important LSD metabolite? Acta Clin Belg 1999; 1(suppl):94–96.

31. Trulson ME. Separation of tolerance to the behavioral effects of LSD from changes in serotonin receptor binding in cats. Eur J Pharmacol 1985;111:385–388.

32. Strassman RJ. Adverse reactions to psychedelic drugs. A review of the literature. J Nerv Ment Dis 1984;172: 577–595.

33. Isbell H, Wolbach AB, Wikler A, Miner EJ. Cross tolerance between LSD and Psilocybin. Psychopharmacology 1961;2:147–159.

34. Wolbech AB, Isbell H, Miner EJ. Cross tolerance between mescaline and LSD. Psychopharmacology 1962;3:1–14.

35. Appel JB, Freedman DX. Tolerance and cross tolerance among psychotomimetic drugs. Psychopharmacologia 1968;13:267–-274.

36. Bonson KR, Buckholtz JW, Murphy DL. Chronic administration of serotonergic antidepressants attenuates the subjective effects of LSD in humans. Neuropsychopharmacology 1996;14:425–436.

37. Bonson KR, Murphy DL. Alterations in responses to LSD in humans associated with chronic administration of tricyclic antidepressants, monoamine oxidase inhibitors or lithium. Behav Brain Res 1996;73:229–233.

38. Halberstadt AL, Geyer MA. Multiple receptors contribute toe the behavioral effects of indoleamine hallucinogens. Neuropharmacology 2011;61:364–381.

39. Appel JB, West WB, Buggy J. LSD, 5-HT (serotonin), and the evolution of a behavioral assay. Neurosci Biobehav Rev 2004;27:693–701.

40. Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A. The pharmacology of lysergic acid diethylamide: a review. CNS Neurosci Ther 2008;14:295–314.

41. Heym J, Jacobs BL. Serotonergic mechanisms of hallucinogenic drug effects. Monogr Neural Sci 1987;13:55–81.

42. Sanders-Bush E, Burris KD, Knoth K. Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. J Pharmacol Exp Ther 1988; 246:924–928.

43. Kuhn DM, White FJ, Appel JB. The discriminative stimulus properties of LSD: Mechanisms of action. Neuropharmacology 1978;17:257–263.

44. Fantegrossi WE, Murnane AC, Reissig CJ. The behavioral pharmacology of hallucinogens. Biochem Pharmacol 2008;75:17–33.

45. Watts VJ, Lawler CP, Fox DR, Neve KA, Nichols DE, Mailman RB. LSD and structural analogs: pharmacological evaluation at D1 dopamine receptors. Psychopharmacology (Berl) 1995;118:401–409.

46. Rinkel M, Hyde RW, Solomon HC, Hoagland H. Experimental psychiatry. II. Clinical and physio-chemical observations in experimental psychosis. Am J Psychiatry 1955;111:881–895.

47. Abraham HD, Aldridge AM, Gogia P. The psychopharmacology of hallucinogens. Neuropsychopharmacology 1996;14:285–298.

48. Blaho K, Merigian K, Winbery S, Geraci SA, Smartt C. Clinical pharmacology of lysergic acid diethylamide: case reports and review of the treatment of intoxication. Am J Ther 1997;4:211–221.

49. Milman DH. An untoward reaction to accidental ingestion of LSD in a 5 year old girl. JAMA 1967;201: 821–825.

50. Ianzito BM, Liskow B, Stewart MA. Reaction to LSD in a two year old child. J Pediatr 1972;80:643–647.

51. Decker WJ, Brandes WB. LSD misadventure in middle age. J Forensic Sci 1978;23:3–4.

52. Samuelsson BO. LSD intoxication in a two year old child. Acta Paediatr Scand 1974;63:797–798.

53. Cohen S. A classification of LSD complications. Psychosomatics 1966;7:182–186.

54. Fuller DG. Severe solar maculopathy associated with the use of lysergic acid diethylamide (LSD). Am J Ophthalmol 1976;81:413–416.

55. Rosen DH, Hoffman AM. Focal suicide: self-enucleation by two young psychotic individuals. Am J Psychiatry 1972;128:1009–1012.

56. Klepfiaz A, Racy J. Homicide and LSD. JAMA 1973;223: 429–430.

57. Knudsen K. Homicide after treatment with LSD. Acta Psychiatr Scand 1964;40(suppl 180):S389–S395.

58. Ungerleider JT. LSD and the courts. Am J Psychiatry 1970; 126:1179.

59. Frosch WA, Robbins ES, Stern M. Untoward reactions to lysergic acid diethylamide (LSD) resulting in hospitalization. N Engl J Med 1965;273:1235–1239.

60. Abraham HD, Aldridge AM. Adverse consequences of lysergic acid diethylamide. Addiction 1993;88: 1327–1334.

61. Vardy MM, Kay SR. LSD psychosis or LSD-induced schizophrenia? A multimethod inquiry. Arch Gen Psychiatry 1983;40:877–883.

62. Roy A. LSD and onset of schizophrenia. Can J Psychiatry 1981;26:64–65.

63. Abraham HD. A chronic impairment of colour vision in users of LSD. Br J Psychiatry 1982;140:518–520.

64. Abraham HD. Visual phenomenology of the LSD flashback. Arch Gen Psychiatry 1983;40:884–889.

65. Gaillard M-C, Borruat F-X. Persisting visual hallucinations and illusions in previously drug-addicted patients. Klin Monatsbl Augenheilkd 2003;220:176–178.

66. Halpern JH, Pope HG Jr. Hallucinogen persisting perception disorder: what do we know after 50 years? Drug Alcohol Depend 2003;69:109–119.

67. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed., text rev. Washington, DC: American Psychiatric Association; 2000.

68. Lerner AG, Skladman I, Kodesh A, Sigal M, Shufman E. LSD-induced hallucinogen persisting perception disorder treated with clonazepam: two cases reports. Isr J Psychiatry Relat Sci 2001;39:133–136.

69. Sobel J, Esponas OA, Friedman SA. Carotid artery obstruction following LSD capsule ingestion. Arch Intern Med 1971; 127:290–291.

70. Rumbaugh CL, Bergeron RT, Fang HC, McCormick R. Cerebral angiographic changes in drug abuse patients. Radiology 1971; 101:335–344.

71. Lieberman AN, Bloom W, Kishore PS, Lin JP. Carotid artery occlusion following ingestion of LSD. Stroke 1974; 5:213–215.

72. Friedman SA, Hirsch SE. Extreme hyperthermia after LSD ingestion. JAMA 1971;217:1549–1550.

73. Behan WM, Bakheit AM, Behan PO, More IA. The muscle findings in the neuroleptic malignant syndrome

associated with lysergic acid diethylamide. J Neurol Neurosurg Psychiatry 1991;54:741–743.

74. Griggs EA, Ward M. LSD toxicity: A suspected cause of death. J Ky Med Assoc 1977;75:172–173.

75. Mercieca J, Brown EA. Acute renal failure due to rhabdomyolysis associated with use of a strait jacket in lysergide intoxication. Br Med J 1984;288:1949–1950.

76. Halpern JH, Pope HG Jr. Do hallucinogens cause residual neuropsychological toxicity? Drug Alcohol Depend 1999;53:247–256.

77. Cohen MM, Shiloh Y. Genetic toxicology of lysergic acid diethylamide (LSD-25). Mutat Res 1977-78;47:183–209.

78. Margolis S, Martin L. Anophthalmia in an infant of parents using LSD. Ann Ophthalmol 1980;12:1378-1381.

79. Apple DJ, Bennett TO. Multiple systemic and ocular malformations associated with maternal LSD usage. Arch Ophthalmol 1974;92:301–303.

80. Bogdanoff B, Rorke LB, Yanoff M, Warren WS. Brain and eye abnormalities. Am J Dis Child 1972;123:145–148.

81. McGlothlin WH, Sparkes RS, Arnold DO. Effect of LSD on human pregnancy. JAMA 1970;212:1483–1487.

82. Jacobson CB, Berlin CH. Possible reproductive detriment in LSD users. JAMA 1972;222:1367–1373.

83. Li J-H, Lin L-F. Genetic toxicology of abused drugs: a brief review. Mutagenesis 1998;13:557–565.

84. Muneer RS. Effects of LSD on human chromosomes. Mutat Res 1978;51:403–410.

85. Cohen MM, Hirschhorn K, Frosch WA. *In vivo* and *in vitro* chromosomal damage induced by LSD-25. N Engl J Med 1967;277:1043–1049.

86. Egozene J, Irwin S, Maruffo CA. Chromosomal damage in LSD users. JAMA 1968;204:214–218.

87. Robinson JT, Chitham RG, Greenwood RM, Taylor JW. Chromosome aberrations and LSD. A controlled study of 50 psychiatric patients. Br J Psychiatry 1974;125:238–244.

88. Tijo JH, Pahnke WN, Kurland AA. LSD and chromosomes. JAMA 1969;210:849–856.

89. Cassells NP, Craston DH, Hand CW, Baldwin D. Development and validation of a non-isotopic immunoassay for the detection of LSD in human urine. J Anal Toxicol 1996;20:409–415.

90. McNally AJ, Goc-Szkutnicka K, Li Z, Pilcher I, Polakowski S, Salamone SJ. An onLine immunoassay for LSD: comparison with GC-MS and the Abuscreen® RIA. J Anal Toxicol 1996;20:404–408.

91. Wu AHB, Feng Y-J, Pajor A, Gornet TG, Wong SS, Forte E, Brown J. Detection and interpretation of lysergic acid diethylamide results by immunoassay screening of urine in various testing groups. J Anal Toxicol 1997;21:181–184.

92. Grobosch T, Lemm-Ahlers U. Immunoassay screening of lysergic acid diethylamide (LSD) and its confirmation by HPLC and fluorescence detection following LSD ImmunElute extraction. J Anal Toxicol 2002;26:181–186.

93. Wiegand RF, Klette KL, Stout PR, Gehlhausen JM. Comparison of EMIT®II, CEDIA®, and DPC® RIA assays for the detection of lysergic acid diethylamide in forensic urine samples. J Anal Toxicol 2002;26:519–523.

94. Kerrigan S, Brooks DE. Indirect enzyme-linked immunosorbent assay for the quantitative estimation of lysergic acid diethylamide in urine. Clin Chem 1998;44:985–990.

95. Cody JT, Valtier S. Immunoassay analysis of lysergic acid diethylamide. 1997;21:459–464.

96. Vidal C, Skripuletz T. Bupropion interference with immunoassays for amphetamines and LSD. Ther Drug Monit 2007;29:373–375.

97. Rohrich J, Zorntlein S, Lotz J, Becker J, Kern T, Rittner C. False-positive LSD testing in urine samples from intensive care patients. J Anal Toxicol 1998;22:393–395.

98. Ritter D, Cortese CM, Edwards LC, Barr JL, Chung HD, Long C. Interference with testing for lysergic acid diethylamide. Clin Chem 1997;43:635–637.

99. Cassells NP, Craston DH. The effects of commonly used adulterants on the detection of spiked LSD by enzyme immunoassay. Sci Justice 1998;38:109–117.

100. Nelson CC, Foltz RL. Chromatographic and mass spectrometric methods for determination of lysergic acid diethylamide (LSD) and metabolites in body fluids. J Chromatogr Biomed Appl 1992;580:97–109.

101. Röhrich J, Zörntlein S, Becker J. Analysis of LSD in human body fluids and hair samples applying ImmunElute columns. Forensic Sci Int 2000;107:181–190.

102. Musshoff F, Daldrup T. Gas chromatographic/mass spectrometric determination of lysergic acid diethylamide (LSD) in serum samples. Forensic Sci Int 1997;88:133–140.

103. Rule GS, Henion JD. Determination of drugs from urine by on-line immunoaffinity chromatography-high-performance liquid chromatography-mass spectrometry. J Chromatogr 1992;582:103–112.

104. Johansen SS, Jensen JL. Liquid chromatography-tandem mass spectrometry determination of LSD, *iso*-LSD, and the main metabolite 2-oxo-3-hydroxy-LSD in forensic samples and application in a forensic case. J Chromatogr B 2005;825:21–28.

105. Cui M, McCooeye MA, Fraser C, Mester Z. Quantitation of lysergic acid diethylamide in urine using atmospheric pressure matrix-assisted laser desorption/ionization ion trap mass spectrometry. Anal Chem 2004;76:7143–7148.

106. Sklerov JH, Kalasinsky KS, Ehorn CA. Detection of lysergic acid diethylamide (LSD) in urine by gas chromatography-ion trap tandem mass spectrometry. J Anal Toxicol 1999;23:474–478.

107. Poch GK, Klette KL, Hallare DA, Manglicmot MG, Czarny RJ, McWhorter LK, Anderson CJ. Detection of metabolites of lysergic acid diethylamide (LSD) in human urine specimens: 2-oxo-3-hydroxy-LSD, a

prevalent metabolite of LSD. J Chromatogr B 1999;724: 23–33.

108. Chung A, Hudson J, McKay G. Validated ultra-performance liquid chromatography-tandem mass spectrometry method for analyzing LSD, *iso*-LSD, *nor*-LSD, and O-H-LSD in blood and urine. J Anal Toxicol 2009; 33:253–259.

109. Paul BD, McKinley RM, Walsh JK Jr, Jamir TS, Past MR. Effect of freezing on the concentration of drugs of abuse in urine. J Anal Toxicol 1993;17:378–380.

110. Francom P, Andrenyak D, Heng-Keang L, Bridges RR, Foltz RL, Jones RT. Determination of LSD in urine by capillary column gas chromatography and electron impact mass spectrometry. J Anal Toxicol 1988;12:1–8.

111. Li Z, McNally AJ, Wang H, Salamone SJ. Stability study of LSD under various storage conditions. J Anal Toxicol 1998;22:520–525.

112. de Kanel J, Vickery WE, Waldner B, Monahan RM, Diamond FX. Automated extraction of lysergic acid diethylamide (LSD) and *N*-demethyl-LSD from blood, serum, plasma, and urine samples using the Zymark RapidTrace with LC/MS/MS confirmation. J Forensic Sci 1998;43:622–625.

113. Klette KL, Horn CK, Stout PR, Anderson CJ. LC-MS analysis of human urine specimens for 2-oxo-3-hydroxy LSD: method validation for potential interferants and stability study of 2-oxo-3-hydroxy LSD under various storage conditions. J Anal Toxicol 2002;26:193–200.

114. Smith RN, Robinson K. Body fluid levels of lysergide (LSD). Forensic Sci Int 1985;28:229–237.

115. McCarron MM, Walberg CB, Baselt RC. Confirmation of LSD intoxication by analysis of serum and urine. J Anal Toxicol 1990;14:165–167.

116. Upshall DG, Wailling DG. The determination of LSD in human plasma following oral administration. Clin Chim Acta 1972; 36:67–73.

117. Fysh RR, Oon MC, Robinson KN, Smith RN, White PC, Whitehouse MJ. A fatal poisoning with LSD. Forensic Sci Int 1985;28:109–113.

118. Reuschel SA, Eades D, Foltz RL. Recent advances in chromatographic and mass spectrometric methods for determination of LSD and its metabolites in physiological specimens. J Chromatogr B 1999;488:145–159.

119. Vu-duc T, Vernay A, Calanca A. Détection de l'acide lysergique diethylamide (LSD) dans l'urine humaine: elimination, dépistage et confirmation analytique. Schweiz Med Wschr 1991;121:1887–1890.

120. Reuschel SA, Percey SE, Liu S, Eades DM, Foltz RL. Quantitative determination of LSD and a major metabolite, 2-oxo-3-hydroxy-LSD, in human urine by solid-phase extraction and gas chromatography-tandem mass spectrometry. J Anal Toxicol 1999;23:306–312.

121. Taunton-Rigby A, Sher SE, Kelly PR. Lysergic acid diethylamide: radioimmunoassay. Science 1973;181:165–166.

122. Berrens Z, Lammers J, white C. Rhabdomyolysis after LSD ingestion. Psychosomatics 2010;51:356–356e.3.

123. Rodin E, Luby E. Effects of LSD-25 on the EEG and photic evoked responses. Arch Gen Psychiatry 1966; 14:435–441.

124. Culver CM, King FW. Neuropsychological assessment of undergraduate marihuana and LSD users. Arch Gen Psychiatry 1974;31:707–711.

125. Woody GE. Visual disturbances experienced by hallucinogenic drug abusers while driving. Am J Psychiatry 1970;127:683–686.

126. Raval MV, Gaba RC, Brown K, Sato KT, Eskandari MK. Percutaneous transluminal angioplasty in the treatment of extensive LSD-induced lower extremity vasospasm refractory to pharmacologic therapy. J Vasc Interv Radiol 2008;19:1227–1230.

127. Consroe PF. Specific pharmacological management of acute toxicity due to "psychedelic" drugs. Ariz Med 1972; 29:920–925.

128. Leikin JB, Krantz AJ, Zell-Kanter M, Barkin RL, Hryhorczuk DO. Clinical features and management of intoxication due to hallucinogenic drugs. Med Toxicol Adverse Drug Exp 1989;4:324–350.

129. Lerner AG, Gelkopf M, Skladman I, Oyffe I, Finkel B, Sigal M, Weizman A. Flashback and hallucinogen persisting perception disorder: clinical aspects and pharmacological treatment approach. Isr J Psychiatry Relat Sci 2002;39:92–99.

130. Lerner AG, Gelkofp M, Skladman I, Rudinski D, Nachshon H, Bleich A. Clonazepam treatment of lysergic acid diethylamide-induced hallucinogen persisting perception disorder with anxiety features. Int Clin Psychopharmacol 2003;18:101–105.

131. Moskowitz D. Use of haloperidol to reduce LSD flashbacks. Mil Med 1971;136:754–756.

132. Schneider S, Kuffer P, Wenning R. Determination of lysergide (LSD) and phencyclidine in biosamples. J Chromatogr B 1998;713:189–200.

Chapter 23

BARBITURATES (AMOBARBITAL, BUTALBITAL, PENTOBARBITAL, SECOBARBITAL)

In 1864, Adolf von Baeyer first synthesized barbituric acid as a product of the condensation of malonic acid (acid derivative of apples) and urea (component of animal excrement). Barbituric acid does not produce central nervous system (CNS) depression, but the addition of alkyl or aryl groups at C5 confers sedative hypnotic properties to these drugs. Conrad and Guthzeit synthesized the first barbituric acid derivative, diethylbarbituric acid (barbital, veronal, malonal, Gardenal) in 1881.[1] In the prebarbiturate era of the latter half of the 19th century, the common hypnotic drugs included bromides, scopolamine, chloral hydrate, paraldehyde, and morphine; Fischer and von Mering published the first scientific report on the hypnotic properties of barbital in 1903.[2] This compound became the first barbiturate introduced into clinical practice as a hypnotic by the German companies, E. Merck (Darmstadt) and F. Bayer and Company (Elberfeld) in 1904. During the 20th century, modification of the barbituric acid molecule resulted in over 2,500 barbiturate derivatives with about 50 of these compounds introduced into clinical practice.[3] In 1911, Horlein synthesized phenobarbital; the following year, F. Bayer and Company introduced this drug into clinical practice as Luminal®. Following World War I, the US Congress passed the Trading with the Enemy Act of 1917 that allowed the manufacture of patented German products by American subsidiaries of German companies by the modification of the generic name. Diethylbarbituric acid became barbital in the United States, whereas this compound was barbitone in the United Kingdom. Thus, the nomenclature of barbiturates persisted as differences in the 2 endings, -al and -one. Alfred Hauptmann serendipitously discovered the anticonvulsant properties of phenobarbital in 1912,

when he administered this barbiturate to epileptic patients as a sedative for sleep.[4] In the early 1920s, Klaesi introduced the use of barbiturates as a treatment for psychotic patients by the induction of deep, prolonged sleep as means to facilitate communication between the patient the psychotherapist.[5] Between the 1920s and the mid-1950s, barbiturates became the major sedatives and hypnotics in clinical practice.[3] By 1930, the use of phenobarbital superseded the use of bromides as anticonvulsants despite reports of tolerance and withdrawal seizures. In 1923, Schonle and Moment at Eli Lilly Company synthesized amobarbital (Amytal®, Eli Lilly Company, Indianapolis, IN); 6 years later, Schonle synthesized secobarbital (Seconal®). Both of these drugs had similar pharmacologic properties as the earlier synthesized barbiturate, butalbital, Volwiler and Tabern synthesized pentobarbital (Nembutal®, Abbott Laboratories, Abbott Park, IL) and thiopental (Pentothal®) in 1930. The latter barbiturate was a sulfur derivative of pentobarbital that became a popular intravenous (IV) anesthetic.[6]

Although many barbiturates were synthesized during the early 20th century, only a few barbiturates became widely used as sedative hypnotics, anticonvulsants, and anesthetics. Pharmacologic differences in the duration of action allows a practical categorization of the common barbiturates into the following: 1) short- and intermediate-acting: hypnotics; 2) long-acting: anticonvulsants, anxiolytics, treatment of withdrawal symptoms; and 3) ultrashort-acting: induction of anesthesia for minor operation and procedures. Table 23.1 displays identifying data for common barbiturates.

Other uses of barbiturates included treatment of essential tremor (primidone) and headache (i.e., in

TABLE 23.1. Classification of Common Barbiturates.

Class	Barbiturate	Trade Name	Chemical Name	CAS Number
Ultrashort	Thiopental	Pentothal	5-ethyl-5-(1-methylbutyl)-thiobarbituric acid	76-75-5
Short	Pentobarbital	Nembutal	5-ethyl-5-(1-methylbutyl)-barbituric acid	76-74-4
	Secobarbital	Seconal	5-allyl-5-(1-methylbutyl)-barbituric acid	76-73-3
Intermediate	Amobarbital	Amytal	5-ethyl-5-isopentylbarbituric acid	57-43-2
	Butalbital	*	5-allyl-5-isobutylbarbituric acid	77-26-9
Long	Phenobarbital	Luminal	5-ethyl-5-phenylbarbituric acid	50-06-6

*Butalbital is usually marketed as a variety of proprietary preparations with several other drugs (e.g., caffeine, aspirin, acetaminophen, or codeine).

combination with other analgesics). Sodium amobarbital and sodium pentobarbital ("truth serum") were adjuncts during narcoanalysis to allow increased interaction of catatonic and schizophrenic patients with their therapists, particularly during the treatment of these patients during the 1930s and 1940s.[7] Following World War II, the use of amobarbital interviews was a popular method to express repressed feelings, particularly for aiding soldiers with posttraumatic stress from combat experiences.[8] In contrast to hypnosis, amobarbital interviews could be administered to all patients rather than only those individuals susceptible to hypnosis.

Smith, Kline, & French introduced Dexamyl® (amobarbital, dextroamphetamine) as an antidepressant in the 1930s. The abuse of this combination (street name: goofballs) became widely publicized in the 1970s and early 1980s when Dr. George C. Nichopoulos was indited (but not convicted) for prescribing this drug and other narcotics to Elvis Presley and Jerry Lee Lewis. Dexamyl® was withdrawn from the US market in 1981. Short- (pentobarbital, secobarbital) and intermediate-acting (amobarbital, butalbital) barbiturates are more frequently associated with drug abuse than the long-acting (phenobarbital) barbiturates based on admissions to drug treatment centers in the 1970s.[9] Typically, the short- and intermediate-acting barbiturates have half-lives ranging from 10–50 hours.

Peak US production of barbiturates occurred in the late 1940s and 1950s prior to the introduction of benzodiazepines. Dependence on barbiturates ("Veronal habit"). was recognized soon after the introduction of first commercial barbiturate, barbitone (Veronal). Despite the synthesis of over 2,500 derivatives of barbituric acid, no barbiturate eliminated the problems of dependence and fatalities associated with barbiturate overdose. Although barbiturates were not included in the US Dangerous Drugs Act of 1920, the Poisons Rules in 1935 required a prescription for barbiturates. Well-documented reports of barbiturate dependence did not appear until the 1950s.[10] By the 1960s, barbiturate abuse was a well-recognized problem.[10] In 1962, Marilyn Monroe died of a barbiturate overdose. In that same year, President Kennedy convened a special drug-dependence committee to investigate the extent of the barbiturate dependence, estimated at that time to involve 250,000 barbiturate addicts in the United States. The introduction of chlorpromazine in 1952 and chlordiazepoxide in 1960 led to the development of more efficacious and safer alternatives to barbiturates. Currently, barbiturates are alternatives for the treatment of epilepsy, insomnia, preoperative sedation, sedative-hypnotic withdrawal, gastrointestinal (GI) functional disorders, and migraine headaches (i.e., as combination formulations).

AMOBARBITAL

IDENTIFYING CHARACTERISTICS

In general, increased lipophilicity is associated with more rapid onset of action, shorter duration of effect, and greater hypnotic properties. Table 23.2 lists some physiochemical properties of amobarbital. Street names for amobarbital include Amys, Birds, Blues, Blue Angels, Blue Birds, Blue Bullets, Blue Clouds, Blue Devils, Blue Dolls, and Blue Heaven. Figure 23.1 displays the chemical structure of amobarbital.

EXPOSURE

Epidemiology

Epidemiologic data indicate that the prevalence of amobarbital abuse began declining in the 1960s and 1970s as benzodiazepines began replacing barbiturates as preferred sedative hypnotic drugs. The yearly average US emergency department visits for amobarbital misuse/abuse reported by DAWN (Drug Abuse Warning Network) decreased from 1,945 in the late 1970s to 439

TABLE 23.2. Some Physiochemical Properties of Amobarbital.

Physical Property	Value
Melting Point	157°C (314.6°F)
pKa	7.84
log P (Octanol-Water)	2.07
Water Solubility	603 mg/L (25°C/77°F)
Vapor Pressure	2.17E-11 mm Hg (25°C/77°F)
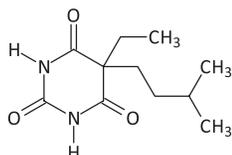
FIGURE 23.1. Chemical structure of amobarbital.

in the mid-1980s.[11] During these times, drug misuse/abuse related deaths reported by DAWN medical examiner facilities in the United States also decreased from 294 to 65. Both amobarbital and secobarbital were among the prescription drugs with the highest reported drug-related deaths per dispensed prescriptions during the 1970s and 1980s. Currently, the estimated emergency department visits related to barbiturate misuse/abuse are approximately 5% (i.e., about 11,000) of the emergency department visits related to benzodiazepine misuse/abuse, based on DAWN data.[12] These rates have been relatively stable over the last few years.

Sources

Tuinal® (Eli Lilly) was a commonly abused barbiturate for sedative and euphoric effects, particularly during the 1960s and 1970s. This drug combination contained equal amounts of amobarbital and secobarbital in total barbiturate doses of 50 mg, 100 mg, and 200 mg.

Methods of Abuse

Typically, amobarbital abuse results from the ingestion of excessive doses of amobarbital. Occasionally, amobarbital is injected; however, the irritancy of barbiturates frequently causes skin ulcers. Rapid acting barbiturates (amobarbital, pentobarbital, secobarbital) are more common drugs of abuse than the long-acting barbiturates (barbital, phenobarbital).

DOSE EFFECT

The adult therapeutic dose of amobarbital ranges from 50–150 mg with doses up to 500 mg for narcoanalysis and adjunctive therapy during anesthesia. Predicting toxicity based on the estimated amobarbital dose is complicated by several variables including tolerance, time of supportive care in relation to the ingestion, and concomitant administration of other CNS depressants (e.g., ethanol, sedative hypnotic drugs). The ingestion of 2–3 g amobarbital is potentially fatal in nontolerant individuals. The ingestion of 20 g amobarbital by a 52-year-old man was associated with coma, apnea, hypotension, and hypothermia.[13] He survived with intensive supportive care. Barbiturate-dependent patients usually ingest supratherapeutic doses of barbiturates, but the daily dose frequently fluctuates. Daily amobarbital doses exceeding 800 mg are associated with dependence and withdrawal upon cessation of use.[14] A 54-year-old woman gradually increased her consumption of amobarbital over 13 years with some fluctuation. At the time of her admission to a detoxification unit, her daily amobarbital consumption was 1,800 mg with reported maximum daily intake up to 3 g.[15]

TOXICOKINETICS

Kinetics

The GI absorption of amobarbital is almost complete with no significant first-pass metabolism.[16] The rate-limiting factors for amobarbital absorption are drug dissolution and dispersal. Distribution of barbiturates depends on lipid solubility, protein binding, and the extent of ionization. The average protein binding is approximately 55–60%. The liver metabolizes most of the amobarbital dose; amobarbital undergoes almost complete biotransformation to 3′-hydroxyamobarbital via *C*-hydroxylation and to *N*-hydroxyamobarbital via *N*-hydroxylation as demonstrated in Figure 23.2.[17] Oxidation of amobarbital to 3′-hydroxyamobarbital accounts for approximately one-half of the amobarbital dose. Minor urinary metabolites include *N*,*N*'-dimethylamobarbital and 5-(3′-carboxybutyl)-5-ethylbarbituric acid.[18] There is substantial interspecies variation in the metabolism of amobarbital with the virtual absence of diol metabolite in humans and the absence of amobarbital-*N*-glucoside in animals (dog, rodents, guinea pig, hamster).[19]

The average serum elimination half-life of amobarbital is approximately 24 hours with substantial interindividual variation.[20,21] In a study of 7 healthy men and 9 healthy women receiving 3.54 mg amobarbital/kg intravenously, the mean serum elimination half-life was

469
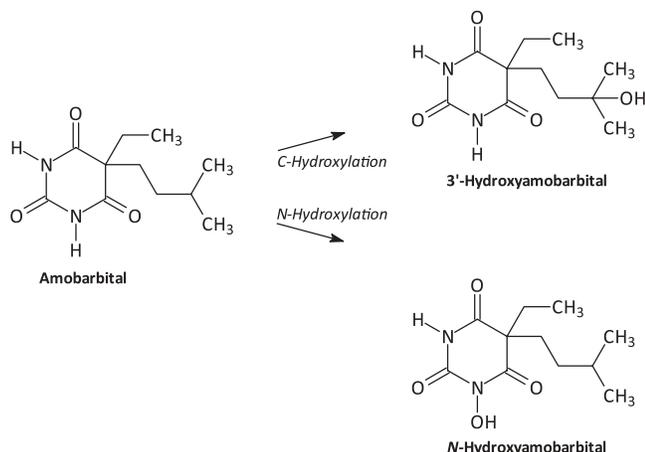
FIGURE 23.2. Biotransformation of amobarbital.

approximately 21 hours (mean, 22.7 ± 1.6 hours; women, 20.0 ± 1.0 hour).[22] In 4 mothers at term receiving 200 amobarbital daily during the last month of pregnancy, the plasma amobarbital elimination half-life ranged from 8.4–19.3 hours, whereas the fifth mother had a prolonged elimination half-life (70.2 hours).[23] Similar variation, but more prolonged elimination occurs in the neonates.[24] In a study of 36 healthy individuals receiving a single therapeutic dose of amobarbital, the mean clearance of amobarbital was 36.7 ± 10.0 mL/minute.[16] Over the first 48 hours, 3′-hydroxyamobarbital accounted for about 34% of the administered dose. Limited pharmacologic studies suggest that 3′-hydroxyamobarbital may exhibit some CNS activity.[25] The kidney excretes very small amounts of amobarbital in the urine unchanged, even in overdose; little amobarbital appears in the bile.[26] In a study of 5 volunteers receiving 200 mg sodium amobarbital, the percentage of the amobarbital dose excreted in the urine over 6 days ranged from about 34–49% compared with 1–3% as unchanged amobarbital measured by gas chromatography.[27] The estimated average amobarbital half-life in blood was 25 hours following nonfatal amobarbital poisoning measured by ultraviolet spectrophotometry.[28]

Maternal and Fetal Kinetics

Amobarbital crosses the placenta and the amobarbital concentration in cord and maternal serum are similar.

Tolerance

Tolerance to barbiturates results from both pharmacokinetic and pharmacodynamic effects. In both humans and animals, the chronic administration of amobarbital

and short-acting barbiturates (pentobarbital, secobarbital) induces hepatic microsomal enzymes and increases the elimination rate of barbiturates.[15,29] A pharmacokinetic study compared the dose of Tuinal® necessary to produce toxicity (drowsiness, slurred speech, ataxia, nystagmus) in 9 patients admitted to a detoxification unit for Tuinal® addiction with healthy medical students and volunteer medical staff.[30] The mean toxic dose of the Tuinal® abusers was about 58% higher than the controls. Induction of CYP450 enzymes occurs in a few days and pharmacodynamic tolerance develops over weeks to months, depending on the dose. Cross-tolerance of barbiturates with alcohol and benzodiazepines also occurs.

Drug Interactions

Similar to other barbiturates, amobarbital induces CYP450 isoenzymes. Following the daily ingestion of 200 mg amobarbital for 3 weeks, the average half-life of antipyrine decreased about 35% (CYP1A2).[31] The metabolism of antipyrine involves at least 10 cytochrome P450 isoenzymes including CYP1A2, CYP2A6, CYP3A4, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, and CYP2E1.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Barbiturates are CNS depressants that suppress neuronal excitability, impulse conduction, and the release of neurotransmitters. These compounds potentiate the inhibitory effects of γ-aminobutyric acid (GABA) in the CNS to cause cognitive and motor impairment characteristic of CNS depression. The GABA_A receptor is a pentameric transmembrane chloride ion channel that binds GABA as well as barbiturates, benzodiazepines, and ethanol. Barbiturates enhance the binding of GABA to the chloride channel receptors, and these drugs promote, rather than displace, the binding of benzodiazepines to these receptors. Only the GABA_A α- and β-subunits rather than the γ-subunit are necessary for the clinical effects of barbiturates.[32] Short- and intermediate-acting barbiturates have similar mechanisms of action, binding to the picrotoxin allosteric site on the GABA receptor-ionophore complex and directly activating the GABA-related chloride channels to enhance presynaptic chloride conductance. These drugs prolong the opening of the chloride channels, whereas benzodiazepines increase the frequency rather than the duration of chloride channel opening. Barbiturates also depress voltage-activated calcium currents and reduce glutamate-induced polarization mediated by the α-amino-3-hydroxy-5-methylisoxazole-4-propionic   acid

(AMPA) receptors.[33] Barbiturates are cardiorespiratory depressants that cause apnea by decreasing the respiratory drive and diaphragmatic movements. Autopsy findings of individuals dying from amobarbital intoxication are nonspecific with features primarily of asphyxia.

CLINICAL RESPONSE

Illicit Use

In general, barbiturates produce clinic effects similar to ethanol intoxication, manifest by dysarthria, ataxia, nystagmus, lightheadedness, impaired cognition, poor judgment, loss of inhibitions, and confusion. Similar to short-acting and other intermediate-acting barbiturates, amobarbital produces CNS and cardiorespiratory depression. Apnea occurs early in the course of severe amobarbital intoxication.

Overdose

Hypotension and hypothermia may complicate large overdoses of amobarbital. Alteration of consciousness ranges from drowsiness to coma with unstable vital signs. Reed et al [34] classified barbiturate coma into 5 stages as defined in Table 23.3. Pupils typically are constricted early in the course of coma; in the later stages of coma, pupil dilate. The clinical features of stage 4 barbiturate coma include nonreactive pupils, loss of brainstem reflexes (corneal, oculovestibular), absent deep tendon reflexes, and evidence of pathologic reflexes (e.g., Babinski sign). This clinical pattern is nonspecific; severe intoxications with other sedative hypnotics (meprobamate, glutethimide, ethchlorvynol, methaqualone) and hypoxic encephalopathy produce similar findings including benzodiazepines. Skin bullae are another nonspecific finding in barbiturate coma. Blanching, erythematous lesion are intra- or subepidermal lesions that appear on pressure points within the first 24 hours of coma and progress to bullae within 2–3 days. Resolution occurs spontaneously in 1–2 weeks in the absence of local complications.

Abstinence Syndrome

Considerable interindividual variation occurs in the clinical features of barbiturate withdrawal including the abstinence syndrome associated with amobarbital. The severity of the withdrawal reaction following cessation of chronic barbiturate use relates directly to the duration and intensity of barbiturate use. Some patients develop only weakness and anxiety, whereas other patients progress to delirium, multiple seizures, hyperthermia, and acute psychosis. Minor symptoms following discontinuation of amobarbital, short-acting, or other intermediate-acting barbiturates begin 8–36 hours after the last dose including anxiety, lightheadedness, tachycardia, diaphoresis, coarse tremor, generalized weakness, insomnia, anorexia, visual hallucinations, and nightmares. Other effects include hyperreflexia, postural hypotension, vomiting, and autonomic hyperactivity. Maximum effects occur within 24–48 hours after the last dose. Serious effects following the abrupt cessation of barbiturate use include hyperpyrexia, delirium, florid hallucinations, and seizures. Convulsions may occur 1–5 days after the last dose; in contrast to withdrawal from ethanol, seizures during barbiturate withdrawal frequently are multiple. Psychosis with visual hallucinations may develop following these seizures, manifest by agitation, insomnia, tremors, confusion, delusions, disorientation, and hallucinations. The delirium typically lasts <5 days and a period of prolonged sleep ensues.[14] Withdrawal symptoms resolve over 2–15 days, depending on the severity of the withdrawal reaction.

Reproductive Abnormalities

There are limited human data on the teratogenicity and fetotoxicity of amobarbital; previous studies on amobarbital and congenital abnormalities are inconsistent. The US Food and Drug Administration (FDA) lists amobarbital in category D (positive evidence of human fetal risk). In a convenience sample of 9 children born to mothers attempting suicide with amobarbital during the first trimester, there were no congenital abnormalities or evidence of fetal growth retardation.[36] A retrospective study of prescription drug use during pregnancy and congenital abnormalities detected a statistically significant increase in major malformation (6.9%) in neonates born to mothers ingesting amobarbital during the first trimester, when compared with controls.[37]

TABLE 23.3. Reed Classification of Barbiturate Coma.[34]

Stage*	Clinical Features
0	Stuporous, responsive to verbal command
1	Responsive to painful stimuli, unresponsive to verbal stimuli
2	Unresponsive to verbal and painful stimuli, reflects intact, vital signs stable
3	Unresponsive, areflexic, stable vital signs
4	Unresponsive, areflexic, unstable vital signs

*Patients in stage 4 coma are not always areflexic and patients in stage 3 coma may be areflexic without changes in the vital signs.[35]

DIAGNOSTIC TESTING

Analytic Methods

Screening for barbiturates in blood involves enzyme-multiplied immunoassay technique (EMIT® tox Serum Barbiturates, Siemens Healthcare Diagnostics, Deerfield, IL) and fluorescence polarization immunoassays (FPIA) including ADx serum barbiturates (Abbott Diagnostics, Abbott Park, IL) and COBAS INTEGRA 700 Barbiturates (Roche Diagnostic Systems, Somerville, NJ). Positive cutoffs for these methods are 3 mg/L, 2 mg/L, and 0.2 mg/L, respectively. The cross-reactivity of these assays vary with the type of barbiturate because the antibodies are raised against secobarbital.[38] Consequently, this assay is highly sensitive to secobarbital, but the cross-reactivity to amobarbital is about 18%.[39] Methods for the quantitation of amobarbital in biologic samples include ultraviolet (UV) spectrophotometry, thin layer chromatography, gas chromatography, high performance liquid chromatography, gas chromatography/mass spectrometry (GC/MS), and liquid chromatography/electrospray/mass spectrometry.[40] The lower limit of quantitation (LLOQ) of amobarbital in serum using the latter method is 0.5 mg/L with an interday coefficient of variation of <7%. The LLOQ for GC/MS with addition of formic acid to the carrier gas is 0.1 mg/L,[41] whereas the use of liquid extraction with methyl-*tert*-butyl ether/chloroform and alkylation to the *N*-ethyl derivative results in a LLOQ near 0.05 mg/L (coefficient of variance, ~5–6%).[42] Both *N*-hydroxyamobarbital and 3′-hydroxyamobarbital are detectable in urine with GC/MS combined with on-column methylation and pyrolysis.[43] Micellar liquid chromatography with UV detection allows the direct inject of plasma samples without preanalytic derivatization.[44] The limit of detection (LOD) for amobarbital in biologic samples ranges from 0.2–0.4 mg/L. Thermal instability of *N*-hydroxyamobarbital limits the detection of this metabolite by gas chromatography. In urine samples, GC/MS allows the detection of amobarbital in the presence of other barbiturates (butalbital, pentobarbital, secobarbital) with limits of detection in the range of 0.01–0.02 mg/L.[45] Micellar liquid chromatography allows the analysis of complex matrices without extraction. Limits of detection for amobarbital using this method with UV detection is approximately 0.07 mg/L.[46] Barbiturates including amobarbital are relatively stable in pH-neutral biologic specimens, even after chemical fixation with formalin. Substantial amounts of amobarbital are detectable in liver tissue and in formalin at least 20 months after death.[47] The postmortem stability of barbiturates in blood and tissues depends on pH with amobarbital being relatively stable in the mildly acidic conditions present in most postmortem blood samples. Amobarbital is relatively stable in unpreserved blood and liver tissue at 4°C (39.2°F) and 25°C (77°F) for 2–3 months at pH 6.4–7.0. Under these storage conditions, amobarbital decreased to 76 ± 5% and 77 ± 9%, respectively, over 2 months of storage.[48]

Biomarkers

After barbiturate poisoning, the liver typically contains the highest barbiturate concentration followed by kidney, spleen, and brain.[47]

Blood

Therapeutic/Recreational Doses.   Twelve hours after the IV administration of 250 mg amobarbital/70 kg to 6 healthy volunteers, the mean amobarbital concentration was 2.49 ± 0.24 mg/L as measured by gas chromatography/flame ionization detection.[25] The mean serum amobarbital concentration 30 minutes after the injection of 3.54 mg amobarbital/kg to 6 healthy volunteers was 5.14 ± 0.11 mg/L.[22] The mean plasma amobarbital concentration in 5 patients a half-hour after receiving 600 mg amobarbital over the preceding 3 hours was 8.7 mg/L, declining to 6.6 mg/L 4 hours later.[49] All of these patients demonstrated signs of drug effect (sedation, unsteady gait, poor balance, and coordination) at the time of initial testing. In a study of newly admitted patients to a psychiatric ward with anxiety, the mean dose of sodium amobarbital administered to 23 patients was 463 mg (range, 286–657 mg). After 7 days of treatment, the mean plasma amobarbital concentration was 4.11 ± 0.30 mg/L (range, 1.68–7.06 mg/L). There was no accumulation of amobarbital over the 7-day study; the response to treatment did not correlate with plasma amobarbital concentrations.

Overdose.   In a case series of 3 amobarbital overdoses requiring intubation and ventilatory support, the maximum serum amobarbital concentrations ranged from 40–67 mg/L as measured by gas chromatography.[50] The duration of coma ranged from 44–59 hours. As measured by UV spectrophotometry, blood amobarbital concentrations of 20–25 mg/L are associated with light coma (reflexes present) and 55–60 mg/L with severe intoxication (comatose with unstable vital signs).[28] A 23-year-old woman presented with coma, absent corneal and gag reflexes, mild hypothermia, and dilated right pupil with increased tone in her left arm.[51] There were no imaging studies reported, and she recovered after 40 hours of coma. The admission blood amobarbital concentration was 63 mg/L (method not reported).

POSTMORTEM   In a series of 55 cases involving amobarbital as the cause of death, the postmortem blood concentrations (site and analytic method not specified [NOS]) ranged from 13–96 mg/L.[52] The report did not include the presence or absence of other drugs other than a comment that amobarbital poisoning was the cause of death. In a convenience series of 17 fatalities, the lowest amobarbital concentration in postmortem blood attributed to the cause of death was 27 mg/L by UV spectrophotometry.[53] The report did not include sample site, clinical details, or the presence of other drugs in postmortem specimens. The mean postmortem amobarbital concentration in blood samples (site NOS) from 6 deaths attributed to amobarbital intoxication was 47 mg/L (range, 29–68 mg/L).[54] In this case series, the mean postmortem liver concentration of amobarbital was 219 mg/kg (range, 106–580 mg/kg). In postmortem blood (site NOS) from 4 amobarbital-related fatalities, the mean amobarbital concentration was 41 mg/L (range, 25.5–78.5 mg/L) compared with a mean of 4.2 mg/L (range, 1.1–8.4 mg/L) for amobarbital concentrations in 11 non-drug related fatalities.[55] The amobarbital-related fatalities were single-drug fatalities with the exception of ethanol, nicotine, and caffeine. The distribution of amobarbital to bone is relatively small compared with blood, liver, and kidney; however, amobarbital does persist in bone for prolonged periods. Remnants of the skeleton from a 33-year-old psychiatric patient were found 1½ years after her disappearance.[56] The concentrations of amobarbital in the skeletal remnants were as follows: rib 9.3 mg/kg; sternum, 25.6 mg/kg; and vertebra, 9.2 mg/kg.

Abnormalities

Rhabdomyolysis with subsequent acute renal dysfunction may complicate prolonged barbiturate-induced coma, manifest by elevated serum creatine kinase, urinary myoglobin, and increasing serum creatinine. Additionally, hypoglycemia may occur during amobarbital intoxication.

TREATMENT

Stabilization

Amobarbital is a CNS depressant that may cause respiratory and cardiovascular depression during severe intoxications. Patients should be evaluated for the presence of hypoxia and respiratory insufficiency; intubation and ventilatory support may be necessary in severe cases. Hypotension initially is treated with IV fluids, but vasopressors (dopamine, norepinephrine) may be necessary in patients unresponsive to saline infusion.

Hemodynamic monitoring (echocardiography, Swan-Ganz catheter) may be necessary in patients with stage 4 coma and hemodynamic instability. Hypoglycemia occurs in a substantial portion of comatose patients with barbiturate intoxication; all patients should be evaluated with point of care testing and glucose and thiamine administered as indicated. Severe hypothermia may occur in deeply comatose patients during severe amobarbital intoxication; these patients may require prolonged cardiopulmonary resuscitation as a result of persistent ventricular fibrillation unresponsive to defibrillation. In rare cases, thoracotomy, internal cardiac massage, and direct rewarming of the mediastinum is necessary to increase the body temperature sufficiently to allow successful defibrillation.[57] Other than hypothermia-associated dysrhythmias, barbiturate poisoning is usually not complicated by rhythm disorders.

Gut Decontamination

In general, if the patient presents within 1–2 hours of ingestion, the administration of a single dose of activated charcoal (1 g/kg body weight) is a therapeutic option; however, there are inadequate data on the clinical outcome of this intervention. *In vitro* studies suggest that the adsorption of amobarbital to activated charcoal is limited (<51 mg/g activated charcoal at pH 1.3 and 37°C) and lower than other barbiturates (secobarbital, 124 mg/g; pentobarbital, 103 mg/g).[58] The actual adsorption of amobarbital during overdose is probably lower as a result of the presence of mucous, bile acids, food, and altered GI motility.

Elimination Enhancement

There are limited data on the use of artificial means to enhance the elimination of amobarbital; however, existing data suggest that the elimination of amobarbital with these methods is very limited. Charcoal hemoperfusion for 3.5 hours at 200 mL/minute removed 4% of the estimated dose of amobarbital from the blood of a comatose, hypotensive 54-year-old man with amobarbital intoxication.[59] Dialysis for 31.5 hours in a 52-year-old man with severe amobarbital intoxication removed only about 3% of the estimated amobarbital dose; he remained intubated for 24 hours after hemodialysis ended.[13] Forced diuresis is contraindicated in barbiturate overdose because of the lack of efficacy and the potential for fluid overload and pulmonary edema. Multiple-dose activated charcoal (MDAC) enhances the elimination of phenobarbital, but there are inadequate data on the efficacy of MDAC for the treatment of life-threatening amobarbital poisoning.[60]

Antidotes

There are no antidotes for barbiturate intoxication.

Supplemental Care

Barbiturate withdrawal requires pharmacotherapy and hospital admission. Oral loading with phenobarbital is a technique developed in the 1970s for the treatment of barbiturate withdrawal. The dose is 120 mg phenobarbital/hour until at least 3 of the following effects occur: nystagmus, dysarthria, ataxia, drowsiness, or emotional lability. Table 23.4 provides a rating scale for the evaluation of these 5 signs. Hourly phenobarbital loading terminates when the score is ≥8. These signs are evaluated each time before the next loading dose along with frequent vital signs and level of consciousness. In a study of 21 barbiturate addicts (daily consumption, 0.5–4 g), the mean phenobarbital loading dose was 23.4 ±7.1 mg/kg with a median postloading phenobarbital concentration of 35.9 mg/L (range, 13.2–71.6 mg/L).[61] Most of these patients were polydrug users (e.g., alcohol, diazepam, methaqualone, amphetamines). Discharge or rehabilitation usually can occur 48 hours after phenobarbital loading begins. Patients becoming intoxicated at ≤7 mg/kg (i.e., about 500 mg) phenobarbital are prob-ably not physically dependent and do not require full phenobarbital loading.[62] High-dose oral diazepam (10–20 mg/h until reduction in withdrawal symptoms or appearance of toxic effects) is an alternative to oral phenobarbital loading, similar to the treatment of ethanol withdrawal; however, there are inadequate clinical data to compare the efficacy of phenobarbital loading and benzodiazepine use in barbiturate withdrawal.

BUTALBITAL

IDENTIFYING CHARACTERISTICS

Butalbital (5-allyl-5-isobutylbarbituric acid, CAS RN: 77-26-9) is a white odorless powder that is slightly soluble in cold water and soluble in boiling water. This compound is also soluble in alcohol and alkaline solutions of hydroxides and carbonates. Figure 23.3 displays the chemical structure of butalbital. Table 23.5 lists some physiochemical properties of butalbital.

TABLE 23.4. Rating Scale for Oral Phenobarbital Loading.[69]

Category	Sign	Score
Nystagmus	Absent	0
	Present on lateral gaze only	1
	Easily elicited and sustained	2
	Coarse, sustained	3
Dysarthria	Absent	0
	Minor slurring over some words	1
	Moderate slurring	2
	Severe slurring, unintelligible	3
Ataxia	Absent	0
	Mildly unsteady on tandem gait	1
	Moderately unsteady on regular gait	2
	Needs support on regular gait	3
Drowsiness	Alert	0
	Awake but drowsy	1
	Asleep but roused easily	2
	Asleep and difficult to arose	3
Emotional lability	Normal, same as before loading	0
	Some mood change	1
	Obvious mood change	2
	Uninhibited, mood swings	3

Note. Score each of the 5 categories before loading and before each hourly dose. Discontinue phenobarbital loading when the score is ≥8. Vital signs are repeated frequently until the patient is alert.
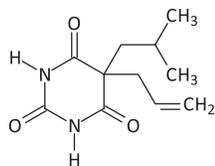
FIGURE 23.3. Chemical structure of butalbital.

TABLE 23.5. Some Physiochemical Properties of Butalbital.

Physical Property	Value
Molecular Weight	224.3 g/mol
Melting Point	138.5°C (281.3°F)
log P (Octanol-Water)	1.870
Water Solubility	1700 mg/L (25°C/77°F)

EXPOSURE

Fiorinal® (Novartis, Basel, Switzerland) is a capsule containing 330 mg acetylsalicylic acid (ASA), 40 mg caffeine, and 50 mg butalbital. Other Fiorinal® preparations may substitute acetaminophen for ASA or may add codeine. Many Fiorinal® abusers use the preparation containing codeine and also abuse other drugs (benzodiazepines, opioids, sedative hypnotics). The FDA approved butalbital-containing medications for the treatment of tension headache, but not for migraine headaches; however, patients with migraine headaches frequently receive prescriptions for these medications.[63] A meta-analysis of placebo-controlled trials of patients with episodic tension-type headaches indicated that butalbital compounded with aspirin, acetaminophen, and/or caffeine is efficacious;[64] however, there are inadequate data to determine the effectiveness of this combination for migraine headache.[65] Abuse of butalbital typically involves the chronic ingestion of butalbital-containing medication; however, abuse of suppositories occurs as a result of the rapid absorption of these preparations and the higher concentrations of butalbital (150 mg vs. 50 mg) in suppositories.[66]

DOSE EFFECT

In a case series of 29 Fiorinal® abusers admitted to a detoxification unit, the mean daily intake of butalbital was 700 mg (i.e., 14 capsules) with a range of 300–1,500 mg.[67] The mean loading phenobarbital dose for this cohort was 1,120 mg, which indicated moderate dependence. Disorientation, agitation, insomnia, tremu-

lousness, and auditory hallucinations occurred in a 59-year-old man after cessation of butalbital use.[68] He had ingested up to 1,500 mg butalbital daily in the preceding 2 years. In another series of 18 butalbital abusers admitted to a detoxification unit, the mean daily consumption of butalbital tablets was 13.4 (range, 8–20).[69] The sedative dose of butalbital in nontolerant individuals is 50–100 mg; the hypnotic dose is 100–200 mg. Predicting toxicity based on the estimated butabarbital dose is complicated by several variables including tolerance, time of supportive care in relation to the ingestion, and concomitant administration of other CNS depressants (e.g., ethanol, sedative hypnotic drugs).

TOXICOKINETICS

Absorption of butalbital is rapid with peak butalbital concentrations in the blood occurring within 1 hour of ingestion. The volume of distribution is approximately 0.8 L/kg; protein binding of butalbital in the blood is relatively small (i.e., 26%). Butalbital undergoes almost complete metabolism by the liver, primarily by C5 oxidation. The major metabolites are 5-isobutyl-5-(2,3-dihydroxypropyl)barbituric acid and 5-allyl-5-(3-hydroxy-2-methyl-1-propyl)barbituric acid. There are limited and somewhat inconsistent pharmacokinetic data on the serum elimination half-life of butalbital. This drug is an intermediate-acting barbiturate with a biologic half-life in the range 30–40 hours based on renal excretion, similar to pentobarbital and secobarbital. In a study of 5 healthy volunteers receiving 100 mg butalbital in combination with aspirin and caffeine, the mean plasma elimination half-life of butalbital was 61 hours (range, 35–87.5 hours).[70]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Butalbital is a CNS depressant that suppresses neuronal excitability, impulse conduction, and the release of neurotransmitters, similar to other barbiturates including amobarbital. The mechanism of action and toxicity is similar to amobarbital (see Amobarbital Histopathology and Pathophysiology).

CLINICAL RESPONSE

As with other barbiturates, the clinical features of butalbital are similar to ethanol intoxication and include cognitive impairment, dysarthria, ataxia, unsteady gait, slurred speech, sedation, poor attention, emotional lability, and inappropriate judgment. Clinical effects associated with chronic butalbital abuse include nausea, restlessness, irritability, asthenia, impaired memory

and concentration, depression, and neurotic behavior. Chronic daily headaches (i.e., rebound headaches) are a complication of the misuse of butalbital-containing analgesics. The clinical features of withdrawal from chronic butalbital abuse is similar to other barbiturate abstinence syndromes with agitation, tachycardia, and diaphoresis within ~48 hours after the last dose.[71] Other clinical effects include insomnia, anorexia, hyper-reflexia, tremor, hypertension, and seizures. In contrast to ethanol withdrawal, seizures often are multiple and occur later (i.e., >48 hours) during the abstinence syndrome. Depending on the duration and magnitude of butalbital use, the clinical course may progress to visual hallucinations, delirium, and acute psychosis. Neonatal abstinence syndrome may occur in children born to mothers abusing butalbital.[72] Clinical features of this syndrome include jitteriness, irritability, rhinitis, muscle jerks, nystagmus, dysconjugate gaze, and poor feeding.

DIAGNOSTIC TESTING

Analytic Methods

Methods for quantitation of butalbital in biologic fluids include UV spectrophotometry, gas chromatography with flame ionization or electron capture detection, micellar electrokinetic capillary chromatography,[73] solid phase extraction with high performance liquid chromatography and UV detection at 220 nm,[74] and gas chromatography/mass spectrometry (GC/MS). The limits of quantitation for butalbital in blood and urine using GC/MS are similar to amobarbital (0.050 mg/L and 0.020 mg/L, respectively) with a coefficient of variance near 8–9%,[42] whereas the LLOQ for high performance liquid chromatography and UV detection is 3.75 mg/L.[74]

Barbiturates including butalbital are relatively stable in pH-neutral biologic specimens, even after chemical fixation with formalin; however, barbiturates diffuse out of tissue into surrounding formalin solutions during storage. The recovery rate of butalbital from liver tissue fixed in formalin and stored for 6 months was about 22% of the initial value at autopsy.[75] Substantial amounts of butalbital diffused from the tissue to the formalin during storage. The surrounding formalin contained approximately 66% of the original butalbital concentration in the liver at autopsy for a total recovery of about 88%. The postmortem stability of barbiturates in blood and tissues depends on pH with butalbital being relatively stable in the weakly acidic conditions usually present in postmortem blood samples.

Biomarkers

The whole blood/plasma ratio of butalbital is near unity. In a study of 5 healthy volunteers receiving 100 mg butalbital in combination with 80 mg caffeine and 660 mg ASA, the mean whole blood/plasma ratio was 0.987 (range, 0.763–1.148).[70] The mean peak butalbital concentration of 2.14 mg/L occurred at 2 hours with the butalbital concentration declining to 1.39 mg/L at 30 hours after ingestion. In postmortem blood (site NOS) from 3 butalbital-related fatalities, the mean butalbital concentration was 13.8 mg/L (range, 1.9–31 mg/L) compared with a mean of 7.1 mg/L (range, 1.6–22 mg/L) for butalbital concentrations in 8 nondrug-related fatalities.[55] The butalbital-related fatalities were single-drug fatalities with the exception of ethanol, nicotine, and caffeine.

Large doses of butalbital may cross react with some urine immunoassays reportedly specific for phenobarbital (e.g., EMIT® 2000 Phenobarbital Assay, Syva Co., San Jose, CA).[76]

TREATMENT

The treatment of butalbital is supportive. This drug is a CNS depressant that may cause respiratory and cardiovascular depression during severe intoxication. The treatment of acute butalbital intoxication is similar to other barbiturates (See Amobarbital Treatment). Common clinical practice of patients chronically using butalbital containing analgesics is to taper the dose by 1 tablet every 2–3 days, when daily use is <10 tablets daily. Supervised withdrawal is usually necessary when the daily consumption exceeds 10 tablets or outpatient withdrawal is unsuccessful as a result of medical or psychiatric conditions. For the use of phenobarbital loading for prevention of barbiturate withdrawal, the conversion ratio of 30 mg phenobarbital to 100 mg butalbital converts to 300 mg phenobarbital for patients ingesting 20 50-mg butalbital tablets daily.

PENTOBARBITAL

IDENTIFYING CHARACTERISTICS

Pentobarbital [5-ethyl-5-(1-methylbutyl)-barbituric acid, CAS RN: 76-74-4] is a racemate; the stereocenter is in the 2-position. Table 23.6 lists some physiochemical properties for pentobarbital. Figure 23.4 displays the chemical structure of pentobarbital. Street names for

TABLE 23.6. Some Physiochemical Properties of Pentobarbital.

Physical Property	Value
Melting Point	129.5°C (265.1°F)
pKa	8.11 (25°C/77°F)
log P (Octanol-Water)	2.1
Water Solubility	679 mg/L (25°C/77°F)
Vapor Pressure	3.02E–10 mm Hg (25°C/77°F)
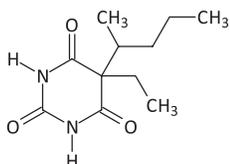
FIGURE 23.4. Chemical structure of pentobarbital.

pentobarbital include Nebbie, Nembie, Yellow Bullet, Yellow Doll, Yellow Football, and Yellow Jacket. Propylene glycol is a constituent of IV preparations of both pentobarbital and phenobarbital.

EXPOSURE

Epidemiology

Similar to amobarbital and secobarbital, epidemiologic data indicate that the prevalence of pentobarbital abuse began declining in the 1960s and 1970s as benzodiazepines began replacing barbiturates as preferred sedative hypnotic drugs. The yearly average US emergency department visits for pentobarbital misuse/abuse reported by the DAWN network decreased from 678 in the late 1970s to 195 in the mid-1980s.[11] During these times, drug misuse/abuse related deaths reported by DAWN medical examiner facilities in the United States also decreased from 214 to 52.

Sources

Pentobarbital (Nembutal) is available as an injectable solution of 50 mg/mL for sedation and the induction of barbiturate coma. This preparation contains 40% propylene glycol and the pH is adjusted to 9.5. This drug is also available as a veterinary drug for euthanasia; additionally, pentobarbital is a drug of choice for assisted suicide.

Methods of Abuse

The abuse liability of pentobarbital is greater than most other prescription hypnotic drugs based on reinforcement, actual abuse, memory impairment, toxicity, and animal studies.[77] Heroin addicts use IV barbiturates as a substitute for heroin when heroin is unavailable; occasionally, both drugs are abused together.[78]

DOSE EFFECT

The adult IV hypnotic dose of pentobarbital is initially 100 mg, followed by repeat doses up to 200–500 mg total dose with close observation and monitoring of vital signs. The IV dose in children is 1–3 mg/kg to a maximum of 100 mg until asleep. The loading dose for barbiturate coma is 5–10 mg/kg given slowly over 1–2 hours with close monitoring of blood pressure and oxygen saturation. The initial maintenance infusion is 1 mg/kg/hour with an increase to 2–3 mg/kg/hour as required for the desired clinical endpoint. The pentobarbital dose that produces intoxication depends on tolerance. Ingestion of 400 mg pentobarbital daily may cause inebriation initially, but signs of intoxication disappear with daily dosing over 2–3 weeks based on studies of healthy volunteers with histories of narcotic violations.[79] In these studies, cessation of 400 mg pentobarbital daily for 90–360 days is not usually associated with abstinence symptoms, whereas all 18 men in a study at an addiction center ingesting 900–2,200 mg pentobarbital daily for over 30 days developed symptoms of withdrawal. Delirium developed in 12 of these men; 14 men had withdrawal seizures. Thirty milligrams of phenobarbital is equivalent to 100 mg pentobarbital.

Predicting toxicity based on the estimated pentobarbital dose is complicated by several variables including tolerance, time of supportive care in relation to the ingestion, and concomitant administration of other CNS depressants (e.g., ethanol, sedative hypnotic drugs). The ingestion of 2–3 g pentobarbital is potentially fatal. A 24-year-old woman was unresponsive to verbal or physical stimulation with stable vital signs (stage 3 coma) after the ingestion of an estimated dose of 3,500 mg pentobarbital, whereas a 20-year-old woman was apneic after an estimated ingestion of 5,000 mg pentobarbital.[80] Both women survived with supportive care. Their medication history was not reported, but no other drugs were detected in their blood.

TOXICOKINETICS

The GI absorption of pentobarbital is relatively rapid, similar to secobarbital. The volume of distribution (V_d)

of pentobarbital is relatively small (0.8–1 L/kg) with protein binding of approximately 65%. Pharmacokinetic studies indicate that protein binding varies between the two enantiomers. In a study of 7 healthy volunteers receiving 100 mg racemic pentobarbital, the mean protein binding of the *R*- and *S*-enantiomers were 63.4% and 73.5%, respectively.[81] The V_d of the *R*-enantiomer was approximately 13% larger than the *S*-enantiomer with a mean volume of distribution (V_d) of 1.24 ± 0.55 L/kg and 1.11 ± 0.52 L/kg, respectively. The liver metabolized almost all of the pentobarbital dose, primarily to 3′-hydroxypentobarbital and *N*-hydroxypentobarbital.

The kidney excretes very little (<1%) of the pentobarbital dose as parent drug.[82] The terminal plasma half-life of pentobarbital following therapeutic doses is about 20–30 hours.[83] The estimated average pentobarbital half-life in blood was 20 hours following nonfatal pentobarbital poisoning, as measured by UV spectrophotometry.[28] Some differences exist in the clearance of enantiomers of pentobarbital. In a study of 7 volunteers receiving 100 mg racemic pentobarbital, the median clearance of the (*R*)-enantiomer was about 25% higher than the (*S*)-enantiomer (43 mL/min and 33 mL/min, respectively).[81]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Pentobarbital interacts with the GABA receptor-ionophore complex, binding the γ-aminobutyric acid type A (GABA$_A$) receptor. Electrophysiologic studies suggest that pentobarbital and other barbiturates exert at least some of their CNS effects by increasing the mean channel open time of the chloride ion channel in the GABA receptor-ionophore complex.[84,85] Aliphatic-substituted barbiturates (e.g., pentobarbital, secobarbital) significantly enhance GABA receptor-coupled responses in comparison to the lack of responses with phenyl-substituted barbiturates (e.g., phenobarbital).[86] At low micromolar concentrations, pentobarbital potentiates GABA$_A$-evoked responses, whereas high micromolar concentrations open the GABA$_A$ receptor downstream of the middle of the M2 domain.[87] At millimolar concentrations, pentobarbital reduces the response of the GABA$_A$ receptor. Pentobarbital is a CNS and cardiovascular depressant similar to other short-acting and intermediate-acting barbiturates.

CLINICAL RESPONSE

Similar to other short-acting and intermediate-acting barbiturates, pentobarbital produces coma, apnea, and hypotension following the ingestion of high doses. During the 1960s and 1970s, pentobarbital and secobarbital accounted for a large percentage of serious drug intoxications.[88] Clinically significant hypothermia may complicate severe pentobarbital intoxication, particularly following prolonged exposure during pentobarbital-induced coma. These patients may survive without clinically significant sequelae despite prolonged ventricular fibrillation and cardiopulmonary resuscitation during the period of severe hypothermia.[89] Prolonged use of pentobarbital and phenobarbital infusions for barbiturate coma may cause propylene glycol toxicity, manifest by lactic acidosis, refractory hypotension, hyperosmolality, hemolysis, and renal failure.[90] The abstinence syndrome associated with pentobarbital is similar to other short-acting (i.e., secobarbital) and intermediate-acting (e.g., amobarbital, butalbital) barbiturates.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the quantitation of pentobarbital in biologic samples include gas chromatography with flame ionization detection,[91] liquid chromatography/electrospray/mass spectrometry,[40] and GC/MS.[45,92] The LOD for pentobarbital in serum using the latter 2 methods is 0.50 mg/L. The LLOQ for pentobarbital in blood and urine using GC/MS is similar to amobarbital (0.050 mg/L and 0.020 mg/L, respectively) with a coefficient of variance near 7–9%.[42] Pentobarbital is detectable in hair following solid phase microextraction and gas chromatography/chemical ionization/tandem mass spectrometry; LOD and LLOQ with this method are 0.07 ng/mg and 0.1 ng/mg, respectively.[93] Pentobarbital is relatively stable in unpreserved blood and liver tissue at 4°C (39.2°F) and 25°C (77°F) for 2–3 months at pH 6.4–7.0. Under these storage conditions, pentobarbital did not deteriorate over 2 months of storage.[48] Increasing degradation of proteins to amines results in a more alkaline environment in the decaying body compared with the first 24 hours after death. *In vitro* studies indicate that pentobarbital is relatively stable in formaldehyde solutions (37%) present in embalming preparations. Over 1 month, degradation of pentobarbital in 37% formaldehyde solution at pH 7 and 9.5 was <13% and 30%, respectively. Substantial amounts of pentobarbital are detectable in liver tissue and in formalin at least 20 months after death.[47] Storage of plasma containing pentobarbital at –20°C (–4°F) resulted in minimal degradation (2.5%) of the pentobarbital as measured by gas chromatography with flame ionization detection.[94]

Biomarkers

The mean plasma pentobarbital concentration in 5 patients a half-hour after receiving 600 mg pentobarbital over the preceding 3 hours was 3.0 mg/L, declining to 2.0 mg/L approximately 8 hours later.[49] All of these patients demonstrated signs of intoxication (sedation, unsteady gait, poor balance, and coordination) during the study. McCarron et al. evaluated 1,140 cases of short-acting barbiturate intoxication and correlated an intoxication score with serum barbiturate concentrations as measured by UV spectrophotometry.[95] Ultraviolet spectrophotometry does not distinguish between the parent barbiturate and metabolites; therefore, this method is not as specific as GC/MS. Table 23.7 outlines the results of their work. This table provides a broad guideline for the interpretation of serum pentobarbital and secobarbital concentrations, depending on tolerance and the concomitant ingestion of other CNS depressants. A 24-year-old woman was comatose with stable vital signs (Reed State 3 coma) after ingesting pentobarbital.[80] Her serum pentobarbital concentration was 13.6 mg/L 4 hours after ingestion with no other drugs detected as measured by high performance liquid chromatography and GC/MS. In the same series, a mildly hypotensive, comatose 31-year-old woman had a serum pentobarbital concentration of 29.82 mg/L 12 hours after pentobarbital ingestion. She also had no other drugs detected in her serum. In 2 comatose patients with pentobarbital intoxication, the maximum serum pentobarbital concentrations were 26 mg/L and 27 mg/L as measured by gas chromatography.[50] The duration of coma was 43 hours and 27 hours, respectively; neither patient required intubation and ventilatory support. As measured by UV spectrophotometry, blood pentobarbital concentrations of 15–25 mg/L are associated with light coma (reflexes present) and >35 mg/L with severe intoxication (comatose with unstable vital signs).[28]

In a convenience series of 15 cases of death associated with pentobarbital intoxication, the lowest pentobarbital concentration in postmortem blood was 12 mg/L by UV spectrophotometry.[53] The report did not include sample site, clinical details, or the presence of other drugs in postmortem specimens. In a series of 43 cases involving pentobarbital as the cause of death, the postmortem blood (site, analytic method NOS) ranged from 12–88 mg/L.[52] The peripheral postmortem pentobarbital concentrations in 2 men found dead after deliberately ingesting veterinary pentobarbital sodium were 8.6 mg/L and 27 mg/L.[96] Stomach samples from these two cases contained high concentrations of pentobarbital (4.3 g and 1.27 g, respectively). The report did not include the presence or absence of drugs other than pentobarbital. Propylene glycol is a diluent for iv pentobarbital preparations that is usually present in postmortem blood of individuals dying of pentobarbital intoxication from IV preparations.[97]

TREATMENT

The treatment of pentobarbital intoxication is supportive, similar to the treatment of amobarbital poisoning. The main life-threatening complication are CNS and cardiopulmonary depression. Dysrhythmias are uncommon during pentobarbital intoxication, and the presence of QTc prolongation suggests the presence of other drugs (e.g., tricyclic antidepressants).[80] In general,

TABLE 23.7. Correlation of Serum Pentobarbital and Secobarbital Concentrations with Clinical Effects in Nonbarbiturate Dependent Individuals.[95]

Clinical Stage	Clinical Features	Serum Concentration (mg/L)*
Alert	No signs of CNS depression	<6
Drowsy	CNS depression between alert and stuporous	8 ± 2
Stuporous	Markedly sedated, responsive to verbal or tactile stimuli	14 ± 3
Coma 1	Responsive to painful stimuli, unresponsive to verbal or tactile stimuli, stable vital signs	18 ± 2
Coma 2	Unresponsive to verbal or painful stimuli, stable vital signs	22 ± 2
Coma 3	Unresponsive to all stimuli or abnormal response to painful stimuli, abnormal spontaneous respirations (slow, shallow, or rapid) and/or low blood pressure with adequate perfusion	26 ± 2
Coma 4	Unresponsive to all stimuli or abnormal response to painful stimuli, apnea or inadequate respirations and/or inadequate blood pressure	34 ± 6

Abbreviation: CNS = Central nervous system.

*Tolerance reduces the response to the listed serum concentration, whereas concomitant ingestion of CNS depressants (e.g., ethanol, other sedative hypnotic drugs) increases the response at a given concentration.

the rate of removal of short- and intermediate-acting barbiturates is slow and the total recovery of pentobarbital in the dialysate is small compared with the absorbed dose. Estimated clearance of pentobarbital during hemodialysis and hemoperfusion is about 20–40 mg/min and 50–120 mL/min compared with a calculated clearance of 7.6 mL/min during 48 hours of continuous venovenous hemodiafiltration for a fatal pentobarbital intoxication.[98] The removal of pentobarbital in this fatal case was about 15% of the estimated pentobarbital dose. During hemodialysis of a 21-year-old woman with severe pentobarbital and secobarbital overdose, the calculated clearance of pentobarbital and secobarbital was 26.6 mL/min with estimated removal of 1.3–3.6 mg/min.[99] She underwent prolonged dialysis (68 hours) with some deterioration in her clinical condition following the temporary cessation of dialysis. There are no antidotes for pentobarbital poisoning.

SECOBARBITAL

IDENTIFYING CHARACTERISTICS

Secobarbital (5-allyl-5-(1-methylbutyl)-barbituric acid, CAS RN: 76-73-3) is a highly lipophilic compound hypnotic drug that is marketed as seconal (secobarbital sodium, CAS RN: 309-43-3). Table 23.8 lists some physiochemical properties of secobarbital (quinalbarbitone). Street names for secobarbital include Blues & Reds, M & Ms, Pinks, Reds & Ripple (R & R), Red Birds, Red Bullets, Reds, Seccy, and Seggy. Figure 23.5 displays the chemical structure of secobarbital.

EXPOSURE

Similar to amobarbital and pentobarbital, epidemiologic data indicate that the prevalence of secobarbital abuse began declining in the 1960s and 1970s as benzodiazepines began replacing barbiturates as preferred sedative hypnotic drugs. The yearly average US emergency department visits for secobarbital misuse/abuse reported by the Drug Abuse Warning Network (DAWN) decreased from 3,626 in the late 1970s to 927 in the mid-1980s.[11] During these times, drug misuse/abuse related deaths reported by DAWN medical examiner facilities in the United States also decreased from 463 to 123. Both secobarbital and amobarbital were among the prescription drugs with the highest reported drug-related deaths per dispensed prescriptions during the 1970s and 1980s. Secobarbital is a schedule II drug in the United States, marketed as 100 mg capsules. Abuse of secobarbital usually involves the ingestion of secobarbital tablets, although occasionally secobarbital causes the death of IV drug abusers.[100]

DOSE EFFECT

In a study at an addiction research center, the daily ingestion of 400 mg secobarbital for 1 year did produce symptoms of withdrawal, whereas minor withdrawal symptoms developed after the daily ingestion of 600 mg pentobarbital for 4 weeks including weakness, tremor, nausea, vomiting, anxiety, and insomnia.[101] More serious withdrawal symptoms occurred after the daily ingestion of 800 mg secobarbital for over 1 month. Thirty milligrams of phenobarbital is equivalent to 100 mg secobarbital. Secobarbital and pentobarbital produce addiction and the abstinence syndrome at equivalent doses.[79]

Predicting toxicity based on the estimated secobarbital dose is complicated by several variables including tolerance, time of supportive care in relation to the ingestion, and concomitant administration of other CNS depressants (e.g., ethanol, sedative hypnotic drugs). The ingestion of 2–3 g secobarbital is potentially fatal in nontolerant individuals. The ingestion 1,750 mg secobarbital by a 47-year-old woman was associated with hypotension, respiratory failure, and death 3 days after ingestion.[88] The report did not include a medication history. Studies of chronically intoxicated healthy adults indicate that some individuals remain ambulatory despite daily secobarbital or pentobarbital doses of 2.2 g.

TABLE 23.8. Some Physiochemical Properties of Secobarbital.

Physical Property	Value
Molecular Weight	238 g/mol
Melting Point	100°C
pKa	7.8
log P (Octanol-Water)	1.97
Water Solubility	550 mg/L (25°C/77°F)
Vapor Pressure	3.36E-10 mm Hg (25°C/77°F)
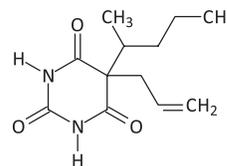
FIGURE 23.5. Chemical structure of secobarbital.

TOXICOKINETICS

Kinetics

The GI absorption of secobarbital is relatively rapid. Following ingestion of 3.3 mg secobarbital/kg by 6 healthy men, the peak whole blood secobarbital concentration occurred between 2–4 hours after ingestion; the mean whole blood secobarbital concentration at 3 hours was 2.01 mg/L.[102] The mean volume of distribution in 7 medical students and staff volunteers was 1.66 L/kg.[103] Volunteer studies suggest that the major urinary metabolite of secobarbital include 5-(2′,3′-dihydroxypropyl)-5-(1′-methylbutyl)-barbituric acid, 5-allyl-5-(3′-hydroxy-1′-methylbutyl)-barbituric acid, and 5-allyl-5-(1′-methyl-3′-carboxypropyl)-barbituric acid.[104] The elimination half-life for secobarbital is relatively long. In a study of medical students and staff volunteers, the mean biologic half-life was 19.3 hours.[103] The calculated biologic half-life in a study of 6 healthy men was 28.9 hours.[102]

Drug Interactions

The drug interactions of secobarbital are similar to other short- and intermediate-acting drugs including the synergistic action of other CNS depressants on the clinical effects of secobarbital. In healthy volunteers receiving 2 mg secobarbital/kg and 0.2 mg morphine/kg intravenously, both drugs alone caused ventilatory depression compared with baseline; the combination caused greater and more prolonged reduction in arterial oxygenation than either drug alone.[105] At 5 minutes after IV administration, the mean arterial oxygen saturations were as follows: morphine alone, 86.6 ± 7.27 mm Hg ($P < .05$); secobarbital alone, 90.1 ± 6.21 mm Hg (P not significant); and morphine/secobarbital together, 80.7 ± 10.64 mm Hg ($P < .01$). Rodent studies indicate that chronic ethanol consumption of ethanol does not produce cross-reactivity with highly lipid soluble barbiturates (e.g., secobarbital) based on responses to hypothermia, tilt-lane, and rotarod.[106] *In vitro* studies indicate that secobarbital is a selective inactivator of CYP2B1.[107]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Similar to other short and intermediate acting barbiturates, secobarbital interacts with the GABA receptor-ionophore complex, binding the γ-aminobutyric acid type A (GABA$_A$) receptor and increasing the mean channel open time of the chloride ion channel in the GABA receptor-ionophore complex. Secobarbital is a cardiorespiratory and CNS depressant. Postmortem findings are nonspecific with signs of asphyxia.

CLINICAL RESPONSE

All short- and intermediate-acting barbiturates produce clinical effects similar to ethanol intoxication, manifest by dysarthria, ataxia, nystagmus, lightheadedness, impaired cognition, poor judgment, loss of inhibitions, and confusion. Complications of serious secobarbital intoxication include apnea, hypotension, and hypothermia. The abstinence syndrome associated with secobarbital is similar to other short-acting (e.g., pentobarbital) and intermediate-acting (e.g., amobarbital, butalbital) barbiturates.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the quantitation of secobarbital include gas chromatography with flame ionization detection,[91] high performance liquid chromatography,[108] gas chromatography/mass spectrometry (GC/MS),[45] and GC/MS in selective ion monitoring mode with barbital as the internal standard.[109] The LOD for secobarbital in serum using the latter method is 0.5 mg/L. The LLOQ for secobarbital in blood and urine using GC/MS is similar to amobarbital (0.050 mg/L and 0.020 mg/L, respectively) with a coefficient of variance near 5–7%.[42] Analysis of the overall precision of GC/MS with solid phase extraction demonstrates a coefficient of variation in the range of 2%.[110] Micellar liquid chromatography allows the analysis of complex matrices for secobarbital without extraction. The LOD for secobarbital using this method with UV detection is approximately 0.064 mg/L.[46] Secobarbital is relatively stable in unpreserved blood and liver tissue at 4°C (39.2°F) and 25°C (77°F) for 2–3 months at pH 6.4–7.0. Under these storage conditions, secobarbital concentrations in samples stored at 4°C and 25°C decreased $91 \pm 12\%$ and $97 \pm 16\%$, respectively over 2 months of storage.[48] Substantial amounts of secobarbital are detectable in liver tissue and in formalin at least 20 months after death.[47] Typically, immunoassays (e.g., enzyme multiplied immunoassay technique, fluorescence polarization immunoassay) are very sensitive to secobarbital because the antibodies for these tests usually are raised against secobarbital.[38]

Biomarkers

The typical therapeutic serum concentration of secobarbital is 1–2 mg/L with toxic symptoms appearing in most

patients with secobarbital concentrations exceeding 8 mg/L. The mean plasma secobarbital concentration in 5 patients a half-hour after receiving 600 mg secobarbital over the preceding 3 hours was 4.3 mg/L, declining to 3.4 mg/L approximately 8 hours later.[49] All of these participants demonstrated signs of intoxication (sedation, unsteady gait, poor balance, and coordination) during the study. As measured by UV spectrophotometry, blood secobarbital concentrations of 12–17 mg/L are associated with light coma (reflexes present) and 35 mg/L with severe intoxication (comatose with unstable vital signs).[28] Serum concentrations of secobarbital and pentobarbital produce similar clinical effects as outlined in Table 23.7.

The highest secobarbital concentrations following fatal overdose occur in the blood, liver, and brain.[111] In a series of 108 cases involving secobarbital as the cause of death, the postmortem blood (site, analytic method NOS) ranged from 11–60 mg/L.[52] The report did not include the presence or absence of other drugs other than a comment that secobarbital poisoning was the cause of death. In postmortem blood (site NOS) from 16 secobarbital-related fatalities, the mean secobarbital concentration was 17.6 mg/L (range, 6.2–37 mg/L) compared with a mean of 5.5 mg/L (range, 1.3–21 mg/L) for secobarbital concentrations in 12 nondrug-related fatalities.[55] The secobarbital-related fatalities were single-drug fatalities with the exception of ethanol, nicotine, and caffeine. Animal studies suggest that substantial postmortem redistribution of secobarbital occurs after death.[112]

TREATMENT

Secobarbital is a CNS depressant that causes respiratory and cardiovascular depression during severe intoxication. The treatment of secobarbital intoxication is similar to other barbiturates (e.g., amobarbital). Similar to other barbiturates, hourly phenobarbital loading is an option for the treatment of secobarbital withdrawal. In a case series of 7 patients undergoing detoxification for secobarbital abuse, the mean phenobarbital loading dose was 31.7 ± 15.0 mg/kg with a mean total phenobarbital dose of 1,560 ± 830 mg.[113]

References

1. Carter MK. The history of barbituric acid. J Chem Educ 1951;28:525–528.
2. Fischer E, von Mering J. Uber ein neue klass von schlafmitteln. Ther Gegenwart 1903;44:97–101.
3. Lopez-Monoz F, Ucha-Udae R, Alamo C. The history of barbiturates a century after their clinical introduction. Neuropsychiatr Dis Treat 2005;1:329–343.
4. Hauptmann A. Luminal bei epilepsie. Munch Med Wochenschr 1912;59:1907.
5. Klaesi J. Uber die therapeutische anwendung der "Dauernarkos" mittels somnifens bei schizophrenen. Zeitsch Gesamte Neurol Psychiatrie 1922;74:557–592.
6. Tabern DL, Volwiler EH. Sulfur-contained barbiturate hypnotics. J Am Chem Soc 1935;57:1961–1963.
7. Hollister LE. The pre-benzodiazepine era. J Psychoactive Drugs 1983;15:9–13.
8. Zonana HV. Hypnosis, sodium Amytal, and confessions. Bull Am Acad Psychiatry Law 1979;7:18–28.
9. Forrest JA, Tarala RA. Abuse of drugs "for kicks": a review of 252 admissions. Br Med J 1973;4(5885):136–139.
10. Brooke EM, Glatt MM. More and more barbiturates. Med Sci Law 1964;4:277–282.
11. Harold Davis, Baum C, Graham DJ. Indices of drug misuse for prescription drugs. Int J Addict 1991;26:777–795.
12. Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Drug Abuse Warning Network, 2006: National Estimates of Drug-Related Emergency Department Visits. DAWN Series D-30, DHHS Publication No. (SMA) 08-4339, Rockville, MD, 2008.
13. Terplan M, Unger AM. Survival following massive barbiturate ingestion. JAMA 1966;198:232–233.
14. Isbell H, White WM. Clinical characteristics of addictions. Am J Med 1953;14:558–565.
15. Watson J, Radford MD. Blood barbiturate levels in a patient dependent upon amylobarbitone sodium. Psychol Med 1970;1:178–180.
16. Inaba T, Tang BK, Endrenyi L, Kalow W. Amobarbital—a probe of hepatic drug oxidation in man. Clin Pharmacol Ther 1976;20:439–444.
17. Tang BK, Inaba T, Kalow W. N-hydroxyamobarbital: the second major metabolite of amobarbital in man. Drug Metab Dispos 1975;3:479–486.
18. Baldeo W, Gilbert JN, Powell JW, Jones LV, Whtehouse MJ. A new metabolite of amylobarbitone: 5-(3'-carboxybutyl)-5-ethylbarbituric acid. J Pharm Pharmacol 1977;29:254.
19. Tang BK, Grey AA, Reilly PA, Kalow W. Species differences of amobarbital metabolism: dihydroxyamobarbital formation. Can J Physiol Pharmacol 1980;58:1167–1169.
20. Endrenyi L, Inaba T, Kalow W. Genetic study of amobarbital elimination based on its kinetics in twins. Clin Pharmacol Ther 1976;20:701–714.
21. Inaba T, Tang BK, Endrenyi L, Kalow W. Amobarbital—a probe of hepatic drug oxidation in man. Clin Pharmacol Ther 1976;20:439–444.
22. Balasubramaniam K, Lucas SB, Mawer GE, Simons PJ. The kinetics of amylobarbitone metabolism in healthy men and women. Br J Pharmacol 1970;39:564–572.

23. Draffan GH, Dollery CT, Davies Ds, Krauer B, Williams FM, Clare RA, et al. Maternal and neonatal elimination of amobarbital after treatment of the mother with barbiturates during late pregnancy. Clin Pharmacol Ther 1976;19:271–275.

24. Krauer B, Draffan GH, Williams FM, Clare RA, Dollery CT, Hawkins DF. Elimination kinetics of amobarbital in mothers and their new born infants. Clin Pharmacol Ther 1973;14:442–447.

25. Balasubramaniam K, Mawer GE, Pohl JE, Simons PJ. Impairment of cognitive function associated with hydroxyamylobarbitone accumulation in patients with renal insufficiency. Br J Pharmacol 1972;45:360–367.

26. Mawer GE, Lee HA. Value of forced diuresis in acute barbiturate poisoning. Br Med J 1968;2(5608):790–793.

27. Grove J, Toseland PA. The prognostic value of the hydroxyamylobarbitone/amylobarbitone ratio. Arch High Rada Toksikol 1970;21:353–362.

28. Sunshine I, Hackett E. Chemical findings in patients with acute nonfatal barbiturate intoxication. Clin Chem 1957;3:125–134.

29. Misra AL, Pontani RB, Mulé SJ. (2-^{14}C) Amobarbital metabolism in tolerant rats. Xenobiotica 1974;4:409–423.

30. Faulkner TP, McGinity JW, Hayden JH, Olson DA, Comstock EG. Pharmacokinetic studies on tolerance to multiple doses of sedative-hypnotics in a polydrug abuse population. II. Secobarbital-amobarbital. J Clin Pharmacol 1979;19:605–616.

31. Stevenson IH, Browning M, Crooks J, O'Malley K. Changes in human drug metabolism after long-term exposure to hypnotics. Br Med J 1972;4:322–324.

32. Thompson SA, Whiting PJ, Wafford KA. Barbiturate interactions at the human GABAA receptor: dependence on receptor subunit combination. Br J Pharmacol 1996;117:521–527.

33. Ffrench-Mullen JM, Barker JL, Rogawski MA. Calcium current block by (−)-pentobarbital, phenobarbital, and CHEB but not (+)-pentobarbital in acutely isolated hippocampal CA1 neurons: comparison with effects on GABA-activated Cl- current. J Neurosci 1993;13:3211–3221.

34. Reed CE, Driggs MF, Foote CC. Acute barbiturate intoxication: a study of 300 cases based on a physiologic system of classification of the severity of the intoxication. Ann Intern Med 1952;37:290–303.

35. Arieff AI, Friedman EA. Coma following nonnarcotic drug overdosage: management of 208 adult patients. Am J Med Sci 1973;266:405–426.

36. Petick D, Timmermann G, Czeizel AE, Acs N, Banhidy F. A study of the teratogenic and fetotoxic effects of large doses of amobarbital used for a suicide attempt by 14 pregnant women. Toxicol Ind Health 2008;24:79–85.

37. Nelson MM, Forfar JO. Associations between drugs administered during pregnancy and congenital abnormalities of the fetus. Br Med J 1971;1(5748):523–527.

38. Charlier CJ, Polmteux GJ. Evaluation of Emit® Tox benzodiazepine and barbiturate assays on the Vitalab Viva analyser and FPIA on the Abbott ADx analyser. Clin Chem Lab Med 2000;38:615–618.

39. Schwenzer KS, Peralman R, Tsilimidos M, Salamone SJ, Cannon RC, Wong SH, et al. New fluorescence polarization immunoassays for analysis of barbiturates and benzodiazepines in serum and urine: performance characteristics. J Anal Toxicol 2000;24:726–732.

40. Hori Y, Fujisawa M, Shimada K, Hirose Y, Yoshioka T. Method for screening and quantitative determination of serum levels of salicylic acid, acetaminophen, theophylline, phenobarbital, bromvalerylurea, pentobarbital, and amobarbital using liquid chromatography/electrospray mass spectrometry. Biol Pharm Bull 2006;29:7–13.

41. Saka K, Uemura K, Shintani-Ishida K, Yoshida K-I. Determination of amobarbital and phenobarbital in serum by as chromatography-mass spectrometry with addition of formic acid to the solvent. J Chromatogr B 2008;869:9–15.

42. Meatherall R. GC/MS confirmation of barbiturates in blood and urine. J Forensic Sci 1997;42:1160–1170.

43. Tang BK, Inaba T, Kalow W. Amobarbital metabolism in man determination of N-hydroxyamobarbital and 3'-hydroxyamobarbital in urine by gas chromatography chemical ionization mass spectrometry. Biomed Mass Spectrom 1977;4:73–76.

44. Quiñones-Torrelo C, Martín-Biosca Y, Sagrado S, Villanueva-Camañas RM, Medina-Hernández MJ. Determination of amobarbital and secobarbital in plasma samples using micellar liquid chromatography. Biomed Chromatogr 2000;14:287–292.

45. Maurer HH. Identification and differentiation of barbiturates, other sedative-hypnotics and their metabolites in urine integrated in a general screening procedure using computerized gas chromatography-mass spectrometry. J Chromatogr 1990;530:307–326.

46. Capella-Peiro ME, Gil-Agusti M, Martinavarro-Dominguez A, Estve-Romero J. Determination of serum of some barbiturates using micellar liquid chromatography with direct injection. Anal Biochem 2002;309:261–268.

47. Sunshine I, Hackett E. Chemical findings in cases of fatal barbiturate intoxication. J Forensic Sci 1957;2:149–158.

48. Levine BS, Blanke RV, Valentour JC. Postmortem stability of barbiturates in blood and tissues. J Forensic Sci 1984;29:131–138.

49. Parker KD, Crim M, Elliott HW, Wright JA, Nomof N, Hine CH. Blood and urine concentrations of subjects receiving barbiturates, meprobamate, glutethimide, or diphenylhydantoin. Clin Toxicol 1970;31:131–145.

50. Forrest JA, Roscoe P, Prescott LF, Stevenson IH. Abnormal drug metabolism after barbiturate and paracetamol overdose. Br Med J 1974;4:499–502.

51. Carroll BJ. Barbiturate overdosage: presentation with focal neurological signs. Med J Aust 1969;1:1133–1135.

52. Gupta RC, Kofoed J. Toxicological statistics for barbiturates, other sedatives, and tranquillizers in Ontario: a 10-year survey. Can Med Assoc J 1966;94:863–865.

53. Gillett R, Warburton FG. Barbiturate blood levels found at necropsy in proven cases of acute barbiturate poisoning. J Clin Pathol 1970;23:435–439.

54. Baselt RC, Cravey RH. A compendium of therapeutic and toxic concentrations of toxicologically significant drugs in human biofluids. J Anal Toxicol 1977;1:81–103.

55. Caplan YH, Ottinger WE, Crooks CR. Therapeutic and toxic drug concentrations in post mortem blood: a six year study in the state of Maryland. J Anal Toxicol 1983; 7:225–230.

56. Benko A. Toxicological analysis of amobarbital and glutethimide from bone tissue. J Forensic Sci 1985; 30:708–714.

57. Linton AL, Ledingham IM. Severe hypothermia with barbiturate intoxication. Lancet 1966;1:24–26.

58. Sellers EM, Khouw V, Dolman L. Comparative drug adsorption by activated charcoal. J Pharm Sci 1977; 66:1640–1641.

59. Iversen BM, Willassen Y, Bakke OM, Wallem G. Assessment of barbiturate removal by charcoal hemoperfusion in overdose cases. Clin Toxicol 1979;15: 139–149.

60. Roberts DM, Buckley NA. Enhanced elimination in acute barbiturate poisoning—a systematic review. Clin Toxicol 2011;49:2–12.

61. Robinson GM, Sellers EM, Janecek E. Barbiturate and hypnosedative withdrawal by a multiple oral phenobarbital loading dose technique. Clin Pharmacol Ther 1981; 30:71–76.

62. Sullivan JT, Sellers EM. Treatment of the barbiturate abstinence syndrome. Med J Aust 1986;145:456–458.

63. Caviness VS Jr, O'Brien P. Current concepts. Headache. N Engl J Med 1980;302:446–450.

64. Silberstein SD, McCrory DC. Butalbital in the treatment of headache: history, pharmacology, and efficacy. Headache 2001;41:953–967.

65. Wenzel RG, Sarvis CA. Do butalbital-containing products have a role in the management of migraine? Pharmacotherapy 2002;22:1029–1035.

66. Raja M, Altavista MC, Azzoni A, Albanese A. Severe barbiturate withdrawal syndrome in migrainous patients. Headache 1996;36:119–121.

67. Bevenyi P, Rideout J, Schneiderman J. Abuse of a commonly prescribed analgesic preparation. Can Med Assoc J 1985;133:294–296.

68. Preskorn SH, Scwin RL, McKnelly WV. Analgesic abuse and the barbiturate abstinence syndrome. JAMA 1980; 244:369–370.

69. Loder E, Biondi D. Oral phenobarbital loading: a safe and effective method of withdrawing patients with headache from butalbital compounds. Headache 2003;43: 904–909.

70. Drost ML, Walter L. Blood and plasma concentrations of butalbital following single oral doses in man. J Anal Toxicol 1988;12:322–324.

71. Romero CDE, Baron JD, Knox AP, Hinchey JA, Roper AH. Barbiturate withdrawal following Internet purchase of Fioricet. Arch Neurol 2004;61:1111–1112.

72. Ostrea EM Jr. Neonatal withdrawal from intrauterine exposure to butalbital. Am J Obstet Gynecol 1982; 143:597–599.

73. Ferslew KE, Hagardorn AN, McCormick WF. Application of micellar electrokinetic capillary chromatography to forensic analysis of barbiturates in biological fluids. J Forensic Sci 1995;40:245–249.

74. Pistos C, Stewart JT. Assay for the simultaneous determination of acetaminophen-caffeine-butalbital in human serum using a monolithic column. J Pharmaceut Biomed Anal 2004;26:737–741.

75. Cingolani M, Cippitelli M, Froldi R, Tassoni G, Mirtella D. Stability of barbiturates in fixed tissues and formalin solutions. J Anal Toxicol 2005;29:205–208.

76. Nordt SP. Butalbital cross-reactivity to an EMIT assay for phenobarbital. Ann Pharmacother 1997;31:254–255.

77. Griffiths RR, Johnson MW. Relative abuse liability of hypnotic drugs: a conceptual framework and algorithm for differentiating among compounds. J Clin Psychiatry 2005;66(suppl 9):S31–S41.

78. Smith De, Wesson Dr, Lannon RA. New developments in barbiturate abuse. Clin Toxicol 1970;3:57–65.

79. Fraser HF, Wikler A, Essig CF, Isbell H. Degree of physical dependence induced by secobarbital or pentobarbital. JAMA 1958;166:126–129.

80. Koyama K, Suzuki R, Yoshida T, Kikuno T. Usefulness of serum concentration measurements for acute pentobarbital intoxication in patients. Chudoku Kenkyo 2007;20:45–53.

81. Cook CE, Seltzman TB, Tallent CR, Lorenzo B, Drayer DE. Pharmacokinetics of pentobarbital enantiomers as determined by enantioselective radioimmunoassay after administration of racemate to humans and rabbits. J Pharmacol Exp Ther 1987;241:779–785.

82. Tang BK, Inaba T, Kalow W. *N*-hydroxylation of pentobarbital in man. Drug Metab Disp 1977;5:71–74.

83. Baldeo WC, Gilbert JN, Powell JW. Multidose studies in the human metabolism of pentobarbitone. Eur J Drug Metab Pharmacokinet 1980;5:75–80.

84. Yakushiji T, Oyama Y, Akaike N. Comparative study on barbiturates using isolated single neurons: GABA-mimetic action and augmentatory action on GABA response. Brain Res 1989;488:357–360.

85. Miller LG, Deutsch SI, Greenblatt DJ, Paul SM, Shader RI. Acute barbiturate administration increases benzodiazepine receptor binding *in vivo*. Psychopharmacology 1988;96:385–390.

86. Iadorola MJ, Fanelli RJ, McNamaa JO, Wilson WA. Comparison of the effects of diphenylbarbituric acid, phenobarbital, pentobarbital and secobarbital on

GABA-mediated inhibition and benzodiazepine binding. J Pharmacol Exp Ther 1985;232:127–133.

87. Serafini R, Bracamontes J, Steinbach JH. Structural domains of the human GABAA receptor β3 subunit involved in the actions of pentobarbital. J Physiol 2000;524:649–676.

88. Greenblatt DJ, Allen MD, Harmatz JS, Noel BJ, Shader RI. Overdosage with pentobarbital and secobarbital: assessment of factors related to outcome. J Clin Pharmacol 1979;19:758–768.

89. Fell RH, Gunning AJ, Bardhan KD, Triger DR. Severe hypothermia as a result of barbiturate overdose complicated by cardiac arrest. Lancet 1968;1:392–394.

90. Bledsoe KA, Kramer AH. Propylene glycol toxicity complicating use of barbiturate coma. Neurocrit Care 2008; 9:122–124.

91. Newton B, Foery RF. Retention indices and dual capillary gas chromatography for rapid identification of sedative hypnotic drugs in emergency toxicology. J Anal Toxicol 1984;8:129–134.

92. Soo VA, Bergert RJ, Deutsch DG. Screening and quantification of hypnotic sedatives in serum by capillary gas chromatography with nitrogen-phosphorus detector and confirmation by capillary gas chromatography-mass spectrometry. Clin Chem 1986;32:325–328.

93. Frison G, Favreto D, Tedeschi L, Ferrara SD. Detection of thiopental and pentobarbital in head and pubic hair in a case of drug-facilitated sexual assault. Forensic Sci Int 2003;133:171–174

94. Howard PJ, Nair SG, Kennedy MS. Plasma pentobarbitone levels estimation by gas-liquid chromatography after clinical doses. Anaesthesia 1976;31:1032–1036.

95. McCarron MM, Schulze BW, Walberg CB, Thompson GA, Ansari A. Short-acting barbiturate overdosage correlation of intoxication score with serum barbiturate concentration. JAMA 1982;248:55–61.

96. Cantrell FL, Nordt S, McIntyre I, Schneir A. Death on the doorstep of a b order community—intentional self-poisoning with veterinary pentobarbital. Clin Toxicol 2010;48:849–850.

97. Poklis A, Hameli AZ. Two unusual barbiturate deaths. Arch Toxicol 1975;34:77–80.

98. Bironneau E, Garrec F, Kergueris MF, Testa A, Nicolas F. Hemodiafiltration in pentobarbital poisoning. Renal Failure 1996;18:299–303.

99. Hudson JB, Dennis AJ Jr, Hobbs DR, Sussman HC. Extended hemodialysis in short acting barbiturate poisoning: case report. South Med J 1969;62:457–460.

100. Noirfalise A, Dodinval P, Quiriny J, Schreiber H. Death through injection of barbiturates. Forensic Sci Int 1987;141–144.

101. Isbell H, White WM. Clinical characteristics of addictions. Am J Med 1953;14:558–565.

102. Clifford JM, Cookson JH, Wickham PE. Absorption and clearance of secobarbital, heptabarbital, methaqualone, and ethinamate. Clin Pharmacol Ther 1974;16:376–389.

103. Faulkner TP, McGinity JW, Hayden JH, Martinez M, Comstock EG. Pharmacokinetic studies on tolerance to sedative-hypnotics in a poly-drug abuse population 1. Secobarbital. Clin Pharmacol Ther 1978;23:36–46.

104. Gilbert JN, Hetherington WL, Powell JW, Whalley WB. Metabolism of quinalbarbitone. J Pharm Pharmacol 1975;27:343–347.

105. Zsigmond EK, Flynn K. Effect of secobarbital and morphine on arterial blood gases in healthy human volunteers. J Clin Pharmacol 1993;33:453–457.

106. Khanna JM, Le AD, Kalant H, Chau A, Shah G. Effect of lipid solubility on the development of chronic cross-tolerance between ethanol and different alcohols and barbiturates. Pharmacol Biochem Behav 1997;57: 101–110.

107. He K, He YA, Szklarz GD, Halpert JR, Correia MA. Secobarbital-mediated inactivation of cytochrome P450 2B1 and its active site mutants. J Biol Chem 1996;271: 25864–25872.

108. Tanaka E, Terada M, Tanno K, Misawa S, Wakasugi C. Forensic analysis of 10 barbiturates in human biological samples using a new reversed-phase chromatographic column packed with 2-micrometre porous microspherical silica-gel. Forensic Sci Int 1997;85:73–82.

109. Johnson LL, Garg U. Quantitation of amobarbital, butalbital, pentobarbital, phenobarbital, and secobarbital in urine, serum, and plasma using gas chromatography-mass spectrometry (GC-MS). Methods Mol Biol 2010;603: 65–74.

110. Liu RH, McKeehan AM, Edwards C, Foster G, Bensley WD, Langner JG, Walia AS. Improved gas chromatography/ mass spectrometry analysis of barbiturates in urine using centrifuge-based solid-phase extraction, methylation, with d5-pentobarbital as internal standard. J Forensic Sci 1994;39:1504–1514.

111. Tracqui A, Kintz P, Mangin P, Lugnier AA, Chaumont AJ. A fatality involving secobarbital, nitrazepam, and codeine. Am J Forensic Med Pathol 1989;10:130–133.

112. Quatrehomme G, Bourret F, Zhioua M, Lapalus P, Ollier A. Postmortem kinetics of secobarbital. Forensic Sci Int 1990;44:117–123.

113. Janecek E, Kapur BM, Devenyi P. Oral phenobarbital loading: a safe method of barbiturate and nonbarbiturate hypnosedative withdrawal. Can Med Assoc J 1987;137: 410–412.

Chapter 24

ETHCHLORVYNOL

HISTORY

Similar to other older sedative-hypnotic drugs (e.g., methaqualone, meprobamate, glutethimide), ethchlorvynol was introduced as a safer, nonaddictive substitute for barbiturates during the 1950s. However, the abuse potential and toxicity of these sedatives were well recognized during the 1960s and 1970s. During the late 1970s, Drug Abuse Warning Network (DAWN) data indicated that ethchlorvynol use was associated with over 300 deaths yearly.

IDENTIFYING CHARACTERISTICS

Ethchlorvynol is a tertiary alcohol (1-chloro-3-ethylpent-1-en-4-yn-3-ol, CAS RN: 113-18-8) as displayed in Figure 24.1. The aspirate from the gastric lavage of patients with ethchlorvynol overdoses frequently is pink. Table 24.1 lists some physiochemical properties of ethchlorvynol.

EXPOSURE

Ethchlorvynol (Placidyl, Abbott Laboratories, Abbott Park, IL) is a sedative-hypnotic drug for the short-term treatment of insomnia. This seldom used drug has been replaced by other, more effective drugs. Product formulations include gelatin capsules in strengths ranging from 100–750 mg. Based on data from the American Association of Poison Control Centers, ethchlorvynol exposure is relatively rare in the United States with no fatalities and few exposures reported in 2009.[1] Although ethchlorvynol abuse typically involves the excessive ingestion of prescription drugs, occasionally drug abusers inject the liquid capsular contents of ethchlorvynol capsules.[2]

DOSE EFFECT

The typical adult dose of ethchlorvynol is 500–750 mg. Mild to moderate ethchlorvynol intoxication begins with ingestions in the 4–10 g range. The response to ethchlorvynol is variable depending on a number of factors including tolerance and the concomitant ingestion of other central nervous system (CNS) depressants. The ingestion of 30–50 g ethchlorvynol was associated with prolonged coma, respiratory insufficiency, and hypotension.[3] Case reports associated survival after intensive supportive care and prolonged coma following the estimated ingestion of 80–125 g ethchlorvynol.[4]

TOXICOKINETICS

Absorption

Following the ingestion of therapeutic doses of ethchlorvynol, gastrointestinal (GI) absorption is rapid with peak serum ethchlorvynol concentrations occurring about 1–1.5 hours after ingestion. In a small study of healthy volunteers, the peak serum ethchlorvynol concentration occurred about 1 hour after the ingestion of 500 mg and 750 mg.[5] The ingestion of fatty foods delays ethchlorvynol absorption.

Distribution

The initial distribution half-life of ethchlorvynol after therapeutic doses is about 1–3 hours with a relatively
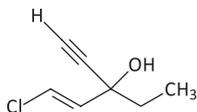
FIGURE 24.1. Chemical structure of ethchlorvynol.

TABLE 24.1. Some Physiochemical Properties of Ethchlorvynol.

Physical Property	Value
Melting Point	<25°C (<77°F)
Boiling Point	174°C (345.2°F)
log P (Octanol-Water)	1.650
Vapor Pressure	0.1 mm Hg (29°C/84.2°F)

large volume of distribution (i.e., 4 L/kg). This highly lipophilic drug distributes widely into fatty tissues. The sedative effects disappear in 3–5 hours as ethchlorvynol distributes into peripheral tissues. A rebound increase in serum ethchlorvynol concentrations can develop 7–10 hours after ingestion as a result of the redistribution of ethchlorvynol from peripheral stores. Ethchlorvynol is approximately 50–60% protein bound.

Biotransformation

The liver probably is the primary site of ethchlorvynol metabolism; however, the exact pathway of ethchlorvynol metabolism remains poorly defined. A potential metabolite includes 1-chloro-3-ethynylpent-1-en-3,4-diol from the hydroxylation of C4.[6]

Elimination

The kidney excretes small amounts (i.e., <0.1%) of a therapeutic ethchlorvynol dose unchanged in the urine.[5] The initial serum elimination half-life of ethchlorvynol after a therapeutic dose is relatively rapid (i.e., about 6–8 hours), but a slow β-elimination phase results from a biphasic elimination pattern and the delayed release of ethchlorvynol from tissue stores as demonstrated in Figure 24.2. During acute ethchlorvynol intoxication, the serum elimination half-life may increase substantially (20–100 hours).[7,8] The serum elimination half-life of ethchlorvynol in an obese, hypothyroid man chronically using ethchlorvynol was 78 hours with a total body clearance of 9.92 mL/min.[9] Renal clearance of ethchlorvynol is relatively small.

Maternal and Fetal Kinetics

Ethchlorvynol rapidly crosses the placenta, producing equilibration of maternal and fetal ethchlorvynol con-
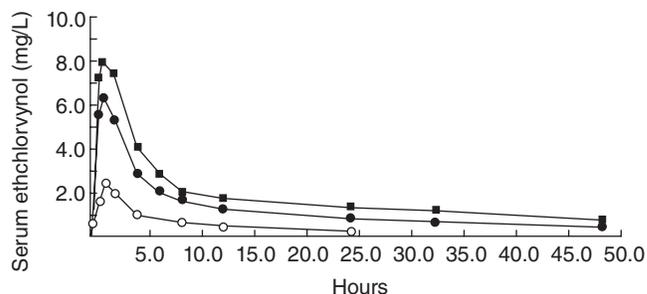
FIGURE 24.2. Mean serum ethchlorvynol concentrations in mg/L following therapeutic doses: single 200 mg (○), single 500 mg (●), single 750 mg (■).[5] The analytic method was gas chromatography with electron capture detection. (Reprinted from LM Cummins, YC Martin, EE Scherfling, Serum and urine levels of ethchlorvynol in man, Journal of Pharmaceutical Sciences, 1971, Fig. 2, p. 262, permission by John Wiley & Sons.)

centrations based on animal studies. In a study involving 5 pregnant mongrel dogs, the oral administration of 300 mg ethchlorvynol/kg produced rapid equilibration of ethchlorvynol concentrations in maternal and fetal blood ranging from 3.5–6.5 mg/L with amniotic and chorionic fluid concentrations about 20–25% of the maternal blood concentration.[10]

Tolerance

Tolerance develops following the chronic, excessive use of ethchlorvynol. A review of cases of chronic ethchlorvynol abuse reported daily ethchlorvynol consumption ranging from 1–4 g.[11]

Drug Interactions

Ethchlorvynol does not induce hepatic microsomal enzymes and hepatic dysfunction does not alter ethchlorvynol metabolism. Porphyria is a contraindication for ethchlorvynol use because ethchlorvynol increases γ-aminolevulinic acid production. Ethanol increases the sedative effect of ethchlorvynol similar to other CNS depressants.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Ethchlorvynol is a CNS depressant similar to barbiturates and ethanol, but the mechanism of action is not well defined. Experimental studies suggest that ethchlorvynol rather than diluents is responsible for the

pulmonary edema associated with ethchlorvynol intoxication.[12] Postmortem findings during examination of individuals found dead following ethchlorvynol overdose are nonspecific with pulmonary and visceral congestion along with cerebral edema. Massive pulmonary edema may be present at autopsy.[13]

CLINICAL RESPONSE

Illicit Use

The clinical features of chronic ethchlorvynol abuse are similar to chronic ethanol abuse. Symptoms associated with chronic abuse of ethchlorvynol include ataxia, dysarthria, diplopia, blurred vision, generalized weakness, tremor, toxic amblyopia, and central scotomata.[11] Clinical effects begin within 15–30 minutes.

Overdose

The clinical features of mild ethchlorvynol overdose are similar to sedative-hypnotic drug overdose with symptoms ranging from slurred speech, ataxia, and nystagmus to lethargy. In addition, a relative bradycardia and excessive salivation often occurs following ethchlorvynol overdose.[3] Symptoms of intoxication also include a mint-like taste, dry cough, and pungent odor on the breath. Severe ethchlorvynol intoxication produces a prolonged deep coma, hypothermia, respiratory depression, bullae, and hypotension. Serious complications of ethchlorvynol intoxication include aspiration pneumonia, respiratory arrest, and pulmonary edema. Although pulmonary edema occurs more commonly after the injection of ethchlorvynol capsules, occasional case reports associated large ethchlorvynol overdose with the development of noncardiogenic pulmonary edema 24–48 hours after ingestion.[2,14] Prolonged coma during ethchlorvynol intoxication may cause an ischemia-induced peripheral neuropathy. The presence of a pink gastric aspirate with prolonged coma, hypotension, and a relative bradycardia suggests ethchlorvynol poisoning.

Abstinence Syndrome

Heavy, chronic use of ethchlorvynol may cause an abstinence syndrome following abrupt cessation similar to other sedative-hypnotic drugs and delirium tremens. Clinical features of withdrawal include agitation, confusion, disorientation, delirium, tremor, hypertension, tachycardia, hallucinations, and seizures that begin from 3–15 days after cessation of use.[15,16] Symptoms usually resolve in 3–8 days, but occasionally symptoms may persist longer.[11] Case reports associate the development

of withdrawal symptoms during the first several days of a neonate born to a mother using high doses of ethchlorvynol during pregnancy.[17] Symptoms included mild hypotonia, poor suck and Moro reflexes, and poor grasp followed by jitteriness and irritability. The pregnancy was complicated by toxemia.

Reproductive Abnormalities

There are few data on the effects of maternal ethchlorvynol use and reproductive abnormalities, but animal data do not suggest that ethchlorvynol is a significant teratogen. A study of female rats exposed to ethchlorvynol doses of 20 mg/kg and 80 mg/kg did not demonstrate significant differences in the gross anatomy or histology of various organs of the offspring, when compared with the offspring of control rats without ethchlorvynol exposure.[18]

DIAGNOSTIC TESTING

Analytic Methods

Methods for the detection of ethchlorvynol include spectrophotometry,[19,20] high performance liquid chromatography,[21] and gas chromatography.[22] Detection limits for these analytic procedures are in the range of 1 mg/L.[23] Some loss of ethchlorvynol can occur during storage. A case report documents the loss of approximately 22% of the initial postmortem blood ethchlorvynol concentration of 66 mg/L following storage for 90 days at 0°C (32°F).[24]

Abnormalities

The chest x-ray in patients with pulmonary edema following ethchlorvynol poisoning typically demonstrates diffuse bilateral alveolar densities without cardiomegaly.

Biomarkers

BLOOD

OVERDOSE. Interpretation of serum ethchlorvynol concentrations requires consideration of a variety of factors including tolerance, concomitant ingestion of other CNS depressants, coexisting disease, and individual variability. Typically, therapeutic concentrations of ethchlorvynol range from about 2–8 mg/L with toxicity developing when the serum ethchlorvynol concentration exceeds 20 mg/L. In pharmacokinetic studies of healthy volunteers, the mean peak serum ethchlorvynol concentration following ingestion of 500 mg and 750 mg

ethchlorvynol was approximately 6.5 mg/L and 8 mg/L, respectively.[5]

Serious toxicity potentially develops when serum ethchlorvynol concentrations exceed 50 mg/L. Serum ethchlorvynol concentrations do not necessarily correlate to the clinical presentation because of the factors listed above. A 33-year-old, hypothyroid, obese man with a history of drug abuse and chronic ethchlorvynol use (i.e., prescribed dose, 1 g daily for 2 years) was oriented, lethargic, and responsive to verbal command.[9] His initial serum ethchlorvynol concentration was 70 mg/L. Survival occurred in patients treated with intensive supportive care despite the presence of peak serum ethchlorvynol concentrations exceeding 200 mg/L.[25]

POSTMORTEM.   In a series of 4 postmortem cases involving only ethchlorvynol, the ethchlorvynol concentration in postmortem blood samples ranged from 29–94 mg/L with a mean of 55 mg/L as measured by UV spectrophotometry.[7] Case reports indicate that high postmortem concentrations of ethchlorvynol occur in the liver and bile compared with the blood and urine.[26,27] The ethchlorvynol concentration in a postmortem blood sample from a chronic ethchlorvynol abuser found dead was 66 mg/L as measured by gas chromatography with a hydrogen flame detector.[24]

Driving

There are few data on the effect of ethchlorvynol and driving skills; however, the CNS depressant properties indicate that abuse of ethchlorvynol would impair driving skills.

TREATMENT

Stabilization

As for all sedative-hypnotic overdoses, stabilization requires careful evaluation of the level of consciousness and the adequacy of respirations. Seizures may occur during withdrawal from ethchlorvynol along with restlessness, agitation, and delirium. All patients with altered levels of consciousness should be evaluated rapidly for serum glucose concentrations and hypoxemia.

Gut Decontamination

The administration of activated charcoal is appropriate for patients with ethchlorvynol overdose presenting <1 hour after ingestion. However, most patients present to a health care facility >1 hour after ethchlorvynol ingestion, and gut decontamination measures are not necessary as a result of the lack of clinical data to support the use of aggressive decontamination measures in these patients. Theoretically, the administration of activated charcoal more than 1–2 hours after ingestion may decrease ethchlorvynol absorption in comatose patients with decreased gastrointestinal motility, but there are inadequate data to indicate that the administration of activated charcoal at this time improves clinical outcome. Gastric lavage in comatose patients is a therapeutic option, particularly with delayed gastric emptying. However, there are few data on the efficacy of gastric lavage in ethchlorvynol overdose. Syrup of ipecac is not recommended because of the lack of efficacy and the potential for aspiration.

Elimination Enhancement

The renal clearance of ethchlorvynol during resin hemoperfusion for ethchlorvynol intoxication is high (140–170 mL/min),[28] but there are few data on the effect of resin hemoperfusion on the clinical outcome of ethchlorvynol intoxication. Substantial rebound of ethchlorvynol from peripheral stores may significantly increase the serum ethchlorvynol concentrations within 6–8 hours after treatment. Factors to consider for the use of resin hemoperfusion include failure to respond to supportive measures, life-threatening complications associated with prolonged coma, and serum ethchlorvynol concentrations that exceed 150 mg/L. Although hemodialysis reduces the plasma ethchlorvynol half-life, the effect of hemodialysis on the clinical outcome of ethchlorvynol intoxication is unclear, in part because of relatively high protein binding and the large redistribution from tissue to blood. Three hours of hemodialysis removed 3 g (i.e., about 3–5% of the total dose) of an estimated 80–125 g ingestion of ethchlorvynol.[29] The estimated clearance of ethchlorvynol during hemodialysis for 2 patients with ethchlorvynol poisoning was 46 mL/min and 82 mL/min.[3] Forced diuresis is contraindicated because of the limited excretion of ethchlorvynol and the tendency of ethchlorvynol intoxication to cause pulmonary edema.

Antidotes

There is no antidote.

Supplemental Care

Baseline laboratory tests during ethchlorvynol intoxication include complete blood count and serum electrolytes, glucose, and creatinine as well as chest x-ray and pulse oximetry. Hemodynamic monitoring may be necessary for patients with hypotension unresponsive to

fluid therapy. Prolonged coma is characteristic of serious ethchlorvynol intoxication and potential complications include pulmonary edema, respiratory arrest, and pneumonia.

References

1. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Giffin SL. 2009 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th Annual Report. Clin Toxicol (Phila) 2010;48:979–1178.

2. Conces DJ Jr, Kreipke DL, Tarver RD. Pulmonary edema induced by intravenous ethchlorvynol. Am J Emerg Med 1986;4:549–551.

3. Teehan BP, Maher JF, Carey JJ, Flynn PD, Schreiner GE. Acute ethchlorvynol (Placidyl®) intoxication. Ann Intern Med 1970;72:875–882.

4. Klock JC. Letter: hemolysis and pancytopenia in ethchlorvynol overdose. Ann Intern Med 1974;81:131–132.

5. Cummins LM, Martin YC, Scherfling EE. Serum and urine levels of ethchlorvynol in man. J Pharm Sci 1971;60:261–263.

6. Horwitz JP, Brukwinski W, Treisman J, Andrzejewski D, Hills EB, Chung HL, Wang CY. Ethchlorvynol: potential of metabolites for adverse effects in man. Drug Metab Disp 1980;8:77–83.

7. Bailey DN, Shaw RF. Ethchlorvynol ingestion in San Diego County: a 14-year review of cases with blood concentrations and findings. J Anal Toxicol 1990;14:348–352.

8. Pochopien DJ. Rate of decrease in serum ethchlorvynol concentration after extreme overdosage—a case study. Clin Chem 1975;21:894–895.

9. Kolpek JH, Parr MD, Marshall ML, Flueck JA. Ethchlorvynol pharmacokinetics during long-term administration in a patient with hyperlipidemia and hypothyroidism. Pharmacotherapy 1986;6:323–327.

10. Hume AS, Williams JM, Douglas BH. Disposition of ethchlorvynol in maternal blood, fetal blood, amniotic fluid, and chorionic fluid. J Reprod Med 1971;6:229–231.

11. Flemenbaum A, Gunby B. Ethchlorvynol (Placidyl) abuse and withdrawal. (Review of clinical picture and report of 2 cases). Dis Nerv Syst 1971;32:188–192.

12. Glauser FL, Smith WR, Caldwell A, Hoshiko M, Dolan GS, Baer H, Olsher N. Ethchlorvynol (Placidyl®) induced pulmonary edema. Ann Intern Med 1976;84:46–48.

13. Algeri EJ, Katsas GG, Luongo MA. Determination of ethchlorvynol in biologic mediums, and report of two fatal cases. Am J Clin Pathol 1962;38:125–130.

14. Schottstaedt MW, Nicotra B, Rivera M. Placidyl abuse: a dimorphic picture. Crit Care Med 1981;9:677–679.

15. Heston LL, Hastings D. Psychosis with withdrawal from ethchlorvynol. Am J Psychiatry 1980;137:249–250.

16. Blumenthal MD, Reinhart MJ. Psychosis and convulsions following withdrawal from ethchlorvynol. JAMA 1964;190:154–155.

17. Rumack BH, Walravens PA. Neonatal withdrawal following maternal ingestion of ethchlorvynol (Placidyl). Pediatrics 1973;52:714–716.

18. Peters MA, Hudson PM. The effects of totigestational exposure to ethchlorvynol on development and behavior. Ecotoxicol Environ Saf 1981;5:494–502.

19. Haux P. Ethchlorvynol (Placidyl) estimation in urine and serum. Clin Chim Acta 1973;43:139–141.

20. Wallace JE, Hamilton HE, Riloff JA, Blum K. Spectrophotometric determination of ethchlorvynol in biologic specimens. Clin Chem 1974;20:159–162.

21. Needham LL, Kochhar MM. Determination of ethchlorvynol by high-pressure liquid chromatography. J Chromatogr 1975;111:422–425.

22. Evenson MA, Poquette MA. Rapid gas chromatographic method for quantitation of ethchlorvynol ("Placidyl") in serum. Clin Chem 1974;20:212–216.

23. Bridges RR, Jennison TA. Analysis of ethchlorvynol (Placidyl®): evaluation of a comparison performed in a clinical laboratory. J Anal Toxicol 1984;8:263–268.

24. Winek CL, Bricker JD, Esposito FM. A death due to ethchlorvynol abuse. A case report. Forensic Sci Int 1981;17:219–224.

25. Kelner MJ, Bailey DN. Ethchlorvynol ingestion: Interpretation of blood concentrations and clinical findings. Clin Toxicol 1983–1984;21:399–408.

26. Winek CL, Wahba WW, Rozin L, Winek CL Jr. Determination of ethchlorvynol in body tissues and fluids after embalmment. Forensic Sci Int 1988;37:161–166.

27. Winek CL, Wahba WW, Winek CL Jr. Body distribution of ethchlorvynol. J Forensic Sci 1989;32:687–690.

28. Lynn RI, Honig CL, Jatlow PI, Kliger AS. Resin hemoperfusion for treatment of ethchlorvynol overdose. Ann Intern Med 1979;91:549–553.

29. Tozer TN, Witt LD, Gee L, Tong TG, Gambertoglio J. Evaluation of hemodialysis for ethchlorvynol (Placidyl) overdose. Am J Hosp Pharm 1974;31:986–989.

Chapter 25

GLUTETHIMIDE

HISTORY

In 1954, glutethimide (Doriden®) was introduced into the US market as a safe substitute for barbiturates. Within 1 year, the first report of glutethimide poisoning appeared in the medical literature.[1] Subsequent experience with glutethimide indicated that the severity of the abuse potential and withdrawal symptoms were similar to those of barbiturates; chronic abuse of glutethimide resulted in tolerance, dependence, and an abstinence syndrome. During the 1980s, the combination of codeine and glutethimide ("packs" or "loads") gained popularity as an aphrodisiac and an oral substitute for heroin. In 1991, glutethimide was given a US Drug Enforcement Administration schedule II classification in response to an upsurge in the prevalence of diversion, abuse, and glutethimide-associated fatalities. Today, there is limited medical use of glutethimide in the United States.

IDENTIFYING CHARACTERISTICS

Glutethimide (CAS RN: 77-21-4, $C_{13}H_{15}NO_2$) is structurally related to phenobarbital and thalidomide as displayed in Figure 25.1. The IUPAC name for glutethimide is 2-ethyl-2-phenylglutarimide (3-ethyl-3-phenyl-2,6-piperidinedione). The poor water solubility and high pKa (9.2) of glutethimide limits the gastrointestinal (GI) absorption of glutethimide, particularly during an overdose. Table 25.1 lists some physiochemical properties of glutethimide. The molecular weight of this highly lipophilic drug is 217 g/mol.

Slang terms for the combination of glutethimide and codeine include Loads and Pancakes and Syrup (codeine cough syrup). Other street names for glutethimide are D's, CB, CD, Eight's, Four Doors, Glue, Hits, and Sets.

EXPOSURE

Exposure to glutethimide is very limited in the United States. The American Association of Poison Control Centers' National Poison Data System Annual Report did not list any exposures to glutethimide in 2009.[2] Glutethimide is a sedative-hypnotic drug used for the treatment of anxiety and insomnia. Other potential uses include essential tremor.[3] The 250- and 500-mg dosages are white tablets. Glutethimide is available as a generic drug or under the trade name Doriden® (USV Pharmaceutical, Mumbai, India). A synonym for glutethimide in France and Belgium is Doridene®.

The combination of glutethimide and codeine reportedly produces euphoria similar to intravenous (IV) heroin. Typical oral doses involved with the abuse of glutethimide are 1 g glutethimide and 240 mg codeine.[4] Desired effects from the use of this combination is the relatively long-lasting effects (i.e., up to 8 hours), the avoidance of the complications of IV drug use, and relatively low cost of the ingredients. However, the combination is highly addictive, and the rapid development of tolerance leads to escalating doses and fatalities.[5]

DOSE EFFECT

The usual adult hypnotic dose of glutethimide is 250–500 mg with a range up to 1,500 mg. Mild to moderate toxicity may develop when the glutethimide dose exceeds 3–7 g. The ingestion of glutethimide doses
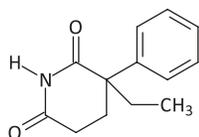
FIGURE 25.1. Chemical structure of glutethimide.

TABLE 25.1. Some Physiochemical Properties of Glutethimide.

Physical Property	Value
Melting Point	84°C (183.2°F)
pKa Dissociation Constant	9.2
log P (Octanol-Water)	1.9
Water Solubility	999 mg/L (30°C/86°F)
Vapor Pressure	1.49E–09 mm Hg (25°C/77°F)

>10–20 g or 150–300 mg/kg are potentially lethal as a result of CNS depression.[6] A variety of factors determine the lethality of a specific dose, and survival after the ingestion of 42.5 g occurred with intensive treatment.[7]

TOXICOKINETICS

Absorption

The poor water solubility of glutethimide results in erratic and delayed GI absorption. Following the ingestion of 500 mg glutethimide by healthy volunteers, the peak plasma glutethimide concentrations ranged from about 3–7 mg/L at 1–6 hours after ingestion.[8]

Distribution

Glutethimide is highly lipophilic, resulting in relatively high glutethimide concentrations in brain and adipose tissue after ingestion. The apparent volume of distribution is about 2.7 L/kg following therapeutic doses of glutethimide. The initial distribution phase into tissues is relatively rapid with a distribution half-life of about 4 hours, whereas the terminal elimination half-life is longer (i.e., about 12 hours). Glutethimide is approximately 50% protein bound in blood.

Biotransformation

Glutethimide (2-ethyl-2-phenylglutarimide) undergoes extensive metabolism in the liver to both conjugated and unconjugated metabolites that the kidney excretes
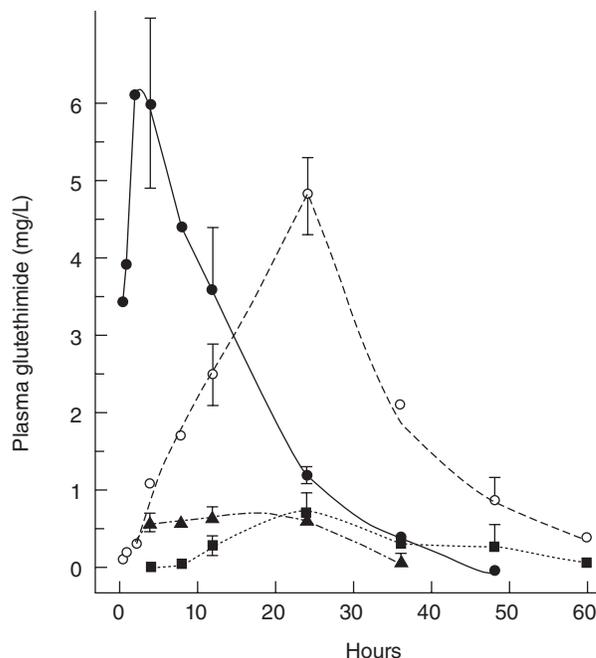
FIGURE 25.2. Mean plasma concentrations (mg/L) of glutethimide and glutethimide metabolites for 3 subjects ingesting 13.5 mg/kg body weight glutethimide. Glutethimide (•), 4-hydroxyglutethimide (○), conjugated 4-hydroxygluthethimide (▪), iso-(1-hydroxyethyl)glutethimide (▲). (Reprinted from KA Kennedy, LJ Fischer, Quantitative and stereochemical aspects of glutethimide metabolism in humans, Drug Metabolism and Disposition, Vol. 7, p. 321, copyright 1979.)

in the urine including 4-hydroxy-2-ethyl-2-phenyl-glutarimide (4-hydroxyglutethimide, major metabolite), 2-(1-hydroxyethyl)-2-phenylglutarimide, and 2-ethyl-2-(4-hydroxyphenyl)glutarimide.[9] Minor urinary metabolites include 2-(2-hydroxyethyl)-2-phenylglutarimide, 2-(2-hydroxyethyl)glutarimide, and dihydroxyl metabolites [e.g., 4-hydroxy-2-(1-hydroxyethyl)-2-phenylglutarimide, 2-ethyl-2-(3,4-dihydroxyphenyl)glutarimide].[10] Racemic glutethimide undergoes stereoselective metabolism. Hydrolysis of the glutarimide ring of the *d*-enantiomer forms 4-hydroxyglutethimide, whereas hydrolysis of the *l*-enantiomer results in the release of acetaldehyde from α-phenyl glutarimide. Peak concentrations of the metabolite, 4-hydroxyglutethimide occurred about 24 hours after ingestion of 13.5 mg racemic glutethimide/kg by 3 volunteers.[11] Although some glutethimide metabolites (e.g., 4-hydroxyglutethimide, phenyl-γ-butyrolactone) demonstrate pharmacologic activity in animals,[12] these metabolites are not definitely associated with human toxicity.[13,14] Peak plasma concentration of 4-hydroxyglutethimide occur substantially after the resolution of the sedative effects of glutethimide as demonstrated in Figure 25.2.

Elimination

The kidney excretes minimal unchanged glutethimide in urine with the vast majority (i.e., 98%) of the glutethimide dose appearing in the urine as conjugated metabolites.[11] The major glutethimide metabolites in the urine are the glucuronic acid conjugates of hydroxy metabolites (e.g., 4-hydroxyglutethimide, [1-hydroxyethyl]-glutethimide). The estimated unaided renal clearance of glutethimide is about 0.5–11 mL/min. In therapeutic doses, the elimination half-life of glutethimide is approximately 8–12 hours. The elimination half-life can increase substantially during serious glutethimide poisonings. In a patient with coma, respiratory failure, and hypotension, the plasma elimination half-life of glutethimide was about 13 hours with a peak plasma glutethimide concentration of 41 mg/L.[13] In a case series of 38 glutethimide poisonings, the mean serum elimination half-life was approximately 40 ± 5 hours; however, each calculation was based on only 2 measurements and there was no confirmation that the glutethimide samples were drawn during the postabsorption phase.[15] Additionally, the presence of hypotension in some of these patients may have prolonged the serum half-life. A majority of the glutethimide metabolites undergo enterohepatic recirculation and slow urinary excretion.

Maternal and Fetal Kinetics

Glutethimide easily crosses the placenta to develop similar glutethimide concentrations in maternal and fetal blood. Relatively small amounts of the unchanged glutethimide occur in breast milk,[8] and breastfeeding following the occasional use of a single dose of glutethimide probably is safe for the infant.[16]

Drug Interactions

Glutethimide induces hepatic microsomal enzyme formation. Rodent studies indicate that the administration of glutethimide with codeine potentiates and prolongs the analgesic effect of codeine by increasing the plasma morphine concentration and reducing the plasma concentration of the inactive metabolite, morphine-3-glucuronide.[17] Similar to other sedative-hypnotics, the congestion of ethanol and glutethimide synergistically depresses the CNS. In a volunteer study, the concomitant administration of ethanol and glutethimide increased the blood ethanol concentration while reducing the plasma glutethimide concentration when compared with the administration of ethanol and glutethimide separately.[18]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Glutethimide is a CNS depressant similar to phenobarbital, but glutethimide does not possess anticonvulsant properties. Glutethimide has prominent anticholinergic properties, manifest during glutethimide intoxication by mydriasis, paralytic ileus, and urinary retention. Cardiovascular depression and shock are relatively more common during severe glutethimide intoxication than during similar stages of coma after poisoning by other older sedative-hypnotic drugs. Postmortem examination of patients dying from glutethimide intoxication usually demonstrates nonspecific findings including visceral congestion, cerebral edema, and pulmonary edema.[6]

CLINICAL RESPONSE

Overdose

The clinical features of glutethimide intoxication are similar to other sedative-hypnotic drugs (ethchlorvynol, meprobamate, methaqualone) except the frequent occurrence of fluctuating levels of coma and prominent anticholinergic signs. Serious complications of glutethimide overdose include coma, papilledema, cerebral edema, pulmonary edema, seizures, hypotension, and respiratory arrest.[19,20] Although respiratory depression is less severe following glutethimide ingestion compared with equivalent doses of barbiturates, hypotension occurs at an earlier stage of coma than a similar stage of barbiturate intoxication.[6] Anticholinergic symptoms are often prominent with mydriasis being the most common sign. Thick, tenacious secretions, ileus, urinary retention, and warm, dry skin may occur. Temperature abnormalities include hypothermia followed by elevated body temperatures. Hepatic dysfunction, visceral hemorrhage, and renal dysfunction are uncommon manifestations of glutethimide intoxication. Factors associated with poor outcome during glutethimide intoxication include age >60 years,[21] high-grade coma, large glutethimide ingestion (>10 g), plasma glutethimide level exceeding 30 mg/L, and concomitant ingestion of other CNS depressants.

Abstinence Syndrome

Abrupt withdrawal of glutethimide following chronic use of high doses may produce an abstinence syndrome similar to other sedative-hypnotic drugs (e.g., meprobamate, methaqualone, ethchlorvynol). Clinical features of withdrawal from glutethimide include nausea, vomiting, anxiety, sleep disturbances, and seizures.

Reproductive Abnormalities

Limited data on glutethimide in pregnancy does not suggest that this drug is fetotoxic or teratogenic in therapeutic doses. In a study of 33 glutethimide overdoses in pregnant women, 16 delivered live infants.[22] Three of these infants had congenital abnormalities (fetal alcohol syndrome, pectus carinatum, atrial septal defect type II). Compared with 16 matched siblings, there was no significant difference in congenital abnormalities, cognition, or behavior. The birth weight of the exposed infants was higher than controls as a result of a relatively longer gestation period.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the quantitation of glutethimide in biologic samples include gas chromatography with flame ionization detection (limit of detection [LOD], 0.2 mg/L)[23,24] and gas chromatography/mass spectrometry (GC/MS).[10] Analysis of biologic samples with gas chromatography/flame ionization detection detects both glutethimide and the active metabolite, 4-hydroxy-2-ethyl-2-phenylglutarimide with a LOD of approximately 0.2 mg/L and 0.5 mg/L, respectively.[25] Separation of glutethimide enantiomers by high performance liquid chromatography (HPLC) on cellulose tris(4-methylbenzoate) as the chiral stationary phase followed by detection with HPLC allows the determination of the stereoselectivity of the metabolites formed by glutethimide enantiomers.[26]

Biomarkers

Blood

Overdose.   Serum glutethimide concentrations do not always correlate to clinical effect because of the presence of other drugs, tolerance, underlying medical disease, individual variability, and the metabolism of glutethimide to a possibly active metabolite. Variations in the depth of coma do not correlate well to serum glutethimide or to serum 4-hydroxyglutethimide concentrations. Therapeutic serum glutethimide concentrations range from about 2–6 mg/L, whereas toxic serum glutethimide concentrations range from 10–80 mg/L. Case reports associate serum glutethimide concentrations near 25 mg/L with altered consciousness and responsiveness only to painful stimuli.[27] Coma may persist despite low serum glutethimide concentrations; case reports suggest that the level of consciousness cor-

relates better to serum glutethimide and 4-hydroxy-2-ethyl-2-phenylglutarimide concentrations rather than serum glutethimide alone.[28] However, there are inadequate data to determine the exact contribution of any active glutethimide metabolite to the clinical features of glutethimide intoxication. Fatalities can occur when the serum glutethimide concentration exceeds 30–100 mg/L. A case report associated coma, hypotension, and apnea with a peak plasma glutethimide concentration of 41 mg/L as measured by GC/MS.[13]

Postmortem.   In a case series of 3 fatalities involving only glutethimide, the postmortem blood glutethimide concentration ranged from 29–60 mg/L as measured by a spectrophotometric method that included the 4-hydroxyglutethimide metabolite.[27] Reviews of fatalities following the combined use of glutethimide and codeine indicate that these two drugs potentiate respiratory depression. Consequently, death occurs at lower concentrations of these 2 drugs than the postmortem concentrations detected following overdose fatalities resulting from these drugs alone. In a case series of 16 deaths resulting from the use of glutethimide and codeine, the postmortem blood glutethimide concentration ranged from 0.7–61 mg/L (mean, 12.7 mg/L), whereas the postmortem blood codeine concentrations ranged from 0.07–2 mg/L (mean, 0.6 mg/L).[29] In another case series of 5 fatal poisonings involving only glutethimide and codeine, the postmortem blood glutethimide and codeine concentrations ranged from 7.2–22.1 mg/L (mean, 14.5 mg/L) and 0.29–1.99 mg/L (mean, 1 mg/L), respectively.[30] Although the distribution of glutethimide is relatively low in bone compared with bile, blood, and the liver, bone samples (sternum, ribs) are alternative biologic samples for the qualitative detection of glutethimide in previously interred corpses.[31]

Abnormalities

Although the frequency and reactivity of an electroencephalogram (EEG) correlate positively with the degree of brainstem depression during glutethimide overdose,[12] complete clinical recovery is possible despite the absence of EEG activity when anoxic brain damage is not present.[32]

Driving

A double-blind study of healthy volunteers ingesting an evening dose of 250 mg glutethimide did not detect decrements in psychomotor performance (i.e., choice reaction time, coordination test, divided attention task) when tested the following morning.[33]

TREATMENT

Stabilization

Similar to other sedative-hypnotic overdoses, stabilization requires careful evaluation of the level of consciousness and oxygenation. Seizures may occur during withdrawal from glutethimide along with restlessness, agitation, and delirium. All patients with altered levels of consciousness should be evaluated rapidly for hypoglycemia and hypoxia. Fluctuating levels of consciousness may result in initial improvement followed by serious deterioration of vital signs.

Gut Decontamination

The administration of activated charcoal is appropriate for patients with glutethimide overdose presenting <1–2 hours after ingestion. However, patients who present to a health care facility >1–2 hours after glutethimide ingestion frequently require no gut contamination measures as a result of the lack of clinical data to support the use of aggressive decontamination measures. Theoretically, the administration of activated charcoal more than 1–2 hours after ingestion can decrease glutethimide absorption in comatose patients with decreased GI motility, but there are inadequate data to indicate that the administration of activated charcoal at this time improves clinical outcome. Gastric lavage in comatose patients is a therapeutic option, particularly when delayed gastric emptying and the formation of drug concretions are suspected. However, there are few data on the efficacy of gastric lavage in glutethimide overdose. Syrup of ipecac is not recommended because of the potential for aspiration and the lack of efficacy. Fluctuating vital signs or level of consciousness suggests the presence of continued glutethimide absorption from drug concretions and, therefore, the need for endoscopy.

Elimination Enhancement

There are limited data on the effect of hemoperfusion and hemodialysis on the clinical outcome of severe glutethimide intoxication. Although both resin hemoperfusion and hemodialysis increase glutethimide clearance about 300 mL/min and 30–110 mL/min, respectively, the large tissue stores of glutethimide limit the total amount of drug removed. Reported *in vitro* dialysance of glutethimide at renal flow rates of 200 mL/min was 80 mL/min.[6] Consequently, the exact role of hemodialysis and hemoperfusion remains unclear; most patients require only supportive care. If used, hemodialysis or hemoperfusion should be limited to potentially severe intoxications. Forced diuresis is contraindicated because of the propensity to produce pulmonary edema.

Antidotes

There is no antidote.

Supplemental Care

Baseline laboratory tests include a complete blood count, serum electrolytes, serum glucose, and measures of renal and hepatic function. Chest x-rays and monitoring of oxygen saturation are necessary during moderate and severe glutethimide intoxication to detect pneumonia and pulmonary edema.

References

1. Gerster P, Scholer H, Zuger M. [Clinical observations in an acute alpha-phenyl-alpha-ethylglutarimide (Doriden) poisoning]. Schweiz Med Wochenschr 1955;85:991–994. [German]
2. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Giffin SL. 2009 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th Annual Report. Clin Toxicol (Phila) 2010;48:979–1178.
3. Aisen ML, Holzer M, Rosen M, Dietz M, McDowell F. Glutethimide treatment of disabling action tremor in patients with multiple sclerosis and traumatic brain injury. Arch Neurol 1991;48:513–515.
4. Shamoian CA. Codeine and glutethimide; euphoretic, addicting combination. N Y State J Med 1975;75:97–99.
5. Bender FH, Cooper JV, Dreyfus R. Fatalities associated with an acute overdose of glutethimide (Doriden) and codeine. Vet Hum Toxicol 1988;30:332–333.
6. Maher JF, Schreiner GE, Westervelt FB Jr. Acute glutethimide intoxication I. Clinical experience (twenty-two patients) compared to acute barbiturate intoxication (sixty-three patients). Am J Med 1962;33:70–82.
7. Phillips WS, Bortin MM. Massive glutethimide (Doriden) intoxication: Successful treatment with dialysis. Wis Med J 1965; 64:440–442.
8. Curry SH, Gordon JS, Riddall D, Simpson P, Binns TB, Rondel RK, McMartin C. Disposition of glutethimide in man. Clin Pharmacol Ther 1971;12:849–857.
9. Kennedy KA, Ambre JJ, Fischer LJ. A selected ion monitoring method for glutethimide and six metabolites: application to blood and urine from humans intoxicated with glutethimide. Biomed Mass Spectrom 1978;5:679–685.
10. Stillwell WG. Metabolism of glutethimide in the human. Res Commun Chem Pathol Pharmacol 1975;12:25–41.

11. Kennedy KA, Fischer LJ. Quantitative and stereochemical aspects of glutethimide metabolism in humans. Drug Metab Dispos 1979;7:319–324.

12. Ambre JJ, Fischer LJ. Identification and activity of the hydroxy metabolite that accumulates in the plasma of humans intoxicated with glutethimide. Drug Metab Dispos 1974;2:151–158.

13. Curry SC, Hubbard JM, Gerkin R, Selden B, Ryan PJ, Meinhart R, Hagner D. Lack of correlation between plasma 4-hydroxyglutethimide and severity of coma in acute glutethimide poisoning. A case report and brief review of the literature. Med Toxicol Adverse Drug Exp 1987;2:309–316.

14. Crow JW, Lain P, Bochner F, Shoeman DW, Azarnoff DL. Glutethimide and 4-OH glutethimide: pharmacokinetics and effect on performance in man. Clin Pharmacol Ther 1977;22:458–464.

15. Maher JF. Determinants of serum half-life of glutethimide in intoxicated patients. J Pharmacol Exp Ther 1970; 174:450–455.

16. Pons G, Rey E, Matheson I. Excretion of psychoactive drugs into breast milk. Pharmacokinetic principles and recommendations. Clin Pharmacokinet 1994;27:270–289.

17. Popa D, Loghin F, Imre S, Curea E. The study of codeine-glutethimide pharmacokinetic interaction in rats. J Pharm Biomed Anal 2003;32:867–877.

18. Mould GP, Curry SH, Binns TB. Interaction of glutethimide and phenobarbitone with ethanol in man. J Pharm Pharmacol 1972;24:894–899.

19. Wright N, Roscoe P. Acute glutethimide poisoning. Conservative management of 31 patients. JAMA 1970;214: 1704–1706.

20. McBay AJ, Katsas GG. Glutethimide poisoning. A report of four fatal cases. N Engl J Med 1957;257:97–100.

21. Greenblatt DJ, Allen MD, Harmatz JS, Noel BJ, Shader RI. Correlates of outcome following acute glutethimide overdosage. J Forensic Sci 1979;24:76–86.

22. Petik D, Acs N, Banhidy F, Czeizel AE. A study of the effects of large doses of glutethimide that were used for self-poisoning during pregnancy on human fetuses. Toxicol Ind Health 2008;24:69–78.

23. Shipe JR, Savory J. A comprehensive gas chromatography procedure for measurement of drugs in biological materials. Ann Clin Lab Sci 1975;5:57–64.

24. Anweiler J, Bender G, Hobel M. Simultaneous determination of glutethimide, methyprylon, and methaqualone in serum by gas liquid chromatography. Arch Toxicol 1976; 35:187–193.

25. Hansen AR, Fischer LJ. Gas chromatographic simultaneous analysis for glutethimide and an active hydroxylated metabolite in tissues, plasma, and urine. Clin Chem 1974; 20:236–242.

26. Weinz C, Blaschke G, Schiebel H-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis. J Chromatogr B 1997;690:233–242.

27. Bailey DN, Shaw RF. Interpretation of blood glutethimide, meprobamate, and methyprylon concentrations in nonfatal and fatal intoxications involving a single drug. J Toxicol Clin Toxicol 1983;20:133–145.

28. Hansen AR, Kennedy KA, Ambre JJ, Fischer LJ. Glutethimide poisoning a metabolite contributes to morbidity and mortality. N Engl J Med 1975;292:250–252.

29. Havier RG, Lin R-L. Deaths as a result of a combination of codeine and glutethimide. J Forensic Sci 1985;30: 563–566.

30. Bailey DN, Shaw RF. Blood concentrations and clinical findings in nonfatal and fatal intoxications involving glutethimide and codeine. Clin Toxicol 1985-86;23:557–570.

31. Benko A. Toxicological analysis of amobarbital and glutethimide from bone tissue. J Forensic Sci 1985;30: 708–714.

32. Myers RR, Stockard JJ. Neurologic and electroencephalographic correlates in glutethimide intoxication. Clin Pharmacol Ther 1974;17:212–220.

33. Saario I, Linnoila M. Effect of subacute treatment of hypnotics, alone or in combination with alcohol, on psychomotor skills related to driving. Acta Pharmacol Toxicol 1976;38:382–392.

Chapter 26

MEPROBAMATE

HISTORY

The development of meprobamate began with the synthesis of mephenesin as a disinfectant for Gram-negative bacteria in the mid-1940s. Discovery of the muscle relaxant properties of mephenesin in animals led to the introduction of this drug as an alternative to tubocurarine during light anesthesia; however, the low potency, short duration of action, and the limited effect on supratentorial structures resulted in attempts to modify the structure of mephenesin. In 1951, Ludwig and Piech reported that meprobamate was an effective alternative to mephenesin as a muscle relaxant.[1] Shortly thereafter, the selective action of meprobamate on anxiety was discovered and this drug soon became a popular sedative.[2] This drug was the first anxiolytic agent and along with chlorpromazine initiated the age of psychopharmacology. In the late 1950s, meprobamate was administered to neurotic patients as a less toxic replacement for the sedative effects of barbiturates, chloral hydrate, bromides, ethanol, opium, and paraldehyde. The first report of meprobamate poisoning appeared in 1956.[3] Although initially marketed as a safe sedative-hypnotic with low abuse potential, later reports of serious overdose and addiction indicated that the adverse effects of meprobamate were similar to other sedative-hypnotics.[4] In a French case series of 141 meprobamate intoxications published in 1968, the mortality rate was 5%, resulting primarily from cardiogenic shock.[5]

IDENTIFYING CHARACTERISTICS

Meprobamate (CAS RN: 57-53-4, $C_9H_{18}N_2O_4$) is a carbamic acid ester of a glycol (2-methyl-2n-propyl-1,3-propanediol dicarbamate) as displayed in Figure 26.1.

Table 26.1 lists some physiochemical properties of meprobamate.

EXPOSURE

Exposure to meprobamate is limited in the United States; however, the exposures reported to American Association of Poison Control Centers for meprobamate are higher than the combined exposures to the other 3 older sedative-hypnotic drugs (ethchlorvynol, glutethimide, methaqualone).[6] The American Association of Poison Control Centers' National Poison Data System Annual Report listed 42 exposures and no fatalities associated with the use of meprobamate in 2009. Meprobamate occurs in a variety of preparations in doses of 200 mg and 400 mg including distribution in the United States under the trade names Equanil® (Wyeth, Madison, NJ) and Miltown® (Wallace Laboratories, El Segundo, CA). Meprobamate remains available in France, primarily as an adjunctive treatment for alcohol withdrawal despite the lack of clinical data to support this indication.

Behavioral studies suggest that the abuse potential of meprobamate is similar to benzodiazepines, but less than barbiturates. In a behavioral study of 9 men with histories of drug abuse housed on a residential research ward, meprobamate had similar abuse potential as lorazepam when compared in a within-subject, double-blind study.[7] Meprobamate produced subjective ratings of drug liking and monetary street value similar to lorazepam, but there was no placebo-control in this study. A study of 14 recreational drug users with histories of prior sedative-hypnotic drug use compared the behavioral and pharmacologic effects of triazolam (0.5 mg), meprobamate (2,400 mg), and butabarbital
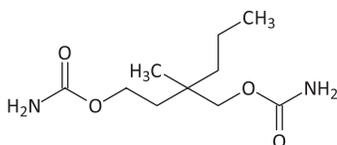
FIGURE 26.1. Chemical structure of meprobamate.

TABLE 26.1. Some Physiochemical Properties of Meprobamate.

Physical Property	Value
Conversion Units	1 mg/L = 0.22 μmol/L
	1 μmol/L = 4.55 mg/L
Melting Point	105°C (221°F)
log P (Octanol-Water)	0.7
Water Solubility	4700 mg/L (25°C/77°F)
Vapor Pressure	3.05E-03 mm Hg (25°C/77°F)

(400 mg) in a double-blind, crossover, placebo-controlled study. Meprobamate was indistinguishable from placebo on the euphoria and abuse potential scales. Based on behavioral economics analysis, the ranking of abuse potential was butabarbital > triazolam ≥ meprobamate.

DOSE EFFECT

The usual adult therapeutic dose is 600–1,600 mg daily in 3–4 divided doses up to a daily maximum of 2,400 mg. Meprobamate doses in the range of 6–10 g produce ataxia and transient hypotension, whereas the ingestion of 10–20 g causes significant hypotension and respiratory insufficiency.[8] The ingestion of 30–40 g meprobamate produced profound coma, hypotension, and respiratory failure.[9]

TOXICOKINETICS

Absorption

Although the ingestion of large doses of meprobamate may delay GI absorption, peak meprobamate concentrations following the administration of therapeutic doses typically occur about 2–4 hours after ingestion.[10] Low water solubility, stability in acid solutions, and reduced gut motility contribute to the formation of drug masses and the subsequent delay in absorption and serious toxicity. Approximately 25 g meprobamate

remained in the stomach of a woman during the post-mortem examination; she died of respiratory arrest after initially regaining consciousness following a large meprobamate overdose.[11] Forty hours after the ingestion of an estimated meprobamate dose of 36 g, gastrotomy removed approximately 25 g meprobamate from the stomach of a 56-year-old woman.[12]

Distribution

The volume of distribution of meprobamate is relatively small (0.75 L/kg) with limited plasma protein binding (i.e., 10–20%). Arterial-venous differences in the plasma meprobamate concentration may develop during meprobamate overdose. In a study of 35 meprobamate intoxications, the mean plasma meprobamate concentration in femoral arterial blood samples was about 10% higher ($P < .01$) than in plasma venous samples.[13] Case reports of the meprobamate concentrations in various postmortem tissues suggest that meprobamate distributes preferentially into muscle, heart, and bile with relatively low concentrations in the brain.[14]

Biotransformation

Meprobamate undergoes biotransformation in the liver by *N*-glucuronidation of the parent drug and by oxidation of the propyl side-chain to hydroxymeprobamate. The latter metabolite is probably inactive. Meprobamate is a metabolite and prodrug of the muscle relaxant, carisoprodol.

Elimination

The kidney excretes about 10% of a therapeutic dose of meprobamate unchanged in the urine. The unaided renal clearance of meprobamate is about 5–30 mL/min. The serum elimination half-life of meprobamate is approximately 8–12 hours following the administration of therapeutic doses of meprobamate to healthy volunteers,[15] but a case report suggests that the elimination half-life may increase during an overdose.[16] However, the propensity of meprobamate to form concretions during an overdose complicates the interpretation of such increases in serum half-life during meprobamate intoxication.

Maternal and Fetal Kinetics

Meprobamate crosses the placenta and produces similar maternal and fetal meprobamate concentrations following the maternal use of meprobamate. Meprobamate is excreted in breast milk.

Tolerance

Animal studies suggest that chronic use of meprobamate causes tolerance to the behavioral effects of meprobamate, similar to other sedative hypnotic drugs.[17]

Drug Interactions

Sedative-hypnotics, antipsychotics, opioids, and ethanol synergistically increase the central nervous system depressant effects of meprobamate. Chronic induction of hepatic CYP450 isoenzymes (e.g., ethanolism) reduces the serum half-life of meprobamate by increasing meprobamate elimination,[18] whereas acute administration of ethanol decreases the meprobamate metabolism.[19] In a study of 4 alcohol abusers and 4 nonabusers, the administration of ethanol for 1 month under metabolic ward conditions (i.e., up to 46% of the caloric intake) reduced the mean serum half-life of meprobamate for these 2 groups from 16.7 ± 2.5 hours to 8.1 ± 1.5 hours and 13.7 ± 1.0 hour to 8.2 ± 0.3 hour, respectively.[20] The administration of meprobamate to patients with acute porphyria is contraindicated because meprobamate increases the concentration of *S*-aminolevulinic acid.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Meprobamate is a CNS depressant that inhibits spinal reflexes and produces limited muscle relaxation. Following the ingestion of high dose of meprobamate, depressed cardiac contractility and slightly reduces systemic vascular resistance may cause hypotension, typically before respiratory depression.[21] An echocardiographic study of hypotensive patients during meprobamate intoxication demonstrated hypokinesis of the heart manifest by a low cardiac index and increased diastolic filling pressures (dilated inferior vena cava).[22] The mechanism of cardiac toxicity during meprobamate poisoning remains unclear. Postmortem findings following fatal meprobamate intoxication are nonspecific (i.e., pulmonary edema, visceral congestion).[11]

CLINICAL RESPONSE

Adverse effects associated with the medical use of meprobamate include drowsiness, headache, weakness, paresthesias, blurred vision, and GI upset.

Overdose

Meprobamate produces a clinical picture of CNS depression that is similar to those of other sedative-hypnotic drugs including phenobarbital. Symptoms range from nystagmus, dysarthria, ataxia, and confusion to deep coma, hypotension, apnea, and pulmonary edema. Serious complications of meprobamate overdose include profound hypotension, shock, hypothermia, and respiratory failure. Because of depressed myocardial contractility during meprobamate intoxication, cardiogenic pulmonary edema may develop, particularly following the administration of substantial amounts of IV fluids.[23] In a review 57 meprobamate overdoses from the files of a clinical laboratory, the most common physical signs associated with meprobamate intoxication were dysarthria (21%) and hypotension (18%) followed by tachycardia, ataxia, miosis and mydriasis, nystagmus, respiratory depression, hallucination, seizures, dysconjugate gaze, and hypothermia.[16] Fluctuating levels of consciousness may result from the delayed release of meprobamate from concretions in the stomach; sudden deterioration may occur after initial improvement because of the release of meprobamate from these concretions. Hypotension usually correlates to the depth of coma, but occasionally hypotension develops during the lighter stages of coma. Convulsions are more common in the recovery phase of meprobamate overdose compared with other sedative-hypnotic drug overdoses.

Abstinence Syndrome

Case reports suggest that the chronic use of high meprobamate doses causes withdrawal symptoms following the abrupt cessation of use. The clinical features of meprobamate withdrawal are similar to barbiturate withdrawal, particularly when the daily dose of meprobamate ranges from 3–6 g. Signs of withdrawal of patients ingesting high doses of meprobamate include insomnia, nausea, vomiting, tremors, anxiety, muscle twitching, headache, ataxia, delirium, and seizures.[24] The abstinence syndrome is much less common in patients ingesting daily meprobamate doses below 1.6 g; however, a 50-year-old man chronically using meprobamate (i.e., estimated 1,200 mg meprobamate daily for 3 years) developed hypertension, sweating, agitation, incoherent behavior, and hallucinations in the hospital 4 days after cessation of use.[25] Although fatalities can occur during meprobamate withdrawal, the contribution of meprobamate withdrawal to these deaths is not well-defined.[26]

Reproductive Abnormalities

There are few data on the effect of meprobamate on human reproduction, and there is no definite evidence that meprobamate use during pregnancy is teratogenic.

An epidemiologic study of 1,870 children of mothers using meprobamate during pregnancy did not detect an increased incidence of fetal demise or abnormalities in these children up to the age of 4 years.[27] In a case series of 42 infants born to mothers ingesting an overdose of meprobamate during pregnancy, 7 infants had congenital abnormalities compared with 4 congenital abnormalities in matched siblings (OR = 1.7; 95% CI: 0.5–4.9).[28] Of the 14 mothers ingesting an overdose of meprobamate during the first trimester, 2 infants had minor congenital abnormalities (undescended testes, congenital hip dysplasia).

DIAGNOSTIC TESTING

Analytic Methods

Analytic methods for the determination of meprobamate concentrations in biologic fluids include thin layer chromatography,[29] liquid chromatography/tandem mass spectrometry with [^{13}C-^{2}H$_3$]-meprobamate as the internal standard,[30] gas chromatography with liquid-liquid[31] or liquid-solid extraction,[32] and gas chromatography with electron impact mass spectrometry.[33] The limit of detection (LOD) for meprobamate using gas chromatography with flame ionization detection or liquid chromatography/tandem mass spectrometry is approximately 1 mg/L.[30,34] For gas chromatography/mass spectrometry with carisoprodol as the internal standard, the LOD and lower limit of quantitation (LLOQ) for meprobamate are 0.58 mg/L and 1.93 mg/L, respectively.[35] Meprobamate is unstable at high temperatures and displays no useful UV spectrum.

Biomarkers

A study of healthy volunteers receiving single therapeutic doses of meprobamate (400 mg, 800 mg, 1,200 mg) indicated that meprobamate is detectable in hair samples beginning about 4–5 days after ingestion with peak concentrations occurring about 7–9 days after exposure.[36] Meprobamate was not detectable in most hair samples 2 weeks after exposure. There are inadequate data to determine the dose and time of ingestion based on meprobamate concentrations in hair samples. Bile is an alternative postmortem sampling site with meprobamate concentrations exceeding 53 mg/L consistent with potentially fatal overdoses.[37]

BLOOD

OVERDOSE. Serum meprobamate concentrations do not always correlate to clinical effects because of the presence of other drugs, tolerance, underlying medical disease, and individual variability. Therapeutic serum concentrations of meprobamate range from approximately 10–20 mg/L with toxic effects occurring in nontolerant individuals when the serum meprobamate concentration approaches 30–50 mg/L. Serum meprobamate concentrations in the range of 80–120 mg/L are associated with significant alteration of consciousness, whereas serum meprobamate concentrations in the range of 150–200 mg/L produce deep coma and hemodynamic instability. Although the level of consciousness does not always correlate to the serum meprobamate concentration,[16] most patients regain consciousness when the meprobamate concentration decreases below 50 mg/L. A 2-year-old child was unconscious, responding only to painful stimuli after ingesting meprobamate tablets that produced a peak plasma meprobamate concentration of 170 mg/L.[38] The patient regained consciousness when the plasma meprobamate concentration was 144 mg/L. The serum meprobamate concentration helps guide management, but these concentrations must be interpreted along with clinical features because of a variety of factors affecting response including tolerance. For example, a serum meprobamate concentration of 67 mg/L was associated with lethargy and responsiveness to verbal stimuli in a nontolerant individual, whereas a tolerant individual was alert when his serum meprobamate concentration was 82 mg/L as measured by a spectrophotometric method that did not include meprobamate metabolites.[39]

POSTMORTEM. Analysis of postmortem blood samples for cases involving meprobamate often demonstrates the presence of other drugs, particularly ethanol. In a series of 19 cases demonstrating meprobamate in postmortem blood samples, the mean whole blood meprobamate concentrations was 182 mg/L with a range of 41–397 mg/L.[40] Only 2 cases involved *only* meprobamate (68 mg/L, 168 mg/L) as measured by gas chromatography/mass spectrometry with solid phase extraction. In a case series of 12 fatalities involving only meprobamate intoxication, the meprobamate concentrations in postmortem blood samples ranged from 142–342 mg/L (mean, 226 mg/L) as measured by colorimetry.[41] In blood samples from 10 fatalities involving both meprobamate and ethanol, the blood meprobamate concentration ranged from 43–155 mg/L with a mean of 117 mg/L.

Abnormalities

The development of cardiac dysrhythmias and bradycardia are rare during meprobamate intoxication.

Driving

There are limited data on the effect of meprobamate on driving skills. Based on the pharmacologic similarity of meprobamate and barbiturates, the ingestion of substantial doses of meprobamate would be expected to impair coordination and slow reaction times. In a study of drivers arrested for driving under the influence with only carisoprodol/meprobamate present in blood samples, inappropriate driving behaviors included weaving, driving slowly, swerving, erratic lane changes, and impacts with both stationary and moving objects.[42] Meprobamate is a metabolite of the muscle relaxant, carisoprodol.

TREATMENT

Stabilization

Similar to other sedative-hypnotic overdoses, stabilization requires careful evaluation of the level of consciousness, oxygenation (pulse oximetry, arterial blood gases), and hemodynamic stability (rhythm, blood pressure). Hypotension may occur with minimal alteration of consciousness, but fluid overload must be avoided because of the depressant effects of meprobamate on myocardial contractility. Vasopressors are a therapeutic option for patients unresponsive to initial fluid replacement (i.e., 2 L of normal saline); however, hemodynamic monitoring (e.g., Swan-Ganz catheter, echocardiography) may be necessary to guide the management of fluids and vasopressors, particularly in the presence of hypotension and hypokinesis of the heart (reduced left ventricular ejection fraction, cardiac index). Case reports of severe meprobamate intoxication monitored by Swan-Ganz catheter indicate that severe meprobamate intoxication produces myocardial depression and vasodilation rather than noncardiac pulmonary edema.[43]

Gut Decontamination

The administration of activated charcoal is appropriate for patients with meprobamate overdose presenting <1–2 hours after ingestion. However, most patients present to a health care facility >1–2 hours after meprobamate ingestion, and these patients frequently require no gut contamination measures as a result of the lack of clinical data to support the use of aggressive decontamination measures. Theoretically, the administration of activated charcoal more than 1–2 hours after ingestion can decrease meprobamate absorption in comatose patients with decreased GI motility, but there are inadequate data to indicate that the administration

of activated charcoal at this time improves clinical outcome. Gastric lavage in comatose patients is a therapeutic option, particularly when delayed gastric emptying and the formation of drug concretions are suspected. However, there are few data on the efficacy of gastric lavage in meprobamate overdose. Syrup of ipecac is not recommended because of the potential for aspiration and the lack of efficacy. Fluctuating vital signs or level of consciousness suggests the presence of co-ingestants or continued meprobamate absorption from drug concretions and therefore, the need for endoscopy.[44]

Elimination Enhancement

There are few clinical data to compare the clinical outcome of various methods of enhancing the elimination of meprobamate during overdose. Case reports indicate that the use of hemoperfusion substantially increases the clearance of meprobamate from the blood. In a series of 4 cases of severe meprobamate intoxication treated with hemoperfusion, the renal clearance of meprobamate ranged from about 150–220 mL/min with the clearance slightly higher for resin cartridges compared with charcoal cartridges.[45,46,47] Hemodialysis also increases the clearance of meprobamate, but the clearance rates are several times less than the clearance rates during hemoperfusion. In a patient with hypotension, coma, and respiratory failure after a meprobamate overdose (serum meprobamate concentration, 500 mg/L), the clearance rate for meprobamate during hemodialysis was about 62 ± 10 mL/min.[9] Continuous arteriovenous hemoperfusion (CAVHP) with coated activated charcoal is an alternative to hemoperfusion and hemodialysis that does not require a pump. The technique of CAVHP is similar to arteriovenous hemofiltration with the exception that a carbon cartridge replaces the hemofilter. Case reports indicate that despite low mean arterial blood pressure (i.e., about 50 mm Hg), CAVHP can achieve meprobamate clearance rates of about 50 mL/min.[48] Following hemodynamic stabilization, the clearance rates of CAVHP are similar (i.e., 170–210 mL/min) to the clearance achieved by charcoal or resin hemoperfusion. Unaided renal clearance of meprobamate is approximately 5–30 mL/min. Factors involved with the decision to use methods to enhance meprobamate elimination include lack of response to supportive care and massive meprobamate ingestion (30–40 g) or serum meprobamate concentrations of 100–200 mg/L with hemodynamic instability.

A case report suggests that the use of serial activated charcoal (50 g every 6 hours for 5 doses) reduces the serum half-life of meprobamate during mild to moderate meprobamate intoxication, but there are inadequate

kinetic and clinical data to verify the efficacy of serial activated charcoal during meprobamate intoxication.[49] The relatively small volume of distribution suggests that rebound increases in blood meprobamate concentrations following the use of measures to enhance elimination are unlikely, and the presence of such increases suggests the presence of continuing meprobamate absorption (i.e., bezoar). Forced diuresis is contraindicated during meprobamate intoxication because of the propensity of meprobamate toxicity to cause pulmonary edema. Peritoneal dialysis is much less effective than hemodialysis.

Antidotes

There is no antidote.

Supplemental Care

Baseline laboratory tests for severe meprobamate ingestion include a complete blood count, serum electrolytes, glucose, creatinine, chest x-ray, and arterial blood gases. Frequent vital signs are necessary during the initial stages of meprobamate intoxication because of the occurrence of hypotension during the early stages of coma. Fluctuating vital signs or coma may indicate a drug mass and gastroscopy should be considered. Pulmonary complications of meprobamate intoxication include pulmonary edema and aspiration pneumonia. Chronic meprobamate users may develop an abstinence syndrome 1–4 days after the cessation of meprobamate use.

References

1. Ludwig BJ, Piech E. Some anticonvulsant agents derived from 1,3-propanediol. J Am Chem Soc 1951;74: 5779–5781.

2. Ramchandani D, Lopez-Munoz F, Alamo C. Meprobamate—tranquilizer or anxiolytic? A historical perspective. Psychiatr Q 2006;77:43–53.

3. Allen AG, Black AV. Near-fatal case of intoxication with meprobamate treated with electro-stimulation and levarterenol. Ohio Med 1956;52:1303.

4. Greenblatt DJ, Shader RI. Meprobamate: a study of irrational drug use. Am J Psychiatry 1971;127:1297–1303.

5. Gaultier M, Fournier E, Bismuth C, Rapin J, Fréjaville JP, Gluckman JC. [Acute poisoning by meprobamate. Apropos of 141 cases]. Bull Mem Soc Med Hop Paris 1968;119:675–705. [French]

6. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Giffin SL. 2009 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th Annual Report. Clin Toxicol (Phila) 2010;48:979–1178.

7. Roache JD, Griffiths RR. Lorazepam and meprobamate dose effects in humans: behavioral effects and abuse liability. J Pharmacol Exp Ther 1987;243:978–988.

8. Allen MD, Greenblatt DJ, Noel BJ. Meprobamate overdosage: a continuing problem. Clin Toxicol 1977;11: 501–515.

9. Lobo PI, Spyker D, Surratt P, Westervelt FB Jr. Use of hemodialysis in meprobamate overdosage. Clin Nephrol 1977;7:73–75.

10. Parker KD, Elliott HW, Wright JA, Nomof N, Hine CH. Blood and urine concentrations of subjects receiving barbiturates, meprobamate, glutethimide or diphenylhydantoin. Clin Toxicol 1970;3:131–145.

11. Jenis EH, Payne RJ, Goldbaum LR. Acute meprobamate poisoning. JAMA 1969;207:361–362.

12. Schwartz HS. Acute meprobamate poisoning with gastrotomy and removal of a drug containing mass. N Engl J Med 1976;295:1177–1178.

13. Sato S, Baud FJ, Bismuth C, Galliot M, Vicaut E, Buisine A. Arterial-venous plasma concentration differences of meprobamate in acute human poisonings. Hum Toxicol 1986;5:243–248.

14. Kintz P, Tracqui A, Mangin P, Lugnier AA. Fatal meprobamate self-poisoning. Am J Forensic Med Pathol 1988; 9:139–140.

15. Hollister LE, Levy G. Kinetics of meprobamate elimination in humans. Chemotherapy 1964;15:20–24.

16. Bailey DN. The present status of meprobamate ingestion: A five year review of cases with serum concentrations and clinical findings. Am J Clin Pathol 1981;75:102–106.

17. Richelle M. A note on behavioral tolerance to meprobamate. J Exp Anal Behav 1965;8:45–46.

18. Misra PS, Lefévre A, Ishii H, Rubin E, Lieber CS. Increase of ethanol, meprobamate and pentobarbital metabolism after chronic ethanol administration in man and in rats. Am J Med 1971;51:346–351.

19. Khanna JM, Chung S, Ho G, Shah G. Acute metabolic interaction of ethanol and drugs. Curr Alcohol 1979;7: 93–108.

20. Misra PS, Lefevre A, Ishii H, Rubin E, Lieber CS. Increase of ethanol, meprobamate and pentobarbital metabolism after chronic ethanol administration in man and in rats. Am J Med 1971;51:346–351.

21. Blumberg AG, Rosett HL, Dobrow A. Severe hypotensive reactions following meprobamate overdosage. Ann Intern Med 1959;51:607–612.

22. Charron C, Mekontso-Dessap A, Chergui K, Rabiller A, Jardin F, Vieillard-Baron A. Incidence, causes and prognosis of hypotension related to meprobamate poisoning. Intensive Care Med 2005;31:1582–1586.

23. Maddock RK, Bloomer HA. Meprobamate overdosage. Evaluation of its severity and methods of treatment. JAMA 1967;201:999–1003.

24. Haizlip TM, Ewing JA. Meprobamate habituation. A controlled clinical study. N Engl J Med 1958;258:1181–1186.

25. George WK, Stubb JB. Meprobamate withdrawal syndrome. Tex State J Med 1965;61:48.

26. Swanson LA, Okada T. Death after withdrawal of meprobamate. JAMA 1963;184:780–781.

27. Hartz SC, Heinonen OP, Shapiro S, Siskind V, Slone D. Antenatal exposure to meprobamate and chlordiazepoxide in relation to malformations, mental development and childhood mortality. N Engl J Med 1975;292:726–728.

28. Timmermann G, Acs N, Banhidy F, Czeizel AE. A study of teratogenic and fetotoxic effects of large doses of meprobamate used for a suicide attempt by 42 pregnant women. Toxicol Ind Health 2008;24:97–107.

29. McConnell DT. Thin layer chromatographic identification of thirteen medicinally important carbamates. J Chromatogr 1967;29:283–287.

30. Delavenne X, Gay-Montchamp JP, Basset T. HPLC MS/MS method for quantification of meprobamate in human plasma: application to 24/7 clinical toxicology. J Chromatogr B 2011;879:215–218.

31. Trenque T, Lamiable D, Millart H, Vistelle R, Choisy H. Gas chromatographic determination of meprobamate in human plasma. J Chromatogr 1993;615:343–346.

32. Gaillard Y, Gay-Montchamp JP, Ollagnier M. Gas chromatographic determination of meprobamate in serum or plasma after solid-phase extraction. J Chromatogr 1992;577:171–173.

33. Kintz P, Mangin P. Determination of meprobamate in human plasma, urine, and hair by gas chromatography and electron impact mass spectrometry. J Anal Toxicol 1993;17:408–410.

34. Trenque T, Lamiable D, Millart H. Gas chromatographic determination of meprobamate in human plasma. J Chromatogr 1993;615:343–346.

35. Daval S, Richard D, Souveine B, Eschalier A, Coudore F. A one-step and sensitive GC-MS assay for meprobamate determination in emergency situations. J Anal Toxicol 2006;30:302–305.

36. Kintz P, Tracqui A, Mangin P. Pharmacological studies on meprobamate incorporation in human beard hair. Int J Leg Med 1993;105:283–287.

37. Fanton L, Bevalot F, Gustin MP, Paultre CZ, Le Meur C, Malicier D. Interpretation of drug concentrations in a alternative matrix: the case of meprobamate in bile. Int J Legal Med 2009;123:97–102.

38. Dennison J, Edwards JN, Volans GN. Meprobamate overdosage. Hum Toxicol 1985;4:215–217.

39. Bailey DN, Shaw RF. Interpretation of blood glutethimide, meprobamate and methyprylon concentrations in nonfatal and fatal intoxications involving a single drug. J Toxicol Clin Toxicol 1983;20:133–145.

40. Gaillard Y, Billault F, Pepin G. Meprobamate overdosage: a continuing problem. Sensitive GC-MS quantitation after solid phase extraction in 19 fatal cases. Forensic Sci Int 1997;86:173–180.

41. Felby S. Concentrations of meprobamate in the blood and liver following fatal meprobamate poisoning. Acta Pharmacol Toxicol 1970;28:334–337.

42. Logan BK, Case GA, Gordon AM. Carisoprodol, meprobamate, and driving impairment. J Forensic Sci 2000; 45:619–623.

43. Eeckhout E, Huyghens L, Loef B, Maes V, Sennesael J. Meprobamate poisoning, hypotension and Swan-Ganz catheter. Intensive Care Med 1988;14:437–438.

44. Davis RN, Rettmann JA, Christensen B. Relapsing altered mental status secondary to a meprobamate bezoar. J Trauma 2006;61:990–991.

45. Freund LG. Severe meprobamate intoxication treated by hemoperfusion over Amberlite resin. Artif Organs 1981; 5:80–81.

46. Jacobsen D, Wiik-Larsen E, Saltvedt E, Bredesen JE. Meprobamate kinetics during and after terminated hemoperfusion in acute intoxications. Clin Toxicol 1987; 25:317–331.

47. Hoy WE, Rivero A, Marin MG, Rieders F. Resin hemoperfusion for treatment of massive meprobamate overdose. Ann Intern Med 1980;93:455–456.

48. Lin J-L, Lim P-S, Lai B-C, Lin W-L. Continuous arteriovenous hemoperfusion in meprobamate poisoning. Clin Toxicol 1993;31:645–652.

49. Hassan E. Treatment of meprobamate overdose with repeated oral doses of activated charcoal. Ann Emerg Med 1986;15: 73–76.

Chapter 27

METHAQUALONE and RELATED COMPOUNDS

METHAQUALONE

HISTORY

Methaqualone originally was developed during the investigation of antimalarial drugs in India during the early 1950s.[1] The first experimental studies of the hypnotic properties of methaqualone began in 1956,[2] and the first controlled clinical trials comparing methaqualone with barbiturate hypnotic drugs were published in 1961.[3] In 1959, methaqualone was introduced in Japan as a nonprescription drug; the following year, this drug was introduced in the British market as an alternative to barbiturate sedative hypnotic drugs.[4] Methaqualone abuse and an associated abstinence syndrome (delirium, seizures) was first reported in Japan soon after the introduction of this drug.[5] In 1965, Rohrer introduced methaqualone into the United States market as a Drug Enforcement Administration (DEA) schedule V drug (i.e., unlimited prescription refills) under the brand name Quaalude™. Methaqualone was marketed as a safe hypnotic drug with low abuse potential, rapid onset of sleep induction, and few adverse effects. However, methaqualone quickly gained popularity as a "street aphrodisiac." By the early 1970s, methaqualone was recognized as major drug of abuse, particularly on college campuses in combination with ethanol ("luding out").[6] The United Nations listed methaqualone under the UN Convention on Psychotropic Substances, leading to the banning of methaqualone in most member countries. Methaqualone use in the United Kingdom was restricted by the British Drug Prevention of Misuse Act in 1971. The DEA reclassified this drug as a schedule I drug in 1975. Subsequently, the legal production of methaqualone ceased in the United States. The oral abuse of methaqualone decreased in Western countries since the late 1980s; however, illicit production of methaqualone continues, particularly in South Africa where smoking methaqualone is a popular form of drug abuse.[7]

IDENTIFYING CHARACTERISTICS

Methaqualone is a structurally unique sedative-hypnotic drug (Fig. 27.1), being a 2,3-bisubstituted quinazalone [CAS RN: 72-44-6, 2-methyl-3-o-tolyl-4-(3H)-quinazolinone]. This drug is a slightly water soluble, highly lipophilic, weak acid with a pKa of 2.4. Table 27.1 lists some physiochemical properties of methaqualone. Street names for methaqualone include Wallbanger, Quads, Ludes, Sopers, Mandies, Dr. Jekyll and Mr. Hyde Drug, Love Drug, and Soapers. Trade names for methaqualone include Quaaludes, Sopor, Mequin, and Mandrax (methaqualone and diphenhydramine). A case report associated a severe necrotizing cystitis manifest by dysuria, frequency, and suprapubic pain with contamination of illicit methaqualone by o-toluidine during the clandestine production of methaqualone.[8]

EXPOSURE

Although legal production of methaqualone ceased in the United States, the American Association of Poison Control Centers' National Poison Data System Annual Report listed 4 exposures and no fatalities associated with the use of methaqualone in 2009.[9]

	R₁	R₂
Nitromethaqualone	OCH₃	NO₂
Methaqualone	CH₃	H
Mecloqualone	Cl	H
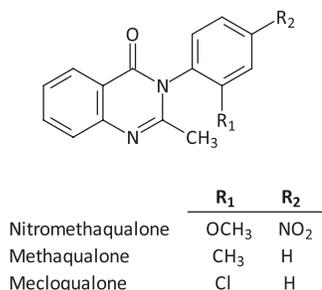
FIGURE 27.1. Chemical structures of methaqualone and related compounds.

TABLE 27.1. Some Physiochemical Properties of Methaqualone.

Physical Property	Value
Melting Point	120°C (248°F)
log P (Octanol-Water)	4.330
Water Solubility	4.730 mg/L (25°C/77°F)
Vapor Pressure	1.16E-07 mm Hg (25°C/77°F)

Sources

Methaqualone is a sedative-hypnotic drug that has been banned in most countries as a result of the abuse potential of this drug. However, illicit production of methaqualone continues in the Middle East, south and central Asia, southern Africa, and particularly in South Africa. Clandestine laboratories synthesize methaqualone and methyl derivatives of methaqualone.[10] Methods of illicit methaqualone synthesis involve 1) preparation of *N*-acetyl anthranilic acid from anthranilic acid and acetic anhydride followed by condensation with *o*-toluidine to form methaqualone with phosphorus trichloride as a dehydrating agent, and 2) refluxing anthranilic acid, *o*-toluidine, and acetic acid (or acetic anhydride) with polyphosphoric acid as a dehydrating agent. The presence of 2-methyl-3-(*o*-carboxyphenyl)-4-quinazolinone indicates the former synthetic process, whereas the presence of *o*-methyl acetanilide indicates the use of the latter synthetic process. Other less common synthetic processes include the cyclization of *o*-acylamino (*N*-substituted) benzamide compounds.[11] Despite the synthesis of numerous quinazolinone derivatives of methaqualone for CNS depressant and anticonvulsant properties, no currently used anticonvulsant contains the 4(3H)-quinazolinone ring system.[12] Previous pharmaceutic formulations of methaqualone included methaqualone base (150-mg and 300-mg tablets), methaqualone hydrochloride (200-mg and 400-mg capsules), and combination tablets (e.g., Mandrax, 250 mg methaqualone, 25 mg diphenhydramine).

Methods to identify routes of illicit methaqualone synthesis include analysis of precursors and by-products by gas chromatography/mass spectrometry and infrared/nuclear magnetic resonance.[13,14] Both the licit and illicit manufacturing of methaqualone produce similar isomers; therefore, analysis of methaqualone enantiomers does not help determine the origin of an illicit methaqualone sample.

Methods of Abuse

The ingestion of 300–450 mg methaqualone with wine (luding out) previously was a popular college pastime. Common methods of methaqualone abuse in South Africa involve smoking a mixture of crushed methaqualone tablets and cannabis called *witpyp* or white pipe, usually in a male-dominated, communal setting.[15,16] Desired effects of methaqualone abuse include euphoria, diminished proprioception, a sense of well-being, ataxia, paresthesias, reduced inhibitions, and increased libido. The popularity of methaqualone as an illicit drug results, in part, from the perception that methaqualone enhances intimate and interpersonal relations through reduction of inhibitions. In a questionnaire of recruited methaqualone users, the percentage of respondents reporting increased libido, enhanced body sensations, paraesthesias, and desire for sexual intercourse was substantially higher than the percentage of respondents expecting these effects prior to drug use.[17] Almost all of these respondents used marijuana concurrently with methaqualone and about one-half ingested alcohol with the methaqualone. Approximately 52% of these respondents were students. Comparative trials indicate that methaqualone produces more euphoria and less sedation compared with benzodiazepines (e.g., lorazepam, alprazolam, diazepam).[18]

DOSE EFFECT

In volunteer studies, the ingestion of 200 mg methaqualone is associated with euphoria and sedation.[19] Typical "street" doses of methaqualone associated with euphoria, paraesthesias, and relaxation range from about 150–500 mg. Chronic methaqualone abusers may use up to 2 g methaqualone daily. Methaqualone doses ranging from 500–1,000 mg cause dizziness, gastrointestinal distress (GI), ataxia, anxiety, and fatigue, whereas methaqualone doses between 1,000–2,000 mg produce increased hyperreflexia and myoclonus. Ingestion of >2 g methaqualone causes serious central nervous system (CNS) toxicity. A 23-year-old man was somnolent and poorly responsive to painful stimuli 2 days after

ingesting an estimated 4–5 g methaqualone during a suicide attempt.[20] Within 2 days, he became comatose with respiratory failure secondary to noncardiogenic pulmonary edema. He recovered completely after 6 days of intubation and ventilatory support. Estimation of the fatal dose of methaqualone is complicated by various factors including tolerance, concomitant drug ingestion, baseline health of the patient, and reporting bias. Although case reports associate death with the ingestion of 8–20 g methaqualone, survival can occur with intensive supportive care following the ingestion of methaqualone doses in the range of 20–25 g.

TOXICOKINETICS

Absorption

The GI tract rapidly adsorbs therapeutic doses of methaqualone with peak methaqualone concentrations occurring about 1–2 hours after ingestion.[21,22] In a study of 7 healthy volunteers administered 300 mg methaqualone, the average peak plasma methaqualone concentration was about 3 mg/L 2 hours after ingestion as measured by gas chromatography with flame ionization detection.[23] Ingestion of 450 mg methaqualone/hour for 4 doses produced a peak serum methaqualone concentration of 9 mg/L 3 hours after administration of the last dose.[24] Based on neuropsychologic testing of healthy volunteers, the maximum effect of methaqualone occurs before peak methaqualone concentrations and the duration of action is <4 hours despite persistent plasma methaqualone concentrations.[25] Ethanol does not significantly delay methaqualone absorption in human volunteers.

Distribution

Methaqualone is highly lipophilic with a large volume of distribution (2.4–6.4 L/kg). The initial distribution phase into peripheral tissues is relatively rapid with an initial distribution half-life of 2–4 hours. Methaqualone is highly protein bound (75–90%).[26]

Biotransformation

Methaqualone undergoes metabolism in the liver to *N*-oxide and hydroxylated metabolites.[27] There is substantial interindividual variability in *C*-oxidation by P450 cytochrome isoenzymes and the formation of hydroxylated metabolites of methaqualone that are subsequently excreted in the urine as glucuronide conjugates.[28,29] Volunteer studies indicate that extensive biotransformation by CYP450 isoenzymes produce at least 5 major hydroxylated metabolites (2′-hydroxy-methaqualone, 3′-hydroxymethaqualone, 4′-hydroxy-methaqualone, 6′-hydroxymethaqualone, and 2-hydroxymethaqualone) as demonstrated in Figure 27.2. Although the specific isoenzymes involved with the biotransformation of methaqualone have not been identified, CYP2D6 and CYP2C19 isoenzymes are probably not involved in this metabolic process.[30] The hydroxylated metabolites are inactive. Dihydroxy, dihydrodiols, and hydroxymethoxy derivatives of methaqualone probably are minor metabolites.

Elimination

The kidney excretes <1–3% of an absorbed dose of methaqualone unchanged in the urine. Most of the methaqualone metabolites appear in the urine as glucuronide conjugates.[31] The terminal elimination following single therapeutic doses of methaqualone is relatively long (i.e., 20–40 hours).[32] In a study of 7 healthy men (mean age, 36 years), the elimination of methaqualone from the plasma was biphasic with an initial and terminal mean plasma elimination half-life of 0.9 hours and 16 hours, respectively.[33]

Maternal and Fetal Kinetics

In rodents, methaqualone crosses the placenta; 1.5 hours after maternal ingestion, the methaqualone concentration in fetal brain and liver is lower than corresponding maternal tissue.[34] The renal clearances of some drugs increase during the third trimester of pregnancy as a result of increased renal blood flow and glomerular filtration rates. In a convenience sample of 10 pregnant women in the third trimester of pregnancy, the mean half-life of methaqualone was 4.36 ± 0.84 hours compared with 6.18 ± 0.92 hours in 13 nonpregnant women.[35]

Tolerance

Animal studies indicate that tolerance develops in rodents after daily administration of methaqualone. The daily intraperitoneal administration of 30 mg methaqualone to female rats produced tolerance to the effects of methaqualone on the Rotorod test as well as cross-tolerance to the effects of phenobarbital.[36] Tolerance to methaqualone also occurs in observational studies of polydrug users, but the degree of tolerance is not well-defined in these studies.[37]

Drug Interactions

In vitro studies indicate that administration of diphenhydramine with methaqualone produces a dose-related inhibition of methaqualone metabolism.[38] The combina-
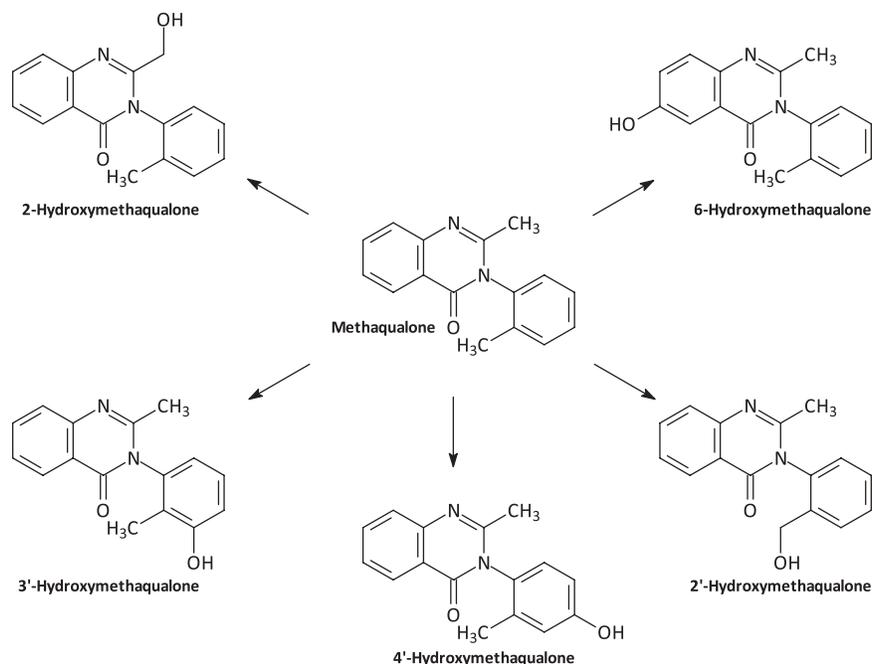
FIGURE 27.2. Major hydroxylated metabolites of methaqualone.[59]

tion of methaqualone and diphenhydramine is a frequently abused drug combination. Ethanol consumption slightly increases blood methaqualone concentrations during elimination and methaqualone produces relatively greater impairment of cognitive and psychomotor tasks when combined with ethanol.[39]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Methaqualone is a sedative-hypnotic drug with euphoriant properties.[4] In contrast to hyporeflexia and muscular depression associated with other sedative-hypnotic drugs, high doses of methaqualone produce selective depression of polysynaptic spinal reflexes with subsequent hypertonia and muscle hyperactivity. Hypotension usually results from myocardial depression. Although hypotension and respiratory insufficiency occurs during serious methaqualone intoxication, the occurrence of hypotension and respiratory insufficiency is relatively less common during methaqualone intoxication compared with most other older sedative-hypnotic overdoses. Pulmonary edema is usually noncardiogenic, resulting from increased capillary permeability. *In vitro* studies of animal and human platelets indicate that methaqualone significantly inhibits the primary phase of adenosine diphosphate (ADP)-induced aggregation as well as platelet adhesiveness.[40] The ingestion of toxic

methaqualone doses also increases the propensity for spontaneous hemorrhage by prolongation of the prothrombin and partial thromboplastin times and reduction in factors V and VII.

CLINICAL RESPONSE

Dermatologic effects associated with the medicinal use of methaqualone include fixed drug eruption, diaphoresis, erythema multiforme, bromhidrosis, and urticaria.[41]

Illicit Use

Ingestion of "street" doses of methaqualone (i.e., 150–500 mg) classically produces euphoria with marked paresthesias followed by a period of deep relaxation. Sleep usually follows the initial period of euphoria. The ingestion of higher doses of methaqualone causes GI distress (nausea, vomiting, diarrhea), dry mouth, lightheadedness, anxiety, restlessness, loss of coordination, blurred vision, fatigue, myoclonus, and painful hyperacusis.

Overdose

Methaqualone is a CNS depressant, which produces toxic effects ranging from GI distress, drowsiness, ataxia, slurred speech, and paresthesias to agitation, convulsions, and coma. Mild methaqualone intoxication

507

produces clinical features similar to barbiturate intoxication including blurred vision, loss of coordination, nystagmus, dilated pupils, salivation, and mild CNS depression. More serious complications of methaqualone overdose include delirium, coma, respiratory depression, increased risk of aspiration with salivation and spontaneous vomiting, seizures, and prominent pyramidal signs (myoclonus, hyperreflexia, hypertonicity). In contrast to most of the older sedative-hypnotic drugs, increased muscle tone frequently occurs during severe methaqualone intoxication,[42] and muscular hyperactivity may be severe enough to require paralytic agents and respiratory support.[43] Although tachycardia is a common feature of methaqualone intoxication, cardiovascular collapse is uncommon except following very large ingestions of methaqualone. Hypotension occurs less commonly in comatose patients compared with other older sedative-hypnotics drugs.[44] The ingestion of large doses (i.e., 4–5 g) of methaqualone can produce respiratory failure and noncardiogenic pulmonary edema several days after ingestion.[20] Spontaneous bleeding (e.g., subconjunctival hemorrhage, retinal hemorrhage, GI hemorrhage) may develop during severe methaqualone intoxication as a result of thrombocytopenia and hypoprothrombinemia.[44,45] Skin lesions developing during serious methaqualone poisoning include bullae, purpura, and petechiae.

Abstinence Syndrome

The methaqualone withdrawal syndrome resembles the clinical features of ethanol and barbiturate withdrawal. Case reports indicate that the daily ingestion of 1.5–2 g methaqualone for several months produces withdrawal symptoms within 1–5 days after cessation of methaqualone use. The most common features of methaqualone withdrawal include headache, tremulousness, anorexia, nausea, vomiting, abdominal pain, and insomnia.[46] Other clinical effects of the abstinence syndrome range from restlessness, confusion, and hyperreflexia to visual hallucination, seizures, and delirium.[47] Brief muscle twitches, sudden diffuse muscle jerks, and tonic-clonic seizures developed in a 20-year-old woman 5 days after stopping the daily ingestion of 300 mg methaqualone.[48]

Reproductive Abnormalities

There are few data on reproductive abnormalities in pregnant women ingesting methaqualone. In rodent studies, fetal abnormalities occur at methaqualone doses (e.g., >25 mg/kg/d) that cause maternal toxicity.[49]

DIAGNOSTIC TESTING

Analytic Methods

Methods for the determination of methaqualone concentrations include immunoassays, ultraviolet (UV) spectrophotometry, gas chromatography with flame ionization, nitrogen phosphorus or electron capture detection, and gas chromatography/mass spectrometry (GC/MS).[50] The limit of detection (LOD) for gas chromatography with nitrogen phosphorus detection is near 0.020 mg/L,[51] whereas the lower limit of quantitation (LLOQ) for GC/MS ranges from about 0.050–0.100 mg/L.[52] Under some gas chromatographic conditions, thermal conversion of the methaqualone-N-oxide metabolite, which represents about 5–9% of methaqualone metabolites, increases the apparent concentration of methaqualone.[53] Gas chromatography/mass spectrometry effectively separates the structurally similar methaqualone compounds (nitromethaqualone, mecloqualone) from methaqualone.[54] Ultraviolet spectrophotometry does not separate unchanged methaqualone from metabolites; therefore, this method measures total methaqualone (methaqualone plus metabolites).[55]

Biomarkers

BLOOD

Interpretation of the serum methaqualone concentrations requires careful evaluation of the clinical setting and the physical evidence of intoxication. Blood methaqualone concentrations do not correlate well to the clinical response because of the presence of tolerance, concomitant ingestion of other respiratory depressant drugs, and the time since drug ingestion.[56] Deep coma and respiratory failure may occur in patients following large ingestions of methaqualone despite the presence of relatively low serum methaqualone concentrations and high concentrations of metabolites.[20] The therapeutic range of methaqualone is 1–5 mg/L. In a study of 7 healthy men receiving 300 mg methaqualone hydrochloride, the mean peak plasma methaqualone concentration was 2.12 ± 0.44 mg/L 3 hours after ingestion.[33] Toxicity is associated with methaqualone concentrations exceeding 10–30 mg/L. In a retrospective review of 60 cases of methaqualone intoxication, serum methaqualone concentrations associated with CNS depression exceeded 9 mg/L.[57] Serious intoxication can occur when the serum methaqualone concentrations exceeds 25–30 mg/L, particularly at serum methaqualone concentration >80 mg/L. There are few data on the methaqualone concentrations in postmortem blood samples

from individuals dying of methaqualone toxicity. Analysis of postmortem blood samples from a 62-year-old man found dead after a massive, intentional ingestion of toquilone compositum (methaqualone and diphenhydramine) demonstrated a methaqualone concentration of 800 mg/L.[58]

URINE

The Roche Abuscreen ONLINE™ (Roche Diagnostics, Indianapolis, IN) and the Syva EMIT II™ (Syva Co., San Jose, CA) are sensitive methods for the detection of methaqualone in the urine.[59] Because of the minimal excretion of unchanged methaqualone in the urine, the sensitivity of these immunoassays depends on the high cross-reactivity of the reagents for the conjugated metabolites of methaqualone.

Abnormalities

Laboratory abnormalities associated with methaqualone intoxication include thrombocytopenia, prolonged prothrombin time/international normalized ratio, and transient elevation of serum hepatic aminotransferases. Nonspecific ST-T wave changes and sinus tachycardia are the most common electrocardiographic changes seen during methaqualone intoxication; serious cardiac abnormalities are rare.[42]

Driving

There are limited data on the effects of methaqualone on driving skills. Analyses of blood samples submitted by law enforcement officers for suspected driving under the influence in Switzerland indicate that the use of methaqualone by impaired drivers is relatively uncommon (i.e., <5%).[60] Ingestion of methaqualone can produce sedation, loss of motor coordination, and slowing of motor responses. A double-blind study of healthy volunteers ingesting an evening dose of 250 mg methaqualone and 25 mg diphenhydramine did not detect decrements in psychomotor performance (i.e., choice reaction time, coordination test, divided attention task) when tested the following morning.[61] In a series of 536 drivers arrested for driving under the influence and testing positive only for methaqualone, the median blood methaqualone concentration was about 4 mg/L with a range of 1–9 mg/L as measured by analytic methods that excluded methaqualone metabolites.[62] Blood methaqualone concentrations above 2 mg/L were frequently associated with obvious intoxication (i.e., staggering, drowsiness, and slurred speech). Methaqualone was detected in 3 of 440 (<1%) blood samples drawn from Swiss drivers arrested for suspected driving while impaired (50 of the 440 blood samples contained no detectable drugs).[63] The methaqualone concentrations ranged from 0.15–2.36 mg/L.

TREATMENT

Stabilization

As for all sedative-hypnotic overdoses, stabilization requires careful evaluation of the level of consciousness and the adequacy of respirations. Patients with methaqualone intoxication frequently have a sinus tachycardia, but hypotension is relatively rare. Seizures may occur during withdrawal from methaqualone along with restlessness, agitation, and delirium. All patients with altered levels of consciousness should be evaluated rapidly for serum glucose concentrations and hypoxia (e.g., pulse oximetry).

Gut Decontamination

The administration of activated charcoal is appropriate for patients with methaqualone overdose presenting <1–2 hours after ingestion. However, most patients present to a health care facility >1–2 hours after methaqualone ingestion, and these patients frequently require no gut contamination measures as a result of the lack of clinical data to support the use of aggressive decontamination measures. Theoretically, the administration of activated charcoal more than 1–2 hours after ingestion can decrease methaqualone absorption in comatose patients with decreased GI motility, but there are inadequate data to indicate that the administration of activate charcoal at this time improves clinical outcome. Syrup of ipecac is not recommended because of the potential for aspiration and the lack of demonstrated efficacy.

Elimination Enhancement

Case reports indicate that hemodialysis reduces plasma methaqualone concentrations, but there are few data on the effect of hemodialysis on clinical outcome during methaqualone intoxication. Hemodialysis produces mean methaqualone clearance of approximately 29 mL/min during severe methaqualone intoxication, whereas the mean clearance during peritoneal dialysis was substantially lower (i.e., about 7 mL/min).[64] The clearance of methaqualone is superior following hemoperfusion (e.g., 179 mL/min) compared with hemodialysis (e.g.,

29 mL/min), but clinical response is limited perhaps because of the large volume of distribution, high protein binding, and large tissue stores of methaqualone during methaqualone intoxication. Blood methaqualone levels >40 mg/L suggest the need for hemoperfusion. Forced diuresis is contraindicated because of the propensity of methaqualone to cause pulmonary edema.

Antidotes

There is no antidote.

Supplemental Care

Most methaqualone overdoses require only supportive care. Baseline laboratory tests for severe methaqualone poisoning include a complete blood count, platelets, prothrombin time, electrolytes, glucose, creatinine, liver function tests, pulse oximetry, and an electrocardiogram. Patients with moderate to severe methaqualone overdoses require observation for the development of aspiration pneumonia and pulmonary edema. Because of the association of thrombocytopenia and reduced prothrombin times following methaqualone overdoses, these patients also should be observed for the development of spontaneous hemorrhage (e.g., GI, retinal). The treatment for persistent hemorrhage during methaqualone intoxication includes platelet packs, vitamin K, and fresh-frozen plasma as indicated by diagnostic testing. Muscular hyperactivity may require muscle relaxants (e.g., diazepam, lorazepam), but these patients should be carefully observed for the development of respiratory depression. Withdrawal symptoms may develop 1–5 days after cessation of methaqualone use. There are inadequate clinical data on specific therapeutic options for the treatment of methaqualone addiction; typically, benzodiazepines (e.g., diazepam) are first-line drugs used for the treatment of methaqualone dependence.[65]

MECLOQUALONE

Mecloqualone (CAS RN: 340-57-8, 2-methyl-3-ortho-chlorophenyl-4(3H)-quinazolinone) is a chlorinated homologue of methaqualone; mecloqualone is also a drug of abuse. The DEA lists both methaqualone and mecloqualone (Nubarene) as schedule I drugs. Clandestine laboratories synthesize mecloqualone and methyl derivatives of methaqualone as well as methaqualone.[66] Mecloqualone undergoes extensive bio-transformation, primarily by hydroxylation.[67] The major metabolite of mecloqualone in the urine is 3-(2-chloro-4-hydroxyphenyl)-2-methyl-4[3H]-quinazolinone. Minor metabolites include 3-(o-chlorophenyl)-2-(hydroxymethyl)-4[3H]-quinazolinone and 3-(o-chlorophenyl)-6-hydroxy-2-methyl-4[3H]-quinazolinone. Approximately one-half of the dose of mecloqualone is eliminated within 2 days and 80% within 5–6 days, primarily in the urine as conjugates.[68] The kidney excretes very small amounts (i.e., <2–3%) of unchanged mecloqualone in the urine. The molecular weight and lack of a strong anionic group suggest that mecloqualone has very limited enterohepatic recirculation. Analytic methods for the separation and detection of quinazolinone compounds include thin layer chromatography, gas liquid chromatography, nuclear magnetic resonance, and gas chromatography/mass spectrometry.[54,67] Ultraviolet spectra identify the quinazolinone nucleus, but UV spectroscopy is incapable of distinguishing between methaqualone, methaqualone metabolites, nitromethaqualone, or mecloqualone.

NITRO-METHAQUALONE

Nitromethaqualone [CAS RN: 340-52-3, 2-methyl-3-(2'-methoxy-4'-nitrophenyl)-4(3H)-quinazolinone] is a non-barbiturate hypnotic drug (Parnox®) introduced in Europe in 1967.[69] In a study of 2 healthy volunteers, peak plasma nitromethaqualone concentrations were 65 ng/mL at 2 hours and 135 ng/mL at 1.5 hours after the ingestion of 25 mg of this drug as measured by gas chromatography with electron capture detection.[70] Based on volunteer studies the most important unconjugated metabolite of nitromethaqualone biotransformation is 2-methyl-3-(2'-methoxy-4'aminophenyl)-4(3H)-quinazolinone as a result of the reduction of the nitro group to the corresponding amino derivative.[69] Excretion of this metabolite occurs partially in the acetylated form. o-Demethylation followed by reduction of the nitro group is a minor metabolic pathway in humans. Cleavage of the quinazolinone nucleus results in formation of 2-methoxy-4-nitroaniline. Volunteer studies indicate that biotransformation is the primary route of elimination with minimal excretion of unchanged nitromethaqualone in the urine.[69] This study suggests that extensive enterohepatic circulation results in the protracted excretion of nitromethaqualone metabolites. Analytic methods for quantitation of nitromethaqualone include gas chromatography with electron capture detection.[70]

References

1. Gujral ML, Kohli RP, Saxena PN. A preliminary report on some new synthetic hypnotics. J Assoc Phys India 1955;2:29–35.

2. Gujral ML, Kohli RP, Saxena PN. Experimental study of hypnotic potency, toxicity and safety margin of 2,3-disubstituted quinozolones. Indian J Med Sci 1956; 10:871–876.

3. Barcelo R. A clinical study of methaqualone, a new non-barbiturate hypnotic. Can Med Assoc J 1961;85: 1304-1305.

4. Ionescu-Pioggia M, Bird M, Orzack MH, Genes F, Beake B, Cole JO. Methaqualone. Int Clin Psychopharmacol 1988;3:97–109.

5. Kato M. An epidemiological analysis of the fluctuation of drug dependence in Japan. Int J Addict 1969;4: 591–621.

6. Falco M. Methaqualone misuse: foreign experience and United States drug control policy. Int J Addict 1976;11: 597–610.

7. Parry CD, Pluddemann A, Louw A, Leggett T. The 3-Metros study of drugs and crime in South Africa: findings and policy implications. Am J Drug Alcohol Abuse 2004;30:167–185.

8. Goldfarb M, Finelli R. Necrotizing cystitis secondary to "bootleg" methaqualone. Urology 1974;3:54–55.

9. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Giffin SL. 2009 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th Annual Report. Clin Toxicol (Phila) 2010;48:979–1178.

10. Angelos SA, Lankin DC, Meyers JA, Raney JK. The structural identification of a methyl analog of methaqualone via 2-dimensional NMR techniques. J Forensic Sci 1993;38:455–465.

11. van Zyl EF. A survey of reported synthesis of methaqualone and some positional and structural isomers. Forensic Sci Int 2001;122:142–149.

12. Wolfe JF, Rathman TL, Sleevi MC, Campbell JA, Greenwood TD. Synthesis and anticonvulsant activity of some new 2-substituted 3-aryl-4(3H)-quinazolinones. J Med Chem 1990;33:161–166.

13. Grove AA, Rohwer ER, Laurens JB, Vorster BC. The analysis of illicit methaqualone containing preparations by gas chromatography-mass spectrometry for forensic purposes. J Forensic Sci 2006;51:376–380.

14. Angelos SA, Meyers JA. The isolation and identification of precursors and reaction products in the clandestine manufacture of methaqualone and mecloqualone. J Forensic Sci 1985;30:1022–1047.

15. Bhana A, Parry CD, Myers B, Plüddemann A, Morojele NK, Flisher AJ. The South African Community Epidemiology Network on Drug Use (SACENDU) project, phases 1-8–cannabis and Mandrax. S Afr Med J 2002;92:542–547.

16. Gillman MA, Harker N, Lichtigfeld FJ. Combined cannabis/methaqualone withdrawal treated with psychotropic analgesic nitrous oxide. Int J Neurosci 2006; 116:859–869.

17. Gerald MC, Schwirian PM. Nonmedical use of methaqualone. Arch Gen Psychiatry 1973;28:627–631.

18. Orzack MH, Friedman L, Dessain E, Bird M, Beake B, McEachern J, Cole JO. Comparative study of the abuse liability of alprazolam, lorazepam, diazepam, methaqualone, and placebo. Int J Addict 1988;23:449–467.

19. Ionescu-Pioggia M, Bird M, Cole JO. Subjective effects of methaqualone. NIDA Res Monogr 1980;95:455.

20. Kurz RW, Hainz R, Gremmel F, Grisold W, Hruby K, Dellert P, Vycudilik W. [Dangerous intoxication from extreme serum concentrations of methaqualone metabolites. Detection and quantification of biosynthesis with gas chromatography-mass spectrometry]. Anaesthesist 1995; 44:863–868. [German].

21. Smyth RD, Lee JK, Polk A, Chemburkar PB, Savacool AM. Bioavailability of methaqualone. J Clin Pharmacol 1973;13:391–400.

22. Nayak RK, Smyth RD, Chamberlain JH, Polk A, DeLong AF, Herczeg T, et al. Methaqualone pharmacokinetics after single- and multiple-dose administration in man. J Pharmacokinet Biopharm 1974;2:107–121.

23. Morris RN, Gunderson GA, Babcock SW, Zaroslinski JF. Plasma levels and absorption of methaqualone after oral administration to man. Clin Pharmacol Ther 1972;13: 719–723.

24. Delong AF, Smyth RD, Polk A, Nayak RK, Reavey-Cantwell NH. Blood levels of methaqualone in man following chronic therapeutic doses. Arch Int Pharmacodyn 1976;222:322–331.

25. Alvan G, Ericsson O, Levander S, Lindgren J-E. Plasma concentrations and effects of methaqualone after single and multiple oral doses in man. Eur J Clin Pharmacol 1974;7:449–454.

26. Wilson K, Burnett D, Oram M, Reynolds CT. The kinetics of the urinary excretion of the N-oxide and glucuronides of methaqualone in man. Eur J Drug Metab Pharmacokinet 1981;6:289–295.

27. Brown SS, Goenechea S. Methaqualone: metabolism, kinetic, and clinical pharmacologic observations. Clin Pharmacol Ther 1973;14:314–324.

28. Burnett D, Reynolds CN, Wilson K, Francis JR. Urinary excretion of C-hydroxy derivatives of methaqualone in man. Xenobiotica 1976;6:125–134.

29. Wilson K, Reynolds CN, Burnett D. Inter- and intra-individual variation in the metabolism of methaqualone in man after a singe oral dose. Eur J Clin Pharmacol 1978;13:291–297.

30. Prost F, Thormann W. Assessment of the stereoselective metabolism of methaqualone in man by capillary electrophoresis. Electrophoresis 2003;24:2598–2607.

31. Ericsson O. Synthesis of sulfate and glucuronide conjugates of methaqualone metabolites. Acta Pharm Suec 1978:15:81–86.

32. Alvan G, Lindgren J-E, Bogentoft C, Ericsson O. Plasma kinetics of methaqualone in man after single oral doses. Eur J Clin Pharmacol 1973;6:187–190.

33. Clifford JM, Cookson JH, Wickham PE. Absorption and clearance of secobarbital, heptabarbital, methaqualone, and ethinamate. Clin Pharmacol Ther 1974;16:376–389.

34. Shah NS, Hixson E, Gulati OD, Kuhn D, Mathur PP. Maternal-fetal distribution of methaqualone in control and SKF 525-A-pretreated pregnant mice. Toxicol Appl Pharmacol 1977;40:497–509.

35. Noschel H, Peiker G, Voigt R, Meinhold P, Muller B, Schroder S, Bonow A. Research on pharmacokinetics during pregnancy. Arch Toxicol 1980;4(suppl):S380–S384.

36. Commissaris RL, Rech RH. Tolerance and cross-tolerance to central nervous system depressants after chronic pentobarbital or chronic methaqualone administration. Pharmacol Biochem Behav 1983;18:327–331.

37. Faulkner TP, Hayden JH, Mehta CM, Olson DA, Comstock EG. Dose-response studies on tolerance to multiple doses of secobarbital and methaqualone in a polydrug abuse population. Clin Toxicol 1979;15:23–37.

38. Hindmarsh KW, Hamon NW, LeGatt DF, Wallace SM. Effect of diphenhydramine on methaqualone metabolism: an *in vitro* study. J Pharm Sci 1978;67:1547–1550.

39. Roden S, Harvey P, Mitchard M. The effect of ethanol on residual plasma methaqualone concentrations and behaviour in volunteers who have taken Mandrax. Br J Clin Pharmacol 1977;4:245–247.

40. Mills DG. Effects of methaqualone on blood platelet function. Clin Pharmacol Ther 1978;23:685–691.

41. Heng MC. Fixed drug eruption due to methaqualone. Australas J Dermatol 1986;27:83–85.

42. Lawson AA, Brown SS. Acute methaqualone (Mandrax) poisoning. Scot Med J 1967;12:63–68.

43. Abboud PT, Freedman MT, Rogers RM, Daniele RP. Methaqualone poisoning with muscular hyperactivity necessitating the use of curare. Chest 1974;65:204–205.

44. Burston GR. Self poisoning with "Mandrax." Practitioner 1967;199:340–344.

45. Trese M. Retinal hemorrhage caused by overdose of methaqualone (Quaalude). Am J Ophthalmol 1981;91:201–203.

46. Inaba DS, Gay GR, Newmeyer JA, Whitehead C. Methaqualone abuse "luding out". JAMA 1973;224:1505–1509.

47. Swartzburg M, Lieb J, Schwartz AH. Methaqualone withdrawal. Arch Gen Psychiatry 1973;29:46–47.

48. Faught E. Methaqualone withdrawal syndrome with photoparoxysmal responses and high amplitude visual evoked potentials. Neurology 1986;36:1127–1129.

49. Petit TL, Sterling JW. The effect of methaqualone on prenatal development in the rat. Experientia 1977;33:1635–1636.

50. Liu F, Liu YT, Feng CL, Luo Y. Determination of methaqualone and its metabolites in urine and blood by UV, GC/FID and GC/MS. Acta Pharmaceut Sinica 1994;29:610–616.

51. Peat MA, Finkle BS. Determination of methaqualone and its major metabolite in plasma and saliva after single oral doses. J Anal Toxicol 1980;4:114–118.

52. Plaut O, Girod C, Staub C. Analysis of methaqualone in biological matrices by micellar electrokinetic capillary chromatography. Comparison with gas chromatography-mass spectrometry. Forensic Sci Int 1998;92:219–227.

53. Reynolds CN, Wilson K, Burnett D. Urinary excretion of methaqualone-*N*-oxide in man. Xenobiotica 1976;6:113–124.

54. Daenens P, van Boven M. The identification of quinazolinones on the illicit market. J Forensic Sci 1976;21:552–563.

55. Bonnichsen R, Marde Y, Ryhage R. Identification of free and conjugated metabolites of methaqualone by gas chromatography-mass spectrometry. Clin Chem 1974;20:230–235.

56. Matthew H, Proudfoot AT, Brown SS, Smith AC. Mandrax poisoning: Conservative management of 116 patients. Br Med J 1968;2:101–102.

57. Bailey DN. Methaqualone ingestion: Evaluation of present status. J Anal Toxicol 1981;5:279–282.

58. Fucci N. A case of lethal intoxication after ingestion of Toquilone Compositum. Am J Forensic Med Pathol 1996;17:231–232.

59. Brenner C, Hui R, Passarelli J, Wu R, Brenneisen R, Bracher K, ElSohly MA, et al. Comparison of methaqualone excretion patterns using Abuscreen ONLINE and EMIT II immunoassays and GC/MS. Forensic Sci Int 1996;79:31–41.

60. Augsburger M, Rivier L. Drugs and alcohol among suspected impaired drivers in Canton de Vaud (Switzerland). Forensic Sci Int 1997;85:95–104.

61. Saario I, Linnoila M. Effect of subacute treatment of hypnotics, alone or in combination with alcohol, on psychomotor skills related to driving. Acta Pharmacol Toxicol 1976;38:382–392.

62. McCurdy HH, Holbrook JM. Incidence of methaqualone in driving-under-the-influence (DUI) cases in the state of Georgia. J Anal Toxicol 1981;5:270–274.

63. Augsburger M, Donbze N, Menetrey A, Brossard C, Sporkert F, Giroud C, Mangin P. Concentration of drugs in blood of suspected impaired drivers. Forensic Sci Int 2005;153:11–15.

64. Proudfoot AT, Noble J, Nimmo J, Brown SS, Cameron JC. Peritoneal dialysis and hemodialysis in methaqualone (Mandrax) poisoning. Scot Med J 1968;13:232–236.

65. McCarthy G, Myers B, Siegfried N. Treatment for methaqualone dependence in adults. Cochrane Database Syst Rev 2005;2:CD004146.

66. Angelos SA, Meyers JA. The isolation and identification of precursors and reaction products in the clandestine manufacture of methaqualone and mecloqualone. J Forensic Sci 1985;30:1022–1047.

67. Van Boven M, Janssen G, Daenens P. Mass spectrometry-gas chromatographic determination of mecloqualone metabolites from urine extracts. Mikrochim Acta 1974; 4:603–610.

68. Dubnick B, Towne CA, Bush MT. Detection, assay and rate of excretion of mecloqualone in animals and man. Toxicol Appl Pharmacol 1969;15:632–641.

69. van Boven M, Daenens P. Biotransformation and excretion of nitromethaqualone in rats and humans. J Pharm Sci 1982;71:1152–1157.

70. van Boven M, Daenens P, Vandereycken G. Determination of nitromethaqualone in blood by electron-capture-gas chromatography. J Chromatogr 1980;182:435–438.

Chapter 28

BUPRENORPHINE

HISTORY

Buprenorphine was first developed as an analgesic with low addiction potential based on animal studies and marketed under the trade name Temgesic® (Schering-Plough, Kenilworth, NJ). This drug was available as a pre-anesthetic medication and as an analgesic for moderate to severe pain in dosages of 0.2 mg. Since 1987, buprenorphine has been marketed in France as an analgesic. During the early 1980s in England and Australia, reports documented the intravenous (IV) abuse of analgesic preparations of buprenorphine,[1] while reports from Scotland documented the IV abuse of illegally diverted buprenorphine as a heroin substitute during the late 1980s, frequently in combination with benzodiazepines.[2] In 1996, a high-dose sublingual formulation under the trade name Subutex® (Reckitt-Benckiser, Slough, United Kingdom) became available in France for the treatment of opioid addiction. A buprenorphine transdermal delivery system was released in Europe during 2001 for the treatment of chronic pain at release rates of 35 µg/h, 52.5 µg/h, and 70 µg/h over 72 hours corresponding to daily buprenorphine doses of 0.8 mg, 1.2 mg, and 1.6 mg, respectively. The US Food & Drug Administration (FDA) approved the use of sublingual buprenorphine for the treatment of opioid addiction in 2002. Although buprenorphine is a relatively safe drug, case series soon associated fatalities with the illicit use of buprenorphine and benzodiazepines including the use of this combination in drug-facilitated sex crimes.[3,4] Abuse of buprenorphine preparations for the treatment of opioid dependence continues both in patients undergoing buprenorphine maintenance treatment and in untreated opioid addicts.[5]

IDENTIFYING CHARACTERISTICS

Buprenorphine is a highly lipid soluble, semisynthetic opioid (Fig. 28.1) that is derived from thebaine. The source of this latter alkaloid is the opium poppy, *Papaver somniferum* L. Buprenorphine is structurally related to morphine. In rodent antinociceptive assays (writhing, tail pressure), parenteral buprenorphine is about 25–40 times more potent than morphine, whereas oral buprenorphine is approximately 7–10 times more potent than morphine.[6] Buprenorphine is a partial agonist at µ-opioid receptors and a weak antagonist at κ-opioid receptors. The slow dissociation of buprenorphine from µ-opioid receptors results in a prolonged duration of action. Table 28.1 lists some physiochemical properties of buprenorphine.

EXPOSURE

Epidemiology

Both the intentional and accidental ingestion of buprenorphine has increased as the clinical use and availability of this drug has increased. In a review of all pediatric admissions (<18 years of age) to a Maine medical center from 1999 to 2009, the number of children admitted for buprenorphine toxicity tripled the last 5 years of the study compared with the first 5 years.[7] The admissions included 6 adolescents (3 suicidal ingestions, 3 recreational ingestions). Overall, the prevalence of abuse of sublingual buprenorphine is relatively low compared with methadone and heroin.[8] Although experimental use of buprenorphine occurs among polydrug users, the use of buprenorphine as a primary drug
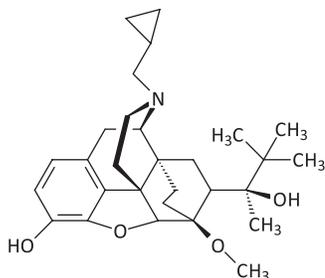
FIGURE 28.1. Chemical structure of buprenorphine.

TABLE 28.1. Physiochemical Properties of Buprenorphine.

Property	Value
CAS Number	52485-79-7
Molecular Formula	$C_{29}H_{41}NO_4$
pKa	8.31
log P (Octanol-Water)	4.98
Water Solubility	0.636 mg/L (25°C/77°F)

of abuse is relatively uncommon depending on the population studied. In a study of >1,000 prescription opioid abusers, buprenorphine was the preferred drug of abuse in <3% of this convenience sample.[9] A cross-sectional survey of 343 French IV drug users attending community pharmacies, vending machines, and needle exchange programs to obtain equipment indicated that ~34% of the polydrug users occasionally injected buprenorphine, whereas ~24% injected only buprenorphine within the last 6 months.[10] A cross-sectional study of 404 individuals in a French buprenorphine maintenance therapy program indicated that about one-half of these opioid addicts had injected buprenorphine during their lifetime and about two-thirds of this group used illicit IV buprenorphine since joining the buprenorphine maintenance program.[11] In a field-recruited Australian cohort of 316 currently injecting drug users, 33 (10%) of the users reported the regular injection of buprenorphine, whereas 101 (32%) of this cohort reported the injection of buprenorphine within the preceding 3 months.[12] A survey of 350 needle exchangers reported the use of buprenorphine by 89% of injecting heroin abusers and 24% of injecting amphetamine abusers.[13] The reported use of buprenorphine by heroin addicts was primarily related to self-detoxification of the treatment of withdrawal, whereas the use of buprenorphine for euphoria was more common in IV amphetamine users.

Sources

Buprenorphine is synthesized from the opiate alkaloid, thebaine, during a 7-step process. In the United States,

buprenorphine is available in the following two sublingual preparations: (1) Subutex®—oval, white tablet in 2 mg (embossed B2) and 8 mg (embossed B8) dosages, and (2) Suboxone®— hexagonal orange tablet in 2 mg buprenorphine/0.5 mg naloxone (embossed N2) and 8 mg buprenorphine/2 mg naloxone (embossed N8) dosages. Because the bioavailability of oral naloxone is poor, the ingestion of the combination of buprenorphine and naloxone produces pharmacologic effects primarily related to buprenorphine. However, the injection of dissolved tablets of the buprenorphine/naloxone can cause opioid withdrawal symptoms.[14] Most abuse of buprenorphine results from the illicit diversion of pharmaceutic preparations of buprenorphine. Consequently, the abuse of buprenorphine typically involves relative pure preparations without significant contaminants or additives. Of the 33 individuals regularly using buprenorphine in an Australian study of currently injecting drug users, about two-thirds obtained their buprenorphine through prescription, and the remainder of this subgroup obtained this drug illicitly (i.e., friends, street purchase).[12] Another Australian study suggests that the prevalence of the diversion of buprenorphine sublingual preparations is higher than the diversion of liquid preparations of methadone.[15]

Methods of Abuse

Consistent with the μ-opioid receptor agonist properties of buprenorphine, this drug has abuse potential although this potential is relatively low compared with full μ-opioid agonists (e.g., heroin, methadone). Typically, the abuse of buprenorphine involves the IV injection of pharmaceutic preparations marketed for analgesia.[16] However, abuse also occurs as a result of the illicit diversion and IV use of buprenorphine during buprenorphine drug maintenance treatment programs. In an Australian study of currently injecting drug users, the primary abusers (i.e., 10% of the cohort) injected buprenorphine approximately 10 times per week as a less-expensive alternative to heroin.[12] The abuse of buprenorphine in opioid-dependent addicts participating in treatment programs is limited by the addition of naloxone to the buprenorphine. This combination produces withdrawal symptoms following the IV injection of the combination drug in some, but not all, addicts.[17,18] In a study of opioid-dependent volunteers, the intramuscular administration of up to 16 mg buprenorphine and 4 mg naloxone produced dose-related increases on indices of opioid antagonist effects consistent with naloxone-precipitated withdrawal that resolved relatively quickly;[14] the IV use of buprenorphine/naloxone combination is frequently associated with dysphoria ("bad experience") in contrast to the use of

buprenorphine alone.[19] Similar doses of sublingual buprenorphine and naloxone did not cause withdrawal symptoms. As a result of the relatively mild euphoria associated with IV buprenorphine use compared with IV doses of heroin, the concomitant use of other drugs by IV drug abusers is frequent, particularly benzodiazepines.[20] Insufflation is an alternative route of buprenorphine abuse by injecting heroin users.[13]

DOSE EFFECT

The final daily sublingual maintenance dose of buprenorphine/naloxone for the treatment of opioid dependence ranges from 4 mg buprenorphine/1 mg naloxone to 24 mg buprenorphine/6 mg naloxone with a typical target dose of 16 mg buprenorphine/4 mg naloxone.[21] The usual dose adjustment of the 4:1 formulation involves the increment or decrement of 2 mg/0.5 mg or 4 mg/1 mg. At doses below 12 mg, buprenorphine produces clinical effects similar to opioid agonists (e.g., methadone, morphine). The typical parenteral dose of buprenorphine for analgesia is 0.2–0.6 mg with the duration of action being about 6 hours. Because buprenorphine is a partial μ-opioid receptor agonist, the maximal effects of buprenorphine are less than full agonist (e.g., heroin, methadone). In general, high buprenorphine doses produce fewer adverse effects than heroin or methadone. Additionally, high doses of buprenorphine produce more prolonged pharmacologic effects rather than more serious adverse reactions.[22] The subcutaneous administration of 2 mg buprenorphine to 10 healthy volunteers with histories of opioid abuse produced miosis and mild euphoria without significant changes in body temperature, blood pressure, respiration, or heart rate.[23] The administration of sublingual buprenorphine doses up to 32 mg to healthy volunteers produced miosis, sedation, positive mood, and respiratory depression.[24] However, there are no dose-related increases in the severity of these symptoms with higher buprenorphine doses despite increasing plasma buprenorphine concentrations. The administration of IV buprenorphine in doses up to 16 mg to nondependent opioid abusers are associated with varying degrees of a positive mood, minimal increase in systolic blood pressure, and no significant change in heart rate or oxygen saturation (no capnometry measurements).[25] Most side effects were mild except the development of severe vomiting in one patient. At doses above 12 mg, buprenorphine produces little additional opioid effects, and the dose-response curve for the μ_1-opioid agonist effect flattens. The administration of 0.001–10 mg buprenorphine/kg body weight to rhesus monkeys produced a reduction in respiratory responses (respiratory rate, minute volume, tidal volume) to 5% CO_2,

when compared with control (placebo) values.[26] The response to buprenorphine was dose-dependent, and the maximal effect on these respiratory parameters was about 40–60% of control (placebo) values at a buprenorphine dose of 1 mg/kg. The respiratory depression did not increase with doses above 1 mg/kg, and the administration of up to 10 mg buprenorphine/kg body weight did not cause clinically significant respiratory depression.

The sublingual administration of buprenorphine in doses of 13.2–39.0 μg/kg/day in 3 divided doses to neonates of opioid–addicted mothers is a treatment for neonatal abstinence syndrome.[27] Children ingesting >4 mg buprenorphine usually develop clinical effects of opioid intoxication. The ingestion of an estimated buprenorphine (Subutex®) dose of 4 mg by a 4-year-old girl was associated with bilateral miosis and slight restlessness that resolved by the next day without complications.[28] A 20-month old boy developed lethargy, cyanosis, and respiratory depression after the ingestion of 12 mg buprenorphine (0.97 mg/kg).[29] Any child ingesting >2 mg buprenorphine or any child <2 years of age ingesting more than a taste should be evaluated in the emergency department for central nervous system (CNS) and respiratory depression.[30]

TOXICOKINETICS

Absorption

The oral bioavailability of buprenorphine is low as a result of a large first-pass effect; however, the bioavailability of sublingual doses is substantially higher than oral doses.[31] The systemic bioavailability of oral buprenorphine is about 15% compared with about 30–55% for sublingual doses of buprenorphine, depending on the formulation of the sublingual preparation (i.e., liquid vs. tablet).[32] Sublingual buprenorphine tablets require 2–10 minutes to completely dissolve in the mouth. Although pharmacokinetic studies suggest that the bioavailability of buprenorphine sublingual tablets is 40–50% less than liquid-filled sublingual formulations,[33] pharmacodynamic studies with positron emission tomography (PET) scanning indicate that the equivalent doses of these two formulations produce similar binding to μ-opioid receptors.[34] In a study of 5 postoperative patients receiving 0.4 mg buprenorphine and 5 patients receiving 0.8 mg buprenorphine sublingually, the mean systemic bioavailability was about 55%.[35] The mean bioavailability of sublingual and buccal doses of 4 mg buprenorphine in 6 healthy volunteers was approximately 51% and 28%, respectively.[36] There is substantial variability in the time to maximum buprenorphine plasma concentration following sublin-

gual administration. In a study of postoperative patients receiving low (0.4 mg, 0.8 mg) sublingual doses of buprenorphine, the mean time to peak to plasma buprenorphine concentration was about 3.3 hours (range, 1.5–6 hours).[36] The average peak plasma buprenorphine concentration for this sublingual dose was about 3.3 ng/mL with an approximate range of 1.9–7.2 ng/mL, whereas the average maximum buprenorphine plasma concentration after buccal administration of 4 mg buprenorphine was 1.98 ng/mL (range, 0.25–3.9 ng/mL). Peak subjective effects of buprenorphine occur after peak plasma concentrations of buprenorphine due to a lag in CNS penetration.[37]

Distribution

Buprenorphine is highly protein bound (i.e., 96%), primarily to α- and β-globulins.[38] The volume of distribution of buprenorphine is relatively large. In a study of nondependent heroin users receiving 1.2 mg buprenorphine intravenously, the mean V_d was 335 L (range, 125–771 L).[36]

Biotransformation

Buprenorphine undergoes extensive biotransformation in the liver by oxidative *N*-demethylation. The primary P450 enzymes involved in the biotransformation of buprenorphine are CYP3A4 and, to a lesser extent, CYP2C8 isoenzymes as determined by *in vitro* human liver microsome studies.[39] The main metabolite of buprenorphine is desalkyl-buprenorphine (norbuprenorphine).[40] Norbuprenorphine is an active metabolite with 1/50th the analgesic potency of buprenorphine based on the tail flick test in rats following IV administration of buprenorphine and norbuprenorphine.[41] Buprenorphine and norbuprenorphine undergo extensive phase II metabolism catalyzed by uridine diphosphate glucuronyltransferase 1A1 (buprenorphine), uridine diphosphate glucuronyltransferase 2B7 (buprenorphine), and uridine diphosphate glucuronyltransferase 1A3 (norbuprenorphine).[42]

Elimination

Animal studies indicate that a majority of the orally administered dose of buprenorphine appears in the feces as a result of biliary excretion. After IV administration of 100 μg [³H]buprenorphine/kg body weight to bile duct-cannulated rats, the bile contained over 90% of the administered radioactivity within 48 hours after dosing.[43] In humans, biliary excretion of buprenorphine and buprenorphine glucuronide conjugates account for about 80% of the elimination of buprenorphine, whereas urinary excretion accounts for the rest of the elimination of buprenorphine from the body.[44] Urinary elimination of buprenorphine results from the excretion of inactive glucuronide conjugates of buprenorphine and norbuprenorphine. Very little free buprenorphine appears in the urine.[45] There is substantial variation in the elimination half-lives of buprenorphine and norbuprenorphine both between different individuals and between different routes of administration. Mean elimination half-life of buprenorphine following the IV administration of 1.2 mg buprenorphine to 5 nondependent heroin users was 3.2 hours (range, 1.6–8.2 hours) compared with a mean of 27.7 hours (range, 5.2–49 hours) following the sublingual administration of 4 mg buprenorphine.[36] In a study of 11 male heroin-dependent volunteers participating in a research study, the mean steady-state elimination half-life of sublingual buprenorphine and norbuprenorphine was 42 hours and 57 hours, respectively.[46]

Maternal and Fetal Kinetics

Buprenorphine crosses the placenta during pregnancy, and the drug is excreted in breast milk. Case reports suggest that serum buprenorphine concentrations in the fetus can exceed maternal serum buprenorphine concentrations.[47] These reports also suggest that the excretion of buprenorphine in breast milk is low enough to produce minimal effects in breast-feeding infants, particularly after the first month when CYP3A4 activity reaches 30–40% of adult values. Breastfeeding does not prevent the mild abstinence syndrome associated with infants born to mothers in buprenorphine maintenance programs, and cessation of breastfeeding from mothers in these programs does not induce abstinence symptoms in these infants.

Tolerance

Studies of chronic pain patients receiving long-term buprenorphine therapy via transdermal patches suggest a low incidence of tolerance.[48]

Drug Interactions

Case reports on fatalities associated with the IV use of buprenorphine and benzodiazepines suggest the possibility that these two types of drugs produce synergistic respiratory depression.[49] This interaction is probably pharmacodynamic rather than pharmacokinetic because the lack of significant alteration of flunitrazepam metabolism in the presence of buprenorphine.[50] Drugs that inhibit (ketoconazole, nefazodone, protease inhibitors)

or induce (carbamazepine, phenytoin, rifampicin) CYP3A4 potentially decrease or enhance the *N*-dealkylation of buprenorphine. The protease inhibitors (ritonavir, indinavir, and saquinavir) are extensively metabolized by CYP3A4 isoenzymes,[51] and the selective serotonin reuptake inhibitors (fluoxetine, fluvoxamine) are CYP3A4 inhibitors *in vitro*.[52] Potentially, the concomitant administration of these drugs could alter buprenorphine metabolism, but the clinical significance of this potential interaction is unclear. A case report associated the development of the serotonin syndrome (clonus, agitation, altered mental status, confusion) following ingestion of Suboxone® (buprenorphine/naloxone) by a 54-year-old insulin-dependent diabetic on doxepin and amitriptyline.[53] He recovered after intubation and treatment with cyproheptadine.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Treatment with sublingual buprenorphine decreases opioid self-administration by humans and animals along with reducing drug-seeking behavior and craving (i.e., motivational measures).[54] The clinical effectiveness of buprenorphine results from the binding of this drug to μ-opioid receptors, thereby decreasing the availability of the μ-opioid receptor to other opioids while alleviating opioid withdrawal symptoms. The slow dissociation of buprenorphine from the μ-opioid receptor results in a long duration of action, whereas the partial agonist effect of buprenorphine causes low intrinsic activity and a plateau in the opioid effects at high doses. Buprenorphine has a high affinity for μ-opioid receptors. In a study of 5 heroin-dependent volunteers, buprenorphine in daily doses of 2 mg, 16 mg, and 32 mg decreased mean whole-brain μ-opioid receptor availability by $41\% \pm 8\%$, $80\% \pm 2\%$, and $84\% \pm 2\%$, respectively, relative to placebo as measured by PET scanning with ^{11}C-carfentanil.[34] Analysis occurred 4 hours after the last buprenorphine dose in areas of the prefrontal cortex, anterior cingulate, thalamus, amygdala, nucleus accumbens, and caudate. This study indicated that buprenorphine rather than the norbuprenorphine metabolite binds to the μ-opioid receptors. The administration of buprenorphine displaces other opioids (e.g., heroin, methadone) from μ-opioid receptors as well as blocking the clinical effects of opioids. Clinical studies suggest that daily doses of 8–12 mg buprenorphine sublingually are more effective than daily doses of 20–35 mg methadone as measured by the risk of discontinuing treatment and positive urine drug screens for opioids.[55] However, buprenorphine may not be as effective as methadone in opioid–dependent individuals requiring high doses (80–100 mg) of methadone.

Mechanism of Toxicity

Buprenorphine is a partial μ-opioid receptor agonist and a weak κ-opioid receptor antagonist that is a relatively safe drug because of the diminished μ_1- and μ_2-opioid agonist actions at high doses. In animal studies, buprenorphine has κ-opioid antagonist activity.[56] The role of κ-opioid receptor in opioid toxicity is unclear, but some κ-opioid agonist produce dysphoria. Some respiratory depression does occur at low doses of buprenorphine. In a study of 10 patients in an intensive care unit, the IV administration of 0.4 mg buprenorphine caused a significant reduction in mean ventilation rate and an increase in mean arterial P_aCO_2 without altering the heart rate, P_aO_2, or pH.[57] Although the partial μ-opioid agonist activity of buprenorphine provides a margin of safety, the concomitant administration of other CNS depressants (e.g., opioids, ethanol, benzodiazepines) can produce serious respiratory dysfunction.

Postmortem Examination

Postmortem examination of fatalities associated with buprenorphine usually demonstrates nonspecific findings of prolonged asphyxiation including pulmonary edema, deep cyanosis, and congestion of multiple organs.[58]

CLINICAL RESPONSE

Adverse effects of buprenorphine are typical of the effects associated with opioids including constipation, nausea, vomiting, and dizziness.[59] Case reports associate the onset of acute icteric hepatitis with buprenorphine treatment of opioid addicts with hepatitis C virus-positive serology.[60] There were no signs of liver failure, and the jaundice resolved with reduction or elimination of the buprenorphine dose. Delayed hypersensitivity reactions may occur following the use of transdermal buprenorphine manifest by generalized skin eruptions and pruritic erythematous plaques.[61]

Illicit Use

In general, buprenorphine intoxication produces less-severe respiratory depression than heroin overdose, but

the coadministration of CNS depressant drugs (e.g., benzodiazepines) with buprenorphine can result in serious respiratory insufficiency.[62] Complications of the parenteral use of crushed buprenorphine tablets include the formation of coagulation necrosis and sterile abscess around the injection site.[63] Cellulitis and phlebitis are potential complications of the parenteral use of crushed buprenorphine tablets as a result of the low water solubility of buprenorphine and the injection of mouth flora and saliva from illicitly diverted pharmaceutic preparations of buprenorphine. High doses of buprenorphine can precipitate prolonged, opioid withdrawal in opioid-dependent individuals. A case report associated the development of severe opioid withdrawal with the parenteral administration of 11 crushed, 8 mg-tablets of buprenorphine.[64] There was no significant respiratory depression; the withdrawal symptoms persisted 4 days. A case report temporally associated the development of a diffuse, symmetrical cystic leukoencephalopathy with the IV injection of crushed buprenorphine tablets dissolved in water.[65] The patient developed bilateral pyramidal dysfunction with spastic quadriparesis, hypertonia, hyperreflexia, and sustained clonus along with diffuse leukoencephalopathic changes in the periventricular, deep, and subcortical white matter of the frontal and parietal lobes. The cerebellum and brainstem were normal. In contrast to methadone, buprenorphine is not usually associated with prolongation of the QTc interval.[66]

Overdose

In a retrospective review of 54 buprenorphine overdoses in children <6 years of age reported to the Research Abuse, Diversion, and Addiction-Related Surveillance System, the most common clinical feature of accidental exposure to buprenorphine was drowsiness or lethargy (55%), followed by vomiting (21%), miosis (21%), respiratory depression (7%), agitation or irritability (5%), pallor (3%), and coma (2%).[30] The onset of symptoms was typically within 1 hour (range, 20 minutes to 3 hours). In general, accidental exposure was well-tolerated by young children; however, significant CNS and respiratory depression occasionally occurs. A case series of 5 toddlers documented the development of respiratory and CNS depression following the accidental ingestion of buprenorphine.[29] The clinical features of buprenorphine in these toddlers were classic of opioid overdose, including miosis, respiratory depression and somnolence. One child in this series developed apnea within 5 hours after ingestion, and the paramedics intubated the child in the field. The report did not indicate that this child received naloxone.

Abstinence Syndrome

The repeated administration of buprenorphine to opioid-dependent individuals produces or maintains physical dependence on opioids; single doses of buprenorphine block μ-opioid receptors for over 24 hours.[67] In a study of 8 opioid-dependent patients on a residential ward, the daily administration of 8 mg buprenorphine sublingually prevented the development of opioid withdrawal symptoms.[68] Withdrawal symptoms developed in these patients following the intramuscular administration of 3 mg naloxone/70 kg body weight. As a partial agonist of the μ-opioid receptor, the withdrawal associated with cessation of buprenorphine therapy is milder in intensity and later in onset than heroin withdrawal. Similarly, in highly opioid dependent patients, the administration of buprenorphine usually, but not always causes milder withdrawal symptoms compared with naloxone.[69] Subjective symptoms and physiologic signs of opioid withdrawal did not develop within 72 hours of the cessation of buprenorphine treatment in 8 buprenorphine-maintained, opioid-dependent patients.[70] Withdrawal from buprenorphine begins more slowly than during heroin withdrawal with peak symptoms occurring about 5 days after cessation of buprenorphine.[71] A majority of neonates born to mothers on chronic buprenorphine maintenance will develop neonatal abstinence syndrome.[72] Compared with infants born to mothers in methadone maintenance programs, neonatal abstinence symptoms are similar or less severe in neonates of mothers in buprenorphine maintenance programs.[73] Analysis of differences in the neonatal abstinence syndrome associated with these 2 heroin substitutes is complicated by the continued abuse of opioid drugs among these mothers. The neonatal abstinence syndrome associated with buprenorphine generally appears within 12–48 hours, peaks at approximately 72–96 hours, and lasts for 120–168 hours.[74]

Reproductive Abnormalities

There are few data on the effects of maternal buprenorphine treatment during pregnancy on birth outcomes and abstinence syndrome in their neonates. A case report on 2 women conceiving on buprenorphine did not reveal any abnormal birth outcomes, such as pregnancy complications, prematurity, low birth weight, or poor neurodevelopmental progress.[75] In a case series of 31 infants with mothers on buprenorphine maintenance treatment, neonatal withdrawal syndrome developed in 13 cases (42%) and 8 of these babies required opioid treatment.[76] A study of 27 full-term neonates born to mothers receiving buprenorphine during their

pregnancy did not detect biochemical evidence (cord artery serum erythropoietin, cardiac troponin T, S100) of asphyxia when compared with substance-abusing and healthy pregnant women.[77] All 27 infants had normal Apgar scores and normal cord blood pH. In general, the neonatal abstinence syndrome is milder in neonates born to mothers on buprenorphine than methadone. In a double-blind, double-dummy, flexible-dosing, randomized, controlled study of 58 pregnant women on buprenorphine and 73 on methadone, the neonates of the former group required less morphine (mean, 1.1 mg and 10.4 mg, respectively; $P < .009$) and shorter duration of treatment (mean, 4.1 days and 9.9 days, respectively; $P < .003$).[78]

DIAGNOSTIC TESTING

Analytic Methods

METHOD

Methods to quantitate buprenorphine and norbuprenorphine in biologic matrices include immunoassays,[79,80] gas chromatography with electron capture (lower limit of quantitation [LLOQ], 0.1 ng/mL),[81] high performance liquid chromatography with fluorescence detection,[82] high performance liquid chromatography with electrochemical detection,[83] gas chromatography/mass spectrometry (GC/MS),[84] liquid chromatography/mass spectrometry,[85] and liquid chromatography/tandem mass spectrometry.[86] The LLOQ for the latter method for buprenorphine and norbuprenorphine is in the range of 0.2 ng/L to 0.5 ng/L,[87] whereas the LLOQ for buprenorphine and norbuprenorphine using GC/MS ranges from 0.2 ng/mL (selective solid-phase extraction by Bond Elut Certify columns and quadrupole mass detector working in EI selected ion monitoring mode)[88] to 4 ng/mL [selective ion monitoring mode with *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane as the derivatizing reagent].[89] The use of liquid chromatography/tandem mass spectrometry eliminates the need for pre-analytic hydrolysis of glucuronide conjugates. These conjugates often have low volatility and require hydrolysis and derivatization when GC/MS is used.

The precision of radioimmunoassay methods depends on the ability of the assay to distinguish between buprenorphine and norbuprenorphine. Some early radioimmunoassay methods for buprenorphine lacked specificity, but newer mass spectrometric methods are able to separate these 2 compounds. Sensitive non-isotopic immunoassays for the detection of buprenorphine include enzyme-linked immunosorbent assays (ELISA) and cloned enzyme donor immunoassays

(CEDIA). These assays have low cross-reactivity with other opioids and with the norbuprenorphine metabolite.[90] With the latter CEDIA method, the sensitivity and specificity of the assay for buprenorphine in 176 unadulterated urine samples from patients receiving buprenorphine was 100% and 87.5%, respectively, as confirmed by liquid chromatography/mass spectrometry.[91] The cutoff for buprenorphine in these urine samples was 5 ng/mL. The LLOQ for buprenorphine and the glucuronide conjugate in urine samples is 5 ng/mL as measured by liquid chromatography/tandem mass spectrometry compared with 25 ng/mL for norbuprenorphine and the related glucuronide conjugate.[92] Intraassay and interassay imprecision with this method is <17%. Following hydrolysis by β-glucuronidase, liquid-liquid extraction at basic pH, and derivatization by *N*-methyl -*N*-(*tert*-butyl-dimethylsilyl)trifluoroacetamide, the LLOQ for buprenorphine and norbuprenorphine in urine samples were 1 ng/mL and 2 ng/mL, respectively, using GC/MS in electron impact mode.[93]

STORAGE

In general, analysis of blood for buprenorphine concentrations should occur within 2 weeks of sampling unless the sample is frozen. Buprenorphine glucuronides are relative stable in refrigerated and frozen samples; however, some degradation occurs after about 3 weeks of storage at 4°C (39.2°F). About 82% of the original buprenorphine concentration remained in the whole blood sample after 3 weeks of refrigerated storage,[86] whereas approximately the same amount remained in a spiked blood sample after 1 year of refrigerated storage in another study.[94]

Biomarkers

BLOOD

THERAPEUTIC/ILLICIT USE.   There is substantial individual variability in the serum buprenorphine concentrations following sublingual administration of buprenorphine. In a study of 14 volunteers receiving 8 mg buprenorphine sublingually, the peak serum buprenorphine concentration ranged from 1–5 ng/mL.[32] The mean peak plasma concentrations of buprenorphine in 5 opioid-dependent patients receiving daily buprenorphine doses of 2 mg, 16 mg, and 32 mg were approximately 0.5–1 ng/mL, 5–6 ng/mL, and 13–14 ng/mL, respectively.[34] In a study of volunteers receiving a sublingual tablet of 8 mg buprenorphine and 2 mg naloxone, detectable concentrations of buprenorphine and norbuprenorphine appeared in the plasma up to 24 hours after administration as measured by liquid chromatography/electrospray ionization/tandem mass
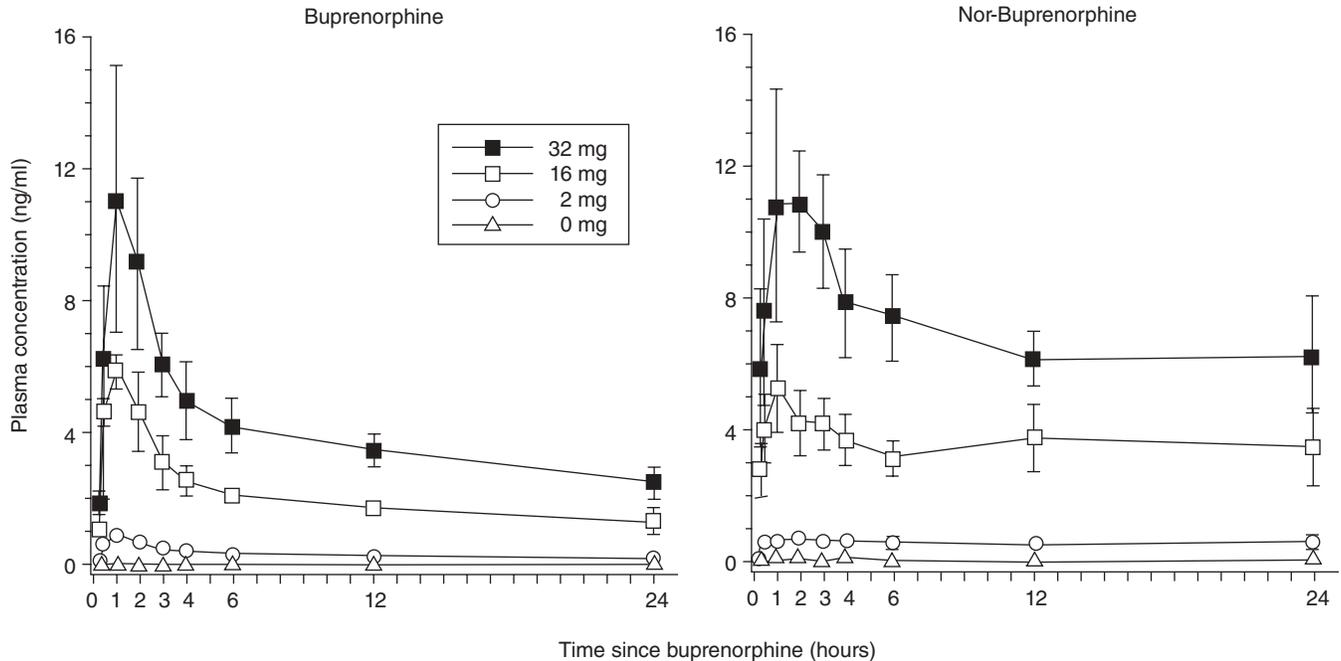
FIGURE 28.2. Mean (±SEM) buprenorphine and norbuprenorphine concentrations over 24 hours following doses up to 32 mg. (Reprinted by permission from Macmillan Publishers Ltd.: Neuropsychopharmacology, MK Greenwald, C Johanson, DE Moody, JH Woods, MR Kilbourn et al., Effects of buprenorphine maintenance dose on [mu]-opioid receptor availability, plasma concentrations, and antagonist blockade in heroin-dependent volunteers, Vol. 28, Issue 11, p. 2006, Fig. 4, copyright 2003.)

spectrometry.[95] Figure 28.2 demonstrates the plasma buprenorphine and norbuprenorphine concentrations over 24 hours after sublingual administration of placebo, 2 mg, 16 mg, and 32 mg buprenorphine doses. During daily buprenorphine maintenance, steady-state plasma buprenorphine concentrations exceeding 0.7 ng/mL are associated with minimal withdrawal symptoms, when the blood samples are drawn immediately prior to the daily buprenorphine dose.[46]

OVERDOSE. Four hours after the ingestion of 4 mg buprenorphine by a 4-year-old girl, the serum buprenorphine and norbuprenorphine concentrations were 0.64 ng/mL and 7.7 ng/mL, respectively.[28] At that time, she was alert and slightly restless with stable vital signs and miosis. The child was discharged the following day with no sequelae.

POSTMORTEM. Most fatalities associated with buprenorphine involve multiple drugs; deaths associated only with buprenorphine are rare. In a case series of 117 postmortem blood samples positive for buprenorphine, only 1 case involved buprenorphine as a sole agent; postmortem examination of this case revealed pulmonary aspiration. The mean buprenorphine concentration in these cases was about 10–12 ng/mL. Typically, fatalities associated with buprenorphine involve multiple drugs, commonly benzodiazepines, antidepressants, and other drugs of abuse.[58,96] A 25-year-old drug addict was found dead with buprenorphine tablets nearby. Postmortem examination of the blood demonstrated buprenorphine and norbuprenorphine concentrations of 3,300 ng/mL (3.3 mg/L) and 400 ng/mL (0.4 mg/L), respectively, in addition to high concentrations of the flunitrazepam metabolite, 7-aminoflunitrazepam.[97] The bile also contained buprenorphine (2,035 mg/L) and norbuprenorphine (536 mg/L) at concentrations a thousand times greater than blood.

URINE

Buprenorphine and the de-alkylated metabolite, norbuprenorphine are excreted in urine, almost exclusively as glucuronide conjugates. Most immunoassays used to screen urine for drugs of abuse do not reliably detect buprenorphine. Evaluation of 16 urine samples pretreated with glucuronidase from 16 patients in buprenorphine maintenance programs revealed total buprenorphine and norbuprenorphine concentrations ranging from 31–1,080 ng/mL and 48–2,050 ng/mL, respectively, as measured by liquid chromatography/electrospray ionization/tandem mass spectrometry.[98] The total buprenorphine and norbuprenorphine concentrations in urine samples from 33 suspected abusers ranged from 2.3–796 ng/mL and 5.0–2,580 ng/mL, respectively. Maximum buprenorphine and norbuprenorphine

521

concentrations occurred 1 hour and 1.5 hours, respectively, after administration.

Eighteen healthy volunteers received a single dose of 0.4 mg buprenorphine sublingually, and urine samples were collected for 96 hours postingestion. As measured by cloned enzyme donor immunoassay (CEDIA), the mean time of continuous positive results for buprenorphine (5 ng/mL cutoff) in urine samples was 9 hours (range, 4–24 hours).[99] The mean detection time for urinary buprenorphine in this study following analysis by liquid chromatography/tandem mass spectrometry was 76 hours (range, 23–96 hours). The mean time for the norbuprenorphine/buprenorphine ratio to exceed 1 was approximately 7 hours. Four hours after the ingestion of 4 mg buprenorphine by a 4-year-old girl, the urine buprenorphine and norbuprenorphine concentrations were 173 ng/mL and 419 ng/mL, respectively.[28]

Driving

Experimental studies in opioid-dependent volunteers in buprenorphine maintenance programs suggest that single therapeutic doses of buprenorphine cause a slight impairment in gross psychomotor performance, but these buprenorphine doses cause little impairment of more complex cognitive functions when administered to opioid-dependent individuals.[14] The administration of sublingual buprenorphine in doses up to 32 mg caused little impairment on most neuropsychologic tests of psychomotor speed (digit symbol substitution, trail-making A and B), time perception, conceptual flexibility (trail-making A and B), focused attention, and working memory with the exception of mild impairment of long-term memory.[100] The IV infusion of buprenorphine to non-opioid-dependent subjects causes some impairment on performance in neuropsychiatric testing of visual information processing, psychomotor speed, choice reaction time, eye-hand coordination, and attention, when compared with baseline values.[101,102]

TREATMENT

Stabilization

The management of buprenorphine intoxication is similar to the treatment of other opioids. Respiratory depression and pulmonary aspiration are the most common serious complications of buprenorphine overdose. Patients should be evaluated for the adequacy of respiration and monitored for the development of hypoxia with pulse oximetry and arterial blood gases as indicated by clinical examination. Intubation should be considered in patients with inadequate ventilation, when respirations do not improve following the admin-

istration of naloxone. Young children typically tolerate accidental exposure to buprenorphine without serious toxic effects; however, occasionally significant CNS and respiratory depression occurs. Because of the prolonged duration of action, asymptomatic patients with significant exposure to buprenorphine should be observed at least 6 hours after ingestion for evidence of CNS or respiratory depression.

Gut Decontamination

Decontamination measures are usually unnecessary, unless indicated by the concomitant ingestion of other substances. The administration of activated charcoal is a therapeutic option in alert patients presenting within 1 hour of an overdose, but there are inadequate data to determine the effect of this therapeutic measure on clinical outcome.

Elimination Enhancement

Although there are few data on methods to enhance the elimination of buprenorphine, the large volume of distribution and high protein binding likely limit the effectiveness of any measure to enhance the elimination of buprenorphine.

Antidotes

Naloxone is the antidote of choice for buprenorphine intoxication. Although buprenorphine overdose produces an opioid syndrome similar to heroin and methadone, limited data on the use of naloxone for buprenorphine overdose suggests that buprenorphine intoxication is less responsive to naloxone than heroin or methadone intoxication.[103] The relatively high affinity of buprenorphine for the μ-opioid receptor potentially limits the effectiveness of naloxone.[104] Additionally, benzodiazepines are frequently co-ingested with buprenorphine;[103] the effect of benzodiazepines is not affected by the administration of naloxone. Because of the mixed agonist-antagonist μ-opioid receptor activity of buprenorphine, optimization of the naloxone dose depends on the buprenorphine dose. In a study of 16 healthy volunteers, an IV naloxone dose of 0.8 mg did not reverse the respiratory depression from 0.2 mg buprenorphine as measured by ventilation (L/min).[105] Increasing naloxone doses (2–4 mg) given over 30 minutes produced full reversal of the depression in ventilation; however, high naloxone doses (i.e., >5–7 mg) reversed the decline in ventilation associated with effective, lower naloxone doses. Similarly in a placebo-controlled, single-blind study of volunteers, the IV administration of 0.3 mg buprenorphine/70 kg body

weight reduced CO_2 responsiveness by about 50%.[106] The IV administration of 5 mg and 10 mg naloxone, but not 1 mg naloxone produced dose-dependent reversal of the effect of buprenorphine on these respiratory parameters. The maximum effect of naloxone occurred 3 hours after administration, indicating that clinical effect of naloxone during buprenorphine intoxication may be delayed compared with heroin intoxication. Symptomatic patients requiring naloxone for reversal of respiratory depression should be observed for 36–48 hours to prevent oxygen desaturation from delayed effects of buprenorphine on the ventilatory rate.

References

1. Quigley AJ, Bredemeyer DE, Seow SS. A case of buprenorphine abuse. Med J Aust 1984;140:425–426.

2. Lavelle TL, Hammersley R, Forsyth A, Bain D. The use of buprenorphine and temazepam by drug injectors. J Addict Dis 1991;10:5–14.

3. Tracqui A, Kintz P, Ludes B. Buprenorphine-related deaths among drug addicts in France: a report on 20 fatalities. J Anal Toxicol 1998;22:430–434.

4. Kintz P, Villain M, Tracqui A, Cirimele V, Ludes B. Buprenorphine in drug-facilitated sexual abuse: a fatal case involving a 14-year-old boy. J Anal Toxicol 2003;27:527–529.

5. Jenkinson RA, Clark NC, Fry CL, Dobbin M. Buprenorphine diversion and injection in Melbourne, Australia: an emerging issue? Addiction 2005;100:197–205.

6. Cowan A, Lewis JW, Macfarlane IR. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. Br J Pharmacol 1977;60:537–545.

7. Martin TC, Rocque M. Accidental and non-accidental ingestion of methadone and buprenorphine in childhood: a single center experience, 1999–2009. Curr Drug Saf 2011;6:12–16.

8. Smith MY, Bailey JE, Woody GE, Kleber HD. Abuse of buprenorphine in the United States: 2003–2005. J Addict Dis 2007;26:107–111.

9. Cicero TJ, Surratt HL, Inciardi J. Use and misuse of buprenorphine in the management of opioid addiction. J Opioid Manag 2007;3:302–308.

10. Obadia Y, Perrin V, Feroni I, Vlahov D, Moatti JP. Injecting misuse of buprenorphine among French drug users. Addiction 2001;96:267–272.

11. Vidal-Trecan G, Varescon I, Nabet N, Boissonnas A. Intravenous use of prescribed sublingual buprenorphine tablets by drug users receiving maintenance therapy in France. Drug Alcohol Depend 2003;69:175–181.

12. Aitken CK, Higgs PG, Hellard ME. Buprenorphine injection in Melbourne, Australia—an update. Drug Alcohol Rev 2008;27:197–199.

13. Hakansson A, Medvedeo A, Andersson M, Berglund M. Buprenorphine misuse among heroin and amphetamine users in Malmo, Sweden: purpose of misuse and route of administration. Eur Addict Res 2007;13:207–215.

14. Stoller KB, Bigelow GE, Walsh SL, Strain EC. Effects of buprenorphine/naloxone in opioid-dependent humans. Psychopharmacology (Berl) 2001;154:230–242.

15. Winstock AR, Lea T, Sheridan J. Prevalence of diversion and injection of methadone and buprenorphine among clients receiving opioid treatment at community pharmacies in New South Wales, Australia. Int J Drug Policy 2008;19:450–458.

16. Sakol MS, Stark C, Sykes R. Buprenorphine and temazepam abuse by drug takers in Glasgow—an increase. Br J Addict 1989;84:439–441.

17. O'Connor JJ, Moloney E, Travers R, Campbell A. Buprenorphine abuse among opiate addicts. Br J Addict 1988;83:1085–1087.

18. Robinson GM, Dukes PD, Robinson BJ, Cooke RR, Mahoney GN. The misuse of buprenorphine and a buprenorphine-naloxone combination in Wellington, New Zealand. Drug Alcohol Depend 1993;33:81–86.

19. Alho H, Sinclair D, Vuori E, Holopainen A. Abuse liability of buprenorphine-naloxone tablets in untreated IV drug users. Drug Alcohol Depend 2007;88:75–78.

20. Singh RA, Mattoo SK, Malhotra A, Varma VK. Cases of buprenorphine abuse in India. Acta Psychiatr Scand 1992;86:46–48.

21. Orman JS, Keating GM. Buprenorphine/naloxone a review of its use in the treatment of opioid dependence. Drugs 2009;69:577–607.

22. Walsh SL, Preston KL, Bigelow GE, Stitzer ML. Acute administration of buprenorphine in humans: partial agonist and blockade effects. J Pharmacol Exp Ther 1995;274:361–372.

23. Jasinski DR, Fudala PJ, Johnson RE. Sublingual versus subcutaneous buprenorphine in opiate abusers. Clin Pharmacol Ther 1989;45:513–519.

24. Walsh SL, Preston KL, Stitzer ML, Cone EJ, Bigelow GE. Clinical pharmacology of buprenorphine: ceiling effects at high doses. Clin Pharmacol Ther 1994;55:569–580.

25. Umbricht A, Huestis MA, Cone EJ, Preston KL. Effects of high-dose intravenous buprenorphine inexperienced opioid abusers. J Clin Psychopharmacol 2004;24:479–487.

26. Kishioka S, Paronis CA, Lewis JW, Woods JH. Buprenorphine and methoclocinnamox: agonist and antagonist effects on respiratory function in rhesus monkeys. Eur J Pharmacol 2000;391:289–297.

27. Kraft WK, Gibson E, Dysart K, Damle VS, LaRusso JL, Greenspan JS, et al. Sublingual buprenorphine for treatment of neonatal abstinence syndrome: a randomized trial. Pediatrics 2008;122:e601–e607.

28. Gaulier J-M, Charvier F, Monceaux F, Marquet P, Lachatre G. Ingestion of high-dose buprenorphine by

a 4 year-old child. J Toxicol Clin Toxicol 2004;42: 993–995.

29. Geib A-J, Babu K, Ewald MB, Boyer EW. Adverse effects in children after unintentional buprenorphine exposure. Pediatrics 2006;188:1746–1751.

30. Hayes BD, Klein-Schwartz W, Doyon S. Toxicity of buprenorphine overdoses in children. Pediatrics 2008;121:e782–e786.

31. Harris DS, Mendelson JE, Lin ET, Upton RA, Jones RT. Pharmacokinetics and subjective effects of sublingual buprenorphine, alone or in combination with naloxone lack of dose proportionality. Clin Pharmacokinet 2004; 43:329–340.

32. Schuh KJ, Johanson CE. Pharmacokinetic comparison of the buprenorphine sublingual liquid and tablet. Drug Alcohol Depend 1999;56:55–60.

33. Nath RP, Upton RA, Everhart ET, Cheung P, Shwonek P, Jones RT, Mendelson JE. Buprenorphine pharmacokinetics: relative bioavailability of sublingual tablet and liquid formulations. J Clin Pharmacol 1999;39:619–623.

34. Greenwald MK, Johanson CE, Moody DE, Woods JH, Kilbourn MR, Koeppe RA, et al. Effects of buprenorphine maintenance dose on mu-opioid receptor availability, plasma concentrations, and antagonist blockade in heroin-dependent volunteers. Neuropsychopharmacology 2003;28:2000–2009.

35. Bullingham RE, McQuay HJ, Porter EJ, Allen MC, Moore RA. Sublingual buprenorphine used postoperatively: ten hour plasma drug concentration analysis. Br J Clin Pharmacol 1982;13:665–673.

36. Kuhlman JJ Jr, Lalani S, Magluilo J Jr, Levine B, Darwin WD, Johnson RE, Cone EJ. Human pharmacokinetics of intravenous, sublingual, and buccal buprenorphine. J Anal Toxicol 1996;20:369–378.

37. Lopatko OV, White JM, Huber A, Ling W. Opioid effects and opioid withdrawal during a 24 h dosing interval in patients maintained on buprenorphine. Drug Alcohol Depend 2003;69:317–322.

38. Elkader A, Sproule B. Buprenorphine clinical pharmacokinetics in the treatment of opioid dependence. Clin Pharmacokinet 2005;44:661–680.

39. Kobayashi K, Yamamoto T, Chiba K, Tani M, Shimada N, Ishizaki T, Kuroiwa Y. Human buprenorphine N-dealkylation is catalyzed by cytochrome P450 3A4. Drug Metab Dispos 1998;26:818–821.

40. Iribarne C, Picart D, Dreano Y, Bail JP, Berthou F. Involvement of cytochrome P450 3A4 in N-dealkylation of buprenorphine in human liver microsomes. Life Sci 1997;60:1953–1964.

41. Ohtani M, Kotaki H, Sawada Y, Iga T. Comparative analysis of buprenorphine- and norbuprenorphine-induced analgesic effects based on pharmacokinetic-pharmacodynamic modeling. J Pharmacol Exp Ther 1995;272:505–510.

42. Meyer MR, Maurer HH. Absorption, distribution, metabolism and excretion pharmacogenomics of drugs of abuse. Pharmacogenomics 2011;12:215–233.

43. Brewster D, Humphrey MJ, McLeavy MA. Biliary excretion, metabolism and enterohepatic circulation of buprenorphine. Xenobiotica 1981;11:189–196.

44. Mendelson JH, Mello NK. Human laboratory studies of buprenorphine. NIDA Res Monogr 1992;121:38–60.

45. Cone EJ, Gorodetzky CW, Yousefnejad D, Buchwald WF, Johnson RE. The metabolism and excretion of buprenorphine in humans. Drug Metab Dispos 1984;12:577–581.

46. Kuhlman JJ Jr, Levine B, Johnson RE, Fudala PJ, Cone EJ. Relationship of plasma buprenorphine and norbuprenorphine to withdrawal symptoms during dose induction, maintenance and withdrawal from sublingual buprenorphine. Addiction 1998;93:549–559.

47. Marquet P, Chevrel J, Lavignasse P, Merle L, Lachatre G. Buprenorphine withdrawal syndrome in a newborn. Clin Pharmacol Ther 1997;62:569–571.

48. Sittl R. Transdermal buprenorphine in the treatment of chronic pain. Expert Rev Neurother 2005;5:315–323.

49. Reynaud M, Tracqui A, Petit G, Potard D, Courty P. Six deaths linked to misuse of buprenorphine-benzodiazepine combinations. Am J Psychiatry 1998;155:448–449.

50. Kilicarslan T, Sellers EM. Lack of interaction of buprenorphine with flunitrazepam metabolism. Am J Psychiatry 2000;157:1164–1166.

51. Iribarne C, Berthou F, Carlhant D, Dreano Y, Picart D, Lohezic F, Riche C. Inhibition of methadone and buprenorphine N-dealkylations by three HIV-1 protease inhibitors. Drug Metab Dispos 1998;26:257–260.

52. Iribarne C, Picart D, Dreano Y, Berthou F: In vitro interactions between fluoxetine or fluvoxamine and methadone or buprenorphine. Fundam Clin Pharmacol 1998;12: 194–199.

53. Isenberg D, Wong SC, Curtis JA. Serotonin syndrome triggered by a single dose of Suboxone. Am J Emerg Med 2008;26:840.e3–840.e5.

54. Greenwald MK, Schuh KJ, Hopper JA, Schuster CR, Johanson CE. Effects of buprenorphine sublingual tablet maintenance on opioid drug-seeking behavior by humans. Psychopharmacology (Berl) 2002;160:344–352.

55. Barnett PG, Rodgers JH, Bloch DA. A meta-analysis comparing buprenorphine to methadone for treatment of opiate dependence. Addiction 2001;96:683–690.

56. Picker MJ, Negus SS, Craft RM. Butorphanol's efficacy at mu and kappa opioid receptors: inferences based on the schedule-controlled behavior of nontolerant and morphine-tolerant rats and on the responding of rats under a drug discrimination procedure. Pharmacol Biochem Behav 1990;36:563–568.

57. Downing JW, Goodwin NM, Hicks J. The respiratory depressive effects of intravenous buprenorphine in patients in an intensive care unit. S Afr Med J 1979;55: 1023–1027.

58. Kintz P. Deaths involving buprenorphine: a compendium of French cases. Forensic Sci Int 2001;121:65–69.

59. Sorge J, Sittle R. Transdermal buprenorphine in the treatment of chronic pain: results of a phase III, multicenter, randomized, double-blind, placebo-controlled study. Clin Ther 2004;26:1808–1820.

60. Herve S, Riachi G, Noblet C, Guillement N, Tanasescu S, Goria O, et al. Acute hepatitis due to buprenorphine administration. Eur J Gastroenterol Hepatol 2004;16: 1033–1037.

61. Vander Hulst K, Amer EP, Jacobs C, Dewulf V, Baeck M, Pujol Vallverdu RM, et al. Allergic contact dermatitis from transdermal buprenorphine. Contact Dermatitis 2008;59:366–369.

62. Boyd J, Randell T, Luurila H, Kuisma M. Serious overdoses involving buprenorphine in Helsinki. Acta Anaesthesiol Scand 2003;47:1031–1033.

63. Feeney GF, Fairweather P. Groin tissue necrosis requiring skin graft following parenteral abuse of buprenorphine tablets. Drug Alcohol Rev 2003;22:359–361.

64. Clark NC, Lintzeris N, Muhleisen PJ. Severe opiate withdrawal in a heroin user precipitated by a massive buprenorphine dose. Med J Aust 2002;176:166–167.

65. Seet RC, Rathakrishnan R, Chan BP, Lim EC. Diffuse cystic leukoencephalopathy after buprenorphine injection. J Neurol Neurosurg Psychiatry 2005;76:890–891.

66. Wedam EF, Bigelow GE, Johnson RE, Nuzzo PA, Haigney MC. QT-interval effects of methadone, levomethadyl, and buprenorphine in a randomized trial. Arch Intern Med 2007;167:2469–2475.

67. Jasinski DR, Pevnick JS, Griffith JD. Human pharmacology and abuse potential of the analgesic buprenorphine: a potential agent for treating narcotic addiction. Arch Gen Psychiatry 1978;35:501–516.

68. Eissenberg T, Greenwald MK, Johnson RE, Liebson IA, Bigelow GE, Stitzer ML. Buprenorphine's physical dependence potential: antagonist-precipitated withdrawal in humans. J Pharmacol Exp Ther 1996;276: 449–459.

69. Rosado J, Walsh SL, Bigelow GE, Strain EC. Sublingual buprenorphine/naloxone precipitated withdrawal in subjects maintained on 100 mg of daily methadone. Drug Alcohol Depend 2007;90:261–269.

70. Eissenberg T, Johnson RE, Bigelow GE, Walsh SL, Liebson IA, Strain EC, Stitzer ML. Controlled opioid withdrawal evaluation during 72 h dose omission in buprenorphine-maintained patients. Drug Alcohol Depend 1997;45:81–91.

71. San L, Cami J, Fernandez T, Olle JM, Peri JM, Torrens M: Assessment and management of opioid withdrawal symptoms in buprenorphine-dependent subjects. Br J Addict 1992;87:55–62.

72. Kahila H, Saisto T, Kivitie-Kallio S, Haukkamaa M, Halmesmaki E. A prospective study on buprenorphine use during pregnancy: effects on maternal and neonatal outcome. Acta Obstet Gynecol 2007;86:185–190.

73. Jones HE, Johnson RE, Jasinski DR, O'grady KE, Chisholm CA, Choo RE, et al. Buprenorphine versus methadone in the treatment of pregnant opioid-dependent patients: effects on the neonatal abstinence syndrome. Drug Alcohol Depend 2005;79:1–10.

74. Johnson RE, Jones HE, Fischer G. Use of buprenorphine in pregnancy: patient management and effects on the neonate. Drug Alcohol Depend 2003;70(suppl 2): S87–S101.

75. Schindler SD, Eder H, Ortner R, Rohrmeister K, Langer M, Fischer G. Neonatal outcome following buprenorphine maintenance during conception and throughout pregnancy. Addiction 2003;98:103–110.

76. Lacroix I, Berrebi A, Chaumerliac C, Lapeyre-Mestre M, Montastruc JL, Damase-Michel C. Buprenorphine in pregnant opioid-dependent women: first results of a prospective study. Addiction 2004;99:209–214.

77. Kahila H, Stefanovic V, Loukovaara M, Alfthan H, Hamalainen E, Halmesmaki E. Prenatal buprenorphine exposure: effects on biochemical markers of hypoxia and early neonatal outcome. Acta Obstet Gynecol Scand 2008;87:1213–1219.

78. Jones HE, Kaltenbach K, Heil SH, Stine SM, Coyle MG, Arria AM, et al. Neonatal abstinence syndrome after methadone or buprenorphine exposure. N Engl J Med 2010;363:2320–2331.

79. De Giovanni N, Fucci N, Scarlata S, Donzelli G. Buprenorphine detection in biological samples. Clin Chem Lab Med 2005;43:1377–1379.

80. Hand CW, Baldwin D, Moore RA, Allen MC, McQuay HJ. Radioimmunoassay of buprenorphine with iodine label: analysis of buprenorphine and metabolites in human plasma. Ann Clin Biochem 1986;23:47–53.

81. Everhart ET, Cheung P, Shwonek P, Zabel K, Tisdale EC, Jacob P 3rd, Mendelson J, Jones RT. Subnanogram-concentration measurement of buprenorphine in human plasma by electron-capture capillary gas chromatography: application to pharmacokinetics of sublingual buprenorphine. Clin Chem 1997;43:2292–2302.

82. Liu SY, Liu KS, Kuei CH, Tzeng JI, Ho ST, Wang JJ. Simultaneous determination of buprenorphine and its prodrug, buprenorphine propionate, by high-performance liquid chromatography with fluorescence detection: application to pharmacokinetic studies in rabbits. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 818:233–239.

83. Schleyer E, Lohmann R, Rolf C, Gralow A, Kaufmann CC, Unterhalt M, Hiddemann W. Column-switching solid-phase trace-enrichment high-performance liquid chromatographic method for measurement of buprenorphine and norbuprenorphine in human plasma and urine by electrochemical detection. J Chromatogr 1993;614: 275–283.

84. Feng S, ElSohly MA, Duckworth DT. Hydrolysis of conjugated metabolites of buprenorphine. I. The quantitative enzymatic hydrolysis of buprenorphine-3-beta-D-glucuronide in human urine. J Anal Toxicol 2001; 25:589–593.

85. Rodriguez-Rosas ME, Lofwall MR, Strain EC, Siluk D, Wainer IW. Simultaneous determination of buprenorphine, norbuprenorphine and enantiomers of methadone and its metabolite (EDDP) in human plasma by liquid chromatography/mass spectrometry. J Chromatogr B 2007;850:538–543.

86. Selden T, Roman M, Druid H, Kronstrand R. LC-MS-MS analysis of buprenorphine and norbuprenorphine in whole blood from suspected drug users. Forensic Sci Int 2011;209:113–119.

87. Polettini A, Huestis MA. Simultaneous determination of buprenorphine, norbuprenorphine, and buprenorphine-glucuronide in plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 2001;754:447–459.

88. Vincent F, Bessard J, Vacheron J, Mallaret M, Bessard G. Determination of buprenorphine and norbuprenorphine in urine and hair by gas chromatography-mass spectrometry. J Anal Toxicol 1999;23:270–279.

89. George S, George C, Chauhan M. The development and application of a rapid gas chromatography-mass spectrometry method to monitor buprenorphine withdrawal protocols. Forensic Sci Int 2004;143:121–125.

90. Miller EI, Torrance HJ, Oliver JS. Validation of the Immunanalysis microplate ELISA for the detection of buprenorphine and its metabolite norbuprenorphine in urine. J Anal Toxicol 2006;30:115–119.

91. Hull MJ, Bierer MF, Griggs DA, Long WH, Nixon AL, Flood JG. Urinary buprenorphine concentrations in patients treated with Suboxone® as determined by liquid chromatography-mass spectrometry and CEDIA immunoassay. J Anal Toxicol 2008;32:516–521.

92. Kacinko SL, Concheiro-Guison M, Shakleya DM, Huestis MA. Development and validation of a liquid chromatography-tandem mass spectrometry assay for the simultaneous quantification of buprenorphine, norbuprenorphine, and metabolites in human urine. Anal Bioanal Chem 2008;392:902–911.

93. Fuller DC. A simple gas chromatography-mass spectrometry procedure for the simultaneous determination of buprenorphine and norbuprenorphine in human urine. J Anal Toxicol 2008;32:626–630.

94. Hadidi KA, Oliver JS. Stability of morphine and buprenorphine in whole blood. Int J Legal Med 1998; 111:165–167.

95. Moody DE, Slawson MH, Strain EC, Laycock JD, Spanbauer AC, Foltz RL. A liquid chromatographic-electrospray ionization-tandem mass spectrometric method for determination of buprenorphine, its metabolite, norbuprenorphine, and a coformulant, naloxone, that is suitable for *in vivo* and *in vitro* metabolism studies. Anal Biochem 2002;306:31–39.

96. Pirnay S, Borron SW, Giudicelli CP, Tourneau J, Baud FJ, Ricordel I. A critical review of the causes of death among post-mortem toxicological investigations: analysis of 34 buprenorphine-associated and 35 methadone-associated deaths. Addiction 2004;99:978–988.

97. Gaulier JM, Marquet P, Lacassie E, Dupuy JL, Lachatre G. Fatal intoxication following self-administration of a massive dose of buprenorphine. J Forensic Sci 2000; 45:226–228.

98. Kronstrand R, Selden TG, Josefsson M. Analysis of buprenorphine, norbuprenorphine, and their glucuronides in urine by liquid chromatography-mass spectrometry. J Anal Toxicol 2003;27:464–470.

99. Kronstrand R, Nystrom I, Andersson M, Gunnarsson L, Hagg S, Josefsson M, Ahlner J. Urinary detection times and metabolite/parent compound ratios after as single dose of buprenorphine. J Anal Toxicol 2008;32:586–593.

100. Mintzer MZ, Correia CJ, Strain EC. A dose-effect study of repeated administration of buprenorphine/naloxone on performance in opioid-dependent volunteers. Drug Alcohol Depend 2004;74:205–209.

101. Zacny JP, Conley K, Galinkin J. Comparing the subjective, psychomotor and physiological effects of intravenous buprenorphine and morphine in healthy volunteers. J Pharmacol Exp Ther 1997;282:1187–1197.

102. Jensen ML, Sjogren P, Upton RN, Foster DJ, Bonde P, Graae C, et al. Pharmacokinetic-pharmacodynamic relationships of cognitive and psychomotor effects of intravenous buprenorphine infusion in human volunteers. Basic Clin Pharmacol Toxicol 2008;103:94–101.

103. Megarbane B, Buisine A, Jacobs F, Resiere D, Chevillard L, Vicaut E, Baud FJ. Prospective comparative assessment of buprenorphine overdose with heroin and methadone: clinical characteristic and response to antidotal treatment. J Subst Abuse Treat 2010;38:403–407.

104. Harris DS, Jones RT, Welm S, Upton RA, Lin E, Mendelson J. Buprenorphine and naloxone coadministration in opiate-dependent patients stabilized on sublingual buprenorphine. Drug Alcohol Depend 2000; 61:85–94.

105. van Dorp E, Yassen A, Sarton E, Romberg R, Olofsen E, Teppema L, et al. Naloxone reversal of buprenorphine-induced respiratory depression. Anesthesiology 2006;105:51–57.

106. Gal TJ. Naloxone reversal of buprenorphine-induced respiratory depression. Clin Pharmacol Ther 1989; 45:66–71.

Chapter 29

DEXTROMETHORPHAN

HISTORY

In 1958, dextromethorphan was approved in the United States as a nonaddictive, safe antitussive, over-the-counter (OTC) replacement for codeine-containing cough preparations. The first tablet form of dextromethorphan, Romilar®, was removed from the US OTC market in the 1970s as a result of abuse.[1] Sporadic episodes of dextromethorphan abuse occurred in Australia during the 1960s[2] and in Sweden during the 1980s. In the early 1990s, dimenhydrinate and cyclizine were popular, nonprescription drugs of abuse among US teenagers.[3] The anticholinergic properties of these drugs produce tachycardia, mild hypertension, and hallucinations similar to dextromethorphan when ingested in excessive doses. During the middle 1990s, caffeine-containing products were popular drugs of abuse among teenagers; later in the 1990s, dextromethorphan became a popular OTC drug of abuse. In 1990, abuse of Robitussin DM by Utah teenagers caused the Utah State Board of Pharmacy to restrict direct access of the public to dextromethorphan. Since the 1980s, abuse of dextromethorphan-containing cough and cold preparation has been relatively common among teenagers as a "poor man's PCP."[4] Analysis of reports to US poison control centers indicate that dextromethorphan abuse among this age group increased significantly in the late 1990s.[3,5]

IDENTIFYING CHARACTERISTICS

Physiochemical Properties

Figure 29.1 displays the chemical structure of dextromethorphan (CAS RN: 125-71-3, d-3-methoxy-N-meth-ylmorphine). This poorly water soluble, white, crystalline substance is the dextrorotatory isomer of the opioid, levorphanol (3-hydroxy-N-methyl morphinan). Levorphanol is the levorotatory isomer of racemic 3-hydroxy-N-methylmorophinan (Dromoran). Levorphanol has significant respiratory depressant, analgesic, and addictive properties.[6] In contrast to levorphanol, dextromethorphan does not stimulate opioid receptors, resulting in the absence of analgesia or respiratory depression at therapeutic doses. Volunteer studies indicate that dextromethorphan is an effective antitussive agent that does not cause euphoria, respiratory depression, or physical dependence to the extent morphine does.[7,8] Other narcotic morphinan derivatives include butorphanol, codeine, levallorphan, and morphine. Commercial dextromethorphan preparations do not contain the (S)-enantiomer.

Terminology

Street names for dextromethorphan-containing medications include the following Coricidin® (skittles, red hots, red devils, triple C) and Robitussin DM® (ro-bowing, robo-copping). Other street names for dextromethorphan include Dex, DXM, and poor man's PCP (phencyclidine).

Form

Abuse of dextromethorphan usually involves the ingestion of cough and cold formulations (i.e., gel capsules, tablets, liquid), which contain other active ingredients including antihistamines (e.g., 2–4 mg chlorpheniramine maleate) and analgesics (325–500 mg acetaminophen).
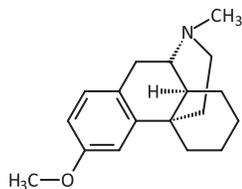
FIGURE 29.1. Chemical structure of dextromethorphan.

Dextromethorphan is available in these medications as the hydrobromide salt, and chronic abuse of large quantities of dextromethorphan hydrobromide increases in the serum bromide concentration. Because the abused dextromethorphan-containing drugs are pharmaceutic preparations, these preparations do not usually contain unknown additives or contaminants.

EXPOSURE

Epidemiology

Dextromethorphan is an ingredient in more than 100 OTC cough and cold preparations in the United States. The abuse of this compound has been recognized since the 1960s; however, abuse of dextromethorphan did not increase significantly until the late 1990s when this compound became a popular drug of abuse with teenagers.[9] Between 1999 and 2004, there was a 10-fold increase in the number of cases of dextromethorphan abuse reported to the California Poison Control System, primarily in the 9- to 17-year-old age group.[10] Similar trends occurred in the data from the American Association of Poison Control Centers (AAPCC) and the Drug Abuse Warning Network (DAWN) databases with the highest frequency among 15- and 16-year-olds. In 2008, the US Substance Abuse and Mental Health Services Administration (SAMHSA) reported that approximately 5% of youth aged 12–25 years used OTC cough and cold preparations for recreational purposes with females overrepresented in the 12–17 year old groups and males overrepresented in the 18–25 year old group.[11] In Korea, zipeprol (CAS No. 34758-84-4) is another centrally acting cough suppressant that is abused with dextromethorphan for stronger hallucinogenic effects, predominantly by teenagers.[12]

Sources

Dextromethorphan hydrobromide (10–30 mg) is present in OTC cough and cold preparations as an antitussive (e.g., NyQuil®, Proctor & Gamble, Cincinnati, OH; Robitussin-DM®, Pfizer Pharmaceuticals, New York, NY; Tylenol-DM®, Ortho-McNeil-Jansson Pharmaceuticals, Titusville, NJ; Coricidin HBP®, Merck & Co., Whitehouse Station, NJ, Vicks Formula 44-D®, Proctor & Gamble). Some liquid dextromethorphan preparations contain ethanol concentrations up to approximately 25%. A novel use of this property is the treatment of drug and alcohol abuse as a means to decrease withdrawal symptoms during acute detoxification and to inhibit the conditioned reactions associated with drug-seeking behavior.[13]

Pure dextromethorphan powder is available on the Internet along with extraction procedures for the manufacture of concentrated dextromethorphan powder from Coricidin HBP™ Cough & Cold tablets.[14] Experienced dextromethorphan abusers use several extraction techniques to eliminate unwanted products (e.g., ethanol, guaifenesin, saccharin, propylene glycol, coloring agents, sweeteners) present in combination cold preparations. These methods include single-phase acid-base extraction with sodium hydroxide into a powder (Crystal Dex) and the two-phase ("Agent Lemon, DXemon Juice") method with ammonia that forms a liquid.[15] The product of the former method is the free-base, crystalline form of dextromethorphan ("Crystal Dex"), whereas the latter method produces liquid dextromethorphan hydrocitrate (DXemon Juice, Agent Lemon). This latter method avoids the use of lye and flammable solvents.

Methods of Abuse

Ingestion is the primary route of dextromethorphan abuse. Rarely, case reports associate dextromethorphan abuse with insufflation.[16]

DOSE EFFECT

Illicit Use

Chronic abusers of dextromethorphan-containing products frequently talk about several levels or plateaus during the ingestion of escalating doses of dextromethorphan. During the first plateau, ingestion of approximately 100–200 mg dextromethorphan produces mild stimulant effects, whereas mild hallucinations develop during the second level following the ingestion of about 200–400 mg dextromethorphan.[17] Dysphoria can occur if dextromethorphan doses exceed 200–250 mg. The third plateau is the "out of body" experience associated with dextromethorphan doses of 300–600 mg; the ingestion of doses > 600 mg are associated with complete disassociation (similar to ketamine). Tolerance develops to the euphoriant properties of dextromethorphan, and chronic abusers can consume 700–1,500 mg daily.[18] A case report documented the daily use of 720 mL of

Tussin DM® (10–15 mg dextromethorphan/5 mL) with daily peak use up to about 1,200 mL (i.e., 2,400–3,600 mg dextromethorphan).[19] Another case reported associated the development of agitation and hallucinations with the chronic ingestion of 3–4 bottles of Robitussin DM® daily for 2 years.[20] The estimated dextromethorphan dose in 3 bottles was 2,160 mg (31 mg/kg body weight). In a retrospective review of 47 patients admitted to an adolescent inpatient psychiatric unit for depression and/or psychosis related to Coricidin HBP® abuse, the number of tablets used per episode ranged from 2–42 with 14% of the patients abusing this product daily.[21] Individual sensitivity to dextromethorphan may vary as a result of pharmacogenetic differences. The ingestion of an estimated 600 mg dextromethorphan hydrobromide within the 12 hours prior to presentation was associated with the development of altered consciousness, seizure, mydriasis, and nystagmus.[22] Phenotyping of this patient demonstrated that he was CYP2D6 deficient (i.e., poor metabolizer of dextromethorphan compared with extensive metabolism of dextromethorphan in normal individuals).

Medical Use

Dextromethorphan is a centrally acting, nonopioid antitussive drug. Recommended dosages are 60–120 mg daily in 3–4 divided doses for adults and children over 12 years of age. Dextromethorphan does not possess expectorant activity, and there are relatively few placebo controlled clinical trials on the efficacy of dextromethorphan as an antitussive agent. As measured by parent questionnaires on the cough-related symptoms during a 3-day upper respiratory tract illness in their children, there was no statistically significant difference in the mean cough and composite symptoms between the dextromethorphan, codeine, and placebo groups.[23]

The administration of a single dose of 2 mg dextromethorphan/kg body weight produced mild cravings as measured by a self-rated visual analog scale in detoxified alcohol abusers, but not in non-alcohol abusers.[24] In a study of opioid addicts on methadone therapy, dextromethorphan (480 mg daily) was associated with mild elevation of blood pressure, heart rate, temperature, and plasma bromide concentration, but none of these effects were clinically significant.[25] The most common adverse effects associated with the highest doses were drowsiness and sleepiness.

Toxicity

Most exposures to dextromethorphan in children do not result in clinically significant symptoms. Patients accidently ingesting more than 7.5 mg dextromethorphan/ kg should be referred to an emergency department for evaluation, whereas patients asymptomatic >4 hours after ingestion can be observed at home.[26]

TOXICOKINETICS

Absorption

Dextromethorphan is rapidly absorbed from the gastrointestinal (GI) tract. As a result of high first-pass metabolism, the concentration of dextromethorphan in the plasma is small compared to the active metabolite, dextrorphan.[27] Following ingestion of dextromethorphan, maximal serum dextromethorphan concentrations occur within about 2–3 hours, whereas peak serum concentrations of dextrorphan occur within approximately 1.5–3 hours.[28,29]

Distribution

Limited data suggest that the volume of distribution of dextromethorphan is relatively large (i.e., about 5–6 L/kg).

Biotransformation

The metabolism of dextromethorphan is extensive based on analysis of urinary metabolites in volunteers receiving the drug orally.[30] Metabolic pathways include O,N-demethylation, O,N-acetylation, and hydroxylation of the phenyl ring with further oxidation. Biotransformation of dextromethorphan results in the formation of the active metabolite, dextrorphan, through CYP2D6-mediated O-methylation and, to a much lesser extent, to 3-methoxymorphinan via CYP3A-mediated N-demethylation. Both of these metabolites are further demethylated to 3-hydroxymorphinan. Dextrorphan has anticonvulsant, sedative, and antitussive properties, as well as an affinity for the phencyclidine site of the ligand-gated channel of the N-methyl D-aspartate (NMDA) receptor complex. The affinity of dextrorphan and ketamine for this receptor is similar.[31] Polymorphism in the expression of the gene for CYP2D6 results in substantial individual variability in the biotransformation of dextromethorphan to dextrorphan. Approximately 7–10% and <1% of the Caucasian and Asians populations, respectively, have deficiencies of CYP2D6 as an autosomal-recessive trait; therefore, these individuals have limited capacity to metabolize dextromethorphan to dextrorphan. In a study of 5 extensive dextromethorphan metabolizers receiving a single oral dose of 30 mg dextromethorphan hydrobromide, the major plasma metabolites were conjugated dextrorphan and conjugated 3-hydroxymorphinan.[32]

529
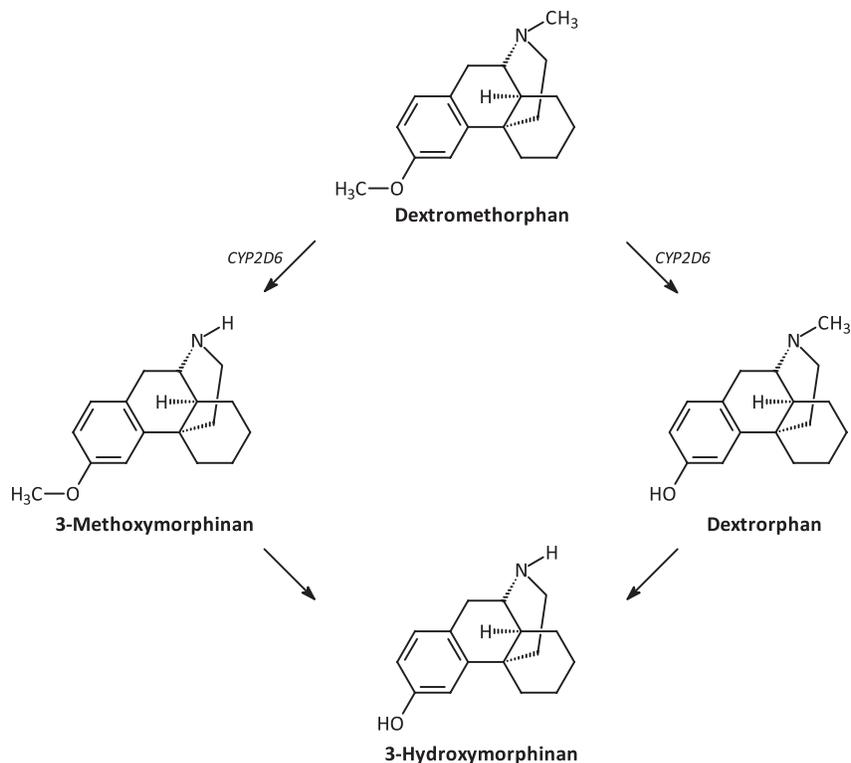
FIGURE 29.2. Biotransformation of dextromethorphan.[17] The biologic activities of 3-methoxymorphinan and 3-hydroxymorphinan are unknown. All biologic activity attributed to dextromethorphan resides in dextrorphan.

Free dextrorphan concentrations were about 100-fold less than conjugated dextrorphan. The rapid glucuronidation of dextrorphan results in very small amounts of free dextromethorphan in the plasma. Dextrorphan glucuronide does not cross the blood–brain barrier. In contrast to extensive metabolizers, dextromethorphan was the major component in the blood after the administration of the same dextromethorphan dose to poor metabolizers, while the plasma concentrations of conjugated dextrorphan was 5–10 times less than plasma dextromethorphan concentrations.

Elimination

In a study of 265 unrelated Swiss participants, 9% were poor metabolizers of dextromethorphan and 91% were extensive metabolizers as defined by an antimode at a metabolic ratio of 0.3.[33] The plasma elimination half-life and the amount of dextromethorphan and dextrorphan excreted in the urine depend on the CYP2D6 phenotype. Urinary excretion of dextrorphan following the ingestion of a single 25 mg-dose of dextromethorphan was <6% in all poor metabolizers compared with about 50% in the extensive metabolizers (i.e., the most common phenotype). The typical plasma elimination half-life of dextromethorphan following the administration of single therapeutic doses of dextromethorphan to subjects with normal CYP2D6 concentrations is about 3–4 hours. In a study of 4 poor metabolizers receiving a single dose of 30 mg dextromethorphan, the mean plasma elimination half-life of dextromethorphan was approximately 29 hours.[32] The concentration of dextromethorphan in plasma from 5 extensive metabolizers was near the level of detection; therefore, the plasma half-life of dextromethorphan in the 5 extensive metabolizers could not be calculated. A majority of dextrorphan excreted in the urine appears as free dextrorphan.[34]

Maternal and Fetal Kinetics

There are no data on the placental transfer of dextromethorphan, although the low molecular weight (i.e., about 271 g/mol) suggests that some placental transfer of dextromethorphan occurs.

Tolerance

As with most opioids, tolerance develops after the long-term abuse of dextromethorphan. Case reports

document the ingestion of escalating doses of dextromethorphan despite the development of adverse effects (e.g., constipation, insomnia, weight gain, psychosis) and disruption of personal life. Over 6 months, the ingestion of dextromethorphan by a 38-year-old woman increased from the occasional use of 3 ounces to 40 ounces daily despite the presence of hallucination, paranoia, and delusional beliefs.[19] Cessation of dextromethorphan use was associated with withdrawal symptoms (i.e., vomiting, diarrhea, myalgias, restlessness, night sweats, insomnia, anxiety).

Drug Interactions

Case reports indicate that the most serious drug interaction with dextromethorphan is the development of the serotonin syndrome during concomitant therapy with monoamine oxidase or selective serotonin reuptake inhibitors, particularly following the ingestion of high doses of dextromethorphan.[35,36] Clinical effects associated with the serotonin syndrome include signs of autonomic instability (hypertension, hyperpyrexia, diaphoresis, tachycardia), muscular hypertonicity (tremor, clonus, myoclonus, hyperreflexia), and mental status changes (agitation, disorientation, confusion). In contrast to the bradykinesia and idiopathic reactions associated with neuroleptic malignant syndrome, serotonin syndrome frequently is dose-related and associated with hyperkinesia, clonus, and hyperreflexia. A case report associated the development of serotonin syndrome following abuse of dextromethorphan by a bipolar patient on fluoxetine and lithium.[37] As a weak serotonin reuptake inhibitor, dextromethorphan potentially may interact with meperidine, tramadol, or propoxyphene to produce serotonin syndrome when ingested with monoamine oxidase inhibitors.[38] Several case reports associate the development of altered consciousness (coma, delirium) with the combined use of dextromethorphan with CYP2D6 inhibitors (amitriptyline, methadone), particularly in poor CYP2D6 metabolizers.[39,40]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The prodrug, dextromethorphan is converted to dextrorphan (CAS No: 125-73-5), which raises the threshold for the cough reflex in the medulla oblongata. The latter compound is a semisynthetic morphine derivative that modulates glutamatergic neurotransmission by noncompetitive antagonism of N-methyl-D-aspartate (NMDA) receptors. These receptors are 1 of 3 types of ionotropic glutamate receptors, which are the primary excitatory neurotransmitter in primates.

Mechanism of Toxicity

The binding affinity (K_i) of the ion channel of the NMDA receptor complex for dextromethorphan is about 3,500 nM compared with 222 nM and 42 nM for dextrorphan and phencyclidine, respectively.[24] Although dextromethorphan binds to NMDA receptors in animal studies, these animal studies suggest that dextrorphan, but not dextromethorphan, has phencyclidine-like properties.[41] NMDA receptors open distinctive membrane channels characterized by voltage-dependent Mg^{++} blockade and high permeability to calcium ions.[42] Activation of NMDA receptors initiate calcium-calmodulin mediated activation of nitric oxide synthase. The active metabolite, dextrorphan, antagonizes these actions, resulting in the enhancement of excitatory neurotransmission and dissociative experiences. Animal studies indicate that dextrorphan and dextromethorphan cause a pronounced enhancement of 5-hydroxytryptamine (5-HT)-induced head-twitch response (HTR) in intact mice, but the clinical relevance of this effect is unclear.[43] Although a pilot study suggested that extensive metabolizers of dextromethorphan have an increased risk of abuse as a result of the positive subjective effects of dextrorphan,[44] there are inadequate data to conclude that extensive metabolizers are overrepresented in samples of dextromethorphan abusers.

Postmortem Examination

The postmortem findings in individuals dying from dextromethorphan intoxication are nonspecific, and similar to opioid poisoning. Postmortem examination of 2 teenagers found dead after ingestion dextromethorphan demonstrated cerebral edema, pulmonary edema, and frothy foam in the major airways without evidence of trauma or antecedent natural disease.[45]

CLINICAL RESPONSE

As an antitussive, dextromethorphan has a wide margin of safety. Adverse drug reactions to therapeutic doses of dextromethorphan are infrequent and mild with recreational abuse of dextromethorphan being the most important drug safety issue associated with this drug.[46] In a study of the neuroprotective effects of dextromethorphan, 181 neurosurgical patients received 212 courses of oral dextromethorphan in doses ranging up to 400 mg.[47] Adverse effects included nystagmus (64%), nausea and vomiting (27%) distorted vision (27%), feeling "drunk" (27%), ataxia (27%), and dizziness (27%). Some patients experienced dysphoria and visual hallucinations.

Illicit Use

Symptoms associated with dextromethorphan abuse include euphoria, altered time perception, lightheadedness, increased perceptual awareness, hallucinations (visual, auditory, tactile), paranoia, visual disturbances, and disorientation. Visual hallucinations involve the appearance of moving swirls and patches of color while the eyes are closed.[14] Case reports indicate that the abuse of dextromethorphan-containing formulations produces nausea, vomiting dizziness, tachycardia, and hypertension along with central nervous system effects (e.g., nystagmus, ataxia, mydriasis, euphoria, lethargy, hallucinations, agitation, seizure, coma).

Dextromethorphan is formulated in cough and cold preparations as the hydrobromide salt. Bromide poisoning is unlikely in episodic, recreational abusers of dextromethorphan hydrobromide. However, chronic, heavy abuse of dextromethorphan may cause chronic bromism presenting with negative anion gap, headache, weakness, lethargy, and altered consciousness.[48] Other clinical effects associated with chronic bromide intoxication include apathy, slurred speech, tremulousness, nystagmus, ataxia, hallucinations, weight loss, acneform rash, and behavioral changes. The differential diagnosis of nystagmus, ataxia and altered mental status includes phencyclidine, lithium, methanol, and anticonvulsant (phenytoin, carbamazepine) intoxication, sedative-hypnotic withdrawal, and thiamine depletion (Wernicke-Korsakoff syndrome). Additionally, chronic dextromethorphan abuse occurs with formulations that include antihistamines (e.g., chlorpheniramine) with anticholinergic effects. Case reports associated dextromethorphan abuse with mania,[49] serotonin syndrome in combination with fluoxetine[50] or chlorpheniramine,[51] and psychosis.[52] Serotonin syndrome may occur during dextromethorphan abuse in patients on therapeutic doses of selective serotonin reuptake inhibitors (e.g., sertraline, escitalopram).[53]

Overdose

Although accidental ingestion of dextromethorphan is a common event, there are few reports of serious toxicity in children following accidental exposure to dextromethorphan; incidental exposures in children may cause mild lethargy, but are typically asymptomatic.[54] Clinical effects commonly associated with mild dextromethorphan overdose include ataxia, drowsiness, hypertension, tachycardia, diaphoresis, mydriasis, vomiting, bidirectional nystagmus, hyperexcitability, and unsteady gait.[17,55] The clinical features of dextromethorphan overdose may simulate the dissociative states associated with ketamine or phencyclidine overdose.

Dextromethorphan intoxication does not simulate classic opioid overdose because this drug lacks strong μ-opioid agonist properties. Miosis and respiratory depression are not a prominent part of dextromethorphan intoxication, which may cause excitation rather than sedation in children.[46] Case reports associate the acute ingestion of dextromethorphan with dystonic reactions (opisthotonus, ataxia, bidirectional nystagmus),[56] psychosis,[57] and the serotonin syndrome (tachycardia, diaphoresis, mydriasis, clonus, hypertonia, confusion, fever).[58]

Dextromethorphan is often formulated in combination with other drugs (e.g., antihistamines, decongestants, expectorants) as part of cough and cold preparations. Consequently, the clinical presentation of intoxication associated with these preparations does not necessarily reflect the classic clinical features of dextromethorphan overdose. Some of these clinical effects also occur following antihistamine intoxication including tachycardia, hypertension, mydriasis, urinary retention, lethargy, agitation, hallucination, and coma. Fatalities due to dextromethorphan intoxication are very rare.

Abstinence Syndrome

A psychologic dependence develops in some patient abusing dextromethorphan over periods ranging from months to years. These patients describe an acute euphoria following dextromethorphan use along with intense craving, insomnia, depression, and dysphoria after cessation of use. Hallucinations and flashbacks can occur within the first few days after the cessation of chronic dextromethorphan abuse.[59] Other clinical features of withdrawal from dextromethorphan include diarrhea, myalgias, restlessness, insomnia, night sweats, chills, and anxiety.[19] Clinical observations suggest that chronic use of dextromethorphan does not produce a classic opioid-like withdrawal syndrome (gooseflesh, yawning, rhinorrhea, lacrimation, arthralgias, tremors, psychomotor agitation);[60] however, minor withdrawal symptoms (nausea, diaphoresis, tachycardia, hypertension) may develop within 24 hours and resolve within 2 days without treatment.[61]

Reproductive Abnormalities

Dextromethorphan is a common antitussive agent for pregnant women. Limited existing data do not indicate that the therapeutic use of dextromethorphan during the first trimester increases the risk of fetotoxicity or congenital malformations. In a study of 128 women ingesting dextromethorphan during the first trimester, there was no statistically significant increase of major

malformation above the expected baseline rate of 1–3%, when compared with pregnant women matched for age, alcohol use, smoking, and upper respiratory tract infection not treated with dextromethorphan.[62] Birth weights and minor malformations were also similar in the two groups. A surveillance study of pharmacy records and pregnancy outcomes in 59 women using dextromethorphan during the first trimester demonstrated similar findings.[63] Dextromethorphan is not generally recognized as teratogenic when used therapeutically during pregnancy (US Food & Drug Administration [FDA] pregnancy category C).

DIAGNOSTIC TESTING

Analytic Methods

Analytic techniques for the quantitation of dextromethorphan and the active metabolite, dextrorphan include high performance liquid chromatography (HPLC) with fluorescence detection,[64] HPLC with ultraviolet detection,[65] gas chromatography with flame ionization detection,[66] and gas chromatography/mass spectrometry (GC/MS).[67] Most of these methods lack the ability to determine the dextromethorphan/dextrorphan ratio. Newer methods provide more accurate determinations of these analytes. The lower limit of quantitation (LLOQ) of dextromethorphan and dextrorphan is 0.002 mg/L and 0.25 mg/L, respectively, following solid-phase extraction of acidified hydrolyzed urine samples and analysis by liquid chromatography/atmospheric pressure chemical ionization/tandem mass spectrometry.[68] The LLOQ for dextromethorphan and metabolites (dextrorphan, 3-methoxymorphinan, 3-hydroxymorphinan) in plasma using ultra performance liquid chromatography/tandem mass spectrometry is near 0.0001 mg/L.[69] The use of capillary electrophoresis on cyclodextrin phases allows the separation of dextromethorphan enantiomers.[70] Analysis of urine or oral fluid samples with enzyme-linked immunosorbent assay (ELISA) and GC/MS allows the determination of dextromethorphan and dextrorphan concentrations with a limit of detection (LOD) and LLOQ of 0.001 mg/L and 0.01 mg/L, respectively.[71] The intraday precision was <5% and the extracted drugs were stable at room temperature for at least 48 hours. The LLOQ for dextromethorphan and dextrorphan in blood samples using dried blood spots technology and HPLC/tandem mass spectrometry is about 0.0002 mg/L.[72] Analysis of cough and cold preparations with HPLC with diode-array UV detection (220 nm) allows the quantitation of various ingredients including dextromethorphan, pseudoephedrine, pheniramine, guaifenesin, pyrilamine, and chlorpheniramine.[73] There are limited human data on the plasma/whole blood ratios of dextromethorphan; however, rodent data suggests that the plasma dextromethorphan concentration is approximately two-thirds the whole blood concentration.[74]

Biomarkers

BLOOD

THERAPEUTIC. The serum dextromethorphan concentrations after oral dextromethorphan doses are relatively low with typical therapeutic concentrations ranging from 0.0005–0.0059 mg/L. The mean peak serum dextromethorphan concentration 2½ hours after administration of 20 mg dextromethorphan to 12 healthy volunteers was 0.0018 ± 0.0022 mg/L when measured as the free dextromethorphan base by gas chromatography.[28] In 181 neurosurgical patients receiving oral dextromethorphan doses ranging from 0.8–9.64 mg/kg (400 mg), the maximum serum dextromethorphan and dextrorphan concentrations were 1.514 mg/L and 0.501 mg/L, respectively. Adverse effects were mild.[47] Figure 29.3 demonstrates the mean plasma concentration of the active metabolite, dextrorphan after the ingestion of 60 mg dextromethorphan in 3 Finnish pharmaceutic cough preparations. In comparison, the mean peak plasma concentrations of the parent compound, dextromethorphan in this study ranged from about 0.002–0.008 mg/L with a mean concentration of approximately 0.0042–0.005 mg/L. Brain dextromethorphan concentrations were up to 68-fold higher than serum dextromethorphan concentrations. In a patient with classic signs of serotonin syndrome following the ingestion of 480 mg dextromethorphan and 64 mg chlorpheniramine, the serum dextromethorphan concentration in an admission blood sample was 0.93 mg/L.[58]

POSTMORTEM. In a case series of 6 infant deaths with detectable dextromethorphan concentrations in postmortem blood, the dextromethorphan concentrations ranged from 0.10–0.95 mg/L.[75] There were insufficient data to separate therapeutic and potentially fatal dextromethorphan concentrations in these cases. In a case series of 8 fatalities involving dextromethorphan, the postmortem dextromethorphan concentration ranged from 7.4–33.55 mg/L.[76] However, the case series did not report specific information on the presence of other drugs or clinical histories. The heart blood of 2 teenagers found dead after abusing dextromethorphan contained dextromethorphan concentrations of 1.89 ng/mL and 3.23 mg/L.[45] One of these teenagers had a therapeutic concentration of diphenhydramine in his postmortem

Dextrorphan concentrations in plasma
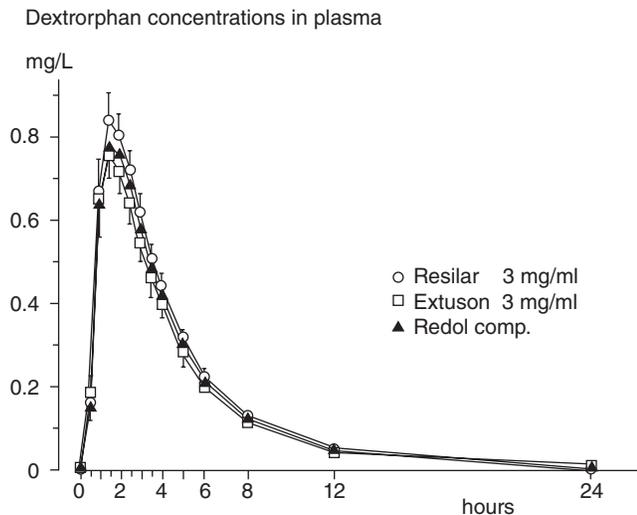
FIGURE 29.3. Dextrorphan concentrations after ingestion of 3 Finnish cough preparations containing 60 mg dextromethorphan. (Reprinted from M Silvasti, P Karttunen, H Tukiainen, P Kokkonen, U Hänninen, S Nykänen, Pharmacokinetics of dextromethorphan and dextrorphan: a single dose comparison of three preparations in human volunteers, International Journal of Clinical Pharmacology and Therapeutic Toxicology, Vol. 25, p. 495, copyright 1987.)

blood. Femoral blood samples from the postmortem examination of an 18-year-old girl found dead in a lavatory with dextromethorphan in her possession demonstrated dextromethorphan and dextrorphan concentrations of 9.2 mg/L (9.2 μg/g) and 2.9 mg/L (2.9 μg/g), respectively, resulting in a dextromethorphan/dextrorphan ratio of 3.17.[77] She attempted suicide 2 weeks prior to this event and she left a suicide note at that time. In the same report, the dextromethorphan/dextrorphan ratio in femoral blood from a 27-year-old man found dead in his bed was 2.20.

A 19-year-old was found unresponsive after abusing dextromethorphan; he was pronounced dead by paramedics after unsuccessful cardiopulmonary resuscitation. His postmortem iliac blood and vitreous humor contained dextromethorphan concentrations of 1.3 mg/L and 0.7 mg/L, respectively (iliac blood/vitreous ratio, 1.86).

URINE

Routine urine toxicology immunoassays do not typically detect the therapeutic use of dextromethorphan as a result of the low cross-reactivity of dextromethorphan and dextrorphan with opioid and phencyclidine immunoassays; detection of dextromethorphan use by ELISA requires specific immunoassays for dextromethorphan and dextrorphan.[78] The ingestion of single, 20 mg, and

40 mg doses of dextromethorphan did not produce positive results in urine samples from 20 adults, when tested 6 hours after ingestion by the EMIT® opioid screen (Syva Company, San Jose, CA).[79] Occasionally, the ingestion of high doses of dextromethorphan produce false-positive results on urine screens for phencyclidine by immunoassay.[80,81] Confirmation of the presence of dextromethorphan in biologic samples requires analysis by more-specific methods (e.g., GC/MS).

Abnormalities

Mild elevation of serum bromide concentrations may or may not occur following the chronic ingestion of large doses of dextromethorphan hydrobromide-containing formulations.[49] Standard clinical laboratory analytic tests do not distinguish between chloride and bromide. Therefore, the ingestion of bromide produces a false elevation in the serum chloride concentrations as well as a decreased anion gap. A common biomarker for bromide toxicity is a negative anion gap resulting from pseudo-elevation of the serum chloride used to calculate the anion gap. For example, Coricidin HBP Cough & Cold tablets contain 6.9 mg bromide, and the ingestion of 8–16 tablets results in the ingestion of 55–110 mg bromide. The acceptable daily intake of bromide is about 1 mg/kg body weight; consequently, the occasional ingestion of this dose of dextromethorphan hydrobromide probably would not produce chronic bromism. The bromide concentration in a serum sample from a 39-year-old man with a manic state and a history of frequent consumption of 1,500 mg dextromethorphan hydrobromide-containing bottles of cough syrup was 3.4 mEq/L.[49] The serum bromide concentration in a 56-year-old man with chronic symptoms of bromism (lethargy, weakness, alteration of consciousness) was 4.0 mEq/L. Analysis of serum chemistry demonstrated a spurious elevation of chloride and a decreased anion gap. He had consumed 60–120 mL of a cold preparation containing dextromethorphan bromide daily for 4–5 years prior to admission to the hospital for loss of consciousness.[82] A rare case report suggests that dextromethorphan overdose may cause QTc prolongation. A 27-year-old man developed QTc prolongation (i.e., 514 msec after potassium repletion) after ingesting 1,920 mg dextromethorphan and ethanol.[83] His previous QTc following an ethanol binge (no dextromethorphan) was 432 msec.

Driving

In a study of 13,439 blood samples submitted to the Wisconsin State Laboratory of Hygiene from drivers suspected of impairment, blood samples from 108

drivers were positive for dextromethorphan.[84] The mean dextromethorphan concentration was 0.207 mg/L (median = 0.051 mg/L) with a range of 0.005–1.8 mg/L. Although evaluation by drug recognition experts indicated that these drivers had poor psychomotor performance on standardized field sobriety tests and horizontal gaze nystagmus, 96% of these samples contained other drugs. In a case series of 5 known dextromethorphan abusers arrested for driving while impaired, the median dextromethorphan concentration was 0.79 mg/L (range, 0.47–1.22 mg/L).[85] Blood samples from most of these drivers contained other drugs including chlorpheniramine.

TREATMENT

Stabilization

The treatment of dextromethorphan intoxication is supportive. Patients with altered consciousness require intravenous (IV) access, pulse oximetry, and rapid evaluation of blood glucose with the administration of intravenous glucose as necessary. The treatment of agitation involves the IV use of benzodiazepines (lorazepam or diazepam). Hypertension and tachycardia usually respond to sedation, reassurance, and a quiet environment. The presence of clinically significant hyperthermia along with hypertonicity suggests the serotonin syndrome as a result of the interaction of monoamine oxidase inhibitors with dextromethorphan. Decontamination measures include the use of activated charcoal within 1–2 hours of ingestion. If oral activated charcoal is administered, the patient should be observed closely for any alteration of consciousness that would predispose the patient to aspiration. Although some patients with altered consciousness following dextromethorphan ingestion respond to naloxone,[86] the clinical response to naloxone is inconsistent. Similarly, the response of acute dystonic reactions to diphenhydramine is also variable during dextromethorphan intoxication, but some case reports document the resolution of extrapyramidal symptoms after administration of IV diphenhydramine.[56]

Supplemental Care

Most patients with minor acute ingestions of dextromethorphan can be released from the emergency department after 4–6 hours of observation as long as the clinical effects have resolved. In a child developing a dystonic reaction following the ingestion of 38 mg dextromethorphan/kg, the administration of diphenhydramine was associated with resolution of opisthotonus, but not ataxia and nystagmus.[56] The administration

of naloxone did not alter the course of the dystonic reaction. Laboratory assessment of patients with moderate to severe dextromethorphan intoxication includes serum electrolytes, acid-base status, serum creatine kinase, urine myoglobin, serum hepatorenal function, urinalysis, and urine drug of abuse screen if required by the clinical presentation. Many dextromethorphan-containing formulations contain acetaminophen or salicylates. Serum acetaminophen and/or serum salicylate concentrations should be analyzed if the product may contain these drugs or if the patient ingested the drug with suicidal intent. Interpretation of the serum acetaminophen and salicylate concentrations requires a history of the time and chronicity of ingestion because dextromethorphan abuse frequently involves multiple ingestions during the period just prior to presentation.

References

1. Shulgin AT. Drugs of abuse in the future. Clin Toxicol 1975;8:405–456.

2. McCarthy JP. Some less familiar drugs of abuse. Med J Aust 1971;2:1078–1081.

3. Crouch BI, Caravati EM, Booth J. Trends in child and teen non-prescription drug abuse reported to a regional poison control center. Am J Health-Syst Pharm 2004;61: 1252–1257.

4. Darboe MN, Keenan GR Jr, Richards TK. The abuse of dextromethorphan-based cough syrup: a pilot study of the community of Waynesboro, Pennsylvania. Adolescence 1996;31:633–644.

5. Baker SD, Borys DJ. A possible trend suggesting increased abuse from Coricidin exposures reported to the Texas Poison Network: comparing 1998 to 1999. Vet Hum Toxicol 2002;44:169–171.

6. Isbell H, Fraser HF. Actions and addiction liabilities of Dromoran derivatives in man. J Pharmacol Ther 1953; 107:524–530.

7. Karttunen P, Tukiainen H, Silvasti M, Kolonen S. Antitussive effect of dextromethorphan and dextromethorphan-salbutamol combination in healthy volunteers with artificially induced cough. Respiration 1987;52:49–53.

8. Jasinski DR. Abuse potential of morphine/dextromethorphan combinations. J Pain Symptom Manag 2000;19(suppl):S26–S30.

9. Banken JA, Foster H. Dextromethorphan an emerging drug of abuse. Ann NY Acad Sci 2008;1139:402–411.

10. Bryner JK, Wang UK, Hui JW, Bedodo M, MacDougall C, Anderson IB. Dextromethorphan abuse in adolescence an increasing trend: 1999–2004. Arch Pediatr Adolesc Med 2006;160:1217–1222.

11. Office of Applied Studies, Substance Abuse and Mental Health Services Administration. (2008a, January 10).

Misuse of over-the-counter cough and cold medications among persons aged 12 to 25. The NSDUH Report. Available at http://oas.samhsa.gov/2k8/cough/cough.pdf. Accessed 2011 March 3.

12. Yoo Y, Chung H, Kim E, Kim N. Fatal zipeprol and dextromethorphan poisonings in Korea. J Anal Toxicol 1996; 20:155–158.

13. Bisaga A, Popik P. In search of a new pharmacological treatment for drug and alcohol addiction: N-methyl-D-aspartate (NMDA) antagonists. Drug Alcohol Depend 2000;59:1–15.

14. Schwartz RH. Adolescent abuse of dextromethorphan. Clin Pediatr 2005;44:565–568.

15. Hendrickson RG, Cloutier RL. "Crystal Dex:" free-base dextromethorphan. J Emerg Med 2007;32:393–396.

16. Fleming PM. Dependence on dextromethorphan hydrobromide. Br Med J (Clin Res Ed) 1986;293(6547):597.

17. Boyer EW. Dextromethorphan abuse. Pediatr Emerg Care 2004;20:858–863.

18. Murray S, Brewerton T. Abuse of over-the-counter dextromethorphan by teenagers. South Med J 1993; 86:1151–1153.

19. Miller SC. Dextromethorphan psychosis, dependence and physical withdrawal. Addict Biol 2005;10:325–327.

20. Wolfe TR, Caravati EM. Massive dextromethorphan ingestion and abuse. J Emerg Med 1995;13:174–176.

21. Dickerson DL, Schaepper MA, Peterson MD, Ashworth MD. Coricidin HBP abuse: patient characteristics and psychiatric manifestations as recorded in an inpatient psychiatric unit. J Addict Dis 2008;27:25–32.

22. Baumann P, Vlatkovic D, Macciardi F. Intoxication with dextromethorphan in an adolescent with genetic cytochrome P450 CYP2D6 deficiency. Therapie 1997;52: 607–614.

23. Taylor JA, Novack AH, Almquist JR, Rogers JE. Efficacy of cough suppressants in children. J Pediatr 1993;122: 799–802.

24. Soyka M, Bondy B, Eisenburg B, Schutz CG. NMDA receptor challenge with dextromethorphan-subjective response, neuroendocrinological findings and possible clinical implications. J Neural Transm 2000;107:701–714.

25. Cornish JW, Herman BH, Ehrman RN, Robbins SJ, Childress AR, Bead V, et al. A randomized, double-blind, placebo-controlled safety study of high-dose dextromethorphan in methadone-maintained male inpatients. Drug Alcohol Depend 2002;67:177–183.

26. Chyka PA, Erdman AR, Manoguerra AS, Christianson G, Booze LL, Nelson LS, et al. Dextromethorphan poisoning: an evidence-based consensus guideline for out-of-hospital management. Clin Toxicol 2007;45:662–677.

27. Silvasti M, Karttuen P, Tukiainen H, Kokkonen P, Hanninen U, Nykanen S. Pharmacokinetics of dextromethorphan and dextrorphan: a single dose comparison of three preparations in human volunteers. Int J Clin Pharmacol Ther Toxicol 1987;25:493–497.

28. Liu D, Chen XY, Zhang YF, Zhong DF, Gu Q, Zhang Y. [Determination of dextrorphan in human plasma and pharmacokinetic study]. Yao Xue Xue Bao 2004;39:449–452. [Chinese]

29. Barnhart JW, Massad EN. Determination of dextromethorphan in serum by gas chromatography. J Chromatogr 1979;163:390–395.

30. Koppel C, Tenczer J, Ibe K. Urinary metabolism of dextromethorphan in man. Arzneimittelforschung 1987;37: 1304–1306.

31. Wong BY, Coulter DA, Choi DW, Prince DA. Dextrorphan and dextromethorphan, common antitussives, are antiepileptic and antagonize N-methyl-D-aspartate in brain slices. Neurosci Lett 1988;85:261–266.

32. Schadel M, Wu D, Otton SV, Kalow W, Sellers EM. Pharmacokinetics of dextromethorphan and metabolites in humans: influence of the CYP2D6 phenotype and quinidine inhibition. J Clin Psychopharmacol 1995;15:263–269.

33. Schmid B, Bircher J, Preisig R, Kupfer A. Polymorphic dextromethorphan metabolism: Co-segregation of oxidative O-demethylation with debrisoquin hydroxylation. Clin Pharmacol Ther 1985;38:618–624.

34. Takashima T, Murase S, Iwasaki K, Shimada K. Evaluation of dextromethorphan metabolism using hepatocytes from CYP2D6 poor and extensive metabolizers. Drug Metab Pharmacokinet 2005;20:177–182.

35. Shamsie SJ, Barriga C. The hazards of use of monoamine oxidase inhibitors in disturbed adolescents. Can Med Assoc J 1971;104:715.

36. Harrison WM, McGrath PJ, Stewart JW, Quitkin F. MAOIs and hypertensive crises: the role of OTC drugs. J Clin Psychiatry 1989;50:64–65.

37. Navarro A, Perry C, Bobo WV. A case of serotonin syndrome precipitated by abuse of the anticough remedy dextromethorphan in a bipolar patient treated with fluoxetine and lithium. Gen Hosp Psychiatry 2006;28:78–80.

38. Gillman PK. Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. Br J Anaesth 2005;95: 434–441.

39. Lotrich FE, Rosen J, Pollock BG. Dextromethorphan-induced delirium and possible methadone interaction. Am J Geriatr Pharmacother 2005;3:17–20.

40. Forget P, le Polain de Waroux B, Wallemaca P, Gala J-L. Life-threatening dextromethorphan intoxication associated with interaction with amitriptyline in a poor CYP2D6 metabolizer: a single case re-exposure study. J Pain Symptom Manage 2008;36:92–96.

41. Szekely JI, Sharpe LG, Jaffe JH. Dextromethorphan inhibits but dextrorphan potentiates behavior induced by PCP and ketamine in rats. NIDA Res Monogr 1991;105: 341–344.

42. Elliott K, Kest B, Man A, Kao B, Inturrisi CE. N-methyl-D-aspartate (NMDA) receptors, mu and kappa opioid tolerance, and perspectives on new analgesic drug development. Neuropsychopharmacology 1995;13:347–356.

43. Kim HS, Park IS, Lim HK, Choi HS. NMDA receptor antagonists enhance 5-HT2 receptor-mediated behavior, head-twitch response, in PCPA-treated mice. Arch Pharm Res 1999;22:113–118.

44. Zawertailo LA, Kaplan HL, Busto, UE, Tyndale RF, Sellers, EM. Psychotropic effects of dextromethorphan are altered by the CYP2D6 polymorphism: a pilot study. J Clin Psychopharmacol 1998;18:332–337.

45. Logan BK, Goldfogel G, Hamilton R, Kuhlman J. Five deaths resulting from abuse of dextromethorphan sold over the internet. J Anal Toxicol 2009;33:99–103.

46. Bem JL, Peck R. Dextromethorphan an overview of safety issues. Drug Saf 1992;7:190–199.

47. Steinberg GK, Bell TE, Yenari MA. Dose escalation safety and tolerance study of the N-methyl-D-aspartate antagonist dextromethorphan in neurosurgery patients. J Neurosurg 1996;84:860–866.

48. Hung YM. Bromide intoxication by the combination of bromide-containing over-the-counter drug and dextromethorphan hydrobromide. Hum Exp Toxicol 2003;22: 459–461.

49. Hinsberger A, Sharma V, Mazmanian D. Cognitive deterioration from long-term abuse of dextromethorphan: a case report. J Psychiatr Neurosci 1994;19:375–377.

50. Navarro A, Perry C, Bobo WV. A case of serotonin syndrome precipitated by abuse of the anti-cough remedy dextromethorphan in a bipolar patient treated with fluoxetine and lithium. Gen Hosp Psychiatry 2006; 28:78–80.

51. Monte AA, Chuang R, Bodmer M. Dextromethorphan, chlorphenamine and serotonin toxicity: case report and systematic literature review. Br J Clin Pharmacol 2010; 70:794–798.

52. Schadel M, Sellers EM. Psychosis with Vicks Formula 44-D abuse. CMAJ 1992;147:843–844.

53. Schwartz AR, Pizon AF, Brooks DE. Dextromethorphan-induced serotonin syndrome. Clin Toxicol 2008;46: 771–773.

54. LoVecchio F, Pizon A, Riley B, Matesick L, O'Patry S. Accidental dextromethorphan ingestion in children less than 5 years old. J Med Toxicol 2008;4:251–253.

55. Pender Es, Parks BR. Toxicity with dextromethorphan-containing preparations: a literature review and report of two additional cases. Pediatr Emerg Care 1991;7: 163–165.

56. Warden CR, Diekema DS, Robertson WO. Dystonic reaction associated with dextromethorphan ingestion in a toddler. Pediatr Emerg Care 1997;13:214–215.

57. Roberge RJ, Hirani KH, Rowland PL 3rd, Berkeley R, Krenzelok EP. Dextromethorphan- and pseudoephedrine-induced agitated psychosis and ataxia: case report. J Emerg Med 1999;17:285–288.

58. Ganetsky M, Babu KM, Boyer EW. Serotonin syndrome in dextromethorphan ingestion responsive to propofol therapy. Pediatr Emerg Care 2007;23:829–831.

59. Ziaee V, Hamed EA, Hoshmand A, Amini H, Kebriaeizadeh A, Saman K. Side effects of dextromethorphan abuse, a case series. Addict Behav 2005;30;1607–1613.

60. Isbell H, Fraser HF. Actions and addiction liabilities of dromoran derivatives in man. J Pharmacol Exp Ther 1953;107:524–530.

61. Mutschler J, Koopmann A, Grosshans M, Hermann D, Mann K, Kiefer F. Dextromethorphan withdrawal and dependence syndrome. Dtsch Arztebl Int 2010;107: 537–540.

62. Einarson A, Lyszkiewicz D, Koren G. The safety of dextromethorphan in pregnancy results of a controlled study. Chest 2001;119:466–469.

63. Aselton P, Jick H, Milunsky A, Hunter JR, Stergachis A. First-trimester drug use and congenital disorders. Obstet Gynecol 1985;65:451–455.

64. Bendriss EK, Markoglou N, Wainer IW. High-performance liquid chromatography assay for simultaneous determination of dextromethorphan and its main metabolites in urine and in microsomal preparations. J Chromatogr B Biomed Sci Appl 2001;754:209–215.

65. Park YH, Kullberg MP, Hinsvark ON. Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high-performance liquid chromatography. J Pharm Sci 1984;73:24–29.

66. Wu YJ, Cheng YY, Zeng S, Ma MM. Determination of dextromethorphan and its metabolite dextrorphan in human urine by capillary gas chromatography without derivatization. J Chromatogr B Analyt Technol Biomed Life Sci 2003;784:219–224.

67. Baumann P, Jonzier-Perey M. GC and GC-MS procedures for simultaneous phenotyping with dextromethorphan and mephenytoin. Clin Chim Acta 1988;171:211–222.

68. Constanzer ML, Chavez-Eng CM, Fu I, Woolf EJ, Matuszewski BK. Determination of dextromethorphan and its metabolite dextrorphan in human urine using high performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry: a study of selectivity of a tandem mass spectrometric assay. J Chromatogr B Analyt Technol Biomed Life Sci 2005;816:297–308.

69. Loos WJ, de Graan A-J, de Bruijn P, van Schaik RH, van Fessem MA, Lam M-H, et al. Simultaneous quantification of dextromethorphan and its metabolites dextrorphan, 3-methoxymorphinan and 3-hydroxymorphinan in human plasma by ultra performance liquid chromatography/ tandem triple-quadrupole mass spectrometry. J Pharm Biomed Anal 2011;54:387–394.

70. Zaugg S, Thormann W. Enantioselective determination of drugs in body fluids by capillary electrophoresis. J Chromatogr A 2000;875:27–41.

71. Rodrigues WC, Wang G, Moore C, Agrawal A, Vincent MJ, Soares JR. Development and validation of ELISA and GC-MS procedures for the quantification of dextromethorphan and its main metabolite dextrorphan in urine and oral fluid. J Anal Toxicol 2008;32:220–226.

72. Liang X, Li Y, Barfield M, Ji QC. Study of dried blood spots technique for the determination of dextromethorphan and its metabolite dextrorphan in human whole blood by LC-MS/MS. J Chromatogr B 2009;877:799–806.

73. Louhaichi MR, Jebali S, Loueslati MH, Adhoum N, Monser L. Simultaneous determination of pseudoephedrine, pheniramine, guaifenesin, pyrilamine, chlorpheniramine and dextromethorphan in cough and cold medicines by high performance liquid chromatography. Talanta 2009; 78:991–997.

74. Witherow LE, Houston JB. Sigmoidal kinetics of CYP3A substrates: an approach for scaling dextromethorphan metabolism in hepatic microsomes and isolated hepatocytes to predict *in vivo* clearance in rat. J Pharmacol Exp Ther 1999;290:58–65.

75. Hanzlick R. National Association of medical examiners pediatric toxicology (PedTox) registry report 3. Am J Forensic Med Pathol 1995;16:270–277.

76. Chung H, Park M, Hahn E, Choi H, Choi H, Lim M: Recent trends of drug abuse and drug-associated deaths in Korea. Ann NY Acad Sci 2004;1025:458-464.

77. Rammer L, Holmgren P, Sandler H. Fatal intoxication by dextromethorphan: a report on two cases. Forensic Sci Int 1988;37:233–236.

78. Farrell LJ, Kerrigan S, Logan BK. Recommendations for toxicological investigation of drug impaired driving. J Forensic Sci 2007;52:1214–1218.

79. Storrow AB, Magoon MR, Norton J. The dextromethorphan defense: dextromethorphan and the opioid screen. Acad Emerg Med 1995;2:791–794.

80. Pugach S, Pugach IZ. Overdose in infant caused by over-the-counter cough medicine. South Med J 2009; 102:440–442.

81. Schier J. Avoid unfavorable consequences: dextromethorphan can bring about a false-positive phencyclidine urine drug screen. J Emerg Med 2000;18:379–381.

82. Ng Y-Y, Lin W-L, Chen T-W, Lin B-C, Tsai S-H, Chang C-C, Huang T-P. Spurious hyperchloremia and decreased anion gap in a patient with dextromethorphan bromide. Am J Nephrol 1992;12:268–270.

83. Kaplan B, Buchanan J, Krantz MJ. QTc prolongation due to dextromethorphan. Int J Cardiol 2011;148: 363–364.

84. Cochems A, Harding P, Liddicoat L. Dextromethorphan in Wisconsin drivers. J Anal Toxicol 2007;31:227–232.

85. Logan BK. Combined dextromethorphan and chlorpheniramine intoxication in impaired drivers. J Forensic Sci 2009;33:54:1176–1180.

86. Shaul WL, Wandell M, Robertson WO. Dextromethorphan toxicity: reversal by naloxone. Pediatrics 1977;59: 117–118.

Chapter 30

FENTANYL ANALOGUES

HISTORY

Paul Janssen and his coworkers synthesized fentanyl in 1960 during their search for analgesics more potent than morphine and meperidine.[1] In 1968, fentanyl was the first of the 4-anilinopiperidine series of opioid agonists introduced into the United States as a synthetic intravenous (IV) anesthetic/analgesic under the trade name Sublimaze® (fentanyl, Janssen-Cilag Ltd, Australia) and Innovar® (fentanyl and droperidol, Janssen-Cilag USA, Titusville, NJ). The latter combination has been discontinued. The rapid onset, short duration of action, and high clinical potency (i.e., about 50–100 times more potent than morphine) led to the acceptance of fentanyl as a commonly used drug for preanesthetic sedation, postsurgical analgesia, and conscious sedation procedures. Fentanyl produces clinical effects (e.g., miosis, bradycardia, respiratory depression, and euphoria) similar to natural opiates. Riley et al. synthesized 3-methylfentanyl from 4-anilino-3-methylpyridine in 1973. In 1979, a powerful, synthetic drug called China White appeared in the illicit drug market; this drug was associated with sudden respiratory arrest and death in opioid addicts.[2] After extensive analysis, the US Department of Justice Special Testing and Research Laboratory identified the drug as the fentanyl derivative, α-methylfentanyl.[3] Later, even more powerful designer opioids appeared in the illicit drug market (e.g., *p*-fluoro fentanyl, 3-methyl fentanyl), which are about 2,000 times more potent than morphine. The illicit use of 3-methyl fentanyl caused at least 100 deaths in California and Pennsylvania during the 1980s.[4,5] Fentanyl derivatives were also involved in the illegitimate administration of doping agents to race horses.[6] Epidemics of fatal 3-methylfentanyl poisonings continue in the Russian Federation, Ukraine, and Estonia.[7]

IDENTIFYING CHARACTERISTICS

Fentanyl and fentanyl derivatives are synthetic opioid analgesics that bind primarily to μ-opioid receptors;[8] there are over 200 fentanyl derivatives.[9] Figure 30.1 displays the chemical structures of fentanyl and common fentanyl analogues. 3-Methyl fentanyl [*N*-(3-methyl-1-(2-phenylethyl)-4-piperidinyl)-*N*-phenylpropanamide, CAS RN: 42045-86-3] is a highly lipid soluble, tertiary amine with a molecular formula of $C_{23}H_{30}N_2O$. The high potency of fentanyl analogues results from the rapid diffusion of these compounds into the brain as well as the high affinity of these drugs for μ-opioid receptors when compared with morphine. There are substantial differences in the potency of the stereoisomers of these drugs. The relative potency of *cis*-(+)-isomer and *trans*-(±)-isomer of 3-methylfentanyl to morphine is approximately 7,000- and 1,000-fold, respectively, based on the tail withdrawal test in rats.[10] Radioreceptor assays indicated that *cis*-3-methylfentanyl is the active isomer of this compound with high affinity ($K_i = 0.24$ nM) for the μ-opioid receptor.[11]

Terminology

China White refers to 3-methyl fentanyl and α-methyl fentanyl as well as to a very pure form of white heroin, often from Southeast Asia. Other street names for these drugs include China Girl, Persian White, Egg White, Crocodile, Dragon, 999, and Synthetic Heroin.
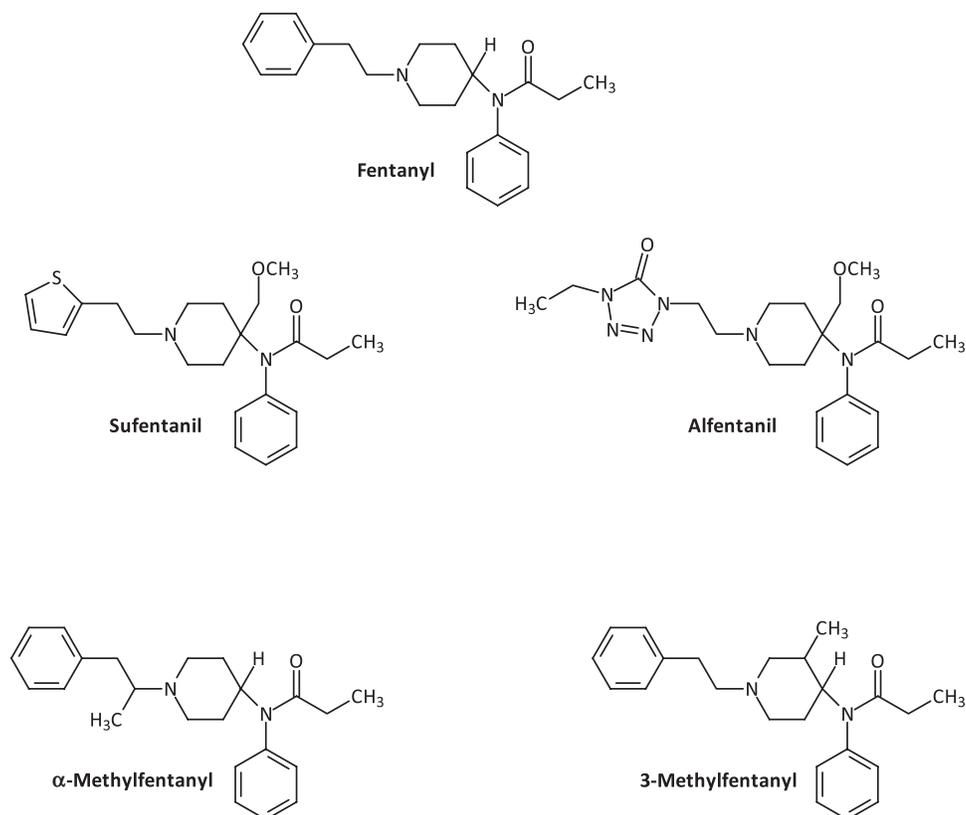
FIGURE 30.1. Chemical structures of fentanyl and related compounds.

Form

The physical appearance of fentanyl and fentanyl analogues is similar to some types of heroin; the range of colors of heroin range from pure white (Persian White) and off white (China White) to light tan (Synthetic Heroin) and light brown (Mexican Brown). The cost and packaging of fentanyl analogues and light-colored heroin are also similar. Fentanyl derivatives mix poorly with diluents (e.g., lactose) and adulterants, resulting in potentially dangerous, nonuniform distribution of the active drug.[12]

EXPOSURE

Epidemiology

Retrospective analysis of data from the Drug Abuse Warning Network (DAWN) suggests that opioid analgesics are a small part of drug abuse despite the increasing medicinal use of opioids (including fentanyl).[13] Occasional fatalities result from the abuse of fentanyl both by IV injection and insufflation,[14] as well as fatalities from the use of 3-methyl fentanyl as an additive in heroin and/or cocaine during sporadic epidemics in California and Pennsylvania.[15,16] Difficulty diluting this potent drug and the general preference of the opioid-dependent population for heroin accounts for the intermittent use of cis-3-methylfentanyl.[17]

Sources

Illicitly synthesized fentanyl analogues for street use include α-methyl fentanyl, p-fluorofentanyl (CAS RN: 90736-23-5, 4-fluorofentanyl), 3-methyl fentanyl, α-methyl acetylfentanyl, acryl α-methylfentanyl, benzylfentanyl (CAS RN: 1474-02-8), and α-methyl-β-hydroxylfentanyl as well as thienyl and hydroxy derivatives of 3-methylfentanyl. These derivatives are typically available through the same distribution channels as heroin. Because of the high potency of fentanyl and fentanyl analogues, dilution of these illicit drugs is difficult. As a result of the potency of fentanyl and fentanyl analogues, most samples of illicit fentanyl and fentanyl analogues analyzed by law enforcement agencies contain up to 99% lactose and other filler materials.[18] Analysis of confiscated samples of p-fluorofentanyl demonstrated large variability in the dose of this illicit analogue with the p-fluorofentanyl content ranging from 33.8–408.7 μg/tablet (0.14–

1.57%).[19] Caffeine was a common adulterant in these formulations.

Methods of Abuse

Abuse of fentanyl analogues primarily involves the IV administration of these drugs, often as a substitute for heroin and usually administered at about the same frequency as heroin. Most habitual heroin users prefer heroin to fentanyl derivatives as a result of the intense "rush" associated with IV heroin use as opposed to the longer, quieter euphoria associated with fentanyl derivatives.[20] Occasionally, fentanyl analogues are insufflated, chewed, or applied to the oral mucosa with fatal effects.

DOSE EFFECT

The sedative and analgesic dose following the IV administration of fentanyl is approximately 50–100 μg. The potency of *p*-fluorofentanyl and fentanyl is similar, but the potency of *cis*-3-methylfentanyl is substantially greater than fentanyl.[21] There are few clinical data on the dose response of illicit fentanyl analogues; in general, fatal street doses are difficult to predict. Animal studies suggest that the potencies of illicit fentanyl analogues are between the potencies of fentanyl and 3-methylfentanyl.[10]

TOXICOKINETICS

Fentanyl is a potent, short-acting opioid analgesic with a large apparent volume of distribution, extensive first-pass hepatic metabolism, a relatively short plasma elimination half-life, and extensive biotransformation. There are limited data on the toxicokinetics of fentanyl analogues, but the toxicokinetics of these compounds is probably similar (but not necessarily identical) to fentanyl.

Absorption

The usual route of fentanyl analogue exposure is by IV because extensive first-pass hepatic metabolism substantially reduces the oral bioavailability of fentanyl analogues. Following IV administration, serum fentanyl concentrations fall rapidly within 5 minutes. The substantial pulmonary absorption of fentanyl suggests that inhalation is a potential route of exposure to fentanyl analogues depending on the efficiency of the delivery system.[22] The systemic pharmacokinetic parameters of the inhaled fentanyl is similar to IV administration (including onset of action). The dermal absorption of fentanyl analogues depends on their octanol water partition coefficient, which varies among fentanyl derivatives.

Distribution

The initial distribution half-life of fentanyl is 1–2 minutes following the IV administration of high-dose (60 μg/kg) fentanyl for surgical anesthesia.[23] The short duration of action of fentanyl analogues results from redistribution from opioid receptors rather than biotransformation. The high lipophilicity of fentanyl and fentanyl analogues suggests that these drugs accumulate in adipose tissue; the volume of distribution of these drugs increase in obesity.[24]

Biotransformation

Fentanyl and illicit fentanyl analogues undergo extensive biotransformation, primarily by oxidative *N*-dealkylation to the inactive nor-metabolite. *In vitro* studies indicate that CYP 3A4 catalyzes the *N*-dealkylation of fentanyl to the nor-metabolite.[25,26] Minor pathways include amide hydrolysis to the despropionyl metabolite and hydroxylation of the piperidine ring, the phenyl rings, or the propionyl side chain.

Elimination

Metabolism accounts for over 90% of the elimination of fentanyl with most studies indicating that the kidney excretes <4–10% of the absorbed therapeutic dose of fentanyl as unchanged drug.[27]

Maternal and Fetal Kinetics

The high lipid solubility of fentanyl analogues suggests that these compounds easily diffuse across the placenta following IV administration to the mother.

Tolerance

There are few clinical data on tolerance to fentanyl analogues. Like heroin, tolerance develops to some, but not all, of the clinical effects of opioids including (respiratory depression, nausea, analgesia, and sedation).

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Although fentanyl derivatives are structurally unrelated to opioids, these drugs exhibit similar pharmacologic and toxicologic properties including a high affinity for the μ-opioid receptor. Binding to μ-opioid receptors causes analgesia, sedation, euphoria, respiratory depression, and hypotension as well as tolerance and physical

withdrawal. *In vitro* studies suggest that μ-, κ-, and δ-opioid receptor selectivity between fentanyl and fentanyl analogues vary. A study in cloned human opioid receptors demonstrated that μ- and δ-opioid receptor selectivities of fentanyl and *p*-fluorofentanyl were similar, but κ-opioid selectivity was greater for *p*-fluorofentanyl than fentanyl.[28]

Postmortem Examination

Postmortem examination of fatalities following overdoses of fentanyl analogues are nondiagnostic (e.g., visceral congestion, pulmonary edema), except for the presence of stigmata of IV drug use (recent puncture wounds, chronic active hepatitis, human immunodeficiency virus [HIV], adenopathy).[29] Death following fentanyl injection occurs rapidly as reflected in the lower postmortem lung weights compared with fatal heroin overdose.[30] Bronchopneumonia and aspiration of gastric contents are uncommon postmortem findings.

CLINICAL RESPONSE

Overdose

Fentanyl analogues produce typical symptoms of opioid overdose manifest by respiratory depression, bradycardia, hypotension, nausea, vomiting, and hypothermia. Seizures occur rarely during fentanyl intoxication, typically resulting from hypoxia rather than drug effect.[31] Death results from respiratory depression and subsequent cardiopulmonary arrest. Frequently, the use of illicit fentanyl analogues produces a very rapid death, and the victim may be found with the needle still in his or her arm. Most fatalities occur in known IV drug abusers, and most of these individuals are polydrug users (e.g., morphine, cocaine, diazepam, amphetamine, codeine, methadone).

Abstinence Syndrome

Tolerance and physical dependence following the habitual use of fentanyl, fentanyl analogues, and opioids are clinically similar. Like other opioids, fentanyl analogues cause downregulation of opioid receptors following prolonged exposure. This property produces tolerance and reduction in the inhibitory pathways during withdrawal.

DIAGNOSTIC TESTING

Analytic Methods

The most common methods for the detection of fentanyl and fentanyl derivatives are radioimmuno-assay, gas chromatography, liquid chromatography/atmospheric pressure ionization/tandem mass spectrometry (lower limit of detection [LLOQ], 0.003–0.027 mg/L),[32] and gas chromatography/mass spectrometry (GC/MS).[21] The latter method can detect nor-metabolites of fentanyl and fentanyl analogues with a sensitivity of 2 ng/mL.[33] Liquid chromatography/tandem mass spectrometry allows the quantitation of 9 fentanyl compounds and 16 opioids commonly used medicinally and abused by drug addicts. This method has LLOQ in the range of approximately 0.1–2 ng/mL. The limit of detection (LOD) for *cis*-3-methylfentanyl using liquid chromatography/tandem mass spectrometry is approximately 0.1 ng/mL.[34] Using liquid chromatography/tandem mass spectrometry with electrospray ionization in positive ionization mode, the LLOQ for fentanyl, norfentanyl, and 3-methylfentanyl is about 0.1 ng/mL.[35] The use of liquid chromatography on a reversed-phase column and a gradient consisting of acetonitrile and ammonium acetate at pH 3.2 along with tandem mass spectrometry (quadrupole/linear ion/trap mass spectrometer with a turbo ion spray interface in positive mode using multiple reaction monitoring) allows the detection of illegal fentanyl analogs.[36] Using this technique, the LLOQ for *cis*- and *trans*-3-methylfentanyl, *α*-methylfentanyl, and *p*-fluorofentanyl is approximately 0.1 ng/mL with a bias of 2–5%. Isolation of fentanyl from biologic matrices involves liquid/liquid and/or solid-phase extraction with ethyl acetate, *n*-butyl chloride or heptane/isoamyl as solvents.[8] Analysis of a postmortem blood sample stored frozen for 16 months after the death of a 28-year-old man from a opioid overdose demonstrated the presence of 3.1 ng/mL α-methylfentanyl.[37]

Biomarkers

The presence of small amounts of fentanyl analogues in the stomach does not necessarily imply ingestion of these drugs because of the excretion of these substances into the acid milieu of the stomach. The aqueous solubility of fentanyl increases exponentially with decreasing pH.

Blood

Fatalities from illicit or surreptitious fentanyl analogue use typically involves younger drug addicts dying at their home with multiple drugs (e.g., benzodiazepines, methadone, morphine or 6-acetyl morphine, oxycodone) present in postmortem blood, when compared with deaths from therapeutic fentanyl use.[38] Tolerance, postmortem redistribution, interindividual variation in response, and the presence of multiple drugs complicate

the interpretation of postmortem fentanyl concentrations. Limited data on the postmortem redistribution of fentanyl analogues suggest that postmortem redistribution is variable, but likely significant based on postmortem data on fentanyl.[39]

Urine

Routine urine drug screens do not typically detect fentanyl and fentanyl analogues. The poor cross-reactivity between fentanyl analogues and opioid antigens in urine drug screens results from the lack of structural similarity, low urinary fentanyl concentrations, and the absence of morphine as a fentanyl analogue metabolite. The concentration of the nor-metabolites of fentanyl analogues may be higher in urine samples than the parent drug. The cross-reactivity of fentanyl analogues with immunoassays designed to detect fentanyl and norfentanyl is variable; the lower limit of detection ranges from 0.25–0.5 ng/mL.[40]

TREATMENT

Stabilization

The classic presentation of fentanyl analogue intoxication is similar to heroin overdose including evidence of IV drug use and altered consciousness along with depressed respiratory rate and miotic pupils. Abuse of fentanyl analogues also occurs by insufflation and inhalation. The onset of symptoms after fentanyl analogue overdose is usually more rapid than heroin overdose. Although miosis develops during acute fentanyl analogue intoxication, the presence of severe hypoxia and metabolic acidosis may alter the pupil size. Initial treatment involves the assessment of ventilation and perfusion. Patients with adequate ventilation and oxygen saturations above 91–92% can be observed with cardiorespiratory monitoring until a normal level of consciousness returns. Patients with inadequate ventilation require bag-value-mask ventilation with 100% oxygen and the administration of naloxone 0.2-0.4 mg IV. Although the rate of subcutaneous absorption of naloxone is slower than the IV route, difficulty obtaining IV access may result in similar onset of action via these 2 routes of administration, at least in the prehospital setting.[41] Endotracheal administration of naloxone is an option to parenteral use of this antidote.[42]

If no improvement occurs within 3–5 minutes, additional doses (1–2 mg) of naloxone should be administered parenterally. Criteria for endotracheal intubation include inability to ventilate with bag-valve-mask, poor oxygenation (i.e., oxygen saturation <90%) despite adequate ventilation and supplemental oxygen, and persistent hypoventilation after doses of naloxone exceeding 2–4 mg. The treatment of overdoses of fentanyl analogues requires the administration of naloxone doses sufficient to reverse respiratory depression. High doses of naloxone may be necessary to reverse large overdoses of the potent opioid receptor agonist action of fentanyl derivatives, but all overdoses of fentanyl analogues respond to adequate doses of naloxone when administered before the onset of irreversible damage from respiratory depression and hypoxia.[4] Lack of response to an adequate dose of naloxone indicates hypoxic brain damage or a diagnosis other than opioid intoxication (e.g., head trauma, sepsis, meningitis, aspiration pneumonia). If pulmonary signs or symptoms (e.g., cough, tachypnea, pink frothy sputum, rales) develop, the patient should undergo a chest x-ray to detect radiographic findings of pulmonary edema and/ or aspiration pneumonia.

Pulmonary Edema

A majority of patients with opioid-induced pulmonary edema require only oxygen supplementation. In a case series of 27 patients with pulmonary edema after resuscitation from heroin overdose, 9 patients required mechanical ventilation with extubation usually occurring within 24 hours after intubation. However, severe hypoxia can develop following the administration of naloxone for fentanyl analogue overdose as a result of the occurrence of pulmonary edema.[43] Patients with other complications (e.g., aspiration pneumonia) in addition to pulmonary edema may require intubation including pressure-cycled ventilation for longer periods.

Hypotension

Hypotension from fentanyl analogue intoxication usually responds to fluids and naloxone.

Gut Decontamination

There are few clinical data to guide gut decontamination therapy during fentanyl analogue intoxication. Methods to prevent absorption of fentanyl are not necessary as abuse of fentanyl and fentanyl analogues is usually via injection or inhalation.

Elimination Enhancement

Methods to enhance elimination (e.g., hemodialysis) are not indicated unless renal failure and hyperkalemia complicate acute fentanyl analogue intoxication, usually as a result of rhabdomyolysis.

Antidotes

Naloxone, a pure opioid antagonist, is the antidote of choice for acute fentanyl analogue intoxication. The initial dose of naloxone usually should not exceed 0.4–0.8 mg IV because of the risk of producing withdrawal in opioid-dependent patients. Larger doses of naloxone (e.g., 2–4 mg) may then be administered if the clinical response is inadequate. After restoration of adequate ventilation, repeat doses of naloxone may be necessary for recurrent, clinically significant hypoventilation, particularly following large doses of fentanyl analogues. Fentanyl analogues are usually relatively short-acting. Mild sedation can recur after the administration of naloxone following fentanyl analogue intoxication, in part as a result of the formation of the naloxone metabolite, 6-α-naloxol, which has agonistic and antagonistic effects on opioid receptors. Although μ-opioid receptors mediate respiratory depression, stimulation of both μ- and κ-opioid receptors causes sedation. Hence, persistent κ-opioid stimulation by 6-α-naloxol can cause recurrence of sedation, but recurrence of respiratory depression does not usually occur.

Supplemental Care

Laboratory testing in patients with prolonged coma from acute fentanyl analogue intoxication include analysis of the blood for complete blood count, electrolytes, creatinine, hepatic aminotransferases, and creatine kinase. The presence of rhabdomyolysis (serum creatine kinase >1000 IU) indicates the need for generous fluid replacement and monitoring of renal function; urinary alkalinization probably should not be considered because of the lack of proven efficacy as well as the potential to increase the penetration of the central nervous system and prolong the urinary elimination of the drug.

References

1. Stanley TH. The history and development of the fentanyl series. J Pain Symptom Manag 1992;7(suppl 3):S3–S7.
2. Henderson GL. Designer drugs: past history and future prospects. J Forensic Sci 1988;33:569–575.
3. Kram TC, Cooper DA, Allen AC. Behind the identification of China White. Anal Chem 1981;53:1379A–1386A.
4. Ziporyn T. A growing industry and menace: Makeshift laboratory's designer drugs. JAMA 1986;256:3061–3063.
5. Martin M, Hecker J, Clark R, Frye J, Jehle D, Lucid EJ, et al. China white epidemic: an eastern United States emergency department experience. Ann Emerg Med 1991; 20:158–164.
6. Frincke JM, Henderson GL. The major metabolite of fentanyl in the horse. Drug Metab Dispos 1980;8:425–427.
7. Ojanpera I, Gergov M, Liiv M, Riikoja A, Vuori E. An epidemic of fatal 3-methylfentanyl poisoning in Estonia. Int J Legal Med 2008;122:395–400.
8. Poklis A. Fentanyl: a review for clinical and analytical toxicologists. Clin Toxicol 1995;33:439–447.
9. Cooper D, Jacob M, Allen A. Identification of fentanyl derivatives. J Forensic Sci 1986;31:511–528.
10. Van Bever WF, Niemegeers CJ, Janssen PA. Synthetic analgesics. Synthesis and pharmacology of the diastereo-isomers of N-(3-methyl-1-(2-phenylethyl)-4-piperidyl)-N-phenylpropanamide and N-(3-methyl-1-(1-methyl-2-phenylethyl)-4-piperidyl)-N-phenylpropanamide. J Med Chem 1974;17:1047–1051.
11. Alburges ME, Hanson GR, Gibb JW, Sakashita CO, Rollins DE. Fentanyl receptor assay II. Utilization of a radioreceptor assay for the analysis of fentanyl analogs in urine. J Anal Toxicol 1992;16:36–41.
12. Hibbs J, Perper J, Winek CL. An outbreak of designer drug-related deaths in Pennsylvania. JAMA 1991;265: 1011–1013.
13. Novak S, Nemeth WC, Lawson KA. Trends in medicinal use and abuse of sustained-release opioid analgesics: a revisit. Pain Med 2004;5:59–65.
14. Henderson GL. Blood concentrations of fentanyl and its analogs in overdose victims. Proc West Pharmacol Soc 1983;26:287–290.
15. Hibbs J, Perper J, Winek CL. An outbreak of designer drug-related deaths in Pennsylvania. JAMA 1991;265: 1011–1013.
16. Center of Disease Control. Nonpharmaceutical fentanyl-related deaths—multiple states, April 2005–March 2007. MMWR Morb Mortal Wkly Rep 2008;57:793–796.
17. Ayres WA, Starsiak MJ, Sokolay P. The bogus drug: three methyl & alpha methyl fentanyl sold as "China white." J Psychoactive Drugs 1981;13:91–93.
18. Brittain JL. China White: The bogus drug. J Toxicol Clin Toxicol 1982–1983;19:1123–1126.
19. de Boer D, Goemans W-PJ, Ghezavat VR, van Ooijen RD, Maes RA. Seizure of illicitly produced para-fluorofentanyl: quantitative analysis of the content of capsules and tablets. J Pharm Biomed Anal 2003;31:557–562.
20. LaBarbera M, Wolfe T. Characteristics, attitudes and implications of fentanyl use based on reports from self-identified fentanyl users. J Psychoactive Drugs 1983;15: 293–301.
21. Kingsbury DP, Makowski GS, Stone JA. Quantitative analysis of fentanyl in pharmaceutical preparations by gas chromatography-mass spectrometry. J Anal Toxicol 1995; 19:27–30.
22. Worsley MH, MacLeod AD, Brodie MJ, Asbury AJ, Clark C. Inhaled fentanyl as a method of analgesia. Anaesthesia 1990;45:449–451.
23. Bovill JG, Sebel PS. Pharmacokinetics of high-dose fentanyl. A study in patients undergoing cardiac surgery. Br J Anaesth 1980;52:795–801.

24. Scholz J, Steinfath M, Schulz M. Clinical pharmacokinetics of alfentanil, fentanyl and sufentanil an update. Clin Pharmacokinet 1996;31:275–292.

25. Labroo RB, Paine MF, Thummel KE, Kharasch ED. Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions. Drug Metab Dispos 1997;25:1072–1080.

26. Tateishi T, Krivoruk Y, Ueng Y-F, Wood AJ, Guengerich FP, Wood M. Identification of human liver cytochrome P-450 3A4 as the enzyme responsible for fentanyl and sufentanil *N*-dealkylation. Anesth Analg 1996;82:167–172.

27. Goromaru T, Matsuura H, Yoshimura N, Miyawaki T, Sameshima T, Miyao J, et al. Identification and quantitative determination of fentanyl metabolites in patients by gas chromatography-mass spectrometry. Anesthesiology 1984;61:73–77.

28. Ules C, van Boven M, Daenens P, Tytgat J. Interaction of *p*-fluorofentanyl on cloned human opioid receptors and exploration of the role of Trp-318 and His-319 in μ-opioid receptor selectivity. J Pharmacol Exp Ther 2000;294:1024–1033.

29. Ferslew KE, Hagardorn AN, McCormick WR. Postmortem determination of the biological distribution of sufentanil and midazolam after an acute intoxication. J Forensic Sci 1989;34:249–257.

30. Henderson GL. Fentanyl-related deaths: demographics, circumstances, and toxicology of 112 cases. J Forensic Sci 1991;36:422–433.

31. Rao TL, Mummaneni N, El-Etr AA. Convulsions: an unusual response to intravenous fentanyl administration. Anesth Analg 1982;61:1020–1021.

32. Wang L, Bernert JT. Analysis of 13 fentanils, including sufentanil and carfentanil, in human urine by liquid chromatography-atmospheric-pressure ionization-tandem mass spectrometry. J Anal Toxicol 2006;30:335–341.

33. Hammargren WR, Henderson GL. Analyzing normetabolites of the fentanyls by gas chromatography/electron capture detection. J Anal Toxicol 1988;12:183–191.

34. Ojanpera I, Gergov M, Rasanen I, Lunetta P, Toivonen S, Tiainen E, Vuori E. Blood levels of 3-methylfentanyl in 3 fatal poisoning cases. Am J Forensic Med Pathol 2006;27:328–331.

35. Cooreman S, Deprez C, Martens F, van Bocxlaer J, Croes K. A comprehensive LC-MS-based quantitative analysis of fentanyl-like drugs in plasma and urine. J Sep Sci 2010;33:2654–2662.

36. Gergov M, Nokua P, Vuori E, Ojanpera I. Simultaneous screening and quantification of 25 opioid drugs in post-mortem blood and urine by liquid chromatography-tandem mass spectrometry. Forensic Sci Int 2009;186:36–43.

37. Gillespie TJ, Gandolfi AJ, Davis TP, Morano RA. Identification and quantification of alpha-methylfentanyl in post mortem specimens. J Anal Toxicol 1982;6:139–142.

38. Hull MJ, Juhascik M, Mazur F, Flomenbaum MA, Behonick GS. Fatalities associated with fentanyl and co-administered cocaine or opiates. J Forensic Sci 2007;52:1383–1388.

39. Olson KN, Luckenbill K, Thompson J, Middleton O, Geiselhart R, Mills KM, Kloss J, Apple FS. Postmortem redistribution of fentanyl in blood. Am J Clin Pathol 2010;133:447–453.

40. Ruangyuttikarn W, Law MY, Rollins DE, Moody DE. Detection of fentanyl and its analogs by enzyme-linked immunosorbent assay. J Anal Toxicol 1990;14:160–164.

41. Wanger K, Brough L, Macmillan I, Goulding J, MacPhail I, Christenson JM. Intravenous vs subcutaneous naloxone for out-of-hospital management of presumed opioid overdose. Acad Emerg Med 1998;5:293–299.

42. Tandberg D, Abercrombie D. Treatment of heroin overdose with endotracheal naloxone. Ann Emerg Med 1982;11:443–445.

43. Pizon AF, Brooks DE. Fentanyl patch abuse: naloxone complications and extracorporeal membrane oxygenation rescue. Vet Hum Toxicol 2004;46:256–257.

Chapter 31

HEROIN and THE OPIUM POPPY PLANT
(*Papaver somniferum* L.)

HISTORY

The opium poppy is one of the oldest known medicinal plants. Although the exact time and place of discovery is unknown, the opium poppy probably originated in Asia minor.[1] Sumerian clay tablets in cuneiform script first described the cultivation of the opium plant about 3000 BC. The Sumerian term for opium was *gil* (happiness), and the opium poppy was called the joy plant (*gil hul*). The Assyrians also collected poppy juice in earthen pots during the early morning by scraping the poppy capsule with an iron scoop. The Persian conquerors of Assyria and Babylonia as well as the ancient Egyptians continued the cultivation of the opium poppy, although the Egyptians restricted opium use to cults that included magicians, priests, and warriors.[1] The writings of Hippocrates (460–377 BC) mentioned the use of poppy juice in wine. Opium and hemlock was a common lethal concoction for the execution of condemned Greeks; the Romans continued the use of opium as a medicinal agent and as a poison. Paracelsus (1493–1541) popularized the use of opium in various mixtures (e.g., laudanum) as an analgesic in Europe during the early 16th century. Later in the 17th century, Thomas Syndenham introduced tincture of opium (i.e., laudanum) into Britain as a treatment for plague.

Filling a pacifier with opium poppy seeds was a popular European custom in the 19th century for quieting a noisy baby; several case reports during the middle 19th century attributed poisoning and death of infants to the use of syrup of poppies.[2,3] In 1874, 100 years following the discovery of morphine, a London researcher, C.R. Wright synthesized heroin (diacetylmorphine, diamorphine) along with a series of acetylated morphine derivatives by boiling anhydrous morphine with acetic anhydride.[4] This compound was originally formulated as a cough suppressant. William Osler first described heroin-induced pulmonary edema in 1880.[5] In the latter part of the 19th century, Felix Hoffman of the Bayer Company isolated heroin. Shortly thereafter, the Bayer company began marketing heroin as a cough suppressant and pain remedy with the expectation that acetylation of the morphine molecule would reduce adverse effects. The addictive potential of heroin was not recognized until the early 20th century. The International Opium Commission was founded in 1909; by 1914, 34 nations had agreed to decrease opium production. The Harrison Narcotic Act of 1914 restricted the use of heroin in the United States. The International Narcotics Control Board of the United Nations now regulates the cultivation of opium poppies with India being the only country involved in the legal international trade of opium.

By the 1920s, the illicit use of heroin, primarily via the nasal route, was associated with crimes in many eastern US cities. Heroin smoking with porcelain bowls and bamboo tubes originated in Shanghai during the 1920s; this technique spread across East Asia and to the United States over the next decade.[6] In 1931, an expert report from the League of Nations recommended replacing the medical use of heroin with other opioids because of the abuse potential of heroin and the availability of alternatives.[7] "Chasing the dragon" (i.e., inhaling vapors released by heating heroin on aluminum foil

with a flame) was a refinement of heroin smoking that originated near Hong Kong in the 1950s. This form of heroin abuse spread to other parts of Southeast Asia, and over the next 20 years, to the Indian subcontinent and parts of Europe. During the early 1980s, case reports associated the development of spongiform leukoencephalopathy with the practice of "chasing the dragon."[8] Except in the United Kingdom, diacetylmorphine (heroin) is not approved for therapeutic use; heroin abuse continues to occur in cyclical patterns depending on availability and illicit drug preferences. Heroin use increased dramatically in the early 1970s, but subsequently declined until availability again increased the use of heroin in the late 1980s and early 1990s. Currently, heroin use is increasing, particularly in adolescents using more concentrated heroin formulations, initially via nasal insufflation.[9]

BOTANICAL DESCRIPTION

The opium poppy plant is one of the approximately 100 plant species in the poppy family. The taxonomy of the poppy family is complex; there are a variety of subspecies and strains as a result of the cultivation of these plants over thousands of years. There are several variations of opium poppy plant and related cultivars as a result of the extensive cultivation of this single plant species over many years. Typically, the Indian variety (*album*) has white flowers with white seeds, whereas the European variety (*nigrum*) has purple flowers and slate gray seeds.[1] The Asia minor variety (*glabrum*) has purple flowers with purplish-black seeds.

Common Name: Opium poppy

Scientific Name: *Papaver somniferum* L.

Botanical Family: Papaveraceae (poppy)

Physical Description: These plants are annual herbs with erect stems and alternate, simple, clasping, toothed leaves. The solitary, terminal flowers have showy petals that vary in color from white to purplish-red depending on the cultivars. The fruit is a capsule with an expanded disc at the top that disperses minute seeds. The dried milky exudate of the unripe seed capsule contains morphine. A poppy plant produces one crop (i.e., 5–8 capsules) over a 3- to 5-month period in sunny, rich, moist areas without frost. In northern India, sowing of seeds begins in November (i.e., late fall, early winter); flowering occurs from April to May with capsules maturing in May or June.

Distribution and Ecology: Legal cultivation of poppy plants occurs principally in northern India (Madhya Pradesh, Rajasthan, Uttar Pradesh)

IDENTIFYING CHARACTERISTICS

Structure

Heroin (diacetylmorphine) is a prodrug, which undergoes rapid hydrolysis in the body first to 6-monoacetylmorphine (6-MAM) and then to morphine, especially at alkaline pH. Figure 31.1 demonstrates the chemical structure of heroin and other major alkaloids potentially in illicit heroin.

Physiochemical Properties

The high lipid solubility of heroin allows the rapid distribution of the drug into the central nervous system (CNS). The pK_a of heroin is 7.6; at physiologic pH, approximately 55% of the drug is ionized and the remainder is accessible for membrane-transport. The smokable form of heroin (i.e., heroin base) contains about 91% of the mass of the hydrochloride salt of heroin used for intravenous (IV) administration.

Pyrolytic products from the heating of heroin result from oxidation, de-acetylation, transacetylation, *N*-demethylation, *O*-methylation, and ring cleavage of heroin. Methanol-dichloromethane extraction and gas chromatography/mass spectrometry (GC/MS) analysis of residues from aluminum foils previously used for street heroin smoking of extracts of residues identify about one-half of the compounds in the residues including 6-MAM, 6-acetylcodeine, papaverine, meconine, diacetylmorphine, 4′-acetoxyacetanilide, 4′-methoxyacetanilide, hydrocotarnine, thebaol (degradation product of thebaine), triacetin, caffeine, cocaine, and paracetamol.[10]

During heating, the thermal decomposition of heroin base is much less than heroin hydrochloride. In a study using an experimental device to heat heroin to 200°C (392°F), the loss of heroin base and heroin hydrochloride during volatilization was 11% and 72%, respectively.[11] The heating of diacetylmorphine base and diacetylmorphine hydrochloride up to 310°C (590°F) for about 25 minutes caused the thermal degradation of about 30% and 70% of these compounds, respectively.[10] 6-Monoacetylmorphine is the major degradation product of these 2 compounds among a variety of degradation products.[12] 6-Monoacetylmorphine, 6-acetylcodeine, and morphine were relatively stable under these conditions.

Terminology

The term *opiate* traditionally refers to opioid-receptor (OR) binding drugs extracted from the opium poppy (*Papaver somniferum* L.) including codeine and
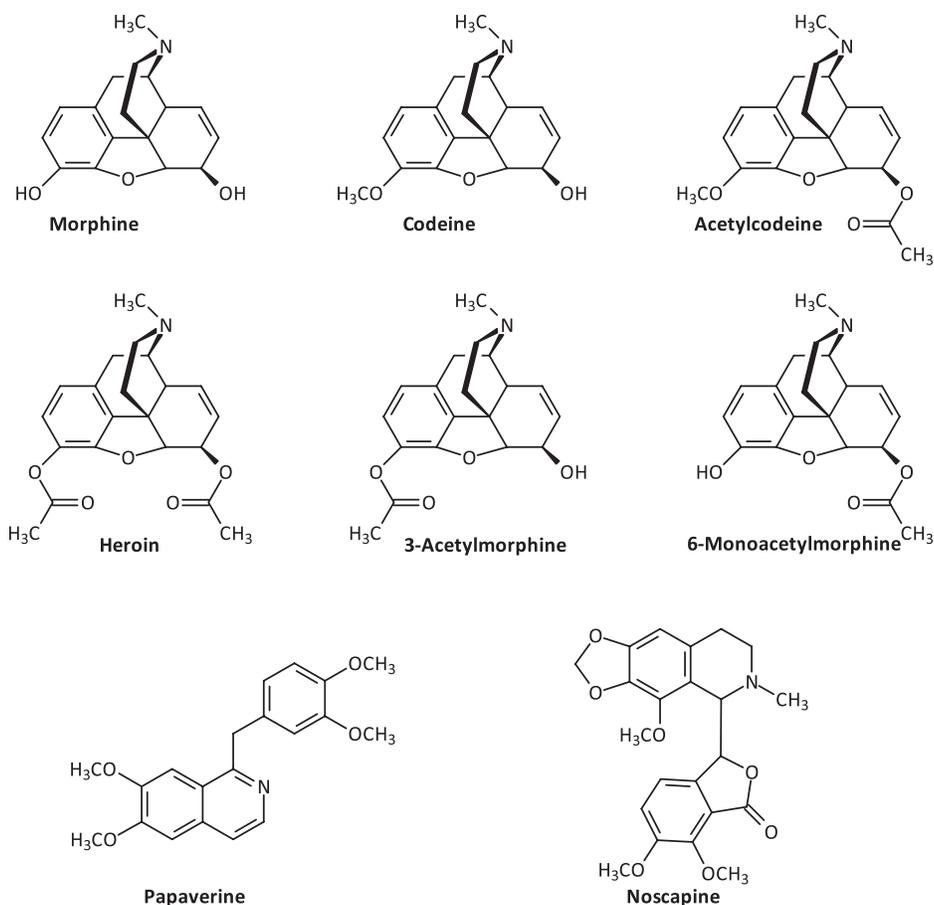
FIGURE 31.1. Chemical structures of heroin and related compounds.

morphine. Opioid is a broader term that includes opium-derived drugs and synthetic substances (fentanyl, propoxyphene) with morphine-like activity as well as substances with OR antagonist activity. "Cheese" is a tan powder suitable for insufflation that is composed of black tar heroin cut with acetaminophen and diphenhydramine to a concentration of approximately 8% heroin.

Form

Heroin is sold in aluminum foil and wax-paper envelopes. A bag (single dose) of heroin typically contains about 100 mg diacetylmorphine with the purity historically ranging from about 1–10%. Recently, the purity of heroin in illicit samples has significantly increased. Illicit heroin varies in chemical and physical appearance depending on the origin of the drug. Figure 31.2 displays confiscated Asian brown and white heroin; Figure 31.3 shows confiscated Mexican black tar heroin.

EXPOSURE

Epidemiology

The estimated number of dependent heroin users among the general Australian population is approximately 7 per 1000 people aged 15–54 years.[13] This prevalence is similar to that of the United Kingdom and within the range (i.e., 3–8 heroin addicts/1000 people) of other European countries. The typical gender distribution of heroin users reveals a male/female ratio of approximately 7:3.[14] In the United States, there are an estimated 200,000 current heroin users (12 years and older) as defined by heroin use within the last month.[15] The estimated lifetime prevalence of heroin use is approximately 3 million (i.e., about 1.4% of Americans). Epidemiologic data indicates that heroin dependence and heroin-related emergency department visits increased substantially within the last 10–20 years in Europe, Australia, and the United States along with

FIGURE 31.2. Confiscated Southwest Asian brown and white heroin. The latter is also produced in Southeast Asia. (Photo courtesy of the US Drug Enforcement Agency)
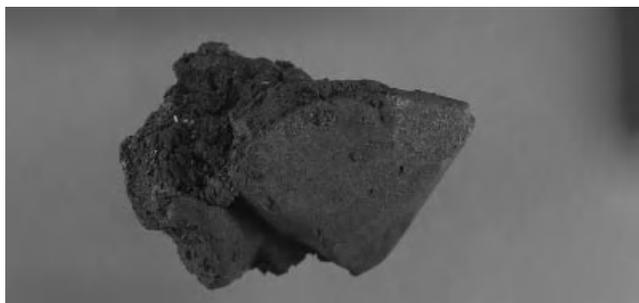
FIGURE 31.3. Confiscated Mexican black tar heroin. (Photo courtesy of the US Drug Enforcement Agency)

heroin-related deaths.[16,17] Although fatal heroin overdoses occur in both sexes, the typical heroin-related fatality involves 25- to 34-year-old male polydrug abusers.[18]

Sources

Heroin is available illegally in the Black Market and legally in the United Kingdom under strict controls. Government-regulated opium is grown on farms primarily in India, and to a lesser extent in Turkey and Australia; some diversion of this opium to clandestine heroin production probably occurs.[19] Previously, most US illicit heroin was a white powder derived from Asian opium that was processed in France. Now, major sources of illicit US heroin include South America (Colombia), Mexico, Southeast Asia (Myanmar, Burma, and to a lesser extent, India), and Southwest Asia/Middle East (Afghanistan, and to a lesser extent, Pakistan, Iran, Lebanon).[20]

Before the 1990s, most heroin transported to the US originated in Southeast Asia with the amount of heroin in street samples ranging from approximately 1–10%. In the last 10 years of US drug trafficking, less-expensive, undiluted forms of heroin replaced the more dilute Southeast Asian heroin, particularly in the eastern United States. A large portion of Southwest Asian heroin is now consumed in western Europe, Pakistan, and Iran. Mexican brown heroin has been transported from Mexico to the Southwest United States for many decades. Black tar heroin and, to a lesser extent brown powdered heroin from Mexico are prominent forms of heroin in the western United States. Black tar heroin is a viscous to hard black substance that originates in the mountainous regions of Northern Mexico (Durango, Sinaloa, Sonora) and in areas southwest of Mexico City.

Origin/Composition

Opium. Opium is the air-dried, white exudate extracted from the poppy plant that contains at least 40 alkaloids in addition to morphine including codeine, thebaine, narcotine (phthalideisoquinoline noscapine), papaverine, and minor alkaloids (aporphine, benzophenanthridine, protoberberine, tetrahydroprotoberbine, tetrahydroisoquinoline). Pharmaceutic preparations of opium contain about 10% morphine, 5–20% water, 20% sugars, and simple organic acids (fumaric, meconic, lactic, oxaloacetic).[1] Meconic acid occurs in various species in the genus, *Papaver*; the presence of this dibasic acid is not limited to the opium poppy plant. According to 2008 US Drug Enforcement Agency statistics, the wholesale purity of Mexican heroin was 40% compared with 57% for South American heroin.[20] The average purity of heroin from different sources changes annually. For example, the highest average purity in 2000 occurring in South American heroin (48%) followed by Southwest Asian heroin (35%) and Mexican heroin (21%). Compared with Southwest Asian heroin, black tar heroin is much less refined due to the production processes (e.g., use of kerosene rather than acetone). The purity of black tar heroin (i.e., average 35%, range, 20–80%) is substantially greater than Southwest Asian heroin.[21]

Opium Poppy Seeds. Figure 31.4 displays an opium poppy field. The seeds from the opium poppy plant are commonly used in foods. Poppy seeds from *Papaver somniferum* contain significant amounts of morphine and codeine. Although thebaine is typically not present in opioid pharmaceutic preparations, this compound is present in poppy seeds and found in heroin users as measured by GC/MS.[22] The content of morphine

FIGURE 31.4. Opium poppy field. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 31.5. Opium harvest: Collection of raw opium gum from the poppy. (Photo courtesy of the US Drug Enforcement Agency)

and codeine in processed poppy seeds varies widely depending on the origin of the poppy seeds, variety, time of harvest, external contamination during harvest, harvest technology, and food-processing techniques.[23] Morphine content in poppy seeds may decrease up to 90% during food processing as a result of washing, grinding, and baking.[24,25] Analysis of 3 samples of poppy seeds confiscated by the Singapore Central Narcotics Bureau contained total morphine and total codeine concentrations ranging from 58.4–62.2 µg/g seeds and 28.4–54.1 µg/g seeds, respectively.[26] Washing the seeds in water for 5 minutes with constant agitation followed by decantation of the aqueous layer removed slightly less than half of the free morphine and codeine.

PRODUCTION PROCESSES

Heroin is the diacetylmorphine product derived from the extraction, acetylation, and crystallization of morphine from opium (i.e., dried sap of the opium poppy).

Opium harvest involves 2 steps: 1) the incision (scoring) of the pod allowing the white latex (raw opium gum) to flow externally where the latex hardens, and 2) collection of the dark, solidified latex the following morning by scraping the solidified latex off the pod. Figure 31.5 displays the collection of the white latex from the opium poppy. The latex is kneaded together into balls or stored in perforated pots; the material is then air-dried. More modern techniques involve drying and processing the entire plant (opium "straw"). The clandestine preparation of heroin requires the separation of morphine from opium, typically by the dissolu-

tion of opium in water that contains lime. After pouring the resultant solution over a coarse filter, the addition of ammonium chloride precipitates the morphine base. This solution is filtered, washed with water, and mixed with charcoal and an acid (hydrochloric acid, sulfuric acid). The subsequent precipitate is dried and contains purified morphine.

The second phase of heroin production involves the acetylation of the morphine that uses acetic anhydride and organic bases along with heat. Alternative chemicals to acetic anhydride include ethylidene diacetate and acetyl chloride. Two acetylation methods include the reaction of morphine with acetic anhydride and the acid-catalyzed cross-condensation of morphine with acetic acid. The latter method is slightly less efficient (i.e., <83%) than the former method as a result of the production of more monoacetylmorphine at the 6- and 3-positions (i.e., 6-MAM, 3-MAM). The treatment of morphine at room temperature with a mixture of trifluoroacetic anhydride (TFAA) and acetic acid also produces slightly less heroin and more 6-MAM and 3-MAM than the traditional acetic anhydride method. In an experimental study comparing the TFAA method with the traditional acetic acid method, the yields were as follows: heroin (76.1% vs. 83.55%); 3-MAM (6.9% vs. 0.75%); and 6-MAM (7.13% vs. 0.63%), respectively.[27] Another method ("homebake") involves the conversion of codeine to heroin by the conversion of codeine to morphine by *O*-demethylation with pyridine followed by acetylation with an anhydride. The homebake method is much less efficient than the former 2 methods, resulting in the conversion of 2 g codeine to only 50–200 mg heroin.

Heroin purification involves the separation of the diacetylmorphine as a free base in alkaline medium with subsequent crystallization as the hydrochloride salt after redissolving the crude heroin in acetone and hydrochloric acid. The mixture is filtered and precipitated with sodium carbonate. To form heroin base instead of the hydrochloride salt of heroin, the purification process involves the use of boiling water that contains citric acid and activated charcoal followed by precipitation with sodium carbonate.

IMPURITIES

Heroin is a semisynthetic opioid derived from morphine extracted from the opium poppy plant. Illicit synthesis of heroin does not produce a pure substance due to the presence of other alkaloids extracted from the opium poppy plant along with morphine. The synthetic process also creates other compounds. During the acetylation stage of heroin production, some opiate alkaloids (e.g., codeine) are converted to acetyl derivatives (i.e., acetyl codeine), whereas other opiate alkaloids (e.g., papaverine, noscapine) remain unchanged. In general, street drugs (e.g., heroin) contain a variety of impurities including substituents, contaminants, diluents, and adulterants.[28] Substitution involves the complete replacement of heroin with another drug. Contaminants are unintentional byproducts of the manufacturing process of heroin. Substances (i.e., diluents, adulterants) added to illicit drug after the completion of the manufacturing process are termed additives. Potential additives in illicit heroin include caffeine, lactose, glucose, paracetamol, clenbuterol, scopolamine, phenobarbital, quinine, mannitol, procaine, sucrose, methaqualone, piracetam, and lidocaine.[29,30] Diluents are inert substances with physical characteristics (e.g., taste, form, texture, color) similar to the illicit drug (i.e., heroin) that are intentionally added to reduce the drug concentration while maintaining bulk substance mass. Common diluents of white heroin include sucrose, starches, quinine, and mannitol. Adulterants are pharmacologically active ingredients that are added to the illicit drug (e.g., heroin) to enhance (e.g., phenobarbital) or antagonize (e.g., caffeine) the effect of drug without the knowledge of the user. Although the quinine content of a bag of heroin may exceed 300 mg, lethal serum concentrations of quinine are not usually present postmortem following fatal heroin overdoses, perhaps as a result of the degradation of quinine during preparation of the illicit drug for injection.[31,32,33] The use of scopolamine as an adulterant of heroin caused an epidemic of severe anticholinergic toxicity among heroin users in the latter 1990s.[34] The IV injection of clenbuterol-adulterated heroin was associated with nausea, chest pain, agitation, anxiety, tachy-cardia, palpitations, hypokalemia, hyperglycemia, hypotension, chest pain, myocardial injury, and lactic acidosis.[35,36] Clenbuterol is a veterinary β_2-adrenergic receptor agonist used illicitly by body builders to enhance muscle mass. Another case series reported the development of tremor, hyperreflexia, myalgia, muscle spasm, and elevated serum creatine kinase after the insufflation or injection of clenbuterol-adulterated heroin; however, the cause (i.e., clenbuterol or an unidentified contaminant) of these effects is unclear.[37] In a study of 106 drug-related deaths during 2007, clenbuterol was detected in 12 cases (11%) as measured by GC/MS in full-scan mode (limit of detection [LOD], 2.5 ng/mL).[38]

PROFILING

Impurity profiling of illicit drugs is an intelligence-gathering tool to support and complement the work of law-enforcement agencies. Heroin signature programs (HSP) analyze seized heroin samples to determine drug trafficking and distribution intelligence. The extraction of morphine from opium is never complete; therefore, heroin contains variable amounts of codeine, papaverine, noscapine, and meconine. The chemical composition of heroin depends on the original composition of the opium, morphine extraction methods, acetylation procedures, addition of different solvents (acetone, acetic acid), purification processes, and the amounts of heroin base and hydrochloride salt.[39,40] The type of contaminants and plant chemicals identify the source and manufacturer of the opium.[41,42] For example, enforcement agencies use analysis of phenanthrene (morphine, codeine, thebaine) and benzyl isoquinoline (papaverine, noscapine) compounds as profiles for the source of the heroin. Using data on principal alkaloids (e.g., total codeine, acetyl codeine, papaverine, noscapine, 6-monoacetylmorphine) analyzed by capillary gas chromatography, high performance liquid chromatography, or GC/MS, HSP identifies the geographic origin of seized heroin samples by the following: Southwest Asia, presence of large quantities of papaverine and noscapine; Southeast Asia, absence of papaverine and noscapine; Mexican heroin, unique profile; and South American heroin, high purity and low acetyl codeine content.[43,44] Tasmanian opium accounts for about 25% of the world's legal supply of opium straw based on the development of a cultivar, *Papaver somniferum* Norman. This cultivar has relatively high concentrations of thebaine and relatively low concentrations of codeine and morphine compared with *Papaver somniferum* L. Additionally, this cultivar contains relatively high concentrations of the thebaine metabolite, oripavine; unique markers in heroin derived from oripavine in

551

Papaver somniferum Norman include 3-acetyl-*N*-acetyldesthebaine, 3-acetyl-6-methoxy-4,5-epoxyphenanthrene,3,4-diacetyl-6-methoxyphenanthrene,and 3,4,6-methoxy-5-[2(*N*-methylacetamido)]ethylphenanthrene.[45]

Similar HSP techniques also provide information regarding the method of heroin synthesis. Standard HSP analysis uses acetic anhydride as the principle acetylating agent. Specific markers indicate the use of other acetylating agents including 1-chloroheroin (acetyl chloride) and 3-[1-(1-carboxymethoxyethyl)]-6-acetylmorphine (ethylene diacetate). Specific markers for the trifluoroacetic anhydride method include *bis*-trifluoroacetylmorphine, 3-trifluoroacetyl-6-acetylmorphine, 3-acetyl-6-trifluoroacetylmorphine, and trifluoroacetylcodeine.[27]

Methods of Abuse

In the past decades, most heroin transported to the United States originated in Southeast Asia as the street content of heroin ranged from approximately 1–10%; the preferred route of heroin abuse was IV injection. Over the last decade, the increased availability of high-purity heroin resulted in the increasing use of insufflation and smoking as an alternative to IV heroin. Although the risk of HIV infection associated with IV drug use was avoided, the use of larger quantities of the drug via snorting resulted in more rapid progression to heroin addiction. In parts of Asia, smoking is the most common route of heroin abuse. Methods of smoking heroin include the inhalation of heroin fumes (i.e., chasing the dragon) and smoking tobacco cigarettes dipped in heroin (i.e., *ack ack*) with IV heroin administration. Chasing the dragon involves the heating of heroin powder on aluminum foil and the inhalation of the vapor with a straw or pipe. The molten heroin moves around the foil during heating, and inhalation of the vapor requires movement of the straw or pipe accordingly. This method of delivery is highly efficient.[46]

Preparation of heroin for injection typically involves mixing the drug with water, lemon juice, or other liquids. The solution is heated on a spoon or other utensil until the powder dissolves; then, the solution is filtered through a cotton ball or cigarette filter and drawn into a syringe. Although addicts use all areas of the skin for IV access, the site of injection varies with the duration and intensity of IV drug use. The antecubital fossa and forearms are the common starting sites following in 3–4 years by the upper arms and hands.[47] After 6 years, use of veins in the neck, foot, and legs are necessary to achieve IV access. By 10 years, the toes, fingers, and groin are the remaining sites of injection. Injection into major vessels is an alternative to poor peripheral venous access including attempts to inject into the groin ("groin shot"), upper arm, and neck ("pocket shot"). Subcutaneous injection ("skin popping") is a method of administration designed to avoid the scarring ("track marks") associated with IV drug abuse.

DOSE EFFECT

Because of tolerance, the typical street dose of heroin varies substantially between addicts. The initial dose of heroin to produce euphoria is usually a few milligrams, but tolerance quickly increases the dose required to produce euphoria. The typical single dose of heroin in drug-dependent individuals is approximately 150–200 mg with a highly dependent heroin abuser using 500–750 mg/day.

TOXICOKINETICS

Absorption

Although the absorption of heroin from various sites (e.g., mucosa, lungs, rectum, vagina, soft tissue) is excellent, the preferred route for most heroin addicts is IV administration. Inhaling heated vapors of heroin (i.e., chasing the dragon) is the most efficient drug-delivery method for non-IV routes of administration with bioavailability of approximately 35–45%.[46]

INTRAVENOUS

In experimental studies of IV infusions of diacetylmorphine to heroin addicts, maximal venous concentrations of diacetylmorphine occurred about 3 minutes after cessation of the infusion.[48] Approximately 4–6 minutes after the infusion, diacetylmorphine concentrations were similar in arterial and venous samples. In a study of 4 heroin addicts administered 12 mg heroin via the intramuscular and nasal routes, peak serum diacetylmorphine concentrations occurred about 5 minutes after administration via both routes.[49] Maximum plasma concentrations developed approximately 45–90 minutes after the administration of heroin.

PULMONARY

Experimental studies indicate that popular forms of smoking heroin deliver absorbed morphine doses ranging from approximately 20% (*ack ack*) to about 40% (chasing the dragon) of the external morphine dose.[50] In a study of 10 heroin addicts smoking heroin in doses ranging from 25–100 mg per session, the bioavailability of heroin ranged from about 35–45% as measured by total urinary morphine concentrations.[46] A study of 9 heroin addicts stabilized in a heroin treatment

program indicated that the bioavailability of heroin following inhalation is substantially less than following IV administration.[51] In this double-blind randomized study using 67%, 100%, and 150% of their regular heroin maintenance dose (maximum single dose, 450 mg), the mean bioavailability of heroin via inhalation was 52% (95% CI: 44–61%).

GASTROINTESTINAL

Volunteer studies indicate that the gastrointestinal (GI) absorption of heroin (diacetylmorphine) is good with maximum mean morphine bioavailability ranging from 64–72%.[52] Extensive first-pass deacetylation of heroin results in negligible concentrations of heroin and 6-monoacetylmorphine (6-MAM) in the blood following the oral administration of heroin, except following massive GI absorption during body packing. Consequently, in volunteer studies, detectable concentrations of heroin and 6-MAM do not usually occur after the oral administration of heroin.[53] Some saturation of first-pass metabolism may occur in chronic heroin users, allowing increased bioavailability of oral heroin when compared with heroin-naïve individuals.[54]

NASAL INSUFFLATION

Although the pharmacokinetic profiles of heroin via nasal and intramuscular administration are similar, the relative potency of intranasal heroin is approximately one-half of the potency of intramuscular heroin. Following intranasal administration of 12 mg heroin via the intranasal route, peak plasma heroin and 6-MAM concentrations occurred about 5 minutes and 5–10 minutes, respectively, after administration.[55]

Distribution

Heroin rapidly crosses the blood–brain barrier and into the CNS. Following the IV administration of heroin, animal studies indicate that heroin metabolites (i.e., 6-MAM) rapidly enter the brain.[56] The volume of distribution of heroin ranges from about 60–100 L.[57] Following deacetylation of heroin to morphine, morphine distributes widely into the liver, lungs, kidney, and brain. Parts of the brain with high concentrations of opioid receptors include the thalamus and the brainstem. Morphine easily traverses the blood–brain barrier, but autopsy studies suggest the distribution of morphine following heroin injection is not consistent between different regions of the brain.[58] The apparent volume of distribution for morphine is approximately 3–5 L/kg compared with about 0.3 L/kg for morphine glucuro-

nides.[59] Similar to morphine, approximately 20–40% of heroin binds to serum albumin.[60]

Biotransformation

Heroin is a prodrug that undergoes rapid hydrolysis to the metabolite, 6-monoacetylmorphine (6-MAM), either spontaneously or by serum cholinesterases; then, 6-MAM undergoes further deacetylation to morphine followed by glucuronidation primarily in the liver and kidneys to morphine-3-glucuronide and morphine-6-glucuronide. Figure 31.6 displays the biotransformation of heroin. Pilot studies indicate that diacetylmorphine (heroin) is detectable only shortly after IV administration; oral or rectal administration of heroin do not typically produce detectable amounts of diacetylmorphine.[61] Pharmacologically active metabolites of heroin include 6-MAM, morphine, and morphine-6-glucuronide (M6G), whereas morphine-3-glucuronide (M3G) is inactive. However, M6G is a minor metabolite of diacetylmorphine (heroin) metabolism; therefore, morphine is the major active metabolite of heroin biotransformation in the human body. A small observational study of 8 current IV heroin users suggests that the M6G/M3G ratio may be higher in IV heroin abusers using heroin than in opioid-naïve volunteers administered IV morphine.[62] Other minor metabolites of heroin metabolism include the 3-ether sulfate of morphine and normorphine.[63] Codeine is not a metabolite of heroin or morphine biotransformation,[64] but codeine often appears in blood samples from heroin abusers as a result of the deacetylation of acetyl codeine. This latter compound is an opiate alkaloid that is a constituent of opium, and therefore present in heroin. Peripheral tissues (e.g., human erythrocyte acetylcholinesterase) probably account for a substantial portion of the deacetylation of diacetylmorphine and 6-MAM to morphine.[65]

Elimination

The conversion of heroin (3,6-diaceytlmorphine) to 6-MAM occurs almost instantaneously with peak 6-MAM concentrations in plasma occurring almost simultaneously with peak diacetylmorphine concentrations. In a study of 8 heroin addicts, the kinetic profile of heroin was linear in IV doses up to 210 mg.[48] In a study of 11 chronic pain patients, the mean elimination $t_{1/2}$ of IV heroin from the blood was 3.0 ± 1.3 minutes.[53] The plasma elimination half-life of 6-MAM is slightly longer (i.e., mean, 5–20 min) than heroin. Following the initiation of a 7-hour infusion of 10 mg heroin/hour, analysis of the urine collected over the subsequent 40 hours indicated the presence of a mean of about 45% of the administered dose (i.e., 70 mg) divided as follows: free
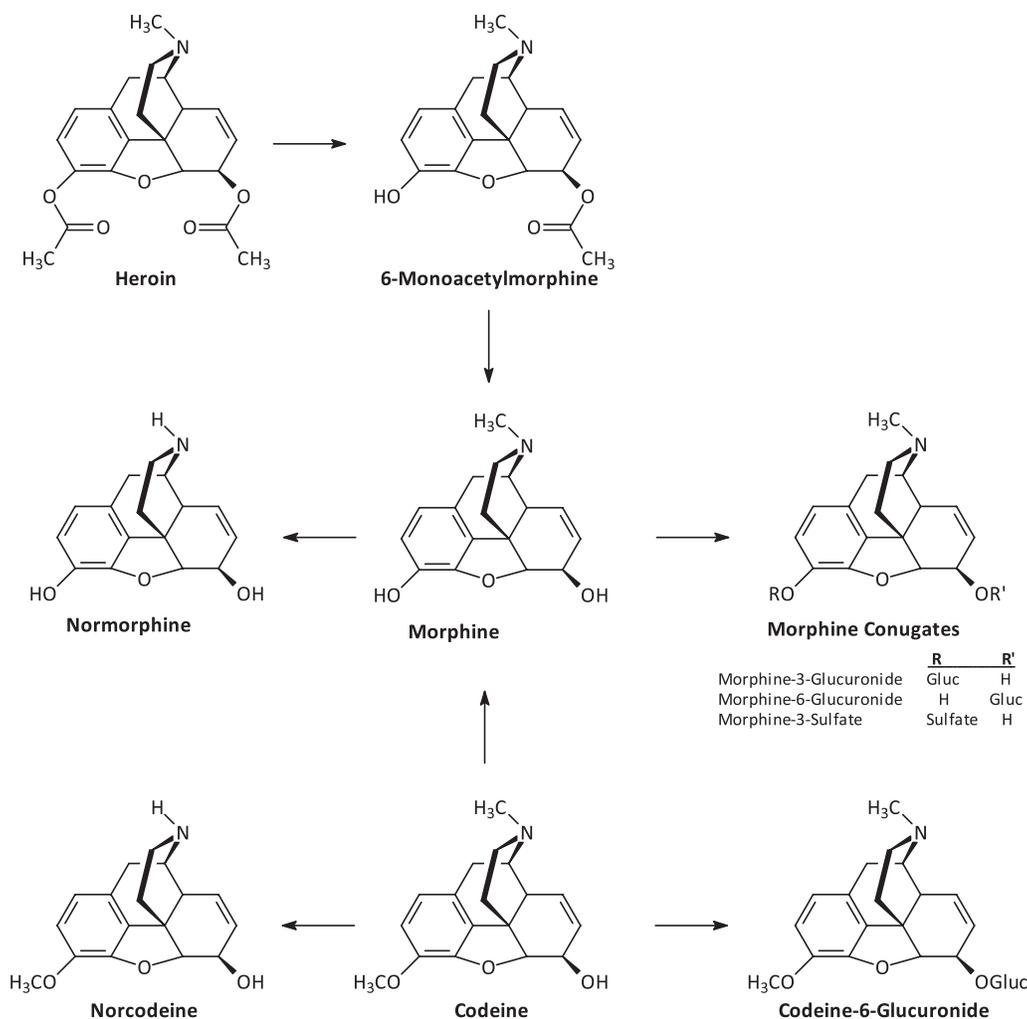
FIGURE 31.6. Biotransformation of heroin, morphine, and codeine.[126]

morphine, 4.2%; conjugated morphine, 38.3%; 6-MAM, 1.3%; and unchanged heroin, 0.1%.[66] The excretion of total morphine in urine is highly variable between individual subjects. In a study of 8 subjects given 3–12 mg IV heroin HCl and 4 subjects administered 3.5–13.9 mg heroin base via inhalation, the total morphine concentration in urine samples as a percentage of the administered heroin dose ranged from 12.8–88.5% with a median of 51%.[67] This variability resulted from large variations in the biotransformation and renal excretion of metabolites as well as the route of exposure.

The biotransformation of heroin to metabolites is virtually complete prior to renal excretion. Following intramuscular administration of 6 mg heroin to healthy volunteers, peak urine concentrations of total morphine occurred about 3–4 hours after administration with peak values ranging from 3.31–12 mg/L.[68] Peak concentrations of 6-MAM occurred in the first voided specimen with concentrations ranging from 0.05–0.24 mg/L. Morphine-3-glucuronide is the main urinary metabolite.

Maternal and Fetal Kinetics

Passive diffusion is the major mechanism of transport of drugs from maternal blood to the fetus. For lipid soluble drugs (e.g., heroin) with molecular weights below 600 g/mol, there is no significant barrier to the transfer of the drug through the placenta.[69] In pharmacokinetic studies in primates, heroin diffuses into breast milk and also crosses the placenta.[70]

Tolerance

The development of tolerance to the clinical effects of heroin is dramatic with a few mg of heroin producing

clinical effects in drug-naïve individuals, whereas illicit use of heroin by drug addicts typically involves the injection of up to 750 mg heroin. Experimental studies suggest that signs of physical dependence in human subjects develop after the infusion of heroin 4 times daily for 2–3 days.[71] Tolerance develops to some, but not all of the clinical effects of opioids including heroin. Tolerance develops to respiratory depression, nausea, analgesia, and sedation, but constipation and miosis continue to occur despite tolerance to other opioid effects. These processes result primarily from pharmacodynamic rather than pharmacokinetic changes.

Drug Interactions

Heroin interacts synergistically with other CNS depressants, particularly ethanol.[72] Several drugs (benzodiazepines, chloramphenicol, tricyclic antidepressants, zidovudine) competitively inhibit the glucuronidation of morphine by UGT2B7 *in vitro*, but the clinical significance of this interaction is unclear.[57]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Heroin is an agonist of the endogenous opioid system with effects on the G-protein-coupled μ-, κ-, and δ-opioid receptors in the brain and spinal cord as well as the nociception (NOR) receptors in the peripheral nervous system. All opioid receptors have endogenous ligands (enkephalins, endorphins). In general, agonists of the μ- (MOP) and δ- (DOP) opioid receptors are analgesic and rewarding, whereas agonists of κ- (KOP) opioid receptors are dysphoric. Table 31.1 summarizes the pharmacologic effects of these opioid receptors. These receptors belong to the large superfamily of

Table 31.1. Pharmacologic Effects of Opioid Receptors.

Opioid Receptor	Pharmacologic Effect
Mu (μ)	Analgesia (supraspinal), euphoria, drowsiness, cognitive impairment, respiratory depression, reduced gastrointestinal mobility (nausea, vomiting, constipation), lowered body temperature, miosis, tolerance, addiction
Kappa (κ)	Analgesia (spinal), sedation, miosis, mild respiratory depression, miosis, dysphoria, diuresis, drug aversion
Delta (δ)	Analgesia, dysphoria, hallucination, delusions

G-protein-coupled receptors involving 7 transmembrane-spanning domains.[73] In addition, these receptors have an extracellular *N*-terminal domain, an intracellular *C*-terminal tail, and 3 extracellular and 3 intracellular loops connecting the 7 transmembrane helical domains. Opioid receptors share about 60% of their structure with the greatest homology in the transmembrane helices and the greatest diversity in extracellular loops and the *N* and *C* termini.[74] Activation of the opioid receptors results in the formation of GTP from GDP and the separation of the G-protein α and βγ subunits. This interaction decreases adenylyl cyclase activity causing a reduction in influx across voltage-gated Ca^{2+} channels and stimulation of G protein-activated inwardly rectifying K^+ channels and phospholipase Cβ. The subsequent hyperpolarization of the cell stops the transfer of afferent nociceptive signals. Activation of the μ-opioid receptor produces calcium influx through the NMDA receptor ion-channel complex, resulting in stimulation of various calcium-dependent, second messenger systems. These μ-opioid receptors are distributed throughout the brain, where they mediate analgesic and addictive effects.

The μ-opioid receptor also displays spontaneous agonist-independent (basal) G protein coupling that causes basal signaling activity in the absence of opioid agonists.[75] The opioid receptors are further divided into subtypes ($μ_1$, $μ_2$, $δ_1$, $δ_2$, $κ_1$, $κ_2$). Mu_1 receptors account for the analgesic effects of heroin, whereas $μ_2$ receptors mediate respiratory depression, miosis, euphoria, physical dependence, and delayed GI motility associated with heroin intoxication. Stimulation of κ-opioid receptors cause analgesia, miosis, respiratory depression, and dysphoria. Delta-opioid receptors are associated with spinal analgesia. The $σ_1$- receptor is not an opioid receptor.[76]

Mechanism of Toxicity

CENTRAL NERVOUS SYSTEM

Heroin produces respiratory depression, and prolonged hypoxia causes postanoxic encephalopathy. The etiology of heroin-induced spongiform encephalopathy remains unclear. The suspected toxin is a thermal degradation product or products of the heating of heroin on aluminum foil. Analysis of residues from aluminum foil do not support triethyl tin as the etiologic agent even though this compound can cause similar neurologic lesions.[77]

RESPIRATORY SYSTEM

Respiratory depression following heroin intoxication results from suppression of brainstem respiratory

centers, primarily via decreases in the ventilatory response to carbon dioxide. Heroin-related pulmonary edema is associated with pulmonary congestion, alveolar edema, pulmonary hemorrhages, and respiratory insufficiency that usually develops following a heroin overdose. The cause of the increased permeability of the alveolar capillary membranes remains unclear. Typical hemodynamic variables during heroin-related pulmonary edema include normal pulmonary capillary pressure, normal or increased cardiac output, and moderately elevated pulmonary artery pressure.[78] Potential mechanisms include hypoxia-induced increase of pulmonary capillary permeability, anaphylactic shock, depressed myocardial contractility, centrally induced respiratory depression, and direct toxic effects on the alveolar capillary membranes. Although pulmonary edema occurs during other opioid overdoses (propoxyphene, codeine, methadone),[79,80] similar degrees of hypoxia occur following barbiturate overdoses without the development of pulmonary edema. In a case-control study of 23 acute heroin fatalities and 12 cases of sudden cardiac death with acute cardiac pulmonary edema, immunohistologic investigation of epithelial and endothelial basal laminae with IgE did not detect a significant difference between the two groups.[81] Although defects of the basal laminae of the alveoli were found in both groups, the poor correlation between IgE-positive cells and heroin use did not suggest an anaphylactic etiology.

TOLERANCE

Potential mechanisms for the development of tolerance and withdrawal include receptor desensitization, endocytosis, functional antagonism, and downregulation of receptors. Receptor desensitization (e.g., phosphorylation of G-protein-coupled receptors followed by binding of regulatory proteins called β-arrestins) involves the alteration of functional coupling of a receptor to the G-protein/second messenger-signaling pathway, whereas endocytosis (internalization) is the translocation of receptors from the cell surface to an intracellular compartment. Extended use of opioids (e.g., heroin) that bind to the μ-opioid receptor result in physical dependence and withdrawal. Chronic use of morphine hypersensitizes norepinephrine receptors in the locus ceruleus, resulting in anxiety, sleep disturbances, and hyperactivity following reduction of chronic morphine doses. Functional antagonism results from the opioid-induced stimulation of the intracellular protein kinase C, which phosphorylates the *N*-methyl-D-aspartate (NMDA) receptor. This effect causes increased Ca^{2+} influx and functional antagonism. NMDA receptor antagonists (ketamine, magnesium, dextromethorphan) and inhibitors of nitric oxide synthase attenuate the effects of

opioid tolerance.[82] Downregulation is the reduction of the number of ligand-binding sites. Downregulation of opioid receptors probably is not solely responsible for the development of tolerance to morphine and heroin.[73]

Postmortem Examination

Heroin is a CNS depressant that causes respiratory arrest and hypoxia following the injection of excessive doses. A wide variety of neuropathologic changes occurs in the postmortem examination of brain specimens from heroin abusers. The primary findings in fatalities following the injection of heroin involve infectious processes that result from bacterial emboli, mycoses, or HIV-1 (human immunodeficiency virus 1) infections.[83] Other complications include cerebral edema and neuronal damage secondary to prolonged heroin-induced respiratory depression and cerebral strokes as a result of thromboembolism, vasculitis, or septic emboli. Autopsies of 18 heroin addicts dying following snorting or smoking of heroin demonstrate nonspecific findings of congestion, edema, and occasionally aspiration of gastric contents, but the complications (e.g. HIV, hepatitis, needle marks) of IV heroin use were absent.[84] Rarely, a toxic spongiform leukoencephalopathy is associated with inhaling heroin via chasing the dragon. Postmortem examination usually reveals the characteristic spongiform degeneration of the white matter along with edema of the myelin sheaths and a vacuolating myelinopathy of the oligodendroglia. Electron microscopy demonstrates that vacuolization in the oligodendroglia forms between the myelin lamellae by splitting at the intraperiod lines.[77] Vascular congestion, ischemic changes, and inflammation of the axons is usually absent, and there is relative sparing of subcortical U-fibers. Pathologic abnormalities in the postmortem examination of hearts from heroin-related deaths include myocardial fibrosis, myocarditis, and contraction bands. These abnormalities do not correlate well to morphine or 6-MAM concentrations in postmortem blood samples.[85]

CLINICAL RESPONSE

Illicit Use

INTOXICATION

Heroin is a highly lipophilic drug that rapidly penetrates the blood–brain barrier; injection of heroin produces an intense euphoria (*rush*) within 1–3 minutes followed by a period of tranquility (*nod*) that persists up to 1 hour. The heroin addict may compare the intense pleasure

and warmth associated with the rush to sexual orgasm. The effects of heroin resolve within 3–5 hours. The addict often injects several times in a day; otherwise, the unpleasant signs and symptoms of opioid withdrawal begin. Heroin addicts are usually polydrug abusers;[112] consequently, the concomitant administration of other drugs of abuse may modify the classic features of heroin intoxication.

MEDICAL COMPLICATIONS

CENTRAL NERVOUS SYSTEM.   Neurologic complications of nonfatal heroin overdoses include acute and sub-acute peripheral mono- and polyneuropathies as a result of prolonged pressure necrosis and compartment syndromes.[86] Rare case reports also associate the use of IV heroin or heroin insufflation with the development of the clinical features of sensorimotor peripheral neuropathy without evidence of compression injury or inflammation.[87] However, most of these cases also had evidence of rhabdomyolysis and dramatic elevations of serum creatine kinase concentrations. Case reports associate a variety of neurologic conditions with IV heroin abuse including spinal cord vasculitis, cerebral vascular accidents, cerebral vasculitis, Brown-Sequard syndrome, neurogenic bladder polyradiculoneuropathy, and transverse myelitis.[88] Infectious neurologic complications of IV heroin use include bacterial meningitis, mycotic aneurysm, mycotic and bacterial brain abscesses, subdural abscess, and epidural abscess. Case reports document the development of wound botulism with the use of parenteral forms of heroin, particularly with the subcutaneous (SC) injection of black tar heroin.[89] Rare case reports associate wound botulism with nasal insufflation.[90] The classical clinical manifestation of botulism includes cranial nerve palsies with bulbar dysfunction (dysarthria, ophthalmoplegia, diminished gag reflex, ptosis) and a symmetrical, descending paralysis (head, chest, upper extremity, lower extremities) in an afebrile patient with a clear sensorium and normal sensation. In severe cases, the muscle weakness progresses to respiratory failure. Blurred vision, diplopia, lethargy, lightheadedness, and dry mouth are early symptoms of botulism.[91] Frequently, these patients have local wound infections following SC injection of the heroin. Heating heroin does not inactivate clostridial spores.

Leukoencephalopathy.   Spongiform leukoencephalopathy is a rare disease characterized by spongiform degeneration of white matter following inhalation of heroin vapors via chasing the dragon; this method is one of several etiologies of this nonspecific neurologic disease.[8] Although less common, spongiform leukoencephalopathy also occurs after insufflation of heroin.[92]

Table 31.2. Stages of Heroin-Induced Encephalopathy.

Stage	Clinical Effects
I	Ataxia, akathisias, apathy, soft (pseudobulbar) speech, paucity of movements, memory loss, inattention
II	Choreoathetoid movements, myoclonus, tremor, spastic paresis, increasing ataxia
III	Hyperpyrexia, paresis, "stretching spasms," hyperventilation, mydriasis, decerebrate posturing, dementia, akinetic mutism, altered consciousness

Prominent clinical features of this disease are progressive motor restlessness, apathy, and cerebellar ataxia. The neurologic deficits are frequently irreversible and the clinical course progresses to death. Rarely, resolution of the white matter lesions may occur along with clinical improvement, but periventricular necrosis may develop.[93] In a case series of 47 cases of heroin pyrolysate-induced spongiform leukoencephalopathy, the clinical presentation of these cases was associated with 3 stages as outlined in Table 31.2.[8] Rarely, case reports associate the development of spongiform leukoencephalopathy with IV heroin use in the absence of hypoxic episodes.[94] Changes in mental status characterized the encephalopathy manifest by neurobehavioral changes ranging from forgetfulness and poor attention span to dementia, coma, and death.[95] In contrast to Alzheimer's disease, language and perception are usually preserved. Cerebellar signs are frequently more prominent than cognitive changes. Muscle strength also remains normal, but marked ataxia may cause an unsteady gait.

Stroke.   Cerebral complications of IV heroin use include hemiplegias, aphasias, hemianopsias, and hemipareses.[96] Potential causes of these complications include cerebral vasculitis with secondary thrombus formation, bacterial or fungal emboli from endocarditis, hypoxic encephalopathy with infarct, acute transverse myelitis, and necrotizing angiitis with rupture of arterial aneurysms. Infarction of the globus pallidus occurred following the insufflation of a large dose of heroin that produced respiratory failure, hypotension, seizures, and hypoxic encephalopathy.[97]

RESPIRATORY SYSTEM.   Common respiratory complications of heroin overdose include acute lung injury, aspiration pneumonia, and hypoxia. Heroin-related noncardiogenic pulmonary edema is an infrequent pulmonary complication (i.e., 1–2%) of overdoses from the

IV, and rarely, intranasal administration of heroin.[98] This syndrome involves the development of diffuse pulmonary infiltrates and hypoxia (oxygen saturation <90% with respiratory rate >12/min) following resuscitation from a clinically apparent heroin overdose. The respiratory insufficiency occurs independent of bronchospasm, pneumonia, or pulmonary embolism. Symptoms of respiratory distress are usually apparent within 1 hour of naloxone reversal of heroin-induced coma and respiratory depression.[99,100] The hypoxia typically resolves within 24–48 hours when other pulmonary complications are absent. Clinical manifestations of heroin-related pulmonary edema in addition to hypoxia include cough, pink frothy sputum, tachypnea, and diffuse rales. Case reports indicate that rarely severe bronchospasm may develop following the IV administration of heroin.[101] Although heroin may contain additives that produce hypersensitivity reactions, case reports indicate that pure heroin can cause acute allergic reactions independent of the direct release of histamine.[102] Other pulmonary complications of IV heroin use include septic emboli, lung abscess, pneumonia, and talc-induced pulmonary hypertension.

GASTROINTESTINAL TRACT.   Heroin body packers may develop intestinal obstruction with perforation and fatal peritonitis. Urine drug screens in these patients may be negative despite the presence of intestinal obstruction secondary to the multiple bags of heroin.[103] Other complications of IV heroin use include hepatitis B, hepatitis C, and chronic active hepatitis.

KIDNEY.   Renal failure may occur as a result of rhabdomyolysis and myoglobin-induced acute tubular necrosis from prolonged pressure necrosis of the limbs during heroin-induced coma. Several renal diseases are associated with the IV use of heroin including glomerulonephritis associated with chronic infections (viral, bacterial, fungal), immune-complex-mediated glomerulonephritis associated with bacterial or fungal endocarditis, HIV-associated nephropathy, and secondary amyloidosis.[104] Case-control studies indicate that heroin users have an increased risk of end-stage kidney disease when adjusted for age, sex, race, socioeconomic status, and history of hypertension or diabetes.[105] Case series from the early 1970s reported a heroin-associated nephropathy that initially was recognized as a complication of IV heroin abuse in the eastern United States. This disease occurred primarily among black men aged 18–45 years.[106] Heroin-associated nephropathy is a nephrotic syndrome that is manifest by massive proteinuria, azotemia, hypertension, renal atrophy, and anemia. Renal biopsies usually demonstrate focal and segmental glomerular sclerosis, variable focal and diffuse interstitial nephritis, membranoproliferative glomerulonephritis, and PAS-positive mesangial deposits (IgA, IgG, IgM, C1q, C3).[107] Progression to end-stage renal disease typically occurs within a few months to a few years. The exact role of heroin, adulterants, hepatitis B and C, and HIV in the etiology of this disease remains unclear. As the purity of heroin increased substantially during the 1990s, the incidence of heroin-associated nephropathy dramatically decreased.[108]

MUSCULOSKELETAL SYSTEM.   Acute rhabdomyolysis associated with life-threatening hyperkalemia, hypocalcemia, and myoglobinuric renal failure may develop after prolonged limb compression secondary to heroin-induced coma. Typical clinical manifestations of heroin-induced rhabdomyolysis are muscle pain and swelling.[109] A compartment syndrome may complicate cases of heroin-induced rhabdomyolysis as a result of severe limb swelling. Vascular insufficiency can cause peripheral neuropathies and transverse myelitis. Infectious complications of IV drug use include osteomyelitis, septic arthritis, infectious sacroiliitis, and Pott disease (i.e., vertebral osteomyelitis secondary to tuberculosis or other infectious agents).

IMMUNE SYSTEM.   HIV infection and opportunistic infections are common complications of the IV use of heroin, particularly in heroin addicts who share needles (e.g., "shooting galleries").[110] Common complications of HIV infection includes oral candidiasis, candida- or cytomegalovirus-induced esophagitis, and *Pneumocystis carinii* pneumonia. Other opportunistic infections include disseminated tuberculosis, atypical mycobacteria, histoplasmosis, viral pneumonia, disseminated herpes zoster or simplex, and cryptococcal encephalitis. Dermatologic diseases include Kaposi sarcoma and cutaneous herpes infections.

SKIN.   Acute cutaneous complications of chronic IV heroin abuse include cutaneous infections, necrotizing fasciitis, necrotizing ulcers (e.g., levamisole-contaminated heroin), thrombophlebitis, intra-arterial injection, and mycotic aneurysm.[111] Edema, peripheral ischemia, and compartment syndrome may result from emboli and vasoconstriction. Hypopigmented or hyperpigmented scars result from chronic IV drug abuse including linear cords ("railroad tracks") and irregular round or oval scars ("pop scars"). The latter is frequently associated with the simultaneous injection of heroin and cocaine ("speedballing"). Tattoos ("shooting tattoos") are often used to hide skin discolorations that result from the deposition of debris in SC tissue.

Overdose

The classic triad of opioid overdose is the combination of abnormal mental status, substantially decreased respiratory rate, and miotic pupils. In a study of patients with heroin intoxication treated in an urban emergency department, this triad demonstrated 92% sensitivity and 76% specificity for the diagnosis of acute heroin intoxication; the response to naloxone did not increase the sensitivity for detecting heroin overdose.[16] Most heroin overdoses occur in experienced users in combination with other drugs (benzodiazepines, ethanol, cannabis); repeat overdoses are common in some heroin addicts.[112] Complications include acute respiratory distress syndrome, pulmonary aspiration, hypoxic encephalopathy, sepsis, shock, and death.[113] Most heroin overdoses are relatively benign if the patient receives supportive care. Other clinical manifestations of serious opioid intoxication include cyanosis, hypotension, hypothermia, and bradycardia.

Fatalities

Acute heroin intoxication is a major cause of death among IV heroin addicts along with HIV.[114] Most deaths following the IV administration of heroin occur as a result of hypoxia in regular, dependent users within 1–3 hours after injection.[115,115] Immediate death following heroin administration is less common than death within 3 hours of administration. In a study of 152 heroin-related deaths in Australia, only 14% were associated with instantaneous death following IV heroin administration.[116] The exact cause of heroin-related overdose deaths is unclear, but risk factors include loss of tolerance, concomitant drug use, and the presence of systemic diseases.[117] The use of naltrexone is probably not a risk factor for fatal heroin overdose.[118] Heroin users often are polydrug users, administering tranquilizers, cocaine, and ethanol in addition to heroin.[119,120] Although autopsy studies suggest that multiple-drug use is a major risk factor for adverse effects of acute heroin intoxication including death, data from a prospective cohort study of heroin overdose patients during the late 1990s did not detect an increased number of complications in heroin overdose with and without multidrug use.[121] Rarely, fatal anthrax is associated with heroin abuse.[122]

Abstinence Syndrome

Acute opioid withdrawal from heroin typically involves autonomic hyperactivity with early signs including tachycardia and hypertension. Symptoms include lacrimation, rhinorrhea, diaphoresis, mydriasis, tremor, goose flesh, restlessness, insomnia, irritability, myalgias, anorexia, yawning, abdominal cramps, vomiting, diarrhea , and feverishness. In contrast to patients experiencing ethanol withdrawal, withdrawal from heroin does not typically cause seizures (except in neonates), dysrhythmias, delirium tremens, or death. The physical symptoms of heroin withdrawal typically begin about 4–6 hours after the heroin injection, peak within about 24–48 hours, and persist up to 7–14 days.[123]

Reproductive Abnormalities

Although there is little evidence for the teratogenic effects of heroin use, obstetrical complications result from the maternal use of heroin.[124] Complications of heroin use by pregnant heroin addicts during labor and delivery include premature rupture of membrane, neonatal sepsis, pre-eclampsia, placenta previa hemorrhage, abruptio placentae, and vaginal hemorrhage. Respiratory depression may occur in the neonate. After birth, withdrawal symptoms occur in almost all infants born to addicted mothers including hyperactivity, hyperirritability, coarse tremors, poor feeding, and poor weight gain. Other symptoms of heroin withdrawal include sneezing, yawning, sweating, vomiting, and diarrhea. Seizures also occur in some infants. The differential diagnosis of seizures in infants from heroin-dependent mothers include hypoglycemia, hypocalcemia, and hypomagnesemia.

DIAGNOSTIC TESTING

Analytic Methods

As with most opioid analyses, analysis of heroin in biologic samples requires both screening technologies (e.g., thin-layer chromatography, immunoassays) and confirmatory techniques (e.g., GC/MS, liquid chromatography/mass spectrometry). The extraction of whole blood with organic solvents yields only free morphine, whereas the same extraction after glucuronide hydrolysis yields both free (unconjugated) and conjugated morphine. Although acid hydrolysis of glucuronides is faster and more effective than hydrolysis with β-glucuronidase, 6-monoacetyl morphine is unstable under these conditions.[125] Confirmation techniques usually require extraction of opioids from biologic matrices either by solid-phase extraction (SPE) or liquid-liquid extraction. Most methods now use SPE. Some current radioimmunoassay (RAI) methods are highly specific for free morphine with low cross-reactivity to other opioids and morphine glucuronides.

METHODS

SCREENING.   Although a variety of methods are available to detect opioids in body fluids, including thin-layer chromatography, gas chromatography with flame ionization or electron capture detection and liquid chromatography, immunologic methods are the initial screening test of choice. Immunoassays reliably detect opioids and metabolites in urine specimens above the cutoff specified by Federal Workplace Drug Testing Programs.[126] In general, urine opioid immunoassays are highly sensitive for compounds (e.g., 6-monoacetylmorphine, codeine, dihydrocodeine) that demonstrate structural similarity to morphine, whereas more structurally dissimilar compounds (e.g., fentanyl, meperidine, methadone, oxycodone) are less reactive. The ingestion of some cough syrups (e.g., ethyl morphine), codeine-containing compounds, and paregoric may cause innocent positive opioid drug screens as a result of the presence of opioid compounds.

CONFIRMATORY.   Although a number of methods exist to quantify opioids and their metabolites, liquid chromatography (LC) does not require pretreatment of the analytes of heroin or heroin metabolites. When used with mass spectrometry (MS) as a detector for liquid chromatography, this analytic method (LC/MS) can separate the lipophilic and hydrophilic analytes of heroin and heroin metabolites in biologic fluids.[127] Isotope-dilution GC/MS or LC/MS are the methods of choice for the quantitation of opioids (6-monoacetylemorphine, morphine, codeine) in biologic fluids.[128] Although acidic hydrolysis is more rapid and technically easier than enzymatic hydrolysis for the determination of total (free plus conjugated) opioid compounds, acid hydrolysis of urine samples destroys biomarker (i.e., 6-MAM) for heroin use.[126] Specific analytic techniques for the determination of 6-MAM in urine include gas chromatography/electron impact/mass spectrometry, which has a LOD near 0.001 mg/L.[129] Analytic methods for the detection of codeine and acetyl codeine as biomarkers of illicit heroin use include liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry[130] and liquid chromatography/tandem mass spectometry (LLOQ, 0.005 mg/L, interassay CV <20%).[131]

STORAGE

In experimental studies, morphine and morphine glucuronide metabolites are stable in spiked fresh blood and plasma samples up to 6 months when stored at −20°C (−4°F), 4°C (39.2°F), and 20°C (68°C).[132] Studies indicate that elevated free morphine concentrations develop in postmortem samples as a result of hydrolysis of morphine glucuronides, particularly M3G at room tempera-

ture (e.g., 20°C/68°F) and to a lesser extent during refrigeration (e.g., 4°C/39.2°F). Freshly collected and refrigerated blood samples stored in sodium fluoride containing M3G produce minimal amounts of free morphine, but increased storage time, room temperature, light, and the present of high concentrations of bacteria (e.g., during putrefaction) cause the hydrolysis of M3G to free morphine, particularly in liver tissue.[133] During prolonged interment, case reports indicate that morphine glucuronides are slowly converted to free morphine in buried corpses, particularly after 3–4 years.[134] Consequently, the free morphine/morphine glucuronide ratio varies depending on the degree of putrefaction and the collection site, when compared with the ratio at the time of death. Heroin remains detectable in corpses after embalming, particularly in bile and liver samples.[135] 6-Monoacetylmorphine is stable in frozen urine samples up to at least 2 years,[136] but 6-MAM undergoes deacetylation to morphine at room temperature with the rate of conversion being pH-dependent.

Biomarkers

Heroin is excreted in saliva transiently following IV administration. In a study of 2 volunteers, heroin remained detectable (i.e., >0.001 mg/L) in saliva for ~1 hour.[137]

BILE

Postmortem concentrations of morphine and morphine glucuronides in bile are typically substantially higher than blood with the bile/blood morphine ratio averaging up to ~100–150. In a case series of 5 postmortem examinations with morphine concentrations in both bile and femoral blood (cause of death not specified), the mean morphine concentration in femoral blood was 0.74 mg/L (range, 0.201–1.71 mg/L) compared with 40.86 mg/L (range, 0.477–181 mg/L) in bile.[138] The presence of high concentrations of morphine in bile suggests chronic heroin use; morphine concentrations may persist in bile following heroin use after blood morphine concentrations are nondetectable as a result of enterohepatic cycling. A 44-year-old heroin addict died in the hospital 144 hours after his last heroin injection.[139] The postmortem bile concentration of morphine was 21.3 mg/L, whereas morphine was not detectable in postmortem blood or urine specimens as measured by GC/MS.

BLOOD

Morphine distributes relatively evenly between plasma and erythrocytes.[140] In a study of 20 heroin-related

deaths, the plasma total morphine concentration was higher than total whole blood morphine concentration in 17 of the 20 cases.[141] These cases included deaths both immediately after injection and within a few hours of injection.

ILLICIT USE.   Heroin has a very short elimination half-life in the blood, and most blood samples from heroin users do not contain detectable amounts of heroin. The heroin metabolite, 6-monoacetylmorphine (6-MAM) has a slightly longer mean half-life of 5–20 minutes; 6-MAM is detectable in blood samples for up to 1–2 hours after heroin administration depending on the dose, analytic method, route of administration, and frequency of heroin use. Because of a variety of factors including tolerance, there is substantial overlap in the morphine concentration in functioning heroin addicts and in blood samples from heroin-related fatalities. Data from case series indicate that some serum samples from heroin addicts in police custody contain total morphine concentrations that exceed the median total morphine concentration in series of postmortem whole blood samples from heroin-related fatalities.[142] In a study of 100 current heroin abusers using heroin within the last 24 hours, the median total morphine concentration in blood samples was about 0.090 mg/L (range, 0.050–1.45 mg/L) compared with 0.350 mg/L (range 0.080–3.20 mg/L) in postmortem blood from 39 heroin-related deaths.[143] Although the median postmortem concentration of total morphine in the 39 heroin-related fatalities was substantially higher than the current heroin users, approximately 7% of the blood samples from the current heroin users contained total morphine concentrations exceeding the median postmortem total morphine concentration (0.35 mg/L) of the 39 heroin-related deaths.

Acetyl codeine is an impurity that is present in opium and in almost all heroin samples, but this compound is not present in pharmaceutical preparations of diacetylmorphine. Deacetylation of this compound in the human body produces codeine. Therefore, acetyl codeine and, to a lesser extent, codeine are biomarkers for the use of illicit heroin, and detectable amounts of codeine commonly occur in the blood samples from heroin users. Analysis of plasma samples from impaired drivers testing positive for 6-MAM indicate that a morphine/codeine ratio greater than unity suggests recent heroin use.[144] In contrast to the recent injection of heroin, the morphine/codeine ratio in plasma is usually less than unity following the recent ingestion of codeine. The volumes of distribution for the morphine glucuronides are approximately 1/10 of the volume of distribution for morphine; therefore, the estimation of absorbed dose based on the total morphine concentration in blood is inappropriate.

POSTMORTEM.
Concentrations.   Heroin is a diester compound that is rapidly hydrolyzed to 6-MAM and subsequently to morphine. Postmortem morphine concentrations vary widely in fatal heroin intoxication as a result of a variety of factors including tolerance, the time between exposure and death, use of supportive care, loss of tolerance from a period of opioid abstinence, and coadministration of other drugs. As a result of these factors, there is frequently an overlap between morphine concentrations in fatal and nonfatal heroin intoxications; consequently, all postmortem morphine and morphine glucuronide concentrations must be interpreted in relation to associated clinical and anatomic factors in the case. Although morphine-6-glucuronide (M6G) is biologically active, the major glucuronide metabolite of morphine is morphine-3-glucuronide (M3G), which is inactive as an opioid and does not cross the blood–brain barrier. There are inadequate data to determine the time of death based on the M3G/M6G ratio. Consequently, interpretation of fatal free (unconjugated) morphine concentrations in postmortem blood samples is more reliable than total morphine concentrations (free or unconjugated plus conjugated morphine). In a study of 87 heroin only-related deaths, the median free morphine and total morphine concentrations in postmortem heart blood samples were 0.170 mg/L (range, nondetected to 2.80 mg/L) and 1.032 mg/L (range, 0.034–4.66 mg/L), respectively, as analyzed by GC/MS with solid-phase extraction.[145] By comparison, the median free morphine concentration in 5 survivors with no other drugs in their blood was 0.065 mg/L (range, 0.028–0.093 mg/L). Free morphine concentrations in postmortem blood exceeding 0.20–0.30 mg/L are often fatal.

When present, 6-MAM concentrations in postmortem samples of femoral blood are usually approximately one order of magnitude lower than free morphine concentrations.[85] Concentrations of 6-MAM and morphine do not correlate well to the time between heroin administration and death.[85] Factors that favor immediate death after heroin administration include detectable concentrations of 6-MAM in postmortem blood, high free morphine concentrations (>0.5 mg/L), high ratio (>0.5–0.6) of free to total morphine, and low urinary concentrations of 6-MAM and free morphine.[146] The morphine glucuronides (M3G, M6G)/morphine ratio increases as the time between administration and blood sampling increases, depending on individual pharmacokinetic parameters (e.g., elimination half-lives, volumes of distribution).[147] This ratio is not dependent on dose. The presence of low free morphine concentrations (<0.2 mg/L) in postmortem blood, total blood morphine >1.0 mg/L, and ratios of free morphine/morphine glucuronides and free morphine plus M6G/M3G in

postmortem blood <1 suggest delayed death (i.e., >3 hours).[148] Codeine frequently occurs in the postmortem blood samples of heroin-related fatalities because codeine is a metabolite of acetyl codeine formed during heroin synthesis. The morphine/codeine ratio in postmortem blood samples is wide, but the ratio is always >1 after use of codeine alone; a morphine/codeine ratio <1 indicates a source of codeine in addition to heroin.

Redistribution.   A several-fold difference in morphine, morphine-3-glucuronide, and morphine-6-glucuronide postmortem blood concentrations may occur between different sampling sites depending in part on the time between injection and death, chemical stability of morphine conjugates, hemoconcentration, diffusion between different compartments, and incomplete distribution of the drug.[149,150] In a study of 32 morphine-related deaths (i.e., mostly IV administration of heroin), the postmortem free morphine concentration in peripheral and central sites did not vary significantly with time based on an average time between death and autopsy <36 hours as measured by a specific radioimmunoassay for free morphine.[151] However, morphine concentrations were consistently higher (i.e., average about double) in heart blood compared with femoral or iliac sites. In this study, the difference between morphine concentrations in central and peripheral samples usually increased as the blood morphine concentration increased, particularly when morphine concentrations in heart blood exceeded 0.30 mg/L. However, there were relatively few samples and no statistical analysis of the trend. Similarly, in a postmortem study of 21 cases with matched postmortem admission and autopsy blood specimens, there was no statistically significant difference between matched samples for concentrations of free morphine, total morphine, M3G, or M6G.[152] The mean postmortem interval was 59 hours. The total morphine concentration in postmortem samples from the femoral vein and the femoral artery is usually similar. In a study of 44 suspected heroin-related deaths with detectable postmortem morphine concentrations, the mean free morphine concentration in femoral vein samples was 0.17 mg/L (95% CI: 0.12–0.22 mg/L) compared with 0.20 mg/L (95% CI: 0.13–0.27 in femoral artery samples.[153] In a study of 50 individuals dying of heroin overdose soon after injection based on urine free morphine concentrations <0.025 mg/L, the mean heart to peripheral blood ratio was 1.4 (range, 0.4–6.9).[154] The ratio of free morphine in peripheral and heart blood does not predict the time between heroin administration and death. Although the metabolism of heroin is too rapid to detect increased concentrations of heroin metabolites in the veins near the injection site, morphine concentrations may remain elevated in the tissue surrounding the site of the fatal injection.[155] The interpretation of the significance of free morphine concentrations requires consideration of storage methods and the time interval between death and analysis because of the hydrolysis of M3G under some storage conditions.[132]

Hair

Hair analysis for illicit drugs potentially provides long-term information about drug use. Heroin and 6-MAM are frequently detectable in hair samples from regular heroin users. However, there are limited data on the length of time heroin and 6-MAM remain detectable in hair samples and on the amount of heroin necessary to produce positive hair samples for heroin or 6-MAM. Following regular heroin use, 6-MAM concentrations in hair samples predominate over heroin and morphine concentrations.[156] However, the dose of administered heroin does not correlate to heroin or 6-MAM concentrations in hair samples collected from the proximal root zone (i.e., about 100 days of hair growth).[157] The presence of morphine/codeine hair ratios >5:1 for low-morphine concentrations (<1 ng/mg hair) or >2:1 for morphine hair concentrations (>1 ng/mg) suggests the use of heroin or morphine.[158] A number of variables complicate the interpretation of the heroin dose based on 6-MAM concentration in hair or route of exposure based on the heroin/6-MAM ratio including site of sampling, technique, and distance from skin.[159] Highly alkaline extractions may degrade heroin and 6-MAM to morphine.[160]

Stomach

Postmortem stomach morphine concentrations are not reliable measurements to distinguish between the IV and oral administration of heroin, probably as a result of postmortem diffusion of morphine from bile in the duodenum to the stomach following the hydrolysis of conjugated morphine present in the bile. In a study of 29 fatal IV heroin overdoses, the mean total morphine concentrations in blood and stomach contents was 0.60 mg/L and 1.16 mg/L (mg/kg), respectively.[161] Detectable morphine concentrations were present in the stomach contents of all 29 cases; the total morphine concentrations in stomach contents were higher than total blood morphine in 83% of the cases.

Urine

6-Monoacetylmorphine.   Analysis of urine samples documents exposure to illicit drugs, but the concentration of specific drugs in the urine do not correlate to

blood concentrations or to impairment. Urine immuno-assays are the most common screening methods for the detection of opioids. Heroin is not usually detectable in urine as a result of the rapid hydrolysis of this drug. Hydrolysis of heroin produces 6-monoacetylmorphine (6-MAM), which is a specific biomarker of heroin use.[162] Although 6-MAM has a short half-life in the blood, this compound is present in urine samples for at least several hours (i.e., first voided urine specimen) after the use of heroin as measured by GC/MS.[163] The CEDIA Heroin Metabolite Immunoassay (Microgenics™, Fremont, CA) is a specific immunoassay for 6-MAM with a confirmation rate of 98% in a study or 525 samples from a criminal justice drug-testing program.[164] Cross-reactivity with opioid analgesics occurred only at high concentrations including morphine at 10 mg/L, oxycodone at 61 mg/L, codeine at 60 mg/L, hydromorphone at 10 mg/L, hydrocodone at 60 mg/L, and pentazocine at 35 mg/L. The cutoff for 6-MAM was 0.01 mg/L. In contrast, morphine is the target analyte for the CEDIA DAU opioid assay with cross-reactivity with other structurally similar opioids including 6-MAM (81%), codeine (125%), hydrocodone (48%), and hydromorphone (57%).[165]

The detection time for 6-MAM and total morphine depends on a variety of factors including the LLOQ of the analytic method, the specific cutoff, the heroin dose, the time since the bladder was emptied, rate of urine formation, and frequency of heroin use. Additionally, there is substantial interindividual variation in the excretion of total morphine after the administration of similar doses of heroin. Consequently, following the chronic use of heroin, the occurrence of a positive urine opioid drug screen within 24 hours following a negative opioid drug screen does not necessarily imply the use of heroin between the two specimens.[166] If the urine sample is collected immediately after heroin use, the urine sample may contain 6-MAM without detectable amounts of some morphine metabolites (e.g., M3G).[167] As a result of individual pharmacogenetics, certain heroin-using individuals may escape detection when urine screening involves only the detection of morphine at the current cutoff.[168]

ACETYL CODEINE. Acetyl codeine is one of the alkaloids present in opium; therefore, this compound occurs in almost all (i.e., about 95%) of heroin samples. The plasma elimination half-life of acetyl codeine is about 4 hours with <1% appearing in urine unchanged.[169] Peak urine concentrations occur ~2 hours after heroin administration. Using GC/MS with a LLOQ of 0.0002 mg/L, acetyl codeine was detectable for about 8 hours after administration. The coadministration of heroin and codeine may result in the formation of detectable concentrations of acetyl codeine.

FREE AND TOTAL MORPHINE. In 1998, the Federal Workplace Drug Testing Program changed urine screening and confirmation cutoff concentrations for opioid testing from 0.300 mg morphine/L to 2.000 mg morphine/L. In addition, positive urine samples require the presence of 6-MAM in concentrations ≥0.001 mg/L as measured by GC/MS. Although this new cutoff decreases detection times, these requirements were necessary to reduce the innocent positive rates for individuals ingesting poppy seed-containing foods. Detection times in urine for heroin use via IV and pulmonary routes are similar.[170] In volunteer studies, the administration of 3–12 mg heroin hydrochloride via inhalation or injection resulted in similar detection times (i.e., ~12 hours) as determined by immunoassay screening and confirmation by the 2.000 mg/L cutoff for morphine and 0.001 mg/L cutoff for 6-MAM.[171] The median detection time (i.e., time to last positive sample) for total morphine after inhalation and IV administration of ~12 mg heroin for the low cutoff (0.300 mg/morphine/L) and the high cutoff (2.000 mg/L) was about 34 hours (range, 10.7–53.5 h) and about 8 hours (range, 2.3–22.3 h), respectively.[67] Changes in urine osmolality can cause fluctuations above and below the cutoff, resulting in positive screens following negative results. Following intramuscular (but not inhalation or IV) administration of heroin, the detection time for free morphine in the urine is substantially shorter than for total morphine. In a study of healthy volunteers administered 3 mg- and 6 mg-doses of heroin intramuscularly, the mean detection time for the 0.300 mg/L cutoff of free and total morphine in urine samples was about 3½ hours and 8 hours, respectively.[68] Although the intranasal route of heroin use produces ~50% of the potency of an intramuscular dose, the pharmacokinetic profiles of the two routes of exposure are similar. Using larger heroin doses (i.e., 6 mg and 12 mg) than the previous study, detection times for total urine morphine by immunoassay and GC/MS confirmation at the 0.300 mg morphine/L- and 2.000 mg/L-cutoff concentrations were about 24–36 hours and <12 hours, respectively.[172] However, the detection times for 6-MAM were highly variable and short with detection times for the ≥0.010 mg/L-cutoff averaging about 2–3 hours (range, 0–6.5 hours) following intranasal administration. Most commercial opioid immunoassays (e.g., Emit® d.a.u.™ Opiate Assay, Syva Company; TDx® Opiates, Abbott Laboratories, Abbott Park, IL; Abuscreen® Radioimmunoassay for morphine, Roche Diagnostic Systems, Inc., Nutley, NJ) detect both free and conjugated morphine as well as free and conjugated codeine. These tests do not distinguish between free and conjugated drug, but codeine use is usually associated with total codeine concentrations exceeding total morphine concentrations.[173] The Coat-A-Count®

Morphine in Urine immunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, CA) is much more specific for free morphine with little cross-reactivity with morphine glucuronides or codeine.[174] Total morphine concentrations in postmortem urine samples from heroin addicts found dead after IV heroin use may be undetectable despite β-glucuronidase or acid hydrolysis as a result of death prior to the excretion morphine and morphine metabolites in the urine.[175]

Cross-Reactivity/False-Positive Urine Opioid Screens. A few compounds cross-react with common opioid urine screening assays including rifampin (i.e., kinetic interaction of microparticles in solution method)[176] and some quinolone compounds (gatifloxacin, levofloxacin, ofloxacin).[177,178] Innocent positive opioid urine screens following ingestion of poppy seeds results from the detection of small amounts of opiates; however, recent reductions in the morphine content of poppy seeds used in foods (e.g., pretreatment of seeds with washing and grinding) and the higher total morphine cutoff have decreased the risk of innocent positive opioid screens, particularly following the ingestion of poppy seed buns.[25] The ingestion of poppy seeds from 1–2 bagels can potentially produce a positive urine drug screen for opioids up to 24 hours after ingestion *depending* on the cutoff.[23] In a study of 3 volunteers consuming as many poppy seeds as possible within approximately 1 hour, the urine drug screen was positive (2.000 mg/L cutoff) up to 4 hours after ingestion in all 3 volunteers and up to 8 hours in one volunteer.[179] Peak urine concentrations of opioids occur within 6–8 hours of ingestion. However, poppy seeds do not contain the synthetic chemical, 6-MAM,[180] and the ingestion of poppy seed-containing food typically does not produce detectable amounts of free morphine in serum samples as analyzed by GC/MS before hydrolysis.[181] There is substantial variation in the content of codeine and morphine in different poppy seeds as well as substantial interindividual variation in urinary excretion of codeine and morphine. Consequently, the presence of a positive urine drug screen after ingesting poppy seeds is highly variable. Other opiates present in poppy seeds based on thin-layer chromatography include thebaine, papaverine, and narcotine.[182] Thebaine is not usually present in the urine of heroin abusers, whereas the consumption of poppy seeds typically produces low concentrations of thebaine detectable in urine up to 36 hours, depending on the dose and individual excretion profile.[183] The absence of thebaine in a urine sample positive for opioids does not exclude the possibility of poppy seed ingestion, particularly when the urine sample is collected 12–24 hours after consumption.

Vitreous Humor

In general, postmortem vitreous humor samples contain substantially lower (i.e., 2- to 3-fold) morphine concentrations than postmortem blood samples following fatal heroin overdoses.[184] In a case series of 10 heroin-related deaths, the mean free morphine concentration in vitreous samples as measured by radioimmunoassay was 0.08 ± 0.04 mg/L compared with 0.32 ± 0.17 mg/L in blood (site not specified).[185] However, the 6-MAM concentration is usually higher in vitreous humor than in blood during postmortem examination of heroin-related deaths.[186] In a study of 25 deaths associated with heroin use, vitreous samples from all 25 cases had detectable concentrations of 6-MAM, whereas only 13 of 25 blood samples were positive for 6-MAM.[187] The mean 6-MAM vitreous humor/blood ratio in these 13 samples was 11.3 (range, 1.7–27). The wide range of values and the lack of data limits correlation of postmortem vitreous humor to blood concentrations following fatal heroin intoxication. Furthermore, there is a lack of correlation between 6-MAM concentrations in urine, vitreous humor, and cerebrospinal fluid.[188] Postmortem cerebrospinal fluid typically contains slightly higher concentrations of morphine compared with vitreous humor, but the morphine concentrations are substantially less than postmortem blood.[189] Because hydrolysis of 6-MAM continues in blood after death, analysis of 6-MAM in vitreous humor and cerebrospinal fluid samples provide an alternative to urine samples for the postmortem detection of heroin use.

Abnormalities

Blood

Numerous laboratory abnormalities occur in IV drug addicts resulting from a variety of diseases including hepatitis, osteomyelitis, cutaneous abscesses, thrombocytopenia, septicemia, endocarditis, tetanus, botulism, tuberculosis, malaria, renal dysfunction, and HIV infections. Laboratory manifestations of heroin-induced rhabdomyolysis include hyperkalemia, hypocalcemia, metabolic acidosis, and increased serum creatinine concentrations. A 5-fold increase in serum creatine kinase concentrations without evidence of cardiac and cerebral damage indicates the presence of rhabdomyolysis. Elevation of serum and urine myoglobin concentrations also suggests the presence of muscle injury, but the rapid clearance of serum myoglobin limits the usefulness of this laboratory abnormality as a measure of ongoing muscle injury.[190]

Cardiac

Acute heroin overdose is associated with nonspecific electrocardiographic changes (e.g., nonspecific ST-T

wave changes, sinus tachycardia, right atrial enlargement), frequently related to hypoxia.[191] More serious dysrhythmias (atrial fibrillation, ventricular tachycardia) occur occasionally in the absence of adulterated heroin (e.g., quinine) usually as a result of acute hypoxia. In contrast to methadone, unadulterated heroin is not usually associated with QT_c prolongation or torsade de pointes. In a study of 15 patients admitted to methadone treatment program after using heroin within the previous 4 hours, 2 patients had increased QT_c intervals as defined by Ashman-Hull criteria.[192] Seven additional patients had bradycardia or tachycardia. By comparison, QT_c prolongation was present in 14 (34%) of the 41 patients on methadone in the treatment program.

ENCEPHALOPATHY

Spongiform leukoencephalopathy differs from other encephalopathies by the histopathologic hallmark of vacuolar degeneration of cerebral white matter. Electron microscopy of the white matter demonstrates fluid entrapment between the myelin lamellae and an absence of demyelination. Heroin-induced toxic leukoencephalopathy is a bilateral, symmetrical disease primarily of white matter; therefore, magnetic resonance imaging (MRI) is the noninvasive diagnostic study of choice because of superior resolution of white matter compared with computerized tomography (CT).[193,194] The head CT scan is usually normal until late in the course of this disease. The lesions typically involve the white matter of cerebellum, cerebrum, and midbrain with selective involvement of the corticospinal tract, the solitary tract, and the lemniscus medialis.[195] Characteristic abnormalities on T2-weighted MRI include symmetric hyperintense lesions of the cerebellar and occipital white matter, corpus callosum, internal capsule, and the medial lemniscus of the brain stem.[196,197] Gray matter is typically preserved with occasional involvement of deep gray matter.[198]

The lack of involvement of the cerebellum in a clinically normotensive patient suggests a diagnosis other than heroin-induced toxic leukoencephalopathy, as spongiform toxic leukoencephalopathy excluding cerebellar and brainstem involvement is a rare complication of the IV or nasal use of heroin.[92] Changes in the frontal lobe are usually minimal to mild. T1-weighted scans demonstrate corresponding hypointensities, and IV gadolinium does not usually enhance the lesions.[94] Restricted diffusion in the cerebral white matter on diffusion-weighted images helps distinguish heroin-induced toxic leukoencephalopathy from other encephalopathies.[199] MR spectroscopy may help differentiate heroin leukoencephalopathy from other encephalopathies associated with drug abuse (e.g., cocaine) or demy-elinating diseases. Demyelination does not typically occur in heroin-induced toxic leukoencephalopathy. The pathologic process of this encephalopathy involves disruption of cellular metabolism, mitochondrial dysfunction, and loss of neurons with gliosis manifest by increased lactic acid and myo-inositol, normal to slightly decreased choline, normal lipid peak, and decreased N-acetyl aspartate and creatine.[200] The reduction of choline and N-acetyl aspartate suggests the presence of axonal injury.

PULMONARY

Radiographic abnormalities following heroin overdose include pulmonary edema, aspiration pneumonia, pulmonary emboli, cardiomegaly, cardiac failure from valvular disease, and rarely hyperaeration from bronchospasm. Heroin-related pulmonary edema typically presents with diffuse, bilateral, fluffy infiltrates that appear symmetric. Occasionally, unilateral or lobar infiltrates may appear, and the pulmonary edema may shift rapidly from lobe to lobe and from one lung to the other.[201] Radiographic evidence of pulmonary edema usually resolve within 24–48 hours. Other pulmonary complications of IV drug use include bronchopneumonia and lung abscesses.

Driving

In blood samples from 5 drivers arrested for driving under the influence, the median free and total morphine concentrations were 0.065 mg/L (range, 0.028–0.093 mg/L) and 0.918 mg/L (range, 0.230–1.451 mg/L), respectively.[132] No other drugs were detected in these blood samples. The presence of 6-MAM indicates the recent use of heroin. When 6-MAM is detected in urine samples from drivers suspected of driving under the influence, urine concentrations of free morphine usually exceed 1 mg/L. Analysis of 212 6-MAM-positive urine specimens from drivers apprehended for driving under the influence of drugs, 92% of these specimens contained morphine concentrations exceeding 1 mg/L as measured by isotope-dilution gas chromatography/mass spectrometry.[202] In an experimental study of 10 heroin addicts, the administration of 25 mg, 50 mg, and 100 mg doses of heroin via inhalation produced a dose-dependent decrease in the simple reaction time as demonstrated in Figure 31.7.[46] The decline in simple reaction times began about 30 minutes after inhalation with peak reduction occurring about 30–60 minutes after inhalation began.

Experimental studies in heroin addicts receiving up to 95 mg IV daily in an observation ward demonstrated that the IV administration of heroin reduced activity and
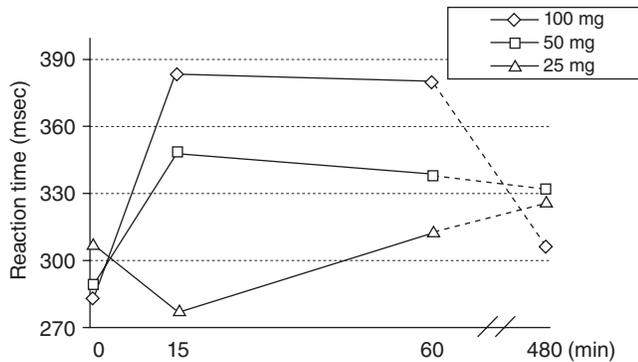
FIGURE 31.7. Mean decline in simple reaction times of 5 volunteer addicts administered various doses of heroin via inhalation. (Reprinted from European Neuropsychopharmacology, Vol. 11, Issue 3, VM Hendriks, W van den Brink, P Blanken, IJ Bosman, JM van Ree, Heroin self-administration by means of 'chasing the dragon': pharmacodynamics and bioavailability of inhaled heroin, p. 250, copyright 2001, with permission from Elsevier.)

responsiveness to ambient stimuli.[203] However, there was no statistically significant decline in performance on pursuit rotor testing when compared to testing during the daily administration of one IV dose of 8 mg heroin.

TREATMENT

Stabilization

The classic hallmarks of heroin intoxication include evidence of heroin use and altered consciousness along with depressed respiratory rate and miotic pupils. Although pinpoint pupils usually occur during acute heroin intoxication, the presence of severe hypoxia and metabolic acidosis may alter the pupil size. Initial treatment of acute heroin intoxication involves the assessment of ventilation and perfusion. Patients with adequate ventilation and oxygen saturations >91–92% can be observed with cardiac monitor and pulse oximetry (capnography if available) until normal level of conscious returns. Patients with inadequate ventilation require bag-value-mask ventilation with 100% oxygen and the administration of naloxone 0.2–0.4 mg IV. Although the rate of SC absorption of naloxone is slower than the IV route, difficulty obtaining IV access may result in similar onset of action via these two routes of administration, at least in the prehospital setting.[204] Atomized intranasal naloxone is available in some emergency settings.

If no improvement occurs within 3–5 min, additional doses (0.4 mg) of naloxone should be administered parenterally titrated to the maintenance of adequate ventilation. Criteria for endotracheal intubation include

inability to ventilate with bag-valve-mask, poor oxygenation (i.e., oxygen saturation <90%) despite adequate ventilation and supplemental oxygen, and persistent hypoventilation after doses of naloxone exceeding 2–4 mg. If patients with acute heroin intoxication ingested oral opioid compounds, larger doses of naloxone or a naloxone drip may be required. Otherwise, the lack of an adequate response to naloxone indicates hypoxic brain damage or another diagnosis (head trauma, sepsis, meningitis, aspiration pneumonia). If pulmonary signs or symptoms (e.g., cough, tachypnea, pink frothy sputum, rales) of pulmonary edema develop, the patient should receive a chest x-ray to detect pulmonary complications of acute heroin intoxication including acute pulmonary edema and aspiration pneumonia.

A majority of patients with heroin-associated pulmonary edema require only oxygen supplementation. In a case series of 27 patients with pulmonary edema after resuscitation from heroin overdose, about one-third of the patients required mechanical ventilation; most of these patients were extubated within 24 hours. Patients with other complication (e.g., aspiration pneumonia) in addition to pulmonary edema may need intubation including pressure-cycled ventilation for longer periods. Hypotension from heroin intoxication usually responds to fluids and naloxone unless sepsis is present.

Gut Decontamination

Methods to prevent absorption of heroin are not usually necessary unless the patient is a body packer (See Supplemental Care, Body Packing).

Elimination Enhancement

Methods to enhance elimination (e.g., hemodialysis) are not indicated for heroin intoxication. The use of dialysis is limited to the presence of renal failure and hyperkalemia, usually as a result of rhabdomyolysis.

Antidotes

Naloxone, a pure narcotic antagonist, is the antidote of choice for acute heroin intoxication. The initial dose of naloxone usually should not exceed 0.4–0.8 mg IV because of the risk of producing withdrawal in opioid-dependent patients. Larger doses of naloxone should be titrated to effect when the clinical response is inadequate. After restoration of adequate ventilation, repeat doses of naloxone are necessary only for recurrent, clinically significant hypoventilation. Mild sedation may recur after the administration of naloxone following heroin intoxication, in part as a result of the formation of the naloxone metabolite, 6-α-naloxol, which pos-

sesses agonistic and antagonistic effects on opioid receptors. Although μ-opioid receptors mediate respiratory depression, both μ- and κ-opioid receptors mediate sedation. Hence, persistent κ-opioid stimulation by 6-α-naloxol may cause recurrence of sedation, but not respiratory depression.

Supplemental Care

Laboratory testing in patients with prolonged coma from acute heroin intoxication include analysis of the blood for complete blood count (CBC), electrolytes, creatinine, hepatic aminotransferases, and creatine kinase. The presence of rhabdomyolysis (serum creatine kinase >1,000 IU) indicates the need for generous fluid replacement with monitoring of renal function and serum potassium. Urine alkalinization is occasionally recommended in the older medical literature, but the efficacy of this method is unclear.

Acute Intoxication

Complications of an IV heroin overdose are usually clinically apparent soon after treatment begins with naloxone. Based on retrospective studies, patients requiring observation for >3–4 hours had intoxication by other drugs (ethanol, methadone, propoxyphene) or medical problems (e.g., pneumonia, pulmonary edema, hypoxic encephalopathy).[205] Clinical characteristics of patients with an adequate response to naloxone and stable medical condition include Glasgow Coma Scale of 15, heart rate 50–100 beats/min, temperature 35.0–37.5°C (95–99.5°F), oxygen saturation >92%, and respiratory rate 10–20 breaths/min.[206] Consequently, following an IV heroin overdose, patients with normal mental status and normal physical examination 4 hours after the administration of naloxone may be discharged.

Body Packing

Figure 31.8 outlines a treatment plan for heroin body packers. In contrast to body packers (swallowers, couriers, mules, internal carriers), body stuffers swallow small amounts of loosely wrapped drugs, usually to avoid arrest by police. Typically, body packers carry about 1 kg of heroin divided into 50–100 packets of about 10 g each.[207] Although previous packets were loosely wrapped, current smuggling of illicit drugs involves an automated process that densely packs the drug into a latex sheath (condom, balloon) covered with several layers of latex and sealed with a hard wax coating.[208] These processes also use aluminum foil, carbon paper, plastic food wrap, or other material to alter the radiodensity of the packet. Frequently, the body packers ingest

constipating agents (loperamide, diphenoxylate) prior to transport with unaided passage of the packets being delayed 1–2 days to 2–3 weeks.[209]

The radiologic detection of heroin packets depends on the number, density, air-packet interface, size, and position in the GI tract. Plain abdominal x-rays are usually sensitive to the presence of heroin packets by demonstrating oval or round soft tissue densities highlighted by a gas halo or direct visualization of the packets. Case reports suggest that the use of contrast-enhanced plain abdominal radiography or contrast-enhanced CT increases the sensitivity and specificity of abdominal imaging for the detection of heroin packets.[210] There are few data on the sensitivity and specificity of CT for the detection of heroin packets. In a study of 124 body packers who passed heroin packets, noncontrast abdominal and pelvic CT detected heroin packets in all 124 body packers, whereas 2 of the 42 body packers undergoing abdominal plain x-rays had false-negative results.[211] However, case reports suggest that contrast-enhanced CT may miss a single packet of heroin, particularly homogeneous isodense packets.[212] The latter packets have Hounsfield units (HU) in the range of −40 to 30 HU compared with water (0–20 HU) and soft tissue (40–60 HU). Altering the typical window width and level settings for abdominal CT imaging to 600–800 HU and −150 to 300 HU, respectively, improves the sensitivity of noncontrast CT for the detection of isodense packets.[211] Opium packets are less dense than heroin packets, typically in the range of 150–200 HU. In a preliminary study of 12 opium body packers, noncontrast CT detected packets in all 12 body packers.[213] The density of these packets ranged from 127–300 HU. The use of contrast-enhanced plain abdominal radiography may interfere with subsequent imaging of the abdomen with CT scans.

There are few data on the sensitivity of ultrasound for the detection of packets of illicit drugs, but in general, both ultrasound and MRI are not sensitive techniques for the detection of heroin packets in the GI tract.[214] The progress of the packets along the GI tract can be monitored by the use of amidotrizoate-meglumine (Gastrografin, 0.9 mL/kg) and plain abdominal films taken at least 5 hours after ingestion of the contrast if CT scans of the abdomen are not indicated.[215]

The lack of sensitivity and specificity of urine drug screens for the detection of body packing limits the usefulness of the urine drug screen for determining the presence of heroin packets in the GI tract. Heroin packets are frequently not accessible to endoscopy and the risk of breakage limits the usefulness of this removal technique. Oral feeding should be restricted until the packets pass into the large intestine. Depending on the size of the packets, the use of syrup of ipecac may be complicated by esophageal obstruction.[216] Alternatives

567
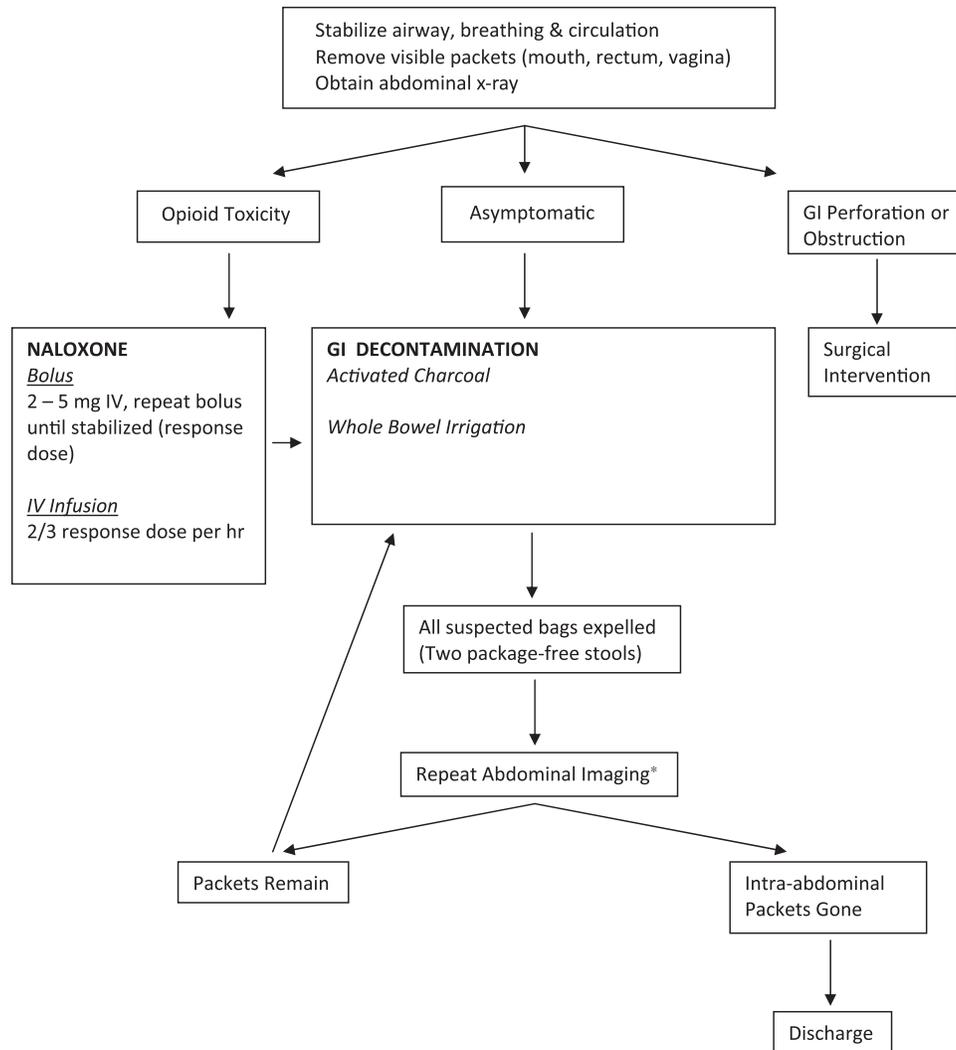
FIGURE 31.8. Treatment algorithm for heroin body packers. *Contrast-enhanced abdominal computed tomography or contrast-enhanced plain abdominal radiography. IV = intravenous; PO = oral; NG = nasogastric.[210]

to the use of whole bowel irrigation include the administration of bisacodyl suppositories (Dulcolax®, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT), activated charcoal mixed with 3% sodium sulfate cathartic, and phosphosoda enemas after passage of the packets into the large intestine.[217] Oil-based laxatives are not recommended because of the potential of these laxatives to reduce the tensile strength of the packets. There are theoretical concerns that the administration of polyethylene glycol solution can increase the GI absorption of heroin either by solubilizing heroin released from packets or suspending the heroin in solution and increasing the surface area; however, there are inadequate clinical data at the present time to determine the clinical significance of these concerns or the efficacy of these 2 regimens. Confirmation of the elimination of packets by contrast-enhanced CT or contrast-enhanced plain abdominal radiography should be done after decontamination measures and following the passage of 2 or 3 stools without packets. Multiple stools devoid of packages or an absence of symptoms after 24 hours of observation are reasonable endpoints for observation of these patients. Rare patients may have retained packets despite negative abdominal imaging.[211]

ADDICTION

Medical detoxification of heroin addicts involves the substitution of long-acting agonists (methadone, buprenorphine) or α_2-adrenergic agonists (clonidine, lofexidine) for heroin as well as psychosocial treatments (behavioral modification, counselling, family therapy).[218,219] Chronic heroin use produces relative endogenous opioid deficiencies as a result of downregu-

lation of opioid production. This opioid deficiency creates an intense craving that causes a preoccupation with obtaining opioids rather than pursuing more productive interests. Methadone relieves these narcotic cravings. Levomethadyl acetate (LAAM) is also an alternative to methadone and buprenorphine for the treatment of heroin addiction, but the concerns about the cardiotoxicity (i.e., torsade de pointes) associated with high doses of LAAM limits the use of this agent.[220] Although ultrarapid or anesthesia-assisted opioid withdrawal and antagonist induction procedures are promoted as relatively symptom-free alternatives to more traditional methods of withdrawal, there are inadequate data to justify the risks associated with these procedures. A randomized clinical trial of 106 healthy (American Society of Anesthesiologists physical status I or II) treatment-seeking, heroin-dependent patients compared 1) anesthesia-assisted, rapid opioid detoxification/naltrexone induction; 2) buprenorphine-assisted, rapid opioid detoxification/naltrexone induction; and 3) clonidine-assisted opioid detoxification with delayed naltrexone induction.[221] The mean withdrawal scores were similar between the 3 groups, and the treatment retention rates at 12 weeks were not statistically different between the 3 groups (20%, 24%, 9%, respectively). There were 3 potentially life-threatening adverse events during the anesthesia procedure.

METHADONE. Methadone is a long-acting μ-opioid receptor agonists that produces peak blood concentrations in about 2–4 hours with a serum elimination half-life of about 24–36 hours. Opioid withdrawal frequently complicates the hospitalization of opioid-dependent addicts for medical illnesses. During inpatient opioid detoxification programs, heroin addicts usually receive an initial daily methadone dose of 5–20 mg orally in 5-mg increments depending on the severity of the withdrawal symptoms and the size of the heroin habit; subsequently, an additional 5–10 mg methadone is added as required to control abstinence symptoms and until a stabilizing dose is reached. Typically, control of abstinence symptoms in hospitalized heroin addicts respond to daily methadone doses of 10–40 mg; however, some highly dependent heroin users require substantially higher doses. Because of the long duration of action and the difficulty determining the appropriate methadone dose based on history, the methadone dose during the first 24–48 hours must be titrated carefully. After about 2–3 days, the methadone dose is tapered ~5 mg/day or 20% per day. Adverse effects associated with methadone use include constipation, irregular menstrual periods, decreased libido, weight gain, and diaphoresis. For maintenance programs, daily methadone doses begin at 25–35 mg with adjustment of the dose to allevi-

ate withdrawal. Typically, the lowest dose of methadone effective for maintaining a heroin addict is about 60 mg with the dose usually increased gradually to 80–120 mg/day.[222] In general, a trough (i.e., 24-h postdose) serum methadone concentration of 0.4 mg/L correlates to adequate methadone dosing, although symptoms of withdrawal may occur in some heroin addicts despite maintenance doses that produce serum methadone concentrations near these trough concentrations.[223]

A major problem with brief opioid detoxification programs is the extremely high rate of relapse to illicit heroin use. Although maintenance therapy with naltrexone is effective in a minority of highly motivated heroin addicts, poor compliance with this regimen limits the efficacy of naltrexone in most opioid addicts.[224] The daily dosage of methadone administered to pregnant women ranges from 10–90 mg with a mean of about 50 mg. Approximately 60% of infants born to women on methadone maintenance demonstrate opioid withdrawal with the severity of these symptoms depending on the maternal methadone dose.[222] Mixed agonist-antagonists (e.g., nalbuphine, butorphanol, pentazocine) should be avoided because of the potential of these drugs to cause opioid withdrawal symptoms. The administration of rifampin, carbamazepine, isoniazid, barbiturates, and phenytoin also reduce the plasma methadone concentrations; therefore, the use of these two drugs with stable methadone doses may precipitate opioid withdrawal. Although dysrhythmias do not usually occur in patients treated with methadone, a case series of 3 patients administered >600 mg daily[225] and a case series of 17 patients (i.e., mean daily dose 397 ± 283 mg)[226] associated torsade de pointes with high-dose methadone therapy. All these patients had markedly prolonged QTc intervals (mean 615 ± 77 msec for series of 17 patients), and most of these patients had predisposing factors (coronary heart disease, hypokalemia, bradycardia) for the development of dysrhythmias. Because of the long half-life of methadone, withdrawal symptoms from the abrupt cessation of methadone treatment beings within 36–72 hours. Peak symptoms methadone withdrawal peak about 4–6 days after cessation of treatment, and the withdrawal symptoms resolve over the subsequent 10–12 days.

BUPRENORPHINE. Buprenorphine is probably more effective than clonidine for the treatment of heroin addiction; the use of buprenorphine may offer some advantages over methadone. A Cochrane review of buprenorphine and methadone suggested that the severity of heroin withdrawal during the use of buprenorphine and methadone was similar, but symptoms resolved more quickly with buprenorphine and patients were more likely to complete withdrawal

569

treatment with buprenorphine.[227] However, there are limited data comparing these 2 treatments and more studies are needed to confirm these preliminary observations. Buprenorphine is a partial opioid agonist-antagonist that requires sublingual administration because of poor oral bioavailability. This drug is a partial μ-opioid receptor agonist and a weak κ-opioid receptor antagonist that has less depressant effects on respiration and no significant effect on the QT_c interval. As the dose of buprenorphine increases, this drug behaves more like an antagonist; withdrawal symptoms can occur, particularly in highly dependent, heroin addicts (i.e., >60 mg methadone/daily). The opioid-antagonist activity limits the maximal analgesic effects as well as respiratory depression associated with buprenorphine use. In the United States, the approved sublingual drug is marketed as Suboxone® (Reckitt Benckiser, Berkshire, UK) in 2 mg and 8 mg tablets combined with naloxone 0.5 mg and 2 mg, respectively. Because the absorption of oral naloxone is poor, the presence of naloxone does not cause withdrawal reactions following sublingual administration, whereas the presence of naloxone theoretically limits diversion of the tablets for IV abuse. The administration of buprenorphine is a treatment both for opioid detoxification and for the maintenance of opioid-dependent users. Clinical studies suggest that the use of buprenorphine-naloxone for the treatment of heroin addiction is more efficacious than rapid detoxification based on lower opioid use and higher retention rate in the treatment program.[228] The typical dose of buprenorphine for detoxification from opioids is 8–12 mg sublingually daily for 5–7 days with some studies using buprenorphine doses as small as 12 mg intramuscularly in 8 divided doses over 24 hours.[229] Limited data in neonates suggest that buprenorphine may be an alternative to methadone or barbiturates for the neonatal abstinence syndrome at doses of 0.5–1.0 µg/kg orally every 6 hours; however, larger trials are required to define the efficacy of this treatment in neonates.[230]

Therapy with buprenorphine-naloxone should not begin until withdrawal symptoms occur. In a randomized clinical trial of buprenorphine for the treatment of concurrent opioid and cocaine dependence, a sublingual buprenorphine dose of 16 mg daily was well tolerated and reduced concomitant opioid and cocaine use.[231] Maintenance doses are 4–32 mg/day with dosing extended to every 2–3 days as tolerated. The initial induction of detoxification with buprenorphine usually occurs under the observation of a physician because of the potential for withdrawal symptoms. For inpatients, the initial dose typically is 4–8 mg sublingually with careful observation for withdrawal symptoms. Recent studies indicate that less-than-daily dosing schedules of buprenorphine are equally efficacious as daily dosing schedules.[232] In a pilot study of 30 heroin users, there was no statistically significant difference in the Clinical Opiate Withdrawal Scale between the high-dose buprenorphine regimen (8 mg, 8 mg, 8 mg, 4 mg, and 2 mg on days 1–5, respectively) and the low-dose regimen (2 mg, 4 mg, 8 mg, 4 mg, and 2 mg on days 1–5, respectively).[233] Both regimens were superior to clonidine without serious treatment-related adverse events.

The slow release of buprenorphine from opioid receptors results in a long duration of action. A buprenorphine dose of 8 mg sublingual is equivalent to an oral methadone dose of 60 mg for the treatment of opioid-dependent patients. Advantages of buprenorphine over methadone include milder withdrawal symptoms, longer duration of action, lack of QT_c prolongation, and lower risk of serious overdose.[234] Clinical studies suggest that buprenorphine and buprenorphine-naloxone have similar efficacy to moderate doses of methadone when used as maintenance therapy for opioid dependence,[235] and buprenorphine-assisted withdrawal from heroin has similar efficacy in specialty clinics and primary care settings.[236] Buprenorphine is probably more effective than clonidine for the reduction of opioid withdrawal symptoms with less adverse effects.[237] There are limited data on adverse effects (e.g., insomnia, vomiting, headache) following excessive doses of buprenorphine. Although there are case reports of deaths associated with buprenorphine use,[238] these fatalities usually occur following the concomitant use of high doses buprenorphine and other drugs (e.g., benzodiazepines).

AMELIORATING AGENTS. Other medications for the treatment of the symptoms of heroin withdrawal include antiemetics, oxazepam (30–60 mg every 6 hours) for muscle spasms, nonsteroidal antiinflammatory drugs for myalgias, benzodiazepines for insomnia, and clonidine for symptoms of excessive sympathomimetic stimulation. Clonidine is an α-adrenergic agonist that effectively blocks much of the autonomic symptoms associated with opioid withdrawal by decreasing norepinephrine secretion in the locus coeruleus. This drug does not alleviate the subjective symptoms of anxiety, muscle cramps, insomnia, or drug craving as much as reducing doses of methadone. A review of the 24 studies of 1,631 opioid addicts concluded that opioid withdrawal following clonidine and reducing doses of methadone are similar; however, adverse effects (hypotension, dizziness, dry mouth, lack of energy) was higher with clonidine than reducing doses of methadone.[239] A typical dose of clonidine is 0.1–0.2 mg orally every 4–6 hours for mild to moderate withdrawal with careful monitor-

ing of blood pressure. Tapering of the clonidine dose begins with reduction of the dose by 0.2 mg/day starting on the third day of cessation of opioid use.

References

1. Schiff PL Jr. Opium and its alkaloids. Am J Pharm Educ 2002;66:188–196.

2. Lodge RT. On a case of poisoning of an infant by syrup of poppies. Lancet 1858;72:7.

3. Anonymous. Death of a child from the administration of syrup of poppies. Lancet 1838;30:239–240.

4. Sneader W. The discovery of heroin. Lancet 1998; 352:1697–1699.

5. Osler W. Oedema of the lung complicating morphine poisoning. Montreal General Hospital Report 1880; 1:291–293.

6. Strang J, Griffiths P, Gossop M. Heroin smoking by "chasing the dragon": origins and history. Addiction 1997;92:673–683.

7. League of Nations. Expert Report on a Technical Committee on Heroin. The heroin habit. Lancet 1931;ii: 55–56.

8. Wolters EC, van Wijngaarden GK, Stam FC, Rengelink H, Lousberg RJ, Schipper ME, Verbeeten B. Leucoencephalopathy after inhaling "heroin" pyrolysate. Lancet 1982;2:1233–1237.

9. Schwartz RH. Adolescent heroin use: a review. Pediatrics 1998;102:1461–1466.

10. Brenneisen R, Hasler F. GC/MS determination of pyrolysis products from diacetylmorphine and adulterants of street heroin samples. J Forensic Sci 2002;47:885–888.

11. Jenkins AJ, Keenan RM, Henningfield JE, Cone EJ. Pharmacokinetics and pharmacodynamics of smoked heroin. J Anal Toxicol 1994;18:317–330.

12. Cook CE, Jeffcoat AR. Pyrolytic degradation of heroin, phencyclidine, and cocaine: identification of products and some observations on their metabolism. NIDA Res Monogr 1990;99:97–120.

13. Hall WD, Ross JE, Lynskey MT, Law MG, Degenhardt LJ. How many dependent heroin users are there in Australia? Med J Aust 2000;173:528–531.

14. Clark MJ, Bates AC. Nonfatal heroin overdoses in Queensland, Australia: an analysis of ambulance data. J Urban Health 2003;80:238–247.

15. Substance Abuse Prevention, Addiction Treatment, and Mental Health Services. 2008 National household survey on drug use and Health (NSDUH).Availabe at http://www.oas.samhsa.gov/nsduh/2k8nsduh/2k8Results.pdf. Accessed 2011 March 9.

16. Sporer KA. Acute heroin overdose. Ann Intern Med 1999;130:584–590.

17. Hall W, Lynskey M, Degenhardt L. Trends in opiate-related deaths in the United Kingdom and Australia, 1985–1995. Drug Alcohol Depend 2000;57:247–254.

18. Preti A, Miotto P, De Coppi M. Deaths by unintentional illicit drug overdose in Italy. Drug Alcohol Depend 2002;66:275–282.

19. Paoli L, Greenfield VA, Charles M, Reuter P. The global diversion of pharmaceutical drugs. India: the third largest illicit opium producer? Addiction 2009;104:347–354.

20. US Department of Justice, Drug Enforcement Agency. Available at http://www.justice.gov/ndic/pubs38/38661/heroin.htm. Accessed 2010, December 18.

21. Sperry K. An epidemic of intravenous narcoticism deaths associated with the resurgence of black tar heroin. J Forensic Sci 1988;33:1156–1162.

22. Lee S, Park Y, Han E, Choi H, Chung H, Oh SM, Chung KH. Thebaine in hair as a marker for chronic use of illegal opium poppy substances. Forensic Sci Int 2011;204:115–118.

23. Pelders MG, Ros JJ. Poppy seeds: differences in morphine and codeine content and variation in inter- and intra-individual excretion. J Forensic Sci 1996;41: 209–212.

24. Sproll C, Perz RC, Lachenmeier DW. Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing as a basis for risk analysis. J Agric Food Chem 2006;54:5292–5298.

25. Lachenmeier DW, Sproll C, Musshoff F. Poppy seed foods and opiate drug testing—where are we today? Ther Drug Monit 2010;32:11–18.

26. Lo DS, Chua TH. Poppy seeds: implications of consumption. Med Sci Law 1992;32:296–302.

27. Odell LR, Skopec J, McCluskey A. A "cold synthesis" of heroin and implications in heroin signature analysis utility of trifluoroacetic/acetic anhydride in the acetylation of morphine. Forensic Sci Int 2006;164:221–229.

28. Shesser R, Jotte R, Olshaker J. The contribution of impurities to the acute morbidity of illegal drug use. Am J Emerg Med 1991;9:336–342.

29. Cunningham EE, Venuto RC, Zielezny MA. Adulterants in heroin/cocaine: implications concerning heroin-associated nephropathy. Drug Alcohol Depend 1984; 14:19–22.

30. Walker JA, Krueger ST, Lurie IS, Marche HL, Newby N. Analysis of heroin drug seizures by Micellar Electrokinetic Capillary Chromatography (MECC). J Forensic Sci 1995;40:6–9.

31. Monforte JR. Some observations concerning blood morphine concentrations in narcotic addicts. J Forensic Sci 1977;22:718–724.

32. Luke JL, Levy ME. Heroin-related deaths-District of Columbia. MMWR Morb Mortal Wkly Rep 1983;32: 321–324.

33. DuPont RL, Greene MH. The dynamics of a heroin addiction epidemic. Science 1973;181:716–722.

34. Hamilton RJ, Perrone J, Hoffman R, Henretig FM, Karkevandian EH, Marcus S, et al. A descriptive study of an epidemic of poisoning caused by heroin adulterated with scopolamine. Clin Toxicol 2000;38:597–608.

35. Hoffman RS, Kirrane BM, Marcus SM, Clenbuterol Study Investigators. A descriptive study of an outbreak of clenbuterol-containing heroin. Ann Emerg Med 2008;52:548–553.

36. Hoffman RS, Nelson LS, Chan GM, Halcomb SE, Bouchard NC, Ginsburg BY et al. Atypical Reactions Associated With Heroin Use—Five States, January–April 2005. MMWR Morb Mortal Wkly Rep 2005;54:793–796.

37. Manini A, Labinson RM, Kirrane B, Hoffman RS, Rao R, Stajic M, Nelson LS. A novel neuromuscular syndrome associated with clenbuterol-tainted heroin. Clin Toxicol 2008;46:1088–1092.

38. Wingert WE, Mundy LA, Nelson L, Wong SC Cutis J. Detection of clenbuterol in heroin users in twelve postmortem cases at the Philadelphia Medical Examiner's Office. J Anal Toxicol 2008;32:522–528.

39. Chiarotti M, Fucci N. Analysis of volatile compounds in heroin samples. Forensic Sci Int 1988;37:47–53.

40. Chiarotti M, Fucci N. comparative analysis of heroin and cocaine seizures. J Chromatogr B 1999;733:127–136.

41. O'Neil PJ, Gough TA. Illicitly imported heroin products: some physical and chemical features indicative of their origin. Part II. J Forensic Sci 1985;30:681–691.

42. Barnfield C, Burns S, Byrom DL, Kemmenoe AV. The routine profiling of forensic heroin samples. Forensic Sci Int 1988;39:107–117.

43. Esseiva P, Dujourdy L, Anglada F, Taroni F, Margot P. A methodology for illicit heroin seizures comparison in a drug intelligence perspective using large databases. Forensic Sci Int 2003;132:139–152.

44. Johnson A, King LA. Heroin profiling: predicting the country of origin of seized heroin. Forensic Sci Int 1998;95:47–55.

45. Odell LR, Skopec J, McCluskey A. Isolation and identification of unique marker compounds from the Tasmanian poppy *Papaver somniferum* L. Implications for the identification of illicit heroin of Tasmanian origin. Forensic Sci Int 2008;175:202–208.

46. Hendriks VM, van den Brink W, Blanken P, Bosman IJ, van Ree JM: Heroin self-administration by means of "chasing the dragon": pharmacodynamics and bioavailability of inhaled heroin. Eur Neuropsychopharmacol 2001;11:241–252.

47. Darke S, Ross J, Kaye S. Physical injecting sites among injecting drug users in Sydney, Australia. Drug Alcohol Depend 2001;62:77–82.

48. Rentsch KM, Kullak-Ublick GA, Reichel C, Meier PJ, Fattinger K: Arterial and venous pharmacokinetics of intravenous heroin in subjects who are addicted to narcotics. Clin Pharmacol Ther 2001;70:237–246.

49. Skopp G, Ganssman Cone EJ, Aderjan R: Plasma concentrations of heroin and morphine-related metabolites after intranasal and intramuscular administration. J Anal Toxicol 1997;21:105–111.

50. Mo BP, Way EL: An assessment of inhalation as a mode of administration of heroin by addicts. J Pharmacol Exp Ther 1966;154:142–151.

51. Rook EJ, van Ree JM, van den Brink W, Hillebrand MJ, Huitema AD, Hendriks VM, Beijnen JH. Pharmacokinetics and pharmacodynamics of high doses of pharmaceutically prepared heroin, by intravenous or by inhalation route in opioid-dependent patients. Basic Clin Pharmacol Toxicol 2006;98:86–96.

52. Girardin F, Rentsch KM, Schwab M-A, Maggiorini M, Pauli-Magnus C, Kullak-Ublick GA, et al. Pharmacokinetics of high doses of intramuscular and oral heroin in narcotic addicts. Clin Pharmacol Ther 2003;74:341–352.

53. Inturrisi CE, Max MB, Foley KM, Schultz M, Shin SU, Houde RW: The pharmacokinetics of heroin in patients with chronic pain. N Engl J Med 1984;310:1213–1217.

54. Halbsguth U, Rentsch KM, Eich-Höchli D, Diterich I, Fattinger K. Oral diacetylmorphine (heroin) yields greater morphine bioavailability than oral morphine: bioavailability related to dosage and prior opioid exposure. Br J Clin Pharmacol 2008;66:781–791.

55. Cone EJ, Holicky BA, Grant TM, Darwin WD, Goldberger BA. Pharmacokinetics and pharmacodynamics of intranasal "snorted" heroin. J Anal Toxicol 1993;17:327–337.

56. Way EL, Kemp JW, Young JM, Grassetti DR. The pharmacological effects of heroin in relationship to its rate of biotransformation. J Pharmacol Exp Ther 1960:129:144–153.

57. Rook EJ, Huitema AD, van den Brink W, van Ree JM, Beijnen JH. Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature. Curr Clin Pharmacol 2006;1:109–118.

58. Spiehler VR, Cravey RH, Richards RG, Elliott HW. The distribution of morphine in the brain in fatal cases due to the intravenous administration of heroin. J Anal Toxicol 1978;2:62–67.

59. Osborne R, Joel S, Trew D, Slevin M. Morphine and metabolite behavior after different routes of morphine administration: demonstration of the importance of the active metabolite morphine-6-glucuronide. Clin Pharmacol Ther 1990;47:12–19.

60. Cohn GL, Cramer JA, McBride W, Brown RC, Kleber HD. Heroin and morphine binding with human serum proteins and red blood cells. Proc Soc Exp Biol Med 1974;147:664–666.

61. Gyr E, Brenneisen R, Bourquin D, Lehmann T, Vonlanthen D, Hug I. Pharmacodynamics and pharmacokinetics of intravenously, orally and rectally administered diacetylmorphine in opioid dependents, a two-patient pilot study within a heroin-assisted treatment program. Int J Clin Pharmacol Ther 2000;38:486–491.

62. Antonilli L, Semeraro F, Suriano C, Signore L, Nencini P. High levels of morphine-6-glucuronide in street heroin addicts. Psychopharmacology 2003;170:200–204.

63. Yeh SY, McQuinn RL, Gorodetzky CW. Identification of diacetylmorphine metabolites in humans. J Pharm Sci 1977;66:201–204.

64. Mitchell JM, Paul BD, Welch P, Cone EJ. Forensic drug testing for opiates. II. Metabolism and excretion rate of morphine in humans after morphine administration. J Anal Toxicol 1991;15:49–53.

65. Salmon AY, Goren Z, Avissar Y, Soreq H. Human erythrocyte but not brain acetylcholinesterase hydrolyses heroin to morphine. Clin Exp Pharmacol Physiol 1999;26:596–600.

66. Elliott HW, Parker KD, Wright JA, Nomof N. Actions and metabolism of heroin administered by continuous intravenous infusion to man. Clin Pharmacol Ther 1971;12:806–814.

67. Smith ML, Shimomura ET, Summers J, Paul BD, Jenkins AJ, Darwin WD, Cone EJ. Urinary excretion profiles for total morphine, free morphine, and 6-acetylmorphine following smoked and intravenous heroin. J Anal Toxicol 2001;25:504–514.

68. Cone EJ, Welch P, Mitchell JM, Paul BD. Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. J Anal Toxicol 1991;15:1–7.

69. Rurak DW, Wright MR, Axelson JE. Drug disposition and effects in the fetus. J Dev Physiol 1991;15:33–44.

70. Hartivig P, Lindberg BS, Lilja A, Lundqvist H, Langstrom B, Rane A. Positron emission tomography in studies on fetomaternal disposition of opioids. Dev Pharmacol Ther 1989;12:74–80.

71. Wikler A, Fraser HF, Isbell H. *N*-allylnormorphine: effects of single doses and precipitation of acute "abstinence syndromes" during addiction to morphine, methadone, or heroin in man (post-addicts). J Pharmacol Exp Ther 1953;109:8–20.

72. Levine B, Green D, Smialek JE. The role of ethanol in heroin deaths. J Forensic Sci 1995;40:808–810.

73. Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. Annu Rev Biochem 2004;73:953–990.

74. Chen Y, Mestek A, Liu J, Yu L. Molecular cloning of a rat kappa opioid receptor reveals sequence similarities to the mu and delta opioid receptors. Biochem J 1993;295:625–628.

75. Wang D, Raehal KM, Bilsky EJ, Sadee W. Inverse agonists and neutral antagonists at μ opioid receptor (MOR): possible role of basal receptor signaling in narcotic dependence. J Neurochem 2001;77:1590–1600.

76. Monnet FP. Sigma-1 receptor as regulator of neuronal intracellular Ca2+: clinical and therapeutic relevance. Biol Cell 2005;97:873–883.

77. Kriegstein AR, Shungu DC, Millar WS, Armitage BA, Brust JC, Chillrud S, Goldman J, Lynch T. Leukoencephalopathy and raised brain lactate from heroin vapor inhalation ("chasing the dragon"). Neurology 1999;53:1765–1773.

78. Otto D, Franke M, Madden JF. Hypothermia accompanied by noncardiogenic pulmonary edema: a case report. Del Med J 1996;68:499–502.

79. Kjeldgaard JM, Hahn GW, Heckenlively JR, Genton E. Methadone-induced pulmonary edema. JAMA 1971;218:882–883.

80. Bogartz LJ, Miller WC. Pulmonary edema associated with propoxyphene intoxication. JAMA 1971;215:259–262.

81. Dettmeyer R, Schmidt P, Musshoff F, Dreisvogt C, Madea B. Pulmonary edema in fatal heroin overdose: immunohistological investigations with IgE, collagen IV and laminin—no increase of defects of alveolar-capillary membranes. Forensic Sci Int 2000;110:87–96

82. Raith K, Hochhaus G. Drugs used in the treatment of opioid tolerance and physical dependence: a review. Int J Clin Pharmacol Ther 2004;42:191–203.

83. Buttner A, Mall G, Penning R, Weis S. The neuropathology of heroin abuse. Forensic Sci Int 2000;113:435–442.

84. Thiblin I, Eksborg S, Petersson A, Fugelstad A, Rajs J. Fatal intoxication as a consequence of intranasal administration (snorting) or pulmonary inhalation (smoking) of heroin. Forensic Sci Int 2004;139:241–247.

85. Fugelstad A, Ahlner J, Brandt L, Ceder G, Eksborg S, Rajs J, Beck O. Use of morphine and 6-monoacetylmorphine in blood for the evaluation of possible risk factors for sudden death in 192 heroin users. Addiction 2003;98:463–470.

86. Warner-Smith M, Darke S, Day C. Morbidity associated with non-fatal heroin overdose. Addiction 2002;97:963–967.

87. Dabby R, Djaldetti R, Gilad R, Herman O, Frand J, Sadeh M, Watemberg N. Acute heroin-related neuropathy. J Peripheral Nerv Sys 2006;11:304–309.

88. Yang C-C, Yang G-Y, Ger J, Tsai W-J, Deng J-F. Severe rhabdomyolysis mimicking transverse myelitis in a heroin addict. Clin Toxicol 1995;34:591–595.

89. Werner SB, Passaro D, McGee J, Schechter R, Vugia DJ. Wound botulism in California, 1951–1998: recent epidemic in heroin injectors. Clin Infect Dis 2000;31:1018–1024.

90. Ricking J, Whittlesey C, Contreras C, Vargas J, Andrews B, Flodin-Jursh D, et al. Wound botulism among black tar heroin users—Washington, 2003. MMWR Morb Mortal Wkly Rep 2003;52:885–886.

91. Cooper JG, Spilke CE, Denton M, Jamieson S. Clostridium botulinum: an increasing complication of heroin misuse. Eur J Emerg Med 2005;12:251–252.

92. Blasel S, Hattingen E, Adelmann Nichtweiß M, Zanella F, Weidauer S. Toxic leukoencephalopathy after heroin abuse without heroin vapor inhalation. Clin Neuroradiol 2010;20:48–53.

573

93. Gacouin A, Lavoue S, Signouret T, Person A, Dinard MD, Shpak N, Thomas R. Reversible spongiform leucoencephalopathy after inhalation of heated heroin. Intensive Care Med 2003;29:1012–1015.

94. Robertson AS, Jain S, O'Neil R. Spongiform leukoencephalopathy following intravenous heroin abuse: radiological and histopathological findings. Australas Radiol 2001;45:390–392.

95. Long H, Deore K, Hoffman RS, Nelson LS. A fatal case of spongiform leukoencephalopathy linked to "chasing the dragon". J Toxicol Clin Toxicol 2003;41:887–891.

96. Brust JC, Richter RW. Stroke associated with addiction to heroin. J Neurol Neurosurg Psychiatry 1976;39:194–199.

97. Zuckerman GB, Ruiz DC, Keller IA, Brooks J. Neurological complications following intranasal administration of heroin in an adolescent. Ann Pharmacother 1996;30:778–781.

98. Duberstein JL, Kaufman DM. A clinical study of an epidemic of heroin intoxication and heroin induced pulmonary edema. Am J Med 1971;51:704–714.

99. Sterrett C, Brownfield J, Korn CS, Hollinger M, Henderson SO. Patterns of presentation in heroin overdose resulting in pulmonary edema. Am J Emerg Med 2003;21:32–34.

100. Sporer KA, Dorn E. Heroin-related noncardiogenic pulmonary edema. A case series. Chest 2001;120:1628–1632.

101. Hughes S, Calverly PM. Heroin inhalation and asthma. Br Med J 1988;297:1511–1512.

102. Shaikh WA. Allergy to heroin. Allergy 1990;45:555–556.

103. Hutchins KD, Pierre-Louis PJ, Zaretski L, Williams AW, Lin R-L, Natarajan GZ. Heroin body packing: three fatal cases of intestinal perforation. J Forensic Sci 2000;45:42–47.

104. Crowe AV, Howse M, Bell GM, Henry JA. Substance abuse and the kidney. Q J Med 2000;93:147–152.

105. Perneger TV, Klag MJ, Whelton PK. Recreational drug use: a neglected risk factor for end-stage renal disease. Am J Kid Dis 2001;38:49–56.

106. Cunningham EE, Brentjens JR, Zielezny MA, Andres GA, Venuto RC. Heroin nephropathy. A clinicopathologic and epidemiologic study. Am J Med 1980;68:47–53.

107. Dettmeyer RB, Preuß J, Wollersen H, Madea B. Heroin-associated nephropathy. Expert Opin Drug Saf 2005;4:19–28.

108. Friedman EA, Rao TK. Disappearance of uremia due to heroin-associated nephropathy. Am J Kidney Dis 1995;25:689–693.

109. Klockgether T, Weller M, Haarmeier T, Kaskas B, Maier G, Dichgans J. Gluteal compartment syndrome due to rhabdomyolysis after heroin abuse. Neurology 1997;48:275–276.

110. Friedland GH, Harris C, Butkus-Small C, Shine D, Moll B, Darrow W, Klein RS. Intravenous drug abusers and the acquired immunodeficiency syndrome (AIDS). Demographic, drug use, and needle-sharing patterns. Arch Intern Med 1985;145:1413–1417.

111. Giudice PD. Cutaneous complications of intravenous drug abuse. Br J Dermatol 2004;150:1–10.

112. Fatovich DM, Bartu A, Daly FF. A prospective study of non-fatal heroin overdose. J Substance Use 2008;13:299–307.

113. Grigorakos L, Sakagianni K, Tsigou E, Apostolakos G, Nikolopoulos G, Veldekis D. Outcome of acute heroin overdose requiring intensive care unit admission. J Opioid Manag 2010;6:227–231.

114. Quaglio G, Talamini G, Lechi A, Venturini L, Lugoboni F. Study of 2,708 heroin-related deaths in north-eastern Italy 1985–98 to establish main causes of death. Addiction 2001;96:1127–1137.

115. Darke S, Zador D. Fatal heroin "overdose": a review. Addiction 1996;91:1765–1772.

116. Zador D, Sunjic S, Darke S. Heroin-related deaths in New South Wales, 1992: toxicological findings and circumstances. Med J Aust 1996;164:204–207.

117. Warner-Smith M, Darke S, Lynskey M, Hall W. Heroin overdose: causes and consequences. Addiction 2001;96:1113–1125.

118. Arnold-Reed DE, Hulse GK, Hansson RC, Murray SD, Basso MR, Holman DJ. Blood morphine levels in naltrexone-exposed compared to non-naltrexone-exposed fatal heroin overdoses. Addict Biol 2003;8:343–350.

119. Levine B, Green D, Smialek JE. The role of ethanol in heroin deaths. J Forensic Sci 1995;40:808–810.

120. Steentoft A, Kaa E, Worm K. Fatal intoxications in the age group 15–34 years in Denmark in 1984 and 1985. Z Rechtsmed 1989;103:93–100.

121. Mirakbari SM, Innes GD, Christenson J, Tilley J, Wong H. Do co-intoxicants increase adverse event rates in the first 24 hours in patients resuscitated from acute opioid overdose? J Toxicol Clin Toxicol 2003;41:947–953.

122. Christie B. Heroin contaminated with anthrax has killed 11 people. BMJ 2010;340:c937.

123. O'Connor PG, Fiellin DA. Pharmacologic treatment of heroin-dependent patients. Ann Intern Med 2000;133:40–54.

124. Wright A, Walker J. Drugs of abuse in pregnancy. Best Pract Res Clin Obstet Gynaecol 2001;15:987–998.

125. Stout PR, Farrell LJ. Opioids—effects on human performance and behavior. Forensic Sci Rev 2003;1:30–59.

126. Wasels R, Belleville F. Gas chromatographic-mass spectrometric procedures used for the identification and determination of morphine, codeine and 6-monoacetylmorphine. J Chromatogr A 1994;674:225–234.

127. Pichini S, Altieri I, Pellegrini M, Zuccaro P, Pacifici R. The role of liquid chromatography-mass spectrometry in the determination of heroin and related opioids in biological fluids. Mass Spectrom Rev 1999;18:119–130.

128. Goldberger BA, Cone EJ. Confirmatory tests for drugs in the workplace by gas chromatography-mass spectrometry. J Chromatogr A 1994;674:73–86.

129. Paul BD, Mitchell JM, Mell LD Jr. Gas chromatography/electron impact mass fragmentometric determination of urinary 6-acetylmorphine, a metabolite of heroin. J Anal Toxicol 1989;13:2–7.

130. Bogusz MJ, Maier RD, Erkens M, Kohls U. Detection of non-prescription heroin markers in urine with liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 2001;25:431–438.

131. Rook EJ, Huitema AD, van den Brink W, Hillebrand MJ, van Ree JM, Beijnen JH. Screening for illicit heroin use in patients in a heroin-assisted treatment program. J Anal Toxicol 2006;30:390–394.

132. Skopp G, Potsch L, Klingmann A, Mattern R. Stability of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in fresh blood and plasma and postmortem blood samples. J Anal Toxicol 2001;25:2–7.

133. Carroll FT, Marraccini J, Lewis S, Wright W. Morphine-3-D glucuronide stability in postmortem specimens exposed to bacterial enzymatic hydrolysis. Am J Forensic Med Pathol 2000;21:323–329.

134. Pounder DJ. The case of Dr. Shipman. Am J Forensic Med Pathol 2003;24:219–226.

135. Alunni-Perret V, Kintz P, Ludes B, Ohayon P, Quatrehomme G. Determination of heroin after embalmment. Forensic Sci Int 2003;134:36–39.

136. Fuller DC. A statistical approach to the prediction of verifiable heroin use from total codeine and total morphine concentrations in urine. J Forensic Sci 1997;42:685–689.

137. Jenkins AJ, Oyler JM, Cone EJ. Comparison of heroin and cocaine concentrations in saliva with concentration in blood and plasma. J Anal Toxicol 1995;19:359–374.

138. Vanbinst R, Koenig J, Di Fazio V, Hassoun A. Bile analysis of drugs in postmortem cases. Forensic Sci Int 2002;128:35–40.

139. Tassoni G, Cacaci C, Zampi M, Froldi R. Bile analysis in heroin overdose. J Forensic Sci 2007;52:1405–1407.

140. Hand CW, Moore RA, Sear JW. Comparison of whole blood and plasma morphine. J Anal Toxicol 1988;12:234–235.

141. Lora-Tamayo C, Tena T, Tena G. Concentrations of free and conjugated morphine in blood in twenty cases of heroin-related deaths. J Chromatogr 1987;422:267–273.

142. Aderjan R, Hofmann S, Schmitt G, Skopp G. Morphine and morphine glucuronides in serum of heroin consumers and in heroin-related deaths determined by HPLC with native fluorescence detection. J Anal Toxicol 1995;19:163–168.

143. Darke S, Sunjic S, Zador D, Prolov T. A comparison of blood toxicology of heroin-related deaths and current heroin users in Sydney, Australia. Drug Alcohol Depend 1997;47:45–53.

144. Ceder G, Jones AW. Concentration ratios of morphine to codeine in blood of impaired drivers as evidence of heroin use and not medication with codeine. Clin Chem 2001;47:1980–1984.

145. Meissner C, Recker S, Reiter A, Friedrich HJ, Oehmichen M. Fatal versus non-fatal heroin "overdose": blood morphine concentrations with fatal outcome in comparison to those of intoxicated drivers. Forensic Sci Int 2002;130:49–54.

146. Goldberger BA, Cone EJ, Grant TM, Caplan YH, Levine BS, Smialek JE. disposition of heroin and its metabolites in heroin-related deaths. J Anal Toxicol 1994;18:22–28.

147. Aderjan R, Hofmann S, Schmitt G, Skopp G. Morphine and morphine glucuronides in serum of heroin consumers and in heroin-related deaths determined by HPLC with native fluorescence detection. J Anal Toxicol 1995;19:163–168.

148. Al-Asmari AI, Anderson RA. Method for quantification of opioids and their metabolites in autopsy blood by liquid chromatography-tandem mass spectrometry. J Anal Toxicol 2007;31:394–408.

149. Moriya F, Hashimoto Y. Distribution of free and conjugated morphine in body fluids and tissues in a fatal heroin overdose: is conjugated morphine stable in postmortem specimens? J Forensic Sci 1997;42:736–740.

150. Skopp G, Lutz R, Ganβmann B, Mattern R, Aderjan R. Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose. Int J Legal Med 1996;109:118–124.

151. Logan BK, Smirnow D. Postmortem distribution and redistribution of morphine in man. J Forensic Sci 1996;41:221–229.

152. Gerostamoulos J, Drummer OH. Postmortem redistribution of morphine and its metabolites. J Forensic Sci 2000;45:843–845.

153. Crandall CS, Kerrigan S, Aguero Blau RL, LaValley J, Zumwalt R, McKinney PE. The influence of site of collection on postmortem morphine concentrations in heroin overdose victims. J Forensic Sci 2006;51:413–420.

154. Levine B, Green-Johnson D, Moore KA, Fowler D, Jenkins A. Assessment of the acuteness of heroin deaths from the analysis of multiple blood specimens. Sci Justice 2002;42:17–20.

155. Druid H, Holmgren P. Fatal injections of heroin. Interpretation of toxicological findings in multiple specimens. Int J Legal Med 1998;112:62–66.

156. Goldberger BA, Caplan YH, Maguire T, Cone EJ. testing human hair for drugs of abuse. III. Identification of heroin and 6-acetylmorphine as indicators of heroin use. J Anal Toxicol 1991;15:226–231.

157. Kintz P, Bundeli P, Brenneisen R, Ludes B. Dose-concentration relationships in hair from subjects in a controlled heroin-maintenance program. J Anal Toxicol 1998;22:231–236.

158. Moeller MR, Fey P, Sachs H. Hair analysis as evidence in forensic cases. Forensic Sci Int 1993;63:43–53.

575

159. Lee S, Cordero R, Paterson S. Distribution of 6-monoacetylmorphine and morphine in head and pubic hair from heroin-related deaths. Forensic Sci Int 2009; 183:74–77.

160. Balíková MA, Habrdová V. Hair analysis for opiates: evaluation of washing and incubation procedures. J Chromatogr B Analyt Technol Biomed Life Sci 2003; 789:93–100.

161. Duflou J, Darke S, Easson J. Morphine concentrations in stomach contents of intravenous opioid overdose deaths. J Forensic Sci 2009;54:1181–1184.

162. Kintz P, Mangin P, Lugnier AA, Chaumont AJ. Identification by GC/MS of 6-monoacetylmorphine as an indicator of heroin abuse. Eur J Clin Pharmacol 1989; 37:531–532.

163. Fehn J, Megges G. Detection of 6-monoacetylmorphine in urine samples by GC/MS as evidence for heroin use. J Anal Toxicol 1985;9:134–138.

164. Jenkins AJ, Lavins ES, Snyder A. Evaluation of the CEDIA® heroin metabolite (6-AM) immunoassay with urine specimens from a criminal justice drug-testing program. J Anal Toxicol 2005;28:201–204.

165. Chronister CW, Gund AL, Goldberger BA. Rapid detection of opioids in vitreous humor by enzyme immunoassay. J Anal Toxicol 2008;32:601–604.

166. Goldberger BA, Loewenthal B, Darwin WD, Cone EJ. Intrasubject variation of creatinine and specific gravity measurements in consecutive urine specimens of heroin users. Clin Chem 1995;41:116–117.

167. von Euler M, Villen T, Svensson J-O, Stahle L. Interpretation of the presence of 6-monoacetylmorphine in the absence of morphine-3-glucuronide in urine samples: evidence of heroin abuse. Ther Drug Monit 2003;25:645–648.

168. Beck O, Bottcher M. Paradoxical results in urine drug testing for 6-acetylmorphine and total opiates: implications for best analytical strategy. J Anal Toxicol 2006;30:73–79.

169. Brenneisen R, Hasler F, Wursch D. Acetylcodeine as a urinary marker to differentiate the use of street heroin and pharmaceutical heroin. J Anal Toxicol 2002; 26:561–566.

170. Narongchai P, Sribanditmonkol P, Thampithung S, Narongchai S, Chitivuthikarn C. The duration time of urine morphine detection in heroin addicts by radioimmunoassay. J Med Assoc Thai 2002;85:82–86.

171. Smith ML, Shimomura ET, Summers J, Paul BD, Nichols D, Shippee R, et al. Detection times and analytical performance of commercial urine opiate immunoassays following heroin administration. J Anal Toxicol 2000; 24:522–529.

172. Cone EJ, Jufer R, Darwin WD, Needleman SB. Forensic drug testing for opiates. VII. Urinary excretion profile of intranasal (snorted) heroin. J Anal Toxicol 1996; 20:379–392.

173. Wolff K, Farrell M, Marsden J, Monteiro MG, Ali R, Welch S, Strang J. A review of biological indicators of illicit drug use, practical considerations and clinical usefulness. Addiction 1999;94:1279–1298.

174. Cone EJ, Dickerson S, Paul BD, Mitchell JM. Forensic drug testing for opiates. V. Urine testing for heroin, morphine, codeine with commercial opiate immunoassays. J Anal Toxicol 1993;17:156–164.

175. Steentoft A, Worm K, Christensen H. Morphine concentrations in autopsy material from fatal cases after intake of morphine and/or heroin. J Forensic Sci Soc 1988; 28:87–94.

176. de Paula M, Saiz LC, González-Revaldería J, Pascual T, Alberola C, Miravalles E. Rifampicin causes false-positive immunoassay results for urine opiates. Clin Chem Lab Med 1998;36:241–243.

177. Straley CM, Cecil EJ, Herriman MP. Gatifloxacin interference with opiate urine drug screen. Pharmacotherapy 2006;26:435–439.

178. Baden LR, Horowitz G, Jacoby H, Eliopoulos GM. Quinolones and false-positive urine screening for opiates by immunoassay technology. JAMA 2001;286: 3115–3119.

179. Rohrig TP, Moore C. The determination of morphine in urine and oral fluid following ingestion of poppy seeds. J Anal Toxicol 2003;27:449–452.

180. Mule SJ, Casella GA. Rendering the "poppy-seed defense" defenseless: identification of 6-monoacetylmorphine in urine by gas chromatography/mass spectroscopy. Clin Chem 1988;34:1427–1430.

181. Moeller MR, Hammer K, Engel O. Poppy seed consumption and toxicological analysis of blood and urine samples. Forensic Sci Int 2004;143:183–186.

182. Preinger VL, Vrublovsky P, Stastny VL. Occurrence of alkaloids in opium poppy seed (*Papaver somniferum*). Pharmazie 1965;207:439–440.

183. Cassella G, Wu AH, Shaw BR, Hill DW. The analysis of thebaine in urine for the detection of poppy seed consumption. J Anal Toxicol 1997;21:376–383.

184. Ziminski KR, Wemyss CT, Bidanset JH, Manning TJ, Lukash L. Comparative study of postmortem barbiturates, methadone, and morphine in vitreous humor, blood, and tissue. J Forensic Sci 1984;29:903–909.

185. Bermejo AM, Ramos I, Fernandez P, Lopez-Rivadulla M, Cruz A. Morphine determination by gas chromatography/ mass spectroscopy in human vitreous humor and comparison with radioimmunoassay. J Anal Toxicol 1992;16:372–374.

186. Antonides HM, Kiely ER, Marinetti LJ. Vitreous fluid quantification of opiates, cocaine, and benzoylecgonine: comparison of calibration curves in both blood and vitreous matrices with corresponding concentration in blood. J Anal Toxicol 2007;31:469–476.

187. Wyman J, Bultman S. Postmortem distribution of heroin metabolites in femoral blood, liver, cerebrospinal fluid, and vitreous humor. J Anal Toxicol 2004;28:260–263.

188. Pragst F, Spiegel K, Leuschner U, Hager A. Detection of 6-acetylmorphine in vitreous humor and cerebrospinal fluid—comparison with urinary analysis for proving heroin administration in opiate fatalities. J Anal Toxicol 1999;23:168–172.

189. Bogusz MJ, Maier R-D, Driessen S. Morphine, morphine-3-glucuronide, morpnhine-6-glucuronide, and 6-monoacetylmorphine determined by means of atmospheric pressure chemical ionization-mass spectrometry-liquid chromatography in body fluids of heroin victims. J Anal Toxicol 1997;21:346–355.

190. Curry SC, Chang D, Connor D. Drug- and toxin-induced rhabdomyolysis. Ann Emerg Med 1989;18:1068–1084.

191. Glauser FL, Downie RL, Smith WR. Electrocardiographic abnormalities in acute heroin overdosage. Bull Narc 1977;29:85–89.

192. Lipski J, Stimmel B Donoso E. The effect of heroin and multiple drug abuse on the electrocardiogram. Am Heart J 1973;86:663–668.

193. Koussa S, Tamraz J, Nasnas R. Leucoencephalopathy after heroin inhalation. A case with partial regression of MRI lesions. J Neuroradiol 2001;28:268–271.

194. Au-Yeung K, Lai C. Toxic leukoencephalopathy after heroin inhalation. Australas Radiol 2002;46:306–308.

195. Tan TP, Algra PR, Valk J, Wolters EC. Toxic leukoencephalopathy after inhalation of poisoned heroin: MR findings. AJNR Am J Neuroradiol 1994;15:175–178.

196. Offiah C, Hall E. Heroin-induced leukoencephalopathy: characterization using MRI, diffusion-weighted imaging, and MR spectroscopy. Clin Radiol 2008;63:146–152.

197. Keogh CF, Andrews GT, Spacey SD, Forkheim KE, Graeb DA. Neuroimaging features of heroin inhalation toxicity: "chasing the dragon". AJR Am J Roentgenol 2003;180:847–850.

198. Weber W, Henkes H, Möller P, Bade K, Kühne D. Toxic spongiform leucoencephalopathy after inhaling heroin vapour. Eur Radiol 1998;8:749–755.

199. Siu JC, Tsui EY. Spongiform leukoencephalopathy after intravenous heroin use: evaluation by diffusion-weighted imaging. J HK Coll Radiol 2004;7:84–87.

200. Bartlett E, Mikulis DJ. Chasing "chasing the dragon" with MRI: leukoencephalopathy in drug abuse. Br J Radiol 2005;78:997–1004.

201. Stern WZ, Spear PW, Jacobson GH: The roentgen findings in acute heroin intoxication. Am J Roentgenol 1968;103:522–532.

202. Ceder G, Jones AW. Concentrations of unconjugated morphine, codeine and 6-acetylmorphine in urine specimens from suspected drugged drivers. J Forensic Sci 2002;47:366–368.

203. Fraser HF, Jones BE, Rosenberg DE, Thompson AK. Effects of addiction to intravenous heroin on patterns of physical activity in man. Clin Pharmacol Ther 1963; 4:188–196.

204. Wanger K, Brough L, Macmillan I, Goulding J, MacPhail I, Christenson JM. Intravenous vs subcutaneous nalox-

205. Smith DA, Leake L, Loflin JR, Yealy DM. Is admission after intravenous heroin overdose necessary? Ann Emerg Med 1992;21:1326–1330.

206. Christenson J, Etherington J, Grafstein E, Innes G, Pennington S, Wanger K, et al. Early discharge of patients with presumed opioid overdose: development of a clinical prediction rule. Acad Emerg Med 2000;7:1110–1118.

207. Bulstrode N, Banks F, Shrotria S. The outcome of drug smuggling by "body packers"—the British experience. Ann R Coll Surg Engl 2002;84:35–38.

208. Pidoto RR, Agliata AM, Bertolini R, Mainini A, Rossi G, Giani G. A new method of packaging cocaine for international traffic and implications for the management of cocaine body packers. J Emerg Med 2002;23:149–153.

209. Jones OM, Shorey BA. Body packers: grading of risk as a guide to management and intervention. Ann R Coll Surg Engl 2002;84:131–132.

210. Traub SJ, Kohn GL, Hoffman RS, Nelson LS. Pediatric body packing". Arch Pediatr Adolesc Med 2003; 157:174–177.

211. Yang R-M, Li L, Feng J, Lai S-S, Lin B-Q, Yu T, et al. Heroin body packing: clearly discerning drug packets using CT. South Med J 2009;102:470–475

212. Hahn IH, Hoffman RS, Nelson LS. Contrast CT scan fails to detect the last heroin packet. J Emerg Med 2004; 27:279–283.

213. Taheri MS, Hassanian-Moghaddam H, Birang S, Hemadi H, Shahnazi M, Jalali AH, et al. Swallowed opium packets: CT diagnosis. Abdom Imaging 2008;33:262–266.

214. Hergan K, Kofler K, Oser W. Drug smuggling by body packing: what radiologists should know about it. Eur Radiol 2004;14:736–742.

215. McCleave RN. Drug smuggling by body packers. Detection and removal of internally concealed drugs. Med J Aust 1993;159:750–754.

216. Dunne JW. Drug smuggling by internal bodily concealment. Med J Aust 1983;2:436–439.

217. Utecht MJ, Stone AF, McCarron MM. Heroin body packers. J Emerg Med 1993;11:33–40.

218. Amato L, Minozzi S, Davoli M, Vecchi S, Ferri MM, Mayet S. Psychosocial and pharmacological treatments versus pharmacological treatments for opioid detoxification. Cochrane Database Syst Rev 2008;(4):CD005031.

219. Lingford-Hughes AR, Welch S, Nutt DJ. Evidence-based guidelines for the pharmacological management of substance misuse, addiction and comorbidity: recommendations from the British Association for Psychopharmacology. J Psychopharmacol 2004;18: 293–335.

220. Krantz MJ, Mehler PS. Treating opioid dependence growing implications for primary care. Arch Intern Med 2004;164:277–288.

221. Collins ED, Kleber HD, Whittington RA, Heitler NE. Anesthesia-assisted vs buprenorphine- or clonidine-assisted

heroin detoxification and naltrexone induction a randomized trial. JAMA 2005;294:903–913.

222. Warner EA, Kosten TR, O'Connor PG. Pharmacotherapy for opioid and cocaine abuse. Med Clin North Am 1997;81:909–925.

223. Hiltunen AJ, Lafolie P, Martel J, Ottosson EC, Boreus LO, Beck O, et al. Subjective and objective symptoms in relation to plasma methadone concentration in methadone patients. Psychopharmacology (Berl) 1995;118: 122–126.

224. Kranzler HR, Amin H, Modesto-Lowe V, Oncken C. Pharmacologic treatments for drug and alcohol dependence. Psychiatr Clin North Am 1999;22:401–423.

225. Walker PW, Klein D, Kasza L. High dose methadone and ventricular arrhythmias: a report of three cases. Pain 2003;103:321–324.

226. Krantz MJ, Lewkowiez L, Hays H, Woodroffe MA, Robertson AD, Mehler PS. *Torsade de pointes* associated with very-high-dose methadone. Ann Intern Med 2002; 137:501–514.

227. Gowing L, Ali R, White JM. Buprenorphine for the management of opioid withdrawal. Cochrane Database Syst Rev 2009;(3):CD002025.

228. Woody GE, Poole SA, Subramaniam G, Dugosh K, Bogenschutz M, Abbott P, et al. Extended vs short-term buprenorphine-naloxone for treatment of opioid-addicted youth. A randomized trial. JAMA 2008;300: 2003–2011.

229. Assadi SM, Hafezi M, Mokri A, Razzaghi EM, Ghaeli P. Opioid detoxification using high doses of buprenorphine in 24 hours: a randomized, double blind, controlled clinical trial. J Substance Abuse Treat 2004;27:75–82.

230. Leikin JB, Mackendrick WP, Maloney GE, Rhee JW, Farrell E, Wahl M, Kelly K. Use of clonidine in the prevention and management of neonatal abstinence syndrome. Clin Toxicol 2009;47:551–555.

231. Montoya ID, Gorelick DA, Preston KL, Schroeder JR, Umbricht A, Cheskin LJ, et al. Randomized trial of buprenorphine for treatment of concurrent opiate and cocaine dependence. Clin Pharmacol Ther 2004;75: 34–48.

232. Marsch LA, Bickel WK, Badger GJ, Jacogs EA. Buprenorphine treatment for opioid dependence: the relative efficacy of daily twice and thrice weekly dosing. Drug Alcohol Depend 2004;77:195–204.

233. Oreskovich MR, Saxon AJ, Ellis ML, Malte CA, Reoux JP, Knox PC. A double-blind, double-dummy, randomized, prospective pilot study of the partial Mu opiate agonist, buprenorphine, for acute detoxification from heroin. Drug Alcohol Depend 2005;77:71–79.

234. Pirnay S, Borron SW, Giudicelli CP, Tourneau J, Baud FJ, Ricordel I. A critical review of the causes of death among post-mortem toxicological investigations: analysis of 34 buprenorphine-associated deaths. Addiction 2004;99: 978–988.

235. Fiellin DA, Pantalon MV, Chawarski MC, Moore BA, Sullivan LE, O'Connor PG, Schottenfeld RS. Counseling plus buprenorphine-naloxone maintenance therapy for opioid dependence. N Engl J Med 2006;355:365–374.

236. Gibson AE, Doran CM, Bell JR, Ryan A, Lintzeris N. A comparison of buprenorphine treatment in clinic and primary care settings: a randomized trial. Med J Aust 2003;179;38–42.

237. Amato L, Davoli M, Ferri M, Gowing L, Perucci CA. Effectiveness of interventions on opiate withdrawal treatment: an overview of systematic reviews. Drug Alcohol Depend 2004;73:219–226.

238. Reynaud M, Petit G, Potard D, Courty P. Six deaths linked to concomitant use of buprenorphine and benzodiazepines. Addiction 1998;93:1385–1392.

239. Gowing L, Farrell M, Ali R, White JM. Alpha2-adrenergic agonists for the management of opioid withdrawal. Cochrane Database Syst Rev 2009;(2):CD002024.

Chapter 32

METHADONE

HISTORY

In the 1930s, scientists at the pharmaceutic laboratories of the I.G. Farbenkonzern, a subsidiary of Farbwerke Hoechst (Frankfurt am Main, Germany) discovered a number of basic, substituted diphenylmethane compounds with analgesic properties. In 1939, these German scientists first synthesized the compound methadone [2-dimethylamino-4,4-diphenylheptanon-(5)], which was then numbered Va 10820. Limited clinical testing of Va 10820 occurred in Germany during World War II under the code name Amidon, but this compound was not distributed to field hospitals or civilians because of side effects during clinical testing.[1] After the war, German patents and research records on Amidon were brought to the United States; in 1947, the Council on Pharmacy and Chemistry in the American Medical Association gave the generic name, methadone, to Amidon.[2] In the same year, Eli Lily initiated commercial production of methadone in the United States under the name Dolophine®. In 1949, Hoechst AG released methadone in Germany under the trade name Polamidon®. Methadone exists under a variety of trade names throughout the world including Adanon®, Adolan®, Althose®, Amidone®, AN-148®, Anadon®, Biodone®, Butalgin®, Diskets®, Dolamid®, Dolophine®, Dopridol®, Eptadone®, Heptadon®, Heptalgin®, Heptanal®, Heptanon®, Ketalgin®, Mephenon®, Metasedin®, Methadone®, Methadose®, Methox, Miadone®, Pallidone®, Petalgin®, Phenadone®, Physeptone®, Sedo Rapide®, Symoron®, Tussol®, and Westadone®.[1] In the 1940s, clinical studies on the analgesic properties of methadone demonstrated the potential for severe respiratory depression and death.[3]

Although the mortality from self-poisoning decreased in the 1980s and 1990s, the total number of deaths from methadone increased as the availability of methadone as a prescription drug increased.[4] However, the increase in methadone-related deaths was substantially lower than the increase in heroin-related deaths.[5] In the first decade of the 21st century, methadone fatalities increased substantially, primarily in the setting of polydrug use along with the dramatic increase in the abuse of prescription opioid analgesics.[6]

IDENTIFYING CHARACTERISTICS

Methadone (CAS RN: 76-99-3, 6-dimethylamino-4,4-diphenyl-3-heptanone) is a synthetic diphenylpropylamine compound with a chemical structure similar to propoxyphene (Figure 32.1). Methadone and morphine differ chemically and pharmacokinetically, but their actions and analgesic properties are similar. The comparative analgesic potency of methadone and morphine is variable, depending on the route of exposure, tolerance (i.e., opioid-naïve or opioid-tolerant), and dose (decreasing at higher doses). In a study of 38 advanced cancer patients switched from oral morphine to oral methadone, the median oral daily dose of morphine before the switch was 145 mg and after the switch the median equianalgesic oral methadone dose was 21 mg (median morphine/methadone ratio = 7.75; range, 2.5–14.3).[7] Methadone is a racemic mixture of R-(-)- and S-(+)-methadone enantiomers in most countries with the exception of Germany.[8] Volunteer experiments indicate that R(-)-methadone (l-methadone) has euphoriant properties and suppresses the physical signs of opioid dependence, whereas the S-(+)-enantiomer
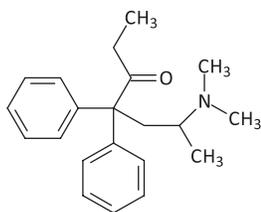
FIGURE 32.1. Chemical structure of methadone.

TABLE 32.1. Physical Properties of Methadone.

Physical Property	Value
pK$_a$ Dissociation Constant	8.94 (25°C/77°F)
log P (Octanol-Water)	3.93
Water Solubility	48.5 mg/L (25°C/77°F)
Vapor Pressure	1.12E-06 mm Hg (25°C/77°F)

(*d*-methadone) is inactive.[9,10] Based on limited clinical data, the analgesic potency of *R*(-)-methadone is about 50 times higher than *S*(+)-methadone as measured by the Hardy-Wolff-Goodell apparatus;[11] there is substantial interspecies variation in experimental tests on the analgesic potency of methadone.[12] Although the (*R*)-enantiomer accounts for most of the opioid effects of racemic methadone, pharmaceutic preparations of methadone are racemates due to cost. Methadone is highly lipid soluble. Clinical studies indicate that blockade of the human ether-à-go-go (hERG) voltage-related potassium channel is isomer-dependent with the (*S*)-methadone isomer being substantially more potent than the (*R*)- methadone isomer.[13]

Methadone is a schedule II drug (US Controlled Substances Act) for use in the treatment of opioid addiction and chronic pain, primarily in oral preparations. An intravenous (IV) formulation of methadone is available for the palliative treatment of cancer pain; however, the propensity of the preservative (i.e., chlorbutanol) in this formulation to enhance the delayed cardiac repolarization associated with methadone limits the use of this preparation.[14] Oral methadone contains sucrose as a means to prevent the IV abuse of oral methadone mixtures.[15] The hypertonic sucrose causes local tissue necrosis if injected. Most illicit methadone is diverted from pharmaceutic uses; therefore, illicit methadone typically contains few impurities.

EXPOSURE

Epidemiology

Methadone maintenance treatment for opioid dependence is an effective method for reducing opioid abuse and criminality, when compared with untreated opioid addicts.[16] Methadone intoxication usually involves multiple drug use in opioid addicts.[17] Accidental methadone poisoning also occurs in children, particularly in households with family members in methadone maintenance programs.[18] Since 1987, methadone-related deaths have increased in the United Kingdom as a result of several factors including the greater availability of medically prescribed drugs and prescribing practices that allow 2 or more prescription sources.[19] Overall, methadone is an uncommon cause of drug-related deaths.[20] In a study of fatal intoxication in Sweden from 1992–2002, methadone ranked 38th out of 50 drugs associated with fatal intoxications.[21] However, among unintentional pharmaceutic overdose fatalities, methadone is common. In a study of unintentional pharmaceutic drug overdoses in West Virginia, methadone was a contributory drug in 112 (40%) of the 295 deaths in 2006.[6] Methadone was a prescribed drug for only 32% of the methadone-related deaths. The mortality of heroin abusers in methadone treatment is about 8 times higher than the general population, but substantially lower than the mortality of heroin abusers without methadone treatment as a result of the relative decrease in death from natural causes and overdose.[22,23]

Sources

Indications for methadone include the detoxification of individuals with physical dependence on opioid drugs and the maintenance of sobriety in opioid addicts.[24] Methadone is a centrally acting opioid analgesic, which is a heroin substitute that significantly reduces the risk of death from accidental heroin overdose for patients participating in a methadone maintenance program.[25] Forms of prescription methadone include methadone tablets (5 and 10 mg methadone hydrochloride), Dolophine 40 mg (methadone hydrochloride diskets), oral solutions (5 mg and 10 mg/5 mL, 10 mg/mL), and parenteral (Dolophine hydrochloride, 10 mg/mL). Methadone is available commercially in the United States as a hydrochloride salt of the racemic mixture. The form of methadone containing only the (*R*)-enantiomer is available in the European Union and is widely prescribed in Germany. The usual source of illicit methadone is the diversion of prescribed methadone.[26] Rarely, methadone contaminates prescription medication as a result of inadvertent errors during the filling of prescriptions.[27]

Methods of Abuse

Abuse of methadone occurs via ingestion and IV injection. Inhalation of methadone is rare. Most cases of

methadone intoxication and abuse are associated with polydrug use. Abuse of benzodiazepines by methadone maintenance patients is very common with a majority of these patients using benzodiazepines in addition to methadone, particularly by patients with concomitant psychiatric disorders and histories of polydrug use.[28,29] In a cross-sectional study of urine drug screens from 2 US drug treatment centers, 65–70% of the drug screens were positive for benzodiazepines.[30] Based on self-reported drug use, the median daily dose of diazepam (i.e., the most common benzodiazepine) was 40–45 mg with the dose usually ingested in the hour before methadone treatment to enhance the effect of methadone.

DOSE EFFECT

Because of the variability in response to methadone, the initial outpatient dose of methadone in maintenance programs is ≤30 mg with an upper range of 25–40 mg in highly tolerant individuals.[15] For patients with uncertain or low tolerance, the initial methadone dose is 10–20 mg. The dose is increased 5–10 mg/day depending on the development of withdrawal symptoms and the craving for heroin with close physician supervision. Patients in methadone maintenance programs typically receive about 50 mg daily, but daily doses range up to 180–300 mg daily. Volunteer studies indicate that 100 mg of methadone blocks the euphoriant effects of 25 mg of heroin in most participants for 48 hours, whereas all participants developed euphoria after receiving 25 mg heroin 72 hours after ingesting 100 mg methadone.[31] The IV administration of 75 mg heroin produced euphoria in 4 of 6 participants despite the ingestion of 100 mg of methadone 48 hours prior to the heroin injection.

Doses greater than 30 mg of oral methadone consistently cause sedation in opioid-naïve individuals.[32] In a study of healthy opioid-naïve volunteers, adverse effects associated with the ingestion of 10–15 mg methadone included nausea, vomiting, and lightheadedness.[33] Only 4 of 17 opioid addicts developed adverse effects (nausea, vomiting, sedation) after receiving a mean methadone dose of 39 ± 17 mg (range, 15–80 mg). Case reports suggest that 1 mg/kg of methadone may cause fatal apnea in children.[34,35] Therefore, the potentially fatal dose in young children is ~10–20 mg. Case reports indicate that some tolerant individuals can receive up to 400 mg methadone subcutaneously without serious adverse effects, whereas some nontolerant adults develop severe respiratory depression after receiving 40 mg methadone intravenously or 70 mg methadone orally.[36] These studies also indicate that opioid-dependent adults lose their tolerance to methadone after 5 weeks of abstinence.

TOXICOKINETICS

Absorption

The absorption of methadone following ingestion is rapid and almost complete with detectable methadone concentrations appearing in plasma within 30 minutes of oral administration.[37] The low hepatic extraction ratio of oral methadone accounts for relatively high bioavailability (i.e. about 70–90%); absorption of methadone is not stereoselective. In a study of 8 opioid abusers receiving 20 mg methadone, the bioavailability of methadone was 0.79 ± 0.21 with a range of 0.41–0.99.[38] The gastric absorption of oral methadone doses is slower in opioid users than opioid-naïve individuals. In a study of 13 opioid-naïve individuals and 17 opioid users receiving oral methadone, the mean absorption half-lives were 0.2 hour and 0.73 hour, respectively.[33] Following the regular daily administration of 100–120 mg methadone to 5 opioid-dependent individuals, the peak plasma methadone concentrations occurred about 2–4 hours after ingestion.[39] Rectal administration of methadone is an alternative to oral use in cancer patients as the absorption of methadone by this route is rapid with high bioavailability. In a study of 7 healthy volunteers receiving 10 mg d_5-methadone-HCl rectally, the mean absolute rectal bioavailability of methadone was 0.76 (95% CI: 0.69–0.82) compared with 0.86 (95% CI: 0.72–0.99) for a similar oral dose.[40] The mean time to peak plasma methadone concentration was more rapid for rectal administration (mean, 1.4 h; 95% CI: 0.83–2.0 h) than ingestion (mean, 2.8 h; 95% CI: 1.3–4.3 h). The bioavailability of methadone via insufflation is sufficient to cause death when methadone is surreptitiously substituted for other drugs of abuse used intranasally.[41] In a cross-over study of 8 healthy volunteers, the mean bioavailabilities of 10-mg doses of methadone administered orally and nasally were approximately 0.85.[42] The maximum plasma methadone concentration and the time of maximum effects as measured by dark-adapted pupil diameter following the nasal dose occurred at 7 minutes and 30 minutes after administration, respectively.

Distribution

Methadone undergoes a rapid distribution phase into a large volume of distribution followed by a slow elimination phase. This drug is widely distributed to fat, lung, liver, kidney, spleen, blood, and urine.[43] The mean apparent volume of distribution was approximately 3.5 L/kg after the IV administration of 10–30 mg methadone to 8 chronic pain patients including 5 cancer patients.[44] A study of 5 opioid addicts receiving daily methadone

doses of 10–60 mg demonstrated a mean steady-state volume of distribution of approximately 6.7 L/kg with range of 4.2–9.2 L/kg.[45] Methadone is highly bound to plasma protein, predominately to the α_1-acid glycoprotein portion with a bound fraction ranging from about 71–89%.[46,47] Methadone binds strongly to orosomucoid 2 (α_1-acid glycoprotein variant) and to a lesser extent to orosomucoid 1.[48] The free fraction of methadone varies directly with plasma α_1-acid glycoprotein concentrations with a range of about 6–14%. Interindividual variation in binding to α_1-acid glycoprotein contributes to the differences in individual response to racemic methadone in addition to variability in cytochrome P450 activity.[49]

Biotransformation

The biotransformation of methadone is stereoselective with large interindividual variation, primarily involving N-demethylation and cyclization of methadone in the liver to inactive pyrrolidine metabolites. Figure 32.2 displays the major metabolites of methadone. Methadone metabolites include 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP), pyrrolidone, pyrroline, hydroxymethadone, hydroxypyrroline, methadol, and hydroxypyrrolidine.[50,51] Methadone also undergoes hydroxylation and dealkylation of the N-methyl groups. The dealkylated metabolites are unstable, resulting in spontaneous cyclization to form EDDP. N-demethylation of EDDP also occurs. Because of the large first-pass effect, the peak EDDP plasma concentration occurs prior to the peak methadone plasma concentration. In a study of 20 opioid-dependent individuals on chronic methadone therapy, the mean time to peak EDDP and methadone concentrations in plasma were about 2.5 hours (range, 1.0–6.7 h) and 3.7 hours (range, 1.8–6.8 h), respectively.[52]

Minor metabolic pathways include the formation of the active metabolites, hydroxymethadone and various methadol metabolites.[53] Figure 32.3 displays the minor metabolites of methadone. Methadone biotransformation involves several cytochrome P450 isoenzymes, but there is a lack of consensus on the exact role of each isoenzyme. Initial *in vitro* studies suggested that CYP3A4 is the primary hepatic CYP450 isoenzyme
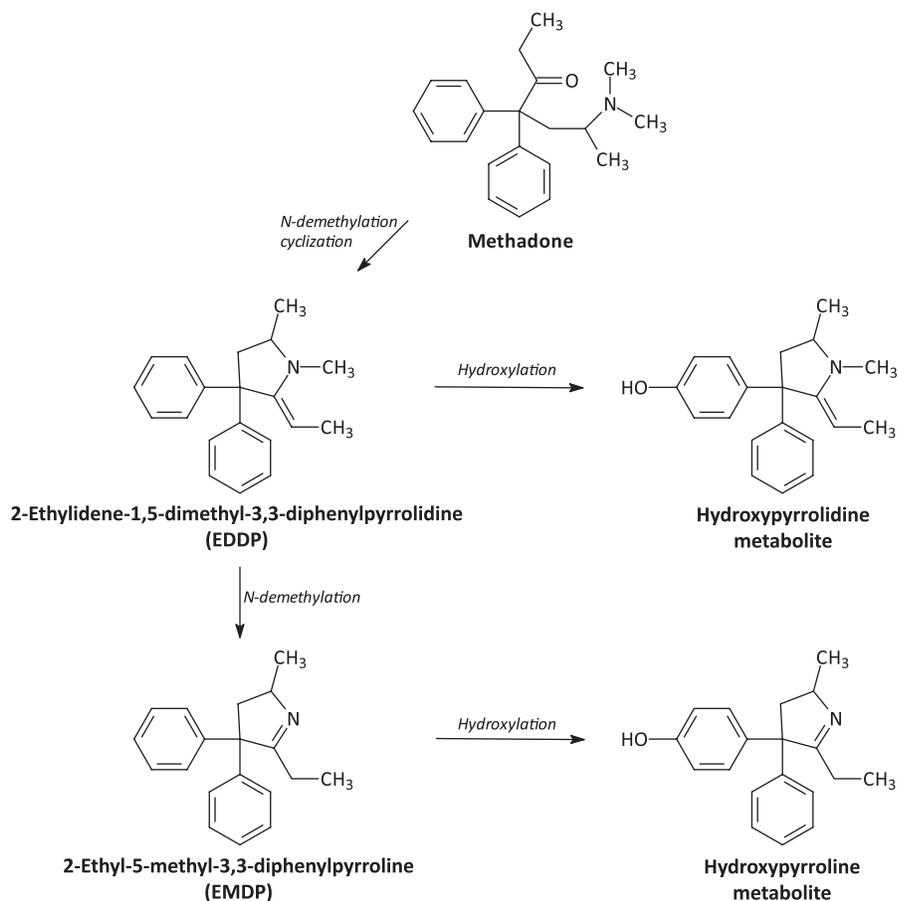
FIGURE 32.2. Major metabolites of methadone (adapted from Kreek, 1976).[53]
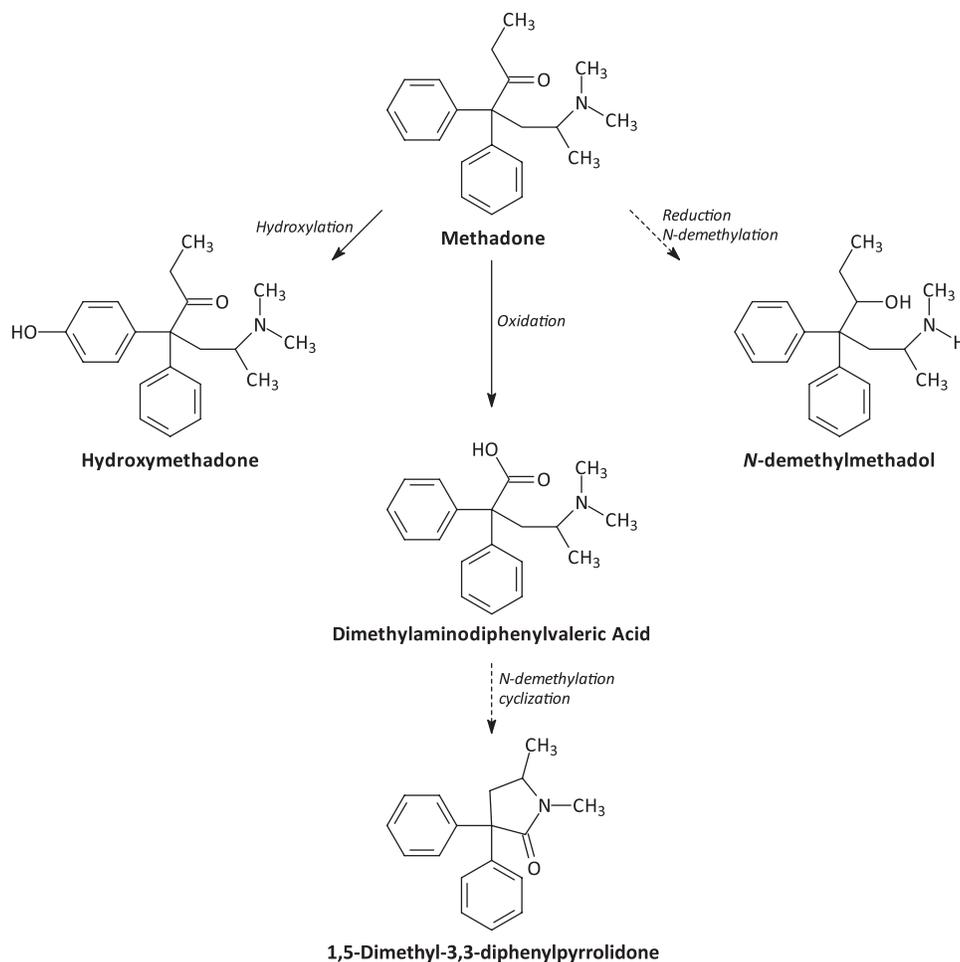
FIGURE 32.3. Minor metabolites of methadone (adapted from Kreek, 1976).[53]

involved with the biotransformation of methadone, whereas other cytochrome P450 isoenzymes (e.g., CYP2B6, CYP2D6, CYP2C9, CYP2C19) participate in the biotransformation of methadone to a lesser extent than CYP3A4.[54,55] However, *in vivo* studies indicate that CYP2B6 has a prominent role in the biotransformation, particularly the chiral *N*-demethylation of methadone.[56] There is substantial variation in the individual expression of CYP3A4; this isoenzyme is inducible resulting in modest individual variation in the biotransformation of methadone. The methadone dose accounts for <50% of the variation in plasma methadone concentrations in studies of methadone maintenance patients, whereas CYP3A4 activity accounts for approximately 20% of the variation in plasma methadone concentrations based on pharmacokinetic studies of CYP probes.[57] CYP3A4 is not stereoselective, whereas CYP2C19 prefers the *R*-(-)-enantiomer. Case reports suggest that CYP3A5 polymorphism may account for rapid methadone clearance and relatively low methadone concentrations in some individuals.[58]

Elimination

The kidney excretes unchanged methadone and glucuronide metabolites with about one-half of a single, methadone dose appearing in the urine as unchanged methadone and metabolites over the first 96 hours after ingestion.[59] Methadone has a long elimination half-life. In a study of healthy volunteers receiving an oral methadone dose of 8–15 mg, the median terminal plasma elimination half-life of methadone ranged from 33–46 hours (depending on the method of calculation).[33] There is wide individual variation (i.e., 15–55 h) in the terminal plasma elimination half-life of methadone. In a study of 5 opioid addicts stabilized on 10–60 mg methadone daily, the mean plasma elimination half-life was about 27 hours (95% CI: 14–39 h).[45] The terminal plasma elimination half-life of the 2 methadone enantiomers was similar in some studies,[60] but the elimination half-life of the (*R*)-enantiomer was slightly longer than the (*S*)-enantiomer in another study (37.5 h and 28.6 h, respectively).[61] In a study of a 44-year old man found

583

comatose with depressed respiration after ingesting an estimated 240 mg methadone, the plasma elimination half-life of the (*R*)- and (*S*)-enantiomers was 16.1 hours and 13.2 hours, respectively.[62]

Increasing plasma methadone concentrations occur when repeat doses of methadone are administered within 6 hours. The induction of P450 isoenzymes requires about 2–3 weeks; therefore, the clearance of methadone gradually increases during this induction period.[63] The urinary excretion of methadone is pH dependent as a result of reduced renal tubular reabsorption of methadone with decreasing urine pH.[59] Hence, the renal clearance of methadone increases in acidic urine. Above a urinary pH of 6, the renal excretion of unchanged methadone is 4% of the absorbed dose, but renal excretion increases substantially when urinary pH is <6.[8] The plasma elimination half-life of methadone in a study of 8 opioid abusers receiving 20 mg methadone orally was 19.5 ± 3.6 hours following urinary acidification and 42.1 ± 8.8 hours following urinary alkalinization.[38] Methadone remains in the plasma long after the analgesic effects of methadone subside. [64]

Maternal and Fetal Kinetics

Methadone crosses the placenta and may cause respiratory depression in the fetus. Methadone metabolism increases during pregnancy, resulting in lower plasma methadone concentrations. Case reports suggest that symptoms of withdrawal can occur in women during pregnancy despite maintaining the same methadone doses that suppressed withdrawal symptoms prior to pregnancy.[65] In general, methadone concentrations in breast milk of lactating mothers in methadone maintenance programs are low and remain stable over time with relative infant dose usually <5% of the maternal weight-adjusted dose.[66] In a study of 12 breast-feeding women on daily methadone doses of 20–80 mg, the mean milk/plasma ratio was 0.44 (95% CI: 0.24–0.64).[67] The mean infant dose as a percentage of the maternal dose was 2.79% (95% CI: 2.07–3.51%) with an estimated mean methadone dose for the infant of 17.4 μg/kg (95% CI: 10.8–24 μg/kg) assuming 100% bioavailability. Case reports suggest that secretion of methadone into breast milk from lactating mothers in methadone maintenance programs is minimal,[68] and the American Academy of Pediatrics lists methadone as a maternal medication usually compatible with breast-feeding.[69] A retrospective study suggested that high-dose (i.e., >100 mg daily) methadone maintenance therapy is not associated with an increased risk of adverse fetal outcomes (neonatal abstinence, infant hospitalization) when compared with low-dose (i.e., <100 mg daily) methadone maintenance therapy.[70]

Tolerance

The development of tolerance to methadone results in cross-tolerance to other opioids with similar properties. However, the cross-tolerance is not complete because methadone acts at several central nervous system (CNS) receptor sites in addition to the μ-opioid receptor including the δ-opioid and NMDA receptors, whereas heroin acts primarily at the μ-opioid receptor. The cross-tolerance of methadone to other opioids varies between individuals and predicting the degree of cross-tolerance is difficult. Consequently, tolerance to heroin does not guarantee tolerance to all the effects of methadone or other opioids.

Partial tolerance develops to the respiratory and pupillary effects of methadone, but the tolerance to the respiratory depressant effects of methadone does not necessarily occur at the same rate as the tolerance to the euphoriant or analgesic effects.[71,72] The development of tolerance requires more time for methadone than for morphine with tolerance to the methadone-induced respiratory effects occurring over 1 year in some individuals.[73] Tolerance develops to reductions in tidal volume, but not to reductions in CO_2 sensitivity.[74]

Drug Interactions

The biotransformation of methadone is complex, involving several CYP450 isoenzymes; therefore, there are a substantial number of potential drug interactions based on *in vitro* studies. A substantial portion of the *in vitro* methadone metabolism occurs by the inducible P450 isoenzyme, CYP3A4, whereas most *in vivo* metabolism of methadone in humans occurs via CYP2B6. The clearance of methadone increases severalfold between the initial dose of methadone and steady-state concentrations.[63] Concomitant administration of certain drugs (e.g., phenytoin, rifampicin, zidovudine, barbiturates, spironolactone, verapamil, diethylstilbestrol, and amitriptyline) induces CYP3A4 and potentially increases methadone clearance. Rifampin lowers plasma methadone concentration, increases methadone urinary excretion, and increases fecal excretion of the major pyrrolidine metabolite.[53,75] The initiation of phenytoin or rifampin accelerates methadone clearance and can cause withdrawal symptoms in the first several days of drug administration.[76] Protease inhibitors (e.g., indinavir, ritonavir) are potent inhibitors of hepatic and intestinal CYP3A isoforms including CYP3A4. However, the clinical significance of this potential drug interaction is unclear. In an *in vivo* study, 12 healthy volunteers received IV and oral methadone alone and in addition to 100 mg ritonavir/800 mg indinavir twice daily.[77] The ritonavir/indinavir dose was sufficient to produce >90%

inhibition of hepatic and first-pass CYP3A activity. There was no statistically significant effects of the administration of ritonavir/indinavir on plasma methadone concentrations, bioavailability, renal methadone clearance, or plasma methadone concentration-effect relationships when compared with baseline values of each volunteer. The lack of a significant drug interaction in this study reflects the importance cytochrome P450 isoenzymes (e.g., CYP2B6) other than CYP3A4 in the biotransformation of methadone. *In vivo* volunteer studies of fosamprenavir 700 mg/ritonavir 100 mg twice daily for 14 days and methadone indicate that this combination causes a small reduction (i.e., mean, 18%) in total plasma (*R*)-methadone concentration without a significant change in free plasma (*R*)-methadone concentration; therefore, no reduction in methadone dosage is recommended when administered with this antiviral agent, consistent with the importance of CYP2B6 compared with CYP3A4.[78]

Ketoconazole, erythromycin, paroxetine, cimetidine, and fluoxetine can increase plasma concentrations of methadone by inhibiting CYP3A4 based on *in vitro* studies, but the relevance of this potential drug interaction is limited because of the importance of other cytochrome P450 isoenzymes (e.g., CYP2B6) in *in vivo* human metabolism of methadone. These potential inhibitory effects last only a few days compared with the 2–3 weeks required for the induction of CYP3A4 isoenzymes. Case reports suggest that potential drug interaction between methadone and fluvoxamine (but not fluoxetine) may be clinically significant.[79,80] Diazepam (10–40 mg) enhances some of the subjective and physiologic effects of methadone (drug-liking, high, goodness) in some opioid addicts.[81,82] However, the concomitant administration of diazepam and methadone does not significantly alter methadone metabolism.[83,84] Central nervous system depressant drugs (e.g., alcohol, sedative-hypnotic, barbiturates) synergistically enhance the respiratory depression of methadone depending on individual tolerance and pharmacogenetics.[85] Although disulfiram enhances *N*-demethylation of methadone and increases urinary excretion of the major pyrrolidine metabolites of methadone, the interaction between methadone and disulfiram is not clinically significant.[86]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Methadone is a central-acting analgesic with high affinity for μ-opioid receptors and some affinity for the δ-opioid receptors. Morphine (K_i, 1.41 nM) has a higher affinity for the μ-opioid receptor than methadone (K_i, 3.51 nM).[87] Stimulation of these opioid receptors increases potassium channel opening by reducing production of cAMP as a result of the inhibition of adenylate cyclase activity. Inhibition of inward Ca^{++} currents results from the opening of voltage-operated calcium channels. The increased K^+ conductance, decreased Ca^{++} conductance, and the subsequent reduction in membrane excitability provide a molecular mechanism for the analgesic activity of methadone and other opioids.[88] Mu and delta receptor agonist activity produce synergistic effects on respiratory depression. Table 32.2 lists the pharmacologic properties of opioid receptor subtypes. Naloxone has lower affinity for the δ-opioid receptors compared with the μ-opioid receptors. The miosis associated with opioid intoxication results from increased cholinergic stimulation of the nucleus of the oculomotor nerve (cranial nerve III). Blockade of acetylcholine release in the reticular activating system by methadone produces CNS depression. In contrast to morphine, hydromorphone, and naltrexone, animal studies suggest that methadone and the *d*- and *l*-enantiomers of methadone have noncompetitive antagonist activity at the NMDA receptor similar to dextromethorphan.[89]

Mechanism of Toxicity

Methadone and heroin are the major opioids associated with respiratory depression and death following opioid overdose. The medullary centers control respiration with peripheral input from chemoreceptors and other sources. Opioids reduce chemoreceptor sensitivity via μ-opioid receptor stimulation and depress medullary centers via μ- and δ-opioid receptor stimulation. Opioids (e.g., methadone) initially reduce tidal volume and decrease CO_2 sensitivity of the respiratory center chemoreceptors. Larger doses of methadone depress both tidal volume and respiratory rate.[90] Glutamate and GABA are the major excitatory and inhibitory neurotransmitters, respectively. Alcohol facilitates opioid-induced respiratory depression by decreasing the excitatory effect of glutamate at NMDA receptors.[91]

During myocardial repolarization, potassium ions exit the myocardial cells via the slow component (I_{Ks}) and rapid component (I_{Kr}) potassium channels. The human ether-a-go-go-related gene (HERG) encodes the rapid channel protein, and methadone blocks the human ether-a-go-go-related gene potassium current (I_{HERG}) with an IC_{50} <3 μM similar to L-α-acetylmethadol.[92] Subsequently, QT_c prolongation occurs because of the reduced potassium efflux during repolarization, similar to over 100 other drugs.[93] Potential factors contributing to the development of

TABLE 32.2. Pharmacologic Properties of Opioid Receptor Subtypes.[15]

Receptor Type	Mu₁ (μ_1)	Mu₂ (μ_2)	Delta₁ (δ_1)	Delta₂ (δ_2)	Kappa (κ)
Analgesia	Supraspinal/spinal	Spinal	Spinal synergistically with μ-opioid receptors	Peripheral independent of μ-opioid receptors	Spinal
Respiratory effect	Decrease tidal volume		Decrease frequency	Decrease frequency	Decrease
Pupil response	Constriction	Miosis	Constriction	Constriction	Dilatation
GI motility	Urinary retention	Constipation			Alters intestinal motility
Smooth muscle	Contraction		Contraction	Contraction	—
Behavior/affect	Euphoria, sedation, indifference	Nausea, vomiting	Sedation		Dysphoria, sedation
Neurologic	Inhibit neuronal activity, open K⁺ channel, close Ca⁺⁺	Inhibits NE, substance P release	Inhibit neuronal activity, adenylate cyclase	Inhibit substance P release	Inhibit DA release
Heart rate	Slows		—	—	
Opioid activity	Reinforcing		Reinforcing		

Abbreviations: GI, gastrointestinal; NE, norepinephrine; DA, dopamine.

sudden death in methadone users with relatively low postmortem methadone concentrations include cardiac dysrhythmias and drug-induced respiratory failure. The cause of death in these situations is probably multifactorial, and the causal role of methadone is not well defined.

Postmortem Examination

Deaths in patients receiving methadone typically involve drug abuse-related causes (overdose), liver failure, pulmonary emboli, sepsis, human immunodeficiency virus (HIV), trauma (self-inflicted, accidents, homicide, burns), and other causes (kidney failure, cancer, infections).[94] The postmortem findings in patients dying from methadone intoxication are nonspecific and usually include evidence of IV drug abuse (e.g., hyperplasia of the lymph nodes of the porta hepatis, hepatic triaditis, cutaneous stigmata).[95] The most frequent postmortem finding during autopsies of methadone-related fatalities is pulmonary congestion and edema.[96] Findings of severe pulmonary edema are typical during postmortem examination of methadone-induced fatalities. In a study comparing 39 methadone-related fatalities with a control group of 75 homicide victims with a history of opioid abuse, the mean lung weights were 1306 g and 914 g, respectively.[97] Other findings include bronchopneumonia, aspiration pneumonia,[98] cerebral edema,[99] and medical complication of IV drug abuse (e.g., viral hepatitis, bacterial endocarditis, pulmonary fibrosis).[100,101] In most, but not all postmortem examinations of methadone-related deaths, the cardiovascular system appears normal, and there is no anatomic evidence of other potential causes of death.[102] The lack of unique pathologic changes complicates the determination of the cause of death in relation to the roles of fatal dysrhythmias and respiratory depression.

CLINICAL RESPONSE

Medical Use

Adverse effects associated with the use of methadone include headache, constipation, pruritus, lightheadedness, dry mouth, anorexia, drowsiness, blurred vision, sweating, myoclonus, confusion, and hallucinations[103] along with hypotension and bradycardia following high methadone doses.[104] Peak effects (e.g., sedation, altered perception, analgesia, CNS depression) occur approximately 3 hours after ingestion of therapeutic doses, but in some cases the onset of peak effects may be delayed. Rare case reports associate high doses of methadone during palliative therapy with myoclonus[105] and toxic leukoencephalopathy manifest by high-intensity changes in the T2-weighted magnetic resonance images of cerebral white matter with sparring of the subcortical U-fibers and posterior fossa structures.[106,107]

Overdose

Methadone overdose produces classic symptoms of opioid intoxication including CNS depression (lethargy, stupor, coma), respiratory depression, and miosis.[108]

Symptoms of intoxication usually develop within 2–3 hours of ingestion; onset of symptoms after 9 hours is unusual. In a retrospective study of poison center data on 44 calls related to methadone, all symptoms developed within 9 hours after ingestion.[109] The mean estimated dose of methadone was about 100 mg; all symptoms resolved within 24 hours. Miosis is not a sensitive sign of methadone poisoning, particularly in children.[34] Normal or increased pupillary diameter can occur with high plasma methadone concentrations and/or hypoxia. The level of consciousness ranges from drowsiness to profound coma. Respiratory depression is the most common, serious complication of methadone intoxication, particularly in weakly tolerant individuals initiating high-dose methadone therapy.[110] Respiratory depression persists several days during methadone intoxication, and severe methadone intoxication frequently requires naloxone administration for several days. A case report of a 22-year-old man, who ingested 420 mg methadone, was observed sleeping with normal respirations 41 hours after ingestion.[111] Shortly thereafter, the naloxone infusion was stopped, and ½ hour later he was observed sleeping. The patient was found dead in his bed 3 hours later. Other complications of opioid overdose include hypotension, bradycardia, hypothermia, rhabdomyolysis, and renal dysfunction.[112] Rarely, case reports associate the development of acute respiratory distress syndrome with methadone toxicity following IV or oral use.[113,114] The development of pulmonary edema occurs in patients during methadone intoxication prior to the use of naloxone. Case reports document transient, acute bilateral sensorineural hearing loss during methadone overdoses that resolved within 24–96 hours.[115,116]

Fatalities

OVERDOSE

Deaths from methadone overdose typically involve one of the following 3 scenarios: 1) excessive or repetitive doses of illicit methadone used to obtain euphoria, 2) drug interaction of licit or illicit methadone and other CNS depressants (ethanol, benzodiazepines, opioids), and 3) accumulation of fatal concentrations of methadone following treatment for dependency or chronic pain.[117] Fatalities related to methadone frequently occur in individuals who obtain the drug without medical supervision.[118,119] Fatalities from the sole ingestion of methadone are relatively rare compared with fatalities from IV heroin use.[120,121] Case reports suggest that the highest risk of death during methadone maintenance for heroin addiction occurs in the first few days to 2 weeks after the initiation of methadone treatment, pri-

marily as a result of individual differences in methadone tolerance and pharmacokinetics. The initial plasma elimination half-life of methadone in opioid addicts can be substantially longer than later in the treatment program, and the initial prolonged elimination half-life allows the accumulation of methadone along with potential adverse effects.[122] Other risk factors for methadone intoxication include persistent use of other illicit drugs, consumption of illicit methadone, poor compliance with methadone dosing schedule, inadequate hepatic clearance of methadone secondary to liver disease, and inappropriate or rapid escalation in methadone dosing.[123,124]

The common scenario of a methadone-related overdose is a methadone-treated heroin addict found dead in the morning after being seen the preceding night appearing intoxicated (euphoria, ataxia, slurred speech).[97] Death is rarely sudden, and the toxicity following oral methadone is delayed in contrast to the more immediate toxicity of IV heroin and fentanyl derivatives.[125] In a study of 87 adult fatalities with positive drug screens for methadone, the mean time between methadone use and death was about 5–6 hours.[126] Polydrug use (e.g., alcohol, benzodiazepines) potentiates the respiratory depressant actions of methadone.[127] Although the use of benzodiazepines by patients in methadone treatment programs is frequent, comparison of unintentional fatal overdoses of methadone and other opioid analgesics does not demonstrate increased benzodiazepine use in the methadone group.[128] Occasionally, fatalities occur in children of patients in methadone maintenance programs, when the child ingests the patient's medication.[129]

SUDDEN DEATH

The risk of fatal cardiac dysrhythmia during methadone maintenance therapy appears relatively low; however, determination of the prevalence of fatal dysthymias is complicated by the lack of clinical data in patients dying while being treated with methadone.[130] Occasional case reports associate the therapeutic postmortem methadone concentrations with sudden death as defined by death within 24 hours of being seen alive and asymptomatic or within 1 hour of the witnessed onset of symptoms. In a case series of 22 sudden cardiac deaths in patients in methadone maintenance programs, the prevalence of detectable cardiac abnormalities was 23% ($n = 5$).[131] All these patients had postmortem blood methadone concentration <1 mg/L; however, the appropriateness of the antemortem doses cannot be accurately determined from postmortem data. The prevalence of cardiac abnormalities (coronary artery narrowing >50%, left ventricular hypertrophy >95% of the

upper limit of normal, dilated cardiomyopathy, myocarditis) was higher than the number of cardiac abnormalities ($n = 64, P = .002$) in a comparison series of 106 cases of sudden death with no evidence of methadone use. Cases with evidence of recreational drug use or drug overdose were excluded from both comparison groups. No other drugs were reported in the toxicology screen for blood samples from 8 of the 17 individuals without evidence of cardiac disease; none of the blood samples had detectable concentrations of benzodiazepines.

Abstinence Syndrome

The onset of withdrawal symptoms following cessation of drug use is slower for methadone than heroin. Additionally, methadone withdrawal is less intense and more prolonged compared with heroin withdrawal. Observational studies indicate that the abstinence symptoms associated with methadone begin about 24–48 hours after cessation of methadone with peak symptoms about 2–6 days after initial withdrawal symptoms of vomiting and diarrhea.[132] The subjective effects of methadone and heroin withdrawal are similar except that muscle cramps and arthralgias are not typically prominent during methadone withdrawal. Manifestations of methadone withdrawal include nausea, vomiting, diarrhea, weakness, anxiety, insomnia, abdominal discomfort, headache, sweating, and hot and cold flashes. Although lethargy may persist for 6–8 weeks, withdrawal symptoms usually subside within 10 days and resolve completely within 2–3 weeks.

Withdrawal symptoms occur 7–10 days after birth in neonates born to mothers chronically receiving methadone, and these withdrawal symptoms typically require treatment for about 1 month.[89] A meta-analysis of the medical literature indicates that the severity of the neonatal abstinence syndrome depends on several factors; the incidence of neonatal withdrawal is not clearly related to the methadone dose.[133] In a study of 450 singleton pregnancies of women prescribed substitute methadone for drug abuse during pregnancy, 48.5% developed neonatal abstinence syndrome requiring morphine therapy.[134] Neonatal withdrawal symptoms include tremors, irritability, hyperactivity, excessive crying, vomiting, and elevated temperature.[135,136] Disturbance of sleep patterns, poor fine motor coordination, and excessive crying can persist in these neonates for several months after withdrawal ceases.

Reproductive Abnormalities

Adverse fetal outcomes associated with opioid-dependent women on methadone during their pregnancy include preterm birth, low birth weight, and neonatal abstinence syndrome.[137] Methadone treatment is continued through pregnancy in opioid-dependent mothers to prevent fluctuating opioid concentrations and withdrawal episodes in the fetus. Birth weights of neonates born to methadone-dependent mothers are lower than neonates born to nonopioid-dependent mothers of similar socioeconomic backgrounds, but are higher than the birth weight of neonates born to opioid-dependent mothers without methadone treatment programs. The risk of preterm delivery and stillbirth for this group of mothers on methadone maintenance is also higher than overall rates in general hospitals.[134] In a study of 141 infants born to opioid-dependent mothers in methadone treatment programs and 127 infants born to nonopioid-dependent mothers, the mean birth weights were $2{,}953 \pm 438$ g and $3{,}210 \pm 488$ g, respectively.[138] The difference was statistically significant ($P < .001$). The control group was matched for socioeconomic status, race, and maternal age. At 6-month follow-up, there was no statistically significant difference between the 2 groups in the scores on the Bayley Scale of Mental Development.[89] Some neonates born to mothers in methadone maintenance programs develop moderate to severe opioid withdrawal symptoms (see Abstinence Syndrome). In general, methadone is not considered teratogenic; no specific congenital abnormalities are associated with methadone treatment during pregnancy with the exception of Pierre Robin sequence in methadone-exposed mothers in a retrospective Irish cohort study of 61,030 singleton births at an urban maternity hospital.[139]

DIAGNOSTIC TESTING

SI Units:

$$1 \text{ ng/mL} \approx 3.75 \text{ nmol/L}$$
$$1 \text{ nmol/L} \approx 0.266 \text{ ng/mL}$$

Analytic Methods

Methods for the quantitation of methadone and the main metabolite, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium (EDDP) in biologic samples include the following: high performance liquid chromatography with UV detection following liquid-liquid extraction (interassay bias <10%; CV <15%),[140] high performance liquid chromatography with diode array detection after solid phase extraction (lower limit of quantitation [LLOQ], 1 ng/mL; accuracy, ±8%),[141,142] liquid chromatography/mass spectrometry after solid-phase extraction and separation with a Sunfire column using a

gradient mode (limit of detection [LOD], 0.5–1 ng/mL; CV <10%),[143] capillary electrophoresis/atmospheric pressure ionization/mass spectrometry,[144] gas chromatography/electron impact mass spectrometry,[145] and gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring with solid phase extraction at pH 6.0.[146] High injector-port temperatures in gas chromatography potentially induce the thermal conversion of methadone to EDDP as an artifact. Reducing the GC injector-port temperature reduces the observed EDDP concentrations, but other methods (e.g., liquid chromatography/mass spectrometry) may be necessary if such conversions occur.[147] Methods to quantitate the enantiomers of methadone and EDDP include chiral liquid chromatography/tandem mass spectrometry.[148,149] The LOD and LLOQ for this method are 0.001 mg/L (mg/kg) and 0.003 mg/L (mg/kg), respectively, with a coefficient of variation <6.3%. Enantioselective liquid chromatography/mass spectrometry allows the quantitation of both free and total enantiomers of methadone and EDDP with accuracy and precision <5–10%.[150,151] Meconium is a more complex media than blood as a result of the presence of bile acids and other excretory products; liquid chromatography/ atmospheric pressure chemical ionization/tandem mass spectrometry allows the quantitation of methadone and EDDP after solid-phase extraction with LLOQ in the range of 5 ng/g.[152] The placenta is an alternative sampling site to meconium for determination of methadone and EDDP concentrations, when meconium is unavailable; however, the placental methadone/ EDDP concentrations and sampling time are substantially less than those in meconium.[153] Methods for the quantitation of methadone and EDDP in breast milk include high performance liquid chromatography with UV detection after liquid-liquid extractions,[67] liquid chromatography/atmospheric pressure chemical ionization/tandem mass spectrometry following protein precipitation and solid-phase extraction,[154] and gas chromatography/electron impact mass spectrometry after protein precipitation (acetonitrile) and solid-phase extraction.[155]

Most of the uncertainty in analytic techniques results from preanalytic (e.g., clotting, heterogeneity, water content, sampling technique, storage) rather than variation in analytic processes. In a study of the preanalytic variation of postmortem methadone measurements from left and right femoral vein samples, preanalytic variation (CV%) for R-(-)- and S-(+)-enantiomers was 3–4% compared with 19–21% for preanalytic variation.[156] For EDDP enantiomers, the CV% was higher (4–6% vs. 30–38%, respectively). Methadone is unstable when stored at room temperature for 24 hours due to the formation of N-oxides.[53] Additionally, pyrrolidine

undergoes degradation to pyrrolidine metabolites when stored at room temperature.

Biomarkers

Some secretion of methadone into the acid environment of the stomach occurs as a result of the diffusion of methadone from surrounding blood vessels in addition to the postmortem diffusion of methadone from fat. Typically, the stomach fluid/postmortem blood methadone ratio is ≤4 following IV methadone use, whereas this ratio is ≥10 following the ingestion of methadone.[125] Hence, a postmortem stomach fluid/blood methadone ratio exceeding 10 suggests antemortem ingestion of methadone. The postmortem methadone concentrations in urine and bile typically exceed the postmortem blood concentration of methadone.[43] In a series of 22 cases with methadone present in postmortem blood samples, the mean bile/femoral blood methadone ratio was 15.25.[157] The concentration of the metabolite, EDDP usually exceeds the methadone concentration in bile.[43,158] Methadone concentrations in vitreous humor do not correlate well to postmortem blood concentrations of methadone.[159]

Blood

Following the administration of IV methadone, the mean whole blood/plasma ratio was 0.75 ± 0.03 as measured by radioimmunoassay.[160]

THERAPEUTIC USE. Following the administration of 15 mg methadone to 5 healthy individuals, the mean peak total methadone plasma concentration was 74 ng/ mL 4 hours after ingestion; the mean plasma methadone concentration decreased to 29 ng/mL 24 hours after ingestion.[59] The mean trough methadone concentration in plasma samples from 6 individuals receiving daily methadone doses of 80 mg in a methadone maintenance program was 420 ± 97 ng/mL.[161] The mean peak plasma methadone concentration 4 hours after daily administration of 100–120 mg methadone was approximately 860 ng/mL.[39] Figure 32.4 displays the methadone timeconcentration from this study. In a study of 50 opioid addicts treated with a mean, daily racemic methadone dose of 95 ± 44 mg, the mean methadone plasma concentration just prior to dispensing the daily methadone dose was highly variable (296 ± 176 ng/mL; range, 97–1,094 ng/mL).[162] In a study of 20 long-term opioid addicts receiving mean daily methadone doses of 60 mg (range, 10–225 mg), the steady-state plasma methadone concentration ranged from 65–630 ng/mL with peak concentrations ranging from 124–1,255 ng/mL as measured by GC/MS after solid-phase extraction.[52] The
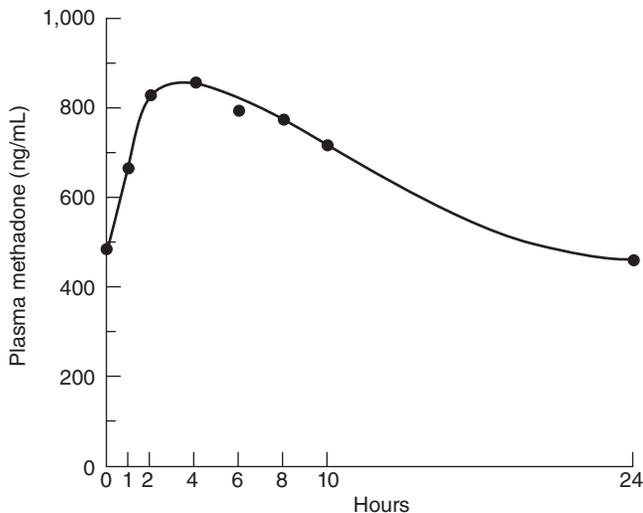
FIGURE 32.4. Time-concentration curve of mean plasma methadone concentration after ingestion of 100–120 mg methadone by 5 individuals in a methadone maintenance program. (Reprinted by permission from Macmillan Publishers Ltd.: Clinical Pharmacology and Therapeutics, Vol. 13, CE Inturrisi, K Verebely, The levels of methadone in the plasma in methadone maintenance, p. 635, 1972.)

plasma concentration of the primary metabolite (EDDP) ranged from 5–55 ng/mL with peak concentrations ranging from 10–301 ng/mL.

In a methadone maintenance program, 31 patients received daily methadone doses ranging from 3–100 mg. The plasma methadone concentration at steady state increased approximately 263 ng/mL for each 1 mg/kg increase in the methadone dose.[163] However, these patients were not under direct observation, and some patients were excluded because of obvious noncompliance. Consequently, this value is an approximation. Plasma methadone concentrations prior to the next methadone dose of 150–200 ng/mL are sufficient to suppress withdrawal symptoms; plasma concentrations below 50 ng/mL are associated with withdrawal symptoms.[164,165] Withdrawal symptoms are unlikely when the plasma methadone concentration exceeds 500–700 ng/mL. Some patients are highly tolerant of methadone. A 25-year-old heroin addict on a daily methadone dose of 360 mg had a plasma methadone concentration of 2,800 ng/mL associated with drowsiness, but no other adverse effects.[166] As a result of the large variability in clinical response to methadone, most patients in methadone maintenance programs are monitored for clinical effects rather than plasma methadone concentrations.

OVERDOSE.   There are few data on the plasma methadone concentration during methadone overdose. In general, treatment is guided by the clinical presentation, and plasma methadone concentrations confirm exposure rather than guide treatment.

POSTMORTEM.   Interpretation of the significance the postmortem methadone blood concentrations requires careful analysis of several factors including the timing and dose of methadone, prior drug use and tolerance, concomitant medications, clinical history immediately prior to death, disease states, postmortem interval and redistribution, condition/storage of blood samples, site of sampling, and analytic methods in addition to the blood methadone concentration. Similar to other lipophilic bases with large volumes of distribution, methadone pharmacokinetics suggest the possibility of substantial postmortem redistribution. Other factors that contribute to uncertainty relating postmortem to antemortem methadone concentrations include interindividual variation in the active/inactive enantiomer (R/S) ratio and preanalytic variation. Although the mean R/S ratio is near unity, the range extends at least from about 0.5–2.5.[167,168] The median R/S ratio in 10 samples of postmortem femoral blood was 1.46 (range, 1.00–2.62).[148] Methadone was a cause or contributing factor to the death of most of these 10 individuals. Additionally, multiple drugs are frequently present during analysis of postmortem blood samples, particularly morphine, benzodiazepines, and cocaine.[169]

Concentrations.   Even though some authors associate postmortem blood concentrations exceeding 400–1,000 ng/mL in nontolerant adults with apnea and death,[170] interpretation of postmortem methadone concentrations must always include an evaluation of the circumstances surrounding the death and the clinical history. Plasma methadone concentrations >1,000–3,000 ng/mL can occur in patients receiving chronic methadone therapy.[15,171] Consequently, determination of the significance of postmortem methadone concentrations in any situation depends on a variety of factors as listed in the first part of the Postmortem section. In 96 postmortem cases with positive drug screens for methadone, the mean postmortem blood concentrations of methadone for methadone-related and nondrug-related deaths in members of methadone maintenance programs were 1,310 ng/mL (range, 180–3,990 ng/mL) and 1,160 ng/mL (range, 180–3,030 ng/mL), respectively.[171] The mean methadone concentration in postmortem blood from chronic pain patients dying of natural causes was 520 ng/mL. Substantial overlap in postmortem methadone concentrations between drug-related and traumatic deaths also occurred in a case series from Palm Beach County, Florida.[172] The mean methadone concentration in postmortem samples from methadone-only related deaths was 559 ng/mL (range, 114–1,949 ng/mL)

compared with a mean methadone concentration of 605 ng/mL (range, 72–2,700 ng/mL) in traumatic deaths with positive drug screen for methadone. In a case series of 11 methadone-related deaths, the mean methadone concentration in postmortem blood was 725 ng/mL with a range of 200–2,400 ng/mL.[173] The mean methadone concentration in postmortem blood samples from 8 deaths in this study involving only methadone and benzodiazepines was similar (mean, 548 ng/mL, range, 200–1,000 ng/mL). Although benzodiazepines are commonly found in blood samples from patients dying from methadone-related causes,[170,174] there are inadequate data to determine a specific postmortem benzodiazepine concentration that contributes significantly to death. Consequently, each case involving both benzodiazepines and methadone must be interpreted considering all the data available.

Methadone concentrations in postmortem samples from children dying of methadone intoxication are usually lower than adult cases. Postmortem blood samples from 5 methadone-induced fatalities of children under the age of 14 years contained blood methadone concentrations ranging from 200–489 ng/mL (mean, 332 ng/mL).[175] A 5-month-old girl was pronounced dead at home after the mother placed methadone in the child's bottle for sedation.[176] The postmortem methadone and EDDP concentrations in femoral blood samples were 1,071 ng/mL and 148 ng/mL, respectively.

The methadone/EDDP ratio does not clearly separate methadone-related and methadone-incidental deaths. 2-Ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) is a less common methadone metabolite in postmortem samples than EDDP; when present, the EMDP concentration in postmortem blood is usually <10 ng/mL. In 46 autopsy cases that screened positive for methadone, no postmortem peripheral blood samples contained detectable EMDP concentrations (LLOQ, 10 ng/mL); however, 17 liver samples contained EMDP concentrations above 40 ng/mL.[177] As during life, the *R*-enantiomer concentrations of methadone in postmortem blood samples exceed the *S*-enantiomer. In a study of 90 postmortem femoral blood samples containing methadone, the *R/S*-enantiomer ratio was 1.38, slightly higher than that in a living person.[178] This study did not detect a significant contribution of polymorphism in the expression of CYP3A4, CYP2B6, or P-glycoprotein on the variability in the response to methadone. There were no statistically significant differences in the *R*-enantiomer/total methadone ratios between deaths associated with methadone or between methadone plus benzodiazepines or other drugs.

Redistribution. Postmortem redistribution accounts for most of the postmortem blood concentration increases over antemortem blood trough concentrations, depending on site-variation,[179] time between death and withdrawal of the sample, and the presence or absence of unabsorbed drug reservoirs in the lungs and stomach.[180] Postmortem methadone concentrations at specific sites can increase severalfold during the postmortem period. In a study of 15 autopsy cases with two sampling sites, the ratio of subclavian/heart blood ranged from 0.30–2.03 (mean, 1.10; *n* = 9) with the highest ratio recorded from a trauma-related death.[181] The inferior vena cava/heart blood ratio for 2 cases was 0.81 and 4.13, whereas the single femoral/heart blood ratio was 0.90. Postmortem methadone concentration in urine and bile typically exceeds the postmortem concentration of methadone in blood.[43] Furthermore, methadone is not distributed evenly between plasma and whole blood; as noted above, the approximate *antemortem* ratio of whole blood/plasma methadone ratio is about 0.75. The postmortem methadone whole blood/plasma ratio will vary substantially based on the water content at the specified site. A prolonged perimortem period between the administration of methadone and death, as suggested by the development of bronchopneumonia, may account for relatively low postmortem concentrations. As a result of these factors, the postmortem methadone blood concentration following methadone overdoses overlap the range of plasma methadone concentrations of healthy individuals in methadone maintenance programs.

Hair

Daily doses of methadone produce the highest hair methadone concentrations in axillary hair followed by pubic hair and scalp hair. In a study of 10 patients receiving daily oral doses of 10–25 mg methadone, methadone concentrations in axillary hair ranged from 1.3–8 ng/mg hair compared with 1–4 ng/mg for pubic hair and 0.5–2.7 ng/mg for head hair as measured by radioimmunoassay.[182] The methadone concentration in a convenience sample of scalp hair from methadone patients in a detoxification center ranged from 0–15 ng/mg as measured by GC/MS after a methanol wash and solid-phase extraction.[183] Stereoselective analyses of methadone in hair samples indicate a relatively higher concentration of the *R*-enantiomer than the *S*-enantiomer. In a study of 9 methadone patients from a detoxification center, the concentrations of *R*- and *S*-enantiomers of methadone ranged from 2.58–10.22 ng/mg and 1.89–9.53 ng/mg, respectively, whereas the *R*- and *S*-enantiomers of EDDP ranged from 0.42–1.73 ng/mg and 0.40–2.10 ng/mg, respectively.[184] Analysis of the enantiomers involved enzymatic hydrolysis, solid phase extraction, and quantitation by liquid chromatography/ion

spray/mass spectrometry. The methadone concentration in hair does not correlate well to the methadone dose or concentration in blood as measured by gas chromatography/ion trap/mass spectrometry after acid hydrolysis and solid phase extraction.[185] EDDP was detectable in only about 50% of 26 hair samples (9 blond, 6 brown, 4 light brown, 4 unknown, 1 red, 1 gray, 1 red/brown) from methadone patients in this study at a LLOQ of 0.2 ng/mg. The location of the hair samples was not reported. Potential external contamination complicates the interpretation of methadone and EDDP in hair samples as a means to determine multiple episodes of prior methadone ingestion.[186]

SALIVA

Methadone accumulates in saliva as a result of the ionization of methadone (pKa = 8.9, 25°C/77°F) in the acidic saliva (pH = 5.6–7.0); however, the salivary pH does not alter the salivary concentration of EDDP. Consequently, changes in salivary pH alter the diffusion of methadone into saliva, resulting in higher methadone concentrations at lower pH and a poor correlation between salivary and plasma methadone concentrations. Studies of saliva/plasma methadone ratios in methadone maintenance patients range from <1–10.[187] Additionally, the type of collection method also alters the saliva/plasma methadone ratio.[188] Although the presence of methadone in oral fluid can document exposure to methadone, the salivary methadone concentration does not imply behavioral or motor impairment.[189]

URINE

As excretion of methadone into urine is pH dependent, the ratio of methadone to the metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) varies substantially between individuals; additionally, the EDDP/creatinine ratio displays inter-individual variation.[190] Urine immunoassays for methadone are specific for methadone; however, many urine drug screens do not include methadone in the urine drug panel. Immunoassays with a methadone cutoff of 300 ng/mL are common methods of screening opioid abusers in methadone maintenance programs. The EMIT-ETS™ d.a.u. (Syva Co., San Jose, CA) immunoassay screens meconium samples specifically for methadone at a cutoff of 200 ng/mL (ng/g) with little cross-reactivity for the methadone metabolites such as EDDP and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP).[191] The CEDIA EDDP immunoassay (Microgenics Corporation, Fremont, CA) is a sensitive and reliable screening technique for determining EDDP in urine samples at a cutoff of 100 ng/mL. In a study of 1,381 urine specimens

screened with CEDIA urinary EDDP immunoassay and the EMIT® (Dade Behring, Siemens Healthcare Diagnostics, Erlangen, Germany; cutoff calibrator concentration of 300 ng/mL) urinary methadone immunoassay, the positive rates for the 2 screening methods were 46% and 39%, respectively.[192] Of the 108 samples positive for EDDP and negative for methadone by immunoassay, GC/MS confirmed the presence of methadone in all 99 samples tested. There are few data on the methadone metabolite/methadone ratio in urine specimens from acute fatalities or chronic methadone therapy. A case series of 4 autopsies of methadone-related fatalities indicated that the methadone metabolite/methadone ratio was <1, whereas the ratio in urine samples from 4 patients on chronic methadone therapy ranged from 1.28–5.45 (mean, 3.8).[193]

The chemical structure of methadone and verapamil are similar, resulting in cross-reaction of some verapamil metabolites with some immunoassays (e.g., Diagnostic Reagents, Inc., Dearborn, MI) for methadone.[194] However, false-positive cross-reactivity does not usually occur if the individual is ingesting methadone therapeutically. A 9-year-old boy with diphenhydramine intoxication had a positive rapid urine drug screen (One Step Multi-Drug, Multi-Line Screen Test Device; ACON Laboratories, San Diego, CA) for methadone; GC/MS failed to confirm the presence of methadone.[195] Case series suggest that false-positive methadone urine drug screens may occur during quetiapine therapy. In a series of 10 inpatients on quetiapine including 2 patients on only quetiapine, the COBAS® Integra Methadone II test kit using KIMS (kinetic interaction of microparticles in solution) methodology (Roche Pharmaceuticals, Nutley, NJ) was positive; GC/MS did not confirm the presence of methadone.[196]

Abnormalities

BLOOD

Rhabdomyolysis, hyperkalemia, hypocalcemia, hyperphosphatemia, and myoglobinuric renal failure can develop from muscle damage secondary to prolonged immobilization and coma. However, case reports suggest that myoglobinuric renal failure can occur during methadone intoxication without coma or prolonged immobilization.[197] Rarely, the chest x-ray demonstrates evidence of pulmonary edema secondary to the acute respiratory distress syndrome after IV or oral administration of methadone.[198] The most common radiologic finding is a bilateral patchy alveolar pattern with nonuniform, asymmetrical infiltrates.[199] The distribution of the infiltrates can be lobar and unilateral, requiring clinical signs/symptoms to differentiate pulmonary edema from

pneumonia. The radiographic abnormalities associated with methadone-induced pulmonary edema usually resolve within 24–48 hours. The development of pulmonary edema is not related to the dose of methadone.

CARDIAC

Cardiac dysrhythmias during methadone intoxication are unusual, and sudden death is rare. The sources of data linking methadone and serious ventricular dysrhythmias are case reports, case series, and small retrospective studies with inadequate data from systematic prospective studies to establish methadone as a sole, specific cause of fatal ventricular dysrhythmias (i.e., torsades de pointes). Current evidence indicates that the occurrence of torsade de pointes in opioid-dependent patients usually occurs in the presence of multiple co-existing risk factors for QT_c prolongation.[200,201] Case reports suggest that these risk factors potentially include the presence of high-dose therapy (>100 mg daily), recent increase in methadone dose,[202] concomitant therapy with drugs (e.g., citalopram, clarithromycin, cocaine, fluconazole, olanzapine, sertraline) that prolong the QT_c interval, unrecognized congenital long QT interval syndrome in individuals without the phenotypic expression of the prolonged QT interval under normal conditions,[203] and associated electrolyte abnormalities (hypokalemia, hypomagnesemia).[204,205] In a 17-week, randomized clinical trial, the mean QT_c interval increased approximately 30 msec in the high-dose methadone group (60–100 mg daily), when compared with baseline at intake.[206] Approximately 23% of the 55 participants in this group had prolonged QT_c intervals at the end of the study as defined by QT_c exceeding 470 msec in men or 490 msec in women. These abnormalities occur most commonly in patients with daily methadone doses of 600–700 mg, particularly in association with hypokalemia. Significant risks of serious ventricular dysrhythmias occurs when the QTc interval exceeds 500 msec; however, clinically significant QTc prolongation (i.e., QTc >500 msec) is unusual, even in patients monitored because of known risk factors (hypokalemia, hypomagnesemia, QT-prolonging drugs, inhibitors of methadone metabolism).[207,208] In a study of 109 patients from an outpatient methadone clinic, 2 patients (1.8%) had clinically significant QTc prolongation (>500 msec) and the methadone dose correlated to QT_c prolongation.[209] All of these patients had received stable doses of methadone for at least 2 months before testing. Serious ventricular dysrhythmias can occur during these episodes of QT_c prolongation including torsades de pointes and ventricular tachycardia.[210,211] Case reports associate the development of ventricular fibrillation following torsades de pointes with severely hypokalemic patients on therapeutic doses of methadone.[212] Episodes of ventricular fibrillation in these cases may spontaneously revert to sinus rhythms.

Driving

Although experimental tests indicate that some impairment of psychomotor skills occurs in chronic methadone users, the reduction in driving skills is probably not sufficient to cause major driving impairment in the absence of excessive or other drug use. In general, opioid-dependent individuals in methadone maintenance programs perform significantly worse than age-, gender-, and education-matched controls in a variety of neuropsychologic domains including information processing, attention, short-term visual memory, delayed visual memory, short-term verbal memory, long-term verbal memory, and problem solving.[213] Interpretation of the significance of these studies is complicated by confounding variables including the concomitant use of drugs (e.g., heroin, benzodiazepines, ethanol) and medical complications (e.g., IV drug use, head injuries) that may impair driving skills. Preliminary studies suggest that at least some of the cognitive impairment associated with methadone results from comparing matched groups of abstinent opioid users and opioid-dependent individuals in methadone maintenance programs.[214] Neuropsychologic testing of patients in methadone maintenance programs suggests some impairment relative to controls in psychomotor speed (digit symbol substitution and trail-making tests), attention, tachistoscopic tasks, sensorimotor coordination, working memory (two-back task), and decision making (gambling task).[215] In general, these changes are within <0.5–0.7 standard deviations of the control groups, and there is no correlation between methadone dose and the test performance of stabilized methadone maintenance patients.[216] Enrollment in a methadone maintenance program was not associated with impairment in time estimation, conceptual flexibility, or long-term memory. In non-opioid users, methadone causes some impairment of psychomotor skills and perception, primarily in reaction time, information processing, and visual acuity. However, most patients, who are stabilized in chronic methadone treatment programs for at least 1 month, do not demonstrate similar impairment, probably due to tolerance.[217]

The driving ability of methadone maintenance patients depends more on the presence of polydrug use and personality (self-control, self-reliance, apprehension, psychiatric disorders) rather than methadone therapy or dose.[187] Examination of driving records suggests that accident rates of abstinent heroin addicts in methadone maintenance programs and age- and sex-matched controls are similar.[218] In a Norwegian study of

635 drivers apprehended for suspected driving under the influence of drugs with methadone present in their blood, methadone was the only psychoactive drug in 10 cases.[219] The mean whole blood methadone concentration in those 10 drivers was 460 ng/mL (range, 190–650 ng/mL) as measured by liquid chromatography/mass spectrometry (LLOQ, 30 ng/mL). Of the 8 drivers receiving clinical tests of impairment soon after apprehension, 5 drivers were considered impaired. There was no correlation between the whole blood methadone concentrations and the test results (impaired/not impaired).

TREATMENT

Stabilization

Treatment of methadone overdose is similar to the general treatment of opioid intoxication (see Heroin Treatment). The main life-threatening complications are respiratory depression and hypotension. Patients should be carefully monitored for the development of respiratory insufficiency with pulse oximetry, capnometry, and arterial or venous blood gases as needed. Patients with inadequate ventilation require bag-value-mask ventilation with 100% oxygen and the administration of naloxone 0.2–0.4 mg IV. Although the rate of subcutaneous absorption of naloxone is slower than the IV route, difficulty obtaining IV access may result in similar onset of action via these 2 routes of administration, at least in the prehospital setting.[220] If no improvement in respiration or hypotension occurs within 3–5 minutes, additional doses (0.4–2 mg) of naloxone should be administered parenterally. Criteria for endotracheal intubation include inability to ventilate with bag-valve-mask, poor oxygenation (i.e., oxygen saturation <90%) despite adequate ventilation and supplemental oxygen, and persistent hypoventilation after doses of naloxone exceeding 2–4 mg. Because of the long half-life of methadone, naloxone infusion (0.2 mg/h or two-thirds of the effective naloxone dose/h) is usually necessary for up to 48–72 hours along with close monitoring of oxygen saturation and mental status. If pulmonary signs or symptoms (e.g., cough, tachypnea, pink frothy sputum, rales) of pulmonary edema develop, a chest x-ray should be obtained to detect pulmonary edema and aspiration pneumonia. Hypotension usually responds to adequate doses of naloxone and IV fluid therapy.

Gut Decontamination

Theoretically, activated charcoal is an option if the patient presents within 1–2 hours of methadone ingestion, but there are few data to guide management. The presence of altered mental status increases the risk of aspiration of the activated charcoal.[221]

Elimination Enhancement

Hemodialysis is unlikely to increase methadone elimination because of high protein binding and the exceedingly large volume of distribution of methadone. Hence, hemodialysis is indicated only in the presence of hyperkalemia and renal failure to treat the complications of renal dysfunction rather than to remove methadone.

Antidotes

Because of the prolonged elimination half-life of methadone, serious methadone intoxication may require continuous naloxone infusion to prevent recurrence of respiratory depression and alteration of consciousness, particularly during the first 12–24 hours.[222] The usual infusion dose is two-thirds of the effective naloxone dose per hour as a continuous infusion. Close monitoring of mental status and oxygenation for at least 2–4 hours in a monitored setting after discontinuation of the naloxone infusion is required to ensure that altered mental status and respiratory depression do not recur.

Supplemental Care

Most patients with serious methadone intoxication should be hospitalized for 48–72 hours to detect delayed respiratory depression, particularly children. Reversal of methadone-induced respiratory depression with naloxone may be followed by recurrence of respiratory depression when the opioid antagonist effects of naloxone are no longer present. Elevated serum creatine kinase, myoglobinuria, and renal dysfunction can complicate methadone intoxication; all seriously intoxicated patients should be evaluated for electrolyte imbalance, renal dysfunction, and myoglobinuria. Treatment options for patients in methadone maintenance programs with QT_c prolongation include reduction of the methadone dose, substitution of buprenorphine, and insertion of an implantable cardioverter-defibrillator.[223]

WITHDRAWAL

Withdrawal symptoms of neonates born to opioid-dependent mothers on methadone are highly variable, ranging from none to severe.[224] In general, the neonatal abstinence syndrome associated with methadone withdrawal is more moderate and prolonged than similar heroin or morphine addiction as a result of the long plasma elimination half-life of methadone. Treatment of

neonatal withdrawal involves the administration of oral doses of methadone from 0.1–0.5 mg/kg/d depending on the severity of withdrawal symptoms. The methadone dose is tapered over 2–4 weeks.

Addiction

Compared to detoxification programs, methadone maintenance treatment more effectively reduces heroin use, decreases risky HIV-associated behavior and criminal activity, and improves life functioning.[225] However, high rates of illicit heroin use and diversion of methadone persist despite enrollment in methadone maintenance treatment programs. There is substantial regional variation in the doses of methadone administered by methadone maintenance programs.[226] In general, low daily doses (20–35 mg) of methadone are less effective than moderate doses (50–80 mg). A study of steady-state, moderate- (40–50 mg) and high-dose methadone (80–100 mg) suggests that the high-dose group had lower rates of positive opioid drug screens (53% and 62%, respectively, $P = .047$).[227] Both doses substantially reduced the rates of illicit opioid use. Federal regulations in the United States discourage the use of methadone doses exceeding 100 mg, although some studies suggest the methadone doses over 100 mg reduce illicit opioid use among heroin abusers unresponsive to moderate methadone doses.[228]

Because of clinically significant variation in the tolerance and individual pharmacokinetics of methadone, the induction dose of methadone for the first few weeks is low (i.e., 20–30 mg up to 40 mg) depending on the estimated degree of previous opioid use and tolerance. For patients with uncertain or low tolerance, the initial methadone dose is 10–20 mg. The dose is increased 5–10 mg daily, depending on the development of withdrawal symptoms and craving for heroin. Adverse effects associated with methadone therapy, particularly at high doses, include sweating, constipation, insomnia, somnolence, decreased libido, and difficulty with orgasm.

References

1. Gerlach R. A Brief overview on the discovery of methadone. Münster, Germany: INDRO e.V.; 2004. Available at www.indro-online.de/discovery.pdf. Accessed 2011 May 9.
2. Council on Pharmacy and Chemistry, American Medical Association. Methadon, generic term for 6-dimethylamino-4,4-diphenyl-3-heptanone. JAMA 1943; 134:1483.
3. Bieter RN, Hirsch SA. Methadone in internal medicine. Ann NY Acad Sci 1948;51:137–144.
4. Neeleman J, Farrell M. Fatal methadone and heroin overdoses: time trends in England and Wales. J Epidemiol Community Health 1997;51:435–437.
5. Bryant WK, Galea S, Tracy M, Piper TM, Tardiff KJ, Valahov D. Overdose deaths attributed to methadone and heroin in New York City, 1990–1998. Addiction 2004;99:846–854.
6. Hall AJ, Logan JE, Toblin RL, Kaplan JA, Craner JC, Bixler D, et al. Patterns of abuse among unintentional pharmaceutical overdose fatalities. JAMA 2008; 300:2613–2620.
7. Ripamonti C, Groff L, Brunelli C, Polastri D, Stavrakis A, De Conno F. Switching from morphine to oral methadone in treating cancer pain: what is the equianalgesic dose ratio? J Clin Oncol 1998;16:3216–3221.
8. Garrido MJ, Troconiz IF. Methadone: a review of its pharmacokinetic/pharmacodynamic properties. J Pharmacol Toxicol 1999;42:61–66.
9. Kristensen K, Christensen CB, Christrup LL. The mu1, mu2, delta, kappa opioid receptor binding profiles of methadone stereoisomers and morphine. Life Sci 1995; 56:PL45–PL50.
10. Isbell H, Eisenman AJ. The addiction liability of some drugs of the methadone series. J Pharmacol Exp Ther 1948;93:305–313.
11. Scott CC, Robbins EB, Chen KK. Pharmacologic comparison on the optical isomers of methadon. J Pharmacol Exp Ther 1948;93:282–286.
12. de Vos JW, Ufkes JG, Kaplan CD, Tursch M, Krause JK, van Wilgenburg H, Woodcock BG, Staib AH. L-Methadone and D,L-methadone in methadone maintenance treatment: a comparison of therapeutic effectiveness and plasma concentrations. Eur Addict Res 1998;4:134–141.
13. Ansermot N, Albayrak O, Schläpfer J, Crettol S, Croquette-Krokar M, Bourquin M, et al. Substitution of (R,S)-methadone by (R)-methadone: impact on QTc interval. Arch Intern Med 2010;170:529–536.
14. Kornick CA, Kilborn MJ, Santiago-Palma J, Schulman G, Thaler HT, Keefe DL, et al. QTc interval prolongation associated with intravenous methadone. Pain 2003; 105:499–506.
15. Wolff K. Characterization of methadone overdose: clinical considerations and the scientific evidence. Ther Drug Monit 2002;24:457–470.
16. Johansson BA, Berglund M, Lindgren A. Efficacy of maintenance treatment with methadone for opioid dependence: a meta-analytical study. Nord J Psychiatry 2007;61:288–295.
17. Persky VW, Goldfrank LR. Methadone overdoses in a New York City hospital. J Am Coll Emerg Med 1976; 5:111–113.
18. Blatman S. Narcotic poisoning in children (1) through accidental ingestion of methadone and (2) *in utero*. Pediatrics 1974;54:329–332.
19. Ghodse H, Oyefeso A, Kilpatrick B. Mortality of drug addicts in the United Kingdom 1967–1993. Int J Epidemiol 1998;27:473–478.

20. Irey NS, Froede RC. Evaluation of deaths from drug overdose a clinicopathologic study. Am J Clin Pathol 1974;61:778–784.

21. Jonsson A, Holmgren P, Ahlner J. Fatal intoxications in a Swedish forensic autopsy material during 1992–2002. Forensic Sci Int 2004;143:53–59.

22. Langendam MW, van Brussel GH, Coutinho RA, van Ameijden EJ. The impact of harm-reduction-based methadone treatment on mortality among heroin users. Am J Public Health 2001;91:774–780.

23. Valmana A, Oyefesco A, Clancy C, Ghodse H. Methadone-related deaths: data from 18 coroners' jurisdictions in England. Med Sci Law 2000;40:61–65.

24. Corkery JM, Schifano F, Ghodse AH, Oyefeso A. The effects of methadone and its role in fatalities. Hum Psychopharmacol 2004;19:565–576.

25. Caplehorn JR, Dalton MS, Haldar F, Petrenas AM, Nisbet JG. Methadone maintenance and addicts' risk of fatal heroin overdose. Subst Use Misuse 1996; 31:177–196.

26. Vormfelde SV, Poser W. Death attributed to methadone. Pharmacopsychiatry 2001;34:217–222.

27. Roland EH, Lockitch G, Dunn GH, Peacock D, Pirie GE. Methadone poisoning due to accidental contamination of prescribed medication. Can Med Assoc J 1984; 131:1357–1358.

28. Man L-H, Best D, Gossop M, Stillwell G, Strang J. Relationship between prescribing and risk of opiate overdose among drug users in and out of maintenance treatment. Eur Addict Res 2004;10:35–40.

29. Brands B, Blake J, Marsh Dc, Sproule B, Jeyapalan R, Li S. The impact of benzodiazepine use on methadone maintenance treatment outcomes. J Addict Dis 2008; 27:37–48.

30. Stitzer ML, Griffiths RR, McLellan AT, Grabowski J, Hawthorne JW. Diazepam use among methadone maintenance patients: patterns and dosages. Drug Alcohol Depend 1981;8:189–199.

31. Zaks A, Fink M, Freedman AM. Duration of methadone induced cross-tolerance to heroin. Br J Addict 1971; 66:205–208.

32. Aylett P. Methadone dose assessment in heroin addiction. Int J Addict 1982;17:1329–1336.

33. Wolff K, Rostami-Hodjegan A, Shires S, Hay AW, Feely M, Calvert R, et al. The pharmacokinetics of methadone in healthy subjects and opiate users. Br J Clin Pharmacol 1997;44:325–334.

34. Aronow R, Brenner SL, Woolley PV Jr. An apparent epidemic: methadone poisoning in children. Clin Toxicol 1973;6:175–181.

35. Aronow R, Paul SD, Woolley PV Jr. Childhood poisoning. An unfortunate consequence of methadone availability. JAMA 1972;219:321–324.

36. Gardner R. Methadone misuse and death by overdosage. Br J Addict 1970;65:113–118.

37. Berkowitz BA. The relationship of pharmacokinetics to pharmacological activity: morphine, methadone and naloxone. Clin Pharmacokinet 1976;1:219–230.

38. Nilsson MI, Meresaar U, Anggard E. Clinical pharmacokinetics of methadone. Acta Anaesth Scand 1982; 74(suppl):66–69.

39. Inturrisi CE, Verebely K. The levels of methadone in the plasma in methadone maintenance. Clin Pharmacol Ther 1972;13:633–637.

40. Dale O, Sheffels P, Kharasch ED. Bioavailabilities of rectal and oral methadone in healthy subjects. Br J Clin Pharmacol 2004;58:156–162.

41. Palmiere C, Brunel C, Sporkert F, Augsburger M. An unusual case of accidental poisoning: fatal methadone inhalation. J Forensic Sci 2011;56:1072–1075.

42. Dale O, Hoffer C, Sheffels P, Kharasch ED. Disposition of nasal, intravenous, and oral methadone in healthy volunteers. Clin Pharmacol Ther 2002;72:536–545.

43. Robinson AE, Williams FM. The distribution of methadone in man. J Pharm Pharmacol 1971;23:353–358.

44. Inturrisi CE, Colburn WA, Kaiko RF, Houde RW, Foley KM. Pharmacokinetics and pharmacodynamics of methadone patients with chronic pain. Clin Pharmacol Ther 1987;41:392–401.

45. Wolff K, Hay AW, Raistrick D, Calvert R. Steady-state pharmacokinetics of methadone in opioid addicts. Eur J Clin Pharmacol 1993;44:189–194.

46. Wilkins JN, Ashofteh A, Setoda D, Wheatley WS, Huigen H, Ling W. Ultrafiltration using the Amicon MPS-1 for assessing methadone plasma protein binding. Ther Drug Monit 1997;19:83–87.

47. Olsen GD. Methadone binding to human plasma proteins. Clin Pharmacol Ther 1973;14:338–343.

48. Eap CB, Cuendet C, Baumann P. Binding of d-methadone, l-methadone, and dl-methadone to proteins in plasma of healthy volunteers: role of the variants of alpha1-acid glycoprotein. Clin Pharmacol Ther 1990;47:338–346.

49. Eap CB, Cuendet C, Baumann P. Binding of d-methadone, l-methadone, and dl-methadone to proteins in plasma of healthy volunteers: role of the variants of alpha 1-acid glycoprotein. Clin Pharmacol Ther 1990;47:338–346.

50. Beckett AH, Taylor JF, Casy AF, Hassan MM. The biotransformation of methadone in man: synthesis and identification of a major metabolite. J Pharm Pharmacol 1968;20:754–762.

51. Pohland A, Boaz HE, Sullivan HR. Synthesis and identification of metabolites resulting from the biotransformation of DL-methadone in man and in the rat. J Med Chem 1971;14:194–197.

52. de Vos JW, Geerlings PJ, van den Brink W, Ufkes JG, van Wilgenburg H. Pharmacokinetics of methadone and its primary metabolite in 20 opiate addicts. Eur J Clin Pharmacol 1995;48:361–366.

53. Kreek MJ, Gutjahr CL, Garfield JW, Bowen DV, Field FH. Drug interactions with methadone. Ann NY Acad Sci 1976;281:350–371.

54. Wu D, Otton SV, Sproule BA, Busto U, Inaba T, Kalow W, Sellers EM. Inhibition of human cytochrome P450 2D6 (CYP2D6) by methadone. Br J Clin Pharmacol 1993;35:30–34.

55. Gerber JG, Rhodes RJ, Gal J. Stereoselective metabolism of methadone *N*-demethylation by cytochrome P4502B6 and 2C19. Chirality 2004;16:36–44.

56. Totah RA, Sheffels P, Roberts T, Whittington D, Thummel K, Kharasch ED. Role of CYP2B6 in stereoselective human methadone metabolism. Anesthesiology 2008; 108:363–374.

57. Shiran M-R, Lennard MS, Iqbal M-Z, Lgundoye O, Seivewright N, Tucker GT, Rostami-Hodjegan A. Contribution of the activities of CYP3A, CYP2D6, CYP1A2 and other potential covariates to the disposition of methadone in patients undergoing methadone maintenance treatment. Br J Clin Pharmacol 2009; 67:29–37.

58. De Fazio S, Gallelli L, De Siena A, De Sarro G, Scordo MG. Role of CYP3A5 in abnormal clearance of methadone. Ann Pharmacother 2008;42:893–897.

59. Inturrisi CE, Verebely K. Disposition of methadone in man after a single oral dose. Clin Pharmacol Ther 1972; 13:923–930.

60. Olsen GD, Wendel HA, Livermore JD, Leger RM, Lynn RK, Gerber N. Clinical effects and pharmacokinetics of racemic methadone and its optical isomers. Clin Pharmacol Ther 1977;21:147–157.

61. Kristensen K, Blemmer T, Angelo HR, Christrup LL, Drenck NE, Rasmussen SN, Sjogren P. Stereoselective pharmacokinetics of methadone in chronic pain patients. Ther Drug Monit 1996;18:221–227.

62. Megarbane B, Decleves X, Bloch V, Bardin C, Chast F, Baud FJ. Case report: quantification of methadone-induced respiratory depression using toxicokinetic/toxicodynamic relationships. Crit Care 2007:11:R5.

63. Rostami-Hodjegan A, Wolff K, Hay AW, Raistrick D, Calvert R, Tucker GT. Population pharmacokinetics of methadone in opiate users: characterization of time-dependent changes. Br J Clin Pharmacol 1999;48:43–52.

64. Beaver WT, Wallenstein SL, Houde RW, Rogers A. A clinical comparison of the analgesic effects of methadone and morphine administered intramuscularly, and of orally and parenterally administered methadone. Clin Pharmacol Ther 1967;8:415–426.

65. Pond SM, Kreek MJ, Tong TG, Raghunath J, Benowitz NL. Altered methadone pharmacokinetics in methadone-maintained pregnant women. J Pharmacol Exp Ther 1985;233:1–6.

66. Glatstein MM, Garcia-Bournissen F, Finkelsein Y, Koren G. Methadone exposure during lactation. Can Fam Physician 2008;54:1689–1690.

67. Wojnar-Horton RE, Kristensen JH, Yapp P, Ilett KF, Dusci LJ, Hackett LP. Methadone distribution and excretion into breast milk of clients in a methadone maintenance programme. Br J Clin Pharmacol 1997;44: 543–547.

68. Geraghty B, Graham EA, Logan B, Weiss EL. Methadone levels in breast milk. J Hum Lact 1997;13:227–230.

69. American Academy of Pediatrics, Committee on Drugs. The transfer of drugs and other chemicals into human milk. Pediatrics 2001;108:776–789.

70. McCarthy JJ, Leamon MH, Parr MS, Anania B. High-dose methadone maintenance in pregnancy: maternal and neonatal outcomes. Am J Obstet Gynecol 2005; 193:606–610.

71. White JM, Irvine RJ. Mechanisms of fatal opioid overdose. Addiction 1999;94:961–972.

72. Marks CE Jr, Goldring RM. Chronic hypercapnia during methadone maintenance. Am Rev Respir Dis 1973; 108:1088–1093.

73. Kreek MJ. Medical complications in methadone patients. Ann N Y Acad Sci 1978;311:110–134.

74. Santiago TV, Goldblatt K, Winters K, Pugliese AC, Edelman NH. Respiratory consequences of methadone: the response to added resistance to breathing. Am Rev Respir Dis 1980;122:623–628.

75. Raistrick D, Hay A, Wolff K. Methadone maintenance and tuberculosis treatment. BMJ 1996;313:925–926.

76. Tong TG, Pond SM, Kreek MJ, Jaffery NF, Benowitz NL. Phenytoin-induced methadone withdrawal. Ann Intern Med 1981;94:349–351.

77. Kharasch ED, Hoffer C, Whittington D, Walker A, Bedynek PS. Methadone pharmacokinetics are independent of cytochrome P4503A (CYP3A) activity and gastrointestinal drug transport insights from methadone interactions with ritonavir/indinavir. Anesthesiology 2009;110:660–672.

78. Cao Y-J, Smith PE, Wire MB, Lou Y, Lancaster CT, Causon RC, et al. Pharmacokinetics and pharmacodynamics of methadone enantiomers after coadministration with fosamprenavir-ritonavir in opioid-dependent subjects. Pharmacotherapy 2008;28:863–874.

79. Bertschy G, Eap CB, Powell K, Baumann P. Fluoxetine addition to methadone in addicts: pharmacokinetic aspects. Ther Drug Monit 1996;18:570–572.

80. Bertschy G, Baumann P, Eap CB, Baettig D. Probable metabolic interaction between methadone and fluvoxamine in addict patients. Ther Drug Monit 1994; 16:42–45.

81. Spiga R, Huang DB, Meisch RA, Grabowski J. Human methadone self-administration: effects of diazepam pretreatment. Exp Clin Psychopharmacol 2001;9:40–46.

82. Preston KL, Griffiths RR, Stitzer ML, Bigelow GE, Liebson IA. Diazepam and methadone interactions in methadone maintenance. Clin Pharmacol Ther 1984; 36:534–541.

83. Preston KL, Griffiths RR, Cone EJ, Darwin WD, Gorodetzky CW. Diazepam and methadone blood levels following concurrent administration of diazepam and methadone. Drug Alcohol Depend 1986;18:195–202.

84. Pond SM, Tong TG, Benowitz NL, Jacob P 3rd, Rigod J. Lack of effect of diazepam on methadone metabolism in

methadone-maintained addicts. Clin Pharmacol Ther 1982;31:139–143.

85. Lasagna L. Drug interaction in the field of analgesic drugs. Proc R Soc Med 1965;58:978–983.

86. Tong TG, Benowitz NL, Kreek MJ. Methadone-disulfiram interaction during methadone maintenance. J Clin Pharmacol 1980; 20:506–513.

87. Blake AD, Bot G, Freeman JC, Reisine T. Differential opioid agonist regulation of the mouse mu opioid receptor. J Biol Chem 1997;272:782–790.

88. Mestek A, Hurley JH, Bye LS, Campbell AD, Chen Y, Tian M, et al. The human μ opioid receptor: modulation of functional desensitization by calcium/calmodulin-dependent protein kinase and protein kinase C. J Neurosci 1995;15:2396–2406.

89. Gorman AL, Elliott KJ, Inturrisi CE. The *d*- and *l*-isomers of methadone bind to the non-competitive site on the *N*-methyl-D-aspartate (NMDA) receptor in rat forebrain and spinal cord. Neurosci Lett 1997;223:5–8.

90. Santiago TV, Edelman NH. Opioids and breathing. J Appl Physiol 1985;59:1675–1685.

91. White JM, Irvine RJ. Mechanisms of fatal opioid overdose. Addiction 1999;94:961–972.

92. Katchman AN, McGroary KA, Kilborn MJ, Kornick CA, Manfredi PL, Woosley RL, Ebert SN. Influence of opioid agonists on cardiac human ether-a-go-go-related gene K(+) currents. J Pharmacol Exp Ther 2002;303:688–694.

93. Wong SC, Roberts JR. Case files of the Drexel University Medical Toxicology Fellowship: methadone-induced QTc prolongation. J Med Toxicol 2007;3:190–194.

94. Watterson O, Simpson DD, Sells SB. Death rates and causes of death among opioid addicts in community drug treatment programs during 1970–1973. Am J Drug Alcohol Abuse 1975;2:99–111.

95. Baden MM. Evaluation of deaths in methadone users. Leg Med Annu 1978;:127–131.

96. Albion C, Shkrum M, Cairns J. Contributing factors to methadone-related deaths in Ontario. Am J Forensic Med Pathol 2010;31:1–7.

97. Greene MH, Luke JL, Dupont RL. Opiate overdose deaths in the District of Columbia. Part II—methadone-related fatalities. J Forensic Sci 1974;19:575–584.

98. Smialek JE, Monforte JR, Aronow R, Spitz WU. Methadone deaths in children a continuing problem. JAMA 1977;238:2516–2517.

99. DiMaio DJ, DiMaio T. Fatal methadone poisoning in children: report of four cases. J Forensic Sci 1973;18: 130–134.

100. Nelson PE, Selkirk RC. The toxicology of twelve cases of death involving methadone: examination of postmortem specimens. Forensic Sci 1975;6:175–186.

101. Chabalko J, LaRosa JC, Dupont RL. Death of methadone users in the District of Columbia. Int J Addict 1973;8:897–908.

102. Gardner R. Deaths in United Kingdom opioid users 1965–1969. Lancet 1970;2:650–653.

103. Ashwath ML, Ajjan M, Culclasure T. Methadone-induced bradycardia. J Emerg Med 2005;29:73–75.

104. Karir V. Bradycardia associated with intravenous methadone administered for sedation in a patient with acute respiratory distress syndrome. Pharmacotherapy 2002;22: 1196–1199.

105. Ito S, Liao S. Myoclonus associated with high-dose parenteral methadone. J Palliat Med 2008;11:838–841.

106. Anselmo M, Campos Rainho A, do Carmo Vale M, Estrada J, Valente R, Correia M, et al. Methadone intoxication in a child: toxic encephalopathy? J Child Neurol 2006;21:618–620.

107. Salgado RA, Jorens PG, Baar I, Cras P, Hans G, Parizel PM. Methadone-induced toxic leukoencephalopathy: NMR imaging and MR proton spectroscopy findings. AJNR Am J Neuroradiol 2010;31:565–566.

108. Glatstein M, Finkelstein, Scolnick D. Accidental methadone ingestion in an infant. Case report and review of the literature. Pediatr Emerg Care 2009;25:109–111.

109. LoVecchio F, Pizon A, Riley B, Sami A, D'Incognito C. Onset of symptoms after methadone overdose. Am J Emerg Med 2007;25:57–59.

110. Drummer OH, Opeskin K, Syrjanen M, Cordner SM. Methadone toxicity causing death in ten subjects starting on a methadone maintenance program. Am J Forensic Med Pathol 1992;13:346–350.

111. Hendra TJ, Gerrish SP, Forrest AR. Fatal methadone overdose. BMJ 1996;313:481–482.

112. Fraser DW. Methadone overdose illicit use of pharmaceutically prepared parenteral narcotics. JAMA 1971; 217:1387–1389.

113. Frand UI, Shim CS, Williams MH Jr. Methadone-induced pulmonary edema. Ann Intern Med 1972;76:975–979.

114. Schaaf JT, Spivack ML, Rath GS, Snider GL. Pulmonary edema and adult respiratory distress syndrome following methadone abuse. Am Rev Respir Dis 1973;107: 1047–1051.

115. Shaw KA, Babu KM, Hack JB. Methadone, another cause of opioid-associated hearing loss: a case report. J Emerg Med 2010. Epub ahead of print.

116. Christenson BJ, Marjala AR. Two cases of sudden sensorineural hearing loss after methadone overdose. Ann Pharmacother 2010;44:207–210.

117. Maxwell JC, Pullum TW, Tannert K. Deaths of clients in methadone treatment in Texas: 1994–2002. Drug Alcohol Depend 2005;78:73–81.

118. Perret G, Deglon J-J, Kreek MJ, Ho A, La Harpe R. Lethal methadone intoxications in Geneva, Switzerland, from 1994 to 1998. Addiction 2000;95:1647–1653.

119. Heinemann A, Iwersen-Bergmann S, Stein S, Schmoldt A, Puschel K. Methadone-related fatalities in Hamburg 1990–1999: implications for quality standards in maintenance treatment? Forensic Sci Int 2000;113:449–455.

120. Graβ H, Behnsen S, Kimont H-G, Staak M, Kaferstein H. Methadone and its role in drug-related fatalities in Cologne 1989–2000. Forensic Sci Int 2003;132:195–200.

121. Schwartz RP, Brooner RK, Montoya ID, Currens M, Hayes M. A 12-year follow-up of a methadone medical maintenance program. Am J Addict 1999;8:293–299.

122. Wolff K, Rostami-Hodjegan A, Hay AW, Raistrick D, Tucker G. Population-based pharmacokinetic approach for methadone monitoring of opiate addicts: potential clinical utility. Addiction 2000;95:1771–1783.

123. Shah N, Lathrop SL, Landen MG. Unintentional methadone-related overdose death in New Mexico (USA) and implications for surveillance, 1998–2002. Addiction 2005;100:176–188.

124. Neale J. Methadone, methadone treatment and non-fatal overdose. Drug Alcohol Depend 2000;58:117–124.

125. Segal RJ, Catherman RL. Methadone—a cause of death. J Forensic Sci 1974;19:64–71.

126. Caplehorn JR, Drummer OH. Fatal methadone toxicity: signs and circumstances, and the role of benzodiazepines. Aust N Z J Pub Health 2002;26:358–362.

127. Hammersley R, Cassidy MT, Oliver J. Drugs associated with drug-related deaths in Edinburgh and Glasgow, November 1990 to October 1992. Addiction 1995;90: 959–965.

128. Paulozzi LJ, Logan JE, Hall AJ, McKinstry E, Kaplan JA, Crosby AE. A comparison of drug overdose deaths involving methadone and other opioid analgesics in West Virginia. Addiction 2009;104:1541–1548.

129. DiMaio DJ, DiMaio T. Fatal methadone poisoning in children: report of four cases. J Forensic Sci 1973;18: 130–134.

130. Butler B, Rubin G, Lawrance A, Batey R, Bell J. Estimating the risk of fatal arrhythmia in patients in methadone maintenance treatment for heroin addiction. Drug Alcohol Rev 2011;30:173–180.

131. Chugh SS, Socoteanu C, Reinier K, Waltz J, Jui J, Gunson K. A community based evaluation of sudden death associated with therapeutic levels of methadone. Am J Med 2008;121:66–71.

132. Martin WR, Jasinski DR, Haertzen CA, Kay DC, Jones BE, Mansky PA, Carpenter RW. Methadone—a reevaluation. Arch Gen Psychiatry 1973;28:286–295.

133. Cleary BJ, Donnelly J, Strawbridge J, Gallagher P, Fahey T, Clarke M, Murphy DJ. Methadone dose and neonatal abstinence syndrome—systematic review and meta-analysis. Addiction 2010;105:2071–2084.

134. Dryden C, Young D, Hepburn M, Mactier H. Maternal methadone use in pregnancy: factors associated with the development of neonatal abstinence syndrome and implications for healthcare resources. BJOG 2009;116: 665–671.

135. Rahbar F. Observations on methadone withdrawal in 16 neonates. Clin Pediatr (Phila) 1975;14:369–371.

136. Reddy AM, Harper RG, Stern G. Observations on heroin and methadone withdrawal in the newborn. Pediatrics 1971;48:353–358.

137. Wouldes TA, Woodward LJ. Maternal methadone dose during pregnancy and infant clinical outcome. Neurotoxicol Teratol 2010;32:406–413.

138. Kaltenbach K, Finnegan LP. Perinatal and developmental outcome of infants exposed to methadone *in-utero*. Neurotoxicol Teratol 1987;9:311–313.

139. Cleary BJ, Donnelly JM, Strawbridge JD, Gallagher PJ, Fahey T, White MJ, Murphy DJ. Methadone and perinatal outcomes: a retrospective cohort study. Am J Obstet Gynecol 2011;204:139.e1–9.

140. Foster DJ, Somogyi AA, Bochner F. Stereoselective quantification of methadone and its major oxidative metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, in human urine using high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2000;744:165–176.

141. Fernandez P, Morales L, Vazquez C, Bermejo AM, Tabernero MJ. HPLC-DAD determination of opioids, cocaine and their metabolites in plasma. Forensic Sci Int 2006;161:31–35.

142. Mercolini L, Mandrioli R, Conti M, Leonardi C, Gerra G, Raggi MA. Simultaneous determination of methadone, buprenorphine and norbuprenorphine in biological fluids for therapeutic drug monitoring purposes. J Chromatogr B 2007;847:95–102.

143. Quintela O, López P, Bermejo AM, López-Rivadulla M. Determination of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine and alprazolam in human plasma by liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2006;834:188–194.

144. Thormann W, Lanz M, Caslavska J, Siegenthaler P, Portmann R. Screening for urinary methadone by capillary electrophoretic immunoassays and confirmation by capillary electrophoresis-mass spectrometry. Electrophoresis 1998;19:57–65.

145. Moore C, Guzaldo F, Hussain MJ, Lewis D. Determination of methadone in urine using ion trap GC/MS in positive ion chemical ionization mode. Forensic Sci Int 2001; 119:155–160.

146. Snozek CL, Bjergum MW, Langman LJ. Gas chromatography-mass spectrometry method for the determination of methadone and 2-ethylidene-1,5-dimethyl-3, 3-diphenylpyrrolidine (EDDP). Methods Mol Biol 2010;603:351–358.

147. Galloway FR, Bellet NF. Methadone conversion to EDDP during GC-MS analysis of urine samples. J Anal Toxicol 1999;23:615–619.

148. Liang HR, Foltz RL, Meng M, Bennett P. Method development and validation for quantitative determination of methadone enantiomers in human plasma by liquid chromatography/tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 806:191–198.

149. Johansen SS, Linnet K. Chiral analysis of methadone and its main metabolite EDDP in postmortem blood by liquid chromatography-mass spectrometry. J Anal Toxicol 2008:32:499–504.

150. Rodriguez-Rosas ME, Medrano JG, Epstein DH, Moolchan ET, Preston KL, Wainer IW. Determination of

total and free concentrations of the enantiomers of methadone and its metabolite (2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine) in human plasma by enantioselective liquid chromatography with mass spectrometric detection. J Chromatogr A 2005;1073: 237–248.

151. Etter ML, George S, Grabiel K, Eichhorst J, Lehotay DC. Determination of free and protein-bound methadone and its major metabolite EDDP: enantiomeric separation and quantitation by LC/MS/MS. Clin Biochem 2005; 38:1095–1102.

152. Choo RE, Murphy CM, Jones HE, Huestis MA. Determination of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, 2-ethyl-5-methyl-3,3-diphenylpyraline and methadol in meconium by liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005;814:369–373.

153. de Castro A, Jones HE, Johnson RE, Gray TR, Shakleya DM, Huestis MA. Maternal methadone dose, placental methadone concentrations, and neonatal outcomes. Clin Chem 2011;57:449–458.

154. Choo RE, Jansson LM, Scheidweiler K, Huestis MA. A validated liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometric method for the quantification of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and 2-ethyl-5-methyl-3,3-diphenylpyroline (EMDP) in human breast milk. J Anal Toxicol 2007;31:265–269.

155. Nikolaou PD, Papoutsis II, Maravelias CP, Spiliopoulou CA, Pistos CM, Calokerinos AC, Atta-Politou J. Development and validation of an EI-GC-MS method for the determination of methadone and its major metabolites (EDDP and EMDP) in human breast milk. J Anal Toxicol 2008;32:478–484.

156. Linnet K, Johansen SS, Buchard A, Munkholm J, Morling N. Dominance of pre-analytical over analytical variation for measurement of methadone and its main metabolite in postmortem femoral blood. Forensic Sci Int 2008; 179:78–82.

157. Vanbinst R, Koenig J, Di Fazio V, Hassoun A. Bile analysis of drugs in postmortem cases. Forensic Sci Int 2002; 128:35–40.

158. Kreek MJ, Kalisman M, Irwin M, Jaffery NF, Scheflan M. Biliary secretion of methadone and methadone metabolites in man. Res Commun Chem Pathol Pharmacol 1980;29:67–78.

159. Ziminski KR, Wemyss CT, Bidanset JH, Manning TJ, Lukash L. Comparative study of postmortem barbiturates, methadone, and morphine in vitreous humor, blood, and tissue. J Forensic Sci 1984;29:903–909.

160. Inturrisi CE, Colburn WA. Pharmacokinetics of methadone. Adv Pain Res Ther 1986;8:191–199.

161. Verebely K, Volavka J, Mule S, Resnick R. Methadone in man: pharmacokinetic and excretion studies in acute and chronic treatment. Clin Pharmacol Ther 1975; 18:180–190.

162. Eap CB, Bertschy G, Baumann P, Finkbeiner T, Gastpar M, Scherbaum N. High interindividual variability of methadone enantiomer blood levels to dose ratios. Arch Gen Psychiatry 1998;55:89–90.

163. Wolff K, Sanderson M, Hay AW, Raistrick D. Methadone concentrations in plasma and their relationship to drug dosage. Clin Chem 1991;37:205–209.

164. Bell J, Bowron P, Lewis J, Batey R. Serum levels of methadone in maintenance clients who persist in illicit drug use. Br J Addict 1990;85:1599–1602.

165. Holmstrand J, Anggard E, Gunne L-M. Methadone maintenance: plasma levels and therapeutic outcome. Clin Pharmacol Ther 1978;23:175–180.

166. Wolff K, Hay A, Raistrick D. High-dose methadone and the need for drug measurements in plasma. Clin Chem 1991;37:1651–1654.

167. Mitchell TB, Dyer KR, Newcombe D, Salter A, Somogyi AA, Bochner F, White JM. Subjective and physiological responses among racemic-methadone maintenance patients in relation to relative (S)- vs. (R)-methadone exposure. Br J Clin Pharmacol 2004;58:609–617.

168. Eap CB, Finkbeiner T, Gastpar M, Scherbaum N, Powell K, Baumann P. Replacement of (R)-methadone by a double dose of (R,S)-methadone in addicts: interindividual variability of the (R)/(S) ratios and evidence of adaptive changes in methadone pharmacokinetics. Eur J Clin Pharmacol 1996;50:385–389.

169. Karch SB, Stephens BG. Toxicology and pathology of deaths related to methadone: retrospective review. West J Med 2000;172:11–14.

170. Mikolaenko I, Robinson CA, Davis GG. A review of methadone deaths in Jefferson County, Alabama. Am J Forensic Med Pathol 2002;23:299–304.

171. Gagajewski A, Apple FS. Methadone-related deaths in Hennepin County, Minnesota: 1992–2002. J Forensic Sci 2003;48:668–671.

172. Wolf BC, Lavezzi WA, Sullivan LM, Flannagan LM. Methadone-related deaths in Palm Beach County. J Forensic Sci 2004;49:1–4.

173. Shields LB, Hunsaker JC, Corey TS, Ward MK, Stewart D. Methadone toxicity fatalities: a review of medical examiner cases in a large metropolitan area. J Forensic Sci 2007;52:1389–1395.

174. Perret G, Déglon JJ, Kreek MJ, Ho A, La Harpe R. Lethal methadone intoxications in Geneva, Switzerland, from 1994 to 1998. Addiction 2000;95:1647–1653.

175. Milroy CM, Forrest AR. Methadone deaths: a toxicological analysis. J Clin Pathol 2000;53:277–281.

176. Kintz P, Villain M, Dumestre-Toulet V, Capolaghi B, Cirimele V. Methadone as a chemical weapon two fatal cases involving babies. Ther Drug Monit 2005; 27:741–743.

177. Danielson TJ, Mozayani A, Sanchez LA. Methadone and methadone metabolites in postmortem specimens. Forensic Sci Med Pathol 2008;4:170–174.

178. Buchard A, Linnet K, Johansen SS, Munkholm J, Fregerslev M, Morling N. Postmortem blood concentrations of *R*- and *S*-enantiomers of methadone and EDDP in drug users: influence of co-medication and P-glycoprotein genotype. J Forensic Sci 2010;55: 457–463.

179. Prouty RW, Anderson WH. The forensic science implications of site and temporal influences on postmortem blood-drug concentrations. J Forensic Sci 1990;35: 243–270.

180. Caplehorn JR, Drummer OH. Methadone dose and postmortem blood concentration. Drug Alcohol Rev 2002;21: 329–333.

181. Levine B, Wu SC, Dixon A, Smialek JE. Site dependence of postmortem blood methadone concentrations. Am J Forensic Med Pathol 1995;16:97–100.

182. Balabanova S, Wolf HU. Methadone concentrations in human hair of the head, axillary and pubic hair. Z Rechtsmed 1989;102:293–296.

183. Goldberger BA, Darraj AG, Caplan YH, Cone EJ. Detection of methadone, methadone metabolites, and other illicit drugs of abuse in hair of methadone-treatment subjects. J Anal Toxicol 1998;22:526–530.

184. Kintz P, Eser HP, Tracqui A, Moeller M, Cirimele V, Mangin P. Enantioselective separation of methadone and its main metabolite in human hair by liquid chromatography/ion spray-mass spectrometry. J Forensic Sci 1997;42:291–295.

185. Girod C, Staub C. Methadone and EDDP in hair from human subjects following a maintenance program: results of a pilot study. Forensic Sci Int 2001;117:175–184.

186. Kintz P, Evans J, Villain M, Cirimele. Interpretation of hair findings in children after methadone poisoning. Forensic Sci Int 2010;196:51–54.

187. Stout Pr, Farrell LJ. Opioids—effects on human performance and behavior. Forensic Sci Rev 2003;15:30–59.

188. Bermejo AM, Lucas AC, Tabernero MJ. Saliva/plasma ratio of methadone and EDDP. J Anal Toxicol 2000; 24:70–72.

189. Gray TR, Dams R, Choo RE, Jones HE, Huestis MA. Methadone disposition in oral fluid during pharmacotherapy for opioid-dependence. Forensic Sci Int 2011; 206:98–102

190. Larson ME, Richards TM. Quantification of a methadone metabolite (EDDP) in urine: assessment of compliance. Clin Med Res 2009;7:134–141.

191. el Sohly MA, Feng S, Murphy TP. Analysis of methadone and its metabolites in meconium by enzyme immunoassay (EMIT) and GC-MS. J Anal Toxicol 2001;25:40–44.

192. George S, Parmar S, Meadway C, Braithwaite RA. Application and validation of a urinary methadone metabolite (EDDP) immunoassay to monitor methadone compliance. Ann Clin Biochem 2000;37:350–354.

193. Garriott JC, Sturner WQ, Mason MF. Toxicologic findings in six fatalities involving methadone. Clin Toxicol 1973; 6:163–173.

194. Lichtenwalner MR, Mencken T, Tully R, Petosa M. False-positive immunochemical screen for methadone attributable to metabolites of verapamil. Clin Chem 1998;44:1039–1041.

195. Rogers SC, Pruitt CW, Crouch DJ, Caravati EM. Rapid urine drug screens diphenhydramine and methadone cross-reactivity. Pediatr Emerg Care 2010;26:665–666.

196. Fischer M, Reif A, Polak T, Pfuhlmann B, Fallgatter AJ. False-positive methadone drug screens during quetiapine treatment. J Clin Psychiatry 2010;71:1696.

197. Nanji AA, Filipenko JD. Rhabdomyolysis and acute myoglobinuric renal failure associated with methadone intoxication. J Toxicol Clin Toxicol 1983;20:353–360.

198. Kjeldgaard MJ, Hahn GW, Heckenlively JR, Genton E. Methadone-induced pulmonary edema. JAMA 1971;218: 882–883.

199. Wilen SB, Ulreich S, Rabinowitz JG. Roentgenographic manifestations of methadone-induced pulmonary edema. Radiology 1975;114:51–55.

200. Modesto-Lowe V, Brooks D, Petry N. Methadone deaths: risk factors in pain and addicted populations. J Gen Intern Med 2010;25:305–309.

201. George S, Moreira K, Fapohunda M. Methadone and the heart: what the clinician needs to know. Curr Drug Abuse Rev 2008;1:297–302.

202. Perrin-Terrin A, Pathak A, Lapeyre-Mestre M. QT interval prolongation: prevalence, risk factors and pharmacovigilance data among methadone-treated patients in France. Fundam Clin Pharmacol 2011;25:503–510

203. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. Circulation 1999; 99:529–533.

204. Routhier DD, Katz KD, Brooks DE. QTc prolongation and *Torsades de Pointes* associated with methadone therapy. J Emerg Med 2007;32:275–278.

205. Piguet V, Desmeules J, Ehret G, Stoller R, Dayer P. QT interval prolongation in patients on methadone with concomitant drugs. J Clin Psychopharmacol 2004;24: 446–448.

206. Wedam EF, Bigelow GE, Johnson RE, Nuzzo PA, Haigney MC. QT-interval effects of methadone, levomethadyl, and buprenorphine in a randomized trial. Arch Intern Med 2007;167:2469–2475.

207. Mayet S, Gossop M, Lintzeris N, Markides V, Strang J. Methadone maintenance, QTc and torsade de pointes: who needs an electrocardiogram and what is the prevalence of QTc prolongation? Drug Alcohol Rev 2011; 30:388–396.

208. Krantz MJ, Martin J, Stimmel B, Mehta D, Haigney MC. QTc interval screening in methadone treatment. Ann Intern Med 2009;150:387–395.

209. Fonseca F, Marti-Almor J, Pastor A, Cladellas M, Farre M, de la Torre R, Torrens M. Prevalence of long QTc interval in methadone maintenance patients. Drug Alcohol Depend 2009;99:327–332.

210. Almehmi A, Malas AM, Yousufuddin M, Rosencrance JG. Methadone-induced torsade de pointes in a patient

with normal baseline QT interval. W V Med J 2004; 100:147–148.

211. Walker PW, Klein D, Kasza L. High dose methadone and ventricular arrhythmias: a report of three cases. Pain 2003;103:321–324.

212. Atkinson D, Dunne A, Parker M. Torsades de pointes and self-terminating ventricular fibrillation in a prescription methadone user. Anaesthesia 2007;62:952–955.

213. Darke S, Sims J, McDonald S, Wickes W. Cognitive impairment among methadone maintenance patients. Addiction 2000;95:687–695.

214. Mintzer MZ, Copersino ML, Stitzer ML. Opioid abuse and cognitive performance. Drug Alcohol Depend 2005; 78:225–230.

215. Mintzer MZ, Stitzer ML. Cognitive impairment in methadone maintenance patients. Drug Alcohol Depend 2002;67:41–51.

216. Specka M, Finkbeiner Th, Lodemann E, Leifert K, Kluwig J, Gastpar M. Cognitive-motor performance of methadone-maintained patients. Eur Addict Res 2000; 6:8–19.

217. Chesher GB. Understanding the opioid analgesics and their effects on skills performance. Alcohol Drugs Driv 1989;5:111–138.

218. Maddux JF, Williams TR, Ziegler JA. Driving records before and during methadone maintenance. Am J Drug Alcohol Abuse 1977;4:91–100.

219. Bernard J-P, Morland J, Krogh M, Khiabani HZ. Methadone and impairment in apprehended drivers. Addiction 2009;104:457–464.

220. Wanger K, Brough L, Macmillan I, Goulding J, MacPhail I, Christenson JM. Intravenous vs subcutaneous nalox-

one for out-of-hospital management of presumed opioid overdose. Acad Emerg Med 1998;5:293–299.

221. Chyka PA, Seger D, Krenzelok EP, Vale JA; American Academy of Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists. Position paper: Single-dose activated charcoal. Clin Toxicol (Phila) 2005;43:61–87.

222. Romac DR. Safety of prolonged high-dose infusion of naloxone hydrochloride for severe methadone overdose. Clin Pharm 1986;5:251–254.

223. Hanon S, Seewald RM, Yang F, Schweitzer P, Rosman J. Ventricular arrhythmias in patients treated with methadone for opioid dependence. J Interv Card Electrophysiol 2010;28:19–22.

224. Offidani C, Chiarotti M, De Giovanni N. Methadone in pregnancy: clinical-toxicological aspects. Clin Toxicol 1986;24:295–303.

225. Sees KL, Delucchi KL, Masson C, Rosen A, Clark HW, Robillard H, et al. Methadone maintenance vs 180-day psychosocially enriched detoxification for treatment of opioid dependence a randomized controlled trial. JAMA 2000;283:1303–1310.

226. D'Aunno T, Vaughn TE. Variations in methadone treatment practices. JAMA 1992;267:253–258.

227. Strain EC, Bigelow GE, Lieson IA, Stitzer ML. Moderate-vs high-dose methadone in the treatment of opioid dependence a randomized trial. JAMA 1999; 281:1000–1005.

228. Fareed A, Casarella J, Roberts M, Selboda M, Amar R, Vayalapalli S, Drexler K. High dose versus moderate dose methadone maintenance: is there a better outcome? J Addict Dis 2009;28:399–405.

Chapter 33

1-METHYL-4-PHENYL-1,2,5,6-TETRAHYDROPYRIDINE (MPTP)

HISTORY

In 1979, Davis et al reported the development of a parkinsonian illness in a 23-year-old graduate student, who injected a synthetic meperidine congener, 1-methyl-4-phenyl-4-N-propionoxypiperidine (MPPP, 4-propyloxy-4-phenyl-N-methylpiperidine) for several months prior to becoming ill.[1] While experimenting with this synthetic drug, the student began increasing the temperatures of the illicit process to reduce reaction times. This change introduced a contaminant (MPTP or 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) that later was identified as the cause of his parkinsonian condition. During the 18-month course of his illness, the drug-induced motor disturbances initially responded to dopamine receptors agonists. Following a fatal cocaine overdose, postmortem examination of this patient revealed marked loss of nigrostriatal cells in the midbrain. In 1983, Langston et al reported the development of a similar parkinsonian illness in 4 intravenous (IV) drug abusers within 1 week of injecting a "synthetic heroin."[2] Subsequent criminal investigations traced the source of this synthetic heroin to a clandestine laboratory that was formulating a structural analog of meperidine and alphaprodine, called MPPP (desmethylprodine). Analysis of samples from the material injected by 2 of these patients demonstrated primarily 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) with trace amounts of MPPP. As a contaminant of MPPP synthesis, MPTP was subsequently identified as the etiologic agent responsible for the development of the parkinsonian illness.

IDENTIFYING CHARACTERISTICS

Figure 33.1 displays the chemical structure of MPTP (CAS RN: 28289-54-5), which has a molecular formula of $C_{12}H_{15}N$. MPTP is a 4-phenyl piperidine derivative that is structurally related to meperidine and alphaprodine. The lipophilic properties of MPTP allow diffusion of this protoxin across the blood–brain barrier before detoxification; once in the brain, MPTP is converted to the toxic metabolite, 1-methyl-4-phenylpyridinium (MPP^+, cyperquat, CAS RN: 48134-75-4). MPTP along with a variety of MPTP analogs display high potency for monoamine oxidase B. However, there is no definite structure-activity relationship for MPTP analogs; most MPTP analogs studied to date have less neurotoxicity than MPTP despite strong affinity for monoamine oxidase B.[3] Table 33.1 lists some of the physical properties of MPTP.

MPTP is a contaminant of the clandestine synthesis of MPPP (CAS RN: 13147-09-6). At that time, MPPP was a potent analgesic that could be synthesized legally from unrestricted chemicals as the reverse ester of meperidine.[1] Figure 33.2 demonstrates the synthetic pathway for MPPP. Phenyl lithium catalyzed the conversion of the starting material N-methyl-4-piperidone (MP) to 4-hydroxy-4-phenyl-N-methylpiperidine (HPMP).[4] The reaction of this intermediate with propionic anhydride produced the esterified end product, MPPP. Although the production of initial batches of this ester was successful, subsequent modifications of the temperatures and reaction times used in this process resulted in dehydration of HPMP and

MPPP to the toxic contaminant, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[1]

EXPOSURE

In 1982, a group of young drug abusers developed a severe parkinsonian illness after the IV injection of a new synthetic heroin that was contaminated by MPTP. Of approximately 300 individuals exposed to this contaminant, only a few developed a severe, permanent form of the illness. Since the 1982 epidemic, there have been no reports of MPTP in street samples of illicit drugs. However, sporadic cases of MPTP-induced parkinsonism developed in individuals injecting material from the attempted synthesis of MPPP.[5] All these cases resulted from the IV injection of MPTP-contaminated MPPP.
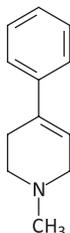
FIGURE 33.1. Chemical structure of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP).

TABLE 33.1. Physical Properties of MPTP.

Physical Property	Value
Melting Point	40°C (104°F)
log P (Octanol-Water)	2.71
Water Solubility	2930 mg/L (25°C/77°F)
Vapor Pressure	9.45E-03 mm Hg (25°C/77°F)

DOSE EFFECT

Animals

There is substantial variability in the sensitivity of different animal species to the neurotoxic effects of MPTP with humans and dogs being very sensitive. Rats do not develop MPTP-induced neurotoxicity. Mice require high parenteral doses of MPTP (100 mg/kg body weight) to produce neurotoxicity; the subsequent neurotoxicity is relatively mild compared with primates receiving parenteral MPTP.[6] In primates, the subcutaneous injection of 2.5 mg MPTP/kg body weight produces motor deficits and a 60–70% reduction in neuronal cells in the substantial nigra within 5–10 days of administration.[7]

Humans

Determination of the amount of MPPP injected by patients developing permanent MPTP-associated parkinsonism is complicated by the lack of analytic samples. Typically, the period of IV injection of MPTP-contaminated material lasted 3 days to 1 month.[8] In a case series of 4 patients with severe MPTP-associated Parkinsonism, the estimated dose of contaminated MPPP material ranged from 5 g over 4 days to 20 g over 5–8 days.[2] Analysis of powder used for injection by 2 patients demonstrated MPPP and MPTP concentrations of 0.3% and 3.2% by weight, respectively, whereas analysis of brown granular material used by the other 2 patients revealed MPPP and MPTP concentrations of 22–27% and 2.5–2.9% by weight, respectively. There is some individual variability in the response to the injection of MPTP-contaminated material because the parkinsonian symptoms resolved in some individuals within 2–3 weeks after injections of this material ceased.

TOXICOKINETICS

Chronic oral administration of relatively low MPTP concentrations (i.e., 2 μg/kg/d) produce low concentra-
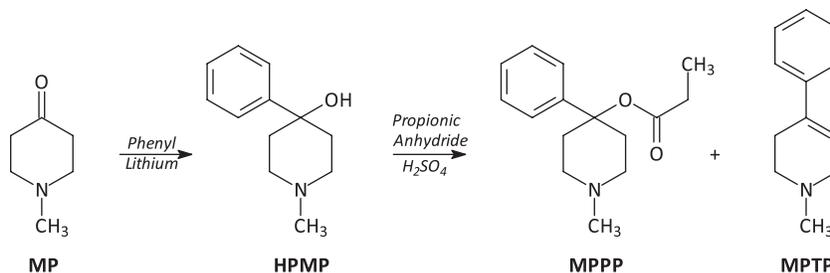
FIGURE 33.2. Chemical synthesis of MPPP. MP = *N*-methyl-4-piperidone; HPMP = 4-hydroxy-4-phenyl-*N*-methylpiperidine; MPPP = 4-propionyloxy-4-phenyl-*N*-methylpiperidine.

tions of the toxic metabolite, MPP[+] in brain tissue as a result of the extensive first pass metabolism of MPTP. Detoxification of MPTP involves the biotransformation of this compound to PTP (4-phenyl-1,2,3,6-tetrahydropyridine). The hepatic *N*-demethylation of MPTP to PTP is mediated at least in part by CYP2D6 based on *in situ* hybridization studies.[9] There is circumstantial evidence linking a deficiency in CYP2D6 with an increased risk of Parkinson disease. A case-control study of 229 patients with Parkinson disease and 720 controls suggested that individuals (i.e., poor metabolizers) with a metabolic defect in the CYP2D6-debrisoquine hydroxylase gene had about a 2.5-fold (95% CI: 1.51–4.28) increased risk of Parkinson disease when compared with individuals with the normal isoenzyme.[10] Inhibitory studies also suggest that CYP1A2 and, to a lesser extent CYP3A4 also contribute to the demethylation of MPTP to PTP. 4-Phenyl-1,2,3,6-tetrahydropyridine is not neurotoxic because the oxidation of PTP does not readily proceed beyond the dihydropyridine structure.[11] MPTP is a lipophilic compound that diffuses across the blood–brain barrier and enters the brain. Once in the brain (i.e., substantia nigra), the formation of the toxic metabolite involves two selective oxidation steps by monoamine oxidase B to yield the intermediate, 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPDP[+]) and then the toxic cation, 1-methyl-4-phenylpyridinium ion (MPP[+]).[12] The primary difference between MPTP-sensitive species (e.g., primates) and MPTP-insensitive species (e.g., rats) is the plasma elimination half-life of MPP[+], which varies from a few hours in the former to 10–28 days in the latter.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

MPTP is a lipophilic protoxin, which traverses the blood-brain barrier and undergoes metabolic transformation to a toxic metabolite.[13] Inside the endothelial cells of the blood–brain barrier, monoamine oxidase B catalyzes the 2-electron transfer from the oxidized flavin that results in the conversion of MPTP to the dihydropyridine, MPDP[+]. Spontaneous oxidation of MPDP[+] results in the formation of the toxic cation, 1-methyl-4-phenyl pyridine (MPP[+]), which cannot diffuse back across the blood–brain barrier. The MPTP not processed by monoamine oxidase enzymes in the endothelial cells of the blood brain barrier enters adjacent glial cells. Like the endothelial cells of the blood–brain barrier, glial cells contain large stores of monoamine oxidases capable of converting MPTP to MPP[+]. The

production of MPP[+] in the astrocyte stimulates the upregulation of TNF-α, interleukin-1β, and interleukin-6 with the subsequent up-regulation of inducible nitric oxide synthase.[14] After the release of MPP[+] into the extracellular space, dopaminergic uptake carriers (i.e., dopamine transporter) actively transfer MPP[+] to the dopamine neurons in the zona compacta region of the nigrostriatal. MPP[+] is a mitochondrial toxin that migrates into the mitochondria of dopaminergic neurons and decreases the production of cellular ATP by inhibiting NADH CoQ1 reductase (complex I) and possibly other electron transport enzymes (Complex III, IV) in the mitochondrial respiratory chain. The complex interaction of mitochondrial dysfunction, depletion of ATP, oxidative stress, excitotoxicity, and activation of the apoptotic pathway ultimately causes cell death. Degeneration of the axons and neuronal terminals follows the death of the cell bodies.

Postmortem Examination

The main anatomic feature of Parkinson disease is the reduction of neuromelanin-containing neurons in the substantia nigra pars compacta with clinical symptoms appearing after loss of over 60% of the neurons in this area of the midbrain.[15] The selective MPP[+]-induced destruction of nigrostriatal dopamine neurons causes the movement disorder associated with the IV injection of MPTP. Pathologic examinations of patients dying of MPP[+]-associated parkinsonism primarily demonstrate a diffusely pale, depigmented substantia nigra with associated loss of nerve cells and nerve fibers. Electron microscopy confirms nerve cell loss and gliosis in the substantia nigra. Although the degree of cell loss is similar to Parkinson disease, the Lewy bodies commonly present in Parkinson disease are absent in MPP[+]-induced parkinsonism. Occasionally, extraneural melanin is present in MPP[+]-induced parkinsonism along with microglial activation suggestive of an active, ongoing process of cell death. Senile plaques and neurofibrillary tangles are absent in MPP[+]-induced parkinsonism; the locus coeruleus is usually unaffected by this disease.[5]

The degenerative process of Parkinson disease destroys neurons in the brain containing norepinephrine. In contrast to Parkinson disease, the areas of the brain (e.g., locus coeruleus) associated with catecholamine production are usually unaffected by MPP[+]-induced illness; increased central noradrenergic activity occurs in MPP[+]-induced parkinsonism. However, animal models of MPP[+]-induced parkinsonism suggest that some damage may occur in the noradrenergic neurons of the locus coeruleus,[16] but the clinical significance of these changes for humans is unclear.

CLINICAL RESPONSE

The injection of MPTP-contaminated material produces dysphoria, hallucinations, blurring or dimming of vision, a metallic taste in the mouth, jerking of the extremities, and a burning pain at the injection site.[8] According to case reports, these symptoms were distinctly different than symptoms associated with heroin injection. Within 1 week, the intermittent muscle jerks resolve and a movement disorder develops characterized by awkward postures and bradykinesias that can progress to lead pipe rigidity and loss of extremity function (i.e., "frozen addict").[17] Physical findings associated with this illness include dysarthria, dysphagia, drooling, increased muscle tone, and cogwheel rigidity of the upper extremity. After 2–3 weeks, resolution of these parkinsonian symptoms occurs in some patients, particularly those with less-severe clinical symptoms. Other exposed patients develop mild symptoms of parkinsonism (bradykinesia, muscle rigidity, postural instability).[18] More severely affected patients develop a chronic, permanent parkinsonian syndrome manifest by bradykinesias, muscle rigidity, loss of postural reflexes and facial expressions, impaired rapid alternating movements, and stooped posture. Widespread central nervous system (CNS) dysfunction is absent due to the preservation of cerebellar, corticospinal, and autonomic function.

In contrast to idiopathic Parkinson disease, dementia and a resting tremor are not prominent parts of the clinical presentation of MPP$^+$-induced parkinsonian illness.[18] However, resting tremor similar to idiopathic Parkinson disease occurs in some patients with more severe forms of MPP$^+$-induced parkinsonian disease. In a study of 7 patients with moderate to severe MPP$^+$-induced parkinsonism, 4 of these patients exhibited a tremor indistinguishable from the characteristic rest tremor of Parkinson disease.[19] Although dopamine agonists initially improve the symptoms associated with MPP$^+$-induced parkinsonism, the efficacy of these drugs decreases over time and the symptoms progress in severity.

DIAGNOSTIC TESTING

Analytic Methods

Highly sensitive methods for the detection of MPTP-contaminated material include the use of high performance liquid chromatography/tandem mass spectrometry. The limit of detection for MPP$^+$ using this method was 1 fmol on columns with a signal-to-noise ratio of 3:1.[20] High performance capillary electrophoresis is an alternative to high performance liquid chromatography for the determination of MPTP.[21]

Abnormalities

In a study of 6 patients with MPP$^+$-induced parkinsonism and 8 patients with Parkinson disease, the cerebrospinal fluid concentrations (CSF) of homovanillic acid (i.e., the major metabolite of dopamine) were decreased in both groups, whereas the concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid were normal in both groups.[22] The main difference in CSF fluid between these 2 illnesses is the concentration of 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), the major metabolite of norepinephrine in the brain. After adjustment for plasma MHPG concentrations, the values were elevated (i.e., >6.0 ng/mL) in MPP$^+$-induced parkinsonism, whereas MHPG levels were reduced (<6.0 ng/mL) in Parkinson disease. Assessment of nigrostriatal dopaminergic function by 6-fluorodopa positron emission tomography (PET) suggests that short-term exposure to MPTP causes a progressive decline in nigrostriatal dopaminergic function similar to the protracted loss of dopaminergic function associated with idiopathic Parkinson disease.[23] A case series suggested that subclinical damage to the nigrostriatal pathway can occur in individuals without overt clinical evidence of MPP$^+$-induced parkinsonian disease based on abnormal PET scans.[24] Typically, these individuals injected lower doses of MPTP than individuals with moderate to severe illness.

TREATMENT

The treatment of MPP$^+$-induced parkinsonism is similar to the treatment of idiopathic Parkinson disease. Even severely affected patients initially respond to L-dopa therapy. However, dyskinesias can begin within several weeks of the initiation of the treatment of severe cases. Typically, these cases become less responsive to carbidopa/L-dopa and bromocriptine with subsequent progression to severe immobility and marked hypophonia.

References

1. Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, Kopin IJ. Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res 1979;1:249–254.

2. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979–980.

3. Mateeva NN, Winfield LL, Redda KK. The chemistry and pharmacology of tetrahydropyridines. Curr Med Chem 2005;12:551–571.

4. Weingarte HL. 1-Methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP): one designer drug and serendipity. J Forensic Sci 1988;33:588–595.

5. Langston JW, Forno LS, Tetrud J, Reeves AG, Kaplan JA, Karluk D. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann Neurol 1999;46:598–605.

6. Johannessen JN. A model of chronic neurotoxicity: long-term retention of the neurotoxin 1-methyl-4-phenylpy-ridinium (MPP+) within catecholaminergic neurons. Neurotoxicology 1991;12:285–302.

7. Irwin I, DeLanney LE, Forno LS, Finnegan KT, Di Monte DA, Langston JW. The evolution of nigrostriatal neuro-chemical changes in the MPTP-treated squirrel monkey. Brain Res 1990;531:242–252.

8. Langston JW. MPTP neurotoxicity: an overview and characterization of phases of toxicity. Life Sci 1985;36:201–206.

9. Gilham DE, Cairnes W, Paine MJ, Modi S, Poulsom R, Roberts GC, Wolf CR. Metabolism of MPTP by cytochrome P4502D6 and the demonstration of 2D6 mRNA in human foetal and adult brain by *in situ* hybridization. Xenobiotica 1997;27:111–125.

10. Smith CA, Gough AC, Leigh PN, Summers BA, Harding AE, Maraganore DM, et al. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. Lancet 1992;339(8806):1375–1377.

11. Finnegan KT, Irwin I, Delanney LE, Ricaurte GA, Langston JW. 1,2,3,6-Tetrahydro-1-methyl-4-(methylpyr-rol-2-yl)pyridine: studies on the mechanism of action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J Pharmacol Exp Ther 1987;242:1144–1151.

12. Tipton KF, McCrodden JM, Sullivan JP. Metabolic aspects of the behavior of MPTP and some analogues. Adv Neurol 1993;60:186–193.

13. Fukuda T. Neurotoxicity of MPTP. Neuropathology 2001;21:323–332.

14. Smeyne RJ, Jackson-Lewis V. The MPTP model of Parkinson's disease. Mol Brain Res 2005;134:57–66.

15. German DC, Manaye K, Smith WK, Woodward DJ, Saper CB. Midbrain dopaminergic cell loss in Parkinson's disease: computer visualization. Ann Neurol 1989;26:507–514.

16. German DC, Liang CL, Manaye KF, Lane K, Sonsalla PK. Pharmacological inactivation of the vesicular monoamine transporter can enhance 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurodegeneration of midbrain dopaminergic neurons, but not locus coeruleus noradrenergic neurons. Neuroscience 2000;101:1063–1069.

17. Ballard PA, Tetrud JW, Langston JW. Permanent human parkinsonism due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): seven cases. Neurology 1985;35:949–956.

18. Tetrud JW, Langston JW, Garbe PL, Rutenber AJ. Mild parkinsonism in persons exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Neurology 1989;39:1483–1487.

19. Tetrud JW, Langston JW. Tremor in MPTP-induced parkinsonism. Neurology 1992;42:407–410.

20. Hows ME, Ashmeade TE, Billinton A, Perren MJ, Austin AA, Virley DJ, et al. High-performance liquid chromatography/tandem mass spectrometry assay for the determination of 1-methyl-4-phenyl pyridinium (MPP+) in brain tissue homogenates. J Neurosci Methods 2004;137:221–226.

21. Quaglia MG, Farina A, Donati E, Cotechini V, Bossu E. Determination of MPTP, a toxic impurity of pethidine. J Pharma Biomed Anal 2003;33:1–6.

22. Burns RS, LeWitt PA, Ebert MH, Pakkenberg H, Kopin IJ. The clinical syndrome of striatal dopamine deficiency. Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). N Engl J Med 1985;312:1418–1421.

23. Vingerhoets FJ, Snow BJ, Tetrud JW, Langston JW, Schulzer M, Calne DB. Positron emission tomographic evidence for progression of human MPTP-induced dopaminergic lesions. Ann Neurol 1994;36:765–770.

24. Calne DB, Langston JW, Martin WR, Stoessl AJ, Ruth TJ, Adam MJ, et al. Positron emission tomography after MPTP: observations relating to the cause of Parkinson's disease. Nature 1985;317:246–248.

Chapter 34

PHENCYCLIDINE and PHENCYCLIDINE ANALOGUES

PHENCYCLIDINE (PCP)

HISTORY

Despite the synthesis of phencyclidine (PCP) in 1926, clinical use of this compound did not begin until the mid-1950s when Parke, Davis and Company chemists discovered the anesthetic properties of PCP during the exploration of chemicals formed by the addition of Grignard reagents to nitriles.[1] The combination of anesthesia and profound analgesia with preservation of normal laryngeal and pharyngeal reflexes initially encouraged the clinical use of PCP. Early primate studies indicated that PCP produced mild stimulation, ataxia, peaceful sedation, and anesthesia without cardiorespiratory depression.[2] The British government issued a patent to Parke, Davis and Company in 1960 for phencyclidine under the trade name Sernyl; in 1963, the US Food and Drug Administration (FDA) approved the clinical use of PCP. However, soon after introduction of PCP into the US markets, reports of adverse psychologic reactions (e.g., hallucinations, delirium, disorientation, dysphoria, muscle rigidity, agitation) following PCP-induced anesthesia resulted in the voluntary withdrawal of PCP by Parke, Davis and Company. The US Drug Enforcement Agency (DEA) reclassified PCP as a schedule II drug (existing medical use, high abuse potential), but there is no current medical indication for the use of PCP. Subsequent to the recognition of the severe emergence psychosis associated with PCP,

the development of a phencyclidine derivative, ketamine, was released for clinical use as a safer substitute for PCP. Until 1979, distribution of PCP for veterinary use continued under the trade name Sernylan (BioCentrics Laboratory, St. Joseph, MO) because of the efficacy of PCP as an animal tranquilizer.

In 1967, phencyclidine (PCP) was sold as the "peace pill" in San Francisco's Haight-Ashbury district; however, the adverse psychologic reactions associated with the ingestion of PCP limited the popularity of PCP as an illicit drug. Reintroduction of PCP during the early 1970s resulted in more widespread use as the route of abuse changed from ingestion to inhalation, particularly in major metropolitan centers (e.g., Los Angeles, New York, Detroit). Frequently, PCP was an adulterant of other popular street drugs (e.g., tetrahydrocannabinol, lysergic acid diethylamide, cocaine, mescaline, psilocybin). By the middle 1970s, the popularity of PCP increased, while the use of PCP as an adulterant in other street drugs substantially decreased.[3] Legal manufacturing of PCP ceased in April 1979; the US federal government is now the only legitimate source in the United States. During the 1980s, PCP use declined along with the use of most other drugs of abuse.[4] Phencyclidine use remained relatively constant in the United State during the 1990s. Despite well-publicized adverse reactions, PCP remains a popular street drug in certain areas of the United States. However, the use of PCP remains relatively infrequent compared with other drugs of abuse (e.g., cocaine, heroin, methamphetamine, club drugs). PCP is not a popular drug of abuse in most other countries.

IDENTIFYING CHARACTERISTICS

Structure

Phencyclidine [1-(1-phenylcyclohexyl)piperidine, PCP] is an arylcycloalkylamine compound structurally related to the less potent and shorter-acting dissociative anesthetic, ketamine. PCP is not a chiral compound, so stereospecific effects will not occur. Figure 34.1 displays the chemical structures of PCP and some PCP analogues.

Physiochemical Properties

PCP is a lipophilic, water soluble, weak base. Table 34.1 lists some physiochemical properties of PCP. The heating of the hydrochloride salt of PCP at 300°C (573°F) for 5 minutes decomposes about 95% of the salt.[5] Pyrolysis of PCP results in elimination of methylamine and deprotonation to produce the thermal degradation

TABLE 34.1. Physiochemical Properties of Phencyclidine (PCP).

Name	Phencyclidine
IUPAC	1-(1-Phenylcyclohexyl) piperidine
CAS RN No.	77-10-1
Molecular Formula	$C_{17}H_{25}N$
Molecular Weight	243.39 g/mol
Melting Point	46–46.5°C (114.8–115.7°F)
pKa	8.5
Log P (Octanol-Water)	4.7
Salts	Hydrochloride (MP = 233–235°C/451.4–455°F, MW = 279.83) Hydrobromide (MP = 214–218°C/417.2–424.4°F, MW = 324.28)

product, 1-phenylcyclohexene (CAS RN: 771-98-2). Experimental studies indicate that pyrolysis of PCP at 400°C (752°F) results in the formation of phenylcyclohexane and 1-phenylcyclohexene with about 60–70% of the PCP remaining intact, whereas with pyrolysis at 600°C (1,112°F) only trace amounts of PCP survive.[6] At these high oven temperatures (i.e., >600°C/1,112°F), pyrolytic products of PCP include a variety of polycyclic aromatic compounds (e.g., styrene, alpha-methylstyrene, naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, cyclohexylbenzene, acenaphthene, phenanthrene, anthracene). However, at the temperatures present during smoking (i.e., 635°C/1,175°F at the burning tip), these polycyclic aromatic compounds do not form.[7]

Terminology

Street names for phencyclidine include angel dust, killer weed, hog, dust, peace, peace weed, peace pill, goon, cyclone, elephant tranquilizer, embalming fluid, rocket fuel, PCP, super grass, super joint, super weed, green tea leaves, aurora borealis, surfer, LBJ, Peter Pan, love boat, horse tranquilizer, mad man, purple rain, mean green, mint weed, crystal joint, zoom, and Polvo de angel (Spanish). The street monikers "Sherman" and "super cool" refer to cigarettes soaked with PCP. Synonyms for liquid preparations that contain PCP dissolved in embalming fluid or other solvents include wet, dry, tecal, dust, illy and matrix.[8] These preparations are added to cigarettes or marijuana sticks and smoked for hallucinogenic effects; however, these preparations may contain other drugs of abuse (e.g., cocaine).[9]

Form

PCP usually appears as the readily soluble hydrochloride salt because PCP base is not water soluble. On the
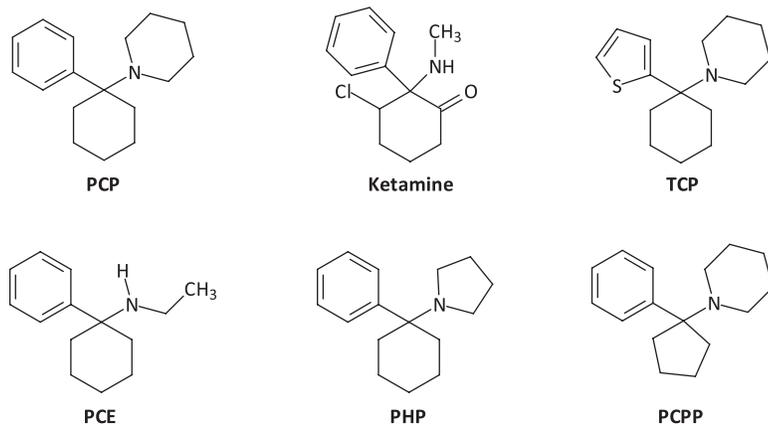
FIGURE 34.1. Chemical structures of phencyclidine (PCP) and some structural analogs.

street, illicit PCP appears as a liquid or solid. Although PCP is frequently sold as a white, crystalline powder containing 1–6 mg PCP, the color of PCP street samples varies substantially including green, yellow, gray, and pink.[10] Rarer street forms of PCP include liquid, rock crystal, paper squares, and paste. Often PCP is incorporated into a leafy mixture of marijuana ("angel dust") or used in combinations with hallucinogens (LSD, mescaline, psilocybin) or cocaine. Figure 34.2 displays a bottle containing PCP and a cigarette (Sherman) used to dip into the liquid PCP.

EXPOSURE

Epidemiology

Among polydrug abusers, phencyclidine use remains high, particularly in urban settings (e.g., Washington, DC,; Philadelphia, PA; Newark, NJ; Baltimore, MD); however, the prevalence of PCP use is relatively low compared with cocaine.[11] Between 1999 and 2002, PCP-related emergency department visits increased 109% based on Drug Abuse Warning Network (DAWN) data.[12] Although the percentage of the population using illicit drugs increased from 6.3% in 1999 to 7.1% in 2001, the overall use of PCP did not increase significantly during this period. By 2002, PCP was the most frequent *hallucinogen* found in patients presenting to the emergency department based on reports to the DAWN, exceeding emergency department visits related to LSD and MDMA (Ecstasy). Between 2004 and 2006, emergency department visits associated with PCP use based on DAWN data decreased from 31,342 to 21,960, but these visits increased to 36,719 in 2009.[13] In compari-

son, ED visits associated with ecstasy use increased from 10,220 in 2004 to 22,816 in 2009.

Sources

ORIGIN/COMPOSITION

The purity of phencyclidine sold as "angel dust" is high (80–100%) compared with the surreptitious adulteration of other street drugs with phencyclidine (range, 10–30%). Typical street doses of PCP range from 1–9 mg with an average of about 5 mg.[14] In a simulated smoking study, 1-phenylcyclohexene and PCP in mainstream smoke accounted for approximately 30% and 40%, respectively, of the inhaled dose of PCP.[15] About 15% of the inhaled PCP dose remained in the cigarette butt and about 16% off-gassed into sidestream smoke.

PRODUCTION PROCESSES

The US government is the only legal source of phencyclidine for research; the diversion of PCP from legal sources remains limited. Synthesis of phencyclidine as originally developed by Parke, Davis and Company chemists in 1965 involves the use of benzene, piperidine, and *p*-toluenesulfonic acid.[16] Description of the synthesis of PCP using 1-piperidinocyclohexanecarbonitrile (PCC, CAS RN: 3867-15-0) as a chemical intermediate is readily available on the Internet, requiring only moderate technical training and readily available precursors. Clandestine phencyclidine synthesis is a relatively simple, inexpensive process that produces large profits from a few chemicals and limited space. An older method for the clandestine synthesis of PCP is the condensation of 1-phenylcyclopentylamine with pentamethylene dibromide (1,5-dibromopentane, CAS RN: 111-24-0).[17] Alternative methods of clandestine PCP production include the direct conversion of piperidine to PCP, and the condensation of piperidinium chloride (CAS RN: 6091-44-7) with cyclohexanone followed by conversion to the PCC intermediate with a cyanide salt as demonstrated in Figure 34.3. Treatment with a phenyl Grignard converts the intermediate to PCP. To reduce the illicit synthesis of PCP, the United States banned PCP precursors (e.g., piperidine). The use of volatile solvents during the synthesis of PCP may cause explosions or fires in clandestine PCP laboratories.

IMPURITIES AND PROFILING

A major contaminant of illicit synthesis of PCP is the chemical intermediate, 1-piperidinocyclohexanecarbonitrile (PCC, CAS RN: 3867-15-0). In a study of the

FIGURE 34.2. Liquid phencyclidine (PCP) and cigarette (Sherman) used to dip into the liquid PCP. (Courtesy of *Drug Bible*)
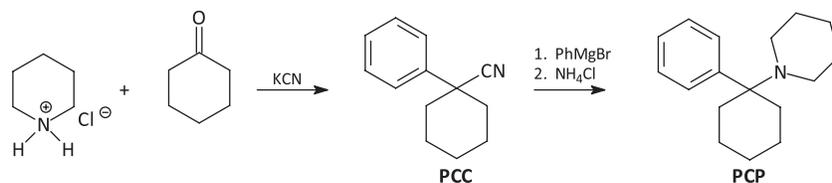
FIGURE 34.3. Chemical synthesis of PCP *via* condensation of piperidinium chloride and cyclohexanone to 1-piperidinocyclohexanecarbonitrile (PCC).

pyrolytic fate of radiolabeled PCC impregnated in marijuana cigarettes, approximately 47% of the PCC appeared unchanged in mainstream smoke. During the smoking study, about 58% of the unchanged PCC in the mainstream smoke was cleaved to form cyanide and 1-(1-piperidino)-1-cyclohexene.[18] The latter compound further decomposed to cyclohexanone, *N*-acetylpiperidine, and piperidine (which has a strong fishy smell). PCC contamination and subsequent cyanide release are not usually clinically significant, except in a massive PCP overdose.[19] Other potentially volatile by-products of PCP synthesis include ether, cyclohexanol, and isopropyl alcohol. Phenyl lithium or phenyl magnesium halides are unstable in atmospheric moisture; these organometal compounds are fire hazards rather than inhalation hazards.

Analytic techniques (e.g., GLC, spot tests) can detect PCC contamination in solid or liquid samples of phencyclidine. However, in a study analyzing 11 confiscated phencyclidine samples with purity ranging from 3–59%, PCC contaminated one specimen.[20] Rapid tests for the presence of PCC with minimum interference from other drugs of abuse include the impregnation of filter paper with sodium picrate that has a detection limit of about 2.5 mg/L.[21] The use of embalming fluid (formaldehyde, methanol, glutaraldehyde, ethanol) as a solvent for dissolving powdered PCP probably does not result in toxicity from smoking the PCP dipped in the PCP-containing liquid ("getting wet") because the volatility of these chemicals during the smoking process substantially reduces exposure to the constituents of the embalming fluid.[22]

Methods of Abuse

Most novice phencyclidine users begin PCP use when introduced to the drug by fellow drug users in a social setting.[23] Frequently, naive users consume phencyclidine while mistakenly believing that the drug was another, more desirable illicit drug. The chronic phencyclidine user is usually a polydrug abuser, who experiments with PCP after using alcohol, sedatives, stimulants, marijuana, and sometimes heroin.[24] Attractive feelings associated with PCP intoxication for these polydrug users include a state of dreamy estrangement and comical incoordination along with feelings of strength, power, and invulnerability. However, increased belligerence, emotional lability, and social isolation complicate chronic PCP use.

The most popular method of using PCP is by smoking thickly wrapped cigarettes in liquid PCP. Alternatively, powdered PCP is mixed with marijuana or tobacco leaves, and rolled into a cigarette (Sherman or Cool brands). Occasionally, the smoking of parsley leaves soaked with PCP is substituted for the use of PCP-soaked marijuana. Less-common methods of abuse include "getting wet" (soaking tobacco or marijuana cigarettes in liquid PCP produced from dissolving powdered PCP in commercial embalming fluid or other solvents), ingestion of PCP-laced food ("dusted food"), nasal insufflation, and IV injection. However, these methods are associated with more undesirable reaction because of the difficulty with titration of illicit PCP doses to desired effects.

DOSE EFFECT

Prediction of adverse reactions based on the PCP dose consumed is complicated by the effect of several variables including the absorbed dose, the route of administration, sample contaminants and adulterants, concomitant drug use, social setting, personality traits, individual variability, and tolerance. The unpredictability of these effects is one of the attractive features for chronic PCP users. Desired effects of illicit PCP use include euphoria, sense of power, analgesia, reduced inhibition, and altered perception of time, space, and body image.[25] Increasing doses of PCP result in amnesia, sensory illusions, altered body images, agitation, panic attacks, obsession with trivial matters, and bizarre behavior. In preclinical drug trials, IV phencyclidine doses of 0.25 mg/kg produced good surgical anesthesia associated with elevated blood pressure and tidal volumes, increased heart rate, occasionally increased muscle tone, bilateral ptosis with decreased pupillary reactivity, impaired visual identification and pinprick sensation, and vertical/horizontal nystagmus.[1]

Postoperatively, most patients were dissociated and euphoric, but approximately 10–20% experienced violent "emergence" reactions. Intravenous PCP doses of 0.5 mg/kg produce agitation in most patients. In healthy volunteers receiving 0.75 mg phencyclidine/kg, perceptual distortions occur rather than hallucinations. The altered mental state resembled schizophrenia and included depersonalization, distortion of body image, slowed time perception, memory loss, and concrete thinking.[26] Intravenous PCP doses of 1 mg/kg resulted in muscle rigidity, catatonia, and convulsions.

The typical street dose of PCP is about 3–5 mg. An approximation of the dose-related effects of PCP depending on the variables listed above is as follows: 1) low doses (1–10 mg): slight elevation of blood pressure and pulse rate may occur along with mild agitation, ataxia, nystagmus, confusion, decreased sensation, euphoria, emotional lability, analgesia, muscle rigidity, blank stare; 2) moderate PCP doses (10–20 mg): profound muscle rigidity, vomiting, hypersalivation, elevated blood pressure, excited or stuporous catatonia, toxic psychosis, bizarre behavior, coma; and 3) high doses (20–100 mg): prolonged coma, convulsions, decerebrate rigidity, opisthotonus, alternating periods of consciousness.[27] Patients emerging from PCP-induced altered consciousness manifest the behavioral toxicity seen at lower phencyclidine doses, characterized by paranoid delusions, agitation, delirium, hypersalivation, and hallucinatory experiences.[28]

TOXICOKINETICS

Table 34.2 lists some approximate toxicokinetic parameters for PCP. These parameters vary with factors such as dose, route of administration, and interindividual differences.

TABLE 34.2. Approximate Toxicokinetic Parameters for Phencyclidine (PCP).

Parameter	Value
Routes of Administration	Oral, Insufflation, Smoking, Intravenous
Typical Dose	Oral: 2–6 mg
	Insufflation, Smoking, Intravenous: 1–3 mg
T_{max}	2.5 hours*
C_{max}	2.7 ng/mL*
$T_{1/2}$	7–46 hours (mean, 17 hours)†
V_d	5.3–7.5 L/kg (mean, ~6 L/kg)
Protein Binding	60–80%
Plasma/Whole Blood Ratio	1.0

Abbreviations: C_{max} peak plasma = PCP concentration; T_{max} = time between exposure and peak plasma PCP concentrations; $T_{1/2}$ = terminal plasma elimination half-life; V_d = volume of distribution.
*1 mg dose.
†↑ up to 4 days during overdose.

as dose, route of administration, and interindividual differences.

Absorption

PCP is well absorbed by all routes with effects beginning within several minutes following insufflation or inhalation.

ORAL

The gastrointestinal absorption of phencyclidine is high. In a study using small doses (i.e., 1 mg) of radiolabeled phencyclidine hydrochloride administered to healthy volunteers, the oral bioavailability was about 72% ± 8% with the average time to peak PCP plasma concentration (2.7 ± 0.4 ng/mL) occurring approximately 1.5–2.5 hours after ingestion.[29] Because PCP is a basic drug, absorption occurs primarily in the small intestine rather than in the acidic medium of the stomach.

INHALATION

The bioavailability of PCP after smoking is high. Case reports indicate that acute PCP intoxication may occur following passive inhalation in both children[30] and adults.[31] During smoking, pulmonary absorption accounts for approximately 30–40% of the PCP dose in a cigarette, whereas heat degrades about 30% of the PCP dose to 1-phenylcyclohexene (PC).[32,33] About one third of the PCP dose remains in the butt or off-gases in the sidestream smoke.[15] Phencyclidine rapidly crosses the alveolar membrane and the blood–brain barrier. In volunteer studies, peak PCP plasma concentrations occur 5–20 minutes after inhalation.[15] A second plasma PCP peak may appear 1–3 hours after inhalation as a result of delayed absorption of PCP depots in the tissues of the upper or lower respiratory tract.

CUTANEOUS

Animal studies in hairless mice suggest that clinically significant percutaneous absorption of phencyclidine hydrochloride may occur within a few hours.[34] Anecdotal experience suggests that percutaneous absorption also occurs in humans (e.g., law enforcement officers handling confiscated material).

Distribution

After equilibrium, PCP distributes widely into the tissues resulting in a large volume of distribution and relatively small plasma concentrations compared with tissue PCP concentrations. Because of high lipid solubility and intracellular ion trapping, phencyclidine is highly

tissue bound. In a study of volunteers administered 1 mg PCP intravenously, the volume of distribution averaged 6.2 ± 0.3 L/kg.[35] Plasma protein binding of PCP ranges from about 60–80%.[35,36] Food deprivation, stress, and weight loss can mobilize phencyclidine from fat stores in experimental animals.[37]

Biotransformation

Biotransformation is the major route of PCP elimination in humans with a variety of metabolic pathways for PCP biotransformation as demonstrated in Figure 34.4.[38] The primary metabolic pathways involve oxidative hydroxylation of the alicyclic (i.e., nonaromatic) rings at several carbon atoms by cytochrome P450 isoenzymes. The major metabolite is the *trans* isomer of 4-phenyl-4-(1-piperidinyl) cyclohexanol (4-PPC). *In vitro* studies of PCP metabolism in human liver microsomes indicate that several CYP isoenzymes contribute

to PCP metabolism, particularly CYP3A.[39] However, there was a large degree of interindividual variation in the metabolite formation. Hydroxylation at C4 of the piperdine or cyclohexane rings forms both mono-hydroxylated metabolites [4-phenyl-4-piperidinocyclohexanol, 1-(1-phenylcyclohexyl)-4-hydroxy-piperidine] as well as the dihydroxy metabolite, 4-(4′-hydroxypiperidine)-4-phenylcyclohexanol. Hydroxylation of PCP at C2 of the piperidine ring forms an unstable carbinolamine that decomposes to form a series of polar, ring-opened compounds.[40] Following the administration of 1 mg PCP to healthy volunteers with normal urinary pH, the ratio of these three metabolites was 6.4/2.5/1, respectively.[29] Primary amine (1-phencyclohexylamine) and amino acid [5-(1-phenylcyclohexylamino)-valeric acid] metabolites are minor products of phencyclidine metabolism in humans.[41] In general, PCP metabolites display less pharmacologic activity than PCP.
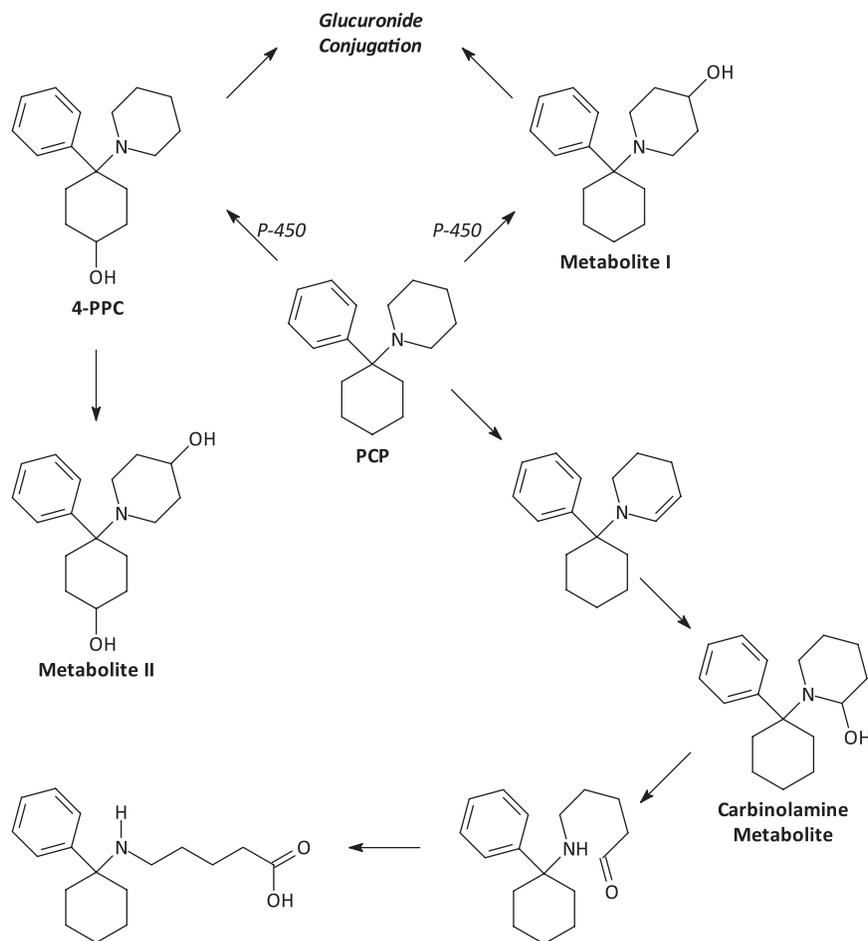
FIGURE 34.4. Biotransformation of PCP. 4-PPC = of 4-phenyl-4-(1-piperidinyl) cyclohexanol; Metabolite I = 1-(1-phenylcyclohexyl)-4-hydroxypiperidine; Metabolite II = 4-(4′-hydroxypiperidine)-4-phenylcyclohexanol.

Elimination

Elimination of PCP occurs primarily by the renal excretion of conjugated hydroxylated metabolites; PCP elimination kinetics are relatively complex. Mono-hydroxylated and di-hydroxylated PCP metabolites are conjugated with glucuronic or sulfuric acid before renal excretion. Renal excretion of these metabolites depends on both urine pH and volume. Following the IV administration of 1 mg PCP, excretion of unchanged PCP in urine with normal pH (i.e., 6.0–7.5) is also relatively small (i.e., <10% of the absorbed dose).[35] Reducing the mean urine pH from 7.4 to 6.1 in human volunteers increases renal excretion of small, IV doses of phencyclidine by 50%.[42] The fecal excretion of PCP metabolites is relatively small (<3–5%) compared with renal elimination.[29]

In human volunteers administered 1 mg PCP, the mean terminal (β) elimination half-life of PCP following inhalation and ingestion were similar averaging 24 ± 7 hours and 21 ± 3 hours, respectively, with a range up to 51 hours.[29,32] In a study of 3 volunteers administered 100 μg ^{3}H-PCP intravenously, the mean α-elimination half-life was 4.6 hours compared with a mean β-elimination half-life of 22 hours based on a three-compartment model.[43] During PCP intoxication, the elimination of PCP displays first-order kinetics over a wide range of doses. The estimated initial PCP plasma elimination half-life (i.e., after he passed the last 2 bags of PCP on hospital day 11) in a body packer with a massive PCP overdose producing PCP concentrations near 1,000 ng/mL was approximately 12.5 hours[44] compared with 11 hours in a patient with PCP-induced psychosis.[45]

Drug Interactions

In vitro studies indicate that PCP inhibits the activity of CYP2B6 and CYP2C19 activity.[46] There was little effect on the activity of other CYP isoenzymes. The clinical relevance of these potential interactions is unclear.

Maternal and Fetal Kinetics

Animal studies indicate that PCP crosses the placenta and distributes into fetal tissues including the brain,[47, 48] whereas *in vitro* studies suggest that some PCP metabolism occurs in the placenta.[49] PCP is detectable in cord blood from neonates born to mothers chronically using PCP. In a study of about 200 cord blood samples drawn from neonates born in a large urban teaching hospital, 12% of the samples contained PCP in concentrations ranging from 0.1–5.8 ng/mL.[50] In animal studies, detect-able concentrations of PCP and PCP metabolites do not persist in the brains of fetuses beyond 24 hours after maternal administration of PCP.[51] Animal studies indicate that PCP also rapidly enters breast milk in concentrations substantially higher (i.e., 10 times) than maternal blood concentrations.[52] Thirty-six days after the hospitalization of a pregnant woman for a toxic psychosis secondary to chronic PCP abuse, she delivered a healthy infant.[53] Five days later her breast milk contained 3.9 ng PCP /mL.

Tolerance

Animal studies including primate studies in monkeys indicate that tolerance to the behavioral effects of PCP develops and dissipates rapidly.[54] Animals will self-administer sufficient amounts of PCP to maintain gross intoxication up to 50 days; following chronic administration, a 2- to 4-fold increase in PCP dose is necessary to produce the original behavioral effects.[55] Following a continuous infusion of 45 mg PCP/kg/d for 7 days to rodents, cessation of the infusion produced withdrawal signs (piloerection, increased audiogenic seizures, reduction in exploratory behavior, impaired performance) in unrestrained rats.[56] Withdrawal signs developed 4 hours after termination of the infusion and resolved by 24 hours. After 3.5 days of infusion, a test dose of 2 mg PCP /kg produced the same motor performance as a 1 mg/ kg dose in drug-naive rats. Tolerance in humans after chronic PCP use is not well defined. Anecdotal accounts suggest that the chronic use of high doses of PCP use produce some tolerance, resulting in the need for substantially larger doses to achieve the same subjective effects.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Although classified pharmacologically as a dissociative anesthetic, phencyclidine has stimulant, depressant, hallucinogenic, and analgesic properties depending on personality, dose, route of administration, and possibly genetic predisposition. Phencyclidine is a potent psychotropic drug that currently provides the best available drug-induced model of schizophrenia. Phencyclidine blocks selected sensory inputs, and interferes with the sensory association pathways in a way that diminishes the user's ability to integrate sensory input into meaningful behavior.[57] Psychoactive doses of phencyclidine produce significant increases in glucose metabolism within areas of the brain associated with emotional behavior including the frontal cortex, the caudate-

putamen, and the hippocampus.[58] Smaller increases in glucose metabolism were detected in the substantia nigra.

Mechanism of Action

Phencyclidine affects several receptors in the brain including the glutamate N-methyl-D-aspartate (NMDA), dopamine D_2, and sigma receptors. Animal studies indicate that this drug is a noncompetitive antagonist of the glutamate NMDA receptor.[59] PCP interacts with several binding sites in the brain including a PCP-binding site involving a NMDA ionophore receptor complex and a sigma binding site with affinity for neuroleptics (e.g., haloperidol).[60] The affinity of classical neuroleptic drugs is much lower for the PCP binding site than the sigma binding site. The sigma receptors (σ_1, σ_2) are single transmembrane-spanning proteins that bind hallucinogenic drugs (PCP, ibogaine), antipsychotic drugs (haloperidol), and antidysrhythmic drugs (amiodarone). These receptors modulate a number of central neurotransmitters including noradrenergic, dopaminergic, and glutamatergic receptors.[61] The functions of sigma receptors involve a variety of cardiovascular effects, but the exact role remains undetermined.[62] PCP selectively reduces the excitatory action of glutamate on spinal neurons mediated by the NMDA receptor subtype. Animal studies suggest that functions of the NMDA receptor include glutamate-activated excitatory synaptic transmission, sensory transduction,[63] learning,[64] coordination of rhythmic movements (e.g., swimming), induction of seizures, and perhaps neuronal plasticity and growth. Sensitivity of the NMDA ion channel to ambient levels of Mg^{++} results in voltage dependence. This voltage sensitivity allows the receptor to respond to intense synaptic activation and the channel to respond to long-term processes (i.e., long-term potentiation) by admitting Ca^{++}.[65] However, not all behavioral effects of PCP result from the interaction of PCP with the NMDA-operated ion channel or the sigma receptor.[66]

In rodent models, PCP has indirect dopaminergic effects that result from the inhibition of striatal dopamine reuptake and the facilitation of dopamine release. *In vitro* studies indicate that the affinity of PCP for the dopamine D_2 receptor is greater than the glutamate NMDA receptor. The dissociation constants (K_i) for these two receptors in an *in vitro* study were 2.7 nM and 313 nM, respectively.[67] Electrophysiologic effects in the nigrostriatal and mesolimbic dopaminergic pathways correlated to enhanced locomotor activity and stereotypical behavior. In addition to indirect dopaminergic properties, PCP possesses mild cholinergic properties resulting from weak inhibition of acetylcholinesterase and binding to the muscarinic acetylcholine receptor.[68] In muscle and electric organ membrane preparations, PCP interacts with the nicotinic receptor, but the structure-activity relationship indicates that this interaction is not behaviorally significant in humans.[69] Although phencyclidine produces a pressor response at low dose,[70] the effect of PCP on norepinephrine synthesis and metabolism in the brain is unclear.[66]

Mechanism of Toxicity

PCP intoxication results from a combination of central nervous system (CNS) effects, anticholinergic/cholinergic effects, and adrenergic agonist activity. During large PCP overdoses, phencyclidine possesses brief, dose-related cholinergic (e.g., hypersalivation, diaphoresis) and anticholinergic (e.g., urinary retention) properties, but the anticholinergic effects of phencyclidine are too weak to account for behavioral abnormalities. Most serious adverse effects associated with PCP use result from trauma when altered behavioral and thought patterns lead to poor judgment and high-risk actions. As the PCP dose increases, medical complications (e.g., catatonia with rhabdomyolysis) develop and major medical effects predominate (e.g., coma, respiratory depression, hyperthermia, renal failure secondary to rhabdomyolysis). In a dog model, fatal doses of PCP cause hyperthermia and primary respiratory failure before cardiovascular collapse.[71] Rhabdomyolysis and myoglobinuria may cause renal dysfunction during severe PCP intoxication, particularly when hypotension and metabolic acidosis develop. The etiology of rhabdomyolysis during PCP intoxication is multifactorial including direct muscle trauma, hyperthermia, prolonged coma, hypotension, and isometric muscle contraction.[72] There are few data to suggest that PCP causes direct muscle toxicity. In a study of rats using a restraint cage with a strain gauge, pathologic damage to skeletal muscle occurred in the restrained, PCP-treated rats. However, these abnormalities did not occur in denervated areas of skeletal muscle in these rats.[73,74] Although delayed psychotic episodes occur in patients abusing PCP, a postmortem study of brains from chronic PCP users did not demonstrate alterations in the affinity or the maximum number of PCP or sigma binding sites.[75]

Postmortem Examination

Postmortem examination of deaths associated with the use of PCP is nonspecific, other than trauma secondary to behavioral effects.[76,77] The pathologic changes associated with rhabdomyolysis during PCP intoxication are

also nonspecific. Typically, muscle biopsies from these patients demonstrate scattered necrosis of muscle fibers and myofibrillar disruption.[78] Hyaline degeneration, edema, and loss of cross striations also occur in the muscle cells.[72]

CLINICAL RESPONSE

Illicit Use

Classically, phencyclidine produces behavioral abnormalities, impaired thought processes, and altered consciousness along with elevated blood pressure, increased pulse rate, and mild temperature increase.[79] Although tachycardia occurs during the acute phase of intoxication, the pulse rate is usually <120–130 beats/minute and pulse rates above these levels suggests the ingestion of other sympathomimetic drugs or concurrent illness. The clinical effects are highly variable depending on a variety of factors including the absorbed PCP dose, sample contaminants and adulterants, concomitant drug use, social setting, personality traits, and tolerance. Additionally, the intensity of the symptoms often waxes and wanes. Table 34.3 lists the common clinical features of PCP intoxication in a case series of 1,000 PCP-intoxicated patients. Adverse effects associated with PCP use include intense paranoia, panic, memory loss, delirium, sensory isolation, and flashbacks.[80] Neuromuscular abnormalities include bizarre posturing, dystonia (torticollis, opisthotonos), and choreoathetosis. Following the smoking of usual illicit doses of PCP, peak effects occur in about 15–30 minutes. Despite the prolonged elimination half-life of PCP, the psychologic effects following usual illicit doses of PCP typically resolve within 4–6 hours as a result of the redistribution of PCP from drug receptors. The persistence of symptoms of PCP intoxication beyond 24 hours after exposure is unusual.

Vertical and/or horizontal nystagmus is a common sign of PCP intoxication with nystagmus being present in a majority of patients in a large case series of PCP intoxication,[81] but the presence of vertical and/or horizontal nystagmus is not a unique sign of PCP use. Hyperacusis may develop. Occasionally, cholinergic signs (miosis, bronchospasm, hypersalivation, diaphoresis) develop during PCP intoxication. The main differential diagnosis of PCP intoxication includes stimulant use (e.g., amphetamine, cocaine), hallucinogen ingestion (e.g., mescaline, LSD, psilocybin) psychologic disorders (e.g., catatonia, bipolar disorder, paranoid schizophrenia), and medical conditions (hyperthermia, neuroleptic malignant syndrome, intracranial hemorrhage, thyrotoxicosis, sepsis, meningitis, hypoglycemia, hypoxia).

TABLE 34.3. Incidence of Clinical Features of Phencyclidine (PCP) Intoxication in 1,000 Patients.[81]

Category	Clinical Effect	%*
Behavior	Violent	35.4
	Agitated	34.0
	Bizarre	28.8
	Hallucinating/delusional	18.5
	Mute and staring	11.7
	Nudism	3.3
	No behavioral effects	3.5
Sensorium	Alert and oriented	45.9
	Acute brain syndrome	36.9
	Unconscious	10.6
	Lethargy/stupor	6.6
Neuromuscular Signs	Nystagmus	57.4
	Generalized rigidity	5.2
	Grand mal seizures	3.1
	Localized dystonias	2.4
	Facial grimacing	1.7
	Athetosis	1.3
Cholinergic Signs	Profuse diaphoresis	3.9
	Bronchospasm	2.1
	Pupils ≤1 mm	2.1
	Hypersalivation	1.7
	Bronchorrhea	0.6
Anticholinergic Signs	Pupils >4 mm	6.2
	Urinary retention	2.4
Vital Signs Abnormalities	Hypertension	57.0
	Tachycardia	30.0
	Hypothermia	6.4
	Apnea/respiratory arrest	2.8
	Hyperthermia	2.6
	Cardiac arrest	0.3

*Percentage of 1,000 patients with this clinical effect of PCP intoxication.

BEHAVIORAL ABNORMALITIES

Exposure to mild to moderate doses of PCP results in a wide spectrum of behavioral responses. Although euphoria similar to mild ethanol intoxication may develop during PCP intoxication, behavior is unpredictable. Intoxicated patients may be agitated with a clear sensorium, ataxia, and increased motor activity. Behavior abnormalities include stereotypical behavior manifest by repetitive motor movements (e.g., sucking, picking), involuntary repetition of words (echolalia), and speechless, blank stare. Unusual behavior (e.g., nudity, obscene gestures, socially uninhibited behavior, unprovoked agitation) may occur without disordered thought process or confusion. The behavioral abnormalities and analge-

sic properties of PCP predispose the patient to traumatic injuries during acute PCP intoxication. Most of the behavioral abnormalities associated with PCP intoxication resolve within a few hours; often these patients are amnesic to the events associated with PCP intoxication.

Most mortality and morbidity associated with PCP intoxication results from accidental and self-inflicted trauma during destructive behavior. The combination of PCP-induced cognitive disorganization, impaired senses, paranoia, and hyperactivity predisposes the user to significant risk of traumatic injury. Delusions of invulnerability and the decreased pain perception contribute to the inability to perceive imminent danger. Traumatic deaths associated with phencyclidine use include falls, automobile accidents, self-inflicted injuries, failure to flee fires, and shallow-water drowning. Although threatening or combative behavior is common even without hallucinations, delusions, or disorientation, violence directed at another person does not usually occur in a PCP user without a violent predisposition.[82] Trauma may be difficult to detect in these patients because of the sensory impairment that precedes psychologic effects. Many phencyclidine users become progressively more irritable, angry, and violent following chronic PCP use.[83]

Mental Disorders

A variety of adverse mental status changes occur during PCP intoxication depending on the dose and individual predisposition. These effects include confusion, disorientation, inappropriate affect, belligerence, delusions, auditory and visual hallucination, agitation, violence, and severe dysphoria. Patients are often lethargic with slow, bizarre responses to verbal or tactile stimuli that include blank stares, mutism, and catatonia. High doses of PCP produce the rapid onset of coma, persisting minutes to days. Case reports indicate that the level of consciousness may fluctuate dramatically with the patient developing coma after an apparent period of recovery.[84] These patients may not respond to painful stimuli, but respiratory depression and apnea do not usually occur, except with concomitant ingestion of respiratory depressant drugs. The whole spectrum of behavioral abnormalities (agitation, hallucination, aggressive behavior, disorientation, catatonia) may develop after emergence from PCP-induced coma in adolescents and adults. Medical complications associated with PCP-induced coma include aspiration pneumonia, rhabdomyolysis, grand mal seizures, and apnea. Severe psychologic reactions associated with PCP intoxication include acute toxic psychosis and catatonia.

Toxic Psychosis. After high doses or chronic use, some PCP abusers develop an acute psychosis that closely resembles schizophrenia.[85] The clinical features of the acute psychosis include distorted body images, estrangement, disorganized thought patterns, delusions, and hallucinations along with behavioral abnormalities excluding catatonia. These psychotic reactions most often occur in young male patients following chronic PCP use. In contrast to amphetamine-induced psychosis, PCP-induced psychosis incorporates both positive (e.g., hallucinations, paranoia) and negative (e.g., emotional withdrawal, motor retardation) symptom of schizophrenia.[86] Additionally, PCP-induced psychosis uniquely incorporates the formal thought disorder and neuropsychologic deficits associated with schizophrenia. These patients demonstrate depersonalization, a sense of unreality, and inability to distinguish between internal and external stimuli. Phencyclidine intensifies thought disorders in schizophrenic patients.[87]

The hallucinations associated with PCP intoxication are both visual and auditory, but are not associated with the vivid imagery common during LSD intoxication.[88] The initial phase of the acute psychosis involves violent, aggressive, and/or disorganized behavior and may last up to several days. Paranoid ideations also can occur. During the first week the patient may remain agitated. Restoration of normal behavior and thought patterns usually begins within 1 week, but in some severe cases of PCP-induced psychosis up to 1 year may be necessary before the return to baseline personality. Case reports suggest that bizarre traumatic events may occur as a result of the psychosis and profound analgesic effects of PCP.[89] Although the presence of an acute psychosis predisposes the patient to traumatic injuries, these mental status changes do not necessarily predispose the patient to criminal behavior, which is goal-directed and which usually occurs in a person with a criminal history.

Catatonia. As a result of the chaotic and disturbing feeling associated with PCP intoxication, a stuporous or excited catatonia may develop.[90] These patients are often motionless with arms and head in bizarre positions. Characteristically, these mute patients are expressionless and do not respond to painful stimuli, but a few of these patients will follow simple commands.[91] Clinical features associated with stuporous catatonia include mutism, grimacing, repetitive and/or bizarre posturing, rigidity, blank stare, constant repetition of nonsense, and echolalia. Excited catatonia involves agitation, impulsiveness, nudism, destructive behavior, incoherent and profuse speech, and unpredictable destructive behavior. Symptoms of catatonia usually resolve in 4–6 hours, but some patients require several days to reintegrate their personalities.

MEDICAL COMPLICATIONS

CENTRAL NERVOUS SYSTEM.   Generalized tonic-clonic seizures are the most common neurologic complication of acute PCP intoxication. In a case series of 1,000 patients presenting to a large urban emergency department with acute PCP intoxication, about 3% developed generalized seizures.[81] Rarely, case reports associated focal seizures and hemiparesis with PCP intoxication.[92] Case reports also associate status epilepticus,[93] spontaneous intracerebral hemorrhage,[94] and acute subarachnoid hemorrhage[95] with evidence of PCP in blood and urine samples, but the causal link between PCP use and these neurologic lesions is unclear. The sensory blockade with blank stare and muscle rigidity during PCP intoxication suggests a toxic rather than a structural lesion as a cause of neurologic and behavioral abnormalities.[96]

CARDIOVASCULAR SYSTEM.   Elevation of blood pressure (systolic >140 mm Hg and/or diastolic >90 mm Hg) occurs in about 50% of PCP intoxications based on a case series of PCP intoxication presenting to a large, urban teaching hospital.[81] Despite the occasional presence of severe hypertension, neurologic and cardiac complications are unusual. Hypertension usually resolves within several hours, particularly when agitation ceases. During severe intoxications, hypertension waxes and wanes similar to behavioral abnormalities with return to normal blood pressures over several days to a week.[97]

HYPERTHERMIA.   Mild to moderate temperature elevations occur during PCP intoxication. In a series of 1,000 patients presenting to a large, urban teaching hospital with PCP intoxication, 20 patients with drug screens positive only for PCP had temperatures above 102°F (38.9°C).[81] The highest recorded temperature in this case series was 107.4°F (41.9°C) rectally. Case reports associate PCP-induced hyperthermia with multiple seizures, hypertension, combative behavior, and secondary rhabdomyolysis.[98] Other serious complications of PCP-induced hyperthermia include multiorgan failure manifest by severe metabolic and respiratory acidosis, acute hepatorenal failure, coma, respiratory insufficiency, hypoglycemia, hyperkalemia, and cardiac arrest.[99] A fatal case of submassive liver necrosis and disseminated intravascular coagulopathy developed in a patient with severe PCP intoxication, respiratory failure, rhabdomyolysis, and hyperthermia (initial rectal temperature of 108°F/42.2°C).[100] Two other patients in this series of PCP-induced hyperthermia and submassive liver necrosis survived.

RHABDOMYOLYSIS AND RENAL FAILURE.   Rhabdomyolysis is the most common serious medical complication of phencyclidine intoxication, but the incidence of rhabdomyolysis-induced acute renal failure during severe PCP intoxication is relatively infrequent (i.e., <1% of PCP cases).[101] The renal failure associated with PCP intoxication results from myoglobinuria rather than direct nephrotoxicity, particularly following the prolonged use of restraints and dramatic elevation of serum creatine kinase concentrations.[102] Hyperuricemia frequently occurs during rhabdomyolysis along with hyperphosphatemia and hypocalcemia, but these abnormalities probably do not contribute to the renal failure associated the rhabdomyolysis.[103] Although hemodialysis may be necessary, spontaneous recovery usually begins within 10–14 days. Recovery of renal function is usually complete.[78]

Overdose

In addition to interindividual differences in response to PCP intoxication, the adverse effects associated with PCP intoxication are dose-related with higher PCP doses causing more severe adverse effects (coma, acute toxic psychosis, bizarre behavior, seizures, rhabdomyolysis). Complications associated with a massive PCP overdose included respiratory failure, hyperthermia, rhabdomyolysis, and protracted seizures. One patient was released from the hospital 24 days after ingesting a leaking plastic bag of PCP.[44] At discharge he had a clear sensorium and normal cognition, but dystonic muscle jerks in the extremities remained 24 days after admission.

Fatalities

Behavioral toxicity accounts for most deaths associated with PCP use, in part, from delusions of invulnerability and the inability to perceive imminent danger. PCP-related traumatic deaths include shallow-water drowning, automobile accidents, self-inflicted trauma, and failure to flee dangerous situations (e.g., fires). In a study of 2 northern California counties from July 1970 to June 1976, 19 coroner's cases were associated exclusively with PCP intoxication.[76] In 13 of these cases, the cause of death was drowning or trauma, whereas PCP overdose accounted for only 2 of these deaths. PCP-intoxicated patients are vulnerable to drowning as a result of PCP-induced reduction in sensation and muscular control. In a Los Angeles County study of 104 PCP-related fatalities, PCP overdose was attributed to only 15 deaths.[104] From 1981 to 1986, PCP was an incidental finding in 96% of 104 deaths reported to the St. Louis Coroner's Office where the vast majority of these

cases involved homicide.[105] The deaths of only 4 of the 104 coroner's cases with detectable concentrations of PCP were attributed to overdose.

Sudden death during apprehension for PCP-induced behavioral abnormalities occurs less commonly than during cocaine and methamphetamine intoxication. The cause of death in these situations is complex, particularly whether or not physical restraint and/or positional asphyxia may be contributory. Potential causes of death in these situations include trauma, illicit drug use, hyperthermia, mental and physical stress, natural diseases, and excited delirium.[106] Unexpected cardiac arrest can occur in patients undergoing psychologic stress without the presence of underlying structural heart disease[107] and in actively psychotic patients without obvious medical explanations.[108] Case reports indicate that death occurs during PCP-induced psychosis without the use of methods (e.g., physical restraint, handcuffs, hog tying, taser) to restrain the patient.[109] Medical causes of death in phencyclidine overdose include status epilepticus, respiratory arrest, hyperpyrexia with cardiovascular collapse, and coma with multiple end-organ failure.

Accidental Exposure

Following accidental PCP intoxication, children usually demonstrate ataxia and nystagmus along with altered mental status (diminished response to tactile and verbal stimuli, depressed sensorium, stupor associated with a blank, expressionless stare).[30] Miosis, choreoathetosis, and seizures are more common during PCP intoxication in children than adults.[110] The triad of a blank stare, miosis, and hypertension in a child is strongly suggestive of phencyclidine intoxication. In contrast to adolescents and adults, severe agitation, aggressive behavior, and psychosis do not usually occur in children with PCP intoxication.[111] Respiratory depression is more common during PCP intoxication in comatose children than comatose adults.[112,113]

Abstinence Syndrome

Although an abstinence syndrome develops in animals chronically administered PCP, a PCP withdrawal syndrome in humans is not well defined. In studies of laboratory animals, physical dependence develops within approximately 1 week of PCP administration as manifest by piloerection, bruxism, lethargy, and seizures.[114] These withdrawal effects begin about 4 hours after cessation of PCP administration with resolution occurring within ~24 hours. Clinical reports suggest that symptoms of depression, increased appetite, hypersomnia, lethargy, and drug craving occur approximately 1 week to 1 month after the cessation of chronic PCP use.[111]

Reproductive Abnormalities

Case-control studies suggest that the incidence of poor attention, hypertonia, and depressed neonatal reflexes may increase in infants born to mothers using PCP during pregnancy.[115] Case reports also suggest that a neonatal withdrawal syndrome can occur following the maternal use of PCP during pregnancy that includes jitteriness, hypertonicity, vomiting, and diarrhea.[116] In a study of 7 mothers with a history of chronic PCP use and 27 drug-free controls, the most characteristic features of the PCP-exposed infants were lability of mood (i.e., sudden outbursts of agitation, rapid changes in level of consciousness) and difficulty consoling the infant.[117] At 3 months of age, scores on the Bayley Scales of Infant Development revealed no significant differences between the 2 groups of infants.

Case reports also associate cerebral palsy, neonatal depression, behavioral abnormalities, and facial dysmorphogenesis with chronic maternal use of PCP.[118] However, reporting bias and the presence of confounding variables (e.g., drug use, poor nutrition) limits conclusions regarding the role of PCP use in these reproductive abnormalities. Another retrospective case-control study did not demonstrate a statistically significant difference in low birth weight (<2500 g) between mothers testing positive for PCP at birth and the control group of mothers without evidence of PCP use based on urine drug screens at the time of obstetrical admission.[119] Most animal and human data do not indicate that PCP is a clinically significant teratogen.[50,120]

DIAGNOSTIC TESTING

Analytic Methods

Table 34.4 lists screening and confirmatory methods for the detection of PCP in various human samples along with associated limits of detection (LOD).

SCREENING

Methods for screening urine for PCP abuse primarily involve the use of enzyme immunoassays including enzyme-linked immunosorbent assay (ELISA; Syva EMIT® II, (Syva Co., San Jose, CA) and polarized immunofluorescence (Abbott TDx FPIA®, Abbott Diagnostics, Abbott Park, IL).[124] Sensitivities and imprecision vary between different immunoassays. In a comparison of ELISA tests (Immunalysis Corp., San Dimas, CA; STC Diagnostics Inc., Bethlehem, PA), the lower limit of quantitation (LLOQ) was <1 ng/mL and 25 ng/mL, respectively.[125] The total coefficient of variance was 26% and 8.8%, respectively. Antibodies for these tests

TABLE 34.4. Methods for the Detection of Phencyclidine (PCP).

Method	LOD	Sample
Spot Test	*	†
Gas chromatography/ FID	5 ng/mL[121]	CSF, urine, serum
Gas chromatography/ MS	10 ng/mL[122]	Urine
Radioimmunoassay	<0.5 ng/mL[123]	Serum

Abbreviations: FID = Flame ionization detector; LOD = limit of detection; MS = mass spectrometry.
*Positive: 3 minutes, 100°C (212°F), red.
†Reagent: *p*-Dimethylaminobenzaldehyde.

are raised against phencyclidine rather than PCP metabolites. PHP is preferred by drug users wishing to escape detection of PCP and PCP analogue use.[126] Alternative methods involve GC with nitrogen-phosphorus detection or capillary electrophoresis.

CONFIRMATORY

Methods for the detection and quantitation of PCP include radioisotopic and enzyme immunoassays, gas chromatography with nitrogen detection,[127] liquid chromatography/mass spectrometry,[128] gas chromatography/ mass spectrometry (GC/MS) following derivation with *N*,*O*)-bis(trimethylallyl)trifluoroacetamide,[129] and GC/ MS using SIM mode with deuterated PCP as internal standard.[130] The limit of detection for PCP by radioimmunoassay (RIA) ranges down to 2 ng/mL.[131] The cross-reactivity of some phencyclidine analogues occasionally limits the correlation between RIA test results and clinical presentation. However, PCP metabolites and most other illicit drugs do not crossreact with RIA assays for PCP. For confirmation of the presence of PCP in urine samples, PCP concentrations above the federally mandated cutoff concentration of 25 ng/mL are required. Gas chromatography/electron impact quadrupole/mass spectrometry is the official method of choice in either selected ion monitoring or full-scan modes for US federal testing mandatory guidelines.[132] GC/MS involves the addition of a deuterated internal standard to the urine, followed by solid-phase or by liquid-liquid extraction and GC/MS analysis. In most chromatographic methods, preanalytic chemical derivation is not necessary.[133]

STORAGE

PCP is stable in storage over at least 3–5 years, even when stored at room temperature and particularly when refrigerated or frozen.[134] Over 6 months, PCP deteriorates (20–40%) in urine specimens when stored at room temperature, but the deterioration in frozen specimens is nominal.[135] A study of 50 urine samples containing a mean PCP concentration of 128 ng/mL demonstrated approximately a 12% decrease in the mean PCP concentration during storage at –20°C (–4°F) for 12 months.[136] In a 3-year study of PCP stored at 4°C (39.2°F) and –20°C (–4°F), samples stored for 1 year did not demonstrate a significant change in PCP concentrations when compared with initial PCP analyses. The average reductions in PCP concentrations in samples stored 2 years and 3 years were approximately 10% and 18%, respectively. Blood phencyclidine samples stored in glass vials with Teflon-lined screw caps, preservative (sodium fluoride), and anticoagulant (potassium oxalate) did not deteriorate significantly over 18 months despite the lack of refrigeration.[137] Eleven of the 45 specimens in this study demonstrated >10% loss of PCP, but PCP was still detectable in all specimens. In another study, most specimens stored over 3 months in gray-top Vacutainer® (BD Diagnostics, Franklin Lakes, NJ) tubes containing sodium fluoride and potassium oxalate at ambient temperature had reduction in PCP concentrations >10%; those stored 4–5 years had losses >70%, but PCP remained detectable in all specimens.[138]

Biomarkers

Urine and blood are the most common fluids used to detect PCP use. Alternate sample matrices for detecting PCP include saliva and cerebrospinal fluid (CSF). Although PCP is usually detectable both in saliva and in blood samples, the PCP concentrations in these 2 samples do not correlate well to one another in animal models.[139] PCP concentrations in CSF often exceed plasma PCP concentrations following complete absorption. The PCP CSF/plasma ratio remains relatively constant for several days after equilibrium, particularly following serious PCP intoxication.[140,141]

BLOOD

The distribution of PCP between plasma and erythrocytes is relatively even with whole blood/plasma ratios near unity.[29,142]

ILLICIT USE. Phencyclidine blood concentrations confirm exposure to PCP, but PCP concentrations in blood samples do not correlate well to behavioral abnormalities. In general, high blood PCP concentrations (i.e., >100 ng/mL) occur in comatose patients, whereas PCP concentrations usually, but not always are lower (<100 ng/mL) in patients with catatonia or toxic

psychosis.[143] In a case series of 46 patients with detectable serum PCP concentrations and abnormal behavioral patterns (lethargy, bizarre, violent, agitation, euphoria), the serum PCP concentrations ranged from 5–300 ng/mL (median, ~25–50 ng/mL).[143] These patients did not have clinically significant abnormalities of their vital signs. Case series suggest that high PCP concentrations in the plasma are associated with increasing risk of serious complications (seizures, hyperthermia, coma, rhabdomyolysis), particularly when the plasma PCP concentration exceeds 400–500 ng/mL.[142] A case series of 10 occasional PCP users suggested that physical findings of PCP intoxication do not correlate to plasma phencyclidine concentrations except for systolic blood pressure.[144] However, the incidence of signs of PCP intoxication (nystagmus, hypertension, hypersalivation, hyperreflexia) increased substantially when the plasma PCP concentration exceeded 100 ng/mL.

OVERDOSE. Massive oral PCP overdoses produced plasma PCP concentrations exceeding 1,000 ng/mL.[44,145] These patients developed severe agitation and coma that required intubation for respiratory support; altered mental status did not improve until the plasma PCP concentrations declined below 100 ng/mL. However, a case report also documented a PCP concentration of 1,100 ng/mL in a postmortem blood sample (location not specified) from a homicide victim.[146]

POSTMORTEM
Blood. Postmortem examinations indicate that the highest postmortem phencyclidine tissue concentrations occur in liver, brain, bile, and kidney while the postmortem blood PCP concentrations are comparatively low.[146] The incidence of fatal PCP intoxications secondary to medical complications is low compared with the number of individuals dying from PCP-related trauma.[105] Although PCP concentrations in postmortem blood samples are usually lower in victims of PCP-induced behavioral toxicity than in fatalities from acute PCP intoxication, some overlap in postmortem PCP concentrations occurs between these two groups. In a case series of 37 autopsies with detectable amounts of PCP in postmortem blood, the demise of 2 individuals found dead was attributed to acute PCP intoxication when alternate explanations for death were not apparent based on autopsy and drug screens.[147] The postmortem blood PCP concentrations of these 2 individuals were 1,500 and 25,000 ng/mL as measured by gas chromatography. In 29 of the 37 cases, the cause of death was attributed to homicide or accidental trauma. The mean blood PCP concentration in postmortem samples from this group was approximately 190 ng/mL (range, 20–700 ng/mL). Two case reports of individuals with histo-

ries of substance abuse associated death from PCP intoxications with postmortem blood PCP concentrations (one femoral, one heart) in the range of 360 ng/mL.[148] Both these individuals were found unresponsive and declared dead in the emergency department. The autopsies revealed mild cardiomegaly and no other cause of death.

In a case series of 5 deaths associated with phencyclidine intoxication and no other detectable drug, the blood PCP concentrations ranged from 300–3,300 ng/mL (mean, 1,000 ng/mL; median, 500 ng/mL) as measured by GC/MS.[149] In 6 additional cases with detectable PCP concentrations in postmortem blood, death was attributed to trauma. The mean PCP concentration in postmortem blood from this group was ~450 ng/mL (range, 60–800 ng/mL). Similarly, in another case series of 10 trauma-related deaths with detectable PCP concentrations in postmortem blood, the mean blood PCP concentration was ~600 ng/mL (range, 100–1,200 ng/mL).[76] There are few data on the postmortem redistribution of PCP, but the large volume of distribution suggests that some elevation (i.e., up to at least 3-fold) may occur in postmortem PCP heart/femoral blood ratio depending on factors related to postmortem redistribution (e.g., amount of tissue degradation, diffusion, postmortem interval).

Liver. In a case series of 5 deaths associated with phencyclidine intoxication and no other detectable drug, the liver PCP concentrations ranged from 0.9–20.5 µg/g (mean, 6.8 µg/g; median, 3.0 µg/g) as measured by GC/MS.[149] Trauma accounts for the death of 6 additional cases with detectable PCP concentrations; the mean concentration of PCP in the liver from these cases was 2.2 µg/g (range, 0.6–3.9 µg/g). The range of PCP concentration in postmortem liver samples from 4 fatalities associated with PCP intoxication and no other discernible cause of death at autopsy was 5–36 µg/g (mean, 13.75 µg/g) compared with 0.32–3.9 µg/g (mean, 1.7 µg/g) for 5 trauma-related deaths with detectable PCP concentrations as measured by gas chromatography.[150] However, occasional case reports of traumatic deaths associate high liver PCP concentrations (e.g., 6.3 µg/g) with substantial PCP concentrations (4,000 ng/mL) in postmortem blood.[76]

VITREOUS HUMOR

Limited data indicate that PCP distributes into vitreous humor with the PCP concentrations in vitreous humor typically being lower than postmortem blood. As measured by gas chromatography/flame ionization detection, the vitreous humor PCP concentrations in a case series of 18 cases testing positive for PCP by

immunoassay (cutoff, 25 ng/mL) ranged from 30–290 ng/mL compared with 50–600 ng/mL in postmortem blood.[151] In a study of 26 deaths involving PCP as an incidental finding, the mean postmortem heart blood/vitreous humor and subclavian blood/vitreous humor ratios were 2.85 ± 1.82 and 2.51 ± 1.49, respectively.[152] There was substantial interindividual variation as reflected in the standard deviations. The equilibrium between vitreous humor and blood is relatively slow compared with other fluid compartments; consequently, some of this variation may reflect difference in the state of equilibrium between blood and vitreous humor.

URINE

Urine PCP concentrations do not correlate to the severity of PCP intoxication.[171] PCP concentrations in urine are high enough during PCP intoxication to detect recent use with commercially available immunoassays, depending in part upon urine pH. Typically, urine PCP concentrations of recent PCP users exceed 100 ng/mL, when measured by RIA techniques that crossreact with PCP and monohydroxylated metabolites.[153] The use of common illicit drugs are unlikely to cause false-positive results for PCP using the EMIT® d.a.u. (Syva Co., San Jose, CA) immunoassay, when the urine concentrations of these drugs are below <100 mg/L.[124,154] Potential false-positive results for the PCP immunoassays can occur as a result of crossreactivity from high urine concentrations of dextromethorphan, ketamine, diphenhydramine,[155] venlafaxine (massive overdose),[156] and tramadol (fatal overdose).[157] Analysis of urine specimens for PCP should include determination of urine pH because the surreptitious addition of sodium bicarbonate or sodium hypochlorite to alter urine pH. The latter reduces the ability to detect PCP concentrations in urine using EMIT®, RIA, or Abbott TDx®, whereas the addition of sodium bicarbonate to urine samples reduces the detection of PCP using Abbott TDx®.[158]

Following PCP use, urine PCP concentration initially declines rapidly during the first 9–10 days; then, urine PCP concentrations decrease more gradually depending on the chronicity of PCP use. On average urine samples remain positive for PCP for about 2 weeks after cessation of chronic PCP use, but the urine from some chronic PCP users may remain positive for 1 month after the last use of PCP.[159] Separation of abstinence from new use during this period may be difficult. Case reports associate surgical procedures within this 30-day period with the development of positive PCP urine drug screens in urine samples from chronic PCP users despite abstinence, probably as a result of the release of PCP from tissue stores.[160]

HAIR

PCP occurs in hair samples from chronic PCP users based on RIA screening techniques and GC/MS confirmation.[161] However, *in vitro* experiments suggest that passive exposure to PCP smoke may produce detectable amounts of PCP in hair samples as measured by gas chromatography/tandem mass spectrometry.[162] As a result of potential external contamination of hair samples following passive exposure to PCP smoke, the detection of PCP metabolites is necessary to confirm the use of PCP using analysis of hair samples. In a study of hair samples from 8 chronic PCP users for PCP metabolites, the major metabolite was *trans*-1-(1-phenyl-4-hydroxycyclohexyl)-4′-hydroxypiperidine (t-PCPdiol), whereas 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP) was a minor metabolite as measured by GC/MS following acidic methanol extraction.[163] The concentrations of PCP, PCHP, and t-PCPdiol in the hair samples ranged from 0.33–14 ng/mg, 0.02–0.12 ng/mg, and 0.09–0.45 ng/mg, respectively.

Driving

Unlike blood–ethanol concentrations, blood PCP concentrations do not correlate well to a specific degree of impairment; the determination of impairment by blood PCP concentrations alone is difficult.[164] Physical evaluation helps confirm the degree of impairment suggested by the blood PCP concentration. In a group of 259 drivers arrested for driving under the influence, 120 blood samples were PCP positive (cutoff, 5 ng/mL) by GC/MS.[165] For the 55 individuals testing positive only for PCP and that were identified as impaired by drug recognition experts (DRE), the mean blood PCP concentration was 51 ± 26 ng/mL (range, 12–118 ng/mL). However, there was no dose-response relationship between the PCP concentration and the degree of impairment. Of the 129 individuals arrested for driving under the influence and testing negative for PCP, 4 (3%) were suspected by the DRE as being under the influence of PCP. In a case series of 50 PCP-intoxicated drivers arrested for driving under the influence (DUI), the blood PCP concentration ranged from 10–180 ng/mL with a similar lack of correlation between blood PCP concentration and the degree of impairment.[166] Urine PCP concentrations confirm exposure, but urine PCP concentrations do not correlate to impairment.

Abnormalities

Abnormalities associated with rhabdomyolysis during PCP intoxication include myoglobinuria, elevated serum creatine kinase, hyperuricemia, hypocalcemia, and

hyperphosphatemia along with renal dysfunction. Hypercalcemia may occur during the diuretic phase of acute renal failure induced by rhabdomyolysis associated with dramatic elevation of serum creatine kinase.[167] These changes typically occur 1–3 days after PCP intoxication develops, particularly in patients with severe agitation requiring restraints.[168] However, dramatic elevation of serum creatine kinase can occur in the absence of restraint.[167] Coagulopathies can develop during severe PCP intoxication associated with coma and hyperthermia, particularly during the terminal phase of excited delirium.[169] During PCP-induced coma, phencyclidine produces a characteristic electroencephalogram (EEG) pattern similar to deep ketamine anesthesia characterized by sinusoidal theta activity interrupted every few seconds by periodic slow-wave complexes.[170] These periodic complexes are unrelated to visual, auditory, or tactile stimuli during coma, but synchronization with repetitive stereotyped movements occurs on emergence. With neurologic recovery, symmetric, reactive 8.5 Hz alpha activity returns.

Neuropsychologic abnormalities present during PCP intoxication include severe impairment of symbolic cognition (e.g., proverb interpretation) and sequential thinking (e.g., serial sevens). Time distortion also occurs during PCP-induced psychosis with these patients consistently underestimating a 30-second time span.[171] Although some studies of chronic PCP users suggest impairment on neuropsychologic tests (abstraction, perceptual motor skills) when compared with national standards,[172] the question of neuropsychologic sequelae following chronic PCP use remains unresolved.

TREATMENT

Stabilization

The most serious early complications of PCP intoxication include coma, respiratory depression, seizures, and trauma to internal organs. Although relatively infrequent, respiratory depression and aspiration pneumonia may complicate the clinical presentation of PCP intoxication. All patients should be evaluated for adequacy of oxygenation with pulse oximetry, capnometry, and arterial/venous blood gases as needed. Although laryngospasm and prolonged duration of succinylcholine-induced paralysis are potential complications of PCP intoxication, intubation should proceed as required with rapid sequence intubation as determined by the physical examination and diagnostic testing. All comatose or catatonic patients should have IV access, IV naloxone (0.4 mg titrated up to 2 mg) if the concurrent use of opioids is suspected, bedside evaluation of glucose, cardiac monitoring, pulse oximetry, and vital signs including a rectal temperature. The patient should be examined for the presence of occult head and abdominal trauma with radiographic and computed tomography imaging performed as clinically indicated.

SEIZURES

Seizures are a relatively rare complication of PCP intoxication; seizures are usually responsive to IV benzodiazepines (diazepam 5–10 mg up to 30 mg, lorazepam 2–5 mg up to 10–15 mg). Continuing seizures necessitate an evaluation of oxygenation, electrolyte balance, acid–base status, blood glucose concentration, core temperature, and rhabdomyolysis. The persistence of seizures despite the administration of anticonvulsants may require rapid sequence intubation and neuromuscular blockade.

HYPERTHERMIA

Mild temperature elevation usually requires no treatment other than reduction of agitation with the use of benzodiazepines (e.g., lorazepam). Rapidly rising temperatures or temperatures exceeding 102–103°F (38.9–39.4°C) should be treated with standard cooling measures (i.e., evaporative cooling) and sedation as needed. Core temperatures above 105°F (40.6°C) require aggressive cooling measures including cooling blankets, ice packs to the groin and axilla, and ice baths with constant monitoring of core temperatures. Concurrent presence of persistent seizures and hyperthermia may require intubation and neuromuscular blockade with monitoring of the EEG. Although the similarity between PCP-associated hyperthermia and neuroleptic malignant syndrome suggest that dantrolene is a therapeutic option, there are no controlled clinical data to indicate that dantrolene controls the hyperthermia associated with severe PCP intoxication. Hyperthermic patients require daily monitoring of serum hepatic aminotransferases, serum creatine kinase, renal function, and appropriate coagulation studies (e.g. INR, aPTT, D-dimer, platelet count, fibrinogen) for disseminated intravascular coagulation at least during the first several days after the development of the hyperthermia.

HYPERTENSION

Hypertension associated with PCP intoxication is usually mild to moderate; complications from PCP-induced hypertension are rare. The elevation in blood pressure is usually self-limited, and the blood pressure typically returns to the normal range following resolution of the agitation with behavioral abnormalities

resolving within several hours. Standard drug treatment of hypertensive crisis (IV nitroprusside, IV labetalol, or esmolol) is appropriate when clinically indicated by the condition of the patient (e.g., presence of end-organ damage).

CARDIAC DYSRHYTHMIAS

Cardiac dysrhythmias are uncommon during PCP intoxication; the presence of dysrhythmias suggests underlying cardiovascular disease or the use of other drugs of abuse (e.g., cocaine, methamphetamine).

Gut Decontamination

The most common route of exposure to PCP is via the lungs; therefore, decontamination measures are usually unnecessary. Agitated and violent behavior during PCP intoxication often limits the use of gastric decontamination measures following the ingestion of PCP. The difficulty associated with the administration of activated charcoal to agitated patients restricts the usefulness of activated charcoal in this setting unless the patient is comatose and has a protected airway. Whole bowel irrigation may be appropriate for PCP body packers with retained packets, but there are inadequate data to determine the efficacy of this procedure in this circumstance.

Elimination Enhancement

The possibility of a significant enterohepatic circulation suggests that serial-activated charcoal may interrupt recirculation, but there are no data to confirm the efficacy of this procedure. Nasogastric suction theoretically removes the phencyclidine secreted into the acid environment of the stomach, but there are inadequate data to indicate that the amount of PCP removed by this technique is clinically significant. The recommendation for acid diuresis during PCP intoxication is based on the enhanced renal excretion of free PCP following reduction of the urine pH rather than clinical data. Although urinary acidification theoretically enhances the renal excretion of PCP by trapping PCP in the renal tubules, the kidney is not the major route of PCP elimination.[173] Additionally, the use of urinary acidification is associated with potentially serious complications (e.g., metabolic acidosis, exacerbation of myoglobin-induced renal failure). Consequently, the use of urinary acidification for PCP intoxication in *not* recommended for the treatment of PCP intoxication. Although there are few clinical data, the toxicokinetics (i.e., large volume of distribution, extensive biotransformation) of PCP indicate that hemodialysis and hemoperfu-

sion will not remove clinically significant amounts of PCP.

Antidotes

There are no specific antidotes for PCP intoxication.

Supplemental Care

Most patients with mild to moderate PCP intoxication can be discharged after their mental status returns to normal and physical examination demonstrates no evidence of significant trauma. Because the symptoms associated with PCP intoxication can wax and wane, patients should be observed at least until their mental status remains normal for several hours. Indications for hospitalization of patients with PCP intoxication include coma, respiratory distress, hyperthermia, seizures, rhabdomyolysis, and clinically significant trauma.

AGITATION

Intravenous diazepam (5–10 mg in an adult) or lorazepam (2–4 mg) are excellent pharmacologic options for the initial management of agitation associated with PCP intoxication. The latter drug may be administered intramuscularly if IV access is impracticable. Medicated patients should be observed for respiratory depression. Haloperidol (5–10 mg intramuscularly) is another option,[174] particularly if significant hypertension is present. Some authors prefer diazepam to haloperidol because of the potential risk of dystonic reactions, decreased seizure thresholds, and orthostatic hypotension associated with the administration of haloperidol, but there are no clinical trials to assess the difference in efficacy or complications associated with the use of these 2 drugs during PCP intoxication. Unexpected aggressive behavior can occur; the use of restraints and a coordinated show of force may be necessary to subdue the patient, along with sedation. Struggling patients in restraints should receive sufficient sedation to limit the agitation and reduce the potential for hyperthermia and rhabdomyolysis. Persistent, prolonged struggling during PCP intoxication may be associated with excited delirium and hyperthermia. Additional treatment measures involve isolation with a minimum of sensory stimuli (quiet room and few procedures) other than the measurement of body temperature. PCP-intoxicated patients do not respond well to attempts by concerned individuals to verbally calm (i.e., "talk-down") them, especially when these individuals are alone with the patient. Medical observation of the phencyclidine-intoxicated patient should continue until the patient remains symptom-free for several hours.

The urine of all patients with coma, toxic psychosis, catatonia, or acute brain syndrome should be evaluated for the presence of heme on the urine dipstick. Heme-positive urine samples that contain no red cells under microscopy should be considered positive for myoglobin, and a urine specimen evaluated for the presence of myoglobin. PCP-intoxicated patients with evidence of rhabdomyolysis should be hospitalized, and blood samples analyzed for serum creatine kinase, electrolytes, calcium, phosphorus, uric acid, and creatinine at least daily for several days. Myoglobinuria should be treated with adequate fluid replacement and the maintenance of good urine output, especially in the hyperthermic, diaphoretic, and agitated patient. In normovolemic patients with normal renal function, initial creatine kinase levels exceeding 14,000 IU/L suggests the need for IV furosemide (40 mg). Although there are limited clinical data, urinary alkalinization for myoglobinuria probably should not be used because of the decreased renal clearance of phencyclidine in alkaline urine. The presence of myoglobinuria is a contraindication to acid diuresis. Bronchospasm is treated with the usual bronchodilators (inhaled albuterol and ipratropium). Generalized muscle rigidity does not usually respond to diphenhydramine. Urinary catheterization may be necessary, particularly in comatose patients because of the increased incidence of urinary retention during PCP intoxication. The clinical presentation of major trauma may be occult because of the analgesia and altered consciousness associated with PCP intoxication. Therefore, each PCP patient should be carefully evaluated for signs of trauma.

Psychosis

Patients with PCP-induced psychosis should be managed in a quiet room with minimal external stimulation. Haloperidol and chlorpromazine are effective neuroleptic agents for PCP-induced psychosis. There are few clinical data on the adverse effects of these antipsychotic drugs during PCP intoxication including newer atypical antipsychotic drugs, but these reactions probably are similar to the treatment of chronic schizophrenia with the potential exception that PCP-intoxicated patients may develop hyperthermia as a result of the reduced ability to dissipate heat; drugs with anticholinergic effects (e.g., diphenhydramine) should be avoided in these patients.[175] The clinical response of patients with PCP-induced psychosis to haloperidol (i.e., dopamine DA_2 receptor antagonist) may be more effective than to chlorpromazine (i.e., mixed DA_1/DA_2 receptor antagonist) based on a clinical trial of 20 PCP-intoxicated patients.[176] Diazepam is a treatment option for the agitation associated with PCP-induce psychosis, and the use of diazepam or other benzodiazepines probably does not prolong phencyclidine toxicity. Physical restraints often are necessary to prevent self-inflicted trauma. Continuous sedation (e.g., IV midazolam or propofol drip) may be necessary to prevent additional muscle injury and rhabdomyolysis. Psychotic patients should be admitted to a psychiatric or medical facility after medical clearance for concomitant overdose and trauma.

PHENCYCLIDINE ANALOGUES

During the late 1970s, synthetic PCP analogues (TCP, PHP, PCC, ketamine) appeared in street samples. There are limited data to determine the incidence of the use of PCP analogues; however, analysis of street samples suggests that the use of PCP analogues is much less than PCP. There are over 30 PCP analogues with behavioral and pharmacologic effects similar, but less intense, than the effects of PCP. At least 6 analogs appear as illicit drugs including TCP (thiophene analog), PCE (*N*-ethyl analog), PHP (phenylcyclohexyl pyrrolidine analog), *N*-(1-phenylcyclohexyl)-propanamine (PCPR),[177] *N*-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA), and *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA).[178] Figure 34.1 displays the chemical structure of some analogs of PCP. Similar to illicit PCP synthesis, the use of volatile solvents during the synthesis of PCP analogues may cause explosions and/or fires in clandestine laboratories. Treatment of PCP analogue intoxication is symptomatic and supportive, similar to PCP.

Dizocilpine

MK-801 (dizocilpine, CAS RN: 77086-21-6) is a potent non-competitive antagonist at the *N*-methyl-D-aspartate (NMDA) family of glutamate receptors in the CNS that shares similar pharmacologic properties with phencyclidine and ketamine. This drug is commonly used as a NMDA receptor antagonist in pharmacological studies. Behavioral sensitization involves persistent enhancement of the locomotor stimulant, stereotypic, and positive reinforcing effects of drugs of abuse following repeated use. Cross sensitization is the enhancement of the effects of drugs of abuse following the use of another drug. Initial animal studies suggested that MK-801

prevented the development of behavioral and cross sensitization;[179] however, subsequent research indicated that the actions of this drug are complex.[180] MK-801 possesses reinforcing properties, induces behavioral sensitization to itself, and induces cross sensitization to other drugs of abuse.[181] A case report associated the death of a 45-year old man found dead with the intentional ingestion of an estimated 25 mg of this compound (heart blood, 150 ng/mL) along with ethanol (heart blood, 20 mg/dL) and diazepam (heart blood, 1,000 ng/mL) plus metabolites (nordiazepam, 1,100 ng/mL; oxazepam, 100 ng/mL; temazepam, 100 ng/mL) as measured by gas chromatography/mass spectrometry.[182]

Ketamine

Ketamine [2-(*o*-Chlorophenyl)-2-(methylamino)cyclohexanone] is a dissociative anesthetic, which is structurally similar to PCP and is marketed in the US under the name Ketalar®. Emergence psychosis (vivid imagery, hallucinations, disorientation, delirium) also occurs in adults after the use of ketamine as an anesthetic. The postanesthesia emergence reactions associated with ketamine are stereospecific with R(-)-ketamine having a stronger association with psychic disturbances. Additional risk factors for emergence reactions include age >16 years, female gender, people who frequently dream during sleep, IV doses of ketamine >2 mg/kg or IV administration rates exceeding 40 mg/min and a history of personality disorders. The effects are typically short-lived (<24 hours) and are not associated with any permanent personality changes. The *S*(+)-isomer is 3–5 times more potent as an anesthetic agent than the *R*(-)-isomer. There are inadequate data to determine if the risk factors associated with ketamine-induced anesthesia emergence are also risk factors for intensified psychic disturbance in PCP users.

1-(1-Phenylcyclohexyl) pyrrolidine (PHP)

1-(1-Phenylcyclohexyl)pyrrolidine (rolicyclidine, CAS RN: 2201-39-0) is a pyrrolidine analogue of PCP with similar adverse behavioral and physiological effects as PCP.[183] PHP is a DEA Schedule I drug. PHP is more difficult to detect in urine drug screens than PCP; determination of the presence of psychoactive phencyclidine analogues (e.g., PHP) requires more rigorous analytic

techniques (gas chromatography/mass spectrometry) than commercial immunoassays for PCP. Analytic methods to detect the presence of PHP include thin layer chromatography[184] and gas chromatography/mass spectrometry.[185] The use of the latter method resulted in the detection of 100 ng PHP/mL in postmortem blood from a 35-year old man shot by police while acting in a bizarre manner and resisting arrest. In a study of 20 patients with PHP-induced psychosis, the administration of 5 mg haloperidol intramuscularly was more effective as a sedative than placebo.[186]

1-(1-[2-Thienyl]cyclohexyl) piperidine (TCP)

1-(1-[2-Thienyl]cyclohexyl)piperidine (TCP, CAS RN: 1867-65-8) is a Schedule I substance in the United States that has been investigated in clinical trials for the treatment of traumatic brain and spinal cord injuries.[187] TCP is not usually an impurity in samples of illicit PCP as analyzed by gas chromatography/chemical ionization/mass spectrometry.[20] Rodent studies suggest that the toxicokinetics of PCP and TCP are similar. In rodent studies, the mean unbound TCP is $42 \pm 6\%$ and mean apparent volume of distribution is large $(27 \pm 17$ L/kg).[188] The clearance of TCP from the blood is rapid with a plasma elimination half-life of about 2 hours. Little (i.e., <1% administered dose) unchanged TCP appears in the urine. Animal data suggest that the physiological properties of TCP and PCP are probably similar in animals and humans, but the behavioral effects of TCP are less intense than PCP or other PCP analogues (dizocilpine, PCE, cyclohexamine).[189] TCP is a high-affinity, non-competitive antagonist of *N*-methyl-ᴅ-aspartate (NMDA) receptors that prevents ion influx through the associated channel. This compound is now the preferred radioligand for the displacement assays of PCP.[190] As measured by a test for ataxia (rotarod) in mice, TCP is about 30–50% more potent than PCP; however, the LD_{50} of TCP was about 1/3 lower than the LD_{50} of PCP.[191] In rodent studies, PCP and TCP have a similar number of high affinity binding sites, but there were twice as many PCP low binding sites as TCP low binding (PCP_3) sites.[192] The number of PCP and TCP high and low binding sites were similar in the cerebellum; however, the density of high binding sites in the cerebrum was 7 times higher than the cerebellum. Methods to detect PCP designer drugs include GC/MS in both electron ionization and positive-ion chemical ionization modes.[193]

References

1. Greifenstein FE, Devault M, Yoshitake J, Gajewski JE. A study of a 1-aryl cyclohexyl amine for anesthesia. Anesth Analg 1958;37:283–294.

2. Collins VJ, Gorospe CA, Rovenstine EA. Intravenous nonbarbiturate, nonnarcotic analgesics: preliminary studies. 1. Cyclohexylamines. Anesth Analg 1960;39: 302–306.

3. Garvey RE. PCP (phencyclidine): an update. J Psychedelic Drugs 1979;11:265–275.

4. Bradley WJ, Jennings N, Jossa D, Rootman I. Recent trends in illicit drug use among young people in Canada. Morb Mortal Wkly Rep MMWR 1985;34:35–37.

5. Cook CE. Pyrolytic characteristics, pharmacokinetics, and bioavailability of smoked heroin, cocaine, phencyclidine, and methamphetamine. NIDA Res Monogr 1991; 115:6–23.

6. Beaver RW, Jones LA. Pyrolysis products of 1-(1-phenycyclohexyl)piperidine (PCP). Can J Chem 1984;62:1022–1027.

7. Lue LP, Scimeca JA, Thomas BF, Martin BR. Identification and quantification of phencyclidine pyrolysis products formed during smoking. J Anal Toxicol 1986;10:81–86.

8. Peters RJ Jr, Kelder SH, Meshack A, Yacoubian GS Jr, McCrimmons D, Ellis A. Beliefs and social norms about cigarettes or marijuana sticks laced with embalming fluid and phencyclidine (PCP): why youth use "fry." Subst Use Misuse 2005;40:563–571.

9. Singer M, Mirhej G, Shaw S, Saleheen H, Vivian J, Hastings E, et al. When the drug of choice is a drug of confusion: embalming fluid use in inner city Hartford, CT. J Ethn Subst Abuse 2005;4:73–96.

10. Morgan JP, Solomon JL. Phencyclidine clinical pharmacology and toxicity. NY State J Med 1978;78:2035–2038.

11. Chan K-M, Mathews WS, Saxena S, Wong ET. Frequency of cocaine and phencyclidine detection at a large urban public teaching hospital. J Anal Toxicol 1993;17: 299–303.

12. U.S. Department of Health and Human Services. The Dawn Report: Trends in PCP-related emergency department visits; January 2004. Available at http://dawninfo.samhsa.gov/old_dawn/pubs_94_02/shortreports/files/TDR_PCPa.pdf. Accessed 2011 May 15.

13. U.S. Department of Health and Human Services. The Dawn Report: National Estimates of Drug-related Emergency Department Visits, 2004 - 2009. Available at https://dawninfo.samhsa.gov/data/report.asp?f=Nation/Illicit/Nation_2009_Illicit_ED_Visits_by_Drug Accessed 2011 April 20.

14. Aronow R, Done AK. Phencyclidine overdose: an emerging concept of management. JACEP 1978;7:56–59.

15. Cook CE, Brine DR, Quin GD, Perez-Reyes M, Di Guiseppi SR. Phencyclidine and phenylcyclohexane disposition after smoking phencyclidine. Clin Pharmacol Ther 1982;31:635–641

16. Maddox VH, Godefroi EF, Purcell RF. The synthesis of phencyclidine and other 1-arylcyclohexylamines. J Med Chem 1965;56:230–235.

17. Kalir A, Edery H, Pelah Z, Balderman D, Porath G. 1-Phenycycloalkylamine derivatives. II. Synthesis and pharmacological activity. J Med Chem 1969;12:473–477.

18. Lue LP, Scimeca JA, Thomas BF, Martin BR. Pyrolytic fate of piperidinocyclohexanecarbonitrile, a contaminant of phencyclidine, during smoking. J Anal Toxicol 1988; 12:57–61.

19. Soine WH, Vincek WC, Agee DT, Boni J, Burleigh GC, Casey TH, et al. Contamination of illicit phencyclidine with 1-piperidinocyclohexanecarbonitrile. J Anal Toxicol 1980;4:217–221.

20. Cone EJ, Vaupel DB, Buchwald WF: Phencyclidine: detection and measurement of toxic precursors and analogs in illicit samples. J Anal Toxicol 1980;4:119–123.

21. Dulik DM, Soine WH. Color test for detection of 1-piperidinocyclohexanecarbonitrile (PCC) in illicit phencyclidine. Clin Toxicol 1981;18:737–742.

22. Morocco AP, Osterhoudt KC. Getting "wet" from recreational use of embalming fluid. Pediatr Case Rev 2003; 3:111–113.

23. Silber TJ, Losefsohn M, Hicks JM, Getson PR, O'Donnell R. Prevalence of PCP use among adolescent marijuana users. J Pediatr 1988;112:827–829.

24. Stillman R, Petersen RC. The paradox of phencyclidine (PCP) abuse. Ann Intern Med 1979;90:428–430.

25. Young T, Lawson GW, Gacono CB. Clinical aspects of phencyclidine (PCP). Int J Addict 1987;22:1–15.

26. Davies BM, Beech HR. The effect of 1-arylcylohexylamine (sernyl) on twelve normal volunteers. J Ment Sci 1960; 106:912–924.

27. Pradhan SN. Phencyclidine (PCP): some human studies. Neurosci Biobehavior Rev 1984;8:493–501.

28. Burns RS, Lerner SE, Corrado R. Phencyclidine—states of acute intoxication and fatalities. West J Med 1975; 123:345–349.

29. Cook CE, Brine DR, Jeffcoat AR, Hill JM, Wall ME, Perez-Reyes M, Di Guiseppi SR. Phencyclidine disposition after intravenous and oral doses. Clin Pharmacol Ther 1982;31:625–634.

30. Schwartz RH, Einhorn A. PCP intoxication in seven young children. Pediatr Emerg Care 1986;2:238–241.

31. Aniline O, Pitts FN Jr, Allen RE, Burgoyne R. Incidental intoxication with phencyclidine. J Clin Psychiatry 1980;41:393–394.

32. Cook CE, Jeffcoat AR. Pyrolytic degradation of heroin, phencyclidine, and cocaine: identification of products and some observations on their metabolism. NIDA Res Monogr 1990;99:97–120.

33. Cook CE, Brine DR, Quin GD, Wall ME, Perez-Reyes M, Di Guiseppi SR. Smoking of phencyclidine: disposition in man and stability to pyrolytic conditions. Life Sci 1981;29:1967–1972.

34. Bailey DN. Percutaneous absorption of phencyclidine hydrochloride *in vivo*. Res Commun Substance Abuse 1980;1:443–450.

35. Cook CE, Perez-Reyes M, Jeffcoat AR, Brine DR. Phencyclidine disposition in humans after small doses of radiolabeled drug. Fed Proc 1983;42:2566–2569.

36. Giles HG, Corrigall WA, Khouw V, Sellers EM. Plasma protein binding of phencyclidine. Clin Pharmacol Ther 1982;31:77–82.

37. James SH, Schnoll SH. Phencyclidine: tissue distribution in the rat. Clin Toxicol 1976;9:573–582.

38. Holsztynska EJ, Domino EF. Biotransformation of phencyclidine. Drug Metab Rev 1985–86;16:285–320.

39. Laurenzana EM, Owens SM. Metabolism of phencyclidine by human liver microsomes. Drug Metab Dispos 1997;25:557–563.

40. Wong LK, Biemann K. Metabolites of phencyclidine. Clin Toxicol 1976;9:583–591.

41. Cohen LS, Gosenfeld L, Wilkins J, Kammerer RC, Tachiki K. Demonstration of an amino acid metabolite of phencyclidine. N Engl J Med 1982;306:1427–1428.

42. Perez-Reyes M, Di Guiseppi S, Brine DR, Smith H, Cook CE. Urine pH and phencyclidine excretion. Clin Pharmacol Ther 1982;32:635–641.

43. Domino SE, Domino LE, Domino EF. Comparison of two and three compartment models of phencyclidine in man. Subst Alcohol Actions Misuse 1982;2:205–211.

44. Jackson JE. Phencyclidine pharmacokinetics after a massive overdose. Ann Intern Med 1989;111:613–615.

45. Marshman JA, Ramsay MP, Seller EM. Quantitation of phencyclidine in biological fluids and application to human overdose. Toxicol Appl Pharmacol 1976;35:129–136.

46. Jushchyshyn MI, Wahlstrom JL, Hollenberg PF, Wienkers LC. Mechanism of inactivation of human cytochrome P450 2B6 by phencyclidine. Drug Metab Dispos 2006;34:1523–1529.

47. Ahmad G, Halsall LC, Bondy SC. Persistence of phencyclidine in fetal brain. Brain Res 1987;415:194–196.

48. Cummings AJ, Jones HM, Cooper JE. Transplacental disposition of phencyclidine in the pig. Xenobiotica 1979;9:447–452.

49. Rayburn WF, Holsztynska EF, Domino EF. Phencyclidine: biotransformation by the human placenta. Am J Obstet Gynecol 1984;148:111–112.

50. Kaufman KR, Petrucha RA, Pitts FN Jr, Kaufman ER. Phencyclidine in umbilical cord blood: preliminary data. Am J Psychiatry 1983;140:450–452.

51. Fico TA, Vanderwende C. Phencyclidine during pregnancy: behavioral and neurochemical effects in the offspring. Ann NY Acad Sci 1989;562:319–326.

52. Nicholas JM, Lipshitz J, Schreiber EC. Phencyclidine: its transfer across the placenta as well as into breast milk. Am J Obstet Gynecol 1982;143:143–146.

53. Kaufman KR, Petrucha RA, Pitts FN Jr, Weekes ME. PCP in amniotic fluid and breast milk: case report. J Clin Psychiatry 1983;44:269–270.

54. Carroll ME: Oral self-administration of phencyclidine (PCP) and PCP analogs and tolerance to PCP's behavioral effects. NIDA Res Monogr 1982;41:74–81.

55. Balster RL, Woolverton WL. Tolerance and dependence to phencyclidine. Psychopharmacol Bull 1980;16:76–77.

56. Spain JW, Klingman GI. Continuous intravenous infusion of phencyclidine in unrestrained rats results in the rapid induction of tolerance and physical dependence. J Pharmacol Exp Ther 1985;234:415–424.

57. Cohen BD, Luby ED, Rosenbaum G, Gottlieb JS. Combined Sernyl and sensory deprivation. Compr Psychiatry 1960;1:345–348.

58. Meibach RC, Glicks D, Cox R, Maayani S. Localisation of phencyclidine-induced changes in brain energy metabolism. Nature 1979;282:625–626.

59. Javitt DC, Jotkowitz A, Sircar R, Zukin SR. Noncompetitive regulation of phencyclidine/sigma-receptors by the *N*-methyl-D-aspartate receptor antagonist D-(-)-2-amino-5-phosphonovaleric acid. Neurosci Lett 1987;78:193–198.

60. Snell LD, Morter RS, Johnson KM. Structural requirements for activation of the glycine receptor that modulates the *N*-methyl-D-aspartate operated ion channel. Eur J Pharmacol 1988;156:105–110.

61. Skuza G, Wedzony K. Behavioral pharmacology of σ-ligands. Pharmacopsychiatry 2004;37(suppl 3):S183–S188.

62. Monassier L, Bousquet P. Sigma receptors: from discovery to highlights of their implications in the cardiovascular system. Fundam Clin Pharmacol 2003;16:1–8.

63. Fox K, Daw N, Sato H, Czepita D. The effect of visual experience on development of NMDA receptor synaptic transmission in kitten visual cortex. J Neurosci 1992;12:2672–2684.

64. Davis S, Butcher SP, Morris RG. The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP *in vivo* at intracerebral concentrations comparable to those that block LTP *in vitro*. J Neurosci 1992;12:21–34.

65. Chizh BA, Headley PM. NMDA antagonists and neuropathic pain—multiple drug targets and multiple uses. Curr Pharm Des 2005;11:2977–2994.

66. Johnson KM, Jones SM. Neuropharmacology of phencyclidine: basic mechanisms and therapeutic potential. Annu Rev Pharmacol Toxicol 1990;30:707–750.

67. Seeman P, Ko F, Tallerico T. Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Mol Psychiatry 2005;10:877–883.

68. Johnson KM, Hillman GR. Comparisons between phencyclidine, its monohydroxylated metabolites, and the stereoisomers of *N*-allyl-*N*-normetazocine (SKF 10047) as inhibitors of the muscarinic receptor and acetylcholinesterase. J Pharm Pharmacol 1982;34:462–464.

69. Albuquerque EX, Adler M, Spivak CE, Aguayo L. Mechanism of nicotinic channel activation and blockade. Ann NY Acad Sci 1980;358:204–238.

70. Bayorh MA, McGee LJ. Cardiovascular and hemodynamic effects of acute and chronic phencyclidine (PCP). Res Commun Subst Abuse 1989;10:27–36.

71. Hackett RB, Obrosky KW, Borne RF, Waters IW. Acute phencyclidine poisoning in the unanesthetized dog: pathophysiologic profile of acute lethality. Toxicology 1981;19:11–20.

72. Reddy SK, Kornblum RN. Rhabdomyolysis following violent behavior and coma. J Forensic Sci 1987;32:550–553.

73. Kuncl RW, Meltzer HY. Pathologic effect of phencyclidine and restraint on rat skeletal muscle structure: prevention by prior denervation. Exp Neurol 1974;45:387–402.

74. Goode DJ, Meltzer HY. The role of isometric muscle tension in the production of muscle toxicity by phencyclidine and restraint stress. Psychopharmacologia 1975;42:105–108.

75. Weissman AD, Casanova MF, Kleinman JE, De Souza EB. PCP and sigma receptors in brain are not altered after repeated exposure to PCP in humans. Neuropsychopharmacology 1991;4:95–102.

76. Burns RS, Lerner SE. Phencyclidine deaths. JACEP 1978;7:135–141.

77. Noguchi TT, Nakamura GR. Phencyclidine-related deaths in Los Angeles County, 1976. J Forensic Sci 1978;23:503–507.

78. Cogen FC, Rigg G, Simmons JL, Domino EF. Phencyclidine associated acute rhabdomyolysis. Ann Intern Med 1979;88:210–212.

79. Barton CH, Sterling ML, Vaziri ND. Phencyclidine intoxication: Clinical experience in 27 cases confirmed by urine assay. Ann Emerg Med 1981;10:243–246.

80. Schwartz RH, Hoffmann NG, Smith D, Hayden GF, Riddile M. Use of phencyclidine among adolescents attending a suburban drug treatment facility. J Pediatr 1987;110:322–324.

81. McCarron MM, Schulze BW, Thompson GA, Conder MC, Goetz WA. Acute phencyclidine intoxication: Incidence of clinical findings in 1000 cases. Ann Emerg Med 1981;10:237–242.

82. Brecher M, Wang B-W, Wong H, Morgan JP. Phencyclidine and violence: clinical and legal issues. J Clin Psychopharmacol 1988;8:397–401.

83. Fauman B, Aldinger G, Fauman M, Rosen P. Psychiatric sequelae of phencyclidine abuse. Clin Toxicol 1976;9:529–538.

84. Fallis RJ, Aniline O, Weiner LP, Pitts FN Jr. Massive phencyclidine intoxication. Arch Neurol 1982;39:316.

85. Allen RM, Young SJ. Phencyclidine-induced psychosis. Am J Psychiatry 1978;135:1081–1084.

86. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 1991;148:1301–1308.

87. Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R. Study of a new schizophrenomimetic drug—Sernyl. Arch Neurol Psychiatry 1959;81:363–369.

88. Showalter CV, Thornton WE. Clinical pharmacology of phencyclidine toxicity. Am J Psychiatry 1977;134:1234–1238.

89. Grove VE Jr. Painless self injury after ingestion of "angel dust." JAMA 1979;242:655.

90. Cohen S. Angel dust. JAMA 1977;238:515–516.

91. McCarron MM, Schulze BW, Thompson GA, Conder MC, Goetz WA. Acute phencyclidine intoxication: clinical patterns, complications and treatment. Ann Emerg Med 1981;10:290–297.

92. Crosley CJ, Binet EF. Cerebrovascular complications in phencyclidine intoxication. J Pediatr 1979;94:316–318.

93. Kessler GF Jr, Demers LM, Berlin C, Brennan RW. Phencyclidine and fatal status epilepticus. N Engl J Med 1974;291:979.

94. Eastman JW, Cohen SN. Hypertensive crisis and death associated with phencyclidine poisoning. JAMA 1975;231:1270–1271.

95. Boyko OB, Burger PC, Heinz ER. Pathological and radiological correlation of subarachnoid hemorrhage in phencyclidine abuse. J Neurosurg 1987;67:446–448.

96. Corales RL, Maull KI, Becker DP. Phencyclidine abuse mimicking head injury. JAMA 1980;243:2323–2324.

97. McMahon B, Ambre J, Ellis J. Hypertension during recovery from phencyclidine intoxication. Clin Toxicol 1978;12:37–40.

98. Thompson TN. Malignant hyperthermia from PCP. J Clin Psychiatry 1979;40:327.

99. Stein GY, Fradin Z, Ori Y, Singer P, Korobko Y, Zeidman A. Phencyclidine-induced multi-organ failure. Isr Med Assoc J 2005;7:535–537.

100. Armen R, Kanel G, Reynolds T. Phencyclidine induced malignant hyperthermia causing submassive liver necrosis. Am J Med 1984;77:167–172.

101. Patel R, Connor G. A review of thirty cases of rhabdomyolysis-associated acute renal failure among phencyclidine users. Clin Toxicol 1986;23:547–556.

102. Lahmeyer HW, Stock PG. Phencyclidine intoxication, physical restrain, and acute renal failure: case report. J Clin Psychiatry 1983;44:184–185.

103. Akmal M, Valdin JR, McCarron MM, Massry SG. Rhabdomyolysis with and without acute renal failure in patients with phencyclidine intoxication. Am J Nephrol 1981;1:91–96.

104. Budd RD, Lindstrom DM. Characteristics of victims of PCP-related deaths in Los Angeles County. J Toxicol Clin Toxicol 1982–1983;19:997–1004.

105. Poklis A, Graham M, Maginn D. Phencyclidine and violent deaths in ST. Louis, Missouri: a survey of medical

examiners' cases from 1977 through 1986. Am J Drug Alcohol Abuse 1990;16:265–274.

106. Pestaner JP, Southall PE. Sudden death during arrest and phencyclidine intoxication. Am J Forensic Med Pathol 2003;24:119–122.

107. Brodsky MA, Sato DA, Iseri LT, Wolff LJ, Allen BJ. Ventricular tachyarrhythmia associated with psychological stress. The role of the sympathetic nervous system. JAMA 1987;257:2064–2067.

108. Chute D, Grove C, Rajasekhara B, Smialek JE. Schizophrenia and sudden death: a medical examiner case study. Am J Forensic Med Pathol 1999;20:131–135.

109. Burns RS, Lerner SE. Causes of phencyclidine-related deaths. Clin Toxicol 1978;2:463–481.

110. Karp HN, Kaufman ND, Anand SK. Phencyclidine poisoning in young children. J Pediatr 1980;97:1006–1009.

111. Carroll ME. PCP and hallucinogens. Adv Alcohol Subst Abuse 1990;9:167–190.

112. Liden CB, Lovejoy FH Jr, Costello CE. Phencyclidine: Nine cases of poisoning. JAMA 1975;234:513–516.

113. Welch MJ, Correa GA. PCP intoxication in young children and infants. Clin Pediatr 1980;19:510–514.

114. Balster RL. Clinical implications of behavioral pharmacology research on phencyclidine. NIDA Res Monogr 1986;64:148–162.

115. Golden NL, Kuhnert BR, Sokol RJ, Martier S, Williams T. Neonatal manifestations of maternal phencyclidine exposure. J Perinat Med 1987;15:185–191.

116. Strauss AA, Modaniou HD, Bosu SK. Neonatal manifestations of maternal phencyclidine (PCP) abuse. Pediatrics 1981;68:550–552.

117. Chasnoff IJ, Burns WJ, Hatcher RP, Burns KA. Phencyclidine: effects on the fetus and neonate. Dev Pharmacol Ther 1983;6:404–408.

118. Golden NL, Sokol RJ, Rubin IL. Angel dust: possible effects on the fetus. Pediatrics 1980;65:18–20.

119. Mvula MM, Miller JM Jr, Ragan FA. Relationship of phencyclidine and pregnancy outcome. J Reprod Med 1999;44:1021–1024.

120. Marks TA, Worthy WC, Staples RE. Teratogenic potential of phencyclidine in the mouse. Teratology 1980;21:541–546.

121. Miceli JN, Bowman DB, Aravind MK. An improved method for the quantitation of phencyclidine (PCP) in biological samples utilizing nitrogen-detection gas chromatography. J Anal Toxicol 1981;5:29–32.

122. Cone EJ, Buchwald W, Yousefnejad D. Simultaneous determination of phencyclidine and monohydroxylated metabolites in urine of man by gas chromatography-mass fragmentography with methane chemical ionization. J Chromatogr 1981;223:331–339.

123. Owens SM, Woodworth J, Mayersohn M. Radioimmunoassay for phencyclidine (PCP) in serum. Clin Chem 1982;28:1509–1513.

124. Caplan YH, Levine B. Abbott phencyclidine and barbiturates abused drug assays: evaluation and comparison of ADx FPIA, TDx FPIA, EMIT, and GC/MS methods. J Anal Toxicol 1989;13:289–292.

125. Kerrigan S, Phillips WH Jr. Comparison of ELISAs for opiates, methamphetamine, cocaine metabolite, benzodiazepines, phencyclidine, and cannabinoids in whole blood and urine. Clin Chem 2001;47:540–547.

126. Budd RD. Phencyclidine (PCP)—structure vs reactivity. Clin Toxicol 1981;18:1033–1041.

127. Bailey DN, Guba JJ. Gas-chromatographic analysis for phencyclidine in plasma, with use of a nitrogen detector. Clin Chem 1980;26:437–440.

128. Tai SS, Christensen RG, Coakley K, Ellerbe P, Long T, Welch J. Certification of phencyclidine in lyophilized human urine reference materials. J Anal Toxicol 1996; 20:43–49.

129. Gole DJ, Pirat J-L, Kamenka J-M, Domino EF. Hydroxy metabolites of phencyclidine identification and quantitation of two novel metabolites. Drug Metab Disp 1988; 16:386–391.

130. Schneider S, Kuffer P, Wennig R. Determination of lysergide (LSD) and phencyclidine in biosamples. J Chromatogr B Biomed Sci Appl 1998;713:189–200.

131. Kaul B, Davidow B. Radioimmunoassay screening test for detection of phencyclidine (PCP, "angel dust") abuse among teenagers. Clin Toxicol 1980;16:7–15.

132. Department of Health and Human Services: Mandatory guidelines for federal workplace drug testing programs. Fed Regist 1988;53:11970–11989.

133. Tai SS, Christensen RG, Coakley K, Ellerbe P, Long T, Welch MJ. Certification of phencyclidine in lyophilized human urine reference materials. J Anal Toxicol 1996; 20:43–49.

134. Mozayani A. Phencyclidine–effects on human performance and behavior. Forensic Sci Rev 2002;15:61–74.

135. Hughes R, Hughes A, Levine B, Smith ML. Stability of phencyclidine and amphetamines in urine specimens. Clin Chem 1991;37:2141–2142.

136. Dugan S, Bogema S, Schwartz RW, Lappas NT. Stability of drugs of abuse in urine samples stored at –20°C. J Anal Toxicol 1994;18:391–396.

137. Clardy DO, Ragle JL. Stability of phencyclidine in stored blood. Clin Toxicol 1981;18:929–934.

138. Giorgi SN, Meeker JE. A 5-year stability study of common illicit drugs in blood. J Anal Toxicol 1995;19:392–398.

139. Bailey DN, Guba JJ. Measurement of phencyclidine in saliva. J Anal Toxicol 1980;4:311–313.

140. Donaldson JO, Baselt RC. CSF phencyclidine. Am J Psychiatry 1979;136:1341–1342.

141. Foster HM, Narasimhachari N. Phencyclidine in CSF and serum: a case of attempted filicide by a mother without a history of substance abuse. J Clin Psychiatry 1986; 47:428–429.

142. Bailey DN. Clinical findings and concentrations in biological fluids after nonfatal intoxication. Am J Clin Pathol 1979;72:795–799.

143. Walberg CB, McCarron MM, Schulze BW. Quantitation of phencyclidine in serum by enzyme immunoassay: results in 405 patients. J Anal Toxicol 1983;7:106–110.

144. Bailey DN, Shaw RF, Buga JJ. Phencyclidine abuse: plasma levels and clinical findings in casual users and in phencyclidine related deaths. J Anal Toxicol 1978; 2:233–237.

145. Young JD, Crapo LM. Protracted phencyclidine coma from an intestinal deposit. Arch Intern Med 1992; 152:859–860.

146. Budd RD, Liu Y. Phencyclidine concentrations in postmortem body fluids and tissues. J Toxicol Clin Toxicol 1982–1983;19:843–850.

147. Caplan YH, Orloff KG, Thompson BC, Fisher RS. Detection of phencyclidine in medical examiner's cases. J Anal Toxicol 1979;3:47–52.

148. deRoux SJ, Sgarlato A, Marker E. Phencyclidine: a 5-year retrospective review from the New York City Medical Examiner's Office. J Forensic Sci 2011;56:656–659.

149. Cravey RH, Reed D, Ragel JL. Phencyclidine related deaths: a report of nine fatal cases. J Anal Toxicol 1979;3: 199–201.

150. Reynolds PC. Clinical and forensic experiences with phencyclidine. Clin Toxicol 1976;9:547–552.

151. Jenkins AJ, Oblock J. Phencyclidine and cannabinoids in vitreous humor. Legal Med 2008;10:201–203.

152. Cox D, Jufer Phipps RA, Levine B, Jacobs A, Fowler D. Distribution of phencyclidine into vitreous humor. J Anal Toxicol 2007;31:537–539.

153. Heveran JE, Anthony M, Ward C. Determination of phencyclidine by radioimmunoassay. J Forensic Sci 1980; 25:79–87.

154. Allen LV Jr., Stiles ML. Specificity of the cannabinoid metabolite and phencyclidine EMIT d.a.u. assays. J Anal Toxicol 1988;12:45–47.

155. Levine BS, Smith ML. Effects of diphenhydramine on immunoassays of phencyclidine in urine. Clin Chem 1990; 36:1258.

156. Bond GR, Steele PE, Uges DR. Massive venlafaxine overdose resulted in a false positive Abbott AxSYM urine immunoassay for phencyclidine. J Toxicol Clin Toxicol 2003;41:999–1002.

157. Hull MJ, Griggs D, Knoepp SM, Smogorzewska A, Nixon A, Flood JG. Postmortem urine immunoassay showing false-positive phencyclidine reactivity in a case of fatal tramadol overdose. Am J Forensic Med Pathol 2006;27: 359–362.

158. Warner A. Interference of common household chemicals in immunoassay methods for drugs of abuse. Clin Chem 1989;35:648–651.

159. Simpson GM, Khajawall AM, Alatorre E, Staples FR. Urinary phencyclidine excretion in chronic abusers. J Toxicol Clin Toxicol 1982;19:1051–1059.

160. Khajawall AM, Simpson GM. Peculiarities of phencyclidine urinary excretion and monitoring. J Toxicol Clin Toxicol 1982–1983;19:835–842.

161. Klein J, Karaskov T, Koren G. Clinical applications of hair testing for drugs of abuse—the Canadian experience. Forensic Sci Int 2000;107:281–288.

162. Kidwell DA. Analysis of phencyclidine and cocaine in human hair by tandem mass spectrometry. J Forensic Sci 1993;38:272–284.

163. Nakahara Y, Takahashi K, Sakamoto T, Tanaka A, Hill VA, Baumbartner WA. Hair analysis for drugs of abuse XVII. Simultaneous detection of PCP, PCHP, and PCPdiol in human hair for confirmation of PCP use. J Anal Toxicol 1997;21:356–362.

164. Poklis A, Maginn D, Barr JL. Drug findings in 'driving under the influence of drugs' cases: a problem of illicit drug use. Drug Alcohol Depend 1987;20:57–62.

165. Kunsman GW, Levine B, Costantino A, Smith ML. Phencyclidine blood concentrations in DRE cases. J Anal Toxicol 1997;21:498–502.

166. Clardy DO, Cravey RH, MacDonald BJ, Wiersema SJ, Pearce DS, Ragle JL. The phencyclidine intoxicated driver. J Anal Toxicol 1979;3:238–241.

167. Milhorn HT Jr. Phencyclidine intoxication: a case report. J Miss State Med Assoc 1990;31:37–40.

168. Patel R, Das M, Palazzolo M, Ansari A, Balasubramaniam S. Myoglobinuric acute renal failure in phencyclidine overdose: report of observations in eight cases. Ann Emerg Med 1980;9:549–553.

169. Hoogwerf B, Kern J, Bullock M, Comty CM. Phencyclidine-induced rhabdomyolysis and acute renal failure. Clin Toxicol 1979;14:47–53.

170. Stockard JJ, Werner SS, Aalbers JA, Chiappa KH. Electroencephalographic findings in phencyclidine intoxication. Arch Neurol 1976;33:200–203.

171. Yesavage JA, Freeman AM, Bourgeois ML. Time distortion in acute phencyclidine (PCP) psychosis. A correlation between 30 seconds estimation and urine drugs levels. Encéphale 1978;4:281–285.

172. Carlin AS, Grant I, Adams KM, Reed R. Is phencyclidine (PCP) abuse associated with organic mental impairment? Am J Drug Alcohol Abuse 1979;6:273–281.

173. Domino EF, Wilson AE. Effects of urine acidification on plasma and urine phencyclidine levels in overdosage. Clin Pharmacol Ther 1977;22:421–424.

174. Castellani S, Giannini AJ, Boeringa JA, Adams PM. Phencyclidine intoxication: assessment of possible antidotes. J Toxicol Clin Toxicol 1982;19:313–319.

175. Walker S, Yesavage JA, Tinklenberg JR. Acute phencyclidine (PCP) intoxication: quantitative urine levels and clinical management. Am J Psychiatry 1981;138: 674–675.

176. Giannini AJ, Eighan MS, Loiselle RH, Giannini MC. Comparison of haloperidol and chlorpromazine in the treatment of phencyclidine psychosis. J Clin Pharmacol 1984;24:202–204.

177. Sauer C, Peters FT, Staack RF, Fritschi G, Maurer HH. Metabolism and toxicological detection of a new designer drug *N*-(1-phenylcyclohexyl)propanamine, in rat urine using gas chromatography-mass spectrometry. J Chromatogr A 2008;1186:380–390.

178. Garey RE, Samuels MS, Daul GC, Heath RG, Hite SA, Minyard F, Giblin V. Phencyclidine abuse in New Orleans: medical, forensic, and laboratory aspects. Subst Alcohol Actions Misuse 1980;1:309–316.

179. Trujillo KA, Akil H. Inhibition of opiate tolerance by non-competitive *N*-methyl-ᴅ-aspartate receptor antagonists. Brain Res 1994;633:178–188.

180. Sripada S, Gaytan O, Swann A, Dafny N. The role of MK-801 in sensitization to stimulants. Brain Res Rev 2001;35: 97–114.

181. Vanderschuren LJ, Schoffelmeer AN, Mulder AH, De Vries TJ. Dizocilpine (MK801): use or abuse? Trends Pharmacol Sci 1998;19:79–81.

182. Mozayani A, Schrode P, Carter J, Danielson TJ. A multiple drug fatality involving MK-801 (dizocilpine), a mimic of phencyclidine. Forensic Sci Int 2003;133: 113–117.

183. Giannini AJ, Castellani S. A case of phenylcyclohexylpyrrolidine (PHP) intoxication treated with physostigmine. J Toxicol Clin Toxicol 1982;19:505–508.

184. Budd RD. Thin-layer chromatographic analysis of 1-(1-henylcyclohexyl)pyrrolidine in urine. J Chromatogr 1982;238:261–263.

185. Nakamura GR, Griesemer EC, Joiner LE, Noguchi TT. Determination of 1-(1-phenylcyclohexyl) pyrrolidine (PHP) in postmortem specimens: a case report. Clin Toxicol 1979;14:383–388.

186. Giannini AJ, Price WA, Loiselle RH, Malone DW. Treatment of phenylcyclohexylpyrrolidine (PHP) psychosis with haloperidol. Clin Toxicol 1985;23:185–189.

187. Hirbec H, Mausset A-L, Kamenka JM, Privat A, Vignon J. Re-evaluation of phencyclidine low-affinity or "non-NMDA" binding sites. J Neurosci Res 2002;68:305–314.

188. Zorbas M, Owens SM, Plunkett LM, Bui H. The pharmacokinetics of [3H]1-(2-thienyl)cyclohexyl]piperidine (TCP) in Sprague-Dawley rats. Drug Metab Dispos 1989;17:641–645.

189. McMillan DE, Wright DW, Wenger GR. Effects of phencyclidine-like drugs on responding under multiple fixed ratio, fixed interval schedules. Behav Pharmacol 1992;3:143–147.

190. al-Deeb OA. New analgesics derived from the phencyclidine analogue thienylcyclidine. Arzneimittelforschung 1996;46:505–508.

191. Vaupel DB, McCoun D, Cone EJ. Phencyclidine analogs and precursors: rotarod and lethal dose studies in the mouse. J Pharmacol Exp Ther 1984;230:20–27.

192. Vignon J, Chaudieu I, Allaoua H, Journod L, Javoy-Agid F, Agid Y, Chicheportiche R. Comparison of [3H] phencyclidine ([3H] PCP) and [3H] N-[1-(2-thienyl) cyclohexyl] piperidine ([3H] TCP) binding properties to rat and human brain membranes. Life Sci 1989;45: 2547–2555.

193. Sauer CK, Peters FT, Staack RF, Fritschi G, Maurer HH. New designer drugs N-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA) and N-(1-phenylcyclohexyl)-2-methoxyethanamine (PCMEA): studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. J Mass Spectrom 2008;43:305–316.

Chapter 35

VOLATILE SUBSTANCE ABUSE

HISTORY

Although inhalants were used before the 1700s and chloroform parties were fashionable in the 19th century, medical reports of volatile substance abuse in the United States first appeared in the 1950s with descriptions of adolescents in remote areas sniffing gasoline as a substitute for ethanol.[1] Volatile substance abuse spread from California to the Midwest and the East Coast; the popularity of glue sniffing increased substantially by the mid-1960s. In the late 1970s, nitrite inhalation became a popular method of enhancing sexual pleasure in male homosexuals.[2] Case reports documented fatalities following the inhalation of volatile substances during acts of atypical autoeroticism.[3] Between 1975 and 1990s, the prevalence of inhalant use by US high school students increased, but the prevalence rate stabilized in the early 2000s.[4] During the last decade, inhalant use among females increased compared with males, but inhalant use among some high-use populations (e.g., American Indian adolescents, Mexican American youth) decreased.[5]

IDENTIFYING CHARACTERISTICS

Structure

The abuse of volatile substances involves pharmacologically diverse liquid or pressurized gas products, which contain a high portion of volatile chemicals that easily vaporize at room temperature.[6] These products are rarely single compounds; the major component in the vapor phase may not be the primary constituent of the liquid. Volatile solvents are the largest group of substances associated with inhalant use. Table 35.1 lists the types of chemicals and sources of substances involved with the abuse of volatile substances.

Physiochemical Properties

The amount of a chemical inhaled during volatile substance abuse depends on several factors including the vapor pressure of the compound, the method of abuse, the physical properties of the product, and minute ventilation. Almost all of the common substances associated with inhalant abuse are lipophilic. The release of vapors from viscous material (e.g., glues) is slower than pure liquids. Pressurized liquids (e.g., butane in cigarette lighter refills) can deliver almost all of the substance with little oxygen. At room temperature, one volume of liquid propellant generates up to 200–300 volumes of vapor.[7] Table 35.2 lists the maximum airborne concentrations of some common volatile substances at room temperature.

Terminology

The National Institute on Drug Abuse defines inhalant use as the deliberate inhalation of volatile substances to induce a psychoactive or mind-altering effect.[8] Table 35.3 lists some street terms associated with volatile substance abuse.

TABLE 35.1 Classification and Sources of Volatile Substances of Abuse.[32]

Class	Common Chemical	Some Sources
Aliphatic Hydrocarbons	Propane	Bottled fuel, aerosol propellants
	Butane	Cigarette lighter fluid, aerosol propellants
Alkyl Halides	1,1,1-Trichloroethane	Correction fluid, dry cleaning products, degreasing agent
	Trichloroethylene	Dry cleaning agent, degreaser, spot remover, PVC cement, gum remover
	Trichlorofluoromethane*	Refrigerant, aerosol propellant (hairspray, spray paint, deodorants, room freshener, medical drugs)
	Bromochlorodifluoromethane	Fire extinguisher
	1,1-Difluoroethane	Dust remover
	Dichloromethane	Paint stripper
Aromatic Hydrocarbons	Gasoline†	Automobile fuel
	Toluene	Adhesives, spray paint, glues, rubber cement, paint thinner, nail polish remover
	Xylene	Wood glues, lacquer thinner
Alkyl Nitrites	Amyl nitrite ("poppers")	Vasodilator
	Butyl nitrite, isobutyl nitrite	Room air freshener
Ethers	Diethyl ether	Laboratory solvent
Esters	Ethyl acetate	Nail polish remover, industrial solvent
Ketones	Acetone	Nail polish, solvent
	Methyl ethyl ketone (MEK)	Adhesives, general solvent
	Methyl n-butyl ketone	Paints
	Methyl isobutyl ketone	Spray paint

*Freon 11 is one of several Freon compounds used as propellants including Freon 12 (dichlorodifluoromethane), Freon 22 (chlorodifluoromethane), and Freon 114 (dichlorotetrafluoroethane).
†Blended product with many aliphatic, aromatic, and cycloalkane compounds as well as additives (methyl tertiary butyl ether, methylcyclopentadienyl manganese tricarbonyl, ethanol).

TABLE 35.2. Maximum Possible Air Concentrations of Some Volatile Compounds at Room Temperature (20°C/68°F).[6]

Compound	Physical State (20°C/68°F)	Maximum Vapor Concentration (mg/L)
Toluene	Liquid	121
Tetrachloroethylene	Liquid	136
Methyl Ethyl Ketone (MEK)	Liquid	324
Trichloroethylene	Liquid	426
Acetone	Liquid	635
1,1,1-Trichloroethane	Liquid	767
Dichloromethane	Liquid	1831
n-Butane	Gas	2412
Dichlorodifluoromethane	Gas	5033
Trichlorofluoromethane	Gas	5719

EXPOSURE

Epidemiology

Intentional inhalation of volatile substances is a worldwide problem, particularly common among youth.[9] Most epidemiologic data on the use of inhalants are derived from cross-sections studies; therefore, the lack of longitudinal studies limits conclusions about the normally episodic course of inhalant use. According to the 2000 and 2001 US National Household Surveys on Drug Abuse, about 3.4% US adolescents aged 12–17 years were inhalant users in the past year during the end of the 1990s, and 0.4% of the adolescent population met the criteria for inhalant use disorder during this time.[27] During the 2000s, the number of US persons aged 12 or older who had used inhalants for the first time within the past 12 months remained relatively stable at approximately 800,000.[10]

TABLE 35.3. Terms Associated with Volatile Substance Abuse.[1]

Term	Definition
Bolt, bullet, climax, locker room, rush	Small bottles butyl nitrite
Poppers, snappers	Ampules amyl nitrite
Whippets	Balloons or small bags nitrous oxide
Texas shoe shine	Toluene-containing shoe shine spray
Torch, fire-breathing	Ignition of exhaled flammable gases

Peak inhalant use occurs at age 14–15 years with use significantly declining at age 17–19 years; the onset of inhalant use is earlier when associated with juvenile delinquency. In a study of 285 adjudicated adolescents, the median age of first-time inhalant use was 13 years.[11] In 2005, about 72% of new volatile substance users between the ages of 12 years and 49 years were under the age of 18 years; the mean age of new inhalant users was 16.1 years.[12] By 2007, the mean age of new volatile substance users increased to 17.1 years and remained near 17 years at the end of the 2000s.[10]

The prevalence of inhalant abuse in certain parts of the population are particularly high. In a study of 723 Missouri adolescents in residential care for antisocial behavior, the prevalence of lifetime computer duster spray use was 14.7%.[13] Although inhalant abuse occurs in many demographic areas (rural, suburban, urban) and among all ethnic groups, inhalant abuse is more prevalent among a geographically isolated and socio-economically disadvantaged population. Such populations include American Indian and Alaska Native youth.[5] In a study of 376 Native Alaskan children aged 10–19 years of age living in 14 isolated villages in the Bering Strait, the reported use of inhalants at least once in this group was 48%.[14] Most inhalant use in this group was sporadic and occurred in social settings; the risk of progression to inhalant substance abuse disorders is highest with the first year of use and much lower thereafter.[15]

In the first decade of 2000, the prevalence of inhalant abuse among adolescents has been relatively stable with a slight decrease in inhalant use among youth aged 12–17 years between 2003 (4.5%) and 2007 (3.8%).[16] Progression from inhalant use to abuse or dependence was associated with early first inhalant use, use of multiple inhalants, and weekly inhalant use. The prevalence of mental illness, antisocial personality, multiple drug abuse, and criminal behavior was particularly high among adolescent inhalant users;[17,18] anecdotal reports associate volatile substance abuse with a variety of criminal behaviors.[19] These reports include a substantial number of cases of recidivism.[20] In a retrospective study

of data from about 73,000 respondents to the 2002 and 2003 US National Survey on Drug Use and Health, the prevalence of inhalant abuse and inhalant dependence among all adults using inhalants in the last year was 6.6% and 1.1%, respectively.[28] This study demonstrated that approximately 1 in 10 adults use inhalants as a psychoactive drug at least once in their lives; about 0.5% of the adult population used inhalants within the last year. Compared with the adult population, the prevalence of inhalant use within the past year was increased in young adults (aged 18–25 years), alcohol abusers, lifetime drug users, Asians, white women, and men with serious mental illnesses. In the young adults (i.e., 18–25 years old), 15% of this group reported inhalant use at least once in their lifetime, and 2% of this group used inhalants in the last year. Approximately 44% of the lifetime adult inhalant users initiated inhalant use after the age of 17 years. Inhalant use disorder was more frequent in adult inhalant users aged 35–49 years, particular in individuals with mental illness, suffer from alcohol abuse, or who had a poor education. The profile of the adult inhalant user is distinct from the adolescent inhalant user. Inhalant use in adults is less often associated with criminal activity than adolescent inhalant use, and these adults tend to use fewer inhalants less often than adolescents do.

Sources

The US National Institute on Drug Abuse classifies volatile substances of abuse in the following 4 categories: 1) volatile solvents (adhesives, cleaning fluids, correction fluids, felt-tip markers, gasoline and other fuels, glues, paint thinners, paint removers, shoe polish); 2) aerosols (household products, personal hygiene products, spray paint); 3) gases (butane lighters, propane tanks, refrigerants, medical anesthetics); and 4) nitrites (amyl nitrite, butyl nitrite, cyclohexyl nitrite).[21] Based on data reported to the Toxic Exposure Surveillance System (TESS) of the American Association of Poison Control Systems from 1996–2001, the top 5 volatile substances of abuse are gasoline (41%), paint (13%), propane/butane (6%), air fresheners (6%), and formalin (5%).[22] The type of inhalant abused varies with the population and time studied. In an analysis of inhalant abuse reported to TESS from 2000–2005, the top 5 volatile substances abuse categories in order of frequency were freon/propellant (15.4%), gasoline (11.4%), paint (9.4%), automotive hydrocarbons (8%), and propane (4.5%).[23] A majority (i.e., about 60%) of past-year inhalant users report the inhalation of multiple types of inhalants.[24] Updating of US Poison Control Center data indicated that propellants, gasoline, and paint remained the most popular volatile substances of abuse with only

TABLE 35.4. Inhalants Used during the Past Year by New Inhalant Adolescent Users.[12]

Type	%
Glue, shoe polish, toluene	30.3
Gasoline, lighter fluid	24.9
Nitrous oxide ("whippets")	24.9
Spray paints	23.4
Correction fluid degreaser, cleaning fluid	18.4
Amyl nitrite ("poppers"), locker room deodorizers ("rush")	14.7
Lacquer thinner, other paint solvents	11.7
Lighter gases (butane, propane)	9.4
Anesthetics (halothane, ether, etc.)	3.4

propellants increasing in popularity.[25] A majority of the propellants were computer or electronic dusters, frequently containing 1,1-difluoroethane. Butane, propane, and air fresheners had the highest fatality rates. Some subsets of the general population prefer specific volatile substances (e.g., nitrite inhalant use by homosexuals).[26] Female adolescents often prefer spray paints, correction fluid, glue and shoe polish, whereas male adolescents frequently use lighter fluid, gasoline, and nitrous oxide.[27] Nitrous oxide and amyl nitrite are the most frequently abused inhalants in adults.[28] Solvent abuse in industry is rare among solvent workers.[29]

Table 35.4 lists the specific type of inhalants most commonly used by first-time inhalant users between the ages of 12–17 years. Typically, products preferred by inhalant abusers contain a relatively high volume of propellants (e.g., topical analgesics, deodorants, fly sprays). Unpopular sources for inhalant abuse include natural gas (methane lacks narcotic properties) and petroleum distillates (most have limited volatility) with the exception of gasoline. The formulation of household products changes over time, and chemical analysis or current material data safety sheets (MSDS) are necessary to confirm the composition of these products.

Methods of Abuse

Methods of inhaling volatile substances include sniffing, snorting, bagging, and huffing. The most common method of volatile substance abuse involves the inhalation of vapors from original containers either through the nose (*snorting*, *sniffing*) or the mouth (*huffing*), primarily in a social setting.[30] Adhesives may be poured into a plastic bag and the vapors inhaled while the top of the bag is placed over the mouth and nose. *Huffing* is also a term for the inhalation of vapors from a rag saturated with the chemical and held over the face. Another method to intensify the effects of inhalants is the placement of small amounts of the volatile substance in a paper or plastic bag and covering the mouth and nose with the bag (*bagging*). Other methods of inhalant abuse include spraying the aerosol directly in the mouth or nose, inhaling vapors from a nearby surface (collar, cuff, fingernail), inhaling air-freshener aerosols (*glading*), and placing the canister straw of aerosol computer or personal electronics cleaning products directly into the mouth or nose (*dusting*). Occasionally, the chemical product is heated (e.g., glue) to increase volatilization, bubbled through water to remove irritants (e.g., aluminum chlorohydrate in antiperspirants), or combined with ethanol prior to inhalation.

DOSE EFFECT

Typically, 10–15 inhalations of a volatile solvent produce euphoria and subsequent drowsiness within seconds to minutes.[31] The rebreathing of exhaled air (e.g., *bagging*) causes hypoxia and hypercarbia that enhance the effect of the inhaled solvent.

TOXICOKINETICS

Absorption

The absorption of most chemicals associated with volatile substance abuse is rapid. Pulmonary uptake of these chemicals depends on a variety of factors including the concentration in inspired air, the air/blood and blood/tissue partition coefficients, ventilation, pulmonary blood flow, and the distribution of fat.

Distribution

Most substances associated with volatile substance abuse are lipophilic; consequently, these compounds distribute rapidly into lipid-rich tissues (e.g., brain, bone marrow, kidney, liver). The volume of distribution of most volatile substances is large because of high lipid solubility.

Biotransformation/Elimination

Most inhaled chemicals are rapidly excreted in the breath; metabolism occurs in the liver, primarily by the cytochrome P450 isoenzymes. Typically, metabolites are more polar and less volatile than the parent compound. The biotransformation of these chemicals produces more hydrophilic compounds that diffuse less easily across membranes, but the kidney excretes the metabolites more easily than the parent compounds. Although

metabolites are usually less toxic than the parent compounds, some toxic metabolites may form from volatile substances (e.g., carbon tetrachloride, chloroform, dichloromethane, *n*-hexane). The biotransformation of volatile substances involves both phase I (oxidation, reduction, hydrolysis) and phase II (conjugation with glucuronic acid, sulfate, amino acids, or acetate) reactions. The metabolic rate depends on age, health, dose, and the presence of other drugs.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

The exact mechanism of action of volatile substances used in inhalant abuse is not well-understood. Potential mechanism of action of volatile substances other than nitrites include the generalized slowing of axonal ion channel transport by alteration of brain membranes and the hyperpolarization of gamma-aminobutyric acid (GABA) receptors.[32] In animal models, the inhibition of the *N*-methyl D-aspartate (NMDA) receptor is a common mechanism of action for several commonly abused inhalants. Prolonged exposure modulates the expression of these glutamatergic receptor subtypes depending on the experimental model, the area of the brain studied, and the dose.[33]

Mechanism of Toxicity

Volatile substances of abuse are simple asphyxiants and pulmonary irritants. The inhalation of these chemicals causes varying degrees of hypoxia as a result of the displacement of oxygen and the subsequent decrease in the partial pressure of oxygen. Pulmonary irritation may cause bronchospasm and reduced oxygen exchange. Potential causes of death during inhalant use include ventricular fibrillation, respiratory depression, asphyxia, pulmonary aspiration of stomach contents, and trauma from risky behavior.

Postmortem Examination

The postmortem changes in the examination of patients dying suddenly from volatile substance abuse are usually minimal including passive congestion of the organs, cerebral edema, and pulmonary edema.[34] Evidence of tracheitis, bronchitis, or marked pulmonary congestion may be present, but these common features are nonspecific.[35] The presence of perioral eczema or the characteristic odor of volatile substances is frequently absent at postmortem examination.

CLINICAL RESPONSE

Illicit Use

Acute intoxication with volatile substances and ethanol is similar. Desirable effects of inhalant use include the rapid onset of a pleasurable sensory experience (*high*) followed by the swift resolution of the central nervous system (CNS) effects with minimal adverse symptoms. The immediate effects of inhaling vapors during volatile substance use are similar to early stages of anesthesia with an initial stimulatory phase involving lightheadedness, excitement, disinhibition, and impulsive behavior. Central nervous system symptoms begin within a minute of inhaling the vapors, and resolve within minutes after exposure ceases. However, the *high* can be maintained for hours by continual inhalation of the vapors.[36] As the dose of the inhalant increases, slurred speech, diplopia, dizziness, ataxia, and disorientation occurs similar to ethanol intoxication. Auditory or visual hallucinations may occur following the use of high doses of inhalants; the presence of terrifying hallucinations helps distinguish inhalant use from ethanol intoxication.[36] Without repeated use of the inhalant, the euphoria fades into drowsiness, sleepiness, and headache. Coma occurs rarely during volatile substance abuse. Adverse effects include mucous membrane irritation (rhinorrhea, salivation, sneezing, cough, conjunctival erythema, nausea, vomiting, abdominal pain) and irritation of the respiratory tract (dyspnea, wheezing).

Neurologic sequelae following inhalant use are relatively rare, and the appearance of neurologic abnormalities depends on a variety of factors including the type of substance abused, duration of use, and individual susceptibility.[37] Peripheral neuropathy following chronic volatile substance abuse is usually associated with the inhalation of *n*-hexane, particularly in association with exposure to methyl *n*-butyl ketone. Cranial nerve abnormalities are rarely associated with volatile substance abuse. Chronic heavy inhalant use is associated with behavioral changes including apathy, malaise, poor school grades, poor hygiene, and changes in social activity and acquaintances along with depression, irritability, hostility, and paranoia.[38,39] Stains or paint may appear on the clothing. Complications of chronic inhalant abuse include halitosis, conspicuous odor on the breath, weight loss, chronic rhinitis or epistaxis, ulcerations of nose or mouth, perioral or paranasal dermatitis with pyoderma (huffer's rash), and tremor.[40] Occasionally, mucosal burns result from the spraying of rapid-cooling aerosols directly into the mouth.

Fatalities

The most serious risk for inhalant abusers is sudden death, and most of these deaths occur in individuals under the age of 20 years, primarily in males.[41,42] Common compounds associated with these deaths include butane, fluorinated alkanes, 1,1,1-trichloroethane, trichloroethylene, and ether. Based on data from the Toxic Exposure Surveillance System (TESS) of the American Association of Poison Control Systems, the most common substances associated with fatalities following inhalant use were gasoline (45%), air fresheners (26%), and propane/butane (11%).[22] In 2009, the annual report of the American Association of Poison Control Centers' National Poison Data System listed 4 deaths in adults from intentional inhalation of volatile substances (butane, fire extinguisher vapors, fluorochlorocarbon propellants).[43] There were no reported deaths from deliberate gasoline inhalation. The risk of sudden death is not related to chronic volatile substance abuse as about one-third of the sudden deaths associated with inhalant use occur in first-time users of these substances.

Abstinence Syndrome

In general, volatile substances are not considered physically addictive, but the effects of volatile substances are strongly self-reinforcing and the chronic use of these substances may cause psychologic addiction.[44] The progression from inhalant use to dependence is associated with early first-time use, weekly inhalant use, use of multiple inhalants, polydrug use, comorbid psychiatric disorders, and delinquent behaviors.[24] Case reports of withdrawal following chronic volatile substance abuse are uncommon.[45] In a study of 162 community-recruited inhalant users as defined by using any type of inhalant >5 times, about 11% of the participants reported some withdrawal symptoms based on structured interviews.[46] The reported symptoms included headaches, nausea or vomiting, hallucination, tearing or rhinitis, anxiety, depressed mood, trembling, fatigue, and trouble concentrating.

DIAGNOSTIC TESTING

Analytic Methods

TECHNIQUES

Methods for the detection of volatile organic compounds in postmortem samples typically involve gas chromatography after extraction with static and dynamic headspace or pulse-heating and solvent extraction.[47] The typical limit of detection (LOD) for volatile substances using headspace gas chromatography is in the range of 0.1 mg/L for biologic samples.[48] Headspace gas chromatography with split flame ionization/electron capture detection is a relative simple analytic method for the screening of biologic samples for volatile substances. The most difficult compounds to separate with this analytic method are isobutane/methanol and paraldehyde/toluene.[49] Other techniques to detect volatile substances in biologic samples include capillary gas chromatography/ Fourier transform infrared spectroscopy. The LOD for the latter method ranges from 0.05–10 mg/L depending on the structure of the analyte.[50] Detection of inhalant abuse (e.g., toluene) with traditional urinary biomarkers (e.g., hippuric acid) may require at 10-fold dilution to maintain linearity and avoid contamination with standard analytic techniques.[51]

STORAGE

Samples should be stored in gas-tight, well-sealed, inert containers with minimal headspace. Some loss of volatile organic compounds may occur by evaporation during handling, storage, or analysis of biologic samples, resulting in reduced or nondetectable concentrations of these compounds.

Biomarkers

The ability to detect volatile substances in biologic samples depends on the time since inhalation, dose, and the handling and storage of the specimen. In a study of 125 samples of suspected exposure to volatile substances, headspace gas chromatography detected the substance or the metabolite in 87% of samples from volatile substance abusers when analysis occurred within 10 hours of exposure.[38] Detection of components of blended petroleum distillates is complicated by the low concentrations of these components even after exposure to massive amounts of gasoline vapors. Routine drug screens do not detect inhalant use. Detection of metabolites is useful for only a few volatile substances (e.g., toluene, trichloroethylene, xylene).[7]

Abnormalities

The chronic inhalation of vapors from household products (e.g., paint remover, some paints) containing methylene chloride may cause increased carboxyhemoglobin concentrations depending on the concentration of methylene chloride in the headspace above the substance. Despite the abuse of large doses of these products, the carboxyhemoglobin concentrations are usually relatively low (i.e., <10%).[52] A 14-year-old boy was found unconscious by his mother.[53] On admission to the

emergency department 1½ hours later, he was drowsy, but oriented with slurred speech and some euphoria. He was placed on 100% FiO$_2$, and his carboxyhemoglobin 4 hours after being found by his mother was 13%. He had no evidence of metabolic acidosis (arterial pH = 7.38, HCO$_3$ = 23 mEq/L); he recovered without sequelae. The inhalation of nitrites is associated with methemoglobinemia that may cause serious clinical effects (hypoxia, altered consciousness). Neuropsychologic testing of chronic adolescent inhalant users suggests some deficits in executive functioning compared with drug-using controls (cannabis); however, establishment of causation is limited by methodologic issues (reporting bias, confounding, acute drug effect, classification bias).[54]

TREATMENT

Stabilization

The treatment of the adverse effects of acute inhalant intoxication is primarily supportive, and symptoms of intoxication typically resolve within ~20 minutes after exposure ceases. The initial treatment of the acutely intoxicated patient involves stabilization of any cardiac or respiratory abnormalities. Patients with depressed consciousness should be monitored for adequacy of respiration, hypoxia, and rhythm disturbances. An intravenous (IV) line should be started and the patient placed on a cardiac monitor and pulse oximeter. Hypotension should be treated with fluid replacement and vasopressors as indicated. Although there are theoretic concerns that the use of vasopressors or bronchodilators (e.g., albuterol) potentially may induce dysrhythmias in these patients, there is no direct evidence that these complications occur during the treatment of inhalant abuse. The development of cardiac dysrhythmias should be treated with conventional antidysrhythmic drugs including the use of epinephrine as indicated during cardiopulmonary resuscitation and the correction of any hypoxia or hypokalemia. Seizures are treated with benzodiazepines; these patients should be evaluated for alternate causes (e.g., head trauma, infection, co-ingestion of seizure-inducing drugs) of seizures. The elimination of volatile substances associated with inhalant use is rapid, and the persistence of symptoms more than a few hours after the cessation of use suggests an etiology other than inhalant use.

Elimination Enhancement

The metabolism of most substances involved with inhalant use is rapid; methods to enhance elimination are usually unnecessary.

Antidotes

There are no specific antidotes for inhalant use with the exception of methylene blue for methemoglobinemia associated with nitrite abuse, high flow oxygen for the carboxyhemoglobin associated with exposure to dichloromethane, and sodium bicarbonate and potassium for certain renal tubular syndromes.

Supplemental Care

INTOXICATION

Routine laboratory tests include complete blood count, serum electrolytes, blood urea nitrogen, blood glucose, serum creatinine, serum hepatic aminotransferases, urinalysis, and urine drug screen for the concomitant use of other drugs of abuse. Chest x-ray, arterial blood gases, and carboxyhemoglobin concentrations should be obtained following exposure to dichloromethane.

ADDICTION

There are limited data on the treatment of inhalant abuse. Consequently, treatment involves the application of methods used to treat other addictive disorders including multisystem and family therapy, 12-step facilitation, motivational enhancement techniques, and cognitive-behavioral therapy.[30]

References

1. Marelich GP. Volatile substance abuse. Clin Rev Allergy Immunol 1997;15:271–289.
2. Lowry TP. Psychosexual aspects of the volatile nitrites. J Psychoactive Drugs 1982;14:77–79.
3. Gowitt GT, Hanzlick RL. Atypical autoerotic deaths. Am J Forensic Med Pathol 1992;13:115–119.
4. Beauvais F. Volatile solvent abuse: trends and patterns. Subst Use Misuse 1997;32:1829–1834.
5. Beauvais F, Wayman JC, Jumper-Thurman P, Plested B, Helm H. Inhalant abuse among American Indian, Mexican American, and non-Latino white adolescents. Am J Drug Alcohol Abuse 2002;28:171–187.
6. Ramsey J, Anderson HR, Bloor K, Flanagan RJ. An introduction to the practice, prevalence and chemical toxicology of volatile substance abuse. Hum Toxicol 1989;8: 261–269.
7. Flanagan RJ, Ruprah M, Meredith TJ, Ramsey JD. An introduction to the clinical toxicology of volatile substances. Drug Saf 1990;5:359–383.
8. National Institute on Drug Abuse. Inhalant abuse (DHHS Publication No. 00-3818). Rockville, MD: US Department

of Health and Human Services, National Institutes of Health; 2000.

9. Ramon MF, Ballesteros S, Martinez-Arrieta R, Jorrecilla JM, Cabrera J. Volatile substance and other drug abuse inhalation in Spain. J Toxicol Clin Toxicol 2003;41: 931–936.

10. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of national findings. Available at http://www.oas. samhsa.gov/NSDUH/2k9NSDUH/2k9ResultsP.pdf. Accessed 2011 April 23.

11. McGarvey EL, Clavet GJ, Mason W, Waite D. Adolescent inhalant abuse: environments of use. Am J Drug Alcohol Abuse 1999;25:731–741.

12. Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2005 National Survey on Drug Use and Health: National Findings (SMA 06-4194). Rockville, MD: Substance SAMHSA Office of Applied Studies; 2006. Available at http://oas.samhsa.gov. Accessed 2011 August 10.

13. Garland EL, Howard MO. Inhalation of computer duster spray among adolescents: an emerging public health threat? Am J Drug Alcohol Abuse 2010;36:320–324.

14. Zebrowski PL, Gregory RJ. Inhalant use patterns among Eskimo school children in western Alaska. J Addict Dis 1996;15:67–77.

15. Perron BE, Howard MO, Maitra S, Vaughn MG. Prevalence, timing, and predictors of transitions from inhalant use to inhalant use disorders. Drug Alcohol Depend 2009;100: 277–284.

16. Substance Abuse and Mental Health Services Administration. National Survey on Drug Use and Health. Rockville, MD: US Health and Human Services, Office of Applied Studies. Available at http://oas.samhsa.gov/ 2k9/inhalantTrends/inhalantTrends.cfm. Accessed 2011 April 23.

17. Young SJ, Longstaffe S, Tenenbein M. Inhalant abuse and the abuse of other drugs. Am J Drug Alcohol Abuse 1999;25:371–375.

18. Wu LT, Pilowsky DJ, Schlenger WE. High prevalence of substance use disorders among adolescents who use marijuana and inhalants. Drug Alcohol Depend 2005;78: 23–32.

19. Perron BE, Howard MO. Adolescent inhalant use, abuse and dependence. Addiction 2009;104:1185–1192.

20. Flanagan RJ, Fisher DS. Volatile substance abuse and crime: data from U.K. press cuttings 1996-2007. Med Sci Law 2008;48:295–306.

21. National Institute on Drug Abuse. Inhalant abuse (NIH Publication No. 00-3818). Rockville, MD: US Department of Health and Human Services, National Institutes of Health; 2000.

22. Spiller HA. Epidemiology of volatile substance abuse (VSA) cases reported to US Poison Centers. Am J Drug Alcohol Abuse 2004;30:155–165.

23. Spiller H, Lorenz DJ. Trends in volatile substance abuse. J Addict Dis 2009;28:164–170.

24. Wu L-T, Pilowsky DJ, Schlenger WE. Inhalant abuse and dependence among adolescents in the United States. J Am Acad Child Adolesc Psychiatry 2004;43:1206–1214.

25. Marsolek MR, White NC, Litovitz TL. Inhalant abuse: monitoring trends by using poison control data, 1992–2008. Pediatrics 2010;125:906–913.

26. Colfax GN, Mansergh G, Guzman R, Vittinghoff E, Marks G, Rader M, Buchbinder S. Drug use and sexual risk behavior among gay and bisexual men who attend circuit parties: a venue-based comparison. J Acquir Immune Defic Syndr 2001;28:373–379.

27. Wu LT, Pilowsky DJ, Schlenger WE. Inhalant abuse and dependence among adolescents in the United States. J Am Acad Child Adolesc Psychiatry 2004;43:1206-1214.

28. Wu L-T, Ringwalt CL. Inhalant use and disorders among adults in the United States. Drug Alcohol Depend 2006; 85:1–11.

29. Cherry N, McArthy TB, Waldron HA. Solvent sniffing in industry. Hum Toxicol 1982;1:289–292.

30. Williams JF, Storck M, and the Committee on Substance Abuse and Committee on Native American Child Health: Inhalant abuse. Pediatrics 2007;119:1009–1017.

31. Brouette T, Anton R. Clinical review of inhalants. Am J Addict 2001;10:79–94.

32. Lorenc JD. Inhalant abuse in the pediatric population: a persistent challenge. Curr Opin Pediatr 2003;15:204–209.

33. Bowen SE, Bais JC, Paez-Martinez N, Cruz SL. The last decade of solvent research in animal models of abuse: mechanistic and behavioral studies. Neurotoxicol Teratol 2006;28:636–647.

34. Kringsholm B. Sniffing-associated deaths in Denmark. Forensic Sci Int 1980;15:215–225.

35. Shephard RT. Mechanism of sudden death associated with volatile substance abuse. Hum Toxicol 1989;8:287–292.

36. Ashton CH. Solvent abuse. BMJ 1990;300(6718):135–136.

37. Lolin Y. Chronic neurological toxicity associated with exposure to volatile substances. Hum Toxicol 1989;8: 293–300.

38. Jacobs AM, Ghodse AH. Depression in solvent abusers. Soc Sci Med 1987;24:863–866.

39. Meredith TJ, Ruprah M, Liddle A, Flanagan RJ. Diagnosis and treatment of acute poisoning with volatile substances. Hum Toxicol 1989;8:277–286.

40. Gautschi OP, Cadosch D, Zellweger R. Postural tremor induced by paint sniffing. Neurol India 2007;55:393–395.

41. Bowen SE, Daniel J, Balster RL. Deaths associated with inhalant abuse in Virginia from 1987 to 1996. Drug Alcohol Depend 1999;53:239–245.

42. Esmail A, Meyer L, Pottier A, Wright S. Deaths from volatile substance abuse in those under 18 years: results from a national epidemiological study. Arch Dis Child 1993; 69:356–360.

43. Bronstein AC, Spyker DA, Cantilena LR, Green JL, Rumack BH, Giffin SL. 2009 Annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th annual report. Clin Toxicol 2010;48:979–1178.

44. Chalmers EM. Volatile substance abuse. Med J Aust 1991;154:269–274.

45. Watson JM. Glue sniffing. Two case reports. Practitioner 1979;222:845–847.

46. Ridenour TA, Bray BC, Cottler LB. Reliability of use, abuse, and dependence of four types of inhalants in adolescents and young adults. Drug Alcohol Depend 2007;91: 40–49.

47. Wille SM, Lambert WE. Volatile substance abuse— postmortem diagnosis. Forensic Sci Int 2004;142:135–156.

48. Broussard LA. The role of the laboratory in detecting inhalant abuse. Clin Lab Sci 2000;13:205–209.

49. Streete PJ, Ruprah M, Ramsey JD, Flanagan RJ. Detection and identification of volatile substances by headspace capillary gas chromatography to aid the diagnosis of acute poisoning. Analyst 1992;117:1111–1127.

50. Ojanperä I, Hyppölä R, Vuori E. Identification of volatile organic compounds in blood by purge and trap PLOT-capillary gas chromatography coupled with Fourier transform infrared spectroscopy. Forensic Sci Int 1996;80: 201–209.

51. Charoun R, Faidi F, Hedhili A, Charbaji K, Nouaigui H, Ben Laiba M. Inhalant abuse detection and evaluation in young Tunisians. J Forensic Sci 2008;53:232–237.

52. Horowitz BZ. Carboxyhemoglobinemia caused by inhalation of methylene chloride. Am J Emerg Med 1986;4: 48–51.

53. Sturmann K, Mofenson H, Caraccio T. Methylene chloride inhalation: an unusual form of drug abuse. Ann Emerg Med 1985;14:903–905.

54. Takagi M, Yucel M, Cotton SM, Baliz Y, Tucker A, Elkins K, Lubman DI. Verbal memory, learning, and executive functioning among adolescent inhalant and cannabis users. J Stud Alcohol Drugs 2010;72:96–105.

A   Anesthetics

Chapter 36

CHLOROFORM

HISTORY

Chloroform and ether were popular euphoriants in the 19th and early 20th centuries. In 1831, Dumas first prepared chloroform, and he purified this substance by 1834. Both chloroform and ether were introduced as anesthetics in 1847. James Simpson, Professor of Midwifery at the University of Edinburgh, published a report in that year on the use of chloroform for general anesthesia.[1] He previously used ether to relieve the pain associated with childbirth, but he was dissatisfied with the technical difficulties (respiratory irritation, unpleasant odor, slow induction/prolonged recovery) and gastrointestinal effects associated with the use of ether.[2] Soon thereafter, a druggist apprentice intentionally became intoxicated by inhaling chloroform vapors, and he subsequently died.[3] Queen Victoria received chloroform for the birth of her eighth child, Prince Leopold, in 1853.[4] Fatalities and complications (cardiac dysrhythmias, hepatotoxicity) from the use of chloroform-induced general anesthesia were reported during the 19th century, resulting in the general preference for ether-induced anesthesia.[5] The first recorded death from chloroform anesthesia occurred in 1848, when a 15-year-old girl died suddenly shortly after receiving chloroform for the removal of an infected toenail. Various authors attributed her death to several factors including an overdose, fatal dysrhythmia, aspiration of food, and complications of the use of water and brandy to revive her; however, review of the data indicated that a definitive conclusion regarding the cause of death was not possible with the available data.[6] Chlorodyne was a popular narcotic that was formulated in 1846 by Dr. John Collis Browne.[4] This patent medicine contained chloroform, morphine tincture of Indian hemp (cannabis), prussic (hydrocyanic) acid, and other substances. In the US medical literature, Schneck reported the chronic inhalation of chloroform for euphoriant effects in 1945.[7] In the same year, Heilbrunn et al collected 29 cases from the medical literature dating back to 1830; he suggested that chloroform addiction resembled chronic alcoholism.[8] Clinical effects reportedly associated with chloroform addiction included emaciation, depression, anxiety, irritability, delusions, hallucinations, tremors, and paresthesias.

IDENTIFYING CHARACTERISTICS

Chloroform is a simple trihalogenated compound (Figure 36.1). This compound is a colorless, volatile liquid with a sweet, burning taste and characteristic odor. Chloroform (CAS RN: 67-66-3, trichloromethane) possesses moderately high vapor pressure, high lipid solubility, and a slight, but comparatively greater, solubility in water compared with other haloalkanes. Table 36.1 displays some physiochemical properties of chloroform. This compound also has irritant effects on the skin, conjunctiva, and mucous membranes.

EXPOSURE

The abuse of chloroform is relatively rare. In some alcohol abusers, the habitual use of chloroform is a substitute for ethanol. Chloroform abusers equate sniffing chloroform with a stiff drink of whiskey or paraldehyde, and the use of chloroform is associated with feelings of tranquility and exhilaration, a sense of well-being ("world seems rosier"), and an inclination to daydream.[7]
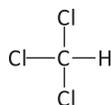
FIGURE 36.1. Chemical structure of chloroform.

TABLE 36.1. Physiochemical Properties of Chloroform.

Parameter	Value
Density	1.48 g/mol
Water Solubility	7.5–9.3 g /L (20°C/68°F)
Viscosity	5.63 millipoises (20°C/68°F)
Boiling Point	61.2°C/142.2°F
Odor Threshold	50–200 ppm
Vapor Pressure	159.6 mm Hg (20°C/68°F)
	199 mm Hg (25°C/77°F)
Vapor Density	4.1 (Air = 1)
Surface Tension	27.1 dynes/cm (20°C/68°F)
Relative Evaporation Rate	11.6 (Butylacetate = 1)
Flash Point	None
Air Partition Coefficient	
Blood	10.3
Water	3.5
Oil	401

With continued chloroform use, drowsiness, irritability, and eventually sleep occur.

DOSE EFFECT

There are few data on plasma chloroform concentrations during the abuse of chloroform. Vertigo, fatigue, and nausea develop in volunteers following inhalation of 1,000 ppm chloroform for several hours; dizziness and salivation occur after exposure to approximately 1,500 ppm for several minutes. Lightheadedness, near syncope, and vomiting appear soon after exposure to 4,000 ppm chloroform. Concentrations of 10,000–20,000 ppm produce anesthesia, but higher levels are associated with an increased incidence of cardiac dysrhythmias and hypotension.[9] In patients breathing spontaneously, 2–2.5% chloroform is necessary for adequate surgical anaesthesia.[10]

TOXICOKINETICS

Absorption

The uptake of chloroform by the pulmonary vasculature depends on the ventilatory rate and the solubility of chloroform (i.e., blood–gas partition coefficient) as well as the inspired chloroform concentration. In a study of 16 patients undergoing surgical anesthesia with chloro-

form, the initial uptake of chloroform was rapid with a plateau after 40–50 minutes.[10] The arterial concentration reached approximately 25% of the inspired concentration in a patient breathing spontaneously after ~1 hour of exposure.

Distribution

Absorbed chloroform rapidly distributes into the tissues based on lipid content and on the blood partition coefficients of individual tissues. The highest concentrations of chloroform appear (i.e., highest first) in fat, brain, kidney, and blood.[11] Hydrophobic sites on blood proteins (e.g. hemoglobin) are probably the main carriers of circulating chloroform.[12]

Biotransformation/Elimination

The oxidative cytochrome P450 pathway in the liver is the principal site of chloroform metabolism. The major end products of chloroform metabolism are carbon dioxide and chloride ion with phosgene identified as an intermediate precursor metabolite from *in vitro* studies via spontaneous dehydrochlorination of trichloromethanol.[13] The primary cytochrome P450 isoenzymes involved with the metabolism of chloroform at lower concentrations are CYP2E1 and to a lesser extent by CYP2A6. The importance of the latter isoenzyme increases following the saturation of CYP2E1-mediated reactions.[14] The biotransformation of chloroform at high concentrations also involves CYP2B1.[15]

Detoxification of phosgene occurs either via reaction with water to produce carbon dioxide or via conjugation with thiols (e.g., glutathione, cysteine). Human studies indicate that metabolism is dose dependent. The elimination of chloroform is rapid.[10] Figure 36.2 displays the elimination of chloroform in 8 patients receiving chloroform anesthesia.

Maternal and Fetal Kinetics

There are few humans data on maternal or fetal kinetics of chloroform. In animal studies, chloroform diffuses across the placental barrier, but fetal uptake is low, primarily as a result of the low fat content in the fetus.[16] Based on these animal studies, there is minimal accumulation of chloroform in the fetus.

Drug Interactions

There are few data in humans on drug interactions during chloroform abuse. The simultaneous exposure of chloroform and central nervous system (CNS) depressants would be expected to cause additive or synergistic effects.
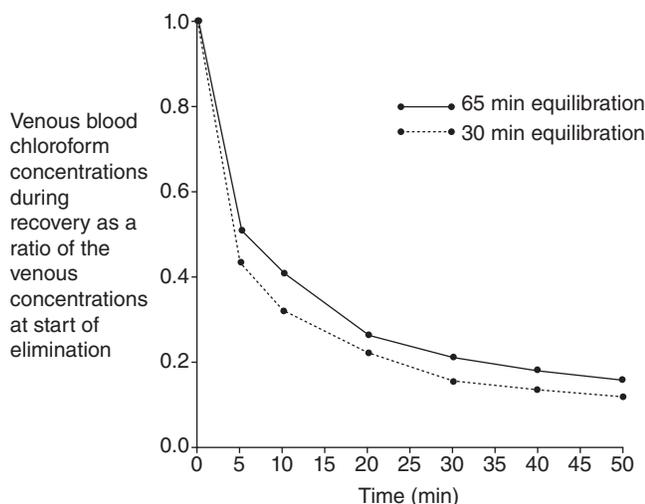
FIGURE 36.2. Elimination of chloroform from central venous blood in 4 patients after receiving 30 minutes of chloroform anesthesia (broken line) and in another 4 patients after receiving 65 minutes of chloroform anesthesia (solid line) as a ratio to the central venous blood at the start of elimination. (Reprinted from N Poobalasingham, JP Payne, The uptake and elimination of chloroform in man, British Journal of Anaesthesia, 1978, Vol. 50, p. 327, by permission of Oxford University Press.)

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

The major target organs of chloroform toxicity are the liver and kidney based on animal studies. The hepatotoxicity of chloroform probably results from the cytochrome P450-dependent production of reactive metabolites (e.g., phosgene) that covalently bind to cellular lipids and proteins.[17,18] At the high chloroform concentrations present during abuse, this compound is a central nervous system (CNS) depressant that may cause respiratory arrest and subsequent hypoxia. Similar to other volatile anesthetic agents, chloroform produces vascular dilatation both by direct effects on the vessels and by depression of the vasomotor center.[9]

Postmortem Examination

Typical pathologic findings during autopsies include submucosal hemorrhage, hyperemia and erosion of the trachea, esophagus, and stomach along with marked congestion of various organs including the lungs and brain. A 39-year-old woman with a history of inhaling chloroform monthly for 7 years died shortly after the inhalation of chloroform from a saturated towel.[19]

Postmortem examination demonstrated a few petechiae in the conjunctiva, moderate submucosal hemorrhage in the trachea and esophagus, mild to moderate edema of the lungs with alveolar hemorrhage, moderate vacuolar degradation of the endocardium, and moderate focal fragmentation of the myocardium. Schmorl staining demonstrated large numbers of lipofuscin pigments in the myocardium and focal lipofuscin pigmentation around the central veins of the liver. A 33-year-old man died of diphtheria after a 12-year addiction to chloroform.[8] The postmortem examination demonstrated moderate pathologic changes in the putamen (lipofuscin in a reduced population of large ganglion cells) and cerebellum (reduced Purkinje cells with poor staining). The autopsy of an elderly woman found dead after inhaling chloroform demonstrated marked pulmonary edema and extensive contraction band necrosis of the cardiac muscles.[20]

CLINICAL RESPONSE

Chloroform is a CNS depressant and anesthetic that causes respiratory depression, altered consciousness, coma, and dysrhythmias. A 23-year-old chemistry student was found unresponsive with respiratory distress.[21] A bottle of laboratory-grade chloroform was next to his bed. In the emergency department, he was reactive only to deep painful stimuli, mitotic, mildly acidotic (pH = 7.26), and hypoxemic (pO$_2$ = 51 mm Hg on 70% O$_2$). His respiratory insufficiency necessitated intubation and ventilatory support. He was extubated on the second hospital day; subsequently, he developed nausea, anorexia, jaundice, and mild intravascular hemolysis (serum free haptoglobin = 29.9 mg/dL with normal <10 mg/dL). Large ingestions of chloroform are associated with hepatotoxicity manifest by elevated serum hepatic aminotransferases, hyperbilirubinemia, jaundice, and hypoprothrombinemia with peak effects occurring about 3–6 days after exposure.[22,23] Chloroform is an irritant of the gastrointestinal tract that causes chest pain, abdominal pain, nausea, and vomiting after ingestion.

Withdrawal symptoms following chronic chloroform abuse are occasionally reported in the medical literature. An emaciated 33-year-old man with chloroform addiction (1–2 ounces daily for 12 years) developed a seizure, hallucinations, and paranoia 3 days after cessation of chloroform use in the hospital.[8] Additionally, he had pronounced cerebellar signs (dysarthria, ataxia, coarse intention tremor, positive Romberg's sign, nystagmus, past-pointing). These cerebellar signs gradually diminished over 6 weeks, but he died of diphtheria.

DIAGNOSTIC TESTING

Analytic Methods

Analytic techniques for the determination of chloroform are similar to other volatile drugs of abuse including headspace gas chromatography with flame ionization detection[24] and headspace solid-phase microextraction followed by gas chromatography/mass spectrometry (GC/MS).[25] Potential causes of perimortem and postmortem loss of chloroform from the body include prolonged resuscitation, improper storage in poorly sealed containers, and long postmortem interval. Chloroform concentrations rapidly decline during storage at room temperature. Over 1 week, chloroform concentrations in glass containers decreased over 80% compared with about 10% at 4°C (39.2°F) and approximately 2% at –20°C (–4°F) as measured by headspace GC/MS.[26] By 1 month, little chloroform remained in the glass bottles stored at room temperature, whereas approximately 60% of the original chloroform concentration remained in the glass vials stored at 4°C (39.2°F). Over approximately 1 year, ~58–65% of the chloroform in glass bottles stored at 4°C (39.2°F) was lost.[27]

Biomarkers

Experimental studies indicate that chloroform may be detected in formalin-fixed tissues at least 14 days after death. A 14-day study of rabbits compared the chloroform concentrations in tissues fixed in formalin immediately after death with chloroform concentrations in unpreserved tissues collected at the same time.[28] Two days after death from a mixture of ether, chloroform, toluene, and ethanol, the chloroform concentrations in formalin-fixed brain and liver tissues were 54% and 73%, respectively, of the chloroform concentrations in unpreserved samples analyzed immediately postmortem. Over the next 12 days, the concentrations of chloroform in the formalin-fixed brain and liver tissues declined to 22% and 17%, respectively, of the concentrations in unpreserved tissues. *In vitro* studies of human erythrocytes exposed to 500 ppm chloroform for 2 hours suggest that the average whole blood/plasma ratio of chloroform is about 3.[12] There are few data on serum chloroform concentrations following exposure. A comatose 19-year-old man with stable vital signs survived the ingestion of 75 mL chloroform after intubation and treatment with *N*-acetylcysteine. He developed mild hepatocellular toxicity; his serum chloroform concentration on admission was 91 mg/L.[22]

BLOOD

ILLICIT USE.   Based on the blood/gas partition coefficient of chloroform, the chloroform concentration at equilibrium after inhaling 1% chloroform is 367 mg/L.[10] In patients undergoing surgical anesthesia with chloroform, the mean chloroform concentration in arterial samples from patients receiving 1 hour of 1% chloroform via artificial ventilation was about 150 mg/L compared with approximately 230 mg/L for surgical patients spontaneously breathing 2–2.5% chloroform. Patients regained consciousness when the chloroform concentrations in venous samples were between 20–40 mg/L.

OVERDOSE.   Ten hours after accidental ingestion, the chloroform concentration in a blood sample from a 19-year-old adolescent was 200 mg/L.[29] At that time, the patient was comatose and intubated for respiratory insufficiency.

POSTMORTEM.   A 26-year-old man was found dead with evidence of the use of chloroform during autoerotic activities.[25] Postmortem heart blood, adipose tissue, and brain specimens contained chloroform concentrations of 150.6 mg/L, 79.4 mg/L, and 107.4 mg/L as determined by headspace solid-phase microextraction and GC/MS. A 39-year-old woman had a cardiopulmonary arrest shortly after inhaling chloroform vapors, and she was pronounced dead after 30 minutes of cardiopulmonary resuscitation.[19] The heart blood drawn about 9 hours after her death contained a chloroform concentration of 32.5 ± 0.9 mg/L. Analysis of heart blood drawn 30 hours and 36 hours after the suicides of an elderly couple demonstrated chloroform concentrations of 29.1 mg/L and 41.4 mg/L, respectively, as measured by headspace gas chromatography with flame ionization detection.[20] Postmortem blood from this couple did not contain clinically significant concentrations of any other drugs.

Abnormalities

Abnormalities of hepatic and renal function may occur following exposure to high concentrations of chloroform. Serum hepatic transaminases peak approximately 3–4 days following exposure and return to normal within 6–8 weeks.[30] In general, the alteration of serum hepatic aminotransferase concentrations and prothrombin times are greater than changes in serum bilirubin or alkaline phosphatase concentrations.

TREATMENT

Respiratory depression and cardiac dysrhythmias represent the immediate life-threatening complications of

chloroform exposure; immediate attention should be directed towards the maintenance of ventilation and blood pressure with intubation, mechanical ventilation, intravenous (IV) fluids, and cardiac monitoring as indicated by history and physical examination.[30] Methods to enhance elimination of chloroform have not been studied. Although no antidotes have been studied for the treatment of chloroform intoxication, the depletion of glutathione stores suggest that *N*-acetylcysteine may be effective similar to poisoning with carbon tetrachloride. However, there are limited clinical data at the present time to support the use of *N*-acetylcysteine in this situation. A 19-year-old man survived the ingestion of 75 mL chloroform without developing severe liver failure after treatment with standard IV doses of *N*-acetylcysteine (150 mg/kg over 1 hour, then 50 mg/kg over 4 hours followed by an IV drip at 6.25 mg/kg/h until a decline in serum hepatic aminotransferases).[22] If severe hepatorenal dysfunction develops, supportive care includes the treatment of renal failure with dialysis and hepatic failure with fresh frozen plasma, vitamin K, low protein diet, neomycin, lactulose, and careful fluid and electrolyte balance. Hepatic and renal function should be followed daily for at least 3 days after serious exposure to chloroform.

References

1. Simpson JY. A new anesthetic agent, more efficient than sulphuric ether. Lancet 1847;ii:549–550.
2. Payne JP. Chloroform in clinical anaesthesia. Br J Anaesth 1981;53(suppl):S11–S15.
3. Anonymous. Another death from chloroform. Lancet 1848;i:218–219.
4. Silberman HC. The many aspects of chlorodyne. Part 1: the years 1846-1884. Pharm Hist (London) 2006;36: 27–31.
5. Colon GA. October 1848 and 1898. J La State Med Soc 1998;150:463–464.
6. Knight PR III, Bacon DR. An unexplained death: Hannah Greener and chloroform. Anesthesiology 2002;96: 1250–1253.
7. Schneck JM. Chloroform habituation. Bull Menninger Clin 1945;9:12–17.
8. Heilbrunn G, Liebert E, Szanto PB. Chronic chloroform poisoning. Clinical and pathologic report of a case. Arch Neurol Psychiatr (Chicago) 1945;53:68–72.
9. Davison MH. Chloroform. Br J Anaesth 1965;37: 655–660.
10. Poobalasingham N, Payne JP. The uptake and elimination of chloroform in man. Br J Anaesth 1978;50:325–329.
11. International Programme on Chemical Safety. Environmental Health Criteria 163 chloroform. Geneva: World Health Organization; 1994.
12. Lam C-W, Galen TJ, Boyd JF, Pierson DL. Mechanism of transport and distribution of organic solvents in blood. Toxicol Appl Pharmacol 1990;104:117–129.
13. Pohl LR, Bhooshan B, Whitaker NF, Krishna G. Phosgene: a metabolite of chloroform. Biochem Biophys Res Commun 1977;79:684–691.
14. Gemma S, Vittozzi L, Testai E. Metabolism of chloroform in the human liver and identification of the competent P450s. Drug Metab Dispos 2003;31:266–274.
15. Meek ME, Beauchamp R, Long G, Moir D, Turner L, Walker M. Chloroform: exposure estimation, hazard characterization, and exposure-response analysis. J Toxicol Environ Health 2002 (Part B);5:283–334.
16. Danielsson BR, Ghantous H, Dencker L. Distribution of chloroform and methyl chloroform and their metabolites in pregnant mice. Biol Res Pregnancy Perinatol 1986;7: 77–83.
17. Kenna JG, Jones RM. The organ toxicity of inhaled anesthetics. Anesth Analg 1995;81(suppl 6):S51–S61.
18. MacDonald TL. Chemical mechanisms of halocarbon metabolism. Crit Rev Toxicol 1983;11:85–120.
19. Harada K, Ichiyama T, Ikeda H, Ishihara T, Yoshida K-I. An autopsy case of acute chloroform intoxication after intermittent inhalation for years. Jpn J Leg Med 1997; 51:319–323.
20. Ago M, Hayashi T, Ago K, Ogata M. Two fatalities associated with chloroform inhalation. Variation of toxicological and pathological findings. Leg Med 2011;13:156–160.
21. Hutchens KS, Kung M. "Experimentation" with chloroform. Am J Med 1985;78:715–718.
22. Schroeder GH. Acute and delayed chloroform poisoning. Br J Anaesthesiol 1965;37:972–975.
23. Dell'Aglio DM, Sutter ME, Schwartz MD, Koch DD, Algren DA, Morgan BW. Acute chloroform ingestion successfully treated with intravenously administered *N*-acetylcysteine. J Med Toxicol 2010;6:143–146.
24. Singer PP, Jones GR. An unusual autoerotic fatality associated with chloroform inhalation. J Anal Toxicol 2006;30:216–218.
25. Musshoff F, Padosch SA, Kroener LA, Madea B. Accidental autoerotic death by volatile substance abuse or nonsexually motivated accidents? Am J Forensic Med Pathol 2006;27:188–192.
26. Riße M, Erdmann F, Schutz H, Weiler G. Multiple homicides as a result of chloroform poisoning: case report and experimental study. Forensic Sci Int 2001;124:209–213.
27. Allan AR, Blackmore RC, Toseland PA. A chloroform inhalation fatality—an unusual asphyxiation. Med Sci Law 1988;28:120–122.
28. Takayasu T, Saito K, Nishigami J, Ohshima T, Nagano T. Toxicological analysis of drugs and poisons in formalin-fixed organ tissues 2. Volatile substances. Int J Leg Med 1994;107:7–12.
29. Storms WW. Chloroform parties. JAMA 1973;225:160.
30. Rao KN, Virji MA, Moraca MA, Diven WF, Martin TG, Schneider SM. Role of serum markers for liver function and liver regeneration in the management of chloroform poisoning. J Anal Toxicol 1993;17:99–102.

Chapter 37

ETHERS

DIETHYL ETHER

HISTORY

The effects of diethyl ether (ether) on the level of consciousness were probably known for centuries before the first documented use of ether in anesthesia in the middle of the 19th century. Paracelsus observed the anesthetic effects of ether on chickens in the 16th century with no apparent harm.[1] The exhilarating effects of inhaled diethyl ether vapors ("ether frolics") have been known since soon after the synthesis of ether in the early 19th century.[2] After Faraday demonstrated that diethyl ether had similar anesthetic properties as nitrous oxide in 1815, ether-parties (ether-jags) became popular both in Europe and in the United States. In the 1830s, ladies in Philadelphia would vaporize a little ether in a bladder; for amusement, they would then inhale the vapors from a tube with a stopcock.[3] Dr. Crawford W. Long used diethyl ether during the first surgical anesthetic procedure in 1842.[4] Shortly thereafter, physicians began dispensing diethyl ether in water as a nonalcoholic libation. In Germany, Hoffmann's drops (1 part diethyl ether to 3 parts alcohol) were a treatment for feeling depressed, whereas *perles d'éther* gelatin capsules were a "morning pick-me-up" for French ladies in high position. Before 1846, diethyl ether was widely available from dealers in pharmaceuticals; recognized effects of this compound included exhilaration and dulling of the senses. Morton first successfully demonstrated diethyl ether anesthesia in the Bullfinch Operating Theater at the Massachusetts General Hospital in October 1846.[5] The use of diethyl ether anesthesia quickly spread across the United States and to the United Kingdom.[6,7] Ether anesthesia typically was delivered by dropping the liquid from a suitable bottle unto a wire frame covered with gauze (Schimmelbusch mask). Later advances included vaporization of ether in a glass container by the flow of air or oxygen (draw-over vaporizer). The major problem with these devices was the difficulty delivering a constant amount of anesthetic because vaporization cooled the liquid (latent heat of vaporization). In general, diethyl ether anesthesia was not widely accepted because of the anesthesia risks resulting in part from the need for frequent adjustments of the anesthetic and religious beliefs that pain resulted from Divine Will as a means to understand suffering, insight, meaning, and salvation.

As the illegal distillation of alcoholic spirits (*poteen*) became more difficult in Ireland, diethyl ether drinking became a substitute for alcoholic beverages in the latter half of the 19th century.[8] The immediate effects of diethyl ether were considered similar and more rapid than ethanol.[9] Adverse effects were also similar including hangover (headache, nausea, malaise), cough, mid-epigastric distress, and gastrointestinal reflux. In 1890, the British government classified diethyl ether as a poison, and the sale of diethyl ether was severely limited. More sophisticated diethyl ether drinkers switched to morphine (morphia), which was more readily available and did not have the unpleasant smell associated with the use of diethyl ether. However, diethyl ether remained popular as an alternative to alcohol in lower economic areas of Ireland, East Prussia, Norway, Russia, France, and Hungary.[3] Diethyl ether dependence was first reported in the latter 19th century; the abuse of diethyl ether remains an uncommon form of volatile substance abuse.[10]

H₃C⌒O⌒CH₃
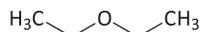
FIGURE 37.1 Chemical structure of diethyl ether.

TABLE 37.1. Some Physiochemical Properties of Diethyl Ether.

Physical Property	Value
Melting Point	–116°C (–178.6°F)
Boiling Point	34.5°C (94.1°F)
log P (Octanol-Water)	0.89
Water Solubility	6.04E + 04 mg/L (25°C/77°F)
Vapor Pressure	538 mm Hg (25°C/77°F)

IDENTIFYING CHARACTERISTICS

Diethyl ether (CAS RN: 60-29-7, ether) is an oxygenated hydrocarbon (Figure 37.1). Diethyl ether is a highly flammable, volatile liquid at room temperature that is relatively insoluble in water. The molecular formula is $C_4H_{10}O$ and molecular weight is 74.12 g/mol. This chemical is not readily ionizable (pKa = –3.59). Table 37.1 lists some of the physiochemical properties of diethyl ether.

The relative high blood/gas solubility of diethyl ether results in slow induction and prolonged recovery from anesthesia. The potency of diethyl ether is relatively low compared with modern inhaled anesthetics; additionally, diethyl ether is a respiratory irritant.

EXPOSURE

The abuse of diethyl ether is relatively rare, and the intentional inhalation of this ether for euphoriant effects usually occurs in the setting of polydrug use (e.g., ethanol). Methods of abuse include both the inhalation of vapors and ingestion. Diethyl ether abuse remains a rare cause of death.[11] Case reports document the inhalation of diethyl ether vapors by soaking a pad with ether and holding the ether-soaked pad by the knees while lying in bed.[12] Over the course of a few hours, the vapors are inhaled until the user becomes unconscious. After sleeping several hours, the user awakens with a sense of elation and hypomania. This phase is followed by a depressive phase that is associated with agitation, irritability, and restlessness. This process is repeated numerous times during the day.

DOSE EFFECT

Following chronic abuse of diethyl ether, daily use of this ether may increase to 0.5–1 liter.[10] In animal studies (e.g., dogs), the administration of 2–2.5 times the

minimum alveolar concentration (MAC) of diethyl ether causes apnea.[13] The mean MAC for this ether in a study of rabbits was about 2.7% ± 0.2%.

TOXICOKINETICS

The absorption of diethyl ether is relatively rapid, and this compound is distributed quickly to the tissues. Hydrophobic sites on blood proteins (e.g., hemoglobin) are probably the main carriers of this ether in the blood.[14] Elimination is also relatively rapid with the lungs excreting the vast majority (i.e., >90%) of an absorbed dose into expired air unchanged. The primary metabolic pathway probably involves the cleavage of ether to acetaldehyde and ethanol followed by oxidation to acetate via cytochrome P450 isoenzymes.[15,16] The formation of acetaldehyde varies substantially between individuals.[17] The urine contains minor amounts of unchanged diethyl ether. The use of escalating doses of diethyl ether during volatile substance abuse indicates the presence of tolerance in the chronic ether abuser.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Diethyl ether is a central nervous system (CNS) depressant similar to other inhaled anesthetics. Experiments in animals suggest that the acute intoxication produced by abused volatile inhalants (e.g., diethyl ether, toluene, 1,1,1-trichloroethane, halothane) and ethanol is similar. Ether is a relatively safe anesthetic because respiratory depression occurs before serious cardiac depression; subsequently, the depth of anesthesia decreases with progressive respiratory depression without supplemental respiratory support.[18] In mice, subanesthetic concentrations of diethyl ether cause ethanol-like discriminative stimulus effects greater than other common anesthetics (i.e., desflurane, isoflurane, enflurane).[19] Animal studies also indicate that diethyl ether activates the hypothalamic–pituitary–adrenal axis similar to physical stressors (e.g., cold, electric shock); thus, ether is used as a research tool to study hormonal parameters associated with stress.[20]

Postmortem Examination

Similar to other volatile substances of abuse, case reports associate the chronic abuse of diethyl ether with fatalities. Typically, these cases involve an individual found dead with evidence of volatile substance abuse.[21] The postmortem findings are nonspecific including

passive congestion of the organs, pulmonary edema, and cerebral edema.

CLINICAL RESPONSE

The high solubility of ether in the blood and tissues results in a slow induction and prolonged recovery from anesthesia compared with newer anesthetics. Clinical effects of diethyl ether intoxication include euphoria, blurred vision, ataxia, lightheadedness, visual illusions, and hallucination. Adverse effects following the intentional inhalation of ether include headache, nausea, and cough. The odor of diethyl ether is commonly present during the chronic use of this compound. Although the classical effects of addiction develop during the chronic abuse of diethyl ether, physical withdrawal symptoms do not usually occur following the cessation of ether use.

DIAGNOSTIC TESTING

Analytic Methods

Techniques to quantitate diethyl ether concentrations in blood include gas chromatography (head space or direct injection)[22] and headspace capillary gas chromatography/mass spectrometry.[23] The limit of detection and lower limit of quantitation for diethyl ether using the latter method are ~2 mg/L and 7 mg/L, respectively. In a stoppered grey-top tube (1% sodium fluoride, 0.2% potassium oxalate) stored at 3°C (37.4°F), diethyl ether concentrations of 100–5,000 mg/L remained stable in blood samples for 2 months.[24]

Biomarkers

Experimental studies indicate that diethyl ether may be detected in formalin-fixed tissues at least 14 days after death. A 14-day study of rabbits compared the concentrations of ether in tissues fixed in formalin immediately after death with concentrations in unpreserved tissues collected at the same time.[25] One day after death from a mixture of diethyl ether, chloroform, toluene, and ethanol, the diethyl ether concentrations in formalin-fixed brain and liver tissues were 22% and 13%, respectively, of the concentrations in unpreserved samples analyzed immediately postmortem. Over the next 13 days, the concentrations of diethyl ether in the formalin-fixed brain and liver tissues declined only slightly compared with the diethyl ether concentrations in the unpreserved tissues. *In vitro* studies of human erythrocytes exposed to 500 ppm diethyl ether for 2 hours indicate that the average whole blood/plasma ratio of diethyl ether is about 1.8.[14]

Typical plasma diethyl ether concentrations during surgical anesthesia range between 500–1,500 mg/L with deep surgical anesthesia occurring at ~1,200 mg/L.[26] Analgesia is associated with plasma diethyl ether concentrations in the range 100–500 mg/L. A 20-year-old man was found unresponsive in his dormitory room with 2 black plastic trash bags secured over his head by a rubber band.[27] Two saturated rags and a clear resealable bag containing diethyl ether were found inside the black trash bags. The postmortem heart blood and peripheral blood contained diethyl ether concentrations of 319 mg/L and 304 mg/L, respectively. A 49-year-old was found dead with a plastic bag over his head and a towel soaked with ether inside the plastic bag.[28] The postmortem blood diethyl ether concentration was 1,277 mg/L.

Driving

Experimental studies indicate that evidential breath analyzers detect only very low concentrations of diethyl ether, depending on the type of analyzer. Interference of diethyl ether with ethanol determination occurs primarily with infrared analyzers exclusively using the 9.5-μm region.[29] A simulated test of the inhalation of 0.044 mg diethyl ether/L was associated with a 4% increase in the estimated breath ethanol concentration.[30]

TREATMENT

Similar to other inhaled anesthetics, respiratory depression and cardiac dysrhythmias potentially represent the most immediate life-threatening complications of diethyl ether exposure; immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, intravenous fluids, and cardiac monitoring as indicated by the history and physical examination. The lungs excrete most of the absorbed dose of diethyl ether unchanged; consequently, there are no methods to enhance elimination.

DIMETHYL ETHER

HISTORY

In 1867, Richardson investigated the anesthetic properties of dimethyl ether in rabbits and pigeons; later he successfully used this compound as an anesthetic agent in humans.[31] Although he considered dimethyl ether an

effective anesthetic agent, this compound was never accepted as a reliable anesthetic drug. In the early 1920s, Brown obtained adequate anesthesia with 65% dimethyl ether in cats, and the administration of 85% dimethyl ether produced profound anesthesia with respiratory arrest.[32] Undesirable properties of dimethyl ether included a feeling of suffocation during induction, excessive salivation, and persistent muscle activity during anesthesia. In 1925, Davidson reported the effects of dimethyl ether anesthesia in humans.[33] The administration of 20% dimethyl ether produced unconsciousness within 17 minutes, but prolonged recovery from the depressant effects of the anesthesia occurred after cessation of administration.

IDENTIFYING CHARACTERISTICS

Dimethyl ether (CAS RN: 115-10-6, methyl ether, MW 46.07 g/mol) is a colorless, inflammable, water-soluble gas with an unpleasant odor resembling chloroform. The molecular formula of dimethyl ether is C_2H_6O; the chemical structure is displayed in Figure 37.2. Table 37.2 lists some physiochemical properties of dimethyl ether. This compound is used as a liquid refrigerant, solvent, aerosol dispersant, rocket fuel, and method to rapidly freeze meat and fish. Dimethyl ether was considered as a substitute for liquid petroleum gas because the physical properties of these 2 compounds are similar. In experimental studies, dimethyl ether produces an explosion more intense than propane with a detonation concentration of 5.5–9.0%.[34]

EXPOSURE

Dimethyl ether is a compound in common household aerosol, spray, and liquid products including arts and

$$H_3C \diagdown O \diagup CH_3$$
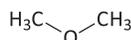
FIGURE 37.2. Chemical structure of dimethyl ether.

TABLE 37.2. Some Physiochemical Properties of Dimethyl Ether.

Physical Property	Value
Melting Point	−142°C (−223.6°F)
Boiling Point	−24.8°C (−12.64°F)
Vapor Density	1.617 (Air = 1)
log P (Octanol-Water)	0.1
Water Solubility	4.60E + 04 mg/L (25°C/77°F)
Vapor Pressure	4450 mm Hg (25°C/77°F)

crafts, auto products, home maintenance, home office, and personal care products (see Table 37.3).

DOSE EFFECT

There are few data on the dose response of dimethyl ether. Older medical studies suggest that the acute toxicity of dimethyl ether is relatively low. In mice, the median lethal concentration (LC_{50}) following 15-minute exposure was 494 ppm.[35] Studies in cats indicate that 65% dimethyl ether produces anesthesia, whereas the administration of 85% causes profound anesthesia with gradual cessation of respiration.[32] The administration of 50% dimethyl ether to volunteers produced unpleasant side effects including a feeling of suffocation.

TOXICOKINETICS

There are limited data on the toxicokinetics of dimethyl ether.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

There are few data on the toxicity of dimethyl ether. In a rodent study, exposure of Wistar rats to 2% v/v ether 6 hours daily for 5 days a week for 30 weeks produced some elevation of serum hepatic alanine aminotransferase as well as a reduction of liver weight compared with unexposed controls.[36] Histologic examination of the organs including the liver demonstrated no abnormalities.

CLINICAL RESPONSE

Dimethyl ether is a CNS depressant and asphyxiant that potentially causes hypoxia following high acute exposure. Although there are few clinical data on the effects of dimethyl ether, potential serious effects include respiratory insufficiency and altered consciousness. There are no human data on dysrhythmias associated with acute dimethyl ether exposure.

DIAGNOSTIC TESTING

Headspace gas chromatography with split flame ionization/electron capture detection is a simple method for screening a wide range of volatile substances in biologic fluids including dimethyl ether.[37] An initial column temperature of 40°C (104°F) followed by programming to 200°C (392°F) allows the differentiation of dimethyl ether from other low-boiling chemicals (e.g., butane, propane, bromochlorodifluoromethane).

TABLE 37.3. Some Household Products Containing Dimethyl Ether.

Brand (Formulation Date)	Form	%
Armor All Detailers Advantage Tire Foam 18 Oz.	liquid	1–5
Armor All Tire Foam Protectant (07/01/1998)	aerosol	1–5
Armor All Tire Foam Protectant (9/07/2000)	aerosol	1–5
Armor All Tire Foam, Aerosol (10/01/2007)	aerosol	1–5
DAP Tex Insulating Foam Sealant	aerosol	1.0–5.0
Duro All Purpose Spray Adhesive	aerosol	50–55
Elmers Spray Adhesive	aerosol	5–10
Farnam Cologne & Deodorant for Pets	aerosol	31
Farnam Cologne & Deodorant for Pets (07/18/2007)	liquid	31
Gaps and Cracks Foam (09/26/2002)	aerosol foam	10–30
Great Stuff Window and Door (09/19/2002)	aerosol foam	10–30
Great Stuff (09/19/2002)	aerosol foam	10-30
Hartz Control Pet Care System Home Fogger (09/27/2004)	aerosol	43.5
HOUSE SAVER Pet Stain & Odor Remover	liquid	31
Krylon Upside Down Marking Paint	aerosol	0–38
Minwax Polycrylic Protective Finish (Aerosol), Clear Gloss	aerosol	35
Minwax Polycrylic Protective Finish (Aerosol), Clear Satin	aerosol	35
Minwax Polycrylic Protective Finish (Aerosol) Clear Semi-Gloss	aerosol	35
Minwax Polycrylic Protective Finish-Clear	liquid	35
Sherwin-Williams White Lightening Stop Gap Insulating Foam Minimal	spray	1–8
Sherwin-Williams White Lightening Stop Gap Insulating Foam Triple	spray	1–8
Spray A Gasket Copper Hi Temp Seal	aerosol	20–45
Sprayway Ammoniated Glass Cleaner No. 43	aerosol	1–5
Sprayway Auto Glass Foaming Windshield Cleaner No. 41	aerosol	1–5
Sprayway Chewing Gum Remover No. 802	aerosol	<70–80
Sprayway Dust It No. 519	aerosol	<70
Sprayway Heavy-Duty Trim Adhesive No. 92	aerosol	20–25
Sprayway Multi-Purpose Spray Adhesive No. 88	aerosol	10–20
Sprayway Spray Adhesive No.66	aerosol	10–15
Sprayway Water-Based Multi-Purpose Adhesive Remover No. 894	aerosol	15
STP One-Step Tire Care, Aerosol	aerosol	1–5

TREATMENT

There are few data on the treatment of dimethyl ether exposure. Treatment is supportive, similar to other inhaled anesthetics with respiratory depression and cardiac dysrhythmias potentially representing the most immediate life-threatening complications of exposure. Immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, intravenous fluids, and cardiac monitoring as indicated by the history and physical examination.

References

1. Whalen FX, Bacon Dr, Smith HM. Inhaled anesthetics: an historical overview. Best Pract Res Clin Anaesthesiol 2005;19:323–330.
2. Strickland RA. Historical vignette ether drinking in Ireland. Mayo Clin Proc 1996;71:1015.
3. Connell KH. Ether drinking in Ulster. Q J Stud Alcohol 1965;26:629–653.
4. Long CW. An account of the first use of sulphuric ether by inhalation as an anaesthetic in surgical operations. South Med Surg J 1849;5:705–713.
5. Campagna JA. The end of religious fatalism: Boston as the venue for the demonstration of ether for the intentional relief of pain. Surgery 2005;138:46–55.
6. Defalque RJ, Wright AJ. An anesthetic curiosity in Philadelphia (March, 1849). Anesthesiology 1999;90:1492–1493.
7. Westhorpe R. Ether inhaler, 1847. Anaesth Intensive Care 1997;25:213.
8. Kerr N. Ether inebriety. JAMA 1891;17:791–794.
9. Hart E. An address on ether-drinking: its prevalence and results. Br Med J 1980;2:885–890.
10. Krenz S, Zimmermann G, Kolly S, Zullino DR. Ether: a forgotten addiction. Addiction 2003;98:1167–1168.
11. Wick R, Gilbert JD, Felgate P, Byard RW. Inhalant deaths in South Australia. A 20-year retrospective autopsy study. Am J Forensic Med Pathol 2007;28:319–322.

12. Bartholomew AA. Two cases of ether addiction/habituation. Med J Aust 1962;49:550–553.

13. Brandstater B, Eger EI II, Edelist G. Effects of halothane, ether and cyclopropane on respiration. Br J Anaesth 1965; 37:890–897.

14. Lam C-W, Galen TJ, Boyd JF, Pierson DL. Mechanism of transport and distribution of organic solvents in blood. Toxicol Appl Pharmacol 1990;104:117–129.

15. Morland J, Aune H, Bessesen A, Renck H. Formation of acetaldehyde from diethyl ether in man. Adv Exp Med Biol 1980;126:453–461.

16. Chengelis CP, Neal RA. Microsomal metabolism of diethyl ether. Biochem Pharmacol 1980;29:247–248.

17. Aune H, Renck H, Bessesen A, Morland J. Metabolism of diethyl ether to acetaldehyde in man. Lancet 1978;2 (8080):97.

18. Bovill JG. Inhalation anaesthesia: from diethyl ether to xenon. Handb Exp Pharmacol 2008;(182):121–142.

19. Bowen SE, Balster RL. Desflurane, enflurane, isoflurane and ether produce ethanol-like discriminative stimulus effects in mice. Pharmacol Biochem Behav 1997;57: 191–198.

20. Glowa JR Behavioral and neuroendocrine effects of diethyl ether exposure in the mouse. Neurotoxicol Teratol 1993;15:215–221.

21. Kringsholm B. Sniffing-associated deaths in Denmark. Forensic Sci Int 1980;15:215–225.

22. Reid PE, Brooks DE, Pang YC, Muelchen R. Routine direct injection gas–liquid chromatographic procedure for the analysis of volatile halogenated anaesthetics in whole blood using a new external injection port. J Chromatogr 1978;146:297–310.

23. Scotter MJ, Roberts DP. Development and validation of a rapid headspace gas chromatography-mass spectrometry method for the determination of diethyl ether and acetone residues in Tween extracts of shellfish intended for mouse bioassay for diarrhetic toxins. J Chromatogr A 2007;1157: 386–390.

24. Sharp M-E, Dautbegovic T. Ether: stability in preserved blood samples and a case of ether-assisted suicide. J Anal Toxicol 2001;25:628–630.

25. Takayasu T, Saito K, Nishigami J, Ohshijma T, Nagano T. Toxicological analysis of drugs and poisons in formalin-fixed organ tissues 2. Volatile substances. Int J Leg Med 1994;107:7–12.

26. Moffat AC (Ed). Clarke's isolation and identification of drugs in pharmaceuticals, body fluids, and postmortem material. 2nd ed. London: The Pharmaceutical Press; 1986:595.

27. Cox D, DeRienz R, Jufer Phipps RA, Levine B, Jacobs A, Fowler D. Distribution of ether in two postmortem cases. J Anal Toxicol 2006;30:635–637.

28. Athanaselis S, Stefanidou M, Karakoukis N, Koutselinis A. Asphyxial death by ether inhalation and plastic-bag suffocation instructed by the press and the internet. J Med Internet Res 2002;4:e18.

29. Bell CM, Gutowski SJ, Young S, Wells D. Diethyl ether interference with infrared breath analysis. J Anal Toxicol 1992;16:166–168.

30. Laakso O, Pennanen T, Himberg K, Kuituenen T, Himberg J-J. Effect of eight solvents on ethanol analysis by Drager 7110 evidential breath analyzer. J Forensic Sci 2004;49: 1113–1116.

31. Seevers MH, Waters RM. Pharmacology of the anesthetic gases. Physiol Rev 1938;18:447–479.

32. Brown WE. Experiments with anesthetic gases propylene, methane, dimethyl-ether. J Pharmacol Exp Ther 1924;23: 485–496.

33. Davidson BM. Studies on intoxication VI. The action of methyl ether. J Pharmacol Exp Ther 1925;26:43–48.

34. Mogi T, Horiguchi S. Explosion and detonation characteristics of dimethyl ether. J Hazard Mater 2009;164: 114–119.

35. Caprino L, Togna G. Toxicological aspects of dimethyl-ether. Eur J Toxicol 1975;8:287–290.

36. Collins CJ, Cobb LM, Purser DA. Effects of chronic inhalation of dimethyl ether in the rat. Toxicology 1978;11: 65–71.

37. Streete PJ, Ruprah M, Ramsey JD, Flanagan RJ. Detection and identification of volatile substances by headspace capillary gas chromatography to aid the diagnosis of acute poisoning. Analyst 1992;117:1111–1127.

Chapter 38

HALOGENATED ETHERS (ENFLURANE, ISOFLURANE, METHOXYFLURANE, SEVOFLURANE)

Robbins first reported the anesthetic properties of several fluorinated hydrocarbons in 1946.[1] In the 1950s, advances in organic fluorine chemistry allowed the development of fluorinated anesthetic alkanes and ethers that replaced the variety of gas and volatile liquids introduced as anesthetics in the first half of the 20th century. Trifluoroethyl vinyl ether (fluroxene, Ohio Medical Products, Cleveland, OH) was the first fluorinated anesthetic and was marketed from 1951 until 1974, when this anesthetic was withdrawn because of the discovery of a toxic metabolite.[2] Halothane was introduced by Ayerst Laboratories (New York, NY) and Imperial Chemical Industries, PLC (London, UK) into clinical practice in 1955–1956, about 5 years after the discovery of halothane by Charles Suckling in 1951; however, the subsequent use of halothane declined dramatically as newer, safer anesthetics became available. Methoxyflurane (Abbott Laboratories, Abbott Park, IL) was introduced into clinical practice in 1960. Within a decade, concerns about dose-related nephrotoxicity limited the use of this anesthetic. Over the next 10–15 years, Louise Croix and Ross Terrell synthesized several hundred new fluorinated compounds at Ohio Medical Products.[3] Terrell synthesized enflurane in 1963, and he introduced this anesthetic into clinical practice in 1966. Enflurane was approved as an anesthetic in the United States in 1972. In 1965, Terrell discovered the enflurane isomer, isoflurane. He introduced isoflurane into clinical practice in 1971, but approval of isoflurane in the United States was delayed until 1980 because of early concerns about carcinogenicity. Desflurane is a relatively new volatile halogenated ether anesthetic with minimal bio-transformation and lower solubility in blood and fat than most of the other volatile anesthetics.[4]

ENFLURANE

IDENTIFYING CHARACTERISTICS

Halogenated ethers represent a group of anesthetic agents along with halogenated hydrocarbons (halothane) and nitrous oxide. Inhalational anesthetics are either gases or vapors from volatile liquids. A substance is a gas when the ambient temperature is above the critical temperature (i.e., the temperature at which the substance cannot be liquified no matter how high the pressure). Below the critical temperature, the substance is a vapor. Enflurane (2-chloro-1,1,2-trifluoroethyldifluoromethyl ether, CAS RN: 13838-16-9) is a colorless, nonflammable, volatile liquid with a pleasant ether odor. The lipid solubility of enflurane is relatively low compared with halothane; therefore, the depth of anesthesia and the recovery from anesthesia occur more rapidly than halothane anesthesia. Figure 38.1 displays the chemical structure of enflurane along with other common halogenated ethers (isoflurane, methoxyflurane), halothane, and nitrous oxide.

The blood/gas partition coefficient is a measure of solubility and the amount of vapor that must be transferred from alveolar gas to the blood to achieve a given tension. The most soluble anesthetics (e.g., halothane,
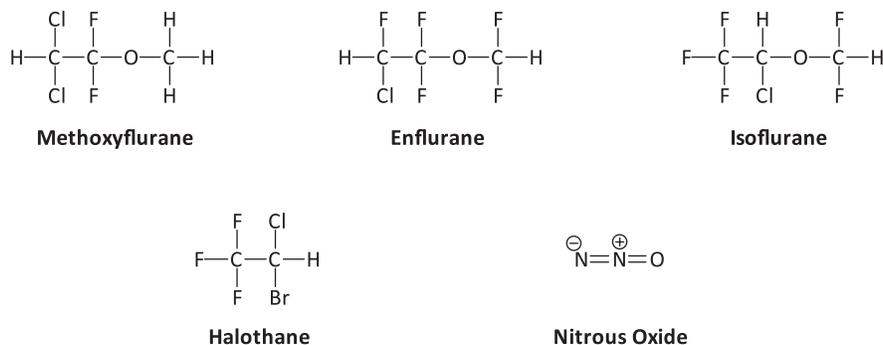
FIGURE 38.1. Chemical structures of halogenated ether anesthetics.

TABLE 38.1. Comparison of the Physical Properties of Halothane, Halogenated Ethers, and Nitrous Oxide.[13]

Anesthetic	MAC (%)	Vapor Pressure(mm Hg at 25°C/77°F)	Blood/Gas Partition Coefficient (37°C/98.6°F)	Oil/Gas Partition Coefficient (37°C/98.6°F)
Methoxyflurane	0.16	49.1	12.0	970
Halothane	0.75	302	2.3	224
Enflurane	1.68	591	1.9	98
Isoflurane	1.40	591	1.4	99
Nitrous Oxide	105	gas	0.47	1.4
Sevoflurane	2.6*	197	0.69	47–54

Abbreviation: MAC = minimum alveolar concentration.
Note. The concept of MAC assumes that the alveolar partial pressure of an anesthetic is the same as arterial blood; however, uniform mixing within the alveolus may not occur for anesthetics with high molecular weight (e.g., flurane/isoflurane).[6]
*Age 25, the MAC decreases with age (MAC = 1.7% at 60 years).

methoxyflurane) are associated with the slowest induction and recovery, assuming the same inspired concentrations and stable cardiac output. The oil/gas partition coefficient is a measure of the uptake of the gas in adipose tissue and the elimination rate of the gas after cessation of the anesthetic; this coefficient correlates to potency and the MAC. Table 38.1 compares these physical properties among halothane, halogenated ethers, and nitrous oxide. In general, a compound with a vapor pressure below 0.76 mm Hg at room temperature will attain an ambient concentration <1,000 ppm, which is below concentrations generally observed for narcotic or anesthetic effects.[5]

Carbon monoxide forms when volatile anesthetics pass through dry CO_2 absorbents, more so with Baralyme® (Allied Healthcare Products, Inc., Stuyvesant Falls, NY) than dry soda lime.[7,8] A structural requirement for the formation of carbon monoxide from halo ether degradation is the presence of a difluoromethoxy group. *In vitro* experimental studies suggest that carbon monoxide production is highest with enflurane, intermediate with isoflurane, and insignificant with sevoflurane and halothane. In a patient simulation model using desiccated soda lime, the peak carbon monoxide produc-

tion was as follows: enflurane, 10,654 ppm; isoflurane, 2,512 ppm; halothane, 210 ppm; and sevoflurane, 121 ppm.[9] The amount of carbon monoxide production depends on the amount of desflurane in contact with the dry absorbent, the absorbent water content, and fresh gas flow.[10] The first anesthetic cases involved enflurane, isoflurane, and desflurane with rare case reports documenting carboxyhemoglobin concentrations exceeding 30%.[11] Carbon monoxide formation may occur during the use of sevoflurane in the presence of dry CO_2 absorbents.

EXPOSURE

Abuse of halogenated ethers typically involves medical or veterinary personnel using halothane or enflurane.[12] The abuse of these volatile compounds usually occurs along with the abuse of other drugs (e.g., ethanol).

DOSE EFFECT

The minimum alveolar concentration (MAC) is a measure of the concentration of an inhaled anesthetic required to block purposeful movement in an individual

patient. A dose of 1 MAC will prevent muscle movement following surgical incision in 50% of patients. Typically, anesthetics are administered in concentrations of 0.3–1.5% along with a carrier gas (e.g., oxygen or oxygen plus nitrous oxide).[13] Table 38.1 compares the minimum alveolar concentration (i.e., potency) of enflurane with other halogenated ethers.

TOXICOKINETICS

The elimination of halogenated ethers depends on the individual compound. Compared with halothane, the biotransformation of enflurane is low (i.e., about 8.5%).[14] The lungs excrete most of the absorbed dose of enflurane unchanged in exhaled air; <10% of the absorbed dose of enflurane undergoes biotransformation with inorganic fluoride being the major metabolic product.[15] In a study of 7 healthy female patients undergoing enflurane anesthesia, 82.7% ± 18.8% of the absorbed drug was excreted unchanged by the lungs.[16] Enflurane elimination followed a 3-compartment model with half-times of 17.8 minutes, 3.2 hours, and 36.2 hours. The biotransformation of enflurane produces substantially less fluoride than methoxyflurane.[17] About 2.4% of the absorbed dose of enflurane appeared in the urine as nonvolatile fluorinated metabolites with inorganic fluoride accounting for 0.5% and organic fluoride for 1.9%. Maximum excretion of inorganic fluoride (20–60 mg/d) occurs 3–7 hours after the cessation of anesthesia. The mean half-time of inorganic and organic fluorine was 1.55 days and 3.69 days, respectively.

All inhaled anesthetics potentially interact with neuromuscular blocking agents (e.g., atracurium, pancuronium, vecuronium) to increase the intensity and duration of neuromuscular blockade.[18] Furthermore, benzodiazepines and opioids may decrease the MAC of inhaled anesthetics. Enflurane displaces diazepam from serum proteins *in vitro*; this interaction potentially increases the pharmacodynamic response of diazepam.[19] However, the clinical relevance of this drug interaction is unclear. The presence of nitrous oxide does not alter the metabolism of enflurane.[20]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

The major molecular targets of halogenated ether anesthetics are protein receptors rather than surrounding lipid membranes.[21] Halogenated ethers affect the GABA and NMDA brain receptor systems similar to ethanol. These anesthetics act nonselectively at a number of molecular targets to produce amnesia, hyp-

nosis, and immobility including the molecular targets of propofol (gamma-aminobutyric acid type A receptors) and nitrous oxide (glutamate and nicotinic receptors, potassium leak channels).[22] Halogenated ethers probably produce anesthesia by some part or combination of enhancing inhibitory postsynaptic channel activity (GABA$_A$, glycine receptors) and inhibiting excitatory synaptic channel activity (glutamate, serotonin, nicotinic acetylcholine receptors). At clinically effective concentrations, certain ion channels are sensitive to volatile anesthetics including the superfamilies of cysteine-loop neurotransmitter receptors (GABA$_A$, serotonin type 3, glycine, nicotinic acetylcholine) and glutamate receptors (i.e., activated by *N*-methyl-D-aspartate or α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid).[23] Volatile halogenated anesthetics enhance GABA$_A$ activity,[24] inhibit glutamate and nicotinic acetylcholine receptors,[25] and activate potassium leak channels (e.g., TREK-1, TREK-2, TASK-1, TASK-2, TASK-3, TRESK).[22] In contrast to hypoxia, volatile halogenated ether anesthetics open potassium channels including the TASK-1 and K$_v$ potassium channels in the membrane of carotid body type I cells.[26] Additionally, volatile halogenated anesthetics act on glycine receptors and mediators of neurotransmitter release. GABA$_A$ receptors are the most abundant inhibitory neurotransmitter receptors in the brain, but alternate pathways to anesthesia probably include the inhibition of excitatory ligand-gated ion channels (NMDA-sensitive glutamate, neuronal nicotinic acetylcholine receptors).[23]

Mechanism of Toxicity

All volatile anesthetics are concentration-dependent myocardial depressants as a result of reductions in trans-sarcolemmal calcium entry and alterations in sarcoplasmic reticulum function.[27] These fluorinated compounds cause respiratory depression manifest by decreased responsiveness to hypoxia and increased carbon dioxide concentrations even at subanesthetic doses.[28] Additionally, these anesthetics depress the central respiratory centers, eliminate conscious respirations, and reduce the responsiveness of peripheral chemoreceptors (oxygen-sensitive glomus type I cells) in the carotid bodies. All halogenated ether anesthetics also reduce pharyngeal reflexes, and relax skeletal muscles following the administration of anesthetic doses.

Postmortem

Autopsy findings in patients dying from the abuse of halogenated ethers are nonspecific (e.g., congestion of viscera, pulmonary edema, cerebral edema) and may demonstrate signs of asphyxia or irritation of the

bronchial mucosa.[29] Autopsy tissues may contain strong odors (e.g., enflurane), depending on the odor threshold of the anesthetic.

CLINICAL RESPONSE

Like other volatile halogenated ethers, enflurane is a central nervous system (CNS) depressant that produces an ethanol-like intoxication that is associated with abuse in some individuals. Malignant hyperthermia is a rare complication of anesthesia with halogenated ethers.[30] Clinical features of malignant hyperthermia include muscle rigidity, elevated body temperature, cardiac dysrhythmias, hypotension, and metabolic acidosis. Rhabdomyolysis also may occur along with renal failure and pulmonary edema.[31] The incidence of hepatotoxicity following enflurane anesthesia is extremely rare (1/800,000).[10]

DIAGNOSTIC TESTING

Analytic Methods

Methods for the analysis of halogenated ethers (e.g., enflurane) in biologic samples include headspace gas chromatography with flame ionization detection or electron capture detection, gas chromatography/Fourier transformation infrared spectroscopy,[32] and headspace gas chromatography/mass spectrometry.[33] The limits of detection and quantitation of the latter method were 1.2 and 4.7 mg/L, respectively. Methods for sampling halogenated ethers include headspace,[34] pulse-heating,[35] solid-phase microextraction,[36] purge-and-trap, and cryogenic oven trapping.[37] Halogenated ethers do not significantly interfere with the determination of breath ethanol concentrations unless the analytic machine uses a single wavelength of 9.5 μm to determine the ethanol concentration in expired air.[38] Like other volatile substances of abuse, storage of the biologic samples in headspace vials that are sealed and stored immediately at −20°C (−4°F) minimizes loss of the halogenated ether, including enflurane, from the container.

Biomarkers

In a study of 10 patients undergoing enflurane anesthesia (0.5–2% v/v), the mean peak blood concentration of enflurane was about 95 mg/L in central venous blood 30 minutes after the beginning of induction; the blood enflurane concentration declined to <0.5 mg/L 90 minutes after the termination of anesthesia.[39] The mean concentration of enflurane in venous blood samples from 12 patients undergoing anesthesia with 1.5% enflurane and 0.75% halothane was 64.8 ± 35.7 mg/L

immediately after the end of anesthesia.[40] A 21-year-old anesthesiology student was found dead with 2 empty bottles of enflurane and a breathing mask nearby. Postmortem blood samples drawn during autopsy 3½ days after death contained 130 mg enflurane/L.[41] Samples of fat and brain tissues also contained high concentrations of enflurane (100 mg/L and 350 mg/L, respectively) as measured by gas chromatography/mass spectrometry. A 29-year-old surgical technician was found dead in his bed with his bedclothes pulled over his head and an empty bottle of enflurane nearby.[42] The postmortem blood enflurane concentration was 71 mg/L as measured by headspace gas chromatography with flame ionization detection. There was no detectable enflurane in the gastric contents.

Abnormalities

Renal dysfunction following enflurane anesthesia is unlikely because serum inorganic fluoride concentrations following enflurane anesthesia seldom exceed the 50 μM concentration associated with renal dysfunction.[43] Isoflurane may prolong the QT_c interval similar to isoflurane and sevoflurane. In a study of 6 healthy adults undergoing anesthesia with only enflurane (2.16 ± 0.13 mM), the QT_c interval increased from 400 ± 10 msec to 460 ± 140 msec. The QRS duration and the PR interval remained unchanged from baseline; there were no dysrhythmias.[44]

TREATMENT

The treatment of complications from enflurane abuse is similar to the abuse of other inhaled anesthetics. Respiratory depression potentially represents the most immediate life-threatening complication of enflurane exposure; immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, intravenous (IV) fluids, and cardiac monitoring as indicated by the history and physical examination. The lungs excrete most of the absorbed dose of enflurane unchanged; consequently, there are no efficacious methods to enhance elimination.

ISOFLURANE

IDENTIFYING CHARACTERISTICS

Isoflurane (1-chloro-2,2,2-trifluoroethyldifluoromethyl ether, CAS RN: 26675-46-7) is a nonflammable, clear, colorless, volatile liquid anesthetic with a low blood/gas

solubility coefficient that produces the rapid induction of anaesthesia with small volumes. Isoflurane is a structural isomer of enflurane with a chemical formula of $C_3H_2ClF_5O$ (Figure 38.1). The vapor has a slightly pungent, ether-like odor that irritates the airways more than other halogenated ether anesthetics.

EXPOSURE

With the increasing use of isoflurane, the abuse of this anesthetic also increased. In volunteer studies, isoflurane can function as a reinforcer and produce abuse liability-related subjective (i.e., pleasant, psychedelic-like, euphoria) effects in some, but not the majority of participants.[45,46] In general, these subjective effects are less intense than nitrous oxide. In abusers, the immediate effects of inhaling anesthetics are feelings of elation and euphoria followed soon thereafter by hypersensitivity to noise.[47] Continued use produces effects similar to ethanol including loss of inhibition, loquaciousness, agitation, and dysarthria. Sleepiness limits the continued use of the anesthetic. Recovery typically occurs within 30 minutes.

DOSE EFFECT

The minimum alveolar concentration (MAC) is a measure of potency as defined by the concentration of an inhaled anesthetic required to block purposeful movement in an individual patient. A dose of one MAC will prevent muscle movement following surgical incision in 50% of patients. Typically, anesthetics are administered in concentrations of 0.3–1.5% along with a carrier gas (e.g., oxygen or oxygen plus nitrous oxide).[48] Table 38.1 compares the minimum alveolar concentration (i.e., potency) of isoflurane with other halogenated ethers. Isoflurane causes minimal cardiovascular depression at MAC values below 2.[10]

TOXICOKINETICS

Volunteer studies indicate that the percutaneous loss of isoflurane is too small to affect pharmacokinetic studies or to produce clinical effects during anesthesia.[49] Compared with halothane, the biotransformation of isoflurane is minimal (i.e., <1%).[14] The lungs excrete most of an absorbed dose of isoflurane unchanged in expired air. Metabolism of isoflurane primarily involves the formation of trifluoroacetic acid and, to a lesser extent, fluoride ions. The kidneys excrete these metabolites in the urine. Volunteer studies of patients undergoing coronary artery bypass grafting surgery indicate the following 2 distinct components: 1) initial 5-minute fast component reflecting the initial washout of isoflurane

from the functional residual capacity of the lungs, and 2) a 15-minute slow component reflecting the passage of isoflurane across the alveolar-pulmonary capillary membrane and the blood–brain barrier.[50] The elimination of isoflurane from the mixed venous blood (pulmonary artery) as a measure of isoflurane elimination from the body was mono-exponential with a mean half-life of 32.2 ± 11.9 minutes. Isoflurane elimination from the well-perfused brain was more rapid as reflected in a mean isoflurane half-life of 18.9 ± 7.2 minutes in jugular bulb venous blood. End-tidal isoflurane concentrations are higher than arterial isoflurane concentrations at a constant 2% inspired isoflurane concentration, but the difference is relatively constant.

Animal studies indicate a lack of tolerance to chronic doses of halogenated ether anesthetics. Mice continuously exposed to 0.15% and 0.30% isoflurane did not demonstrate either autotolerance or cross-tolerance.[51] However, these studies suggest that some cross-tolerance to halogenated ethers develops following the chronic administration of nitrous oxide or ethanol.[52] Cross-tolerance to isoflurane in these mice persisted 80 days following the cessation of ethanol.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The mechanism of action of isoflurane is similar to enflurane (see the Histopathology and Pathophysiology section under Enflurane). Isoflurane is a respiratory tract irritant in high doses that can cause laryngospasm; this property limits use of this agent for anesthesia induction. *In vitro* studies suggest that isoflurane is a potent modulator of extrasynaptic $GABA_A$ receptors in the ventrobasal thalamus of mice. Isoflurane enhances fast synaptic inhibition in the brain mediated by $GABA_A$ receptors at anesthetic concentrations similar to other halogenated ether anesthetics. These receptors also occur at extrasynaptic sites in the CNS, such as the thalamocortical relay neurons. The thalamo-corticothalamic loop is a key target for the induction of sedation and hypnosis for anesthetic effects. These extrasynaptic $GABA_A$ receptors contain primarily α_4, β_2, and δ subunits in contrast to α_1, β_2, and γ_2 in synaptic $GABA_A$ receptors. The extrasynaptic $GABA_A$ receptors are particularly sensitive to low isoflurane concentrations.[53]

CLINICAL RESPONSE

Like other volatile halogenated ethers, isoflurane is a CNS depressant that produces an ethanol-like intoxication and is associated with abuse in some individuals. The clinical effects of isoflurane are similar to enflurane with the exception of some difference in potency. Rare case

reports associate fulminant malignant hyperthermia with combined nitrous oxide and isoflurane anesthesia.[54]

DIAGNOSTIC TESTING

In a study of isoflurane anesthesia, the concentration of isoflurane in venous blood ranged between 85.2 mg/L at the beginning of induction and 104.9 mg/L during the operation.[33] A 36-year-old operating room assistant was found dead in a hospital locker room with a plastic bag over his head and an almost empty bottle of isoflurane in his locker.[29] No resuscitation was attempted. The mean concentration of 6 samples of cardiac blood stored in sealed headspace vials was 47.9 ± 0.9 mg/L. The postmortem blood also contained a nordiazepam concentration similar to nordiazepam concentrations in patients with therapeutic plasma diazepam concentrations; the postmortem gastric contents contained unexpectedly high isoflurane concentrations (252 ± 16.05 mg/L [mg/kg]). Renal dysfunction following isoflurane anesthesia is unlikely because serum inorganic fluoride concentrations following isoflurane anesthesia seldom exceed the 50 µM concentration associated with renal dysfunction.[55] Isoflurane may prolong the QT_c interval similar to enflurane and sevoflurane. In a study of 8 healthy adults undergoing anesthesia with only isoflurane (1.04 ± 0.11 mM), the QTc interval increased from 420 ± 10 msec to 470 ± 140 msec. The difference in the QRS duration, the PR interval and the QT interval during anesthesia were not statistically different than baseline.[56]

TREATMENT

The treatment of complications from isoflurane abuse is similar to the abuse of other inhaled anesthetics. Respiratory depression potentially represents the most immediate life-threatening complication of isoflurane exposure; immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, IV fluids, and cardiac monitoring as indicated by the history and physical examination. The lungs excrete most of the absorbed dose of isoflurane unchanged; consequently, there are no efficacious methods to enhance elimination.

METHOXYFLURANE

IDENTIFYING CHARACTERISTICS

Methoxyflurane (2,2-dichloro-1,1-difluoroethylmethyl ether, CAS RN: 76-38-0) is highly soluble in blood; con-

sequently, the induction of anesthesia is slower than halothane and many other halogenated ether anesthetics. Methoxyflurane is also highly fat soluble.

EXPOSURE

Methoxyflurane is now primarily a general anesthetic for animals because of the nephrotoxicity associated with the use of this compound as an anesthetic.

DOSE EFFECT

The mean alveolar concentration of methoxyflurane necessary to induce the first plane of surgical anesthesia is about 0.3%. A 39-year-old gynecologist developed hepatitis after sniffing about 2 mL methoxyflurane once or twice daily for 6 weeks (i.e., total 125 mL) as a treatment for insomnia.[57] His clinical symptoms and laboratory abnormalities resolved completely following cessation of use.

TOXICOKINETICS

The elimination of methoxyflurane primarily involves biotransformation. Compared with other halogenated ethers, a relatively large part of the absorbed dose of methoxyflurane is metabolized. In a study of 6 Japanese patients undergoing anesthesia with nitrous oxide and 0.31% methoxyflurane, the lungs excreted about 35% of the absorbed methoxyflurane dose unchanged in expired air.[58] The kidneys excreted approximately 46% of the dose in the urine, primarily as organic fluoride, and to a lesser extent inorganic fluoride. Peak serum fluoride concentrations occurred about 6 hours after the end of anesthesia; serum fluoride concentrations may exceed the 50 µM concentration associated with renal dysfunction.[59] *In vitro* studies of microsomes from human livers indicate that CYP2E1 is the principal cytochrome P450 isoenzyme involved in methoxyflurane biotransformation.[60] However, these studies indicate that there are several other isoenzymes involved including CYP1A2, CYP2C9/10, and CYP2D6.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The chronic abuse of methoxyflurane is associated with hepatorenal damage. A needle biopsy of the liver from 39-year-old man with methoxyflurane-induced hepatitis demonstrated centrilobular to midzonal confluent hepatic necrosis with few inflammatory cells.[57] Portal tract changes were minimal. On electron microscopy, there were many irregularly shaped vacuoles with partially enveloped structures resembling fat droplets and

increased lysosomes in the hepatocytes. Nephrocalcinosis may develop in patients abusing methoxyflurane.[61] The most striking feature seen on renal biopsy of these patients is the deposition of birefringent crystals within tubular lumens, particularly in the cortical lumens. Thickening of the Bowman capsule and focal widening of the mesangial spaces occurs with some glomeruli becoming sclerotic. These changes are similar to the renal damage caused by secondary oxalosis.

A 42-year-old veterinary assistant developed fatal hepatic failure with submassive hepatic necrosis after sniffing methoxyflurane for the preceding 2 months.[62] Postmortem examination of the liver revealed centrilobular liver necrosis with some mononuclear cells infiltrates. There were regenerating liver cells in the peripheral and periportal areas.

CLINICAL RESPONSE

Like other volatile halogenated ethers, methoxyflurane is a CNS depressant that produces an ethanol-like intoxication and is associated with abuse in some individuals. Hepatorenal damage may occur following the chronic inhalation of methoxyflurane. Case reports associate jaundice, hepatic encephalopathy, and fulminant hepatic failure with the chronic abuse of methoxyflurane.[57,62] The clinical features of the hepatitis associated with methoxyflurane are typical of acute hepatitis including general malaise, nausea, vomiting, pruritus, scleral icterus, confusion, encephalopathy, and hepatic failure. A toxic nephropathy is associated with elevated serum fluoride concentrations following methoxyflurane anesthesia.[63] The chronic abuse of methoxyflurane may cause a progressive renal and bone disease similar to fluoride toxicity. A 27-year-old nurse developed painful periostitis, osteosclerosis, hypertension, and renal dysfunction following the long-term, intermittent abuse of methoxyflurane.[64] Symptoms included severe bone pain, headache, polyuria, polydipsia, and epigastric distress. Nephrocalcinosis and secondary oxalosis may complicate the chronic abuse of methoxyflurane as a result of the metabolism of this compound to oxalic acid and fluoride.[61] These changes may cause renal dysfunction, hypertension, edema, anemia, and electrolyte imbalance.

DIAGNOSTIC TESTING

Radiographic abnormalities associated with bone changes from chronic abuse of methoxyflurane resemble periostitis deformans associated with fluorosis from drinking fluorinated wine.[65] These individuals develop multiple crops of hyperostotic periosteal nodules, particularly in the phalanges, as a result of osteoblastic stimulation caused by excessive fluoride concentrations. In severe cases of chronic methoxyflurane abuse, sequelae of hepatic and renal damage may occur including elevation of serum hepatic aminotransferases, hyperbilirubinemia, hyperammonemia, hyperphosphatemia, chronic anemia, and coagulation disorders.

TREATMENT

Respiratory depression represents the most immediate life-threatening complication of methoxyflurane exposure; immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, IV fluids, and cardiac monitoring as indicated by the history and physical examination. Methods to enhance elimination of methoxyflurane have not been studied. Although *N*-acetylcysteine is a potential antidote for carbon tetrachloride-induced liver damage, there are no clinical data currently available to determine of efficacy of *N*-acetylcysteine for the treatment of methoxyflurane-induce liver damage. If severe hepatorenal dysfunction develops, supportive care includes the treatment of renal failure with dialysis and hepatic failure with fresh frozen plasma, vitamin K, low protein diet, neomycin, lactulose, and careful fluid and electrolyte balance. Hepatic and renal function should be followed daily for at least 3 days after serious abuse of methoxyflurane.

SEVOFLURANE

IDENTIFYING CHARACTERISTICS

Sevoflurane is a methyl isopropyl ether anesthetic that is poorly soluble in blood. This volatile anesthetic has a less pungent odor and less airway irritation than other halogenated anesthetics. Sevoflurane is a colorless, nonflammable liquid, which has a vapor pressure of 160 mm Hg at room temperature. Compound A [fluoromethyl-2,2-difluoro-1-(trifluoromethyl) vinyl ether] is a degradation product of sevoflurane resulting from the interaction of sevoflurane with absorbents used to remove carbon dioxide during anesthesia.

EXPOSURE

Like isoflurane, sevoflurane is a reinforcer in volunteer studies; this anesthetic produces abuse liability-related subjective (i.e., pleasant, psychedelic-like, euphoria) effects in some individuals. In general, these subjective effects are less intense than nitrous oxide. Continued

sniffing of sevoflurane causes effects similar to the ingestion of ethanol including loss of inhibition, loquaciousness, agitation, and dysarthria. Sleepiness limits the continued use of halogenated anesthetics.

DOSE EFFECT

Inspired concentrations of sevoflurane during the induction of anesthesia range from 1–8%. The minimum alveolar concentration of sevoflurane necessary to prevent movement in 50% of patients (MAC) is about 1.7–2.1%.[66] In a study of 42 elective adult surgical patients, the estimated MAC was 1.58% (95% CI: 1.14–1.98%).[67] Rapid induction of anesthesia occurs following the inhalation of 6–8% sevoflurane. The administration of 1.5–3% sevoflurane maintains surgical anesthesia. Compound A is nephrotoxic in rats; mild, reversible renal abnormalities (e.g., transient albuminuria, enzymuria) occur in humans when the dose of Compound A exceeds 240 ppm-hour (e.g., >30 ppm for 8 hours).[68]

TOXICOKINETICS

The alveolar equilibration of sevoflurane is rapid (i.e., 85% complete within 30 minutes), similar to desflurane (90%) and more rapid than isoflurane (73%) and halothane (58%).[69] The lungs excrete most of an absorbed dose of sevoflurane unchanged into expired air with a mean total body clearance (3.5 L/minute) similar to isoflurane. Like all fluorinated volatile anesthetics, sevoflurane undergoes limited biotransformation to organic and inorganic fluoride metabolites. Similar to isoflurane and enflurane, about 2–5% of an absorbed dose of sevoflurane is metabolized via CYP2E1 isoenzymes.[70] In a study of 32 healthy Japanese patients undergoing elective surgery, the estimated mean degradation rate of sevoflurane was 3.3%.[71] The major metabolites are hexafluoroisopropanol (HFIP) and fluoride; these 2 metabolites form in equimolar concentrations. Peak serum fluoride concentrations following sevoflurane anesthesia are about 1.5–2 times higher than those following enflurane anesthesia, but the decline of the fluoride concentrations is much more rapid (i.e., a few hours) following sevoflurane anesthesia than after the administration of enflurane. The metabolism of sevoflurane does not produce trifluoroacetylated liver proteins, which are associated with halothane-induced hepatitis. After conjugation of hexafluoroisopropanol with glucuronic acid, the kidney excretes the glucuronide metabolite with a urinary elimination half-life of about 20 hours. Unlike methoxyflurane, sevoflurane undergoes minimal defluorination in the kidney.[72] The terminal plasma elimination half-life of sevoflurane is approximately 1 day. The renal clearances of the metabolites, fluoride and HFIP are prolonged with apparent elimination half-lives of 21.4 ± 2.8 hours and 20.1 ± 2.6 hours, respectively, in a study of 10 patients receiving 1.3 MAC (2.7% end-tidal) sevoflurane in oxygen for 3 hours.[73] There is substantial variation in the biotransformation of sevoflurane between animal species; therefore, extrapolation of animal data to humans is limited.[60]

In volunteer studies, the administration of disulfiram, which is a potent CYP2E1 inhibitor, substantially decreases the formation of fluoride ion and hexafluoroisopropanol.[74] Other drugs induce the formation of CYP2E1 including phenobarbital, phenytoin, isoniazid, and chronic ethanol use. However, the clinical significance of these potential interactions remains unclear, in part because of the limited metabolism.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Sevoflurane is a CNS depressant that causes respiratory depression and hypotension in high doses (i.e., >1 MAC). The dose-dependent cardiac, respiratory, and CNS depressant effects are similar to the effects of isoflurane.[69] The use of sevoflurane during surgery prolongs the QTc interval similar to isoflurane.[75] In a study of 18 women undergoing sevoflurane anesthesia with prior medications, the QTc interval increased from 434 ± 5 msec at baseline to 459 ± 6 msec 10 minutes after anesthesia began.[76] Although case reports associate sevoflurane abuse with ventricular fibrillation,[77] the role of QTc prolongation in these cases of sudden death is unclear. Sevoflurane produces no significant reactive intermediates; therefore, sevoflurane is not associated with the immune-mediated hepatotoxicity that occurs following halothane anesthesia.

CLINICAL RESPONSE

Like other volatile halogenated ethers, sevoflurane is a CNS depressant that produces an ethanol-like intoxication and is associated with abuse in some individuals. The cessation of these effects is rapid (i.e., within 30 minutes).

DIAGNOSTIC TESTING

Following the 3-hour administration of 2.7% sevoflurane to 10 patients, blood samples were collected 1 hour and 8 hours after cessation of anesthesia.[73] The mean sevoflurane concentrations were 38 mg/L and 7.2 mg/L, respectively.

The postmortem sevoflurane concentration depends on a number of factors including losses during perimor-

tem resuscitation, postmortem interval, collection, storage, and analysis (e.g., absorption into plastic, head-space losses). Comparison of postmortem and anesthetic data is complicated by the ventilatory support surgical patients receive during and after the cessation of anesthesia. A 31-year-old anesthetist was found dead in the break room of the surgery floor near a bottle of sevoflurane.[78] No drugs were detected in postmortem femoral blood except the estimated sevoflurane concentration of 15 mg/L. The autopsy was normal except for pulmonary edema and 50% occlusion of the left main coronary artery. A 44-year-old man was found dead with an oxygen mask containing a gauze pad connected to his face and a variety of medications near his body.[79] The postmortem femoral blood samples contained a high concentration of methamphetamine (7.02 mg/L) and a valproic acid (60.8 mg/L) concentrations consistent with therapeutic concentrations. The concentrations of sevoflurane in postmortem femoral blood and vitreous humor were 26.2 mg/L and 86.7 mg/L, respectively. The author suggested that the concentration of sevoflurane was higher at the time of death because of the high sevoflurane vitreous humor/femoral blood ratio of 3.31. A 47-year-old man was found dead in bed with an empty container of sevoflurane near him.[80] He was last seen alive by his mother 4 days earlier. Postmortem heart and subclavian blood samples contained sevoflurane concentrations of 16 mg/L and 8 mg/L, respectively.

TREATMENT

Similar to other inhaled anesthetics, respiratory depression potentially represents the most immediate life-threatening complication of sevoflurane exposure; immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, IV fluids, and cardiac monitoring as indicated by the history and physical examination. The lungs excrete most of the absorbed dose of sevoflurane unchanged; consequently, there are no efficacious methods to enhance elimination.

References

1. Robbins GH. Preliminary studies of the anesthetic activity of fluorinated hydrocarbons. J Pharmacol Exp Ther 1946;86:197–204.
2. Whalen FX, Bacon Dr, Smith HM. Inhaled anesthetics: an historical overview. Best Pract Res Clin Anaesthesiol 2005;19:323–330.
3. Terrell RC. The invention and development of enflurane, isoflurane, sevoflurane, and desflurane. Anesthesiology 2008;108:531–533.
4. Caldwell JE. Desflurane clinical pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 1994;27:6–18.
5. Dahl AR. Contemporary issues in toxicology. Dose concepts for inhaled vapors and gases. Toxicol Appl Pharmacol 1990;103:185–197.
6. Landon MJ, Matson AM, Royston BD, Hewlett AM, White DC, Nunn FJ. Components of the inspiratory-arterial isoflurane partial pressure difference. Br J Anaesth 1993;70:605–611.
7. Baxter PJ, Garton K, Kharasch ED. Mechanistic aspects of carbon monoxide formation from volatile anesthetics. Anesthesiology 1998;89:929–941.
8. Baxter PJ, Kharasch ED. Rehydration of desiccated Baralyme prevents carbon monoxide formation from desflurane in an anesthesia machine. Anesthesiology 1997;86:1061–1065.
9. Keijzer C, Perez RS, De Lange JJ. Carbon monoxide production from five volatile anesthetics in dry soda lime in a patient model: halothane and sevoflurane do produce carbon monoxide; temperature is a poor predictor of carbon monoxide production. BMC Anesthesiol 2005;5:6.
10. Bovill JG. Inhalation anaesthesia: from diethyl ether to xenon. Handb Exp Pharmacol 2008;182:121–142.
11. Coppens MJ, Versichelen LF, Rolly G, Mortier EP, Struys MM. The mechanisms of carbon monoxide production by inhalational agents. Anaesthesia 2006;61:462–468.
12. Yamashita M, Matsuki A, Oyama T. Illicit use of modern volatile anaesthetics. Can Anaesth Soc J 1984;31:76–79.
13. Heusler H. Quantitative analysis of common anaesthetic agents. J Chromatogr 1985;340:273–319.
14. Carpenter RL, Eger EI 2nd, Johnson BH, Unadkat JD, Sheiner LB. The extent of metabolism of inhaled anesthetics in humans. Anesthesiology 1986;65:201–205.
15. Dale O, Brown BR Jr. Clinical pharmacokinetics of the inhalational anaesthetics. Clin Pharmacokinet 1987;12:145–167.
16. Chase RE, Holaday DA, Fiserova-Bergerova V, Saidman LJ, Mack FE. The biotransformation of ethrane in man. Anesthesiology 1971;35:262–267.
17. Cousins MJ, Greenstein LR, Hitt BA, Mazze RI. Metabolism and renal effects of enflurane in man. Anesthesiology 1976;44:44–53.
18. Stachnik J. Inhaled anesthetic agents. Am J Health-Syst Pharm 2006;63:623–634.
19. Dale O, Nilsen OG. Binding and distribution of restrictively and non-restrictively eliminated drugs to serum and liver cell cytosol: effects of volatile anaesthetics. Br J Anaesth 1986;58:55–62.
20. Cahalan MK, Johnson BH, Eger EI II. Relationship of concentrations of halothane and enflurane to their metabolism and elimination in man. Anesthesiology 1981;54:3–8.
21. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994;367:607–614.

22. Solt K, Forman SA. Correlating the clinical actions and molecular mechanisms of general anesthetics. Curr Opin Anaesthesiol 2007;20:300–306.

23. Campagna JA, Miller DW, Forman SA. Mechanisms of actions of inhaled anesthetics. N Engl J Med 2003;348: 2110–2124.

24. Westphalen RI, Hemmings HC Jr. Volatile anesthetic effects on glutamate versus GABA release from isolated rat cortical nerve terminals: basal release. J Pharmacol Exp Ther 2006:316:208–215.

25. Cheng G, Kendig JJ. Enflurane decreases glutamate neurotransmission to spinal cord motor neurons by both pre- and postsynaptic actions. Anesth Analg 2003;96: 1354–1359.

26. Patel AJ, Honoré E. Anesthetic-sensitive 2P domain K+ channels. Anesthesiology 2001;95:1013–1021.

27. Connelly TJ, Coronado R. Activation of the Ca2+ release channel of cardiac sarcoplasmic reticulum by volatile anesthetics. Anesthesiology 1994;81:459–469.

28. Knill RL, Manninen PH, Clement JL. Ventilation and chemoreflexes during enflurane sedation and anaesthesia in man. Can Anaesth Soc J 1979;26:353–360.

29. Pavlic M, Haidekker A, Grubwieser P, Rabl W. Fatal accident caused by isoflurane abuse. Int J Leg Med 2002;116: 357–360.

30. Allen GC, Brubaker CL. Human malignant hyperthermia associated with desflurane anesthesia. Anesth Analg 1998; 86:1328–1331.

31. Hosoya N, Miyagawa K, Mimura T, Hoshide S, Akazawa H, Kanda Y, et al. Malignant hyperthermia induced by general anesthesia for bone marrow harvesting. Bone Marrow Transplant 1997;19:509–511.

32. Ojanperä I, Pihlainen K, Vuori E. Identification limits for volatile organic compounds in the blood by purge-and-trap GC-FTIR. J Anal Toxicol 1998;22:290–295.

33. Yang NC, Hwang KL, Hung DZ, Wuhh HH, Ho WM. Reliable gas chromatographic-mass spectrometric method combined with a headspace autosampler for isoflurane determination in blood. J Chromatogr B Biomed Sci Appl 2000;742:277–282.

34. Maruyama K, Takatsu A, Obata T. The quantitative analysis of inhalational anaesthetics in forensic samples by gas chromatography/mass spectrometry/selected ion monitoring. Biomed Chromatogr 1995;9:179–182.

35. Saito K, Takayasu T, Nishigami J, Kondo T, Ohtsuji M, Lin Z, Ohshima T. Determination of the volatile anesthetics halothane, enflurane, isoflurane, and sevoflurane in biological specimens by pulse-heating GC-MS. J Anal Toxicol 1995;19:115–119.

36. Poli D, Bergamaschi E, Manini P, Andreoli R, Mutti A. Solid-phase microextraction gas chromatographic-mass spectrometric method for the determination of inhalation anesthetics in urine. J Chromatogr B Biomed Sci Appl 1999;732:115–125.

37. Kojima T, Ishii A, Watanabe-Suzuki K, Kurihara R, Seno H, Kumazawa T, et al. Sensitive determination of four general anaesthetics in human whole blood by capillary gas chromatography with cryogenic oven trapping. J Chromatogr B 2001;762:103–108.

38. Verheecke G, Troch E, Moerman E. Can inhaled anesthetics influence the breath analyser Alcotest®. Acta Anaesthesiol Belg 1982;33:39–42.

39. Corall IM, Knights KM, Strunin L. Enflurane (Ethrane) anaesthesia in man. Metabolism and effects on biochemical and haematological variables. Br J Anaesth 1977;49: 881–885.

40. Imbenotte M, Erb F, Goldstein P, Erb C, Scherpereel P: Halothane and enflurane metabolite elimination during anaesthesia in man. Eur J Anaesthesiol 1987;4:175–182.

41. Jacob B, Heller C, Daldrup T, Burrig KF, Barz J, Bonte W. Fatal accidental enflurane intoxication. J Forensic Sci 1989;34:1408–1412.

42. Walker FB, Morano RA. Fatal recreational inhalation of enflurane. J Forensic Sci 1990;35:197–198.

43. Maduska AL. Serum inorganic fluoride levels in patients receiving enflurane anesthesia. Anesth Analg 1974;53: 351–353.

44. Schmeling WT, Warltier DC, McDonald DJ, Madsen KE, Atlee JL, Kampine JP. Prolongation of the QT interval by enflurane, isoflurane, and halothane in humans. Anesth Analg 1991;72:137–144.

45. Walker DJ, Beckman NJ, Zacny JP. Reinforcing and subjective effects of the volatile anesthetic, sevoflurane. Drug Alcohol Depend 2004;76:191–201.

46. Beckman NJ, Zacny JP, Walker DJ. With-subject comparison of the subjective and psychomotor effects of a gaseous anesthetic and two volatile anesthetics in healthy volunteers. Drug Alcohol Depend 2006;81:89–95.

47. De Francisco CP. Pentrane dependence: a case report. Br J Psychiatr 1971;119:609–610.

48. Heusler H. Quantitative analysis of common anaesthetic agents. J Chromatogr 1985;340:273–319.

49. Lockhart SH, Yasuda N, Peterson N, Laster M, Taheri S, Weiskopf RB, Eger EI II. Comparison of percutaneous losses of sevoflurane and isoflurane in humans. Anesth Analg 1991;72:212–215.

50. Lu C-C, Tsai C-S, Hu OY, Chen R-M, Chen T-L, Ho S-T. Pharmacokinetics of isoflurane in human blood. Pharmacology 2008;81:344–349.

51. Smith RA, Winter PM, Smith M, Eger EI. Tolerance to and dependence on inhalational anesthetics. Anesthesiology 1979;50:505–509.

52. Johnstone RE, Kulp RA, Smith TC. Effects of acute and chronic ethanol administration on isoflurane requirement in mice. Anesth Analg 1975;54:277–281.

53. Jia F, Yue M, Chandra D, Homanics GE, Goldstein PA, Harrison NL. Isoflurane is a potent modulator of extrasynaptic GABAA receptors in the thalamus. J Pharmacol Exp Ther 2008;324:1127–1135.

54. Bross T, Steinmann D. Fulminant malignant hyperthermia. Acta Anaesthesiol Scand 2008;52:164–165.

55. Dobkin AB, Kim D, Choi JK, Levy AA. Blood serum fluoride levels with enflurane (Ethrane®) and isoflurane (Forane®) anaesthesia during and following major abdominal surgery. Can Anaesth Soc J 1973;20:494–498.

56. Schmeling WT, Warltier DC, McDonald DJ, Madsen KE, Atlee JL, Kampine JP. Prolongation of the QT interval by enflurane, isoflurane, and halothane in humans. Anesth Analg 1991;72:137–144.

57. Okuno T, Takeda M, Horishi M, Okanoue T, Takino T. Hepatitis due to repeated inhalation of methoxyflurane in subanaesthetic concentrations. Can Anaesth Soc J 1985; 32:53–55.

58. Sakai T, Takaori M. Biodegradation of halothane, enflurane and methoxyflurane. Br J Anaesth 1978;50:785–791.

59. Churchill D, Knaack J, Chirito E, Barré P, Cole C, Muehrcke R, Gault MH. Persisting renal insufficiency after methoxyflurane anesthesia. Report of two cases and review of literature. Am J Med 1974;56:575–582.

60. Kharasch ED, Thummel KE. Identification of cytochrome P450 2E1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. Anesthesiology 1993;79:795–807.

61. Brennan RP, Pearlstein AE, Miller SA. Computed tomography of the kidneys in a patient with methoxyflurane abuse. J Comput Assist Tomogr 1988;12:155–156.

62. Min K-W, Cain GD, Sabel JS, Gyorkey F. Methoxyflurane hepatitis. South Med J 1977;70:1363–1364.

63. Churchill D, Yacoub JM, Siu KP, Symes A, Gault MH. Toxic nephropathy after low-dose methoxyflurane anesthesia: drug interaction with secobarbital? CMAJ 1976; 114:326–333.

64. Klemmer PJ, Hadler NM. Subacute fluorosis a consequence of abuse of an organofluoride anesthetic. Ann Intern Med 1978;89:607–611.

65. Soriano M, Manchón F. Radiological aspects of a new type of bone fluorosis, periostitis deformans. Radiology 1966; 87:1089–1094.

66. Scheller MS, Saidman LJ, Partridge BL. MAC of sevoflurane in humans and the New Zealand white rabbit. Can J Anaesth 1988;35:153–156.

67. Kimura T, Watanabe S, Asakura N, Inomata S, Okada M, Taguchi M. Determination of end-tidal sevoflurane concentration for tracheal intubation and minimum alveolar anesthetic concentration in adults. Anesth Analg 1994;79: 378–381.

68. Goldberg ME, Cantillo J, Gratz I, Deal E, Vekeman D, McDougall R, et al. Dose of compound A, not sevoflurane, determines changes in the biochemical markers of renal injury in healthy volunteers. Anesth Analg 1999;88: 437–445.

69. Patel SS, Goa KL. Sevoflurane a review of its pharmacodynamic and pharmacokinetic properties and its clinical use in general anaesthesia. Drugs 1996;51:658–700.

70. Kharasch ED. Biotransformation of sevoflurane. Anesth Analg 1995;81(suppl 6):S27–S38.

71. Shiraishi Y, Ikeda K. Uptake and biotransformation of sevoflurane in humans: a comparative study of sevoflurane with halothane, enflurane, and isoflurane. J Clin Anesth 1990;2:381–386.

72. Kharasch ED, Hankins DC, Thummel KE. Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. Anesthesiology 1995;82:689–699.

73. Kharasch ED, Karol MD, Lanni C, Sawchuk R. Clinical sevoflurane metabolism and disposition. I. Sevoflurane and metabolite pharmacokinetics. Anesthesiology 1995;82:1369–1378.

74. Kharasch ED, Armstrong AS, Gunn K, Artru A, Cox K, Karol MD. Clinical sevoflurane metabolism and disposition II. The role of cytochrome P450 2E1 in fluoride and hexafluoroisopropanol formation. Anesthesiology 1995;82: 1379–1388.

75. Paventi S, Santevecchi A, Ranieri R. Effects of sevoflurane versus propofol on QT interval. Minerva Anesthesiol 2001;67:637–640.

76. Kuenszberg E, Loeckinger A, Kleinsasser A, Lindner KH, Puehringer F, Hoermann C. Sevoflurane progressively prolongs the QT interval in unpremedicated female adults. Eur J Anaesthesiol 2000;17:662–664.

77. Cantrell FL. A fatal case of sevoflurane abuse. Clin Toxicol 2008;46:918–919.

78. Rosales CM, Young T, Laster MJ, Eger EI II, Garg U. Sevoflurane concentrations in blood, brain, and lung after sevoflurane-induced death. J Forensic Sci 2007;52: 1408–1410.

79. Burrows DL, Nicolaides A, Stephens GC, Ferslew KE. The distribution of sevoflurane in a sevoflurane induced death. J Forensic Sci 2004;49:394–397.

80. Levine B, Cox D, Jufer-Phipps RA, Li L, Jacobs A, Fowler D. A fatality from sevoflurane abuse. J Anal Toxicol 2007; 31:534–536.

Chapter 39

HALOTHANE

HISTORY

Halothane was introduced into clinical practice in 1956 and by the 1970s, this volatile chemical was the most common general anesthetic agent. In the 1960s, cases reports of postanesthetic jaundice following halothane anesthesia appeared in the medical literature; soon thereafter, the National Halothane Study began an investigation the mechanism of the hepatotoxicity associated with halothane.[1] The incidence of massive hepatic necrosis in the National Halothane Study was 1:10,000.[2] The cause of the liver damage remains controversial with several proposed mechanism including hypersensitivity reaction, toxic metabolites, and nutritional factors. The use of halothane as an anesthetic has dramatically declined because of the introduction of safer drugs.

IDENTIFYING CHARACTERISTICS

Figure 39.1 displays the chemical structure of halothane (2-bromo-2-chloro-1,1,1-trifluoroethane, CAS RN: 151-67-7). This compound is the only modern volatile anesthetic that is not a halogenated methyl ethyl ether. This compound (Freon 123B1) is a nonflammable, colorless liquid with a pleasant, sweet, chloroform-like odor. The vapor pressure at 20°C (68°F) is 241 mm Hg. Table 39.1 lists some physiochemical properties of halothane. Halothane is the most soluble modern anesthetic; the blood/gas solubility of halothane is 2.3, resulting in slow induction and recovery characteristics. This paradoxical property causes the slow equilibrium of alveolar-arterial tension as a result of the rapid removal of halothane from alveolar air and the prolonged equilibration of inspired and alveolar air.[3] The minimum alveolar concentration (MAC) of halothane is 0.74%, but this concentration decreases in the presence of 70% nitrous oxide to 0.29%.[4]

Halothane is less stable in light than enflurane and isoflurane; consequently, the storage of halothane requires the use of a stabilizing agent (e.g., thymol). Ultraviolet light oxidizes halothane to free chlorine and bromine ions, HCl, HBr, and phosgene ($COCl_2$). Rubber readily absorbs halothane, resulting in the decreased delivery of halothane through rubber tubing.

EXPOSURE

Halothane abuse occurs primarily among medical personnel. Reported desired effects of halothane abuse include euphoria similar to sniffing glue; the route of exposure typically involves sniffing from a rag soaked in halothane or inhalation of vapors from a plastic bag containing halothane.[5] The abuse of halothane may continue until the individual becomes unconscious. Severe hepatic necrosis is a rare, immune-related complication of halothane anesthesia that occurs at a ratio of about 1:35,000.[6]

DOSE EFFECT

The minimum alveolar concentration (MAC) is a measure of the concentration of an inhaled anesthetic required to block purposeful movement in an individ-
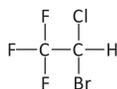
FIGURE 39.1. Chemical structure of halothane.

Table 39.1. Physiochemical Properties of Halothane.

Physical Property	Value
Melting Point	–118°C (–180.4°F)
Boiling Point	50.2°C (122.36°F)
log P (Octanol-Water)	2.3
Water Solubility	4070 mg/L (25°C/77°F)
Vapor Pressure	302 mm Hg (25°C/77°F)

ual. This measure is the accepted standard measure of the clinical potency of inhaled anesthetics. A dose of 1 MAC will prevent muscle movement following surgical incision in 50% of patients. Typically, anesthetics are administered in concentrations of 0.3–1.5% along with a carrier gas (e.g., oxygen or oxygen plus nitrous oxide). The MAC for the first plane of surgical anesthesia with halothane is approximately 0.7% compared with 1.4% for isoflurane and 0.16% for methoxyflurane. In animal studies, the administration of 2–2.5 times the minimum alveolar concentration (MAC) of halothane causes apnea.[7]

TOXICOKINETICS

Biotransformation

The liver metabolizes up to approximately 20–40% of the absorbed dose of halothane via an oxidative pathway and, to a much lesser extent, via a reductive pathway.[8] Under sufficient oxygen tension, metabolites of oxidative dehalogenation of halothane include trifluoroacetic acid, trifluoroacetyl chloride, inorganic bromide, and chloride. Following acetylation, trifluoroacetyl chloride reacts with nucleophilic centers near the site of generation, forming covalent bonds with liver proteins and phospholipids. The cytochrome P450 isoenzymes, CYP2E1 and to a lesser extent CYP2A6, catalyze the oxidative pathway. A minor metabolic pathway (i.e., 1–6%) involves the reduction of halothane via CYP2A6 and to a lesser extent CYP3A4.[9] *In vitro* studies indicate that CYP inhibitors do not reduce the formation of reductive metabolites.[10] The metabolites formed by the reductive pathway include inorganic fluoride, 2-chloro-1,1,1-trifluoroethane, and 2-chloro-1-1-difluoroethylene.[11] The reductive biotransformation of halothane is more extensive in women than in men.[12] Hypoxia substan-

tially increases the reductive biotransformation of halothane.[13] During halothane anesthesia, the concentration of fluoride in venous samples remains relatively stable.[14]

Elimination

The elimination of halothane is relatively rapid following cessation of exposure, primarily by pulmonary excretion in expired air. About 20–50% of the absorbed dose of halothane is metabolized in a dose-dependent manner. In a study of 5 patients undergoing halothane anesthesia (mean, 0.93%), the mean percentage of halothane recovered in expired air was 72.8% ± 9.2%.[15] The mean percentage of the absorbed dose of halothane recovered in the urine as metabolites was 17.7%. At lower alveolar concentrations, more halothane is metabolized, but the percentage of unchanged halothane excreted in expired air increases as the metabolic enzymes become saturated.[8] Saturation of these metabolic enzymes occurs at about 2–10% of the MAC.[16] In a case series of 10 patients undergoing halothane anesthesia (0.75%), the mean halothane concentration in venous blood decreased from 68.2 ± 35.7 mg/L at the end of anesthesia to 7.3 ± 5.0 mg/L 3 hours later.[14] Peak concentrations of halothane metabolites occur about 24 hours after the cessation of anesthesia.[17]

Drug Interactions

Synergistic depression of the central nervous system (CNS) may occur following the concomitant use of CNS depressants (e.g., ethanol) and halothane.[18] *In vitro* and *in vivo* animal experiments suggest halothane alters the pharmacokinetics of many drugs by inducing enzyme activity and changing drug-protein binding. Enzyme-inducing drugs (e.g., phenytoin, phenobarbital) enhance the reductive biotransformation of halothane, whereas the ingestion of ethanol does not alter reductive halothane metabolism.[19] Potentially, the halothane metabolite, trifluoroacetic acid may displace drugs (e.g., phenytoin, diazepam) from binding sites on serum albumin and increase the amount of free (active) drug.[20] The presence of nitrous oxide does not significantly affect the pharmacokinetics of halothane.[21]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

The administration of halothane causes a dose-dependent reduction in systemic arterial pressure as a result of a 20–50% reduction in cardiac output with

665

a corresponding reflex tachycardia.[22] There is minimal reduction in the total peripheral vascular resistance. Halothane depresses the ventilatory response to carbon dioxide and hypoxia, resulting in carbon dioxide retention and decreased sensitivity of peripheral chemoreceptors.[23]

Volatile halogenated anesthetics affect most of the molecular targets of general anesthetics including enhancement of $GABA_A$ activity, inhibition of glutamate and nicotinic acetylcholine receptors, and activation of potassium leak channels (e.g., TREK-1).[24] Additionally, volatile halogenated anesthetics act on glycine receptors and mediators of neurotransmitter release. Animal studies indicate that halothane depresses glutamatergic synaptic transmission in the hippocampus by the presynaptic inhibition of transmitter release and postsynaptic depression of glutamate currents.[25]

Mechanism of Toxicity

Halothane exhibits the most negative inotropic and myocardial sensitizing effects of all the modern inhaled anesthetics. *In vitro* studies indicate that halothane inhibits muscarinic receptor regulation of myocardial adenylyl cyclase activity and stimulates G protein-dependent adenylyl cyclase activity.[26,27] The most common injury in halothane-treated patients is mild, transient hepatocellular injury usually manifest by asymptomatic elevation of serum hepatic aminotransferase concentrations that reflect alterations of cellular integrity seen by electron microscopy. Halothane anesthesia is not associated with renal impairment except in the setting of fulminant hepatic failure. The hepatic lesions result from reactive intermediates (e.g., trifluoroacetyl chloride) produced by the oxidative and reductive pathways of halothane in combination with local hypoxia. The generation of reactive intermediates via CYP2E1-mediated oxidation of halothane causes trifluoroacetylation of intracellular proteins. In animal studies, the histologic damage is dose-dependent.[28] In susceptible individuals, the formation of antibodies directed toward the acetylated neoantigens (i.e., trifluoroacetylated proteins in the endoplasmic reticulum) produced by reactive intermediates causes the more severe form of hepatic injury. Current evidence suggests that exposure to halothane anesthesia causes the formation of a wide variety of trifluoroacetylated proteins, but only a small subset of susceptible individuals produce an immunologic reaction to these proteins.[29] Histologic examinations of liver biopsies from patients with severe halothane anesthesia-associated hepatitis demonstrate massive hepatocellular necrosis. The pathologic findings are virtually indistinguishable from fulminant viral hepatitis.[30]

Halothane is a respiratory depressant that reduces tidal volume as the concentration of halothane increases. This compound is also a cardiac depressant that reduces myocardial contractility in a dose-dependent manner. Dysrhythmias result primarily from hypercarbia secondary to respiratory depression.[30]

Postmortem Examination

Postmortem findings of patients dying following halothane abuse are nonspecific, typical of volatile substance abuse. The most common abnormalities in these patients are pulmonary congestion and edema without evidence of hepatorenal damage.[5,31]

CLINICAL RESPONSE

Illicit Use

Halothane anesthesia is associated with the following 2 types of liver damage: 1) type I halothane hepatitis—a common asymptomatic elevation of serum hepatic aminotransferases, and 2) type II halothane hepatitis—a rare, idiopathic, severe hypersensitivity-mediated hepatitis directed against hepatocyte.[32] The more severe form occurs in about 1 in 35,000 halothane exposures, whereas the mild form of hepatitis occurs in up to one-fourth of halothane-induced anesthesia.[33] Major risk factors for the hypersensitivity hepatitis are repeated exposure to halothane anesthesia and the use of halothane in middle-aged, obese women. Clinical features of halothane-associated hepatitis are typical of acute hepatitis including fever, anorexia, vomiting, malaise, jaundice, light-colored stools, tea-colored urine, and right upper quadrant tenderness.[34] Signs and symptoms of an immune reaction (e.g., fever, arthralgias, rash, peripheral eosinophilia) may also occur. Typically, symptoms appear about 5–21 days after anesthesia with the abrupt onset of fever and chills followed in several days by jaundice. Cirrhosis does not usually develop following type II halothane hepatitis. Case reports associated both fulminant hepatic failure and asymptomatic elevation of serum hepatic aminotransferases with halothane abuse.[35]

Malignant hyperthermia is a rare complication of halothane anesthesia.[36] This pharmacogenetic disorder of skeletal muscle presents as a hypermetabolic response to potent volatile anesthetic gases including halothane. Classical features of malignant hyperthermia are muscle rigidity, hyperthermia, tachycardia, tachypnea, hypercarbia, increased oxygen consumption, and acidosis. Rhabdomyolysis may also occur along with renal failure and pulmonary edema.

Reproductive Abnormalities

There are few data on the effect of halothane abuse on reproductive outcomes. Animal studies do not indicate that halothane is teratogenic in typical anesthetic doses. There were no major or minor teratologic effects in the offspring of pregnant Sprague-Dawley rats exposed to 0.8% halothane 6 hours daily for 3 consecutive days in 1 of 3 periods (i.e., pregnancy days 14–16, 11–13, or 8–10).[37] In a study of Swiss/ICR mice, the incidences of major malformations and minor anomalies did not increase following exposure to subanesthetic concentrations of halothane.[38] Daily anesthetic exposure to 4.0 MAC hours was lethal to both dams and embryos, and this exposure resulted in major developmental malformations in surviving fetuses.

DIAGNOSTIC TESTING

Analytic Methods

Techniques to quantitate halothane and other halogenated ether anesthetics include clinical infrared gas analyzer,[39] gas chromatography with flame ionization detection (GC/FID),[40] headspace solid-phase or capillary gas chromatography/mass spectrometry,[41,42] and gas chromatography/mass spectrometry in selected ion monitoring mode.[43] The detection limit of simple gas chromatography ranges from 0.010–0.100 mg/L, whereas the accuracy (% CV) of GC/FID ranges between 0.8–9.5%.

Biomarkers

In a case series of 10 patients undergoing halothane anesthesia (0.75%), the mean halothane concentration in venous samples at the end of 1 hour of anesthesia was 68.2 ± 35.7 mg/L.[14] Paramedics on the scene began cardiopulmonary resuscitation of a 19-year-old man who was found comatose. On admission to the emergency department, he had clinical features of severe hypoxic encephalopathy and the blood halothane concentration was 200 mg/L.

Following analysis by gas chromatography, the halothane concentrations in postmortem blood samples from 2 men abusing halothane were 0.36% and 0.50% compared with halothane concentrations of 1–2% during halothane anesthesia.[5] Both men were found in cardiopulmonary arrest; resuscitation measures continued for 1 hour and 2 hours, respectively. A 26-year-old anesthetist was found dead holding a polyvinyl bag with a gauze pad in his mouth. The postmortem blood halothane concentration was 200 mg/L.[44] Similar to other volatile substances of abuse, the halothane concentration present on analysis depends on the analytic method, storage conditions (container, temperature), and postmortem interval. The concentration of halothane in erythrocytes is slighter greater than plasma; the whole blood/plasma ratio of halothane is ~1 based on *in vitro* studies.[45]

Abnormalities

Halothane hepatitis is associated with elevations of hepatic serum aminotransferases and bilirubin along with variable increases in serum alkaline phosphatase concentrations. Hepatic encephalopathy may occur along with severe liver failure, elevated serum ammonia, coagulation abnormalities, and hypoglycemia. A case series of patients undergoing oral surgery with halothane-nitrous-oxide anesthesia reported the development of cardiac dysrhythmias in 44 of the 103 procedures.[46] There were 2 episodes of transient ventricular tachycardia that did not require treatment. Two other patients required treatment for cyanosis that promptly resolved with ventilation. The study did not report potential confounding factors including pre-existing heart disease, electrolyte imbalance, pH, or oxygen saturation. Anesthetic doses of halothane prolong the action potential of the heart. In a study of 8 healthy adults undergoing anesthesia with only halothane (0.81 ± 0.06 mM), the mean QT interval increased from 380 ± 10 msec to 450 ± 10 msec, while the mean QTc interval increased from 390 ± 10 msec to 440 ± 20 msec.[47] Halothane administration did not significantly alter the PR interval or QRS duration. There were no clinically significant dysrhythmias. Inorganic fluoride concentrations are not direct measures of the reductive biotransformation of halothane because of variation in the dietary fluoride intake and the equilibrium of fluoride between bone stores and the systemic circulation.

Driving

Although high concentrations of halothane are detectable by some breathalyzers, these readings are unlikely to interfere with the estimation of breath alcohol concentration at halothane concentrations likely to be present in ambulatory individuals. For example, under experimental conditions a 1% concentration of halothane produced an alcohol reading of 0.029 mg/dL on an older Camic breathalyzer.[48]

TREATMENT

Respiratory depression and cardiac dysrhythmias represent the most immediate life-threatening

complications of halothane exposure; immediate attention should be directed toward maintenance of ventilation and blood pressure with intubation, mechanical ventilation, intravenous fluids, and cardiac monitoring as indicated by the history and physical examination. Methods to enhance elimination of halothane have not been studied. If severe liver dysfunction develops, supportive care includes fresh frozen plasma, vitamin K, low protein diet, neomycin, lactulose, careful fluid and electrolyte, and the use of dialysis as needed for associated renal dysfunction. Hepatic and renal function should be followed daily for at least 3 days after serious abuse of halothane. Liver transplantation (orthotopic, auxiliary partial orthotopic, heterotopic) is the only therapeutic option for advanced fulminant hepatic failure.

References

1. Bunker JP, Blumenfeld CM. Liver necrosis after halothane anesthesia. Cause or coincidence? N Engl J Med 1963;268:531–534.

2. Subcommittee on the National Halothane Study. Summary of the national Halothane Study. Possible association between halothane anesthesia and postoperative hepatic necrosis. JAMA 1966;197:775–788.

3. Fukui Y, Smith NT. Interactions among ventilation, the circulation, and the uptake and distribution of halothane—use of a hybrid computer multiple model: II. Spontaneous vs. controlled ventilation, and the effects of CO2. Anesthesiology 1981;54:119–124.

4. Saidman LJ, Eger EI 2nd. Effect of nitrous oxide and of narcotic premedication on the alveolar concentration of halothane required for anesthesia. Anesthesiology 1964;25:302–306.

5. Spencer JD, Raasch FO, Trefny FA. Halothane abuse in hospital personnel. JAMA 1976;235:1034–1035.

6. Reichle FM, Conzen PF. Halogenated inhalational anaesthetics. Best Prac Res Clin Anaesthesiol 2003;17:29–46.

7. Brandstater B, Eger EI II, Edelist G. Effects of halothane, ether and cyclopropane on respiration. Br J Anaesth 1965;37:890–897.

8. Carpenter RL, Eger EI 2nd, Johnson BH, Unadkat JD, Sheiner LB. The extent of metabolism of inhaled anesthetics in humans. Anesthesiology 1986;65:201–205.

9. Spracklin DK, Kharasch ED. Human halothane reduction in vitro by cytochrome P450 2A6 and 3A4: identification of low and high KM isoforms. Drug Metab Dispos 1998; 2:605–607.

10. Kharasch ED, Hankins DC, Fenstamaker K, Cox K. Human halothane metabolism, lipid peroxidation, and cytochromes P4502A6 and P4503A4. Eur J Clin Pharmacol 2000;55:853–859.

11. Maiorino RM, Sipes IG, Gandolfi AJ, Brown BR Jr, Lind RC. Factors affecting the formation of chlorotrifluoroethane and chlorodifluoroethylene from halothane. Anesthesiology 1981;54:383–389.

12. Jenner MA, Plummer JL, Cousins MJ. Halothane reductive metabolism in an adult surgical population. Anaesth Intensive Care 1990;18:395–399.

13. Gourlay GK, Adams JF, Cousins MJ, Sharp JH. Time-course of formation of volatile reductive metabolites of halothane in humans and an animal model. Br J Anaesth 1980;52:331–336.

14. Imbenotte M, Erb F, Goldstein P, Erb C, Scherpereel P. Halothane and enflurane metabolite elimination during anaesthesia in man. Eur J Anaesthesiol 1987;4:175–182.

15. Sakai T, Takaori M. Biodegradation of halothane, enflurane and methoxyflurane. Br J Anaesth 1978;50:785–791.

16. Sawyer DC, Eger EI 2nd, Bahlman SH, Cullen BF, Impelman D. Concentration dependence of hepatic halothane metabolism. Anesthesiology 1971;34:230–235.

17. Dale O, Brown BR Jr. Clinical pharmacokinetics of the inhalational anaesthetics. Clin Pharmacokinet 1987;12: 145–167.

18. Caranasos GJ. Drug reactions and interactions in patient undergoing surgery. Med Clin North Am 1979;63: 1245–1255.

19. Knights KM, Gourlay GK, Hall PD, Adams JF, Cousins MJ. Halothane hepatitis in an animal model: time course of hepatic damage. Br J Exp Pathol 1987;68:613–624.

20. Dale O, Gandolfi AJ, Brendel K, Schuman S. Rat liver slices and diazepam metabolism: *in vitro* interactions with volatile anaesthetic drugs and albumin. Br J Anaesth 1988; 60:692–696.

21. Cahalan MK, Johnson BH, Eger EI. Relationship of concentrations of halothane and enflurane to their metabolism and elimination in man. Anesthesiology 1981;54:3–8.

22. Filner BE, Karliner JS. Alterations of normal left ventricular performance by general anesthesia. Anesthesiology 1976;45:610–621.

23. Knill RL, Gelb AW. Ventilatory responses to hypoxia and hypercapnia during halothane sedation and anesthesia in man. Anesthesiology 1978;49:244–251.

24. Solt K, Forman SA. Correlating the clinical actions and molecular mechanisms of general anesthetics. Curr Opin Anaesthesiol 2007;20:300–306.

25. Perouansky M, Kirson ED, Yaari Y. Mechanism of action of volatile anesthetics: effects of halothane on glutamate receptors *in vitro*. Toxicol Lett 1998;100-101:65–69.

26. Böhm M, Schmidt U, Gierschik P, Schwinger RH, Böhm S, Erdmann E. Sensitization of adenylate cyclase by halothane in human myocardium and S49 lymphoma wild-type and cyc-cells: evidence for inactivation of the inhibitory G protein Gi alpha. Mol Pharmacol 1994;45:380–389.

27. Vulliemoz Y, Verosky M. Halothane interaction with guanine nucleotide binding proteins in mouse heart. Anesthesiology 1988;69:876–880.

28. Lind RC, Gandolfi AJ, Hall PD. Subanesthetic halothane is hepatotoxic in the guinea pig. Anesth Analg 1992;74:559–563.

29. Gut J. Molecular basis of halothane hepatitis. Arch Toxicol 1988;20(suppl):3–17.

30. Corbett TH. Pharmacology and toxicology of halogenated anesthetics. Adv Pharmacol Chemother 1979;16:195–212.

31. Block S, Rosenblatt R. A halothane-abuse fatality. Anesthesiology 1980;52:524.

32. Wright R, Eade OE, Chisholm M, Hawksley M, Lloyd B, Moles TM, et al. Controlled prospective study of the effect on liver function of multiple exposures to halothane. Lancet 1975;1(7911):817–820.

33. Neuberger J. Halothane hepatitis. Eur J Gastroenterol Hepatol 1998;10:631–633.

34. Tucker SC, Patteson TE. Hepatitis and halothane sniffing. Ann Intern Med 1974;80:667–668.

35. Kaplan HG, Bakken J, Quadracci L, Schubach W. Hepatitis caused by halothane sniffing. Ann Intern Med 1979;90:797–798.

36. Rosenberg H, Davis M, James D, Pollock N, Stowell K. Malignant hyperthermia. Orphanet J Rare Dis 2007;2:21.

37. Mazze RI, Fujinaga M, Rice SA, Harris SB, Baden JM. Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane in Sprague-Dawley rats. Anesthesiology 1986;64:339–344.

38. Wharton RS, Wilson AI, Mazze RI, Baden JM, Rice SA. Fetal morphology in mice exposed to halothane. Anesthesiology 1979;51:532–537.

39. Peyton PJ, Chong M, Stuart-Andrews C, Robinson GJ, Pierce R, Thompson BR. Measurement of anesthetics in blood using a conventional infrared clinical gas analyzer. Anesth Analg 2007;105:680–687.

40. Atherley RJ, Antognini JF. A rapid and simple method for determination of halothane, isoflurane and sevoflurane in blood using gas chromatography. Biomed Chromatogr 2004;18:714–718.

41. Kojima T, Ishii A, Watanabe-Suzuki K, Kurihara R, Seno H, Kumazawa T, et al. Sensitive determination of four general anaesthetics in human whole blood by capillary gas chromatography with cryogenic oven trapping. J Chromatogr B Biomed Sci Appl 2001;762:103–108.

42. Musshoff F, Junker H, Madea B. Rapid analysis of halothane in biological samples using headspace solid-phase microextraction and gas chromatography-mass spectrometry—a case of a double homicide. J Anal Toxicol 2000;24:372–376.

43. Accorsi A, Morrone B, Raffi GB, Violante FS. High-speed capillary gas chromatography for determination of inhalation anesthetics. J Chromatogr A 2005;1071:81–84.

44. Yamashita M, Matsuki A, Oyama T. Illicit use of modern volatile anaesthetics. Can Anaesth Soc J 1984;31:76–79.

45. Pang YC, Reid PE, Brooks DE. Solubility and distribution of halothane in human blood. Br J Anaesth 1980;52:851–862.

46. Fisch C, Oehler RC, Miller JR, Resish CH. Cardiac arrhythmias during oral surgery with halothane-nitrous-oxide-oxygen anesthesia. JAMA 1969;208:1839–1842.

47. Schmeling WT, Warltier DC, McDonald DJ, Madsen KE, Atlee JL, Kampine JP. Prolongation of the QT interval by enflurane, isoflurane, and halothane in humans. Anesth Analg 1991;72:137–144.

48. Dunbar JA, Macrae WA, Murphie JH, Whittet D, Mather AM. Evidential breath testing of drivers—day surgery and halothane anaesthesia. Med Sci Law 1985;25:162–164.

Chapter 40

NITROUS OXIDE

HISTORY

Priestley discovered nitrous oxide in 1776. In 1868, pressurized liquid nitrous oxide was available in metal cylinders, and the use of nitrous oxide as an adjunct to diethyl ether anesthesia began. Humphry Davy recognized the anesthetic and euphoric properties of this compound in the latter part of the 18th century.[1] Some of his study participants experienced pleasurable effects from the inhalation of nitrous oxide. However, Humphry Davy also reported that chronic heavy use of nitrous oxide was associated with sensory abnormalities and a mental disorder. The lack of availability and the low potency of nitrous oxide limited the abuse of this compound. In the latter 19th century, this compound was inhaled at social gatherings similar to diethyl ether (ether).[2] Of the early anesthetics (chloroform, diethyl ether), nitrous oxide is the only anesthetic remaining in regular use; however, the use of nitrous oxide has decreased in recent years.

IDENTIFYING CHARACTERISTICS

Nitrous oxide (CAS RN: 10024-97-2, N_2O) is a nonflammable, stable, colorless, odorless gas that is heavier than air (vapor density = 1.53, air = 1). The molecular weight of nitrous oxide is 44.01 g/mol. This gas has a slightly sweet taste. The low blood/gas solubility of nitrous oxide results in rapid equilibration between alveoli and blood along with rapid penetration of the blood–brain barrier. Nitrous oxide achieves approximately 90% equilibration between inspired gas and alveoli within 10 minutes.[3]

Whippets refers to whipped cream dispensers used recreationally for the abuse of nitrous oxide.

EXPOSURE

Epidemiology

Nitrous oxide is an occasional drug of abuse among medical and dental personnel. Adolescents and young adults also abuse the nitrous oxide present as a propellant in dairy products (e.g., whippets). In a study of juveniles in a correctional facility, nitrous oxide was 1 of the 5 most abused volatile substances after gasoline, Freon, butane lighter fluid, and glue.[4]

Sources

Nitrous oxide is an anesthetic and analgesic gas that has been investigated as a drug (psychotropic analgesic nitrous oxide) to reduce the cravings associated with withdrawal from alcohol, cocaine, and methaqualone.[5,6] This gas is the only inorganic gas used for clinical anesthesia. Nitrous oxide is also a propellant in whipped cream.

Methods of Abuse

Abuse of nitrous oxide typically involves the inhalation of the contents of nitrous oxide-containing containers or inhalation from a balloon filled with the gas. The euphoria usually begins within 15–30 seconds of the beginning of inhalation with peak effects occurring in

~2–3 minutes.[7] The euphoria is associated with a sensation of warmth in the head and auditory illusions. Rebreathing the nitrous oxide prolongs these effects. Case series suggest that chronic nitrous oxide use is associated with medical and psychiatric complication of addiction, but the incidence of addiction to nitrous oxide is extremely low.[8]

DOSE EFFECT

Illicit Use

The minimum alveolar concentration (MAC) is a measure of anesthetic potency as reflected in the concentration or percent of inspired gas at 1 atmosphere (atm) necessary to render 50% of patients unresponsive to surgical stimulus. Because the MAC reflects an adequate dose for only 50% of the patient, successful clinical anesthesia requires 0.5–2 MAC for an individual patient. Nitrous oxide is the least potent anesthetic in modern practice with a MAC of 105; however, MAC is a measure of anesthetic potency rather than a precise measure of respiratory or cardiac effects. The effect of chronically inhaling nitrous oxide depends, in part, on the current intake and the pool of vitamin B_{12} at the time of abuse. The inhalation of approximately 40–60 whipped cream bulbs daily for the preceding 6 months was associated with the development of paresthesias, gait disturbance, and signs of an axonal peripheral neuropathy.[9] In a case series of 13 dentists with nitrous oxide abuse and myeloneuropathy, the reported period of abuse ranged from 3 months to several years.[10]

Toxicity

Subanesthetic concentrations of nitrous oxide for sedation, amnesia, and analgesia in dentistry and minor surgical procedures range from about 20–30% atm. At these concentrations, human behavioral studies indicate that some impairment of psychomotor function (tapping rate, choice reaction time),[11] learning,[12] cognition (word recall, arithmetic tests.),[13] and memory[14] occurs. These effects resolve rapidly (i.e., within minutes) after cessation of use. The anesthetic potency of nitrous oxide is low, and satisfactory anesthesia is not possible without simultaneous anoxia. At least 30% oxygen under normobaric conditions are necessary to overcome ventilation perfusions changes associated with general anesthesia; typical concentrations of nitrous oxide for anesthesia with controlled ventilation is 65–70%.[15] The maximum tolerable mixture of nitrous oxide and oxygen for prolonged inhalation is 65% and 35%, respectively. The inhalation of 85% nitrous oxide with oxygen is tolerable only for brief exposures.

TOXICOKINETICS

Kinetics

The rate of uptake of a gas depends on the following factors: 1) ventilation, 2) blood/gas solubility coefficient, 3) alveolar/blood partial pressure difference, 4) cardiac output, and 5) diffusion of the gas across the alveolar membrane.[16] Based on measurements of nitrous oxide at the mouth, the estimated uptake of nitrous oxide is inversely proportional to the square root of the time elapsed from the start of administration.[17] Using this equation, the estimated uptake of nitrous oxide during the first minute is about 1L compared with 129 mL/min after 60 minutes and 90 mL/min after 2 hours of anesthesia.[18] The lungs excrete nitrous oxide unchanged in exhaled air; the rate of elimination is rapid after inhalation ceases. The nitrous oxide concentration in expired air drops rapidly to about 25% of the initial value; then, the decline is more gradual. Cardiac output has little effect on the elimination of nitrous oxide because of the low solubility of this gas. The placental transfer of nitrous oxide is time-dependent and slower than the maternal uptake of nitrous oxide.[19]

Tolerance

There are few human data on tolerance to the chronic use of nitrous oxide. In rodent studies, tolerance develops to the effects of nitrous oxide in the animals after prolonged exposure (e.g., 18 h exposure to 70% nitrous oxide in Plexiglas chambers followed by a 30-minute recovery before testing).[20]

Drug Interactions

Nitrous oxide is an effective adjunct to the administration of other anesthetics because the concomitant inhalation of these anesthetics with nitrous oxide reduces the MAC of the other anesthetics. However, the reduction of MAC is not consistent between studies, and the effect of nitrous oxide on the MAC of other anesthetics does not correspond to a simple additive relationship.[21] In very high concentrations, nitrous oxide is a cardiorespiratory depressant; consequently, exposure to other central nervous system depressants would be expected to enhance these depressant effects. Nitrous oxide activates supraspinal opioid receptors via corticotropin-releasing factor; however, existing studies are inadequate to determine if the potential interaction of nitrous oxide and opioids is clinically significant.[22]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

The anaesthetic action of nitrous oxide remains unclear. The mechanism of action of nitrous oxide probably involves the release of β-endorphins and the binding of μ-, δ-, and κ-opiate receptors. Animal studies suggest that the analgesic effects of nitrous oxide result from opioid peptide release in the periaqueductal gray area of the midbrain and the subsequent activation of the descending inhibitory pathways and noradrenergic neurons.[23] This action modulates the nociception process in the spinal cord. Implicated receptors include dopamine receptors, benzodiazepine (GABA) receptors, α_2-adrenoceptors, and N-methyl-D-aspartate (NMDA) receptors.[24] Experimental studies indicate that nitrous oxide inhibits NMDA-activated currents, while GABA-activated currents are unaffected.[25] Nitrous oxide may also be a dopamine agonist.[26] Positron emission tomography (PET) scans of volunteers receiving nitrous oxide demonstrate activation in the anterior cingulate cortex (psychomotor and cognitive processes), and deactivation in the posterior cingulate, hippocampus, parahippocampal gyrus, and visual association cortices in both hemispheres.[27] The former two regions mediate learning and memory.

Mechanism of Toxicity

Nitrous oxide is an anesthetic and respiratory depressant. This gas is a mechanical asphyxiant that causes hypoxia by displacing oxygen. In addition, nitrous oxide increases the alveolar-arterial difference of oxygen in the alveoli. Both of these properties cause hypoxia; therefore, prolonged inhalation of pure nitrous oxide will cause asphyxia. Nitrous oxide abuse may cause a posterior column myelopathy (e.g., subacute combined neurodegeneration of the spinal cord) by inactivation of vitamin B_{12} dependent enzymes. Nitrous oxide oxidizes the monovalent cobalt in cobalamin to the bivalent ion, resulting in the inactivation of the vitamin B_{12}-dependent enzyme, methionine synthase. This enzyme catalyzes the formation of tetrahydrofolate and methionine from homocysteine and methyltetrahydrofolate. The purpose of these chemical reactions is the formation of 10-formyl tetrahydrofolate for DNA synthesis and the replenishment of the one-carbon pool. The effect of reduced methionine synthase activity depends, in part, on the pre-existing pool of vitamin B_{12}; individuals with vitamin B_{12} deficiency will experience more adverse effects from the use of nitrous oxide than patients with normal vitamin B_{12} stores.[28] Recovery of methionine synthase activity requires the synthesis of new enzymes (i.e., 3–4 days); however, full recovery may be delayed several weeks.[29] Animal studies suggest that the euphoric effects of nitrous oxide are mediated by the partial opioid agonist properties of this gas.[30]

Postmortem Examination

The postmortem examinations of individual dying during the abuse of nitrous oxide are nonspecific including contraction band necrosis in the myocardium.[31] In a case series of 4 fatalities related to the use of nitrous oxide, the only postmortem finding was visceral congestion.[32]

CLINICAL RESPONSE

The clinical response to nitrous oxide varies with some patients experiencing euphoria, while others experience little or even unpleasant effects. Descriptions of the euphoric effects associated with nitrous oxide include numbness, lightheadedness, tingling, warmth, stiffness, and auditory hallucinations. In a study of volunteers receiving 30% nitrous oxide, inhalation was associated with a dreamy, detached state characterized by euphoric mood, changes in body image, and alteration of time perception.[33] Adverse effects of nitrous oxide anesthesia include hypoxia, claustrophobia, and nausea.

Complications

Nitrous oxide abuse is associated with a sensorimotor polyneuropathy characterized by broad-based gait, ataxia, and sensory loss (vibration, position sense). The clinical presentation of these neurologic changes is virtually identical to subacute combined degeneration with posterior and lateral column injury associated with pernicious anemia. Case reports associate chronic abuse of nitrous oxide (whippets) with an ascending sensorimotor neuropathy and gait disturbances similar to Guillain-Barré syndrome.[34] Clinical features include paresthesias in the extremities, loss of coordination, gait disturbances, generalized weakness, brisk reflexes in the distal portion of the extremities, a positive Romberg sign, impotence, sphincter disturbances, and reduced proprioception with preservation of temperature and pain sensation.[9,35] Occasionally, Lhermitte sign (i.e., an electric sensation transmitted down the spine during neck flexion or rotation) is present. Cognitive ability is usually unaffected in the absence of acute intoxication. However, some case reports of nitrous oxide abuse document both impairment of cognitive function (recall, word list generation) and reduced temperature and pain sensation in a stocking-glove distribution.[36] Typically, the peripheral neuropathy gradually improves with cessation of exposure.

Abstinence Syndrome

Withdrawal symptoms are usually not associated with nitrous oxide abuse.

Reproductive Abnormalities

Some epidemiologic studies of medical personnel suggest that spontaneous abortions may increase following chronic low-level exposure to nitrous oxide in surgical or dental operating suites.[37] However, these studies are limited by failure to control for confounding variables and poor exposure data (e.g., the use of job title as a surrogate for nitrous oxide exposure). More recent studies in hospitals using scavenger equipment to reduce nitrous oxide concentrations have not detected an increased incidence of reproductive abnormalities in exposed women.[38,39] There are few data on the effect of nitrous oxide abuse in pregnant women.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the determination of nitrous oxide in biologic samples include gas chromatography with microionization cross-section detection,[40] gas chromatography with thermal conductivity detection,[41] capillary gas chromatography/mass spectrometry in selected ion monitoring,[42] and headspace gas chromatography/mass spectrometry.[43]

Biomarkers

The nitrous oxide concentration in operating theater personnel depends on the effectiveness of scavenging devices.[44] The mean nitrous oxide concentrations in the breathing zones of anesthesiologists practicing in operating rooms with and without scavenging devices were 57 ppm (range, 6–203 ppm) and 534 ppm (range, 52–2,385 ppm), respectively.[45] The mean nitrous oxide concentrations in the blood of the anesthesiologists in rooms with scavenging devices was ~12 mg/L (~12 ppm), whereas the nitrous oxide concentration in the blood of anesthesiologists in the operating rooms without scavenging devices was up to 10-fold higher. In a study of 80 operating theater personnel, the mean nitrous oxide concentration in air at the end of the operation was 0.049 mg/L as measured by personal passive samplers at collar height.[46] The mean concentrations of nitrous oxide in blood and urine samples collected simultaneously with the air samples was 0.023 mg/L and 0.026 mg/L, respectively. Isolated urine samples of exceptionally high urinary nitrous oxide were attributed to asymptomatic urinary tract infections.

In a case series of 4 individuals found dead following the use of nitrous oxide inside a car, the concentration of nitrous oxide in postmortem blood samples (location not specified) ranged from 46,000 to 93,000 ppm (reported as 4.6–9.3 mL/dL) as measured by headspace gas chromatography with thermal conductivity detection.[32] A 26-year-old man in the same series was found dead in bed with a tube connected to his mouth from a tank of nitrous oxide. His postmortem blood nitrous oxide concentration (site not specified) was 89,700 ppm as measured by the same method.[41]

Abnormalities

Electrodiagnostic testing of patients with peripheral neuropathy associated with nitrous oxide abuse is consistent with patients who have an axonal peripheral neuropathy. Nerve conduction studies demonstrate reduced amplitude and delayed motor/sensory conduction along with prolonged F wave latencies and absent H reflexes.[47] Sensory evoked potentials reveal prolonged latency of scalp-evoked potentials from tibial nerve stimulation, but median nerve values may be normal. Denervation potentials may also appear. The somatosensory evoked potentials from tibial nerve stimulation may be normal to the lumbar spine, but slow conduction often occurs to the cortex.[9] Substantial clinical improvement in these parameters occurs within a year, but minor conduction delays, loss of H reflexes, denervation potentials, or reduced posterior tibial sensory evoked potentials may remain.[48] Abnormalities on sural nerve biopsy are nonspecific, characterized primarily by axonal degeneration.[49] Magnetic resonance imaging (MRI) of the brain is usually normal. MRI findings in patients with neurologic changes suggestive of a demyelinating process include symmetrical T2-weighted hyperintensities and T1-weighted hypointensities of the posterior and lateral columns of the spinal cord, primarily in the cervical and thoracic regions.[50] These MRI changes are not specific to nitrous oxide abuse as these changes occur in other diseases (e.g., human immunodeficiency virus [HIV], lupus, syphilis, Devic disease). Other abnormalities include megaloblastic anemia with increased mean red cell volume, reduced serum vitamin B_{12} concentrations, and low serum folate; however, serum vitamine B12 concentrations may be normal despite the presence of a prominent sensorimotor neuropathy.[34]

Driving

Based on volunteer studies, the effect of nitrous oxide on driving skills diminishes rapidly after cessation of exposure. In a study of 10 dental students, increased errors on a driving simulator were demonstrated within

15 minutes of cessation of exposure to 50–70% nitrous oxide with oxygen.[51] However, the driving of some students improved compared with no exposure. Following the inhalation of self-administered nitrous oxide/oxygen as a sedative/analgesic medication for colonoscopy, scores on psychomotor function returned to normal within 30 minutes.[52] These tests included multiple-choice reaction time, hand–eye coordination, and letter deletion tests. In a study of 80 patients undergoing colonoscopy with a 50% nitrous oxide/50% oxygen mixture, the scores on the adaptive tracking task were compared with baseline scores prior to self-administered analgesia.[53] The mean time between screening and re-testing was approximately 11 minutes (range, 2–30 minutes). There was no statistically significant decline in test scores, and improvement in testing scores of the exposure group was similar to the age-matched control group (no nitrous oxide).

TREATMENT

The treatment of acute exposure to nitrous oxide is supportive with primary focus on the detection and treatment of any respiratory insufficiency. Case reports suggest that supplementation with methionine and vitamin B_{12} may improve the clinical course of the neuropathy associated with nitrous oxide abuse.[54] The dose of methionine is 1 gram 3 times daily. Suggested regimens for the peripheral neuropathy include vitamin B_{12} 100–1,000 µg intramuscularly (IM) daily for 5 days followed by 100–1,000 µg weekly for 5 weeks, then 100 µg to 1,000 µg monthly by mouth until resolution or stabilization of the patient.[55] Other regimens include IM vitamin B_{12} 100 µg daily for 5 days followed by 250 µg daily by mouth for 1 month.[36] Folate supplementation is necessary only when there is a documented folate deficiency.

References

1. Davy H. Researches, chemical and philosophical, chiefly concerning nitrous oxide. London: Butterworth; 1977.
2. Gillman MA. Nitrous oxide abuse in perspective. Clin Neuropharmacol 1992;15:297–306.
3. Becker DE, Rosenberg M. Nitrous oxide and the inhalation anesthetics. Anesth Prog 2008;55:124–131.
4. McGarvey EL, Clavet GJ, Mason W, Waite D. Adolescent inhalant abuse: environments of use. Am J Drug Alcohol Abuse 1999;25:731–741.
5. Gillman MA, Lichtigfeld FJ, Young TN. Psychotropic analgesic nitrous oxide for alcoholic withdrawal states. Cochrane Database Syst Rev 2007;(2):CD005190.
6. Gillman MA, Harker N, Lichtigfeld FJ. Combined cannabis/methaqualone withdrawal treated with psychotropic analgesic nitrous oxide. Int J Neurosci 2006;116: 859–869.
7. Lynn EJ, James M, Dendy R, Harris LA, Walter RG. Nonmedical use of nitrous oxide: a preliminary report. Mich Med 1971;70:203–204.
8. Gillman MA. Nitrous oxide, an opioid addictive agent. Review of the evidence. Am J Med 1986;81:97–102.
9. Butzkueven H, King JO. Nitrous oxide myelopathy in an abuser of whipped cream bulbs. J Clin Neurosci 2000;7: 73–75.
10. Layzer RB. Myeloneuropathy after prolonged exposure to nitrous oxide. Lancet 1978;2(8102):1227–1230.
11. McMenemin IM, Parbrook GD. Comparison of the effects of subanesthetic concentrations of isoflurane or nitrous oxide in volunteers. Br J Anaesth 1988;60:56–63.
12. Mewaldt SP, Ghoneim MM, Choi WW, Korttila K, Peterson RC. Nitrous oxide and human state-dependent memory. Pharmacol Biochem Behav 1988;30:83–87.
13. Korttila K, Ghoneim MM, Jacobs L, Mewaldt SP, Petersen RC. Time course of mental and psychomotor effects of 30 per cent nitrous oxide during inhalation and recovery. Anesthesiology 1981;54:220–226.
14. Block RI, Ghoneim MM, Pathak D, Kumar V, Hinrichs JV. Effects of a subanesthetic concentration of nitrous oxide on overt and covert assessments of memory and associative processes. Psychopharmacology (Berl) 1988;96: 324–331.
15. Leighton KM, Koth B. Some aspects of the clinical pharmacology of nitrous oxide. Can Anaesth Soc J 1973;20: 94–103.
16. Lin C-Y. Nitrous oxide uptake in man: a new concept of uptake of inhalation anesthetics. Acta Anaesthesiol Taiwan 2004;42:127–134.
17. Severinghaus JW. The rate of uptake of nitrous oxide in man. J Clin Invest 1954;33:1183–1189.
18. Stenqvist O. Nitrous oxide kinetics. Acta Anaesthesiol Scand 1994;38:757–760.
19. Karasawa F, Takita A, Fukuda I, Kawatani Y. Nitrous oxide concentrations in maternal and fetal blood during Caesarean section. Eur J Anaesthesiol 2003;20:555–559.
20. Berkowitz BA, Finck AD, Ngai SH. Nitrous oxide analgesia: reversal by naloxone and development of tolerance. J Pharmacol Exp Ther 1977;203:539–547.
21. Christensen LQ, Bonde J, Kampmann JP. Drug interactions with inhalational anesthetics. Acta Anaesthesiol Scand 1993;37:231–244.
22. Sanders RD, Weimann J, Maze M. Biologic effects of nitrous oxide: a mechanistic and toxicologic review. Anesthesiology 2008;109:707–722.
23. Fujinaga M, Maze M. Neurobiology of nitrous oxide-induced antinociceptive effects. Mol Neurobiol 2002;25: 167–189.

24. Ohashi Y, Guo T, Orii R, Maze M, Fujinaga M. Brain stem opioidergic and GABAergic neurons mediate the antinociceptive effect of nitrous oxide in Fischer rats. Anesthesiology 2003;99:947–954.

25. Jevtović-Todorović V, Todorović SM, Mennerick S, Powell S, Dikranian K, Benshoff N, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neuro-toxin. Nature Med 1998:4:460– 463.

26. Murakawa M, Adachi T, Nakao S, Seo N, Shingu K, Mori K. Activation of the cortical and medullary dopaminergic systems by nitrous oxide in rats: a possible neurochemical basis for psychotropic effects and postanesthetic nausea and vomiting. Anesth Analg 1994;78:376–381.

27. Gyulai FE, Firestone LL, Mintun MA, Winter PM. *In vivo* imaging of human limbic responses to nitrous oxide inhalation. Anesth Analg 1996;83:291–298.

28. Landon MJ, Creagh-Barry P, McArthur S, Charlett A. Influence of vitamin B12 status on the inactivation of methionine synthase by nitrous oxide. Br J Anaesth 1992;69:81–86.

29. Bovill JG. Inhalation anaesthesia: from diethyl ether to xenon. Handb Exp Pharmacol 2008;(182):121–142.

30. Hynes MD, Berkowitz BA. Nitrous oxide stimulation of locomotor activity: evidence for an opiate-like behavioral effect. J Pharmacol Exp Ther 1979;209:304–308.

31. Chadly A, Marc B, Barres D, Durigon M. Suicide by nitrous oxide poisoning. Am J Forensic Med Pathol 1989;10:330–331.

32. DiMaio VJ, Garriott JC. Four deaths resulting from abuse of nitrous oxide. J Forensic Sci 1978;23:169–172.

33. Block RI, Ghoneim MM, Kumar V, Pathak D. Psychedelic effects of a subanesthetic concentration of nitrous oxide. Anesth Prog 1990;37:271–276.

34. Tatum WO, Bui DD, Grant EG, Murtagh R. Pseudo-Guillain-Barré syndrome due to "whippet"-induced myeloneuropathy. J Neuroimaging 2010;20:400–402.

35. Diamond AL, Diamond R, Freedman SM, Thomas FP. "Wets"-induced cobalamin deficiency manifesting as cervical myelopathy. J Neuroimaging 2004;14:277–280.

36. Waters MF, Kang GA, Mazziotta JC, DeGiorgio CM. Nitrous oxide inhalation as a cause of cervical myelopathy. Acta Neurol Scand 2005;112:270–272.

37. Cohen EN, Bellville JW, Brown BW Jr. Anesthesia, pregnancy, and miscarriage: a study of operating room nurses and anesthetists. Anesthesiology 1971;35:343–347.

38. Hemminki K, Kyyrönen P, Lindbohm ML. Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs, and other potential hazards in hospitals, based on registered information of outcome. J Epidemiol Community Health 1985;39:141–147.

39. Ericson HA, Källén AJ. Hospitalization for miscarriage and delivery outcome among Swedish nurses working in operating rooms 1973–1978. Anesth Analg 1985;64:981–988.

40. Saloojee Y, Cole P. Estimation of nitrous oxide in blood. Gas chromatographic analysis of trace or analgesic levels. Anaesthesia 1978;33:779–783.

41. Garriott J, Petty CS. Death from inhalant abuse: toxicological and pathological evaluation of 34 cases. Clin Toxicol 1980;16:305–315.

42. Accorsi A, Morrone B, Raffi GB, Violante FS. High-speed capillary gas chromatography for determination of inhalation anesthetics. J Chromatogr A 2005;1071:81–84.

43. Ishimura Y, Gao YT, Panda SP, Roman LJ, Masters BS, Weintraub ST. Detection of nitrous oxide in the neuronal nitric oxide synthase reaction by gas chromatography-mass spectrometry. Biochem Biophys Res Commun 2005;338:543–549.

44. Hillman KM, Saloojee Y, Brett II, Cole PV. Nitrous oxide concentrations in the dental surgery. Anaesthesia 1981;36:257–262.

45. Krapez JR, Saloojee Y, Hinds CJ, Hackett GH, Cole PV. Blood concentrations of nitrous oxide in theatre personnel. Br J Anaesth 1980;52:1143–1148.

46. Brugnone F, Perbellini L, Cerpelloni M, Soave C, Cecco A, Giuliari C. Nitrous oxide in blood and urine of operating theatre personnel and the general population. Int Arch Occup Environ Health 1995;68:22–26.

47. Heyer EJ, Simpson DM, Bodis-Wollner I, Diamond SP. Nitrous oxide: clinical and electrophysiologic investigation of neurologic complications. Neurology 1986;36:1618–1622.

48. Vishnubhakat SM, Beresford HR. Reversible myeloneuropathy of nitrous oxide abuse: serial electrophysiological studies. Muscle Nerve 1991;14:22–26.

49. Sahenk Z, Mendell JR, Couri D, Nachtman J. Polyneuropathy from inhalation of N2O cartridges through a whipped-cream dispenser. Neurology 1978;28:485–487.

50. Pema PJ, Horak HA, Wyatt RH. Myelopathy caused by nitrous oxide toxicity. AJNR Am J Neuroradiol 1998;19:894–896.

51. Moyes D, Cleaton-Jones P, Lelliot J. Evaluation of driving skills after brief exposure to nitrous oxide. S Afr Med J 1979;56:1000–1002.

52. Trojan J, Saunders BP, Woloshynowych M, Debinsky HS, Williams CB. Immediate recovery of psychomotor function after patient-administered nitrous oxide/oxygen inhalation for colonoscopy. Endoscopy 1997;29:17–22.

53. Martin JP, Sexton BF, Saunders BP, Atkin WS. Inhaled patient-administered nitrous oxide/oxygen mixture does not impair driving ability when used as analgesia during screening flexible sigmoidoscopy. Gastrointest Endosc 2000;51:701–703.

54. Stacy CB, Di Rocco A, Gould RJ. Methionine in the treatment of nitrous-oxide-induced neuropathy and myeloneuropathy. J Neurol 1992;239:401–403.

55. Green R, Kinsella LJ. Current concepts in the diagnosis of cobalamin deficiency. Neurology 1995;45:1435–1440.

B   Fluorinated Alkanes

Chapter 41

FLUORINATED ALKANES

HISTORY

Medgley and Henne first reported the synthesis of fluorinated aerosol propellants (i.e., dichlorodifluoromethane, Freon 12) in 1930. These compounds were introduced as a less-toxic refrigerant gas to replace ammonia and sulfur dioxide. Aerosol insecticides (Freon 12) were introduced by the military during World War II, and these insecticides were released to the civilian market in 1947.

IDENTIFYING CHARACTERISTICS

Structure

Trichloromonofluoromethane (FC11) and trichlorotrifluoroethane (FC113a) are liquids at room temperature and standard pressure, whereas dichlorodifluoromethane (FC12) is highly volatile under these conditions. The lipid solubility of FC11 and FC113 are similar and greater than the lipid solubility of FC12 and FC114. The boiling point of chlorodifluoromethane (FC22) is −40.7°C (−41.3°F); this compound is typically stored as a liquid at a pressure of 150 psi. The temperature of the vapors released from these containers is below the boiling point; therefore, exposure to the vapors may cause severe frostbite. At 25°C (77°F) and 760 mm Hg, the relative density of air saturated with FC22 (42.1%) is 3.3 (air = 1), and the vapor pressure is 334 mm Hg. Bromochlorodifluoromethane (FC1211) is a volatile liquid with a boiling point of −3.7°C (25.3°F) that forms a noncombustible cloud over fires and interferes with the chemical reactions within the fire. The boiling point of 1,1-difluoroethane is −24.9°C (−12.8°F), and the rapid evaporation of the liquid causes frostbite as the tissues are cooled below 0°C (32°F). This compound is colorless, odorless, water insoluble, and highly flammable.

Table 41.1 lists identifying data and the boiling points of common fluorocarbons.

Terminology

Fluorinated alkanes are frequently identified by number (e.g., Freon 113). The first digit on the right indicates the number of fluorine atoms, the second digit from the right is one less than the number of hydrogen atoms, and the third digit (absent if zero) is one less than the carbon atoms. Letters identify isomers with "a" denoting the isomer containing the smallest mass difference on each carbon atom. Other letters are added as masses of other isomers diverge from the "a" isomer. For example, Freon 113a is 1,1,1-trichloro-2,2,2-trifluoroethane (trichlorotrifluoroethane).

EXPOSURE

Epidemiology

Fluorinated alkane propellants were popular volatile substances of abuse during the late 1960s and early 1970s, particularly FC11, FC12, and FC114.[1] Abuse of fluorocarbons includes the chronic inhalation of freon-containing refrigerant as well as the abuse of medical inhalers containing Freon propellants.[2] The excessive use of these inhalers even involves children as young as 4 years of age.[3] The abuse of products containing fluo-

TABLE 41.1. Fluorocarbon Identification.

Fluorocarbon	CAS Number	Names	Molecular Formula	Boiling Point
Bromochlorodifluoromethane	353-59-3	FC1211, Freon 12B1, Halon 1211, Fluorocarbon 1211,	$CBrClF_2$	−3.7°C
Bromotrifluoromethane	75-63-8	FC 13B1, Fluorocarbon 1301, Halon 1301, Freon 13B1		
1-Chloro-1,1-difluoroethane	75-68-3	FC 142b, Freon 142, Fluorocarbon 142b, Gentron 142b, CFC 142b	CH_3CClF_2	−10.0°C
Chlorodifluoromethane	75-45-6	FC 22, Freon 22, fluorocarbon 22, CFC 22, monochlorodifluoromethane	$CHClF_2$	−40.7°C
Chloropentafluoroethane	76-15-3	FC115, Freon 115, Halocarbon 115,	C_2ClF_5	−37.9°C
Dichlorodifluoromethane	75-71-8	FC12, Freon 12, Halon 122, F-12, Arcton 12, CFC 12	CCl_2F_2	−29.8°C
Dichlorofluoromethane	75-43-4	FC 21,Freon 21, Arcton 7, Genetron 21, Halon 112	$CHCl_2F$	8.9°C
1,2-Dichloro-1,1,2,2-tetrafluoroethane (Dichlorotetrafluoroethane)	76-14-2	FC114, Freon 114, CFC114, Halocarbon 114, Arcton 114	$C_2Cl_2F_4$	3.8°C
1,1-Difluoroethane	75-37-6	FC 152a, Freon 152a, fluorocarbon 152a, Halocarbon 152a	$C_2H_4F_2$	−24.9°C
1,1,2,2-Tetrachloro-1,2-difluoroethane	76-12-0	FC 112, CFC 112, Halon 112, Halocarbon 112	$C_2Cl_4F_2$	26°C
Trichloromonofluoromethane	75-69-4	FC11, Freon 11, Halon 11, fluorocarbon 11, F11, Arcton 11, CFC 11	CCl_3F	23.7°C
1,1,2-Trichloro-1,2,2-trifluoroethane	76-13-1	FC 113, Freon 113, Frigen 113 TR, CFC-113, Arcton 63	$C_2Cl_3F_3$	47.7°C
1,1,1-Trichloro-2,2,2-trifluoroethane (Trichlorotrifluoroethane)	354-58-5	FC 113a, Freon 113a, fluorocarbon 113a	$C_2Cl_3F_3$	46.1°C

rinated alkanes propellants has declined as other compounds (e.g., alkanes) have replaced these products.

Sources

Halogenated fluorocarbons are used as solvents, aerosol propellants, refrigerants, plastics manufacturing, foam blowing, and components of fire extinguishers with the fluorocarbon concentration varying depending on the use. Aerosols contain propellants, solvents, and active ingredients along with additives (e.g., perfumes, emollients). The use of halogenated hydrocarbons as propellants has decreased because of concern about the effect of these relatively inert compounds on the ozone layer. Bromochlorodifluoromethane and bromotrifluoromethane are constituents of fire extinguishers used to suppress electrical fires. 1,1-Difluoroethane is a component of airbrush propellants and refrigerants.

Methods of Abuse

Methods for abusing volatile halogenated hydrocarbons primarily involve the pouring of the substance on a rag or in a container followed by the inhalation of the vapors. Alternate methods include attempting to inhale vapors from air conditioner fluid (chlorodifluoromethane) directly from the air conditioner unit.[4] At times, a plastic bag is placed over the person's head to increase the concentration of the vapors. Dusting is the inhalation of halogenated hydrocarbons (e.g., difluoroethane, tetrafluoroethane) found in office products containing compressed air. Volatile substance abuse of bromochlorodifluoromethane typically involves the discharge of the fire extinguisher into a plastic bag and the subsequent inhalation of the vapors from the bag.

DOSE EFFECT

Animals

Animal studies demonstrate observable narcotic effects (loss of coordination, tremor, disequilibrium, irregular breathing, lacrimation) at concentrations around 50,000 ppm FC113, whereas anesthetic effects (unconsciousness, convulsions, and death) occur after exposure to ~100,000 ppm FC113 for several hours.[5] Other animal

studies indicate that guinea pigs develop disequilibrium after a 1-minute exposure to 165,000 ppm FC113, whereas rats exposed to 220,000 ppm FC113 developed convulsions, coma, cyanosis, and death within 3–15 minutes; however, these concentrations are sufficient to reduce the oxygen concentration to levels causing asphyxia.[6]

Humans

In volunteer studies, short-term exposure (i.e., <30 minutes) to 40,000 ppm FC1301 caused lightheadedness, confusion, ringing in the ears, and impaired coordination; exposure to 60,000 ppm was associated with increased heart rate, depressed T waves, and premature ventricular contractions.[7,8]

TOXICOKINETICS

Absorption

Halogenated hydrocarbons are rapidly absorbed following inhalation. However, the relatively low peak arterial concentrations of fluorocarbons compared with the total doses administered suggest the amount of fluorocarbons absorbed by the lungs is low. In a study of asthmatic patients inhaling bronchodilators with fluorinated hydrocarbon propellants, peak concentrations of trichlorofluoromethane (FC11) in venous blood occurred 30–90 seconds after inhalation.[9] Similarly, the peak concentration of FC11 in arterial blood from 6 volunteers occurred with 30 seconds after inhalation of this substance.[10] The absorption of FC12 and FC114 are relatively slow compared with FC11 and FC113.[11] Based on [38]Cl-labeled fluorocarbon studies in volunteers following the single-breath inhalation, the retention of fluorocarbons 30 minutes after inhalation ranged from 10% for FC114 to 23% for FC11 as demonstrated in Table 41.2. In a study of 3 volunteers, the lungs absorbed only about 2% of the inhaled dose of FC22 during the

TABLE 41.2. Percentage of Inhaled Fluorocarbon Vapors Retained by Three Volunteers 30 Minutes after Inhalation.[11]

Fluorocarbon	Retention Range (%)	Retention Mean ± Standard Deviation (%)
FC11	19.8–26.6	23.0 ± 2.2*
FC12	8.8–14.2	10.3 ± 2.2
FC113	18.7–21.0	19.8 ± 0.9
FC114	9.2–18.1	12.3 ± 4.1

*Four volunteers.

4 hours of the study.[12] The estimated pulmonary retention of FC113 was about 14% (95% CI: 12–16%) following exposure of 7 volunteers to 1,000 ppm FC113 for 4 hours.[13] Following exposure of 4 pairs of volunteers to 10,000 ppm FC1301 for 24 hours, steady-state concentrations of FC1301 occurred about 2 hours after the beginning of exposure.[14]

Distribution

In volunteer studies, the estimated fat/blood partition coefficient of FC22 was ~10 with a mean blood/air partition coefficient of 0.76 at 20 mg/L,[12] whereas the fat/blood partition coefficient of FC113 was approximately 146 at 20 mg/L.[13] Rodent studies indicate that FC152a is distributed to the brain and blood within seconds after the inhalation of high concentrations of FC152a with intoxication occurring within 20 seconds.[15]

Biotransformation/Elimination

In general, the metabolism of fluorinated alkanes is minimal. Because of low lipid solubility, most inhaled fluorocarbon vapors are exhaled without diffusing across alveolar membranes. Animal and human studies indicate that the elimination of fluorocarbons from the blood is rapid.[16] The blood elimination half-life of trichlorofluoromethane (FC11) in 5 asthmatic patients and 1 control ranged between 0.3–1.5 minutes. In a study of 3 dogs receiving FC11, the mean terminal half-life of FC11 in arterial blood was approximately 3–5 minutes.[17] The elimination of FC22 from venous blood was rapid following exposure of 3 volunteers to 518 ppm FC22 for 4 hours, characterized by a mean initial half-life of 0.23 hour and a mean terminal half-life of 2.8 hours.[12] The lack of an increase in serum fluoride suggests that the biotransformation of FC22 is minimal. In this study, the amount of FC22 excreted in the urine was small. The mean initial and terminal elimination half-lives following exposure of 4 pairs of volunteers to 10,000 ppm FC1301 were 4.5 minutes (range, 2.5–8.1 minutes) and 200 minutes (range, 131–347 minutes), respectively.[14]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Close contact with the vapors of halogenated hydrocarbons released from containers containing pressurized liquid may cause severe local tissue damage. The temperature of these vapors close to the container is typically below the boiling point of the substance, which is

substantially lower than freezing. Therefore, direct contact with these vapors causes severe frostbite.

There are few human data on the mechanisms associated with sudden death during volatile substance abuse. Animal studies suggest that halogenated alkanes may sensitize the heart to the development of dysrhythmias.[18,19] Ventricular fibrillation was an uncommon rhythm during these experiments as the most common dysrhythmia in mice that were asphyxiated after inhaling fluorocarbon propellants was a bradyarrhythmia initiated during asphyxia.[18] There were marked differences in the dysrhythmogenic potential of fluorocarbons between animal species and between different fluorocarbons.[20,21] These studies used anesthetics to depress consciousness, oxygen deprivation, and large doses of epinephrine (5–6 µg epinephrine/kg by central venous catheter over 10 seconds) that substantially exceed the maximum endogenous rate of human epinephrine secretion. Typically, these changes occur only a few minutes after the administration of the epinephrine.[22,23] In a study of 16 anesthetized dogs receiving intravenous pentobarbital, normal arterial oxygen tensions, carbon dioxide tension, and pH were maintained during exposure to high concentrations of fluoroalkane propellants (FC11, FC12).[24,25] The most common scenario was an immediate, dramatic slowing of the heartbeat (sinus bradycardia), followed by A-V dissociation with progressively lower escape rhythms and ultimately electrical and mechanical asystole. Only 2 of the dogs developed ventricular fibrillation. Consequently, the conditions of these studies do not necessarily simulate the conditions present during volatile substance abuse. Animal studies indicate that there is substantial variation in the cardiac effects between both animal species and fluorocarbon compounds.[26] Other possibilities for the effects of fluorocarbons include the general phenomenon of disruption of normal cellular function by chemically inert lipid-soluble compounds.

Postmortem Examination

The postmortem findings in cases of sudden death associated with the abuse of halogenated hydrocarbons are usually nonspecific (pulmonary congestion, cerebral edema, hyperemia of the trachea). A 15-year-old adolescent with a history of inhaling fumes from electrical fire extinguishers (bromochlorodifluoromethane) collapsed and died later that day in the hospital.[27] Postmortem examination confirmed exposure to bromochlorodifluoromethane along with cerebral edema, anoxic brain damage, pulmonary congestion, and blood-stained foam in the airways. Postmortem examination may reveal alternate causes of death (e.g., aspiration of stomach contents, laryngeal obstruction)[28,29] or evidence of cold injuries on the hands or mouth.[4]

CLINICAL RESPONSE

Illicit Use

Adverse effects associated with the inhalation of vapors from halogenated hydrocarbons include nausea, vomiting, disorientation, altered consciousness, and dysrhythmias including ventricular fibrillation. A 15-year-old adolescent discharged a fire extinguisher into a plastic bag and then placed the bag over his head. He inhaled vapors from the bag numerous times, then, he collapsed.[30] Medical personnel found him pulseless and apneic. Cardiac monitoring demonstrated ventricular fibrillation; he was defibrillated and intubated. He regained consciousness in the hospital; by the sixth day he was conversant without focal neurologic signs.

Complications

Complications of the intentional abuse of halogenated products include loss of consciousness, seizures, frostbite (2nd/3rd degree burns), and dysrhythmias.[31] Severe local burns of the hand may require amputation of the affected digits.[32] Local tissue damage to the mouth and airway may cause edema and obstruction that requires intubation or tracheostomy, particularly following exposure to liquid freon when the container is inverted.[33,34] A 16-year-old adolescent presented to the emergency department after losing consciousness while inhaling vapors from an open container that was releasing a propellant containing 1,1-difluoroethane.[35] After regaining consciousness, he noted that his lips were cold and blistered. About 4 hours after presentation to the emergency department, he developed respiratory distress that required nasotracheal intubation. Endoscopy revealed first- and second-degree burns of the larynx with involvement of the vocal cords along with first-degree burns of the proximal esophagus and trachea. Second- and third-degree burns were present on the lips, tongue, and oral cavity. Fluorosis (diffuse sclerosis, increased bone density) of the boney skeleton is a potential complication of chronic fluorocarbon abuse. Most common fluorinated alkanes are listed by the International Agency for Research on Cancer (IARC) as Group 3 compounds (not classifiable as to its carcinogenicity to humans).[36,37,38]

Fatalities

Death immediately following volatile substance abuse with halogenated alkanes may result from respiratory

depression, ventricular fibrillation, suffocation, airway obstruction (laryngeal spasm/edema), or aspiration of gastric contents. Case reports document sudden death following the intentional inhalation of fluorinated alkanes, primarily involving dichlorodifluoromethane (FC11)[39,40] and trichlorofluoromethane (FC12).[41] In a few of these cases, ventricular fibrillation was documented as the initial rhythm during the course of resuscitation.[42] Typically, these individuals feel unwell shortly after inhaling the vapors and then collapse. A case series documented the sudden deaths of 5 youth following the inhalation of vapors from fire extinguishers (bromochlorodifluoromethane).[43] Postmortem examinations did not demonstrate natural causes of death, and postmortem blood confirmed exposure to bromochlorodifluoromethane. Animal studies and case reports suggest that the risk of sudden death occurs during or shortly (i.e., <5–10 minutes) after cessation of exposure to fluorinated alkanes.[44]

DIAGNOSTIC TESTING

Analytic Methods

Methods for the detection of halogenated propellants in biologic samples include headspace gas chromatography with flame ionization or electron capture detection,[45] direct injection gas chromatography/mass spectrometry (GC/MS), headspace mass spectrometry without separation,[46] and headspace GC/MS.[47] The lower limit of quantitation for 1,1-difluoroethane following analysis with headspace GC/MS with flame ionization detection was approximately 4 mg/L (4,000 µg/L).[48]

Proper specimen collection in sealed containers within a timely manner after death is necessary to detect fluorocarbons in postmortem samples. The high volatility of halogenated hydrocarbons requires careful handling of tissue samples with storage in air-tight containers at −30°C (−22°F). Separation of samples, standards, and glassware in separate freezers helps prevent cross-contamination.

Biomarkers

PHARMACEUTIC USE

FC11 was detectable in blood samples 1 hour after the use of an inhaler containing FC11 at a concentration of ~0.120 mg/L, as measured by gas chromatography with electron capture detection.[10] By 2 hours after the use of the inhaler, the FC11 concentration was nondetectable. Following exposure of 3 volunteers to 92 ppm and 518 ppm FC22 for 4 hours, the mean peak FC22 con-

centrations in venous blood samples were 0.25 mg/L and 1.36 mg/L, respectively.[12] Peak concentrations in venous blood occurred approximately 1 hour after exposure began. Following exposure to 10,000 ppm FC1301, the steady concentration of FC1301 was about 3–4.5 mg/L beginning 2 hours after exposure began.[14]

POSTMORTEM

The dichlorodifluoromethane concentration in postmortem blood samples from a man found dead following autoerotic activities was 3.2 mg/L.[49]

1,1-DIFLUOROETHANE. Analysis of postmortem blood samples from 2 adolescents, who died in a crash immediately after their car crossed the median of a four-lane highway, detected 1,1-difluoroethane (FC152a) concentrations of 78 mg/L (driver) and 35 mg/L (passenger).[50] The blood sample from the driver also contained ethanol (13 mg/dL). A 42-year-old man was found dead in his car surrounded by approximately 40 canisters of aerosol duster (difluoroethane).[51] His postmortem femoral blood contained difluoroethane (136.3 mg/L), and the postmortem examination demonstrated myocardial fibrosis and an acute infarction in the left ventricle. A 20-year-old man was found dead with dust remover next to his body, and his postmortem femoral blood contained 1,1-difluoroethane (83.5 mg/L) as measured by headspace gas chromatography with flame ionization detection.[52] In contrast to the blood sample from the previous man, the postmortem blood sample from this man was not sealed in headspace vials at autopsy. A 24-year-old woman died immediately after a single-vehicle accident.[53] The passenger survived, and reported that the driver was inhaling Dust Off Duster (Dupont, Wilmington, DE) while driving shortly before the accident. The femoral blood and vitreous humor contained 1,1-difluoroethane concentrations of 29.8 mg/L and 21.3 mg/L, respectively. The postmortem distribution of difluoroethane in the body suggested that she was in the distribution phase after inhaling this compound. The chest cavity and aortic blood contained 1,1-difluoroethane concentrations of 79.9 mg/L and 85.6 mg/L, respectively.

CHLORODIFLUOROMETHANE. A 22-year-old man developed coughing and wheezing after inhaling fluorocarbon vapors; he presented to the emergency department in ventricular fibrillation.[54] He was pronounced dead 1 hour after being found by his roommates, and the autopsy demonstrated only nonspecific findings. The cans of fluorocarbons found near him contained a 50:50 mixture of chlorodifluoromethane (FC22) and chloropentafluoroethane (FC115). The concentrations of FC22

and FC115 in postmortem blood samples were 71 mg/L and 0.30 mg/L, respectively. The difference in the concentrations of these 2 fluorocarbons may reflect differences in pharmacokinetics, inhaled concentrations, and/or vapor pressures in alveolar blood. Two crew members collapsed while entering a compartment filled with FC22, and they died at the scene.[55] At autopsy 16 hours later, blood samples from the 2 victims contained FC22 concentrations of 37,100 ppm (reported as 37.1 μL/mL) and 26,000 ppm. The concentrations of FC22 in postmortem blood samples were approximately 37 times higher than in vitreous humor.

Dichlorofluoromethane.   A 29-year-old man with a history of fluorocarbon abuse was found dead with a tube in his mouth connected to a Freon dispenser.[56] The postmortem blood contained 8,600 ppm FC21.

Trichlorofluoromethane.   A   23-year-old   factory worker was found dead in a room, which contained a leaking reservoir of FC11.[57] The postmortem examination did not demonstrate a cause of death other than FC11 intoxication. The postmortem blood sample contained 62.8 ppm FC11 compared with 108.9 ppm in the brain and 406.6 ppm in the heart tissue, as measured by gas chromatography/mass spectrometry.

Urine

Detection of fluorocarbons in the urine depends on the dose and time since exposure. In a study of 3 volunteers exposed to 518 ppm FC22, this compound was detectable in urine samples for at least 22 hours, as determined by gas chromatography/flame ionization detection (limit of detection, 0.003 mg/L).[12] FC113 concentrations in urine samples following exposure of volunteers to 1,000 ppm FC113 was below the limit of detection (<1 mg/L).[13]

TREATMENT

First Aid

Animal studies indicate that ocular contact with FC113 produces only mild conjunctivitis and minimal corneal dullness 24 hours after exposure.[58] Splashes to the eye of FC113 should be irrigated copiously with water and medical   attention   sought   if   redness   persists. Decontamination measures are usually unnecessary following oral exposures because FC113 is not well absorbed from the gastrointestinal tract. The main danger results from the inhalation of large concentrations of FC113 or other fluorocarbons that produce narcosis and rarely dysrhythmias. The victim should be removed from the contaminated environment as soon

as safely feasible. Respiration and pulses should be immediately checked and artificial respiration and chest compression begun as necessary. Frostbite of the mouth may cause airway obstruction in severe cases. All patients with altered mental status should receive supplemental oxygen. The primary treatment for frostbite is rapid rewarming, such as a 30-minute warm water bath   (38°C–43°C/100°F–108°F)   for   frostbite on the extremities. Manipulation of tissue in the area of the frostbite should be avoided because this tissue is very sensitive to trauma unless necessary to secure an airway.

Acute Management

Respiratory depression and dysrhythmias represent the principal threat to patients abusing fluorinated alkanes, particularly FC11, FC12, and FC113. The adequacy of ventilation should be evaluated immediately and supplemental oxygen and/or intubation initiated as needed. The patient should be monitored for dysrhythmias. Patients in cardiac arrest should be resuscitated with the standard advanced cardiopulmonary resuscitation protocols including the use of epinephrine; there are inadequate clinical data to recommend specific dysrhythmic drugs. Patients usually recover rapidly as the body quickly eliminates these compounds within 6–24 hours following acute exposure. Hepatorenal dysfunction does not usually result from exposure to fluorinated alkanes. There are no methods to enhance elimination other than ensuring adequate ventilation because the lungs excrete almost all of the absorbed dose of fluorinated alkanes. Blood gases or pulse oximetry should be measured in all patients with altered mental status or symptoms of dyspnea. Usually, no direct sequelae result from exposure to fluorinated alkanes; however, sustained respiratory depression may produce hypoxic damage to the brain or heart.

Supplemental Care

Treatment is supportive. Asymptomatic patients or patients with mild symptoms that resolve may be discharged several hours after they become asymptomatic. Patients with evidence of dysrhythmia should be monitored for 4–6 hours. If all abnormalities resolve, asymptomatic patients may be discharged. Patients remaining symptomatic after observation should be observed overnight with cardiac monitoring.

References

1. Giovacchini RP. Abusing the volatile organic chemicals. Regul Toxicol Pharmacol 1985;5:18–37.

2. Prasher VP, Corbett JA. Aerosol addiction. Br J Psychiatry 1990;157:922–924.

3. O'Callaghan C, Milner AD. Aerosol treatment abuse. Arch Dis Child 1988;63:70.

4. Phatak DR, Walterscheid J. Huffing air conditioner fluid a cool way to die? Am J Forensic Med Pathol 2010;31:1–4.

5. Ravantos J, Lemon PG. The impurities in fluothane: their biological properties. Br J Anaesth 1965;37:716–737.

6. Anonymous. Hygienic guide series 1,1,2-trichloro-1,2,2-trifluoroethane (Trifluorotrichloroethane, fluorocarbon No. 113). Am Ind Hyg Assoc J 1968;19:521–525.

7. Committee on Toxicology, National Research Council (NRC). Emergency and continuous exposure limits for selected airborne contaminants, Vol. 3, Bromotrifluoromethane. Washington, DC: National Academy of Sciences; 1984.

8. Committee on Toxicology, National Research Council (NRC). Bromotrifluoromethane–a literature review. Washington, DC: National Academy of Sciences; 1978.

9. Paterson JW, Sudlow MF, Walker SR. Blood-levels of fluorinated hydrocarbons in asthmatic patients after inhalation of pressurised aerosols. Lancet 1971;2(7724): 565–568.

10. Dollery CT, Draffan GH, Davies DS, Williams FM, Conolly ME. Blood concentrations in man of fluorinated hydrocarbons after inhalation of pressurised aerosols. Lancet 1970; 2:1164–1166.

11. Morgan A, Black A, Walsh M, Belcher DR. The absorption and retention of inhaled fluorinated hydrocarbon vapours. Int J Appl Radiat Isotopes 1972;23:285–291.

12. Woollen BH, Marsh JR, Mahler JD, Auton TR, Makepeace D, Cocker J, Blain PG. Human inhalation pharmacokinetics of chlorodifluoromethane (HCFC22). Int Arch Occup Environ Health 1992;64:383–387.

13. Woollen BH, Guest EA, Howe W, Marsh JR, Wilson HK, Auton TR, Blain PG. Human inhalation pharmacokinetics of 1,1,2-trichloro-1,2,2-trifluoroethane. Int Arch Occup Environ Health 1990;62:73–78.

14. Lam C-W, Weir FW, Williams-Cavender K, Tan MN, Galen TJ, Pierson DL. Toxicokinetics of inhaled bromotrifluoromethane (Halon 1301) in human subjects. Fundam Appl Toxicol 1993;20:231–239.

15. Avella J, Kunaparaju N, Kumar S, Lehrer M, Zito SW, Barleta M. Uptake and distribution of the abused inhalant 1,1-difluoroethane in the rat. J Anal Toxicol 2010;34: 381–388.

16. Azar A, Trochimowicz HJ, Terrill JB, Mullin LS. Blood levels of fluorocarbon related to cardiac sensitization. Am Ind Hyg Assoc J 1973;34:102–109.

17. McClure DA. Failure of fluorocarbon propellants to alter the electrocardiogram of mice and dogs. Toxicol Appl Pharmacol 1972;22:221–230.

18. Taylor GJ 4th, Harris WS. Cardiac toxicity of aerosol propellants. JAMA 1970;214:81–85.

19. Reinhardt CF, Azar A, Maxfield ME, Smith PE Jr, Mullin LS. Cardiac arrhythmias and aerosol "sniffing". Arch Environ Health 1971;22:265–279.

20. Aviado DM, Belej MA. Toxicity of aerosol propellants on the respiratory and circulatory systems I. Cardiac arrhythmia in the mouse. Toxicology 1974;2:31–42.

21. Belej MA, Smith DG, Aviado DM. Toxicity of aerosol propellants in the respiratory and circulatory systems IV. Cardiotoxicity in the monkey. Toxicology 1974;2:381–395.

22. Mullin LS, Azar A, Reinhardt CF, Smith PE Jr, Fabryka EF. Halogenated hydrocarbon-induced cardiac arrhythmias associated with release of endogenous epinephrine. Am Ind Hyg Assoc J 1972;33:389–396.

23. Beck PS, Clark DG, Tinston DJ. The pharmacologic actions of bromochlorodifluoromethane (BCF). Toxicol Appl Pharmacol 1973;24:20–29.

24. Flowers NC, Horan LG. Nonanoxic aerosol arrhythmias. JAMA 1972;219:33–37.

25. Flowers NC, Horan LG. The electrical sequelae of aerosol inhalation. Am Heart J 1972;83:644–651.

26. Aviado DM. Toxicity of aerosol propellants in the respiratory and circulatory systems IX. Summary of the most toxic: trichlorofluoromethane (FC11). Toxicology 1975;3:311–319.

27. Heath MJ. Solvent abuse using bromochlorodifluoromethane from a fire extinguisher. Med Sci Law 1986;26:33–34.

28. Poklis A. Determination of fluorocarbon 11 and fluorocarbon 12 in post-mortem tissues: a case report. Forensic Sci 1975;5:53–59.

29. Baselt RC, Cravey RH. A fatal case involving trichloromonofluoromethane and dichlorodifluoromethane. J Forensic Sci 1968;13:407–410.

30. Steadman C, Dorrington LC, Kay P, Stephens H. Abuse of a fire-extinguishing agent and sudden death in adolescents. Med J Aust 1984;141:115–117.

31. Elliott MC. Frostbite of the mouth: a case report. Mil Med 1991;156:18–19.

32. Wegener EE, Barraza KR, Das SK. Severe frostbite caused by freon gas. South Med J 1991;84:1143–1146.

33. Kuhn JJ, Lassen LF. Acute upper airway obstruction after recreational inhalation of a hydrofluorocarbon propellant. Otolaryngol Head Neck Surg 1999;120:587–590.

34. Kurbat RS, Pollack CV Jr. Facial injury and airway threat from inhalant abuse: a case report. J Emerg Med 1998; 16:167–169.

35. Kuspis DA, Krenzelok EP. Oral frostbite injury from intentional abuse of a fluorinated hydrocarbon. Clin Toxicol 1999;37:873–875.

36. International Agency for Research on Cancer (IARC). Chlorodifluoromethane. IARC Monogr Eval Carcinog Risks 1999;71:1339–1343.

37. International Agency for Research on Cancer (IARC). Chlorofluoromethane. IARC Monogr Eval Carcinog Risks 1999;71:1351–1353.

38. International Agency for Research on Cancer (IARC). 2-Chloro-1,1,1-Trifluoroethane. IARC Monogr Eval Carcinog Risks 1999;71:1355–1357.

39. Luckstead EF, Jordan FB, Prouty RW. Sudden death following fluorohydrocarbon aerosol "sniffing". J Okla State Med Assoc 1978;71:117–119.

40. Crawford WV. Death due to fluorocarbon inhalation. South Med J 1976;69:506–507.

41. Standefer JC. Death associated with fluorocarbon inhalation: report of a case. J Forensic Sci 1975;20:548–551.

42. Brady WJ jr, Stremski E, Eljaiek L, Auferheide TP. Freon inhalation abuse presenting with ventricular fibrillation. Am J Emerg Med 1994;12:533–536.

43. Smeeton WM, Clark MS. Sudden death resulting from inhalation of fire extinguishers containing bromochlorodifluoromethane. Med Sci Law 1985;25:258–262.

44. Mullin LS, Reinhardt CF, Hemingway RE. Cardiac arrhythmias and blood levels associated with inhalation of halon 1301. Am Ind Hyg Assoc J 1979;40:653–658.

45. Street PJ, Ruprah M, Ramsey JD, Flanagan RJ. Detection and identification of volatile substances by headspace capillary gas chromatography to aid the diagnosis of acute poisoning. Analyst 1992;117:1111–1127.

46. Urich RW, Wittenberg PH, Bowerman DL, Levisky JA, Pflug JL. Electron impact mass spectrometric detection of Freon® in biological specimens. J Forensic Sci 1977; 22:34–39.

47. Hamill J, Kee TG. The detection of aerosol propellants in body fluids and tissue by gas chromatography-mass spectrometry. J Forensic Sci Soc 1991;31:301–307.

48. Broussard LA, Broussard A, Pittman T, Lafferty D, Presley L. Headspace gas chromatographic method for the measurement of difluoroethane in blood. Clin Lab Sci 2001; 14:3–5.

49. Gowitt GT, Hanzlick RL. Atypical autoerotic deaths. Am J Forensic Med Pathol 1992;13:115–119.

50. Broussard LA, Brustowicz T, Pittman T, Atkins KD, Presley L. Two traffic fatalities related to the use of difluoroethane. J Forensic Sci 1997;42:1186–1187.

51. Avella J, Wilson JC, Lehrer M. Fatal cardiac arrhythmia after repeated exposure to 1,1-difluoroethane (DFE). Am J Forensic Med Pathol 2006;27:58–60.

52. Xiong Z, Avella J, Wetli CV. Sudden death caused by 1,1-difluoroethane inhalation. J Forensic Sci 2004; 49:627–629.

53. Hahn T, Avella J, Lehrer M. A motor vehicle accident fatality involving the inhalation of 1,1-difluoroethane. J Anal Toxicol 2006;30:638–641.

54. Fitzgerald RL, Fishel CE, Bush LL. Fatality due to recreational use of chlorodifluoromethane and chloropentafluoroethane. J Forensic Sci 1993;38:476–482.

55. Kintz P, Baccino E, Tracqui A, Mangin P. Headspace GC/MS testing for chlorodifluoromethane in two fatal cases. Forensic Sci Int 1996;82:171–175.

56. Goldsmith RJ. Death by Freon. J Clin Psychiatry 1989;50:36–37.

57. Groppi A, Polettini A, Lunetta P, Achille G, Montagna M. A fatal case of trichlorofluoromethane (Freon 11) poisoning. Tissue distribution study by gas chromatography-mass spectrometry. J Forensic Sci 1994;39:871–876.

58. Duprat P, Delsaut L, Gradiski D. [Irritant power of the principal aliphatic chlorinated solvents on the skin and ocular mucosa of the rabbit]. Eur J Toxicol Environ Hyg 1976;9:171–177. [French]

C   Industrial Hydrocarbons

Chapter 42

BUTANE, ISOBUTANE, and PROPANE

BUTANE AND ISOBUTANE

IDENTIFYING CHARACTERISTICS

n-Butane and isobutane are colorless, odorless, and flammable gases at room temperature. Odorants are added to natural gas and liquified petroleum gas (LPG) as a means of identifying potentially explosive concentrations. *n*-Butane and isobutane are the 2 isomers of the 4-carbon aliphatic hydrocarbon, butane (C_4H_{10}). Butane is highly lipid soluble. Table 42.1 lists some physiochemical properties of *n*-butane and isobutane.

EXPOSURE

Epidemiology

Butane is 1of the 5 most common volatile substances of abuse;[1] additionally, this compound is 1of 3 compounds most often associated with sudden death secondary to volatile substance abuse along with propane.[2]

Sources

Items containing butane include cigarette or charcoal lighter fluid (75%–89%), pressurized fuel tanks, liquefied petroleum gas, natural gas, hairspray, and aerosol antiperspirants. Liquefied petroleum gas (LPG) is a mixture of butane and propane that varies in composition with season and type of product. Common LPG mixtures contain 60% propane and 40% butane with the relative butane percentage increasing in summer and the relative propane percentage increasing in winter.

Methods of Abuse

The abuse of butane occurs both in solitary and social situations. Methods of butane abuse include using the cover of the gas canister as a mask, filling a plastic bag with the liquid ("bagging"), and spraying the liquid on a towel before inhaling ("huffing").[3] Inhaled concentrations of volatile substances, including butane, depends on the mode of administration. Sniffing delivers the lowest concentration of butane and bagging the highest. Huffing these chemicals produces an inhaled butane concentration between that of sniffing and bagging. The desired effects of abusing volatile products with butane are euphoria, loss of inhibition, altered perception of body form, sense of slowing of time, illusions, lightheadedness, ataxia, and hallucinations.[4] These effects persist up to 15–20 minutes.[5] The rapid resolution of these effects results in repeated inhalation to maintain the desired effects; often, this pattern of use continues for 3–4 hours.

DOSE EFFECT

Animal studies indicate that *n*-butane and isobutane are central nervous system (CNS) depressants at very high concentrations; these compounds possess relatively less toxicity compared with pentanes.[6] These studies also

TABLE 42.1. Some Physiochemical Properties of *n*-Butane and Isobutane.

Physical Characteristic	*n*-Butane	Isobutane
CAS Registry Number	106-97-8	75-28-5
Molecular Formula	$C_4–H_{10}$	$C_4–H_{10}$
Molecular Weight	58.12 g/mol	58.12 g/mol
Melting Point	−138.4°C (−217.1°F)	−159.4°C (−254.9°F)
Boiling Point	−0.5°C (31.1°F)	−11.73°C (10.9°F)
Water Solubility	61 mg/L (Relatively Insoluble, 25°C/77°F)	49 mg/L (20°C/68°F)
Vapor Density	2.07 (Air = 1 @ 20°C/68°F)	2.07

suggest that the CNS depressive effects of *n*-butane are somewhat greater than the effects of isobutane. Exposure of human volunteers to 10,000 ppm butane for 10 minutes causes some drowsiness, but no other systemic symptoms.[7] Exposure of volunteers to 500 ppm isobutane 8 hours a day, 5 days per week for 4 weeks did not produce any abnormalities on continuous monitoring of the cardiac rhythm, electrocardiogram, neurologic exam, visual evoked response, memory tests, pulmonary function tests, or serum hepatic transaminase concentrations.[8]

TOXICOKINETICS

There are limited data on the toxicokinetics of butane isomers. Because butane isomers are gases, pulmonary absorption is the primary route of exposure. Dermal absorption is limited by the volatility of butane isomers. Based on similarities in animal studies with other alkanes, elimination of butane isomers is probably rapid with little accumulation in tissues.[9] *In vitro* studies indicate that hydrolysis of *n*-butane produces 2-butanol, which may be conjugated with glucuronic acid, oxidized to methyl ethyl ketone, or expired as 2-butanol in air.[10] These studies also demonstrate that *tert*-butanol is the primary hydrolytic product of isobutane metabolism; elimination of *tert*-butanol occurs either by conjugation with glucuronic acid or by excretion unchanged in expired air and urine. In rodent studies, metabolism of *n*-butane yields *sec*-butanol and methyl ethyl ketone, whereas the biotransformation of isobutane produces *tert*-butanol.[11] Presumably, microsomal enzymes oxidize butane to the corresponding secondary or tertiary alcohol; then, alcohol dehydrogenase catalyzes the oxi-

dation of these alcohols to the corresponding ketones. In mice, *tert*-butanol is the primary metabolite following inhalation of isobutane; there is no oxidation of *tert*-butanol to a ketone by alcohol dehydrogenase.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

n-Butane and isobutane are simple asphyxiants and mild CNS depressants with a very narrow safety margin between anesthetic and lethal concentrations. Potential mechanisms for sudden death following the inhalation of butane or isobutane vapors include asphyxia, trauma after dangerous behavior, respiratory depression, aspiration, and cardiac dysrhythmia. Although some authors suggest that myocardial sensitization causes sudden death during butane or isobutane abuse based on experimental studies and case reports, this theory is difficult to prove in individual cases because of the presence of confounders (e.g., hypoxia, respiratory and myocardial depression, concomitant drug use). Animal studies indicate that high doses of epinephrine and high concentrations of isobutane (50,000 ppm)[12] predispose dogs to develop cardiac dysrhythmias. However, the clinical relevance of these abnormalities is unclear because of the difference in blood concentrations between these experiments and volatile substance abuse with the exception of bagging. Aspiration of liquid petroleum by a performer (e.g., during placement of flaming objects in their mouth) may cause hydrocarbon pneumonitis.

Postmortem Examination

Postmortem examinations of victims dying while inhaling products containing butane typically demonstrate nonspecific findings, similar to other volatile substances of abuse, such as findings consistent with suffocation (pulmonary edema, cerebral edema, general congestion of organs) or aspiration.[13] These abnormalities include conjunctival petechiae, edema of the lungs with subpleural hemorrhages, laryngeal edema, cerebral edema, passive congestion of the viscera, white to reddish frothy fluid in the airways, deep purple lividity, and terminal aspiration of stomach contents.[14] Vomiting is a common complication of butane abuse, and postmortem examination often demonstrates evidence of gastric aspiration.[13] Diffuse interstitial fibrosis of the myocardium may also be present on postmortem examination along with intrasarcolemmal accumulation of fibronectin and intracellular loss of the cardiac antigen, troponin C in myocardial fibers.[15] Herniation of the

685

cerebellar tonsils into the foramen magnum may also occur.[16]

CLINICAL RESPONSE

Illicit Use

Adverse effects associated with the acute inhalation of butane include eye, nose, and throat irritation, nausea, vomiting, abdominal pain, anorexia, ataxia, diplopia, fatigue, hallucination, confusion, weakness, and headache. Case reports associate encephalopathy with butane abuse, both as a result of chronic abuse[17] and postresuscitation sequelae of cardiopulmonary arrest during inhalant use.[18] The acute encephalopathy (i.e., disorientation, drowsiness) associated with high butane doses may persist for several days after cessation of use.[17] Psychiatric abnormalities include behavioral disturbances, mood swings, impulsive actions, social isolation, dependency, violence, irritability, delusions of persecution, and hallucination.[19] In some case reports, these psychotic symptoms persist months after the cessation of butane use.[20] A 16-year-old adolescent developed confusion, lethargy, hallucination, and agitation within 2 days after inhaling vapors from butane lighter fluid.[21] He was noncommunicative and ambulated slowly. Magnetic resonance imaging revealed severe bilateral thalamic injury; he remained profoundly mute and apathetic 3 months after the event.

Case reports associate myocardial infarction and ventricular fibrillation with butane abuse in adolescents.[22,23] Ventricular fibrillation may develop immediately following butane abuse in the absence of myocardial infarction.[24,25] Typically, these patients have normal coronary arteries and the presumed etiology of the myocardial infarction is butane-induced coronary artery vasospasm.[26] Asystole may occur during volatile substance abuse. A 16-year-old adolescent collapsed while surreptitiously inhaling butane in his bedroom; he was found unconscious by his parents.[27] The initial rhythm obtained by medical personnel on the scene about 5–10 minutes after his collapse was asystole. He was resuscitated at the scene, and he developed no other dysrhythmias in the hospital prior to discharge.

Ignition of butane during volatile substance abuse may cause severe burns of the hands, arms, and face. In a case series of 48 patients hospitalized at a Korean burn unit for injuries related to butane abuse, 22 patients required split-thickness skin grafts.[28] Five patients with major burns died of respiratory failure and/or septicemia. Flash fires in enclosed spaces (e.g., car) during butane abuse may cause inhalation injuries resulting in adult respiratory distress syndrome, septicemia, and death.[29]

Fatalities

Sudden death is a well-described complication of volatile substance abuse with butane.[30] Most deaths occur outside the hospital, and most of these cases involve asphyxia.[31] Causes of death during volatile substance abuse besides asphyxia include respiratory depression, aspiration of gastric contents,[32] asystole, and trauma (drowning, falls, motor vehicle accidents).[33] In several cases, ventricular fibrillation was the initial rhythm documented by medical personnel in case reports of intentional inhalation of butane; however, ventricular fibrillation is a rare complication of the intentional abuse of butane.[18] A 14-year-old boy inhaled butane gas from a portable fuel canister in a vacant lot for about 1½ hours.[34] He fled when police arrived; he was found collapsed about 15 minutes later. The initial cardiac rhythm obtained by medical personnel on the scene was ventricular fibrillation. Resuscitation was unsuccessful and postmortem examination 12 hours after death revealed no evidence of asphyxia.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the analysis of butane and isobutane in biologic specimens include headspace gas chromatography with flame ionization detection,[35] headspace gas chromatography/mass spectrometry,[16] and solid-phase microextraction gas chromatography/mass spectrometry.[36] Because of loss during storage, samples should be analyzed as soon as possible after storage in gas-tight, well-sealed, inert containers with minimal headspace.

n-Butane and isobutane vaporize at room temperature and standard atmospheric pressures. However, samples stored in properly sealed containers designated for volatile substance and stored at −30°C (−22°F) will retain these gases within the coefficient of variation (10%) for 2 weeks.[34] Suitable containers include a preservative-free Vacutainer® or a tightly closed glass vial with a screw cap lined with Teflon septum.[37]

Biomarkers

The presence of butane and isobutane in postmortem tissue confirms exposure to this compounds antemortem, but interpretation of postmortem concentrations is complicated by perimortem and postmortem changes. These changes include volatilization during resuscitation efforts, loss during storage, postmortem losses (i.e., postmortem interval), and postmortem contamination. 2-Methyl-2-propanol is a metabolite of isobutane; the presence of this metabolite in blood samples confirms

the antemortem presence of isobutane. Following completion of the distribution phase, the highest butane concentrations occur in adipose tissues and brain. A 16-year-old boy was found dead at his home with a gas cylinder of cigarette lighter fluid near his body.[38] Analysis of subclavian blood revealed the presence of 0.090 mg/L *n*-propane, 0.246 mg/L isobutane, and 0.846 mg/L *n*-butane. A 13-year-old boy collapsed suddenly after sniffing the vapors of a gas lighter refill fluid from a vinyl bag; he was pronounced dead in the emergency department after unsuccessful resuscitative efforts.[14] During postmortem examination the following day, the concentrations of butane and propane were highest in fatty tissue and lowest in the lungs. The concentrations of *n*-butane and isobutane in blood samples (site not specified) were 0.94 mg/L (reported as 0.94 μL/g) and 0.37 mg/L (0.37 μL/g), respectively.

TREATMENT

Patients with altered mental status should be monitored for respiratory insufficiency and cardiac dysrhythmias. All symptomatic patients should receive supplemental oxygen. Depressed consciousness is an indication for intubation to protect the airway because of the risk of vomiting and aspiration associated with volatile substance abuse. Although some authors suggest that epinephrine should not be administered during the resuscitation of cardiopulmonary arrests associated with butane abuse,[39] case reports document the successful resuscitation of these patients with protocols that included the use of intravenous epinephrine.[18,22,23,24] Paramedics found a 15-year-old girl in coarse ventricular fibrillation and cardiopulmonary arrest after collapsing following 2 hours of intermittent inhalation of vapors from butane gas lighter refills.[40] After a single 200 J-direct current shock, she developed asystole that responded to 1 mg of intravenous epinephrine with conversion to sinus tachycardia. There were no further dysrhythmias reported. Antiarrhythmic agents for the treatment of recurrent ventricular dysrhythmias include amiodarone and lidocaine.

Myocardial infarction may complicate the course of volatile substance abuse with butane along with hypotension and dysrhythmias. Standard treatment for hypotension includes fluid infusions and dopamine despite the concerns about the use of catecholamines after volatile substance abuse. There are few data on the use of other inotropic agents (e.g., glucagon) or on the use of atropine and glucagon for hemodynamically significant bradycardia in these patients. Seizures should be treated with standard doses of benzodiazepines. Rhabdomyolysis, electrolyte imbalance, or renal dysfunction may complicate the course of severely intoxicated patients. Patients

who remain asymptomatic for 4 hours with no cardiac abnormalities may be discharged.

PROPANE

IDENTIFYING CHARACTERISTICS

n-Propane is a colorless, odorless, and flammable gas at room temperature. Odorants are added to natural gas and to LPG as a means of identifying potentially explosive concentrations of these gases. Propane concentrations of 2–10% are highly explosive. Table 42.2 lists some physical and chemical properties of *n*-propane.

EXPOSURE

Propane abuse is substantially less common than butane abuse. In a survey of 285 adolescents in juvenile correctional facilities, the reported intentional use of propane as inhalant was 6.4% compared with 38.3% for butane.[1]

Sources

Products that may contain propane include cigarette or charcoal lighter fluid, pressurized fuel tanks, LPG, natural gas, hairspray, and aerosol antiperspirants. Charcoal lighter fluid contains relatively small concentrations (i.e., <6%) of *n*-propane, whereas propane gas bottles contain mostly propane (95%) along with ethane (4%) and trace amounts (<1%) of butane and isobutane plus ethyl mercaptan for odor warning properties. Propane occurs in natural gas at concentrations of approximately 3–18%.

TABLE 42.2. Physical and Chemical Characteristics of *n*-Propane.

Physical Characteristic	*n*-Propane
CAS Registry Number	74-98-6
Molecular Formula	$C_3–H_8$
Molecular Weight	44.09 g/mol
Melting Point	−189.7°C (−309.5°F)
Boiling Point	−42.1°C (−43.8°F)
Water Solubility	62.4 ppm (25°C/77°F)
Vapor Density	1.56 (Air = 1 @ 20°C/68°F)
Vapor Pressure	760 mm Hg

Methods of Abuse

The abuse of propane occurs both in solitary and social situations, similar to butane and isobutane. Propane abuse involves inserting a line from the bottle to the mouth and inhaling the gas for 10–15 seconds during inspiration.[41] The breath is held for up to 1 minute before expiration. The metal valve may freeze and cause burns if the value has direct contact with the skin. The desired effects of propane abuse are similar to butane including euphoria, loss of inhibitions, altered perception of body form, sense of slowing of time, illusions, lightheadedness, ataxia, and hallucinations. Repeat inhalation is necessary as a result of the rapid resolution of effects following cessation of use; frequently a session of propane abuse continues 3–4 hours. Propane gas contains ethyl mercaptan to improve the odor warning properties of propane. The presence of ethyl mercaptan in inhaled gas causes noxious taste and unpleasant odors; propane inhalant abusers frequently drink sodas and expectorate to relieve the effects of inhaling ethyl mercaptan.

DOSE EFFECT

Propane is a simple asphyxiant like *n*-butane, but animal studies indicate that the CNS depressant effects of propane are substantially less than the effects of *n*-butane.[42] Exposure of volunteers to 10,000 ppm propane for 10 minutes was not associated with any adverse symptoms; however, exposure of these volunteers to 100,000 ppm propane for 2 minutes caused vertigo.[7] Exposure of volunteers to 1,000 ppm propane 8 hours per day 5 days per week for 4 weeks did not produce any abnormalities on continuous monitoring of the cardiac rhythm, electrocardiogram, neurologic exam, visual evoked response, memory tests, pulmonary function tests, or serum hepatic transaminase concentrations.[43]

TOXICOKINETICS

There are little data on the kinetics of propane in humans or animals. Absorption of propane vapors through the skin is probably small compared with pulmonary absorption. The metabolism and elimination of propane is probably similar to the kinetics of butane isomers.[10] In mice, inhalation of propane yields isopropanol and acetone as a result of oxidation by microsomal enzymes and alcohol dehydrogenase.[11] Case reports suggest that tolerance to the effects of propane may develop after continued, high-dose abuse based on the use of increasing quantities of propane and/or butane.[44]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

n-Propane is a simple asphyxiant and mild CNS depressant that displaces oxygen. The mechanisms of toxicity of *n*-propane and butane are similar. Although some authors suggest that myocardial sensitization causes sudden death during *n*-propane or butane abuse based on experimental studies and case reports, this theory is difficult to prove in individual cases because of the presence of confounders (e.g., hypoxia, respiratory and myocardial depression, concomitant drug use). Animal studies indicate that high doses of epinephrine and high concentrations of *n*-propane (150,000 ppm)[45] predispose dogs to develop cardiac dysrhythmias. However, the clinical relevance of these abnormalities is unclear because the doses are substantially higher than the doses achieved during *n*-propane or butane abuse. Most fatalities associated with propane abuse involve asphyxia rather than dysrhythmias,[46] and postmortem examinations are consistent with asphyxia (e.g., petechiae of the conjunctiva, epicardium, pleural surfaces).[47] Aspiration of liquid petroleum during a maneuver such as "fire-eating" may cause a hydrocarbon pneumonitis. Liquid propane may produce cold injuries to the skin without ignition as a result of evaporative heat loss.[48]

CLINICAL RESPONSE

Complications of propane and butane abuse are similar, although case reports of butane abuse are more common. Ignition of propane during volatile substance abuse may cause severe burns of the hands, arms, and face; flash fires following ignition of propane may cause severe inhalation injuries of the upper airways and lung. Sudden death is a less common complication of propane abuse than butane abuse.[49] Most deaths occur outside the hospital and usually involves asphyxia. These fatalities include the intentional use of propane during auto-erotic activities.[50,51]

DIAGNOSTIC TESTING

Analytic methods for the detection and quantitation of propane and butane are similar. *n*-Propane vaporizes at room temperature and standard atmospheric pressures similar to butane; proper storage of samples requires sealed container designated for volatile substance and stored at −30°C (−22°F). The presence of propane in postmortem tissue confirms antemortem exposure to this gas, but interpretation of postmortem concentrations is complicated by perimortem and postmortem changes including volatilization during resuscitation efforts, loss during storage, postmortem losses (i.e., post-

mortem interval), and postmortem contamination. A 23-year-old man was found dead with a plastic bag over his head and a propane cylinder near him.[52] Postmortem blood propane concentration was 2.8 mg/L. Charcoal lighter fluid contains higher concentrations of butane and isobutane than propane; consequently, the propane concentrations in postmortem samples from deaths associated with charcoal lighter fluid are lower than butane or isobutane concentrations.[38] Following completion of the distribution phase, the highest propane concentrations occur in adipose tissues and brain, similar to butane.

TREATMENT

The treatment of propane intoxication is supportive and similar to the treatment of butane and isobutane intoxication (see Butane and Isobutane—Treatment).

References

1. McGarvey EL, Clavet GJ, Mason W, Waite D. Adolescent inhalant abuse: environments of use. Am J Drug Alcohol Abuse 1999;25:731–741.

2. Spiller HA. Epidemiology of volatile substance abuse (VSA) cases reported to US poison centers. Am J Drug Alcohol Abuse 2004;30:155–165.

3. Mathew B, Kapp E, Jones TR. Commercial butane abuse: a disturbing case. Br J Addict 1989;84:563–564.

4. Evans AC, Raistrick D. Phenomenology of intoxication with toluene-based adhesives and butane gas. Br J Psychiatry 1987;150:769–773.

5. Gunn J, Wilson J, Mackintosh AF. Butane sniffing causing ventricular fibrillation. Lancet 1989;1(8638):617.

6. Stoughton RW, Lamson PD. The relative anaesthetic activity of the butanes and pentanes. J Pharmacol Exp Ther 1936;58:74–77.

7. Patty FA, Yant WP. Odor intensity and symptoms produced by commercial propane, butane, pentane, hexane and heptane vapor (Report 6820). Washington, DC: United States Department of Interior, Bureau of Mines,;1929:1–10.

8. Stewart RD, Herrmann AA, Baretta ED, Forster HV, Sikora JJ, Newton PE, Soto RJ. Acute and repetitive human exposure to isobutane. Scan J Work Environ Health 1977;3:234–243.

9. Filser JG, Bolt HM, Muliawan H, Kappus H. Quantitative evaluation of ethane and *n*-pentane as indicators of lipid peroxidation *in vivo*. Arch Toxicol 1983;52:135–147.

10. Low LK, Meeks JR, Mackerer CR. The aliphatic hydrocarbons (C1-C12). In: Snyder R (Ed.), Ethel Browning's toxicity and metabolism of industrial solvents. Vol. 1. Hydrocarbons. 2nd ed,. Amsterdam: Elsevier; 1987.

11. Tsukamoto S, Chiba S, Muto T, Ishikawa T, Shimamura M. Study on the metabolism of volatile hydrocarbons in mice. J Toxicol Sci 1985;10:323–332.

12. Reinhardt CF, Azar A, Maxfield ME, Smith PE, Mullin LS. Cardiac arrhythmia and aerosol "sniffing". Arch Environ Health 1971;22:265–279.

13. Ago M, Ago K, Ogata M. A fatal case of *n*-butane poisoning after inhaling anti-perspiration aerosol deodorant. Leg Med 2002;4:113–118.

14. Nishi K, Ito N, Mizumoto J, Wada K, Yamada T, Mitsukuni Y, Kamimura S. Death associated with butane inhalation: report of a case. Jpn J Leg Med 1985;39:214–216.

15. Pfeiffer H, Al Khaddam M, Brinkmann B, Kohler H, Beike J. Sudden death after isobutane sniffing: a report of two forensic cases. Int J Leg Med 2006;120:168–173.

16. Jackowski C, Romhild W, Aebi B, Bernhard W, Krause D, Dirnhofer R. Autoerotic accident by inhalation of propane-butane gas mixture. Am J Forensic Med Pathol 2005;26:355–359.

17. Harris D, Mirza Z. Butane encephalopathy. Emerg Med J 2005;22:676–677.

18. Döring G, Baumeister FA, Peters J, von der Beek J. Butane abuse associated encephalopathy. Klin Padiatr 2002;214:295–298.

19. Gomibuchi K, Gomibuchi T, Kurita H. Treatment and 9-year outcome of butane-induced psychotic disorder in a butane-dependent Japanese male adolescent. Psychiatr Clin Neurosci 2001;55:163.

20. Jung I-K, Lee H-J, Cho B-H. Persistent psychotic disorder in an adolescent with a past history of butane gas dependence. Eur Psychiatry 2004;19:519–520.

21. Kile SJ, Camilleri CC, Latchaw RE, Tharp BR. Bithalamic lesions of butane encephalopathy. Pediatr Neurol 2006;35:439–441.

22. O'Neill JO, McCarthy C. Myocardial infarction in a 14 year old boy after butane inhalation. Isr Med J 1999;92:344.

23. LoVecchio F, Flton SE. Ventricular fibrillation following inhalation of Glade Air Freshner™. Eur J Emerg Med 2001;8:153–154.

24. Girard F, Le Tacon S, Maria M, Pierrard O, Monin P. [Ventricular fibrillation following deodorant spray inhalation]. Ann Fr Anesth Reanim 2008;27:83–85. [French]

25. Edwards KE, Wenstone R. Successful resuscitation from recurrent ventricular fibrillation secondary to butane inhalation. Br J Anaesth 2000;84:803–805.

26. El-Menyar AA, El-Tawil M, Al Suwaidi J. A teenager with angiographically normal epicardial coronary arteries and acute myocardial infarction after butane inhalation. Eur J Emerg Med 2005;12:137–141.

27. Roberts MJ, McIvor RA, Adgey AA. Asystole following butane gas inhalation. Br J Hosp Med 1990;44:294.

28. Oh S-J, Lee S-E, Burm J-S, Chung C-H, Lee J-W, Chang Y-C, Kim D-C. Explosive burns during abusive inhalation of butane gas. Burns 1999;25:341–344.

29. Huston BM, Lamm KR. Complications following butane inhalation and flash fire. Am J Forensic Med Pathol 1997; 18:140–143.

30. Rohrig TP. Sudden death due to butane inhalation. Am J Forensic Med Pathol 1997;18:299–302.

31. Oritani S, Zhu BL, Ishida K, Quan L, Taniguchi M, Fujuta M, et al. Two autopsy cases involving asphyxia by butane inhalation. Jpn J Forensic Toxicol 2001;19:257–262.

32. Anderson HR, Macnair RS, Ramsey JD. Deaths from abuse of volatile substances: a national epidemiological study. Br Med J (Clin Res Ed) 1985;290(6464):304–307.

33. Beasley M, Frampton L, Fountain J. Inhalant abuse in New Zealand. N Zl Med J 2006;119:U1952.

34. Fuke C, Miyazaki T, Arao T, Morinaga Y, Takaesu H, Takeda T, Iwamasa T. A fatal case considered to be due to cardiac arrhythmia associated with butane inhalation. Leg Med 2002;4:134–138.

35. Streete PJ, Ruprah M, Ramsey JD, Flanagan RJ. Detection and identification of volatile substances by headspace capillary gas chromatography to aid the diagnosis of acute poisoning. Analyst 1992;117:1111–1127.

36. Park J, Min JS, Heo S, Lim MA, Park SW. Quantification of propane in biological materials by head-space GC. Forensic Sci Int 2005;151:165–170.

37. Winek CL, Wahba WW, Huston RM. Accidental death due to inhalation of butane. J Anal Toxicol 1997;21:323.

38. Bouche M-P, Lambert WE, van Bocxlaer JF, Piette MH, de Leenheer AP. Quantitative determination of *n*-propane, *iso*-butane, and *n*-butane by headspace GC-MS in intoxications by inhalation of lighter fluid. J Anal Toxicol 2002:26:35–42.

39. Adgey AA, Johnston PW, McMechan S. Sudden cardiac death and substance abuse. Resuscitation 1995;29: 219–221.

40. Williams DR, Cole SJ. Ventricular fibrillation following butane gas inhalation. Resuscitation 1998;37:43–45.

41. Wheeler MG, Rozycki AA, Smith RP. Recreational propane inhalation in an adolescent male. Clin Toxicol 1992;30:135–139.

42. Stoughton RW, Lamson PD. The relative anesthetic activity of the butanes and pentanes. J Pharmacol Exp Ther 1936;58:74–77.

43. Stewart RD, Newton PE, Baretta ED, Herrmann AA, Forster HV, Soto RJ. Physiological response to aerosol propellants. Environ Health Perspect 1978;26:275–285.

44. Grosse K, Grosse J. [Propane abuse. Extreme dose increase due to development of tolerance]. Nervenarzt 2000;71:50–53. [German]

45. Krantz JC, Carr CJ, Vitcha JF. Anesthesia. 31. A study of cyclic and noncyclic hydrocarbons on cardiac automaticity. J Pharmacol Exp Ther 1948;94:315–318.

46. Sugie H, Sasaki C, Hashimoto C, Takeshita H, Nagai T, Nakamura S, et al. Three cases of sudden death due to butane or propane gas inhalation: analysis of tissues for gas components. Forensic Sci Int 2004;143:211–214.

47. Haq MZ, Hameli AZ. A death involving asphyxiation from propane inhalation. J Forensic Sci 1980;25:25–28.

48. Hicks LM, Hung JL, Baxter CR. Liquid propane cold injury: a clinicopathologic and experimental study. J Trauma 1979;19:701–703.

49. Siegel E, Wason S. Sudden death caused by inhalation of butane and propane. N Engl J Med 1990;323:1638.

50. Tsoukali H, Dimitriou A, Vassiliades N. Death during deliberate propane inhalation. Forensic Sci Int 1998; 93:1–4.

51. McLennan JJ, Skeula-Perlman A, Lippstone MB, Callery RT. Propane-associated autoerotic fatalities. Am J Forensic Med Pathol 1998;19:381–386.

52. Garriott J, Petty CS. Death from inhalant abuse: toxicological and pathological evaluation of 34 cases. Clin Toxicol 1980;16:305–315.

Chapter 43

ETHYL CHLORIDE

HISTORY

The anesthetic properties of ethyl chloride were described by Flourens in 1847, and the following year Heyfelder anesthetized 3 patients with ethyl chloride.[1] However, he abandoned the use of ethyl chloride for this purpose because of high volatility and cost. McCardie reintroduced ethyl chloride as an anesthetic in the early 1900s after a Swedish dentist inadvertently produced unconsciousness in a patient while spraying the gums during a dental extraction.[2] Ethyl chloride was administered as a convenient and easy-to-use general anesthetic in the United Kingdom from 1945 to 1964; however, the use of ethyl chloride as an anesthetic was abandoned as safer anesthetics became available.[1]

IDENTIFYING CHARACTERISTICS

Ethyl chloride (chloroethane, C_2H_5Cl) is a colorless, flammable gas with a pungent, somewhat unpleasant, ether-like odor at ambient pressure and temperature. This volatile liquid has a boiling point of 12.3°C (54.1°F) and a vapor pressure of >1,000 mm Hg at 20°C (68°F). Table 43.1 lists some the values for the physical properties of ethyl chloride. At room temperature and ambient pressure, the release of 1 mL of ethyl chloride produces 560 mL of vapor.[1] Evaporation of ethyl chloride from the surface of the skin produces rapid cooling (−20°C/−4°F) of the surrounding area; rapid surface cooling also limits the concentration of ethyl chloride in inspired air.

Like other volatile substances of abuse, ethyl chloride is highly lipid soluble. Street terminology for ethyl chloride includes head cleaner, ethyl gaz, and ethyl four star.

EXPOSURE

There are relatively few case reports in the medical literature involving volatile substance abuse associated with ethyl chloride. The abuse of ethyl chloride typically occurs in polydrug abusers (alcohol, marijuana) including use a substitute for amyl nitrite in homosexual men.[3] Ethyl chloride is used as a solvent for the manufacture of perfumes, as well as a refrigerant, alkylating agent, topical anesthetic, and chemical intermediate in the production of tetraethyl lead. In the past, ethyl chloride was an anesthetic agent. Ethyl chloride is a constituent of VCR head cleaners, and these products are intentionally inhaled for euphoria and the enhancement of sexual pleasure.[4] Brand names for some of these products reflect these uses including Maximum Impact, Jungle Juice Plus, Macho, Ethyl Four Star, Head Cleaner, Ethyl Gas, Ethyl Gaz, Rush, and Black-Jac. These products are available in stores and over the Internet.

DOSE EFFECT

Pre-World War II anesthesiology literature indicate that 4% (40,000 ppm) ethyl chloride produces rapid general anesthesia within 1–4 minutes.[1] Case reports associate the daily sniffing of an estimated 100–300 mL ethyl chloride over 4 months with the development of cerebellar dysfunction and hallucinations that resolved over several weeks.[5,6]

TOXICOKINETICS

The high lipid solubility of ethyl chloride results in the rapid absorption of ethyl chloride from the lungs. The biotransformation of ethyl chloride is not well studied in humans. Rodent studies suggest that there are 2 major pathways: 1) cytochrome P450-dependent dechlorination resulting in the formation of acetaldehyde and acetic acid, and 2) glutathione-*S*-transferase-dependent conjugation of ethyl chloride followed by metabolism.[7,8] These pathways are dose- and species-dependent. Oxidation of ethyl chloride in rodents primarily involves CYP2E1. Figure 43.1 displays the proposed pathways of ethyl chloride metabolism.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Ethyl chloride is a central nervous system depressant with anesthetic properties. Autopsy finding in individuals dying during ethyl chloride abuse are nonspecific. Postmortem examination of a 30-year-old man found dead with rag over his mouth and empty cans of ethyl

TABLE 43.1. Physical Properties of Ethyl Chloride.

Physical Property	Value
CAS Number	75-00-3
Density	0.9214 g/cm^3 (0°C)
Melting Point	−139°C (−218.2°F)
Boiling Point	12.3°C (54.1°F)
log P (Octanol-Water)	1.43
Water Solubility	6710 mg/L (25°C/77°F)
Vapor Pressure	1010 mm Hg (20°C/68°F)
Conversion Factor	1 ppm = 2.64 mg/m^3 (25°C/77°F)

chloride containing-VCR head cleaner near him demonstrated general visceral congestion and cerebral edema without a specific cause of death other than ethyl chloride abuse.[9]

CLINICAL RESPONSE

Illicit Use

The effects of the acute inhalation of ethyl chloride vapors resemble ethanol intoxication with behavioral disinhibition, euphoria, and perceptual changes. The intentional inhalation of ethyl chloride vapors may cause lightheadedness, confusion, impaired short-term memory, elation, slurred speech, auditory and visual hallucinations, ataxia, lower extremity weakness, gait spasticity, diplopia, blurred vision, and altered consciousness.[10] Abnormal neurologic signs include positive Romberg test, poor heel-to-shin, and hyperreflexia. Other adverse effects of the abuse of ethyl chloride include disorientation, paranoia, and unpleasant hallucinations.[5] A 41-year-old man with human immunodeficiency virus (HIV) presented to the emergency department with tremor, ataxia, dysarthria, ataxia, gait disturbances, hallucinations, and generalized weakness.[4] He had been inhaling 2 canisters of VCR head cleaner (ethyl chloride concentration not reported) several times weekly including the day of admission. The weakness, hallucinations, and ataxia resolved over 5 days of hospitalization; he was lost to follow-up after hospital discharge.

Fatalities

Several case reports associate fatalities with the abuse of ethyl chloride. A college student was found unconscious with a metal canister containing ethyl chloride near his right hand.[11] On arrival of the paramedics, his
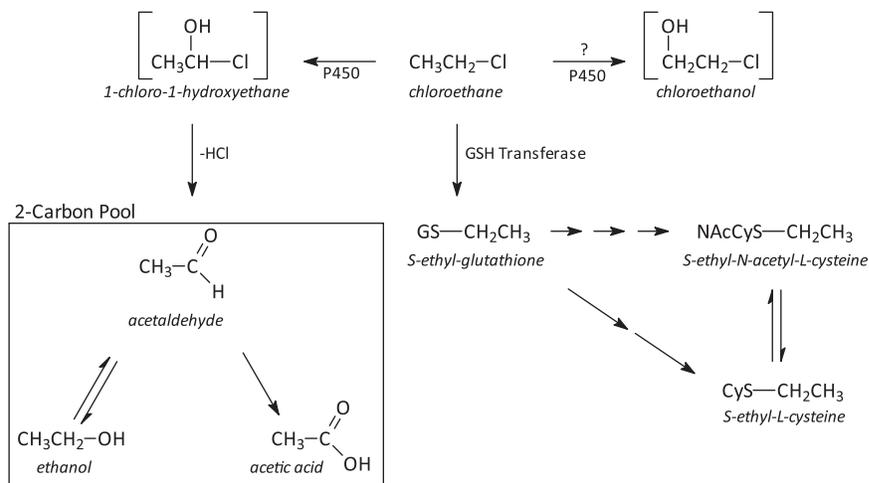
FIGURE 43.1. Proposed pathways of ethyl chloride (chloroethane) metabolism.[8]

initial rhythm was ventricular fibrillation. He was transported to the hospital where he was pronounced dead after 1 hour of resuscitative efforts including intubation and advance cardiac life support.

Abstinence Syndrome

Several case reports associate cessation of chronic abuse of high doses of ethyl chloride with withdrawal-like symptoms including ataxia, tremor, weakness, dysarthria, gait disturbances, and hallucinations.[5] These symptoms abate over the first few days after cessation of the abuse of ethyl chloride, but the authors were unable to determine if the etiology of these effects were related to withdrawal, intoxication, or other withdrawal syndromes. A 52-year-old man developed hypotension, impaired consciousness, and suffered a grand mal seizure within approximately 12 hours after admission to the hospital for confusion and disorientation.[6] Over the next 10 days, disorientation, short-term memory impairment, visual hallucinations, ataxia, gait disturbances, amnesia with confabulation, and visual hallucinations occurred. By 2 weeks, most of these abnormalities resolved. He had a 30-year history of ethyl chloride, ethanol, and benzodiazepine abuse. Although the clinical course suggests withdrawal from ethyl chloride, withdrawal from ethanol or benzodiazepine may also have contributed to these abnormalities.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the quantitation of ethyl chloride primarily involve headspace gas chromatography.[9] The co-elution of ethanol with ethyl chloride or the internal standard, *t*-butyl methyl ether, indicated the importance of using a second complementary analytic technique for ethyl chloride analysis. Using gas chromatography/flame ionization detection, ethyl chloride may be indistinguishable from ethanol because the peaks may co-elute. This result may be particularly confusing when the presence of ethyl chloride abuse is not obvious.[12] Analysis with gas chromatography/mass spectrometry allows the separation of these 2 compounds.

The low boiling point of ethyl chloride requires the storage of biologic samples in tightly sealed, glass containers that are refrigerated or frozen. Blood may be collected in gray top tubes (sodium fluoride, potassium oxalate) with minimal headspace. Repeated opening and recapping of stored samples may result in the loss of ethyl chloride from the sample through volatilization, particularly at room temperature.

Biomarkers

A 30-year-old man was found dead in a locked apartment with a rag held loosely in his mouth and 3 empty cans of VCR head cleaner near his body.[9] Postmortem blood samples contained ethyl chloride concentration of 423 mg/L along with diazepam (0.064 mg/L) and nordiazepam (0.126 mg/L). Analysis of vitreous humor also demonstrated the presence of ethyl chloride (12 mg/L), but loss of ethyl chloride may have occurred because the vial was opened several times for prior analysis. Hospital blood from a college student found unconscious with a canister of ethyl chloride next to him contained 200 mg ethyl chloride/L.[11] He was pronounced dead after about 1 hour of resuscitation; the postmortem heart blood ethyl chloride concentration was 650 mg/L. Some loss of ethyl chloride from the hospital blood sample may have occurred during storage because of repeated opening of the antemortem blood. The ethyl chloride concentration in vitreous humor was 410 mg/L.

TREATMENT

Acute intoxication with ethyl chloride is associated with central nervous system depression and possibly with cardiac dysrhythmias. Most patients with altered consciousness recover rapidly by the time of presentation to the emergency department. The lack of improvement in the level of consciousness suggests the presence of other drugs of abuse, underlying trauma, or hypoxic brain damage. These patients should be carefully evaluated and monitored for dysrhythmias, hypoxia, and electrolyte imbalance. Treatment is supportive; there are no antidotes or methods to enhance the elimination of ethyl chloride. Frequently, volatile substance abuse involves the use of other drugs of abuse. Rehabilitation includes referral to chemical dependence centers and counseling.

References

1. Lawson JI. Ethyl chloride. Br J Anaesth 1965;37: 667–670.
2. McCardie WJ. Ethyl chloride as a general anaesthetic. Lancet 1903;i:952–957.
3. Hersh R. Abuse of ethyl chloride. Am J Psychiatry 1991; 143:270–271.
4. Finch CK, Lobo BL. Acute inhalant-induced neurotoxicity with delayed recovery. Ann Pharmacother 2005;39: 169–172.
5. Hes JP, Cohn DF, Streifler M. Ethyl chloride sniffing and cerebellar dysfunction (case report). Isr Ann Psychiatr Relat Discip 1979;17:122–125.

6. Nordin C, Rosenqvist M, Hollstedt C. Sniffing of ethyl chloride—an uncommon form of abuse with serious mental and neurological symptoms. Int J Addict 1988;23: 623–627.

7. Fedtke N, Certa H, Ebert R, Wiegand HJ. Species differences in the biotransformation of ethyl chloride. I. Cytochrome P450-depenent metabolism. Arch Toxicol 1994;68:158–166.

8. Fedtke N, Certa H, Ebert R, Wiegand HJ. Species differences in the biotransformation of ethyl chloride. II. GSH-dependent metabolism. Arch Toxicol 1994;68:217–223.

9. Broussard LA, Broussard AK, Pittman TS, Lirette DK. Death due to inhalation of ethyl chloride. J Forensic Sci 2000;45:223–225.

10. Soult TA, Walker JS. Ethyl chloride intoxication. Am J Emerg Med 1993;11:313–315.

11. Yacoub I, Robinson CA, Simmons GT, Hall M. Death attributed to ethyl chloride. J Anal Toxicol 1993;17: 384–385.

12. Tarnovski G, Hayashi T, Igarashi K, Ochi H, Matoba R. Misidentification of ethyl chloride in the routine GC-FID analysis for alcohol. Forensic Sci Int 2009;188:e7–e9.

Chapter 44

GASOLINE

HISTORY

Medical reports on the intentional inhalation of gasoline first appeared in the English medical literature in 1941, when a review of gasoline by Machle mentioned the intentional inhalation of gasoline for pleasurable effects.[1] In the early 1950s, case reports documented the abuse of gasoline by children[2] and adults.[3] The popularity of gasoline abuse is primarily limited to remote areas (e.g., Indian reservations, Australian aborigines).[4,5] Lead has been an octane-boosting additive in gasoline since the 1920s. The abuse of leaded gasoline by Aboriginal peoples in Northern Canada began in the late 1960s and intensified in the 1970s.[6] The organolead encephalopathy from leaded gasoline abuse was first recognized in 1968.[7] The misuse of leaded gasoline declined in the early 1980s, and the use of leaded gasoline in the United States and Canada was banned by the 1990s.

IDENTIFYING CHARACTERISTICS

Structure

In formulating gasoline, the primary goal is specified physical characteristics rather than a specific chemical composition. Therefore, gasoline (CAS RN: 8006-61-9) is a petroleum distillate containing a variable mixture of at least 150–200 C_4–C_{12} alkane, cycloalkane, alkene, and aromatic hydrocarbons obtained by "cracking" heavy fractions of crude oil in the boiling range 40–225°C (104–437°F).[8] Regular-grade gasoline contains substantial portions of C_8 and C_9 alkenes and cycloalkanes. Appreciable amounts of aromatic hydrocarbons

(e.g., xylene) are found in commercial fuels from California and Texas, especially those with high octane ratings. The exact mixture of this volatile liquid depends on the origin of the petroleum, the boiling point used to refine the petroleum, and the compounds added to the mixture during the refining process. Because of the greater volatility of low-molecular-weight hydrocarbons, the composition of vapor from gasoline differs from the composition of the liquid. Gasoline vapor contains up to 90% C_3–C_5 nonaromatic hydrocarbons with only about 2% C_6–C_8 aromatic hydrocarbons.[9] Tetraethyl lead (CAS RN: 78-00-2, MW: 323.44) was previously added in the United States as an antiknocking agent; newer additives to boost the octane rating include methyl tertiary-butyl ether (MTBE) and alcohols. Tetraethyl lead is a colorless, oily liquid that frequently has dyes (blue, orange, red) added. This compound has a musty odor; tetraethyl lead is soluble in most organic solvents and is minimally soluble in water. In the United States, the use of tetraethyl lead as an additive routinely occurred from 1921 until 1986. Over the next 6 years, the use of this compound was gradually eliminated; however, the use of tetraethyl lead still occurs in some developing countries.

Gasoline has a distinctive odor that is detectable by most people in the range of 1–2 ppm. The odor threshold depends, in part, on the composition of the gasoline and the odor of gasoline is identifiable in the range of 0.15–0.25 ppm.[10] The vapor density of gasoline is 3–4 times heavier than air, and the vapor pressure ranges from 400–775 mm Hg at 20°C (68°F). The volatility of gasoline depends on the physical characteristics of the particular grade of gasoline. In general, gasoline

possesses intermediate volatility and low viscosity compared with other hydrocarbons.

EXPOSURE

Gasoline sniffing remains a major form of substance misuse, particularly in isolated communities (e.g., Aboriginal communities across Australia and Canada, Native American communities in the United States).[11] Aboriginal victims accounted for about one-quarter of the deaths associated with volatile substance abuse in a retrospective study (1983–2002) of South Australia.[12] In a study of cases of volatile substance abuse reported to the Toxic Exposure Surveillance System (TESS) of the American Association of Poison Control Systems from 1996–2001, gasoline was the most commonly abused volatile substance.[13] The top 5 categories of volatile substances abused were gasoline (41%), paint (13%), propane/butane (6%), air fresheners (6%), and formalin (5%). Among adolescents, gasoline remained one of the most popular volatile substances of abuse in the latter 2000s.[14] Gasoline was the most common volatile substance of abuse among incarcerated youth (mean age, 15.5 years) in a Missouri study based on questionnaires; about one-third of these youth reported lifetime use of inhalants, and 22% of these youth reported gasoline sniffing.[15] Leaded gasoline from the 1970s contained 0.8–2.65 g of organic lead compounds/gallon, primarily as tetraethyl lead. Canada phased out leaded gasoline in 1990, whereas the United States eliminated lead from gasoline in 1996. Gasoline sniffers are typically polydrug users, who use other drugs of abuse when gasoline is not available.[16]

DOSE EFFECT

The toxicity of gasoline depends on the composition of hydrocarbons within this mixture. The C_6–C_8 alkanes and cycloalkanes have relatively stronger narcotic properties (e.g., ataxia, lightheadedness, altered consciousness, headache) and some irritant properties, whereas the corresponding alkenes are relatively weak anesthetics. Eye and throat irritation is the most sensitive symptom of exposure to gasoline. Exposure to ambient concentration of gasoline vapors exceeding 900 ppm causes central nervous system (CNS) symptoms within a few minutes. Volunteer studies indicate that exposures to 500–1,000 ppm gasoline vapors for 30–60 minutes causes mild mucous membrane irritation (eyes, nose, throat) and lightheadedness, whereas exposure to 1,000–3,000 ppm for the same duration causes nausea, lightheadedness, headache, numbness, and impaired coordination.[17] During this study, exposure to gasoline vapors above 10,700 ppm for <5 minutes caused the rapid onset of anesthetic effects including dizziness, ataxia, confusion, and altered consciousness. Exposures to gasoline vapors above 1,000 ppm produced more severe ocular symptoms including lacrimation and conjunctival erythema.[18] Volatile substance abusers typically inhale 15–20 breaths of vapor from open gasoline containers or from gasoline saturated rags to experience hallucinations and euphoria.[19,20] During binges, gasoline abusers inhale for 3–5 minutes every 3–4 hours over 48 hours.[21]

TOXICOKINETICS

Absorption

In general, the highly volatile C_5–C_7 compounds (alkanes, cycloalkanes, aromatic hydrocarbons) readily cross alveolar membranes. Animal studies suggest the absorption of higher-numbered hydrocarbons by the lungs is more limited and the effects of these compounds are primarily local.[22] In the range of C_3–C_9, the absorption of unsaturated hydrocarbons (alkenes, aromatics) through the lungs is greater compared with similar saturated compounds.[23] The lungs readily absorb organic tetraethyl lead.

Distribution

There is substantial differences in the distribution pattern between aliphatic, alicyclic, and aromatic components of petroleum distillates. Although aromatics produce the highest concentration in blood, their concentration in brain is lower than the concentration of aliphatic and alicyclic hydrocarbons.

Biotransformation

The metabolism of most petroleum distillates is complex and involves hepatic biotransformation. The metabolic fate of individual compounds is difficult to predict because of potential saturation of substrates and competitive inhibition or enhancement of various enzyme systems. Monooxygenases in the liver oxidize aliphatic hydrocarbons to their respective alcohols. In rodents, cytochrome P450 isoenzymes rapidly convert tetraethyl lead to the relatively stable compound, triethyl lead.[24] Within 24 hours, triethyl lead accounted for 40–50% of lead in internal organs and 70% of the lead in muscle. Ultimately, most triethyl lead is converted to inorganic lead and stored in bone. Excretion of organic lead in urine and feces is small. The elimination half-life of tetraethyl lead in blood is a few days.[25] In a case of occupational organolead exposure, the elimination of triethyl lead followed a 2-compartment model with an elimination half-life of 35 days and ~100 days.[26]

Elimination

In a patient found unconscious in his gasoline vapor-filled car, the estimated half-life of gasoline was about 17 hours over the week prior to his death from multiorgan failure.[27] Generally, the kidney excretes a portion of alkanes unchanged, whereas most aromatic hydrocarbons appear in the urine as metabolites. Because of high vapor pressure and low blood/air coefficients, alkanes and cycloalkanes with low molecular weight appear in relatively high concentrations in expired air compared with aromatic compounds.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Chronic exposure to gasoline (i.e., sniffing) produces cerebellar abnormalities and intellectual impairment; organic and inorganic lead encephalopathy accounts for most of these changes, but the exact causal role of organolead in gasoline-induced encephalopathy is not well-defined.[4,28] Most chronic studies of animals exposed to unleaded gasoline vapors at doses up to 2,000 ppm do not demonstrate behavioral changes or histopathologic abnormalities in the central and peripheral nervous system.[29] Tetraethyl lead and triethyl lead (metabolite) are much more lipid soluble than inorganic lead.[30] Consequently, tetraethyl lead can accumulate in the CNS much more rapidly than inorganic lead, causing the rapid onset of neurologic dysfunction and encephalopathy. The contribution of tetraethyl lead to the short-term effects of gasoline intoxication is unclear. Although inhalation of tetraethyl lead alone may cause hallucinations and behavioral changes,[31] tetraethyl lead may potentiate the effects of other volatile hydrocarbons in gasoline. Chronic inhalation of petroleum distillates (e.g., gasoline sniffing) does not usually produce a chemical pneumonitis, perhaps because of relatively low alveolar concentration and limited duration of exposure.

Postmortem Examination

Postmortem findings in patients dying suddenly following gasoline sniffing are usually nonspecific.[32] The most common abnormalities on postmortem examination are pulmonary and cerebral edema. A 21-year-old man died in the hospital after his fourth admission for toxic psychosis associated with gasoline sniffing; the blood lead concentration at admission during this visit was 197 μg/dL.[31] Postmortem examination revealed mild cortical atrophy with ventricular dilation and patchy cerebellar atrophy. Histologic examination of the brain demonstrated loss of pyramidal neurons from the hippocampus and patchy loss of Purkinje cells along with dentate neurons. There was also mild, diffuse astrocytic gliosis of the pons, striatum, pallidum, and thalamus.

CLINICAL RESPONSE

Illicit Use

Gasoline sniffing causes 2 distinct clinical syndromes: 1) an acute intoxication, and 2) an organolead-associated encephalopathy.[33] The acute intoxication associated with gasoline sniffing is similar to ethanol intoxication, manifest by giddiness, excitement, restlessness, dizziness, loss coordination and ataxia, feelings of numbness, confusion, disorientation, hallucination, and loss of consciousness. These effects persist for several hours (i.e., up to 5–6 hours).[16] Organic lead may contribute to the acute effects of gasoline sniffing, and serious gasoline abusers often prefer high-octane-leaded gasoline, when available, because of the perception of the more rapid onset of intoxication.[34]

Complications

Complications associated with gasoline sniffing include organic lead encephalopathy, aspiration pneumonia, and burns from the ignition of gasoline vapors or clothes soaked in gasoline.[35] The encephalopathy (i.e., alteration of consciousness or orientation) associated with the chronic abuse of leaded gasoline vapors is distinct from the classic features of inorganic lead poisoning and similar to the encephalopathy associated with tetraethyl lead intoxication following occupational exposure.[30,36] In mild cases of encephalopathy from chronic leaded gasoline sniffing, symptoms include anorexia, nausea, vomiting, insomnia, headache, fatigue, irritability, anxiety, agitation, and emotional lability.[37] Other neurologic complications of chronic leaded gasoline abuse include abnormal jaw jerk, poor memory, nystagmus, hyperreflexia, gait disturbances, movement disorders (ataxia, myoclonus, choreoathetosis, postural or intention tremor), confusion, impaired thinking, depression, poor abstraction, and short attention span.[38,39] In contrast to solvent abuse, acute and persistent organic psychosis is a frequent complication of leaded gasoline sniffing.[6] Psychiatric symptoms are particularly common in organolead toxicity compared with inorganic lead poisoning. More severe cases of organolead-associated encephalopathy involve frank psychosis, delirium, cerebellar ataxia, seizures, and coma along with fluctuating levels of consciousness, illusions, auditory and visual hallucination, paranoia, and delusions.[7,40] Although most of the chronic effects of leaded gasoline sniffing resolve with abstinence, case reports associate recurrent

or progressive encephalopathy with the chronic abuse of leaded gasoline.[31] Continued leaded gasoline sniffing may cause progressive dementia and cerebellar ataxia that results in permanent dysmetria, dysarthria, ataxic gait, and severe impairment of thought processes.[31] Although the encephalopathy associated with leaded gasoline sniffing typically presents with gastrointestinal and psychiatric disturbances, occasionally cerebellar signs (chorea, ataxia, resting tremor) and myoclonus are the prominent parts of the clinical presentation.[41,42]

Nausea, vomiting, abdominal pain, and anorexia may occur during organolead intoxication; however, gastrointestinal symptoms are not as prominent in organolead intoxication compared with inorganic lead poisoning. Rare case reports of chronic gasoline sniffing are associated with myopathy, elevated serum creatine kinase, and myoglobinuria in the absence of CNS effects.[43] Symptoms included muscle weakness and myalgias. Rare case reports also associate gasoline sniffing with sudden death; case series of death related to volatile substance abuse include fatalities attributed to gasoline sniffing.[44] The 2009 Annual Report of the American Association of Poison Control Centers' National Poison Data System did not list any deaths from intentional inhalation of gasoline.[45]

Abstinence Syndrome

There are few data on the existence of an abstinence syndrome in gasoline sniffers. Physical dependence and withdrawal symptoms are not usually associated with gasoline sniffing.[46] However, in a case series of 9 adolescents inhaling gasoline fumes daily, all subjects reported a withdrawal syndrome including irritability, psychomotor retardation, anhedonia, dry mouth, sleep disturbances, craving, and increased lacrimation.[47] Other complaints included headache and palpitations. Leaded gasoline use remained legal during the time of this study. Although some observations of gasoline sniffers suggest that some tolerance develops to the effects of gasoline sniffing based on the need to use larger amounts of gasoline to achieve the desired effects,[48] other case reports do not document tolerance to the hallucinogenic effects of gasoline sniffing.[49]

DIAGNOSTIC TESTING

Analytic Methods

TECHNIQUES

Gasoline is a mixture of hydrocarbons that varies depending on the refinery techniques; quantitation of gasoline is complicated by the complexity of this mixture.

However, gasoline does have a characteristic aromatic, aliphatic, and naphthalenic profile. Methods for analysis of gasoline in biologic samples include gas chromatography with flame ionization detection (GC/FID),[32] and gas chromatography/mass spectrometry (GC/MS) after liquid-liquid extraction.[50] The limit of detection and the lower limit of quantitation were 0.3 mg/L and 1.0 mg/L, respectively, for the latter method. Different authors select different peaks in the chromatograph to estimate the concentration of gasoline in blood or postmortem tissues including isopentane, *n*-pentane, 2-methylpentane, 3-methylpentane, *n*-hexane, benzene, toluene, *m,o*-xylene, *m,p*-xylene, and propylbenzene. The gasoline concentration is estimated by comparing the peak area of the selected marker with the concentration of that marker in the gasoline standard. Selection of the peak depends on the volatility of substance (i.e., risk of loss during storage) and the abundance of the substance in gasoline; the estimated concentration of gasoline will vary depending on the substance selected. A study of the estimated gasoline concentration in heart blood compared the following 6 markers: isopentane, *n*-pentane, 2-methyl-2-butene, 2-methylpentane, 3-methylpentane, and *n*-hexane.[51] The calculated gasoline concentration ranged from 0.051 mg/L (isopentane) to 0.447 mg/L (*n*-hexane). The use of higher boiling point compounds (e.g., C_0–C_2 naphthalene profiles) facilitates comparison of samples with substantial amount of evaporation compared with the original sample.[52]

Factors that affect the chemical composition of gasoline include the source (e.g., refinery), season (i.e., winter blend vs. summer blend), location, time of month, and the amount evaporated from the original sample. Analytic methods are available to determine the similarity of gasoline samples include visual inspection of target compounds in the profile of the gas chromatograph, covariance mapping and GC/MS, and principal component analysis of the GC/MS profiles of the gasoline samples.[53] Covariance mapping and GC/MS can discriminate the source of 2 samples following analysis of 3 replicate samples with test power of >99% ($\beta < 0.01$) at a significance level (α) of 0.05.[54] Collection of blood samples (e.g., gray-top tube with minimal headspace) for analysis of gasoline content is similar to other volatile substances. The choice of an analytic method for gasoline analysis in biologic specimens should be done in consultation with an analytic toxicologist to determine the optimal technique for the specific clinical situation.

Biomarkers

Lipophilic substances (e.g., gasoline) do not easily diffuse into the vitreous humor; therefore, the concen-

trations of substances used to estimate the gasoline concentration are low compared with blood.[50]

BLOOD

ILLICIT USE.   Blood lead concentrations reflect exposure to both organic and inorganic lead; the trend in these blood concentrations is more useful than a single value for determining the extent of leaded gasoline abuse. High blood lead concentrations (i.e., >100 μg/dL) suggest recent, high-dose exposure to leaded gasoline because of the rapid decline over hours to days.[6] Moderately elevated blood lead levels (30–50 μg/dL) remaining relatively stable over several days suggest the presence of inorganic lead and the absence of organic lead exposure in the previous few days to a few weeks, depending on the amount of bone lead stores. These moderately elevated blood lead concentrations decline rapidly in patients with limited leaded gasoline exposure and no significant bone lead stores. Free erythrocyte protoporphyrin (FEP) concentrations do not correlate well to blood lead concentrations in gasoline sniffers.[55]

OVERDOSE.   The estimated blood gasoline concentration in a 44-year-old man found unconscious inside a gasoline vapor-filled car was 247 mg/L, as measured by headspace gas chromatography/mass spectrometry.[27] He died 9 days later of multiorgan failure.

POSTMORTEM.   A 15-year-old boy collapsed at home with several substances next to his body including gasoline, solvents, paint, glue, and turpentine.[50] Postmortem analysis of peripheral and heart blood demonstrated gasoline concentrations of 22.1 mg/L and 38.3 mg/L, respectively, based on GC/FID analysis using *m*, *p*-xylene as the reference peak for gasoline quantitation. The heart blood also contained high concentrations of toluene (12.5 mg/L) and 6% carboxyhemoglobin. A 34-year-old man was found dead in his bed with a small quantity of liquid fuel near his nose.[56] The estimated gasoline concentration was 4.259 ppm (μg/g) based on GC/MS using several constituents as reference peaks for gasoline quantitation.

Abnormalities

In contrast to inorganic lead poisoning, organolead toxicity is not associated with lead lines in the long bones, nail bed changes, or gingival lead lines. Anemia and basophilic stippling does not typically occur with organolead intoxication. In a case series of 50 patients referred to a pediatric neurology clinic for gasoline sniffing, the most common abnormality on electroencephalography

was very low voltage and diffuse slow wave activity.[39] Evaluating the effect of chronic gasoline sniffing on cognitive function is complicated by multiple confounding factors including education, age, gender, baseline intelligence quotient, polydrug use, acute intoxication, and the effect of lead in the gasoline. A cross-sectional study of 50 current indigenous Australian gasoline sniffers and 96 health indigenous Australians suggested that gasoline sniffers performed below controls on complex psychomotor tasks, learning, executive function, and spatial awareness after controlling for demographic factors.[57] The size of the deficit ranged from 0.7–1.5 standard deviations. Simple psychomotor tasks and working memory were less affected by gasoline sniffing. The groups were not matched for gender (i.e., more women in the control group), and most of the gasoline sniffers inhaled daily (i.e., up to 12 hours prior to testing).

TREATMENT

Stabilization

The treatment of acute gasoline intoxication is supportive. Respiratory depression may complicate the clinical course of organolead poisoning associated with gasoline sniffing, particularly if seizures occur. These patients should be evaluated for the presence of hypoxemia and dysrhythmias. Aspiration pneumonia is a common complication of severe organolead toxicity; sepsis may occur.[58]

Elimination Enhancement

There are no clinically effective methods to enhance the elimination of lead other than chelation.

Antidotes

Case reports associate clinical improvement with the use of chelating agents,[59] and some authors recommend the use of chelating agents to remove inorganic lead.[4] However, the improvement in the abnormalities associated with the encephalopathy does not closely correlate to blood lead concentrations, and there are inadequate data to determine whether or not the use of chelating agents alters clinical outcome of gasoline-induced encephalopathy. Most patients improve over several weeks to months with abstinence from gasoline sniffing. The use of chelating agents increases the excretion of inorganic lead, but inorganic lead is not clearly responsible for the psychosis associated with organolead toxicity.[6] In a series of 25 patients admitted with intentional gasoline sniffing and altered mental status (delirium, drowsiness, stupor), 18 of 20 patients were chelated for

lead intoxication.[38] Despite high blood lead concentrations, chelation of inorganic lead did not appear to alter outcome significantly.

References

1. Machle W. Gasoline intoxication. JAMA 1941;117: 1965–1971.

2. Clinger OW, Johnson NA. Purposeful inhalation of gasoline vapors. Psychiatr Q 1951;25:557–567.

3. Faucett RL, Jensen RA. Addiction to the inhalation of gasoline fumes in a child. J Pediatr 1952;41:364–368.

4. Fortenberry JD. Gasoline sniffing. Am J Med 1985; 79:740–744.

5. Boeckx RL, Postl B, Coodin FJ. Gasoline sniffing and tetraethyl lead poisoning in children. Pediatrics 1977; 60:140–145.

6. Tenenbein M. Leaded gasoline abuse: the role of tetraethyl lead. Hum Exp Toxicol 1997;16:217–222.

7. Law WR, Nelson ER. Gasoline-sniffing by an adult report of a case with the unusual complication of lead encephalopathy. JAMA 1968;204:144–146.

8. Weaver NK. Gasoline toxicology implications for human health. Ann NY Acad Sci 1988;534:441–451.

9. International Agency for Research on Cancer: Gasoline. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Occupational Exposures in Petroleum Refining; Crude Oil and Major Petroleum Fuels. Vol 45. Lyon, World Health Organization, 1989:159–201.

10. Anonymous. Toxicology update. Gasoline. J Appl Toxicol 1989;9:203–210.

11. Preuss K, Brown JN. Stopping petrol sniffing in remote Aboriginal Australia: key elements of the Mt Theo Program. Drug Alcohol Rev 2006;25:189–193.

12. Wick R, Gilbert JD, Felgate P, Byard RW. Inhalant deaths in South Australia a 20-year retrospective autopsy study. Am J Forensic Med Pathol 2007;28:319–322.

13. Spiller HA. Epidemiology of volatile substance abuse (VSA) cases reported to US poison centers. Am J Drug Alcohol Abuse 2004;30:155–165.

14. Substance Abuse and Mental Health Services Administration. National Survey on Drug Use and Health. US Health and Human Services, Office of Applied Studies. Available at http://oas.samhsa.gov/2k8/inhalants/inhalants.pdf. Accessed 2011 April 28.

15. Howard MO, Balster RL, Cottler LB, Wu LT, Vaughn MG. Inhalant use among incarcerated adolescents in the United States: prevalence, characteristics, and correlates of use. Drug Alcohol Depend 2008;93:197–209.

16. Poklis A, Burkett CD. Gasoline sniffing: a review. Clin Toxicol 1977;11:35–41.

17. Drinker P, Paglou CP, Warren MF. The threshold toxicity of gasoline vapour. J Ind Hyg Toxicol 1943;25:225–232.

18. Davis A, Schafer LJ, Bell ZG. The effects on human volunteers of exposure to air containing gasoline vapor. Arch Environ Health 1960;1:548–554.

19. Black PD. Mental illness due to the voluntary inhalation of petrol vapour. Med J Aust 1967;2:70–71.

20. Bartlett S, Tapia F. Glue and gasoline "sniffing," the addiction of youth. Mo Med 1966;63:270–272.

21. Edwards RV. A case report of gasoline sniffing. Am J Psychiatry 1960;117:555–557.

22. Gerarde HW. Toxicologic studies on hydrocarbons. IX. The aspiration hazard and toxicity of hydrocarbons and hydrocarbon mixtures. Arch Environ Health 1963;6: 329–341.

23. Dahl AR, Damon EG, Mauderly JL, Rothenberg SJ, Seiler FA, McClellan RO. Uptake of 19 hydrocarbon vapors inhaled by F344 rats. Fundam Appl Toxicol 1988; 10:262–269.

24. Bolanowska W. Distribution and excretion of triethyllead in rats. Br J Ind Med 1968;25:203–208.

25. Grandjean P, Nielsen T. Organolead compounds: environmental health aspects. Residue Rev 1979;72:97–148.

26. Yamamura Y, Takakura J, Hirayama F, Yamauchi H, Yoshida M. Tetraethyl lead poisoning caused by cleaning work in the aviation fuel tank. Jap J Ind Health 1975; 17:223–235.

27. Matsumoto T, Koga M, Sata T, Kadoya T, Shigematsu A. The changes of gasoline compounds in blood in a case of gasoline intoxication. Clin Toxicol 1992;30:653–662.

28. Valpey R, Goble GJ, Copass MK, Sumi SM. Acute and chronic progressive encephalopathy due to gasoline sniffing. Neurology 1978;28:507–510

29. Agency for Toxic Substances and Disease Registry. ATSDR's toxicological profiles: automotive gasoline. Boca Raton, FL: Lewis Publishers; 1997.

30. Beattie AD, Moore MR, Goldberg A. Tetraethyl-lead poisoning. Lancet 1972;ii(7766):12–15.

31. Valpey R, Sumi SM, Copass MK, Goble GJ. Acute and chronic progressive encephalopathy due to gasoline sniffing. Neurology 1978;28:507–510.

32. Poklis A. Death resulting from gasoline "sniffing": a case report. J Forensic Sci Soc 1976;16:43–46.

33. Edminster SC, Bayer MJ. Recreational gasoline sniffing: acute gasoline intoxication and latent organolead poisoning case reports and literature review. J Emerg Med 1985;3:365–370.

34. Hansen KS, Sharp FR. Gasoline sniffing, lead poisoning, and myoclonus. JAMA 1978;240:1375–1376.

35. Schmitt RC, Goolishian HA, Abston S. Gasoline sniffing in children leading to severe burn injury. J Pediatr 1972;80:1021–1023.

36. Sanders LW Sr. Tetraethyllead intoxication. Arch Environ Health 1964;8:270–277.

37. Bethell MF. Toxic psychosis caused by inhalation of petrol fumes. Br Med J 1965;2(5456):276–277.

38. Goodheart RS, Dunne JW. Petrol sniffer's encephalopathy. A study of 25 patients. Med J Aust 1994;160:178–181.

39. Seshia SS, Rjani KR, Boeckx RL, Chow PN. The neurological manifestations of chronic inhalation of leaded gasoline. Dev Med Child Neurol 1978;20:323–334.

40. McGrath J. Petrol "sniffing" and lead encephalopathy. Med J Aust 1986;14:221.

41. Goldings AS, Stewart RM. Organic lead encephalopathy: behavioral change and movement disorder following gasoline inhalation. J Clin Psychiatry 1982;43:70–72.

42. Young RS, Grzyb SE, Crismon L. Recurrent cerebellar dysfunction as related to chronic gasoline sniffing in an adolescent girl. Clin Pediatr 1977;16:706–708.

43. Kovanen J, Somer H, Schroeder P. Acute myopathy associated with gasoline sniffing. Neurology (Cleveland) 1983;33:629–631.

44. Bass M. Sudden sniffing death. JAMA 1970;212: 2075–2079.

45. Bronstein AC, Spyker DA, Cantilena LR, Green JL, Rumack BH, Giffin SL. 2009 Annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 27th annual report. Clin Toxicol 2010;48:979–1178.

46. Clinger OW, Johnson NA. Purposeful inhalation of gasoline vapors. Psychiatr Q 1951;25:557–567.

47. Shah R, Vankar GK, Upadhyaya HP. Phenomenology of gasoline intoxication and withdrawal symptoms among adolescents in India: a case series. Am J Addict 1999; 8:254–257.

48. Lawton JJ Jr, Malmquist CP. Gasoline addiction in children. Psychiatr Q 1961;35:555–561.

49. Bartlett S, Tapia F. Glue and gasoline "sniffing," the addiction of youth. Mo Med 1966;63:270–272.

50. Martinez MA, Ballesteros S. Investigation of fatalities due to acute gasoline poisoning. J Anal Toxicol 2005; 29:643–651.

51. Ikebuchi J, Kotoku S, Yashiki M, Kojima T, Okada K. Gas chromatographic and gas chromatographic-mass spectrometric determination gasoline in a case of gasoline vapor and alcohol poisoning. Am J Forensic Med Pathol 1986;7:146–150.

52. Sandercock PM, Du Pasquier E. Chemical fingerprinting of gasoline 2. Comparison of unevaporated and evaporated automotive gasoline samples. Forensic Sci Int 2004; 140:43–59.

53. Doble P, Sandercock M, Du Pasquier E, Petocz P, Roux C, Dawson M. Classification of premium and regular gasoline by gas chromatography/mass spectrometry, principal component analysis and artificial neural networks. Forensic Sci Int 2003;132:26–39.

54. Sigman ME, Williams MR, Ivy RG. Individualization of gasoline samples by covariance mapping and gas chromatography/mass spectrometry. Anal Chem 2007;79: 3462–3468.

55. Coulehan JL, Hirsch W, Brillman J, Sanandria J, Welty TK, Colaiaco P, et al. Gasoline sniffing and lead toxicity in Navajo adolescents. Pediatrics 1983;71:113–117.

56. Kimura K, Nagata T, Hara K, Kageura M. Gasoline and kerosene components in blood—a forensic analysis. Hum Toxicol 1988;7:299–305.

57. Dingwall KM, Lewis MS, Maruf P, Cairney S. Assessing cognition following petrol sniffing for indigenous Australians. Aust N Z J Psychiatry 2010;44:631–639.

58. Currie B, Burrow J, Fisher D, Howard D, McElver M, Burns C. Petrol sniffer's encephalopathy. Med J Aust 1994; 160:800.

59. Kurt TL, McAnalley BH, Garriott JC. Lead encephalopathy from gasoline sniffing: successful treatment with chelation. Texas Med 1982;78:52–54.

Chapter 45

n-HEXANE

IDENTIFYING CHARACTERISTICS

Commercial hexane, often labeled as the plural "hexanes," is a mixture of hexane isomers including *n*-hexane, cyclohexane, and methyl cyclopentane along with small amounts of pentane isomers, heptane isomers, ketones (acetone, methyl ethyl ketone), dichloromethane, and trichloroethylene.[1] The concentration of *n*-hexane in commercial mixtures generally ranges from about 20–80%. Small amounts (<0.3%) of phthalate esters (dimethyl, diethyl), adipate esters (dibutyl), and organophosphorus compounds may also contaminate some commercial hexanes.[2] *n*-Hexane is an aliphatic alkane with a structural formula of $CH_3(CH_2)_4CH_3$; the "*n*" refers to the "normal" or unbranched isomer. This compound is a colorless, highly volatile liquid with a mild gasoline-like odor. Table 45.1 lists some physical properties of neat *n*-hexane.

EXPOSURE

n-Hexane is a constituent of some glues, rubber cement, stove and lantern fuels, and gasoline. This substance occurs naturally in petroleum products (natural gas, crude oil) and petroleum distillates (gasoline, jet fuel, naphtha, benzine).

DOSE EFFECT

Acute

The acute toxicity of *n*-hexane in animals is relatively low. Short-term, high-dose *n*-hexane exposure produces narcosis with headache, mild nausea, and eye irritation occurring following exposure to 1,500 ppm; confusion and dizziness develops in volunteers exposed to 5,000 ppm *n*-hexane for 10 minutes.[3] Older studies in humans suggest that exposure of individuals to 1,000–5,000 ppm *n*-hexane for 10–60 minutes produces drowsiness, vertigo, and giddiness.[1,4] Exposure of volunteers to 5,000 ppm for 10 minutes produces vertigo, whereas volunteers remain asymptomatic following exposure to 2,000 ppm for 10 minutes.[5] In animal studies, the intratracheal instillation of 0.2 mL *n*-hexane produces a fatal chemical pneumonitis.[6]

Chronic

Case reports document the development of peripheral neuropathies in Japanese sandal workers, Taiwanese press proofers, and Italian shoemakers following chronic occupational exposure to *n*-hexane concentrations ranging from 30–2,500 ppm.[1] Accurate exposure data are not available because many of the measurements did not reflect the cumulative exposure of the affected workers. However, these data suggest that most of the workers were chronically exposed to *n*-hexane in poorly ventilated rooms with *n*-hexane concentrations in air that substantially exceeded 500 ppm.[7] Additionally, most of these industrial exposures involved the use of mixtures of hydrocarbons including methyl ethyl ketone, toluene, acetone, ethyl acetate, and other aliphatic hydrocarbons (pentanes, hexane isomers). Case reports suggest that a peripheral neuropathy may develop after the chronic inhalation of naphtha containing 20% *n*-hexane[8] and high doses of gasoline containing at least 5% *n*-hexane.[9]

TABLE 45.1. Physiochemical Properties of *n*-Hexane.

Property	Value
CAS Number	110-54-3
Molecular Formula	C_6H_{14}
Molecular Weight	86.177 g/mol
Relative Density	0.659 (Water = 1 @ 20°C)
Vapor Pressure	
20°C/68°F	124 mm Hg
25°C/77°F	150 mm Hg
Vapor Density	2.97
Saturated Air (760 mm Hg)	
20°C (68°F)	16.3%
25°C (77°F)	19.7%
Boiling Point	68.74°C (155.73°F)

TOXICOKINETICS

Absorption

Volunteer studies indicate that the steady-state pulmonary retention of *n*-hexane is about 15–30% as measured by the difference between inhaled and expired air at concentrations up to 200 ppm.[10,11] There are few data on retained concentrations of *n*-hexane associated with volatile substance abuse.

Distribution

Studies in animals indicate that *n*-hexane tissue concentrations are highest in the brain followed by liver, kidneys, and adrenal glands.[12] *n*-Hexane does not accumulate easily in tissues; however, the high partition coefficient and the relative long half-life of *n*-hexane in fat suggests that some accumulation may occur following exposure to the high doses of *n*-hexane associated with misuse of *n*-hexane containing products.

Biotransformation

The metabolism of *n*-hexane in humans and rats is qualitatively similar;[1] however, metabolism of *n*-hexane in rats becomes saturable and nonlinear following exposure to *n*-hexane concentrations exceeding 300 ppm. Saturation of metabolic enzymes and the resulting nonlinear kinetics in humans are not well defined, but limited data suggest nonlinear kinetics begin above *n*-hexane concentrations of 300 ppm.[13] The cytochrome P-450 system metabolizes *n*-hexane first to 2-hexanol and then to 2,5-hexanedione, which is a common neurotoxic metabolite shared with methyl *n*-butyl

ketone (MBK) as displayed in Figure 45.1. Animal studies suggest that the metabolism of *n*-hexane involves several cytochrome P450 isoenzymes;[14] CYP2B6 catalyzes the formation of 2-hexanol, whereas CYP2E1 is the major isoenzyme responsible for the formation of 2,5-hexanedione.[15,16] 2,5-Hexanedione, 2,5-dimethylfuran, and γ-valerolactone are the major metabolites present in the urine of humans exposed to *n*-hexane.[17] The kidneys excrete these metabolites primarily in the form of conjugates (glucuronides, alcoholic sulfate esters).[18] 2-Hexanol, free 2,5-hexanedione, and free 2,5-dimethylfuran are minor metabolites that are detectable in urine after exposure to *n*-hexane.[19] Following exposure of Wister rats to 2,000 ppm *n*-hexane, 4,5-dihydroxy-2-hexanone is a major urinary metabolite that is excreted primarily as a glucuronide; acid hydrolysis of the glucuronide metabolite of 4,5-dihydroxy-2-hexanone increases the presence of 2,5-dimethylfuran and 2,5-hexanedione.[20]

Elimination

The lungs excrete about 10% of the absorbed dose of *n*-hexane unchanged based on occupational studies of *n*-hexane exposure.[21] In both animals and humans, the elimination of *n*-hexane from the lungs and the blood is relatively rapid with a biphasic elimination half-life of ~0.2 and 1.5–2 hours, respectively.[11,22] The calculated half-life of *n*-hexane in fat was about 64 hours based on a mathematical model of *n*-hexane distribution.[23] The urinary elimination half-life of 2,5-hexanedione is ~13–14 hours, and the maximum urinary excretion of 2.5-hexanedione occurs about 3–5 hours following cessation of exposure.[1] Animal studies indicate that the formation of 2,5-hexanedione is not proportional to dose following exposure to high *n*-hexane concentrations (500–1,000 ppm) associated with volatile substance abuse.[22]

Chemical Interactions

Methyl ethyl ketone (MEK), methyl isobutyl ketone, and lead acetate enhance the neurotoxicity of *n*-hexane, whereas exposure to toluene reduces the neurotoxicity of *n*-hexane. Animal experiments indicate that MEK enhances the neurotoxicity of *n*-hexane;[24] however, this effect is dose-dependent. Following chronic exposure (12 h/d, 6 d/wk, for 20 weeks) of Wistar rats to 2,000 ppm MEK and 2,000 ppm *n*-hexane, changes in the urinary excretion of 2,5-hexanedione is biphasic with the initial excretion rate of this compound being below the excretion rate of the positive control (2,000 ppm *n*-hexane alone).[25] After about 4 weeks, the 2,5-hexanedione concentration in the urine of rats exposed to both *n*-hexane

FIGURE 45.1. Proposed metabolic pathway for *n*-hexane in animals.[1]

and MEK was twice as high as the urinary concentration of 2,5-hexanedione in rats exposed to *n*-hexane alone. Following a 15-minute exposure to MEK (200–300 ppm) and *n*-hexane (60 ppm), volunteer studies indicate that MEK inhibits the biotransformation of an intermediate step in the conversion of *n*-hexane to the neurotoxic metabolite, 2,5-hexanedione.[26] These results suggest that the interaction of *n*-hexane and MEK may decrease the toxicity associated with exposure to *n*-hexane at concentration near ambient air concentration associated with workplace standards; however, animal studies suggest that enhancement of *n*-hexane neurotoxicity occurs following exposure to MEK at concentrations associated with volatile substance abuse. Animal and human studies indicate that toluene may inhibit the metabolism of *n*-hexane, and thus reduce the excretion of the neurotoxic metabolite, 2,5-hexanedione.[27] Exposure to acetone and *n*-hexane may increase the urinary concentration of free 2,5-hexanedione, but there are few data on the clinical consequence of these metabolic changes.[28]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The neurotoxic substance causing the development of *n*-hexane neuropathy is the toxic metabolite, 2,5-hexanedione.[29] *n*-Hexane neuropathy is primarily an axonal polyneuropathy with secondary demyelination.[30] Similar pathologic findings occur in other toxic neuropathies (e.g., acrylamide, triorthocresyl phosphate, inherited giant axonal neuropathy, vinca alkaloids, colchicine). Sural nerve biopsies demonstrate axonal swelling, loss of large myelinated fibers with focally enlarged "giant" axons containing neurofilaments, widening of nodes of Ranvier, and thinning of the overlying myelin secondary to retraction by axonal swelling.[31] Occasionally, segmental demyelination may occur. Paranodal demyelination occurs at the sites of swelling, and Wallerian degeneration of the axons develops distal to the swollen sites.[32] Aspiration with subsequent chemical pneumonitis is a potential complication of the ingestion of *n*-hexane based on animal studies; however, there is a lack of case reports documenting the pulmonary toxicity of *n*-hexane following aspiration. There are few data on postmortem examination of chronic *n*-hexane abusers.

CLINICAL RESPONSE

Peripheral neuropathy is a rare complication of hexane-containing glue sniffing. Glue-sniffer's neuropathy is a subacute, symmetrical, ascending, predominately motor polyneuropathy associated with the chronic intentional inhalation of *n*-hexane containing products.[33] The neurologic manifestations of this disease are uniform and similar to the sensorimotor neuropathy resulting from chronic occupational exposure to excessive concentrations of *n*-hexane. Weakness begins in the distal lower extremities along with a stocking-glove distribution of paresthesias and hypesthesias; neurologic effects may progress to gait disturbances, difficulty ambulating, and tetraplegia.[34,35] Abnormalities on neurologic examination include diminished deep tendon reflexes, muscle weakness, atrophy, and sensory loss (i.e., reduced pinprick, light touch, temperature, position, and vibration).[36] Often, the earliest symptom of *n*-hexane peripheral neuropathy is the gradual onset of distal numbness in the toes and fingers.[37] Paresthesias rarely spread beyond the knees or wrists. For 2–4 months after cessation of exposure to these products, the neuropathy progresses (i.e., "coasting"). Recovery is slow and variable with some persistent effects (e.g., foot drop) following severe intoxications. Associated symptoms include muscle cramps, anorexia, and weight loss. *n*-Hexane is a mild skin irritant.

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantitation of *n*-hexane in biologic samples includes headspace capillary gas chromatography with flame ionization detection and cryogenic oven trapping,[38] liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry,[39] and headspace with gas chromatography/mass spectrometry (GC/MS) solid-phase microextraction.[40] The latter method allows the determination of *n*-hexane in the general population with a limit of detection (LOD) of 0.089 ng/mL. The LOD for *n*-hexane using gas chromatography with flame ionization detection is about 2.4 and 1 ng/mL for whole blood and urine, respectively, whereas the LOD using liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry is about 50 ng/mL. *n*-Hexane is an ubiquitous chemical found in many laboratory materials and processes including the collecting of blood in a Vacutainer®. Boiling the Vacutainer® stoppers in methanol followed by vacuum baking removes the residue of *n*-hexane in these containers.[40] Gas chromatography with flame ionization detection and GC/MS are the most common analytic methods for the quantitation of the toxic metabolite, 2.5-hexanedione in urine samples. Using headspace solid-phase microextraction and gas chromatography with flame ionization detection, the LOD for this metabolite is 25 ng/mL with the coefficient of variation <7%.[41]

Biomarkers

BLOOD

n-Hexane is frequently detectable in blood samples from the general population without known exposure to *n*-hexane. In a study of patients presenting to an infirmary without known occupational exposure to *n*-hexane, about 86% of the blood samples had detectable concentrations of *n*-hexane.[42] The mean blood concentration of *n*-hexane was 0.61 ng/mL (range, 0.015–7.68 ng/mL); 95% of the blood samples contained *n*-hexane concentrations <1.47 ng/mL. The *n*-hexane concentration is higher in whole blood than plasma because of the uneven uptake of *n*-hexane by erythrocytes. *In vitro* studies indicate that the uptake of *n*-hexane from whole blood by the human erythrocyte fraction was about 66%.[43] Following exposure to low *n*-hexane concentrations (<10 ppm) in ambient air, the serum *n*-hexane concentration was about 30% of the whole blood *n*-hexane concentration.[44]

URINE

The neurotoxic metabolite, 2,5-hexanedione, is a biomarker for the biologic monitoring of exposure to *n*-hexane from inhalant abuse.[45] In human urine, 2,5-hexanedione is present as 2 chemical species: a free form that represents about 8% of the total and the remainder as 4,5-dihydroxy-hexan-2-one. Acid hydrolysis increases the concentration of 2,5-hexanedione in the urine because of the formation of 2,5-hexanedione from 4,5-dihydroxy-hexan-2-one. Small quantities of 2,5-hexanedione may occur in the urine of individuals without occupational exposure to *n*-hexane. Environmental exposure to *n*-hexane accounts for maximum excretion of ~500–800 ng 2,5-hexanedione/mL urine.

Abnormalities

n-Hexane polyneuropathy is one of the few toxic neuropathies that causes electrophysiologic features of both axon loss and demyelination with abnormal spontaneous activities (positive sharp waves, fibrillation potentials, long polyphasic motor unit potentials) and decreased motor and sensory conduction velocities.[46,47] Although *n*-hexane neuropathy is primarily an axonal neuropathy, electrophysiologic abnormalities often meet the criteria for demyelinating neuropathy as a result of secondary demyelination.[31] Electrophysiologic findings depend on the severity of the neuropathy. Nerve conduction studies typically demonstrate reduced conduction velocities, prolonged distal latencies, and decreased amplitudes of both motor and sensory responses that are characteristic of a mixed axonal and demyelinating process.[9] Motor conduction velocities usually increase steadily from distal to proximal segments (e.g., wrist to upper arm); the slowing of proximal conduction velocities is usually mild. F waves are usually absent. Occasionally, nerve conduction studies demonstrate partial conduction block in the median and ulnar nerves.[37] Abnormalities on electrophysiology testing typically resolve within 1 year.[48]

TREATMENT

Because of the limited data on the human toxicity of *n*-hexane, treatment of toxicity is supportive. Exposure to very high *n*-hexane concentrations may cause obtundation and coma. The victim should be removed to fresh air after adequate ventilation of the site. *n*-Hexane is a mild to moderate central nervous system depressant; thus, the adequacy of respiration should be evaluated and assisted ventilation and supplemental oxygen administered as needed. Contaminated clothing should be removed and the skin washed with soap and lukewarm water. Eye contact should be treated with warm saline or water irrigation for 15 minutes.

After the patient has been removed from the source, medical attention should be directed toward evaluation and support of cardiorespiratory function including the potential development of aspiration pneumonia. Ingestion of pure *n*-hexane is rare. Gastrointestinal decontamination is not recommended because of the low acute toxicity of *n*-hexane and the aspiration potential of this liquid. There are no antidotes to *n*-hexane toxicity. The body eliminates *n*-hexane rapidly; therefore, no measures to enhance elimination are indicated. Peripheral and central nervous system symptoms resulting from chronic exposure are treated symptomatically.

References

1. International Programme on Chemical Safety. Environmental health criteria 122 n-hexane. Geneva: World Health Organization; 1991.
2. Vicedo JL, Pellin M, Villanova E. Phthalates and organophosphorous compounds as cholinesterase inhibitors in fractions of industrial hexane impurities. Arch Toxicol 1985;57:46–52.
3. Jorgensen NK, Cohr KH. *n*-Hexane and its toxicologic effects: a review. Scand J Work Environ Health 1981;7:157–168.
4. Yamada S. [Intoxication polyneuritis in workers exposed to *n*-hexane]. Jap J Ind Health 1967;9:651–659. [Japanese]

5. Patty FA, Yant WP. Odor intensity and symptoms produced by commercial propane, butane, pentane, hexane and heptane vapor. Bureau of Mines Report 6820. Washington, DC: United States Department of Interior; 1929:1–10.

6. Gerarde HW. Toxicologic studies on hydrocarbons. Arch Environ Health 1963;6:329–341.

7. Sobue I, Iida M, Yamamura Y, Takayanagui T. *n*-Hexane polyneuropathy. Int J Neurol 1978;11:317–330.

8. Tenenbein M, de Groot W, Rajani KR. Peripheral neuropathy following intentional inhalation of naphtha fumes. Can Med Assoc J 1984;131:1077–1079.

9. Kuwabara S, Kai MR, Nagase H, Hattori T. *n*-Hexane neuropathy caused by addictive inhalation: clinical and electrophysiological features. Eur Neurol 1999;41: 163–167.

10. Brugnone F, Perbellini L, Grigolini L, Apostoli P. Solvent exposure in a shoe upper factory. I. *n*-Hexane and acetone concentration in alveolar and environmental air and in blood. Int Arch Occup Environ Health 1978;42:51–62.

11. Veulemans H, van Vlem E, Janssens H, Masschelein R, Leplat A. Experimental human exposure to *n*-hexane. Study of the respiratory uptake and elimination, and of *n*-hexane concentration in peripheral venous blood. Int Arch Occup Environ Health 1982;49:251–263.

12. Bohlen P, Schlunegger UP, Lauppi E. Uptake and distribution of hexane in rat tissues. Toxicol Appl Pharmacol 1973;25:242–249.

13. Filser JG, Csanady GA, Dietz W, Kessler W, Kreuzer PE, Richter M, Stormer A. Comparative estimation of the neurotoxic risks of *n*-hexane and *n*-heptane in rats and humans based on the formation of the metabolites 2,5-hexanedione and 2,5-heptanedione. Adv Exp Med Biol 1996;387: 411–427.

14. Toftgård R, Haaparanta T, Eng L, Halpert J. Rat lung and liver microsomal cytochrome P-450 isozymes involved in the hydroxylation of *n*-hexane. Biochem Pharmacol 1986;35:3733–3738.

15. Crosbie SJ, Blain PG, Williams FM. Metabolism of *n*-hexane by rat liver and extrahepatic tissues and the effect of cytochrome P-450 inducers. Hum Exp Toxicol 1997; 16:131–137.

16. Iba MM, Fung J, Gonzalez FJ. Functional CYP2E1 is required for substantial *in vivo* formation of 2,5-hexanedione from *n*-hexane in the mouse. Arch Toxicol 2000;74:582–586.

17. Iwata M, Takeuchi Y, Hisanaga N, Ono Y. A study on biological monitoring of *n*-hexane exposure. Int Arch Occup Environ Health 1983;51:253–260.

18. Fedtke N, Bolt HM. Detection of 2,5-hexanedione in the urine of persons not exposed to *n*-hexane. Int Arch Occup Environ Health 1986;57:143–148.

19. Walker R, Flanagan RJ, Lennard MS, Mills GA, Walker V. Solid-phase microextraction: investigation of the metabolism of substances that may be abused by inhalation. J Chromatogr Sci 2006;44:387–393.

20. Fedtke N, Bolt HM. The relevance of 4,5-dihydroxy-2-hexanone in the excretion kinetics of *n*-hexane metabolites in rat and man. Arch Toxicol 1987;61:131–137.

21. Mutti A, Falzoi M, Lucertini S, Arfini G, Zignani M, Lomardi S, Franchini I. *n*-Hexane metabolism in occupationally exposed workers. Br J Ind Med 1984;41:533–538.

22. Baker TS, Rickert DE. Dose-dependent uptake, distribution, and elimination of inhaled *n*-hexane in the Fischer-344 rat. Toxicol Appl Pharmacol 1981;61:414–422.

23. Perbellini L, Mozzo P, Brugnone f, Zedde A. Physiologico-mathematical model for studying human exposure to organic solvents: kinetics of blood/tissue *n*-hexane concentrations and of 2,5-hexanedione in urine. Br J Ind Med 1986;43:760–768.

24. Takeuchi Y, Ono Y, Hisanaga N, Iwata M, Aoyama M, Kitoh J, Sugiura Y. An experimental study of the combined effects of *n*-hexane and methyl ethyl ketone. Br J Ind Med 1983;40:199–203.

25. Ichihara G, Saito I, Kamijima M, Yu X, Shibata E, Toida M, Takeuchi Y. Urinary 2,5-hexanedione increases with potentiation of neurotoxicity in chronic coexposure to *n*-hexane and methyl ethyl ketone. Int Arch Occup Environ Health 1998;71:100–104.

26. van Engelen JG, Rebel-de Haan W, Opdam JJ, Mulder GJ. Effect of coexposure to methyl ethyl ketone (MEK) on *n*-hexane toxicokinetics in human volunteers. Toxicol Appl Pharmacol 1997;144:385–395.

27. Takeuchi Y, Hisanaga N, Ono Y, Shibata E, Saito I, Iwata M. Modification of metabolism and neurotoxicity of hexane by co-exposure of toluene. Int Arch Occup Environ Health 1993;65(suppl 1):S227–S230.

28. Cardona A, Marhueda D, Prieto MJ, Marti J, Periago FJ, Sanchez JM. Behaviour of urinary 2,5-hexanedione in occupational co-exposure to *n*-hexane and acetone. Int Arch Occup Environ Health 1996;68:88–93.

29. Graham DG. Hexane neuropathy: a proposal for pathogenesis of a hazard of occupational exposure and inhalant abuse. Chem Biol Interact 1980;32:339–345.

30. Oh SJ, Kim JM. Giant axonal swelling in "huffer's" neuropathy. Arch Neurol 1976;33:583–586.

31. Smith AG, Albers JW. *n*-Hexane neuropathy due to rubber cement sniffing. Muscle Nerve 1997;20:1445–1450.

32. Shirabe T, Tsuda T, Terao A, Araki S. Toxic polyneuropathy due to glue-sniffing. Report of two cases with a light and electron-microscopic study of the peripheral nerves and muscles. J Neurol Sci 1974;21:101–113.

33. Towfighi J, Gonatas NK, Pleasure D, Cooper HS, McCree L. Glue sniffer's neuropathy. Neurology 1976;26:238–243.

34. Becker CE, Lee DE, Troost BT. Glue sniffing polyneuropathy: an under recognized aspect of a public health hazard. J Adolesc Health 2004;34:94–96.

35. Altenkirch H, Mager J, Stoltenburg G, Helmbrecht J. Toxic polyneuropathies after sniffing a glue thinner. J Neurol 1977;214:137–152.

36. Korobkin R, Asbury AK, Sumner AJ, Nielsen SL. Glue-sniffing neuropathy. Arch Neurol 1975;32:158–162.

37. Chang AP, England JD, Garcia CA, Sumner AJ. Focal conduction block in *n*-hexane polyneuropathy. Muscle Nerve 1998;21:964–969.

38. Kondo K, Lee XP, Kumazawa T, Sato K, Watanabe-Suzuki K, Seno H, Suzuki O. Sensitive determination of *n*-hexane and cyclohexane in human body fluids by capillary gas chromatography with cryogenic oven trapping. J AOAC Int 2001;84:19–23.

39. Andreoli R, Manini P, Mutti A, Bergamaschi E, Niessen WM. Determination of *n*-hexane metabolites by liquid chromatography/mass spectrometry. 1. 2,5-Hexanedione and other phase I metabolites in untreated and hydrolyzed urine samples by atmospheric pressure chemical ionization. Rapid Commun Mass Spectrom 1998;12:1410–1416.

40. Chambers DM, Blount BC, McElprang DO, Waterhouse MG, Morrow JC. Picogram measurement of volatile *n*-alkanes (*n*-hexane through *n*-dodecane) in blood using solid-phase microextraction to assess nonoccupational petroleum-based fuel exposure. Anal Chem 2008;80: 4666–4674.

41. Oliveira AF, Maia PP, Paiva MJ, Siqueira ME. Determination of 2,5-hexanedione in urine by headspace solid-phase microextraction and gas chromatography. J Anal Toxicol 2009;33:223–228.

42. Brugnone F, Maranelli G, Romeo L, Giulaiari G, Gobbi M, Malesani F, et al. Ubiquitous pollution by *n*-hexane and reference biological levels in the general population. Int Arch Occup Environ Health 1991;63:157–160.

43. Lam C-W, Galen TJ, Boyd JF, Pierson DL. Mechanism of transport and distribution of organic solvents in blood. Toxicol Appl Pharmacol 1990;104:117–129.

44. Kawai T, Yasugi T, Mizunuma K, Horiguchi S, Iguchi H, Uchida Y, et al. Comparative evaluation of urinalysis and blood analysis as means of detecting exposure to organic solvents at low concentrations. Int Arch Occup Environ Health 1992;64:223–234.

45. Chakroun R, Faidi F, Hedhili A, Charbaji K, Nouaigui H, Laiba MB. Inhalant abuse detection and evaluation in young Tunisians. J Forensic Sci 2008;53:232–237.

46. Sendur OF, Turan Y, Bal S, Gurgan A. Toxic neuropathy due to *n*-hexane: report of three cases. Inhal Toxicol 2009;21:210–214.

47. Iida M, Yamamura Y, Sobue I. Electromyographic findings and conduction velocity on *n*-hexane polyneuropathy. Electromyography 1969;9:247–261.

48. Kutlu G, Gomceli YB, Sonmez T, Inan LE. Peripheral neuropathy and visual evoked potential changes in workers exposed to *n*-hexane. J Clin Neurosci 2009;16: 1296–1299.

Chapter 46

METHANOL

IDENTIFYING CHARACTERISTICS

At room temperature, methanol (CAS RN: 67-56-1, CH_4O) is a clear, colorless liquid with a faint odor resembling ethanol and a boiling point of 64.6°C (148.3°F). Methanol is soluble in water, ethanol, and many organic solvents. The concentration of methanol in the air above a liquid product is not the same as the methanol concentration in the liquid phase, and the composition of the vapor phase of a liquid mixture containing methanol does not usually match the vapor concentration above the liquid because of differences in vapor pressures. For example, the partial pressure of each solvent in the vapors inhaled from carburetor cleaner depends on saturated vapor pressures rather than the molar concentrations of the liquid in the container. The vapor pressure of methanol is 127 mm Hg at 25°C (77°F) compared with 28 mm Hg at 25°C (77°F) for toluene. Consequently, the vaporized state of the carburetor cleaner at room temperature contains higher concentrations of methanol than toluene.[1] Analysis of a glue used for sniffing contained 82.5% toluene and 6.6% methanol; however, the vapor phase above the liquid contained 3,067 ppm toluene and 12,350 ppm methanol.[1]

EXPOSURE

Epidemiology

Most cases of serious methanol poisoning result from ingestion rather than inhalation, particularly from the substitution of methanol-containing solutions for etha-nolic beverages.[2] Rare cases of significant pulmonary exposure to methanol include exposure to methanol vapors during firefighting[3] and spraying methanol-containing material.[4] Deliberate abuse of methanol-containing products is relatively rare.

Sources

Major commercial applications of methanol include use as an intermediate in chemical manufacturing, an octane booster in gasoline, and a solvent. Other sources of methanol include cleaning solutions, printing and duplicating solutions, varnish, shellac, dyes, stains, paint removers, antifreeze, canned heating fuel for camping stoves and chafing dishes, de-icing products, windshield washer fluid, gasoline additives, formalin, and adhesives. Methanol is a common ingredient in carburetor cleaners along with toluene, methylene chloride, and butane/propane. The typical composition of carburetor cleaner is about 5–25% methanol, 45–80% toluene, 20% methylene chloride, and propellants (isobutane, propane).

Methods of Abuse

The abuse of methanol-containing compounds typically involves the inhalation of vapors from a plastic bag containing the liquid portion of the product, primarily from carburetor cleaning fluid. The central nervous system (CNS) effects of methanol are relatively mild, but the effect on consciousness depends on the dose and concomitant ingestion of other CNS-altering substances. Consequently, the desired effects associated with the intentional inhalation of methanol-containing

products are primarily the result of toluene rather than methanol.

DOSE EFFECT

Exposure of volunteers to 200 ppm methanol for 4 hours does not cause a statistically significant increase in serum formate concentrations or a decrease in blood pH.[5]

TOXICOKINETICS

There are substantial differences in the toxicity of methanol between various species. Primates are particularly sensitive to methanol toxicity, whereas rodents detoxify methanol via a catalase-peroxidase system that prevents the formation of toxic metabolites (formaldehyde, formic acid).[6] Consequently, extrapolation of rodent dose-response data on methanol to primates is inappropriate.

Absorption

The lungs absorb methanol rapidly; the absorption rate constant (i.e., the percentage of the methanol in the lungs absorbed per hour of time) for a 4-hour exposure of volunteers to 200 ppm was 0.87 ± 0.67/hour.[7] In a study of volunteers exposed to 200 ppm for 2 hours, the relative uptake of methanol was approximately 50%.[8] The bioavailability of methanol is limited, in part, by the adsorption of this water-soluble compound onto mucous in the upper respiratory tract.

Distribution

Similar to ethanol, the volume of distribution (V_d) of methanol approximates total body water. Based on volunteer studies, the V_d of methanol is about 0.60–0.77 L/kg, depending on the amount of body fat.[9] The distribution of methanol into tissues is rapid with peak serum methanol concentrations occurring within 1 hour.

Biotransformation

Endogenous metabolism involves the oxidation of methanol to carbon dioxide and water, primarily in the liver. Alcohol dehydrogenase catalyzes the formation of formaldehyde from methanol. The former compound is rapidly converted to formic acid via formaldehyde dehydrogenase within a few minutes of formation.[10] Formaldehyde does not accumulate in blood after the absorption of methanol, whereas formate is the toxic metabolite that accumulates as a result of the low rates of formate oxidation in humans. The conversion rate of formate to carbon dioxide and water depends on the activity of the hepatic tetrahydrofolate-dependent enzyme, 10-formyl-tetra-hydrofolate dehydrogenase. The activity of this enzyme is high in rodents; therefore, methanol toxicity is minor in rodents compared with humans.[11]

Elimination

Following exposure to methanol concentrations associated with volatile substance abuse, zero-order (saturation) kinetics best describe the elimination of methanol.[12] As a result of the saturation of metabolic pathways involved with the oxidation of formate in humans, the plasma elimination half-life of methanol ranges between a few hours at low methanol concentrations and approximately 24 hours in methanol-poisoned patients. Following exposure of volunteers to 800 ppm methanol for 2 hours, the mean plasma elimination half-life of methanol was 1.44 ± 0.33 hours.[13] In an otherwise healthy, methanol-poisoned patient, the total body clearance rate of methanol was 11.3 mL/minute, while methanol metabolism was blocked by the concomitant ingestion of high doses of ethanol.[14] The pulmonary and renal clearance rates in this patient were 5.6 mL/minute and 5.7 mL/minute, respectively. These data indicate that the pulmonary clearance of methanol is low even in poisoned patients. The plasma elimination half-life of the metabolite, formate increases up to ~20 hours in methanol-poisoned patients, but hemodialysis reduces this half-life to 2–3 hours.[15]

Maternal and Fetal Kinetics

Case reports indicate that methanol diffuses across the placenta and into the fetal circulation. Three days after birth by emergency cesarean section because of methanol poisoning in the mother, the plasma methanol concentration of the neonate was 61.6 mg/dL.[16] The child had a persistent metabolic acidosis with an initial pH of 6.9. He died the following day after developing an intraventricular hemorrhage. The mother died 10 days after delivery from complications of methanol poisoning.

Drug Interactions

At serum concentrations >20 mg/dL, ethanol causes almost complete blockade of methanol metabolism.[17] Consequently, the amount of methanol from endogenous sources increases following the consumption of ethanol at doses sufficient to increase the blood ethanol concentration above this concentration. In a study of 5 volunteers with blood ethanol concentrations near 80 mg/dL, the blood methanol concentration increased to 0.12–0.34 mg/dL from baseline methanol concentrations of 0.04–0.08 mg/dL as measured indirectly by analysis of end-expired alveolar air with gas chromatog-

raphy.[18] Below ethanol concentrations of 20 mg/dL, the elimination of methanol becomes exponential (i.e., first-order) rather than linear with an elimination half-life of approximately 1.5–3 hours.[19] Case reports suggest that the simultaneous ingestion of methyl ethyl ketone with methanol also inhibits methanol metabolism.[20]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

The metabolic acidosis and visual changes associated with methanol toxicity result from the biotransformation of methanol to the toxic metabolite, formic acid.[21] The serum formate concentration correlates closely to the metabolic acidosis and the severity of methanol poisoning.[22] 10-Formyl tetrahydrofolate dehydrogenase catalyzes the oxidation of formate; the rate of oxidation depends on the presence of adequate stores of folic acid and the regeneration of tetrahydrofolate during the oxidation of formate. *In vitro* studies indicate that formate inhibits cytochrome c oxidase activity in intact mitochondria and isolated cytochrome aa_3.[23] Inhibition of the cytochrome oxidase complex at the terminal end of the respiratory chain in the mitochondria leads to cellular hypoxia, similar to other cellular toxins (carbon monoxide, cyanide, hydrogen sulfide).[21] Both formate and lactic acid contribute to the metabolic acidosis associated with methanol poisoning. Formate accounts for the early metabolic acidosis, but lactic acidosis occurs later as a result of cellular hypoxia and inhibition of cellular respiration.[24] Direct toxicity by formate probably causes the optic nerve damage associated with methanol toxicity. Undissociated formic acid produces edema, the breakdown of the myelin sheaths, and lesions in the optic disk and retrolaminar section of the optic nerve.[25] Neurotoxicity also results from damage in the basal ganglia (e.g., putamen) and subcortical white matter. Imaging studies suggest that local cellular edema secondary to inhibition of the Na-K ATPase pump and cytochrome oxidase causes the observed edema and necrotic lesions.[26]

Postmortem Examination

The pathologic findings in postmortem examination of fatal cases of methanol poisoning are not unique.[27] Autopsies of patients dying of methanol intoxication typically demonstrate congestion of the lungs and brain, as well as cerebral edema and petechial hemorrhages of the brain and cerebellum.[28] Classic neuropathologic findings include pallor of the myelin of the retrolaminar

optic nerves, and acute hemorrhage and necrosis in the putamen and subcortical white matter.[29,30]

CLINICAL RESPONSE

Illicit Use

Intentional inhalation of methanol may cause clinically significant metabolic acidosis, visual disturbances, CNS depression, altered mental status, and respiratory impairment depending on the duration of use and the absorbed dose of methanol.[31] Symptoms of methanol poisoning include headache, nausea, vomiting, abdominal pain, lethargy, and dyspnea. The development of methanol toxicity is typically delayed 12–18 hours during the metabolism of methanol, and the clinical effects may be delayed over 24 hours as a result of the concomitant ingestion of ethanol. Products that contain methanol usually contain other compounds (e.g., toluene, methylene chloride, acetone) that contribute to the CNS and renal toxicity following abuse. Occasional case reports indicate that metabolic acidosis and decreased visual acuity may occur following the intentional inhalation of methanol-containing household products, but the occurrence of visual disturbances following the abuse of these products is uncommon.[32] A 21-year-old habitual glue-sniffer began inhaling vapors from a new paint thinner that contained 20% methanol, 20% methyl acetate, and 57% toluene.[33] Within 1 week, he developed central vision defects, central scotomata, visual loss (i.e., unable to see a moving hand), dysesthesias of the feet, ataxia, gait disturbances, and loss of coordination. Improvement in the visual defects occurred after hospitalization. The case report did not document the serum methanol concentration, acid-base status, or imaging studies. Other case reports associate the abuse of methanol-containing solvent with nausea, vomiting, thirst, sleeplessness, weakness, fatigue, and blurred vision that resolved within 4 days after cessation of solvent abuse.[1] Ophthalmologic exam of this patient did not detect any abnormalities. An observational study of 7 people presenting to an emergency department after abuse of methanol-containing products did not detect visual abnormalities; the highest formate concentration was 25 mg/dL.[34] Genotoxicity studies and oncogenicity studies in rodents indicate that the inhalation of methanol is unlikely to produce cancer in humans.[35]

Fatalities

A 26-year-old itinerant was found pulseless with several cans of a methanol-containing carburetor cleaner (43.8% toluene, 23.2% methanol, 20.5% methylene chloride, 12.5% propane) and soaked rags nearby.[44] As

reported by companions, he inhaled approximately 9 cans in the 2 days preceding his death. Analysis of postmortem blood indicated that he died of respiratory depression secondary to combined toxicity from very high ethanol concentration (440 mg/dL) in combination with methanol (90 mg/dL), and toluene (5.6 mg/L).

Reproductive Abnormalities

There are few data on the reproductive abnormalities associated with intentional inhalation of methanol-containing products. These products usually contain a variety of other compounds in addition to methanol. Metabolic acidosis, dehydration, and electrolyte disturbances potentially contribute to adverse fetal effects. Consequently, the determination of the causal role of volatile substance abuse and methanol in the development of reproductive abnormalities is difficult. Case reports associate chronic maternal abuse of methanol-containing products with premature delivery[36] and bilateral frontal cortical leukomalacia with cerebral infarcts.[37] In both case reports, the mothers were hospitalized multiple times during their pregnancies for complications of volatile substance abuse including metabolic acidosis, electrolyte imbalance, anemia, and toxic serum methanol concentrations requiring hemodialysis and ethanol infusion.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the quantitation of formic acid primarily involve the use headspace gas chromatography with flame ionization detection.[38] Similar to other volatile substances, storage requires a tightly sealed container along with refrigeration or freezing. Substantial loss of methanol may occur following repeated opening and recapping of the container.

Biomarkers

Some endogenous production of methanol occurs in humans. In a study of volunteers exposed to 200 ppm methanol for 4 hours, the mean serum methanol concentration at the end of the exposure period was 0.65 ± 0.27 mg/dL compared with 0.09 ± 0.06 mg/dL for the sham exposure group.[7] Following exposure of 26 volunteers to 200 ppm methanol for 4 hours, there was no statistically significant difference in the mean serum formate concentration between the methanol exposure (1.43 ± 0.89 mg/dL) and the sham exposure (1.27 ± 0.64 mg/dL).[5]

The intentional inhalation of methanol-containing products may cause clinically significant metabolic aci-

dosis, serum methanol concentrations, and serum formic acid concentrations.[39] In an observational study of 7 patients using methanol-containing carburetor cleaner as a volatile substance of abuse, 4 patients had serum methanol concentrations exceeding 24 mg/dL and 5 patients had elevated serum formic acid concentrations (>12 mg/dL).[34] The anion gap for these patients ranged between 13–31 mEq/L. The methanol concentration in blood samples drawn 4 hours after arrival to the emergency department ranged between 10–86 mg/dL. The patient with a serum methanol concentration of 86 mg/dL had a serum formate concentration of 25 mg/dL and an anion gap of 16 mEq/L.

Abnormalities

In a case series of methanol-abusing patients presenting to an emergency department with elevated serum methanol concentrations (50.4 mg/dL, 128.5 mg/dL), other abnormalities included elevated blood formic acid concentrations (12 mg/dL, 19.3 mg/dL, 48 mg/dL) and metabolic acidosis (pH 7.19).[44] The severity of the acidosis depends on the methanol dose, duration of use, the presence of ethanol, and the time since exposure. A normal anion gap does not necessarily exclude potentially significant serum methanol concentrations, particularly in the period shortly after inhalation. Exposure to methanol also may cause a significant osmolal gap. For each milligram of methanol per deciliter, the osmolal gap increases by about 0.34 mOsm; a methanol concentration of 50 mg/dL increases the osmolal gap by about 17 mOsm/kg H_2O.[21] The presence of severe metabolic acidosis with increased anion and osmolar gaps strongly suggests the presence of methanol or ethylene glycol intoxication.[21] The maximum osmolal gap correlates to the peak absorption of methanol. During the metabolism of methanol, the osmolal gap decreases as the anion gap increases and the metabolic acidosis worsens. Consequently, the osmolal gap does not correlate to the severity of methanol poisoning late in the clinical course, and the absence of an osmolal gap at that time does not exclude the diagnosis of methanol poisoning. The differential diagnosis of this set of laboratory abnormalities includes chronic renal failure, critical illness, diabetic ketoacidosis, and alcoholic ketoacidosis.[40] Visual outcomes do not correlate well to computed tomography (CT) abnormalities during hospitalization for methanol poisoning.[41]

Driving

Experimental studies indicate that evidential breath analyzers (e.g., Dräger 7110 Evidential breathalyzer;

Draeger Safety UK Ltd., Blyth, Northumberland, UK) detect methanol at low concentrations, but the presence of methanol triggers the interfering compound alert. A simulated test of the inhalation of 0.019 mg methanol/L was associated with an 8% increase in the estimated breath ethanol concentration.[42]

TREATMENT

Stabilization

The most common serious complications of methanol poisoning are metabolic acidosis, visual changes, and CNS depression. Initial attention should be directed at evaluating and correcting any cardiopulmonary and acid–base abnormalities. Poor prognostic factors include severe metabolic acidosis and acidemia (pH < 7.0), coma on admission, and >24 hours delay from exposure to admission.[43] Patients with an arterial pH < 7.3 should receive intravenous (IV) sodium bicarbonate to correct the acidosis back to the normal range (7.35–7.45). Most patients presenting after intentionally abusing methanol-containing products respond well to supportive measures alone. Volatile substance abuse associated with methanol-containing products (i.e., huffing) rarely causes toxic methanol concentrations (>50 mg/dL) and metabolic acidosis (pH < 7.2) requiring therapeutic interventions (sodium bicarbonate, ethanol infusion/fomepizole, hemodialysis).[31,44]

Elimination Enhancement

Hemodialysis during methanol intoxication effectively removes formate and methanol, while correcting the metabolic acidosis associated with methanol poisoning. The mean clearance rate of methanol during this procedure is high (i.e., ~125–215 mL/min).[45] Potential indications for hemodialysis in this setting include persistent metabolic acidosis (<7.25–7.30) or electrolyte imbalance unresponsive to conventional therapy, visual changes, renal failure, and deteriorating vital signs despite intensive supportive care.[21] The use of fomepizole obviates the need for dialyzing high methanol concentration (>50 mg/dL) unless the rapid elimination of methanol is necessary. In this situation, folate should be administered and the acid–base status closely monitored. The typical dose of folate is 1 mg/kg/body weight, up to a total dose of 50 mg, administered intravenously over 30–60 minutes in dextrose and water, every 4–6 hours until normalization of the acid–base status and the elimination of methanol. The endpoint of dialysis is a methanol concentration below 20 mg/dL and normalization of the acid–base status.

Antidotes

Both ethanol and fomepizole inhibit alcohol dehydrogenase activity. If administered soon after exposure, ethanol and fomepizole reduce the formation of toxic metabolites and limit the development of metabolic acidosis.[46] Ethanol or fomepizole should be administered as soon as possible after methanol ingestion to prevent the development of formate and metabolic acidosis. There are inadequate data to determine the exact serum methanol concentration necessary to cause toxicity, in part as a result of the dynamic kinetic process involved with the metabolism of methanol to formate. Table 46.1 lists the recommendations of the American Academy of Clinical Toxicology for the administration of antidotes during methanol poisoning.

The loading dose of fomepizole is 15 mg/kg administered intravenously followed by IV doses of 10 mg/kg every 12 hours for 4 doses. After 48 hours, the bolus doses are increased to 15 mg/kg every 12 hours as a result of the self-induction of fomepizole metabolism. This dose of fomepizole is administered until the methanol concentration is <20 mg/dL. There are few clinical data to confirm the superiority of fomepizole over ethanol in terms of efficacy or cost-effectiveness for the treatment of methanol poisonings. Although the acquisition cost of fomepizole is high, the administration of fomepizole does not necessarily require intensive care or frequent laboratory monitoring because of the lack of the metabolic and CNS depressant effects associated with ethanol use. The exact serum methanol concentration that requires treatment with fomepizole is not well defined. Some patients (i.e., huffers) with serum methanol concentrations exceeding 20 mg/dL following inhalation may respond to supportive care only.[34] The presence of visual abnormalities or persistent metabolic

TABLE 46.1. American Academy of Clinical Toxicology Clinical Guidelines on Antidote Administration.

Criteria
Documented plasma methanol concentration >20 mg/dL (>200 mg/L)
or
Documented recent history of ingesting toxic amounts of methanol and osmolal gap >10 mOsm/kg H_2O*
or
History or strong clinical suspicion of methanol poisoning *and* at least two of the following criteria: A. Arterial pH < 7.3 B. Serum bicarbonate <20 mEq/L(mmol/L) C. Osmolal gap >10 mOsm/kg H_2O*

*Laboratory analysis by freezing point depression method only.

713

acidosis is an indication for the treatment of these patients with fomepizole or ethanol, if fomepizole is unavailable.

Supplemental Care

Diagnostic testing for patients exposed to toxic concentrations of methanol include complete blood count, serum electrolytes and anion gap, urinalysis, arterial blood gases to assess acid–base status, serum calcium, serum lipase, serum amylase, serum creatinine, serum creatine kinase, serum osmolality, and serum ethanol. If available, serum methanol concentrations help guide management decisions regarding hemodialysis and the administration of fomepizole. Patients with elevated serum methanol concentrations should be followed for the development of persistent or worsening metabolic acidosis and visual symptoms. All patients should be evaluated with a funduscopic examination and visual acuity. The presence of neurologic signs including altered mental status indicates the need for CT or magnetic resonance imaging of the brain.

References

1. Kira S, Ogata M, Ebara Y, Hourii S, Otsuki S. A case of thinner sniffing: relationship between neuropsychological symptoms and urinary findings after inhalation of toluene and methanol. Ind Health 1988;26:81–85.

2. Paasma R, Hovda KE, Tikkerberi A, Jacobsen D. Methanol mass poisoning in Estonia: outbreak in 154 patients. Clin Toxicol (Phila) 2007;45:152–157.

3. Aufderheide TP, White SM, Brady WJ, Stueven HA. Inhalational and percutaneous methanol toxicity in two firefighters. Ann Emerg Med 1993;22:1916–1918.

4. Kudo Y, Kubo T, Nakamura I, Nunomura K, Takada M, Hukuyama J. Methanol-induced health disturbance in a worker engaged in antimold spraying. Int Arch Occup Environ Health 1996;68:513–515.

5. d'Alessandro A, Osterloh JD, Chuwers P, Quinlan PJ, Kelly TJ, Becker CE. Formate in serum and urine after controlled methanol exposure at the threshold limit value. Environ Health Perspect 1994;102:178–181.

6. Röe O. Species differences in methanol poisoning. Crit Rev Toxicol 1982;10:275–286.

7. Osterloh JD, D'Alessandro A, Chuwers P, Mogadeddi H, Kelly TJ. Serum concentrations of methanol after inhalation at 200 ppm. J Occup Environ Med 1996;38:571–576.

8. Ernstgård L, Shibata E, Johanson G. Uptake and disposition of inhaled methanol vapor in humans. Toxicol Sci 2005;88:30–38.

9. Graw M, Haffner HT, Althaus L, Besserer K, Voges S. Invasion and distribution of methanol. Arch Toxicol 2000;74:313–321.

10. Eells JT, McMartin KE, Black K, Virayotha V, Tisdell RH, Tephly TR. Formaldehyde poisoning. Rapid metabolism to formic acid. JAMA 1981;246:1237–1238.

11. Johlin FC, Swain E, Smith C, Tephly TR. Studies on the mechanism of methanol poisoning: purification and comparison of rat and human liver 10-formyltetrahydrofolate dehydrogenase. Mol Pharmacol 1989;35:745–750.

12. Jacobsen D, Webb R, Collins TD, McMartin KE. Methanol and formate kinetics in late diagnosed methanol intoxication. Med Toxicol Adverse Drug Exp 1988;3:418–423.

13. Batterman SA, Franzblau A, D'Arcy JB, Sargent NE, Gross KB, Schreck RM. Breath, urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. Int Arch Occup Environ Health 1998;71:325–335.

14. Jacobsen D, Ovrebø S, Arnesen E, Paus PN. Pulmonary excretion of methanol in man. Scand J Clin Lab Invest 1983;43:377–379.

15. Jacobsen D, Ovrebø S, Sejersted OM. Toxicokinetics of formate during hemodialysis. Acta Med Scand 1983;214:409–412.

16. Belson M, Morgan BW. Methanol toxicity in a newborn. J Toxicol Clin Toxicol 2004;42:673–677.

17. Haffner HT, Banger M, Graw M, Besserer K, Brink T. The kinetics of methanol elimination in alcoholics and the influence of ethanol. Forensic Sci Int 1997;89:129–136.

18. Jones AW, Skagerberg S, Yonekura T, Sato A. Metabolic interaction between endogenous methanol and exogenous ethanol studied in human volunteers by analysis of breath. Pharmacol Toxicol 1990;66:62–65.

19. Haffner HT, Wehner HD, Scheytt KD, Besserer K. The elimination kinetics of methanol and the influence of ethanol. Int J Leg Med 1992;105:111–114.

20. Price EA, D'Alessandro A, Kearney T, Olson KR, Blanc PD. Osmolar gap with minimal acidosis in combined methanol and methyl ethyl ketone ingestion. Clin Toxicol 1994;32:79–84.

21. Barceloux DG, Bond GR, Krenzelok EP, Cooper H, Vale JA; American Academy of Clinical Toxicology Ad Hoc Committee on the Treatment Guidelines for Methanol Poisoning. American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning. J Toxicol Clin Toxicol 2002;40:415–446.

22. Brent J, McMartin K, Phillips S, Aaron C, Kulig K, Methylpyrazole for Toxic Alcohols Study Group. Fomepizole for the treatment of methanol poisoning. N Engl J Med 2001;344:424–429.

23. Nicholls P. The effect of formate on cytochrome aa3 and on electron transport in the intact respiratory chain. Biochim Biophys Acta 1976;430:13–29.

24. Sejersted OM, Jacobsen D, Ovrebø S, Jansen H. Formate concentrations in plasma from patients poisoned with methanol. Acta Med Scand 1983;213:105–110.

25. Martin-Amat G, McMartin KE, Hayreh SS, Hayreh MS, Tephly TR. Methanol poisoning: ocular toxicity produced by formate. Toxicol Appl Pharmacol 1978;45:201–208.

26. Server A, Hovda KE, Nakstad PH, Jacobsen D, Dullerud R, Haakonsen M. Conventional and diffusion-weighted MRI in the evaluation of methanol poisoning. Acta Radiol 2003;44:691–695.

27. Yayci N, Agritmis H, Turlan A, Koc S. Fatalities due to methyl alcohol intoxication in Turkey: an 8-year study. Forensic Sci Int 2003;131:36–41.

28. Pla A, Hernandez AF, Gil F, Garcia-Alonso M, Villanueva E. A fatal case of oral ingestion of methanol. Distribution in postmortem tissues and fluids including pericardial fluid and vitreous humor. Forensic Sci Int 1991;49:193–196.

29. Wu Chen NB, Donoghue ER, Schaffer MI. Methanol intoxication: distribution in postmortem tissues and fluids including vitreous humor. J Forensic Sci 1985;30:213–216.

30. Feany MB, Anthony DC, Frosch MP, Zane W, De Girolami U. August 2000: two cases with necrosis and hemorrhage in the putamen and white matter. Brain Pathol 2001;11:121–122.

31. Wallace EA, Green AS. Methanol toxicity secondary to inhalant abuse in adult men. Clin Toxicol 2009;47:239–242.

32. LoVecchio F, Sawyers B, Thole D, Beuler MC, Winchell J, Curry SC. Outcomes following abuse of methanol-containing carburetor cleaners. Hum Exp Toxicol 2004;23:473–475.

33. Ogawa Y, Takatsuki R, Uema T, Seki Y, Hiramatsu K, Okayama A, et al. Acute optic neuropathy induced by thinner sniffing: inhalation of mixed organic solvent containing methyl alcohol and methyl acetate. Ind Health 1988;26:239–244.

34. Bebarta VS, Heard K, Dart RC. Inhalational abuse of methanol products: elevated methanol and formate levels without vision loss. Am J Emerg Med 2006;24:725–728.

35. Cruzan G. Assessment of the cancer potential of methanol. Crit Rev Toxicol 2009;39:347–363.

36. Kuczkowski KM, Le K. Substance use and misuse in pregnancy: peripartum anesthetic management of a parturient with recent methanol toluene and isopropanol intake. Acta Anaesth Belg 2004;55:53–55.

37. Bharti D. Intrauterine cerebral infarcts and bilateral frontal cortical leukomalacia following chronic maternal inhalation of carburetor cleaning fluid during pregnancy. J Perinatol 2003;23:693–696.

38. Ferrari LA, Arado MG, Nardo CA, Giannuzzi L. Postmortem analysis of formic acid disposition in acute methanol intoxication. Forensic Sci Int 2003;133:152–158.

39. McCormick MJ, Mogabgab E, Adams SL. Methanol poisoning as a result of inhalational solvent abuse. Ann Emerg Med 1990;19:639–642.

40. Schelling JR, Howard RL, Winter SD, Linas SL. Increased osmolal gap in alcoholic ketoacidosis and lactic acidosis. Ann Intern Med 1990;113:580–582.

41. Sanaei-Zadeh H, Zamani N, Shadnia S. Outcomes of visual disturbances after methanol poisoning. Clin Toxicol (Phila) 2011;49:102–107.

42. Laakso O, Pennanen T, Himberg K, Kuituenen T, Himberg J-J. Effect of eight solvents on ethanol analysis by Drager 7110 evidential breath analyzer. J Forensic Sci 2004;49:1113–1116.

43. Hassanian-Moghaddam H, Pajoumand A, Dadgar SM, Shadnia SH. Prognostic factors in methanol poisoning. Hum Exp Toxicol 2007;26:583–586.

44. Frenia ML, Schauben JL. Methanol inhalation toxicity. Ann Emerg Med 1993;22:1919–1923.

45. Swartz RD, Millman RP, Billi JE, Bondar NP, Migdal SD, Simonian SK, et al. Epidemic methanol poisoning: clinical and biochemical analysis of a recent episode. Medicine (Baltimore) 1981;60:373–382.

46. Brent K. Fomepizole for ethylene glycol and methanol poisoning. N Engl J Med 2009;360:2216–2223.

Chapter 47

NAPHTHALENE and *para*-DICHLOROBENZENE (MOTHBALLS)

NAPHTHALENE

IDENTIFYING CHARACTERISTICS

Similar to *para*-dichlorobenzene (*p*-dichlorobenzene, 1,4-dichlorobenzene), naphthalene is a clear, colorless, lipid-soluble crystalline compound. Figure 47.1 displays the chemical structure of naphthalene. Placing a mothball in a water bath heated to 60°C (140°F) will separate naphthalene and *p*-dichlorobenzene as a result of the melting of *p*-dichlorobenzene (melting point, 52.7°C/126.9°F); naphthalene melts at a higher temperature (melting point, 80.2°C/176.4°F) as displayed in Table 47.1. In addition, the difference in densities between these 2 compounds allows separation. Camphor floats in water, whereas both *p*-dichlorobenzene and naphthalene sink.[1] However, naphthalene mothballs float in a saturated solution of table salt, whereas *p*-dichlorobenzene-containing mothballs sink. Failure to stir the salt solution sufficiently to produce a saturated salt solution may result in suspension the naphthalene mothballs in the solution, leading to false interpretation. A 50% dextrose solution is an alternative to the saturated salt solution because of similar specific gravities.[2] The odor threshold of naphthalene is about 0.44 mg/m³. This compound is soluble in alcohol, benzene, acetone, and ether. Naphthalene is moderately volatile with low water solubility as demonstrated in Table 47.2; the affinity for lipid tissue is also moderate.

EXPOSURE

Distillation and fractionation of coal tar is the most common source of naphthalene; production of naphthalene also results from the dealkylation of methyl naphthalenes.[3] The principal use of naphthalene is feedstock in the production of phthalic anhydride for the synthesis of phthalate plasticizers and resins, pharmaceuticals, insect repellents, and dyes. Other uses include the production of surface-active agents (naphthalene sulfonates), organic chemicals, carbaryl (1-naphthyl-*N*-methylcarbamate), alkylated naphthalene solvents, and synthetic leather tanning agents. There are few data on the prevalence of the use of mothballs as a volatile substance of abuse, but abuse of mothballs is relatively rare. Naphthalene is a constituent of ambient air as a result of the combustion of wood and fossil fuels as well as cigarette smoking, particularly in urban areas.

DOSE EFFECT

Inhalation

The minimum risk level (MRL) for chronic exposure to naphthalene in air is 0.0007 ppm based on an uncertainty factor of 300 and lowest-observed-adverse-effect level (LOAEL) of 10 ppm for hyperplasia of the nasal olfactory and respiratory epithelium in chronic rodent studies.[3] Exposure of rats to 60 ppm naphthalene (6 h/d, 5 d/wk) for 2 years did not affect survival, when compared with unexposed controls.[4] Approximately 300–500

TABLE 47.1. Distinguishing Physical Characteristics of Mothballs.

Physical Characteristic	Naphthalene	*para*-Dichlorobenzene	Camphor
Water bath 60°C (140°F)	Solid	Liquid	Solid
Water bath 25°C (77°F)	Sinks*	Sinks*	Floats

*p-Dichlorobenzene is more dense than naphthalene. In a salt solution, p-dichlorobenzene sinks, whereas naphthalene floats.
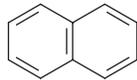
FIGURE 47.1. Chemical structure of naphthalene.

TABLE 47.2. Physical Characteristics of Naphthalene.

Physical Characteristic	Naphthalene
CAS Registry Number	91-20-3
Molecular Formula	$C_{10}H_8$
Molecular Weight	128.16 g/mol
Density	1.16 g/cm^3 (20°C/68°F)
Conversion Factors	1 ppm = 5.24 mg/m^3
	1 mg/m^3 = 0.191 ppm
Melting Point	80.2°C (176.4°F)
Vapor Density	4.42 (Air = 1)
Water Solubility	31 mg/L (25°C/77°F)
Vapor Pressure	0.085 mm Hg (25°C/77°F)

naphthalene-containing mothballs were distributed around an apartment; analysis of air samples from the apartment by gas chromatography with flame ionization detection demonstrated naphthalene concentrations of about 0.020 ppm.[5] The naphthalene concentration was potentially higher when the mothballs were initially distributed. Visitors to the apartment complained of headache, nausea, and vomiting. Individuals with G-6-PDase deficiency are substantially more susceptible to hemolytic anemia following naphthalene exposure than individuals with normal G-6-PDase activity.[6]

Ingestion

A case report associated the ingestion of 2 naphthalene mothballs by a 2-year-old with the development of acute hemolytic anemia and slight jaundice.[7] The G-6-PDase status of the patient was not reported. A case report associated the intentional ingestion of 6 g naphthalene (estimate, 109 mg/kg) by a 16-year-old girl with hemolytic anemia.[8] She developed vertigo, abdominal and flank pain, hemolysis, jaundice, and fever (41.6°C/106°F); she eventually recovered. The G-6-PDase status of the patient was not reported. A 19-year-old woman developed hemolysis, anemia (7.5 g/dL) and methemoglobinemia (13%) after ingesting 12 mothballs containing naphthalene (percentage not reported).[9] Her hemoglobin electrophoresis suggested hemoglobin E α-thalassemia (glucose-6-phosphate dehydrogenase was present).

TOXICOKINETICS

There are few data on the toxicokinetics of naphthalene. The extensive biotransformation of naphthalene produces a variety of reactive and nonreactive metabolites including 1-naphthol (α-naphthol), 2-naphthol (β-naphthol), 1,4-naphthoquinone (α-naphthoquinone), and 1,2-naphthoquinone (β-naphthoquinone). Cytochrome P450 isoenzymes catalyze the first step in naphthalene metabolism by producing the reactive electrophilic arene epoxide intermediate, 1,2-naphthalene oxide as demonstrated in Figure 47.2. There are multiple CYP450 isoenzymes involved with the biotransformation of naphthalene. *In vitro* studies indicate that CYP2E1 enhances the conversion of naphthalene to 1- and 2-naphthol; however, further metabolism of 1- and 2-naphthol requires other P450 isoenzymes.[10] Based on *in vitro* and rodent studies, these isoenzymes include CYP1A1, CYP1A2, CYP1B1, CYP3A7, CYP3A5, CYP2F2, and CYP2B4.[11,12] These studies indicate that there are both gender and species differences in the metabolism of naphthalene.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Although naphthalene does not possess hemolytic properties, the naphthalene metabolite, α-naphthol can produce hemolysis, particularly in G-6-PDase-deficient patients. Glucose-6-phosphate dehydrogenase deficient patients have a diminished capacity to produce the NADPH necessary for the stability of the red cell membrane. These patients are susceptible to increased red
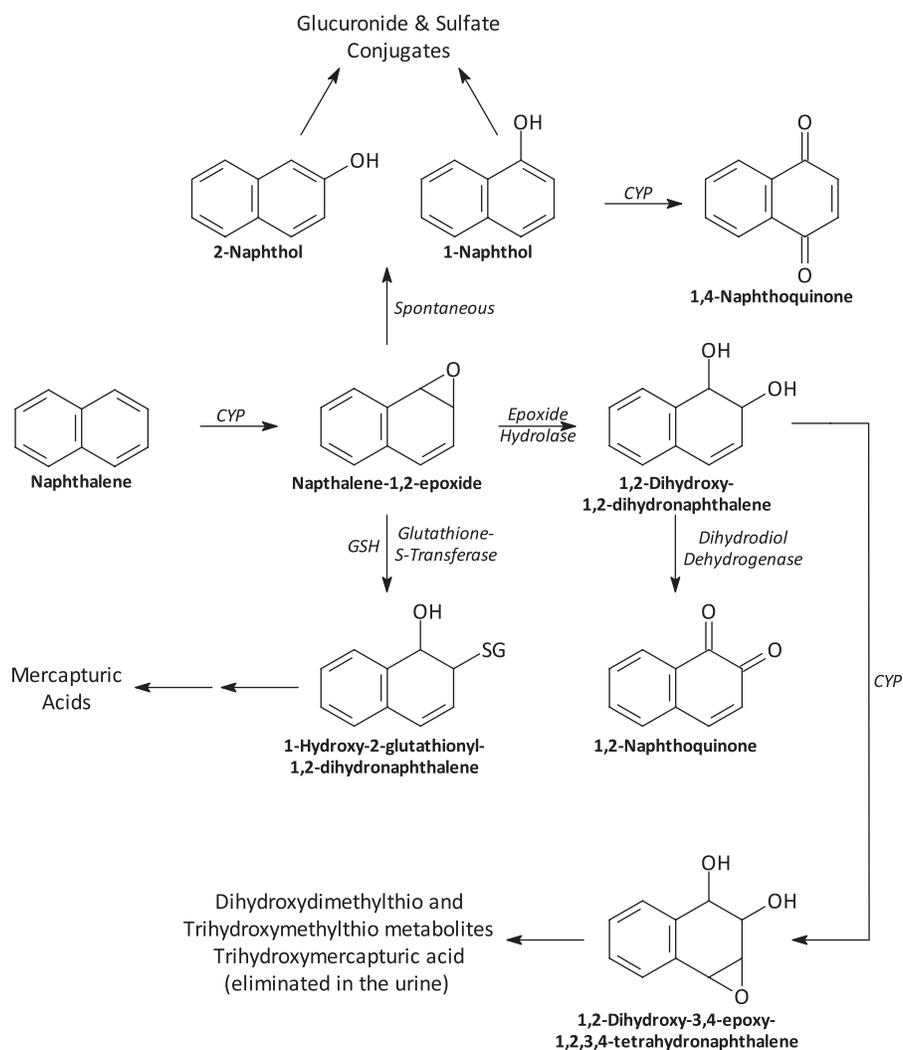
FIGURE 47.2. Proposed metabolic pathways of naphthalene.[3] CYP = cytochrome P450 enzyme(s); GSH = reduced glutathione; SG = glutathione.

cell hemolysis following exposure to oxidizing agents. Oxidation of iron in the heme results in the formation of Heinz bodies, and the formation of free radicals increases red cell membrane fragility and lysis. The destruction of red blood cells causes anemia, increased hematopoiesis (i.e., increased reticulocyte count), and increased bilirubin (jaundice). The exact role of naphthalene in causing hemolysis is unclear. Potential mechanisms include direct oxidation of hemoglobin and reduction of glutathione stores. The development of hemolysis following exposure to naphthalene is highly variable between species with hemolysis occurring in dogs, but not in rodents.[3] A 30-year-old woman presented to the emergency department 2 days after the reported ingestion of 40 mothballs; she died 3 days later.[13] Antemortem cultures demonstrated peritonitis and Gram-negative sepsis. Postmortem examination demonstrated 25 intact mothballs in the stomach, bilat-

eral pleural effusions, pulmonary congestion, and renal tubular dilation and necrosis; however, no necrosis or perforation of the gastrointestinal tract was detected.

CLINICAL RESPONSE

There are limited data on the clinical effects of chronically inhaling vapors from mothballs; most of these data are case reports. Often, these reports rely on historical accounts of exposure, and do not contain documentation of the composition of the mothballs. The primary toxic effects of exposure to mothballs in these case reports are hepatic injury and hemolytic anemia, particularly in neonates with fetal hemoglobin or patients with G6PDase deficiency or sickle cell anemia.[6] Clinical manifestations of naphthalene intoxication include nausea, vomiting, diarrhea, abdominal pain, headache, and dysuria. Clinical evidence of hemolysis typically

appears 1–3 days after exposure with weakness, fatigue, pallor, tachycardia, jaundice, and dyspnea. Hemolysis may persist up to ~1 week.[9] A 26-year-old polydrug user (alcohol, cocaine) presented for a preoperative visit with a history of inhaling vapors from mothballs daily for several years.[14] She had mild anemia, hypokalemia (2.6 mEq/L), and minimal elevation of her serum hepatic aminotransferases. Repeat testing 2 weeks after cessation of mothball use demonstrated a normal serum potassium and normal serum hepatic aminotransferases. This case report did not include the chemical composition of the mothballs or laboratory analysis for hemolysis. Another case report describes a 54-year-old woman with a long history of sniffing and chewing mothballs; she did not report the abuse of any other drugs.[15] She had multiple medical problems including insulin-dependent diabetes, chronic renal failure requiring hemodialysis, hypertension, and peripheral neuropathy (gait disturbance, hyporeflexia, symmetrical motor weakness with upper extremities < lower extremities). The chemical composition of the mothballs could not be determined; the causal role of chronic abuse of mothballs was unclear because of the presence of medical diseases capable of causing these abnormalities. A 10-year-old Native American boy developed nausea, vomiting, abdominal distention, and jaundice after a 2-month episode of sniffing naphthalene mothballs.[16] He died 6 months later with extensive centrilobular necrosis of the liver and no evidence of fibrosis or regenerative nodules. The International Agency for Research on Cancer (IARC) lists naphthalene as a possible human carcinogen (2B), whereas the US National Toxicology Program lists this compound as reasonably anticipated to be a carcinogen.

DIAGNOSTIC TESTING

Analytic Methods

Methods to quantitate naphthalene in biologic materials involve gas chromatography with flame ionization detection, gas chromatography with electron capture detection,[17] and gas chromatography/mass spectrometry (GC/MS).[18] The use of headspace capillary gas chromatography with flame ionization detection allows the detection of naphthalene in whole blood and urine at concentrations of 50 ng/mL and 10 ng/mL, respectively.[19] The interday precision for analysis of 5 mg naphthalene/L in these 2 media were <3% and <5%, respectively. For the analysis of naphthalene in residues, analytic methods include gas chromatography after supercritical fluid extraction, GC/MS combined with a purge and trap thermal desorption extraction,[20] and high performance liquid chromatography (HPLC)

coupled with a diode array detector at 220 nm with confirmation by GC/MS.[21] The limit of detection (LOD) and lower limit of quantitation (LLOQ) for naphthalene in honey using HPLC was 23 ppb (0.023 μg/g honey) and 78 ppb (0.078 μg/g), respectively. Naphthalene metabolites are less lipophilic than the parent compound. Methods for the quantitation of naphthalene metabolites (α-naphthol, α-naphthylglucuronide, and β-naphthylsulfate) include gas chromatography with flame ionization detection,[22] and liquid chromatography/electrospray mass spectrometry in selected ion monitoring mode.[23]

Biomarkers

Naphthalene is detectable in the blood of US citizens without known occupational exposure to naphthalene. 1-Naphthol, 2-naphthol, and 1,4-naphthoquinone appear in the urine as a result of the hydroxylation of naphthalene present in ambient air.[24] Following occupational exposure to naphthalene during the distillation of naphthalene oil, the range of 1-naphthol concentration in end of work shift urine samples from these workers was 400–34,600 ng/mL.[25] The maximum excretion rate for 1-naphthol occurs 1 hour after the end of exposure with a urinary elimination half-life of ~4 hours.

Abnormalities

Naphthalene mothballs are substantially less radioopaque than *p*-dichlorobenzene mothballs. Other tests to distinguish *p*-dichlorobenzene from naphthalene include the addition of chloroform along with a small amount of anhydrous aluminum chloride powder to the mothballs and heating a copper wire dipped in the mothball crystals.[26] In the former method, naphthalene produces an intense blue color immediately, whereas *p*-dichlorobenzene produces no reaction. Both compounds produce a fleeting yellow-orange flame with the latter method, but within the next few seconds *p*-dichlorobenzene produces a brief, intense bright green flame. Naphthalene produces no color. Abnormalities associated with exposure to naphthalene include elevated serum hepatic aminotransferases, hyperbilirubinemia, electrolyte imbalance from fluid loss, renal dysfunction, mild methemoglobinemia, and hemolytic anemia with Heinz bodies and schistocytes.[27]

TREATMENT

The primary risk following acute exposure to high doses of naphthalene is central nervous system (CNS) depression and suppression of the respiratory drive. Patients should be removed from further exposure;

contaminated skin should be washed thoroughly with soap and water after removal of contaminated clothing. Symptomatic patients should be monitored for respiratory depression and cardiac dysrhythmias. Patients with altered consciousness may require respiratory support (supplemental oxygen, assisted ventilation). There are no antidotes for naphthalene exposure. If hemolysis is suspected, a blood smear, urinalysis, and serum hepatic aminotransferases should be obtained. Asymptomatic patients may be discharged after 4 hours observation with repeat testing for hemolysis and liver dysfunction 24–48 hours after discharge.

*para-*DICHLOROBENZENE

IDENTIFYING CHARACTERISTICS

Figure 47.3 displays the chemical structure of *p*-dichlorobenzene. This compound is a lipophilic, volatile crystal with a characteristic odor. *p*-Dichlorobenzene is insoluble in water and soluble in diethyl ether. Table 47.3 lists some physiochemical properties of *p*-dichlorobenzene. Volatilization of *p*-dichlorobenzene varies with mothballs from different manufacturers depending on the specific formulation. In a study of Japanese mothballs, emission rates of *p*-dichlorobenzene from these mothballs ranged from 0.0033–0.035 g/h at 25°C (77°F).[28] Even pure grade *p*-dichlorobenzene (i.e., >99.8%) may contain minor amounts of chlorobenzene, *o*-dichlorobenzene, and *m*-dichlorobenzene. The odor
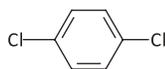
FIGURE 47.3. Chemical structure of *para*-dichlorobenzene.

TABLE 47.3. Physical Characteristics of *p*-Dichlorobenzene.

Physical Characteristic	*para*-Dichlorobenzene
CAS Registry Number	106-46-7
Molecular Formula	$C_6H_4Cl_2$
Molecular Weight	147.01 g/mol
Conversion	1 mg/m³ = 6.01 ppm
Melting Point	52.7°C (126.9°F)
Density	1.46 g/cm³ (20°C/68°F)
Vapor Density	5.07 (Air = 1)
Water Solubility	81.3 mg/L (25°C/77°F)
Vapor Pressure	1.74 mm Hg (25°C/77°F)

threshold of *p*-dichlorobenzene in liquids is about 0.01–0.03 mg/L. Occupational surveys suggest that a faint odor appears at *p*-dichlorobenzene concentrations in ambient air from 15–30 ppm; *p*-dichlorobenzene concentrations in ambient air from 30–60 ppm produce a strong odor, whereas eye and nose irritation occurs at 80–160 ppm.[29]

EXPOSURE

The deodorizing and insecticidal properties of *p*-dichlorobenzene result in the use of this compound as a constituent of mothballs, toilet bowl, and diaper pail deodorizers. Mothballs are relatively pure compounds that are produced as flakes, crystals, and round balls. *p*-Dichlorobenzene is now the primary constituent of mothballs because of the lower toxicity of this compound compared with naphthalene. The treatment of parasitic infections was an antiquated use for *p*-dichlorobenzene.[30] The abuse of *p*-dichlorobenzene by the chronic inhalation of vapors is relatively rare.

DOSE EFFECT

There are few human data on the dose response of *p*-dichlorobenzene; the extrapolation of animal data from different species and strains is limited by substantial differences in the biotransformation of *p*-dichlorobenzene to reactive metabolites including benzoquinone compounds. A serious adverse reaction following exposure to *p*-dichlorobenzene may occur in sensitive individuals (e.g., neonates with fetal hemoglobin, G-6-PDase-deficient patients).

TOXICOKINETICS

There are few quantitative data on the toxicokinetics of *p*-dichlorobenzene in humans. Limited pharmacokinetic studies suggest that the lungs rapidly absorb *p*-dichlorobenzene; rapid distribution into tissues occurs, particularly adipose tissues.[31] Analysis of blood samples from the general population indicate that this compound is absorbed from the environment; in a study of 1,000 adults living throughout the United States, 96% of the samples had detectable concentrations (LOD, 1 ng/mL) of *p*-dichlorobenzene.[32] Biotransformation of *p*-dichlorobenzene primarily involves oxidation by CYP2E1 isoenzymes to 2,5-dichlorophenol.[33,34] Rodent studies indicate that elimination occurs through urinary excretion of this metabolite as the sulfate (50–60%), glucuronide (20–30%), and the free form (5–10%) with minor metabolites accounting for the remaining 10%.[35] These studies suggest that the biliary excretion of glucuronide conjugates of 2,5-dichlorophenol is relative

minor at low doses, but biliary excretion increases with increasing dose. There are substantial species differences in the biotransformation of *p*-dichlorobenzene to reactive metabolites with this conversion being least in human microsomes and greatest in mice; rats were intermediate in conversion rates as reflected in the variable carcinogenic response of this species to *p*-dichlorobenzene.[36]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The mechanisms of toxicity of *p*-dichlorobenzene or naphthalene exposures in rare cases of the hemolytic anemia are likely similar. The clinical finding of Heinz-body formation in red blood cells and methemoglobinemia during hemolytic anemia suggest the presence of oxidative stress; however, the exact mechanism causing these abnormalities is unclear. Potentially, oxidative metabolites of *p*-dichlorobenzene may inhibit glucose-6-phosphate dehydrogenase (G6PD), resulting in the production of hemolysis, methemoglobinemia, and Heinz bodies.

CLINICAL RESPONSE

Hemolytic Anemia

Rare case reports associate hemolytic anemia with the ingestion or chronic inhalation of vapors from *p*-dichlorobenzene-containing mothballs; symptoms from hemolysis are often delayed several days.[37,38] Three days after the ingestion of an unknown amount of *p*-dichlorobenzene, a 19-month-old boy presented to an emergency department with tachycardia, anemia (hemoglobin, 6.4 g/dL), mild methemoglobinemia (2.5%), and jaundice (total bilirubin, 12.0 mg/dL).[39] He was transfused with 150 mL packed cells; subsequent to discharge, he was asymptomatic. A newborn required a blood transfusion for a Coombs-negative hemolytic anemia after the mother inhaled the vapors from mothballs during her pregnancy.[40] The erythrocyte glucose-6-phosphate dehydrogenase activity for the child was 1.4 units/g hemoglobin compared with 2.8 units/g hemoglobin for the mother. Twenty days later the mother was admitted for a blood transfusion (hematocrit, 23%).

Central Nervous System

Several case reports associated chronic exposure to *p*-dichlorobenzene with neurologic abnormalities (e.g., leukoencephalopathy with gait disturbances and brady-kinesias), but determination of causation in these case reports is limited by poor exposure data and the presence of confounding factors. A case report associated the development of mental slowing, unsteady gait, urinary retention, cerebellar syndrome, and pyramidal signs with the inhaling of mothball (*p*-dichlorobenzene) vapors 10 minutes daily for the preceding 4–6 months.[41] The patient recovered within 6 months following the cessation of *p*-dichlorobenzene abuse. She also had a 1-month history of an ichthyosis-like dermatosis on her lower extremities, elbows, and hands. In a similar case report, a 44-year old man presented to an emergency department with an atypical aromatic body odor; anorexia; cachexia; flat affect; social withdrawal; hyperreflexia; cogwheel rigidity in the lower extremities; and dry, scaly, ichthyotic skin.[42] He continued to deteriorate during the first month of hospitalization with the development of clonus, upper extremity cogwheel rigidity, catatonia, and inadequate food intake. The family confirmed that the patient ingested and inhaled vapors from *p*-dichlorobenzene containing mothballs including the heating of these mothballs to increase vapor formation for at least 4 months prior to admission. The serum *p*-dichlorobenzene concentration ~1 month after hospitalization was 1,200 ng/mL. The patient was transferred to a skilled nursing facility after 3 months of hospitalization with slightly increased responsiveness.

Several other case reports associate the chronic inhalation of *p*-dichlorobenzene-containing mothballs with ataxia, gait disturbances, rigidity, dystonia, bradykinesias, dementia, and mutism.[43,44] In some cases, the resumption of *p*-dichlorobenzene abuse resulted in deterioration in the neurologic abnormalities of the patient.[45] However, the presence of multiple confounding factors (e.g., polysubstance abuse, psychiatric illness) and poor exposure data limit definitive conclusions regarding the causal connection between chronic *p*-dichlorobenzene abuse and neurologic abnormalities. Chronic exposure to high doses of *p*-dichlorobenzene for 6 years was associated with the development of severe cerebellar ataxia, dysarthria, moderate weakness, and hyporeflexia that mostly resolved 6 months after cessation of exposure.[46] The chronic ingestion of mothballs for 7 months by a 21-year-old homeless woman was associated with the development of an acute cerebellar syndrome (ataxia, nystagmus) with stupor and a progressive encephalopathy.[47] Magnetic resonance imaging demonstrated a diffuse leukoencephalopathy with predominance in the periatrial deep white mater and the splenium. She developed diffuse cerebral atrophy, and required a percutaneous gastrostomy tube for nutrition. The patient had a history of heroin abuse. There are inadequate data to link chronic exposure to *p*-dichlorobenzene with cataracts.[29]

Carcinogenesis

The International Agency for Research on Cancer (IARC) lists *p*-dichlorobenzene as a possible human carcinogen (Group 2B) based on sufficient evidence in rodent studies and insufficient human epidemiology data on carcinogenicity.[48] The production of liver cancer in B6C3F$_1$ mice by gavage and in BDF$_1$ mice by inhalation probably results from nongenotoxic (promotional) effects by forcing the growth of spontaneous precancerous lesions.[49]

DIAGNOSTIC TESTING

Analytic Methods

Methods to quantitate *p*-dichlorobenzene in biologic materials include GC/MS combined with a purge and trap thermal desorption extraction.[20] The LOD for *p*-dichlorobenzene in honey using GC/MS was 0.15 μg/kg honey. Methods to quantitate *p*-dichlorobenzene in ambient air and solid material include solid-phase microextraction or ultrasonic extraction coupled to GC/MS[50,51] and gas chromatography with flame ionization detection.[28] The LOD of *p*-dichlorobenzene in honey using GC/MS in selected ion monitoring mode after headspace solid-phase microextraction is 1 μg/kg (ppb) honey with precision below 6.3%.[52]

Biomarkers

There are limited data on the concentration of *p*-dichlorobenzene (1,4-DCB) in blood, but the concentrations range from nondetectable to a few ppb. In a convenience sample of blood from 982 adult participants in the Third National Health and Nutrition Examination Survey (NHANES III), the median 1,4-DCB concentration in whole blood samples was 0.33 ng/mL (ppb).[53] In a study of 1,000 adults from throughout the United States, the concentration of 1,4-DCB in their blood averaged 2.1 ng/mL with a range up to 49 ng/mL.[32] Smoking does not alter the concentration of *p*-dichlorobenzene in the blood.[54] The School Health Initiative: Environment, Learning, Disease (SHIELD) Study of low-income neighborhoods in Minneapolis, Minnesota measured multiple chemicals in children's blood samples 4 times over 2 years.[55] About 65–89% of the samples contained detectable concentrations of 1,4-DCB (LOD, 0.04 ng/mL) with the median and 95th percentile of multiple sampling times ranging from 0.12–0.22 ng/mL and 12–27 ng/mL, respectively.

The *p*-dichlorobenzene metabolite, 2,5-dichlorophenol commonly occurs in urine samples from the general population. In a Japanese study of 119 adults living in an urban area (i.e., Osaka), 2,5-dichlorophenol was detectable in 99% of the urine samples as measured by gas chromatography with electron capture detection (LOD, 20 ng/mL).[56] The median urinary 2.5-dichlorophenol concentration was 0.39 mg/g creatinine with a maximum concentration of 3.32 mg/g creatinine.

Abnormalities

p-Dichlorobenzene mothballs are strongly radiopaque. In a study of 10 commercial products of solid mothballs, deodorizers, and bathroom cleaners containing 99–100% *p*-dichlorobenzene and 0.5% cetrimonium bromide, all 10 substances were strongly radiopaque (5/5) in air overlaid by plexiglas.[57] Case reports associate the chronic inhalation of mothball vapors with anemia, hypokalemia, hyponatremia, and mild elevation of serum hepatic aminotransferases that resolves within a few weeks after cessation of abuse.[58]

TREATMENT

The treatment of both *p*-dichlorobenzene and naphthalene exposure is primarily supportive. The primary risk following acute exposure to high doses of *p*-dichlorobenzene is CNS depression and suppression of the respiratory drive. Patients should be removed from further exposure, and contaminated skin should be washed thoroughly with soap and water after removal of contaminated clothing. Symptomatic patients should be monitored for respiratory depression and cardiac dysrhythmias. Patients with altered consciousness may require respiratory support (supplemental oxygen, assisted ventilation). There are no antidotes for *p*-dichlorobenzene exposure.

References

1. Koyama K, Yamashita M, Ogura Y, Ando Y, Fukuda T, Matsuzaki Y. A simple test for mothball component differentiation using water and a saturated solution of table salt: its utilization for poison information service. Vet Hum Toxicol 1991;33:425–427.

2. Tang KY, Chan CK, Lau FL. Dextrose 50% as a better substitute for saturated salt solution in mothball float test. Clin Toxicol 2010;48:750–751.

3. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene. Atlanta, GA: US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry; 2005.

4. Abdo KM, Grumbein S, Chou BJ, Herbert R. Toxicity and carcinogenicity study in F344 rats following 2 years of

whole-body exposure to naphthalene vapors. Inhal Toxicol 2001;13:931–950.

5. Linick M. Illness associated with exposure to naphthalene in mothballs—Indiana. MMWR Morbid Mortal Wkly Rep 1983;32:34–35.

6. Valaes T, Doxiadis SA, Fessas P. Acute hemolysis due to naphthalene inhalation. J Pediatr 1963;63:904–915.

7. Macgregor RR. Naphthalene poisoning from the ingestion of moth balls. Can Med Assoc J 1954;70:313–314.

8. Gidron E, Leurer J. Naphthalene poisoning. Lancet 1956;270(6910):228–231.

9. Lim HC, Poulose V, Tan HH. Acute naphthalene poisoning following the non-accidental ingestion of mothballs. Singapore Med J 2009;50:e298–e301.

10. Wilson AS, Davis CD, Williams DP, Buckpitt AR, Pirmohamed M, Park BK. Characterisation of the toxic metabolite(s) of naphthalene. Toxicology 1996;114:233–242.

11. Buckpitt A, Chang AM, Weir A, Van Winkle L, Duan X, Philpot R, Plopper C. Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV. Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. Mol Pharmacol 1995;47:74–81.

12. Van Winkle LS, Gunderson AD, Shimizu JA, Baker GL, Brown CD. Gender differences in naphthalene metabolism and naphthalene-induced acute lung injury. Am J Physiol Lung Cell Mol Physiol 2002;282:L1122–L1134.

13. McCausland Jurz J. Naphthalene poisoning: critical care nursing techniques. Dimens Crit Care Nurs 1987;6:264–270.

14. Kong J-T, Schmiesing C. Concealed mothball abuse prior to anesthesia: mothballs, inhalants, and their management. Acta Anaesthesiol Scand 2005;49:113–116.

15. Weintraub E, Gandhi D, Robinson C. Medical complications due to mothball abuse. South Med J 2000;93:427–429.

16. Pysher T, Olson A, Fredrick DL, Ford D, Randall A. Fatal hepatopathy due to chronic inhalation of naphthalene. Lab Invest 1984;50:10P.

17. Weistrand C, Jakobsson E, Noren K. Liquid-gel partitioning using Lipidex in the determination of polychlorinated biphenyls, naphthalenes, dibenzo-*p*-dioxins and dibenzofurans in blood plasma. J Chromatogr B 1995;669:207–217.

18. Singh AK, Spassova D, White T. Quantitative analysis of polychlorinated biphenyls, organochlorine insecticides, polycyclic aromatic hydrocarbons, polychlorinated hydrocarbons and polynitrohydrocarbons in spiked samples of soil, water and plasma by selected-ion monitoring gas chromatography-mass spectrometry. J Chromatogr B Biomed Sci Appl 1998;706:231–244.

19. Watanabe K, Hasegawa K, Yamagishi I, Nozawa H, Suzuki O. Simple analysis of naphthalene in human whole blood and urine by headspace capillary gas chromatography with large-volume injection. Forensic Toxicol 2009;27:98–102.

20. Tananaki C, Zotou A, Thrasyvoulou A. Determination of 1,2-dibromoethane, 1,4-dichlorobenzene and naphthalene residues in honey by gas chromatography–mass spectrometry using purge and trap thermal desorption extraction. J Chromatogr A 2005;1083:146–152.

21. Beyoğlu D, Omurtag GZ. Occurrence of naphthalene in honey consumed in Turkey as determined by high-pressure liquid chromatography. J Food Prot 2007;70:1735–1738.

22. Bieniek G. Urinary naphthols as an indicator of exposure to naphthalene. Scand J Work Environ Health 1997;23:414–420.

23. Andreoli R, Manini P, Bergamaschi E, Mutti A, Franchini I, Niessen WM. Determination of naphthalene metabolites in human urine by liquid chromatography-mass spectrometry with electrospray ionization. J Chromatogr A 1999;847:9–17.

24. Bieniek G. Urinary naphthols as an indicator of exposure to naphthalene. Scand J Work Environ Health 1997;23:414–420.

25. Bieniek G. The presence of 1-naphthol in the urine of industrial workers exposed to naphthalene. Occup Environ Med 1994;51:357–359.

26. Ambre J, Ruo TI, Smith-Coggins R. Mothball composition: three simple tests for distinguishing paradichlorobenzene from naphthalene. Ann Emerg Med 1986;15:724–726.

27. Ojwang PJ, Ahmed-Jushuf IH, Abdullah MS. Naphthalene poisoning following ingestion of moth balls: case report. East Afr Med J 1985;62:71–73.

28. Shinohara N, Ono K, Gamo M. *p*-Dichlorobenzene emission rates from moth repellents and leakage rates from cloth storage cases. Indoor Air 2008;18:63–71.

29. Hollingsworth RL, Rowe VK, Oyen F, Hoyle HR, Spencer HC. Toxicity of paradichlorobenzene: determinations of experimental animals and human subjects. AMA Arch Ind Health 1956;14:138–147.

30. Dikmans G. Paradichlorobenzene as an anthelmintic. J Agric Res 1927;35:645–649.

31. Yoshida T, Andoh K, Kosaka H, Kumagai S, Matsunaga I, Akasaka S, et al. Inhalation toxicokinetics of *p*-dichlorobenzene and daily absorption and internal accumulation in chronic low-level exposure to humans. Arch Toxicol 2002;76:306–315.

32. Hill RH Jr, Ashley DL, Head SL, Needham LL, Pirkle JL. *p*-Dichlorobenzene exposure among 1,000 adults in the United States. Arch Environ Health 1995;50:277–280.

33. Nedelcheva V, Gut I, Soucek P, Frantik E. Cytochrome P450 catalyzed oxidation of monochlorobenzene, 1,2- and 1,4-dichlorobenzene in rat, mouse, and human liver microsomes. Chem Biol Interact 1998;115:53–70.

34. Hsiao P-K, Lin Y-C, Shih T-S, Chiung Y-M. Effects of occupational exposure to 1,4-dichlorobenzene on hematologic, kidney, and liver function. Int Arch Occup Environ Health 2009;82:1077–1085.

35. Hissink AM, Dunnewijk R, van Ommen B, van Bladeren PJ. Kinetics and metabolism of 1,4-dichlorobenzene in

male Wistar rats: no evidence for quinone metabolites. Chem Biol Interact 1997;103:17–33.

36. Hissink AM, Oudshoorn MJ, Van Ommen B, Van Bladeren PJ. Species and strain differences in the hepatic cytochrome P450-mediated biotransformation of 1,4-dichlorobenzene. Toxicol Appl Pharmacol 1997;145: 1–9.

37. Hallowell M. Acute haemolytic anaemia following the ingestion of *para*-dichlorobenzene. Arch Dis Child 1957;173:74–75.

38. Campbell DM, Davidson RJ. Toxic haemolytic anaemia in pregnancy due to a pica for paradichlorobenzene. J Obstet Gynaecol 1970;77:657–659.

39. Sillery JJ, Lichenstein R, Barrueto F Jr, Teshome G. Hemolytic anemia induced by ingestion of paradichlorobenzene mothballs. Pediatr Emerg Care 2009;25:252–254.

40. Athanasiou M, Tsantali C, Trachana M. Hemolytic anemia in a female newborn infant whose mother inhaled naphthalene before delivery. J Pediatr 1997;130:680–681.

41. Feuillet L, Mallet S, Sparari M. Twin girls with neurocutaneous symptoms caused by mothball intoxication. N Engl J Med 2006;355:423–424.

42. Hernandez SH, Wiener SW, Smith SW. Case files of the New York City Poison Control Center: paradichlorobenzene-induced leukoencephalopathy. J Med Toxicol 2010;6:217–229.

43. Cheong R, Wilson RK, Cortese IC, Newman-Toker DE. Mothball withdrawal encephalopathy—case report and review of paradichlorobenzene neurotoxicity. Subst Abuse 2006;27:63–67.

44. Reygagne A, Garnier R, Chataigner D, Echenne B, Efthymiou ML. [Encephalopathy due to repeated voluntary inhalation of paradichlorobenzene]. J Toxicol Clin Exp 1992;12:247–250. [French]

45. Kumar N, Dale LC, Wijdicks EF. Mothball mayhem: relapsing toxic leukoencephalopathy due to *p*-dichlorobenzene neurotoxicity. Ann Intern Med 2009;150; 362–363.

46. Miyai I, Hirono N, Fujita M, Kameyama M. Reversible ataxia following chronic exposure to paradichlorobenzene. J Neurol Neurosurg Psychiatry 1988;51:453–454.

47. Avila E, Schraeder P, Belliappa A, Faro S. Pica with paradichlorobenzene mothball ingestion associated with toxic leukoencephalopathy. J Neuroimaging 2006;16: 78–81.

48. International Agency for Research on Cancer. Dichlorobenzenes. IARC Monogr Eval Risk Cancer 1999;73: 223–276.

49. Butterworth BE, Aylward LL, Hays SM. A mechanism-based cancer risk assessment for 1,4-dichlorobenzene. Regul Toxicol Pharmacol 2007;49:138–148.

50. De Coensel N, Desmet K, Sandra P, Górecki T. Domestic sampling: exposure assessment to moth repellent products using ultrasonic extraction and capillary GC-MS. Chemosphere 2008;71:711–716.

51. Harizanis PC, Alissandrakis E, Tarantilis PA, Polissiou M. Solid-phase microextraction/gas-chromatographic/mass spectrometric analysis of *p*-dichlorobenzene and naphthalene in honey. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008;25:1272–1277.

52. Tsimeli K, Triantis TM, Dimotikali D, Hiskia A. Development of a rapid and sensitive method for the simultaneous determination of 1,2-dibromoethane, 1,4-dichlorobenzene and naphthalene residues in honey using HS-SPME coupled with GC-MS. Anal Chim Acta 2008;617:64–71.

53. Churchill JE, Ashley DL, Kaye WE. Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Arch Environ Health 2001;56:147–166.

54. Lin YS, Egeghy PP, Rappaport SM. Relationships between levels of volatile organic compounds in air and blood from the general population. J Expo Sci Environ Epidemiol 2008;18:421–429.

55. Sexton K, Adgate JL, Church TR, Ashley DL, Needham LL, Ramachandran G, et al. Children's exposure to volatile organic compounds as determined by longitudinal measurements in blood. Environ Health Perspect 2005;113:342–349.

56. Yoshida T, Andoh K, Fukuhara M. Urinary 2,5-dichlorophenol as biological index for *p*-dichlorobenzene exposure in the general population. Arch Environ Contam Toxicol 2002;43:481–485.

57. Woolf AD, Saperstein A, Zawin J, Cappock R, Young-Jin S. Radiopacity of household deodorizers, air fresheners, and moth repellents. J Toxicol Clin Toxicol 1993;31: 415–428.

58. Kong J-T, Schmiesing C. Concealed mothball abuse prior to anesthesia: mothballs, inhalants, and their management. Acta Anaesthesiol Scand 2005;49:113–116.

Chapter 48

TOLUENE

HISTORY

Reports of glue sniffing were rare before 1960.[1] Glue sniffing became a popular method of volatile substance abuse in the 1960s, when the practice of inhaling fumes from glue and nail-polish remover became popular among American and Canadian teenagers.[2]

IDENTIFYING CHARACTERISTICS

Toluene is a clear, colorless, moderately volatile, flammable liquid.[3] Table 48.1 outlines the physicochemical characteristics of toluene. Pure toluene has a sweet, pungent aromatic odor detectable at a concentration near 0.8 ppm, whereas the odor threshold for a mixture containing 46% toluene along with other short-chained alkanes and cycloalkanes was approximately 2.5 ppm.[4]

Figure 48.1 displays the chemical structure of toluene. The composition of a liquid mixture containing toluene does not usually match the vapor concentration above the liquid because of differences in vapor pressures. Analysis of a glue used for sniffing contained 82.5% toluene and 6.6% methanol; however, the vapor phase above the liquid contained 3,067 ppm toluene and 12,350 ppm methanol.[5]

EXPOSURE

Toluene is one of the most commonly abused volatile substances. Often, toluene is the exclusive volatile substance of abuse. Toluene is an excellent solvent for paints, lacquers, thinners, coatings, and glues; this chemical is a component of glues, paints, lacquer, and varnish as well as a pure commercial solvent. Toluene is part of the production process for polyurethanes, nylon, plastic bottles, pharmaceutics, nail polish, dyes, and synthetic chemicals. Desired effects of toluene abuse include mild euphoria, excitation, delusions, and distorted perception of space and images.[6] Toluene is a constituent of many abused glues, particularly in older case reports; however, the toluene content varies substantially with different glues. Glue sniffing typically involves the inhaling of vapors from a glue-soaked rag held to the nose, a glue-containing bag held to the mouth, or from glue heated in a pan.[1]

DOSE EFFECT

There are few data on the effects of exposure to toluene concentrations associated with volatile substance abuse. A case report associated chronic inhalation of toluene vapors at work with episodes of headache, inappropriate speech, transient memory loss, and loss of consciousness; however, there were no documented abnormalities of heart, liver, or kidney function.[7] Exposure to an estimated 600–800 ppm toluene causes confusion, altered auditory and visual perception, loss of inhibitions, and poor coordination; estimated toluene exposures in chronic toluene abusers may attain 5,000 ppm.[8,9] Acute exposure of animals to toluene produces rapid excitatory effects at moderate concentrations and depressant effects at high concentrations. In mice, motor activity increased following acute exposure to 560 ppm toluene; decreased activity occurred following acute exposure to 3,000 ppm toluene.[10] Brief exposure to high concentrations of toluene also limits the ability of animals to

TABLE 48.1. Physicochemical Characteristics of Toluene.

Physical Characteristic	Data
CAS Registry Number	108-88-3
Chemical Formula	$C_6H_5CH_3$
Molecular Weight	92.1 g/mol
Boiling Point	110.6°C (231.1°F)
Density	0.8669 g/mL (20°C/68°F)
Water Solubility	534.8 mg/L (25°C/77°F)
Vapor Pressure	28.4 mm Hg (25°C/77°F)
	59.3 mm Hg (40°C/104°F)
	291.5 mm Hg (80°C/176°F)
Odor Threshold	
Water	0.04–1 ppm
Air	8 ppm
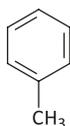
FIGURE 48.1. Chemical structure of toluene.

perform conditioned neuromuscular responses. The exposure of monkeys to 2,000 ppm toluene for <1 hour increased response time and reduced the accuracy of the response to conditioned stimulus, but there were no overt neurological signs (e.g., ataxia, tremor).[11] Recovery from the effects of toluene is also rapid, although there is some tissue accumulation following repeated high-dose exposures. Following exposure of mice to 12,000 ppm toluene for 5 minutes, the results of unconditioned performance and reflex testing returned to normal by 10 minutes after cessation of exposure.[12] However, a cycle of 5 exposures to the same concentration of toluene separated by 30 minutes of fresh air caused abnormalities on these tests until 60 minutes after the cessation of toluene exposure.

Characteristic white matter changes associated with toluene abuse include atrophy, loss of gray matter-white matter boundaries, and callosal thinning.[13] These changes are typically symmetrical and occur after 5–7 years of inhalant abuse. In a series of 24 toluene abusers (mean age 23 ± 4.4 years) admitted to a Canadian drug-treatment unit, 11 patients had cerebellar abnormalities on neurologic examination.[14] The estimated mean daily dose of toluene for this cohort was 425 ± 366 mg toluene for 6.3 ± 3.9 years.

TOXICOKINETICS

Absorption

The average relative uptake of toluene from alveolar air is rapid, ranging from approximately 40–60% depending on several factors (e.g., ventilation rate, fat distribution).[15] Following exposure of 12 healthy male volunteers to about 80 ppm toluene, the uptake of toluene averaged approximately 50% of the inspired amount at rest.[16] Arterial blood contains detectable amounts of toluene within 10 seconds of airborne exposure to toluene.[17] In 5 incarcerated toluene abusers, the peak whole blood toluene concentrations occurred about 20 minutes after cessation of toluene use.[18]

Distribution

Adipose tissue and bone marrow provide depots for toluene; the storage of toluene in fat accounts for the long terminal elimination half-life.

Biotransformation

The cytochrome P450 isoenzymes catalyze the oxidation of most (i.e., ~80%) of absorbed toluene to benzyl alcohol via side-chain oxidation of the methyl group.[19] In studies of rats, the metabolism of toluene involves at least 6 cytochrome P450 isoenzymes (CYP1A1/2, CYP2A1, CYP2B1/2, CYP2C6, CYP2C11, CYP2E1).[20] *In vitro* studies using human liver microsomes indicate that CYP2E1 is the major isoenzyme involved with the methyl hydroxylation of toluene to form benzyl alcohol; whereas CYP1A2 catalyzes the formation of minor phenolic metabolites.[21] Alcohol and aldehyde dehydrogenases oxidize benzyl alcohol to benzoic acid. Conjugation of benzoic acid with glycine produces hippuric acid, which the kidneys subsequently excrete in the urine. *ortho-*, *meta-*, and *para-*cresols are minor metabolites of toluene metabolism. The kidney excretes these minor metabolites as conjugated cresols. Figure 48.2 outlines the metabolism of toluene.

Elimination

The lungs excrete about 7–14% of an absorbed dose of toluene unchanged following exposure to about 80 ppm toluene for 2 hours.[16] In a study of workers exposed to 50 ppm toluene for 2 hours, the mean excretion of toluene in exhaled air was 13 ± 6.2%.[22] Hippuric acid in the urine accounted for 75 ± 6.4% of the total toluene dose. The kidney excretes minimal amounts of unchanged toluene in the urine. The elimination of toluene following inhalation has a rapid initial distribution phase, an
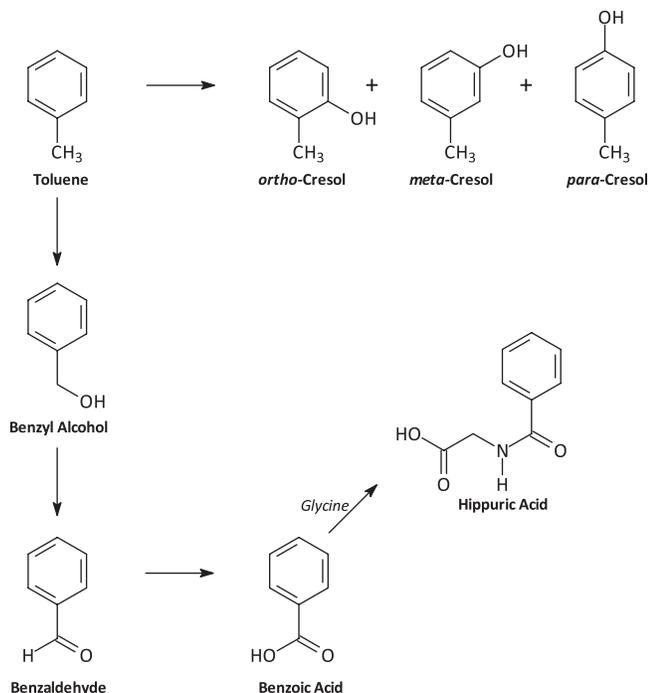
FIGURE 48.2. Toluene metabolism.[22]

intermediate phase, and a slow phase involving the elimination of a relatively small portion of the initial dose from fat stores. The elimination half-lives of toluene from the blood in a small study of incarcerated toluene abusers ranged from about 35–54 minutes during the first hour after cessation of toluene use to 80–110 minutes during the second hour after toluene inhalation.[18] Following exposure of 2 workers to concentrations of a toluene-containing solvent mixture that was sufficient to produce coma, the elimination half-life of toluene from the blood of these 2 workers was approximately 17 hours and 27 hours.[23] In a cross-sectional study of 37 male printers exposed to toluene, the toluene elimination from venous blood followed a 3-compartment model with the terminal median elimination toluene half-time being 90 hours (range, 50–324 hours).[24] The estimated median percentage of toluene elimination for the slow component was about 17% (range, 3–26%) compared with 42% (range, 22–63%) for the intermediate phase. The median time to reduce the plasma toluene concentrations by 50% during the fast and intermediate components were 9 minutes and 2 hours, respectively.

Maternal and Fetal Kinetics

Toluene readily crosses the placenta. Rodent studies suggest that about 10% of an inhaled dose of toluene

rapidly crosses the placenta and distributes into fetal tissue depending on gestational age.[25] Toluene persists in the fetus at least 24 hours after exposure.

Tolerance

Tolerance to the neurobehavioral changes does not usually develop in animals following repeated exposure to toluene.[26] Case reports suggest that tolerance to the effects of inhaling toluene vapors during glue sniffing may develop within 3 months after weekly toluene abuse.[8,27] In a study of young, glue-sniffing adolescents admitted to a pediatric psychiatric service, some patients reported using up to 25 tubes of glue daily.[28] Toluene is a major constituent of glues in older case reports of volatile substance abuse; however, there is substantial variation in the chemical content of vapors from different glues.

Drug Interactions

Although induction of P450 isoenzymes is unlikely at toluene concentrations commonly present in the workplace, enzyme induction may occur in a toluene abuser.[29] Experimental studies suggest that metabolic interaction may occur between toluene, trichloroethylene, and ethanol as a result of alteration of CYP2E1 isoenzymes that oxidize these compounds;[30] however, the clinical significance of these potential interactions is undefined. The administration of moderate doses of ethanol about 1 hour after exposure to toluene near the Swedish occupational limit increased the peak toluene concentration in blood about 33%.[31] The ethanol concentrations with and without toluene exposure were not significantly different.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Central Nervous System

In vitro studies suggest that toluene alters neuronal excitation by producing compensatory responses in the functional expression of ion channels. In a study of primary cultures of rat hippocampal neurons, toluene inhibited NMDA-mediated currents (IC_{50}, 1.5 mM) in a dose-dependent relationship, whereas the non-NMDA agonist kainic acid had no effect on GABA-mediated currents.[32] Pathologic changes reported in case studies of toluene abuse include 1) diffuse atrophy of the cerebellum, brainstem, and cerebrum, and ventricular dilation and widening of sulci in the affected areas; 2)

demyelination of white matter; 3) degeneration and gliosis of ascending and descending long-tracts; and 4) ill-defined myelin pallor and discoloration of the white matter.[33] Neuroimaging and neuropsychologic studies of chronic toluene misuse indicate that toluene abuse preferentially affects regions of the brain in a lipid-dependent distribution.[34] These changes are relatively greater in the periventricular and subcortical (e.g., thalamus, basal ganglia) than in cortical areas; abnormalities in white matter regions are greater than gray matter regions.

Analysis of the brains of toluene abusers by proton magnetic resonance spectroscopy suggests that the white matter changes result from axonal damage and gliosis with secondary demyelination rather than direct neuronal damage and primary demyelination.[35] Characteristic white matter changes include atrophy, loss of gray matter–white matter boundaries, and callosal thinning.[13] Case reports of encephalopathic chronic toluene abusers document the reduction in presynaptic dopamine D_2 receptor density as measured by dopamine transporter scans.[36]

CARDIOVASCULAR SYSTEM

Although cardiac dysrhythmias are a suspected cause of some fatalities associated with toluene abuse and sudden death, there is a lack of convincing evidence in animals that toluene causes direct cardiac effects. In a study of 25 dogs breathing lethal toluene concentrations (30,000 ppm) from a plastic bag attached to their endotracheal tube, respiratory movements continued twice as long as death by suffocation using a plastic bag of the same size; there were no terminal respirations.[37] Transient dysrhythmias with fluctuations in blood pressure were observed in a few animals including ventricular fibrillation, but these dysrhythmias were associated with the terminal changes of hypoxia in the electrocardiogram (e.g., depressed ST segment, inverted T wave, widening of the QRS, reduction of the R wave). The authors concluded that most cases of sudden death during toluene abuse result from severe hypoxia secondary to toluene narcosis; however, they suggested that fatal ventricular fibrillation might occur in sensitized individuals. No serious cardiac dysrhythmias developed in 2 workers found stuporous after exposure to high toluene ambient air concentrations (>7,000 mg/m³).[38] The administration of toluene in near lethal concentrations (66,276 ppm for 30 minutes) was devoid of untoward electrocardiographic effect in chloralose-anesthetized rat.[39] Both animal studies and case reports suggest that high toluene concentrations may cause suppression of cardiac conduction (sinus bradycardia, atrioventricular block); however, these dysrhythmias typically resolve within a few hours after toluene exposure ceases.[40,41]

RENAL SYSTEM

The renal tubule is the primary site of injury to the kidneys following volatile substance abuse of toluene and toluene-containing products. Most toluene abusers with renal dysfunction have a reversible, distal renal tubular acidosis that results from impaired hydrogen ion secretion in the distal renal tubule.[42] The metabolic abnormalities associated with renal tubular acidosis depend on the affected portion of the renal tubule (i.e., proximal or distal). Diseases associated with proximal renal tubular acidosis include nephrotic syndrome and the use of acetazolamide, whereas renal transplants and lithium therapy are associated with distal renal tubular acidosis. The proximal tubule is the main site for bicarbonate absorption. Impaired hydrogen ion secretion at this level limits the resorption of bicarbonate and increases the excretion of bicarbonate in the urine. The proximal tubule absorbs the decreased filtered load of bicarbonate, and excessive bicarbonate is not present in the distal renal tubule. However, decreased hydrogen ion secretion in the distal tubule impairs the regeneration of bicarbonate.

Animal studies indicate that toluene decreases proton conductance in the active transport pathway without affecting the force of the proton pump or back-diffusion of hydrogen ions.[43] Reduced urinary ammonium and titratable acid limits net acid excretion and progressive acidosis develops when net acid excretion decreases below the fixed acid production of the body. Impaired sodium-hydrogen exchange in the distal nephrons causes sodium depletion, secondary hyperaldosteronism, and potassium wasting. These changes result in the combination of hypokalemia, dehydration, and acidosis. Overproduction of hippuric acid from the metabolism of large toluene doses may contribute to the formation of the wide anion gap metabolic acidosis by the accumulation of hippurate and the enhancement of sodium excretion with a corresponding decrease in the glomerular filtration rate.[44] Table 48.2 lists the laboratory abnormalities that aid in the diagnosis of proximal and distal renal tubular acidosis.

Postmortem Examination

A chronic toluene abuser died of acute toluene intoxication; postmortem examination demonstrated diffuse, ill-defined myelin pallor in the brain that was maximal in cerebellar, periventricular, and deep cerebral white matter.[45] Neurons were preserved with no axonal swelling or beading; gliosis was minimal. Occasional, scant

TABLE 48.2. Diagnostic Features of Proximal and Distal Renal Tubular Acidosis.[42]

Laboratory Values	Proximal	Distal
Serum Bicarbonate	>15 mEq/L	<15 mEq/L
Serum Chloride	High	High
Serum Potassium	Normal to low	Normal to Low
Anion Gap	Normal	Normal
Urine pH (Morning)	May be <6.0	>6.0
Bicarbonate Requirement	High	Low

perivascular macrophages were present. Postmortem examination of toluene abusers may demonstrate the smell of volatile substance; a red, scaly perioral rash, and/or paranasal rash; but typically the examination detects only congestion of the organs (e.g., lungs, brain).[46]

CLINICAL RESPONSE

Illicit Use

Toluene produces an initial excitatory phase followed by central nervous system (CNS) depression similar to the ingestion of ethanol. Desirable effects of the deliberate inhalation of toluene include exhilaration, euphoria, disinhibition, and lack of coordination. Other effects associated with the inhalation of toluene include the sensation that time passes quickly, auditory and visual hallucination, illusions, and delusional ideas.[47] Adverse effects include malodorous breath, excessive oral secretions with frequent expectoration, slurred speech, tinnitus, dizziness, impaired judgement, lethargy, gastrointestinal irritation, cough, sneezing, depression, fear, and dangerous delusions (swimming, flying). The effects of toluene inhalation typically resolve within about 30–45 minutes after inhalation ceases. A 14-year-old girl was brought to the emergency department with confusion, disorientation, and agitation after inhaling toluene daily for at least 5 days.[48] These symptoms resolved within 3 hours, but she complained of headache, weakness, and dizziness.

Complications

In a case series of 20 patients with chronic volatile substance abuse (primarily toluene) for over 2 years, 13 of these patients had neurologic abnormalities when examined at least 4 weeks after the cessation of use.[49] These adverse neurologic effects included cognitive (60%), pyramidal (50%), cerebellar (45%), and brainstem/cranial nerve (25%) abnormalities. Case

reports associate cerebellar damage, tremor, and ataxia with toluene abuse.[50] In a series of 24 toluene abusers (mean age 23 ± 4.4 years) admitted to a Canadian drug treatment unit, 11 patients had cerebellar abnormalities on neurologic examination.[14] The severity of the cerebellar signs correlated to the evidence of volume loss in the cerebellum as measured by a computed tomography (CT) scan. There is little evidence that toluene abuse causes peripheral or cranial nerve pathology.[51] A case report associated a history of chronic, heavy abuse of toluene-containing glue with a multiple sclerosis-like syndrome.[52] A 31-year-old man developed progressive incoordination, lower limb weakness, paresthesias, diplopia, urinary incontinence, and diffuse hyperintensities of cerebral white matter on T2-weighted magnetic resonance images (MRIs) that improved with abstinence. During one of his relapses, an acute encephalopathy appeared that also improved after cessation of glue sniffing.

Type I (distal) renal tubular acidosis is a recognized complication of toluene abuse.[53] Symptoms include nausea, vomiting, muscle weakness, and altered mental status. Rhabdomyolysis and myoglobinuria may occur along with severe hypokalemia and hypophosphatemia. Quadriparesis and respiratory failure may occur as a result of severe hypokalemia.[54] The renal tubular acidosis and hypokalemia associated with toluene abuse may mimic familial periodic paralysis. Other renal abnormalities associated with toluene abuse include Fanconi's syndrome. Rarely, case reports associate the development of ureteral calculi with toluene abuse and distal renal tubular acidosis.[42] Hepatotoxicity is not usually associated with toluene abuse.

Case reports associate chronic toluene abuse with dilated cardiomyopathy without coronary artery disease,[55] myocardial infarction with ventricular fibrillation,[56] and recurrent non-Q-wave myocardial infarction.[57] However, the contribution of toluene abuse to the morbidity and mortality associated with these case reports is unclear because of the confounding factors (e.g., ethanol abuse, preexisting congestive heart failure, strong family history of cardiac disease) present in these patients along with the poor documentation of exposure.

Abstinence Syndrome

Sleep disturbances, irritability, tremor, and nausea may occur within 2–5 days after cessation of toluene abuse.[58] However, case reports of withdrawal following the cessation of the inhalation of toluene are rare despite the continued chronic abuse of toluene by patients with serious neurologic and psychiatric abnormalities.[59] A 13-year-old boy reported that his 2-year history of glue

sniffing required progressively larger amounts of glue (i.e., up to 1 pint adhesive/4 h).[60] When hospitalized, he complained of abdominal cramps, myalgias, fatigue, and nausea beginning 36 hours after admission. These symptoms resolved after 5 days as the same symptoms had during a previous episode of abstinence. Withdrawal from the abuse of thinner containing toluene was associated with irritability, agitation, anxiety, irritability, cognitive impairment, tremor, vertigo, nausea, anorexia, insomnia, and paresthesias; however, these case reports do not include analysis of the thinner or confirmation of other drug use.[61,62]

Reproductive Abnormalities

The risk of developmental delay, growth retardation, preterm delivery, and perinatal death increases in infants born to mothers abusing inhalants.[63] Renal tubular acidosis may occur in pregnant mothers or in premature infants born to mothers chronically abusing toluene.[64] In a case series of 5 infants born to women abusing toluene during their pregnancy, 3 infants had growth retardation, 2 infants had hyperchloremic metabolic acidosis, and 2 had congenital anomalies.[65] Case reports associate self-reported toluene abuse with the development of phenotypic facial abnormalities, behavioral abnormalities, and growth deficits in children born to mothers without self-reported ethanol abuse.[66,67] These abnormalities are similar to those of the fetal alcohol syndrome and include growth deficits, microcephaly, attentional impairments, developmental delays (e.g., language), and minor craniofacial and limb defects (small midface, short palpebral fissures, low-set ears, narrow bifrontal diameter, micrognathia, blunted fingertips, small fingernails).[68,69] The causal role of toluene in these congenital and behavioral abnormalities is difficult to determine from case reports based on self-reports and the lack of control of confounding factors.

DIAGNOSTIC TESTING

Analytic Methods

TECHNIQUES

Analytic methods for the quantitation of toluene in biologic samples includes headspace gas chromatography with flame ionization detection or split flame ionization/electron capture detection,[70,71] gas chromatography with Fourier transform infrared detection, and gas chromatography/mass spectrometry (GC/MS).[72] The limit of detection (LOD) and lower limit of quantitation (LLOQ) of toluene in blood samples using gas chromatography with flame ionization detection were 0.030 and

0.10 mg/L, respectively.[73] Interference may occur with some substances (e.g., paraldehyde, n-propanol, degradation products of fatty acids including hexenol). Methods to overcome this interference in the detection of toluene in headspace analysis include the conversion of toluene to benzoic acid via the formation of benzotrichloride and subsequent detection of the trimethylsilyl derivatives with GC/MS.[74] Scanning electron microscopy of lung tissue from patients chronically inhaling paint may reveal particles of inorganic paint pigments, and dispersive x-ray spectral analysis of these particles confirms the presence of pigments (e.g., titanium).[46]

STORAGE

Substantial loss of toluene may occur during storage, even if stored in tightly sealed containers. Following storage of toluene standards in blood containing 2.16 mg/L in glass headspace vials, no significant toluene losses (i.e., <5%) occurred during storage at room, refrigeration, or freezing temperatures for the first week; however, a 19.48-mg/L standard lost 26%, 16%, and 24%, respectively, at these storage temperatures. At 2 weeks, the losses of these 2 standards increased to 40–45% at room temperature (24°C/75°F) and 35–40% at freezing temperature (−18°C/0°F).[75] After 4 years of storage, the losses were 90–95% at room temperature and 83–91% during refrigeration; therefore, toluene was still detectable in blood samples stored 4 years under refrigeration if the initial concentration exceeded 0.40 mg/L. The blood toluene concentrations in frozen samples were not analyzed during this period.

Samples should be stored in gas-tight, well-sealed, inert containers with minimal headspace; transfers of blood should occur under refrigerated conditions with the number of transfers minimized.[76] Examples of suitable containers include glass tubes fitted with a screw-topped cap, a metal foil liner, and an anticoagulant (e.g., EDTA). The tube should be filled up as much as possible with blood to eliminate a large gap between the cap and the blood in the container. Soft rubber tubes are highly permeable to toluene.[77] Teflon bags are unsuitable for the storage of breath samples for the detection of toluene as 1 study documented the loss of 87% of the toluene content over 9 days.[18] Long-term storage at temperatures below −5°C (23°F) should be avoided because of the formation of n-hexenol from the degradation of fatty acids at these temperatures.[70] The production of n-hexanal interferes with the analysis of low concentrations of toluene. False-positive results for toluene and other aromatic hydrocarbons (ethyl benzene, xylene) may potentially result from the use of tubes containing gel separators manufactured with aro-

matic hydrocarbons.[78] Xylene and ethylbenzene may contaminate some containers (e.g., Sarstedt Monovette serum gel blood-collection tubes; Sarstedt, Numbrecht, Germany) as a result of the leaching of these compounds from the tubes. Other sources of positive bias include contamination of butyl rubber materials used in sample preparation consumables (e.g., Vacutainer stoppers, syringe plungers, sample vial septa).[79]

Biomarkers

Toluene is detectable in saliva after experimental and occupational exposure to toluene in ambient air. In a study of 36 synthetic leather industry workers exposed to approximately 5–7 mg/m³ as a time-weighted average (TWA), the geometric mean toluene concentration in their saliva was 0.00096 mg/L (range, 0.00012–0.00549 mg/L) as measured by GC/MS.[80]

BLOOD

Toluene is detectable in the blood of non-occupationally exposed members of the general US population. In a convenience sample of 982 participants from the Third National Health and Nutrition Examination Survey (NHANES III), the median toluene concentration in blood samples from this group was 0.00028 mg/L (0.28 ppb) with an 95th percentile of 0.0015 mg/L (1.5 ppb).[81] Toluene is a component of cigarette smoke and only about one-half of this group were nonsmokers (current smoking status not reported).

Blood toluene concentrations do not correlate well to clinical effects because of the rapid distribution of toluene into the tissues and the rapid toluene elimination from the body. In general, the blood toluene concentration increases as the severity of toluene intoxication increases. Two amnesic workers were found at the bottom of a small swimming pool after using toluene to remove excess glue from tiles; they were stuporous and unable to walk, but their vital signs were normal.[38] The estimated toluene concentration in their ambient air was >7,000 mg/m³. Ninety minutes after the exposure ceased, the blood toluene concentrations were 4.1 mg/L and 2.2 mg/L as measured by headspace gas chromatography with flame ionization detection. Within 1–2 hours, they became fully alert and ambulatory; they were discharged from the emergency department 5 hours after exposure ceased. In a case series of 110 patients referred to a poison control center for volatile substance abuse, 10 patients with blood toluene concentrations exceeding 10 mg/L were either asymptomatic or mildly intoxicated (headache, drowsiness, nausea, vomiting).[82] In a study of 6 habitual toluene abusers, the blood toluene concentrations about 20 minutes after

cessation of inhalation ranged from 9.8–31.2 mg/L.[18] At the time of the sampling, these habitual toluene abusers demonstrated clinical features of moderate intoxication (slurred speech, poor coordination, impaired concentration). Substantial tolerance may develop following the chronic abuse of toluene. In a convenience sample of 62 homeless adolescents from a shelter that provided housing only from 7 PM to 7 AM each day, all admitted to volatile substance abuse and 57 of 62 adolescents had detectable toluene concentrations in their blood (sample time not specified). The blood toluene concentrations ranged from the LOD (0.5 mg/L) to 83.7 mg/L (median, 15.3 mg/L) as measured by gas chromatography with flame ionization detection.[83] Although most of these adolescents displayed signs of intoxication (restlessness, drowsiness, poor focus), this study did not report clinical features of severe toluene intoxication despite high blood toluene concentrations.

The postmortem blood toluene concentration as measured by headspace gas chromatography exceeded 5 mg/L in 22 of 25 deaths attributed to toluene.[82] In a case series of 33 suspected toluene-containing glue sniffers based on clinical history or evidence found at the death scene, the range of the toluene concentration in postmortem blood from suicidal individuals dying of trauma was 1–38 mg/L.[70] The postmortem toluene concentration in samples from individuals dying of drowning ranged from 1.7–54 mg/L, whereas the postmortem toluene concentration in 2 cases of hanging was 8.1 mg/L and 25 mg/L.

URINE

A 14-year-old girl presented to the emergency department after inhaling toluene daily for 5 days.[48] Four hours after presentation, she was alert and cooperative with headache and dizziness. The urinary hippuric acid at that time was 93.9 g/g creatinine. A 32-year-old man was admitted twice for chronic abuse of toluene-containing glue. The initial urinary hippuric acid concentrations at each admission were 24,400 mg/L and 21,800 mg/L.[5] Hippuric acid is a metabolite of toluene, and the concentration of hippuric acid in urine is a biomarker of toluene exposure in environmental and occupational settings.[84] However, hippuric acid is a metabolic product of some dietary components (e.g., benzoate preservatives, prunes, cranberries, plums, black tea); therefore, the presence of hippuric acid in the urine is not specific for toluene exposure. Hippuric acid concentrations up to 1.5 g/g creatinine may occur as a result of dietary exposure, but high concentrations of hippuric acid in the urine indicate toluene abuse. In a study of homeless adolescents in Brazil, urine hippuric acid concentrations exceeding 3 g/g creatinine was associated with intentional toluene exposure.[83] This cutoff had a sensitivity

of ~98% and specificity of 100%, and was below the urine hippuric acid concentrations following occupational exposure to 200 ppm (i.e., 4 times the current occupational limit for toluene in the work environment). The urine concentration of hippuric acid may be <1 g/g creatinine despite the presences of substantial amounts of toluene in the blood.[82]

Abnormalities

NEUROPSYCHOLOGIC TESTING

Presently, there is limited evidence that toluene abuse causes specific or persistent psychiatric or neuropsychologic testing deficits. In general, neuropsychologic tests are highly sensitive, but the specificity of these tests is poor, particularly without premorbid data. Evaluation of the performance of chronic volatile substance abusers on these tests is complicated by small sample sizes, inadequate controls, abuse of multiple drugs and several volatile substances, poor history, residual effects of acute intoxication, and confounding factors (age, educational history, socioeconomic status, psychiatric disorders, poor motivation, delinquency, learning and reading deficits, comorbid mental disorders). A high percentage of chronic toluene abusers have impaired performance on neuropsychologic testing relative to the general population, but there are few data on the premorbid baseline of these patients prior to the onset of volatile substance abuse. Within a cohort of 25 chronic solvent abusers, the patients with white matter changes had a statistically lower performance IQ (but not the verbal IQ) on the Wechsler Adult Intelligence Scale revised compared with other members of the group, particularly in the Digit Symbol Test (sustained attention, response speed, visuomotor coordination).[92] This latter test is less affected by intellectual level and educational background than most WAIS-R tests. There were no data on the premorbid intelligence of this group.

The presence of abnormalities on neurologic examination of chronic volatile substance abusers is often, but not always, associated with impaired performance on neuropsychologic testing including decreased IQ (verbal > performance) compared with population norms, impaired attention/concentration, deficits in short-term memory, and poor insight and judgment.[34] These neuropsychologic deficits are consistent with the white matter pathology detected by MRI. Although earlier studies suggested that toluene did not cause neuropsychologic impairment following mostly experimental use,[85] later studies of more heavily exposed, older adolescents suggests significant impairments in cognitive functioning including speed of information processing, visuospatial reasoning, learning, psychomotor

coordination, sustained attention, and executive abilities (e.g., working memory).[86,87]

A study of 55 volatile substance abusers (mean age 30.1 ± 8.0 years with a mean duration of abuse of 10.7 ± 8.2 years) and 61 chronic drug users (mean age 29.4 ± 6.1 years with cocaine being the drug of choice for 47% of these drug abusers) indicated that both groups scored below the population mean on most neuropsychologic measures.[88] The volatile substance abuse group performed particularly poorly on measures of working memory and executive cognitive functions when compared to the other drug abuse group. There was no clear dose-response relationship between cumulative dose of volatile substances and abnormalities on neuropsychologic tests. MRI data from this study demonstrated a high prevalence of abnormalities in both the inhalant group (44% abnormal) and the control drug group (25.5% abnormal). These abnormalities occurred in the basal ganglia, cerebellum, pons, and thalami of both groups, albeit higher in the inhalant group.

IMAGING STUDIES

Conventional MRI typically demonstrates multifocal white matter T2 hyperintensities and brain atrophy in the subcortical and periventricular white matter, internal capsule, brainstem, cerebellum, and upper cervical cord. Less common MRI abnormalities associated with toluene abuse involve prominent T2 hypointensities in the gray matter of the cortex and cerebellum including the basal ganglia, red and dentate nuclei, substantia nigra, and thalamus.[89,90] In MRI scans, chronic severe toluene abuse is associated with increased cerebellar, basal cistern, and cerebral sulci along with periventricular white matter signal hyperintensity on T2-weighted MR images.[91] In a study of 25 chronic solvent abusers with a mean age of 22.1 ± 4.3 years and mean duration of abuse of 7.5 ± 3.9 years, 10 patients had hyperintensities in cerebral white mater, brainstem, and cerebellum on T2-weighted MR images.[92] The main substance of abuse in 88% of these patients was toluene, occasionally with lacquer thinner and glues. Of these abusers, 9 also had hypointensities in the thalami. However, regression analysis did not demonstrate a statistically significant correlation between atrophy indices and duration of volatile substance abuse. Single photon-emission computed tomography (SPECT) may demonstrate hypohyperperfusion foci and nonhomogeneous uptake of radiopharmaceuticals in the brain.[93]

BLOOD

Multiple metabolic abnormalities may occur following chronic toluene abuse. These metabolic disturbances

include hypokalemia, hyperchloremia, hypophosphatemia, and metabolic acidosis.[94] Debilitating muscle weakness along with rhabdomyolysis may result from profound hypokalemia due to chronic toluene abuse.[95] Metabolic acidosis may occur with either a normal anion gap or a high anion gap. Typically, renal tubular acidosis is associated with a normal anion gap and hyperchloremic metabolic acidosis as a result of impaired renal excretion of ammonium ion; excessive excretion of potassium and sodium in inappropriately alkaline urine (i.e., pH >5.5 in the presence of metabolic acidosis) also occurs.[96] Excessive urinary excretion of hippuric acid may also cause a normal anion gap metabolic acidosis as a result of the rapid excretion of the unmeasured anion, hippurate. Occasionally, a high anion gap acidosis develops along with excessive sodium excretion, prerenal azotemia, and impaired renal excretion of hippuric acid. Although transient distal renal tubular acidosis is a prominent occurrence during these abnormalities, this syndrome does not explain all of the metabolic and renal changes in chronic toluene abusers. These abnormalities may include renal dysfunction. In a case series of 16 episodes of hospitalization for paint sniffing (30–35% toluene), there were 6 episodes of rapidly reversible renal insufficiency with peak serum creatinine concentrations ranging from 1.7–5.4 mg/dL.[97] The urinalysis of chronic toluene abusers may demonstrate glucosuria, proteinuria, or myoglobinuria.[98] An elevated serum osmolal gap may occur in the presence of toluene in the serum.[99]

Driving

In a Norwegian study of 114 drivers arrested for driving under the influence with detectable toluene concentrations in their blood samples, the blood toluene concentration collected 30–90 minutes after apprehension ranged from 0.09–21.5 mg/L.[100] Most of the 29 drivers with blood toluene concentrations exceeding 9.3 mg/L (109 µM) and no other detectable drugs were impaired as determined by a physician's examination. Some decline in the toluene concentration between apprehension and analysis probably occurred because of the rapid toluene elimination rate and the delay between sampling and analysis. In a case series of 6 drivers arrested for suspected driving under the influence with no other drugs of abuse in their blood, the mean blood toluene concentration was 25 ± 12.1 mg/L (median, 23 mg/L, range, 12–45 mg/L).[101] All these drivers displayed moderate intoxication (slurred speech, ataxia, poor concentration) without serious signs of CNS depression. The average time between arrest and collection of the blood sample was approximately 2 hours. The mean toluene blood concentration for glue sniffers

arrested for volatile substance abuse was 10.8 ± 3.9 mg/L (median, 2.4 mg/L; range, 0.2–74.7 mg/L).[102]

TREATMENT

Stabilization

The primary risk following severe acute exposure to high doses of toluene is CNS depression and suppression of the respiratory drive. Severe hypokalemia and profound muscle weakness may result from chronic toluene abuse. Patients should be removed from further toluene exposure; contaminated skin should be washed thoroughly with soap and water after removal of contaminated clothing. Other decontamination measures are usually unnecessary. Symptomatic patients should be monitored for respiratory depression and cardiac dysrhythmias. Patients with altered consciousness may require respiratory support (supplemental oxygen, assisted ventilation). There are no antidotes for toluene exposure.

Supportive Care

The treatment of medical abnormalities associated with toluene abuse is supportive. As a result of the rapid initial elimination of toluene, resolution of CNS symptoms typically occurs within a few hours. Failure of these symptoms to improve within several hours after toluene exposure ceases suggests the presence of other pathology. Correction of renal abnormalities associated with chronic toluene abuse may require longer therapy with most patients responding to fluid and electrolyte replacement within a few days. Blood from these patients should be analyzed for electrolyte (hypokalemia, hypophosphatemia, hypocalcemia) and acid-base disturbances as well as rhabdomyolysis. Patients with a history of chronic toluene abuse should be referred for psychiatric care to encourage abstinence. There is no specific treatment for chronic toluene exposure.

References

1. Glaser HH, Massengale ON. Glue-sniffing in children. Deliberate inhalation of vaporized plastic cements. JAMA 1962;181:300–303.
2. Gellman V. Glue-sniffing among Winnipeg school children. Can Med Assoc J 1968;98:411–413.
3. International Agency for Research on Cancer. Toluene. IARC Monogr 1989;47:79–123.
4. Carpenter CP, Geary DL Jr, Meyers RC, Nachreiner DJ, Sullivan LJ, King JM. Petroleum hydrocarbon toxicity studies. XIII. Animal and human responses to vapors of

toluene concentrate. Toxicol Appl Pharmacol 1976;36: 473–487.

5. Kira S, Ogata M, Ebara Y, Horii S, Otsuki S. A case of thinner sniffing: relationship between neuropsychological symptoms and urinary findings after inhalation of toluene and methanol. Ind Health 1988;26:81–85.

6. Press E, Done AK. Solvent sniffing physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. I. Pediatrics 1967;39:451–461.

7. Satran R, Dodson VN. Toluene habituation. Report of a case. N Engl J Med 1963;268:719–721.

8. Brozosky M, Winkler EG. Glue sniffing in children and adolescents. NY State Med J 1965;65:1984–1989.

9. Ron MA. Volatile substance abuse: a review of possible long-term neurological, intellectual and psychiatric sequelae. Br J Psychiatry 1986;148:235–246.

10. Wood RW, Colotla VA. Biphasic changes in mouse motor activity during exposure to toluene. Fundam Appl Toxicol 1990;14:6–14.

11. Taylor JD, Evans HL. Effects of toluene inhalation on behavior and expired carbon dioxide in macaque monkeys. Toxicol Appl Pharmacol 1985;80:487–495.

12. Bruckner JV, Peterson RG. Evaluation of toluene and acetone inhalant abuse II. Model development and toxicology. Toxicol Appl Pharmacol 1981;61:302–312.

13. Borne J, Riascos R, Cuellar H, Vargas D, Rojas R. Neuroimaging in drug and substance abuse part II opioids and solvents. Top Magn Reson Imaging 2005;16: 239–245.

14. Fornazzari L, Wilkinson DA, Kapur BM, Carlen PL. Cerebellar, cortical and functional impairment in toluene abusers. Acta Neurol Scand 1983;67:319–329.

15. Nomiyama K, Nomiyama H. Respiratory retention, take and excretion of organic solvents in man. benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int Arch Arbeitsmed 1974;32: 75–83.

16. Carlsson A. Exposure to toluene: Uptake, distribution and elimination in man. Scand J Work Environ Health 1982;8:43–55.

17. Astrand I, Ehrner-Samuel H, Kilbom A, Ovrum P. Toluene exposure I. Concentration in alveolar air and blood at rest and during exercise. Work Environ Health 1972;9:119–130.

18. Garriott JC, Foerster E, Juarez L, de la Garza F, Mendiola I, Curoe J. Measurement of toluene in blood and breath in cases of solvent abuse. Clin Toxicol 1981;18:471–479.

19 Toftgard R, Gustafsson JA. Biotransformation of organic solvents. A review. Scand J Work Environ Health 1980;6:1–18.

20. Nakajima T, Wang R-S, Elovaara E, Park SS, Gelboin HV, Hietanen E, Vianio H. Monoclonal antibody-directed characterization of cytochrome P450 isozymes responsible for toluene metabolism in rat liver. Biochem Pharmacol 1991;41:395–404.

21. Tassaneeyuakul W, Birkett DJ, Edwards JW, Veronese ME, Tassaneyakul W, Tukey RH, Miners JO. Human cytochrome P450 isoform specificity in the regioselective metabolism of toluene and o-, m- and p-xylene. J Pharmacol Exp Ther 1966;276:101–108.

22. Pierce CH, Chen Y, Dills RL, Kalman DA, Morgan MS. Toluene metabolites as biological indicators of exposure. Toxicol Lett 2002;129:65–76.

23. Brugnone F, Perbellini L, Apostoli P, Locatelli M, Mariotto P. Decline of blood and alveolar toluene concentration following two accidental human poisonings. Int Arch Occup Environ Health 1983;53:157–165.

24. Nise G, Attewell R, Skerfving S, Orbaek P. Elimination of toluene from venous blood and adipose tissue after occupational exposure. Br J Ind Med 1989;46: 407–411.

25. Ghantous H, Danielsson BR. Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. Biol Res Pregnancy Perinatol 1986;7:98–105.

26. Moser VC, Balster RL. The effects of acute and repeated toluene exposure on operant behavior in mice. Neurobehav Toxicol Teratol 1981;3:481–485.

27. Press E, Done AK. Solvent sniffing physiologic effects and community control measures for intoxication from the intentional inhalation of organic solvents. II. Pediatrics 1967;39:611–622.

28. Brozovsky M, Winkler EG. Glue sniffing in children and adolescents. NY State J Med 1965;65:1984–1989.

29. Nakajima T, Wang R-S. Induction of cytochrome P450 by toluene. Int J Biochem 1994;26:1333–1340.

30. Baelum J, Molhave L, Hansen SH, Vaeth M. Metabolic interaction between toluene, trichloroethylene and n-hexane. Scand J Work Environ Health 1998;24:30–37.

31. Walllen M, Naslund PH, Nordqvist MB. The effects of ethanol on the kinetics of toluene in man. Toxicol Appl Pharmacol 1984;76:414–419.

32. Bale AS, Tu Y, Carpenter-Hyland EP, Chandler LJ, Woodward JJ. Alterations in glutamatergic and gabanergic ion channel activity in hippocampal neurons following exposure to the abused inhalant toluene. Neuroscience 2005;130:197–206.

33. Filley CM, Halliday W, Kleinschmidt-DeMasters BK. The effects of toluene on the central nervous system. J Neuropathol Exp Neurol 2004;63:1–12.

34. Yucel M, Takagi M, Walterfang M, Lubman DI. Toluene misuse and long-term harms: a systemic review of the neuropsychological and neuroimaging literature. Neurosci Biobehav Rev 2008;32:910–926.

35. Aydin K, Sencer S, Ogel K, Genchellac H, Demir T, Minareci O. Single-voxel proton MR spectroscopy in toluene abuse. Magn Reson Imaging 2003;21:777–785.

36. Papageorgiou SG, Arantoni E, Pandis D, Kouzoupis AV, Kalfakis N, Limouris GS. Severe dopaminergic pathways damage in a case of chronic toluene abuse. Clin Neurol Neurosurg 2009;111:864–867.

37. Ikeda N, Takahashi H, Umetsu K, Suzuki T. The course of respiration and circulation in "toluene sniffing." Forensic Sci Int 1990;44:151–158.

38. Meulenbelt J, de Groot G, Savelkul TJ. Two cases of acute toluene intoxication. Br J Ind Med 1990;47:417–420.

39. Vidrio H, Magos GA, Lorenzana-Jimenez M. Electrocardiographic effects of toluene in the anesthetized rat. Arch Int Pharmacodyn Ther 1986;279:121–129.

40. Taylor GJ, Harris WS. Glue sniffing causes heart block in mice. Science 1970;170:866–868.

41. Einav S, Amitai Y, Reichman J, Geber D. Bradycardia in toluene poisoning. J Toxicol Clin Toxicol 1997;35:295–298.

42. Kroeger RM, Moore RJ, Lehman TH, Giesy JD, Skeeters CE. Recurrent urinary calculi associated with toluene sniffing. J Urol 1980;123:89–91.

43. Batlle DC, Sabatini S, Kurtzman NA. On the mechanism of toluene-induced renal tubular acidosis. Nephron 1988;49:210–218.

44. Carlisle EJ, Donnelly SM, Vasuvattakul S, Kamel KS, Tobe S, Halperin ML. Glue-sniffing and distal renal tubular acidosis: sticking to the facts. J Am Soc Nephrol 1991;1:1019–1027.

45. Rosenberg NL, Kleinschmidt-DeMasters BK, Davis KA, Dreisbach JN, Hormes JT, Filley CM. Toluene abuse causes diffuse central nervous system white matter changes. Ann Neurol 1988;23:611–614.

46. Byard RW, Gilbert JD, Terlet J. Death associated with volatile substance inhalation—histologic, scanning electron microscopic and energy dispersive X-ray spectral analyses of lung tissue. Forensic Sci Int 2007;171:118–121.

47. Evans AC, Raistrick D. Phenomenology of intoxication with toluene-based adhesives and butane gas. Br J Psychiatry 1987;150:769–773.

48. Raikhlin-Eisenkraft B, Hoffer E, Baum Y, Bentur Y. Determination of urinary hippuric acid in toluene abuse. Clin Toxicol 2001;39:73–76.

49. Hormes JT, Filley CM, Rosenberg NL. Neurologic sequelae of chronic solvent vapor abuse. Neurology 1986;36:698–702.

50. Boor JW, Hurtig HI: Persistent cerebellar ataxia after exposure to toluene. Ann Neurol 1977;2:440–442.

51. King MD: Neurological sequelae of toluene abuse. Hum Toxicol 1982;1:281–287.

52. Davies MB, Weatherby SJ, Haq N, Ellis SJ. A multiple-sclerosis-like syndrome associated with glue-sniffing. J Roy Soc Med 2000;93:313–314.

53. Taher SM, Anderson RJ, McCartney R, Popovtzer MM, Schrier RW. Renal tubular acidosis associated with toluene "sniffing." N Engl J Med 1974;290:765–768.

54. Kao K-C, Tsai Y-H, Lin M-C, Huang C-C, Tsao C-Y, Chen Y-C. Hypokalemic muscular paralysis causing acute respiratory failure due to rhabdomyolysis with renal tubular acidosis in a chronic glue sniffer. Clin Toxicol 2000;38:679–681.

55. Vural M, Ogel K. Dilated cardiomyopathy associated with toluene abuse. Cardiology 2006;105:158–161.

56. Cunningham SR, Dalzell GW, McGirr P, Khan MM. Myocardial infarction and primary ventricular fibrillation after glue sniffing. Br Med J (Clin Res Ed) 1987;294:739–740.

57. Hussain TF, Heidenreich PA, Benowitz N. Recurrent non-Q-wave myocardial infarction associated with toluene abuse. Am Heart J 1996;131:615–616.

58. Evans AC, Raistrick D. Phenomenology of intoxication with toluene-based adhesives and butane gas. Br J Psychiatry 1987;150:769–773.

59. Lewis JD, Moritz D, Mellis LP. Long-term toluene abuse. Am J Psychiatry 1981;138:368–370.

60. Watson JM. Glue sniffing two case reports. Practitioner 1979;222:845–847.

61. Kouzoupis AV, Konstantakopoulos G, Oulis P, Kalfakis N, Papageorgiou SG. A case of severe toluene withdrawal syndrome treated with clonazepam. J Neuropsychiatry Clin Neurosci 2010;22:e16–e17.

62. Nylander I. "Thinner" addiction in children and adolescents. Acta Paedopsychiatr 1962;29:273–283.

63. Wilkins-Haug L, Gabow PA. Toluene abuse during pregnancy: obstetric complications and perinatal outcomes. Obstet Gynecol 1991;77:504–509.

64. Erramouspe J, Galvez R, Fischel DR. Newborn renal tubular acidosis associated with prenatal maternal toluene sniffing. J Psychoactive Drugs 1996;28:201–204.

65. Goodwin TM. Toluene abuse and renal tubular acidosis in pregnancy. Obstet Gynecol 1988;71:715–718.

66. Arnold GL, Kirby RS, Langendoerfer S, Wilkins-Haug L. Toluene embryopathy: clinical delineation and developmental follow-up. Pediatrics 1994;93:216–220.

67. Pearson MA, Hoyme HE, Seaver LH, Rimsza ME. Toluene embryopathy: delineation of the phenotype and comparison with fetal alcohol syndrome. Pediatrics 1994;93:211–215.

68. Toutant C, Lippmann S. Fetal solvents syndrome. Lancet 1979;1(8130):1356.

69. Hersh JH. Toluene embryopathy: two new cases. J Med Genet 1989;26:333–337.

70. Streete PJ, Ruprah M, Ramsey JD, Flanagan RJ. Detection and identification of volatile substances by headspace capillary gas chromatography to aid the diagnosis of acute poisoning. Analyst 1992;117:1111–1127.

71. Chao TC, Lo DS, Koh J, Ting TC, Quek LM, Koh TH, et al. Glue sniffing deaths in Singapore—volatile aromatic hydrocarbons in post-mortem blood by headspace gas chromatography. Med Sci Law 1993;33:253–260.

72. Jone CM, Wu AH. An unusual case of toluene-induced metabolic acidosis. Clin Chem 1988;34:2596–2599.

73. Martínez MA, Ballesteros S. An unusual case of drug-facilitated sexual assault using aromatic solvents. J Anal Toxicol 2006;30:449–453.

74. El-Haj BM, Al-Amri AM, Hassan MH, Bin-Khadem RK, Al-Hadi AA. A GC-MS method for the detection of toluene and ethylbenzene in volatile substance abuse. J Anal Toxicol 2000;24:390–394.

75. Saker EG, Eskew AE, Panter JW. Stability of toluene in blood: its forensic relevance. J Anal Toxicol 1991;15:246–249.

76. Gill R, Hatchett SE, Osselton MD, Wilson HK, Ramsey JD. Sample handling and storage for the quantitative analysis of volatile compounds in blood: the determination of toluene by headspace gas chromatography. J Anal Toxicol 1988;12:141–146.

77. Dyne D, Cocker J, Streete PJ, Flanagan RJ. Toluene, 1-butanol, ethylbenzene and xylene from Sarstedt Monovette serum gel blood collection tubes. Ann Clin Biochem 1996;33:355–356.

78. Streete PJ, Flanagan RJ. Ethylbenzene and xylene from Sarstedt Monovette serum gel blood-collection tubes. Clin Chem 1993;39:1344–1345.

79. Chambers DM, McElprang DO, Waterhouse MG, Blount BC. An improved approach for accurate quantitation of benzene, toluene, ethylbenzene, xylene, and styrene in blood. Anal Chem 2006;78:5375–5383.

80. Ferrari M, Negri S, Zadra P, Ghittori S, Imbriani M. Saliva as an analytical tool to measure occupation exposure to toluene. Int Arch Occup Environ Health 2008;81:1021–1028.

81. Churchill JE, Kaye WE, Ashley DL. Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Arch Environ Health 2001;56:157–166.

82. Meredith TJ, Ruprah M, Liddle A, Flanagan RJ. Diagnosis and treatment of acute poisoning with volatile substances. Hum Toxicol 1989;8:277–286.

83. Thiesen FV, Noto AR, Barrow HM. Laboratory diagnosis of toluene-based inhalants abuse. Clin Toxicol 2007;45:557–562.

84. Baelum J, Dossing M, Hansen SH, Lundqvist GR, Andersen NT. Toluene metabolism during exposure to varying concentration combined with exercise. Int Arch Occup Environ Health 1987;59:281–294.

85. Chadwick OF, Anderson HR. Neuropsychological consequences of volatile substance abuse: a review. Hum Toxicol 1989;8:307–312.

86. Allison WM, Jerrom DW. Glue sniffing: a pilot study of the cognitive effects of long-term use. Int J Addict 1984;19:453–458.

87. Korman M, Matthews RW, Lovitt R. Neuropsychological effects of abuse of inhalants. Percept Mot Skills 1981;53:547–553.

88. Rosenberg NL, Grigsby J, Dreisbach J, Busenbark D, Grigsby P. Neuropsychologic impairment and MRI abnormalities associated with chronic solvent abuse. Clin Toxicol 2002;40:21–34.

89. Caldemeyer KS, Armstrong SW, George KK, Moran CC, Pascuzzi RM. The spectrum of neuroimaging abnormalities in solvent abuse and their clinical correlation. J Neuroimaging 1996;6:167–173.

90. Kamran S, Bakshi R. MRI in chronic toluene abuse: low signal in the cerebral cortex on T2-weighted images. Neuroradiology 1998;40:519–521.

91. Filley CM, Heaton RK, Rosenberg NL. White matter dementia in chronic toluene abuse. Neurology 1990;40:532–534.

92. Yamanouchi N, Okada S, Kodama K, Sakamoto T, Sekine H, Hirai S, et al. Effects of MRI abnormalities on WAIS-R performance in solvent abusers. Acta Neurol Scand 1997;96:34–39.

93. Kucuk NO, Kilic EO, Ibis E, Aysev A, Gencoglu EA, Aras G, et al. Brain SPECT findings in long-term inhalant abuse. Nucl Med Commun 2000;21:769–773

94. Tang HL, Chu KH, Cheuk A, Tsang WK, Chan HW, Tong KL. Renal tubular acidosis and severe hypophosphatae-mia due to toluene inhalation. Hong Kong Med J 2005;11:50–53.

95. Baskerville JR, Tichenor GA, Rosen PB. Toluene induced hypokalemia: case report and literature review. Emerg Med J 2001;18:514–516.

96. Carlisle EJ, Donnelly SM, Vasuvattakul S, Kamel KS, Tobe S, Halperin ML. Glue-sniffing and distal renal tubular acidosis: sticking to the facts. J Am Soc Nephrol 1991;1:1019–1027.

97. Voigts A, Kaufman CE Jr. Acidosis and other metabolic abnormalities associated with paint sniffing. South Med J 1983;76:443–452.

98. Kamijima M, Nakazawa Y, Yamakawa M, Shibata E, Hisanaga N, Ono Y, et al. Metabolic acidosis and renal tubular injury due to pure toluene inhalation. Arch Environ Health 1994;49:410–413.

99. Dickson RP, Luks AM. Toluene toxicity as a cause of elevated anion gap metabolic acidosis. Respir Care 2009;54:1115–1117.

100. Gjerde H, Smith-Kielland A, Normann PT, Morland J. Driving under the influence of toluene. Forensic Sci Int 1990;44:77–83.

101. Capron B, Logan BK. Toluene-impaired drivers: behavioral observations, impairment assessment, and toxicological findings. J Forensic Sci 2009;54:486–489.

102. Park SW, Kim N, Yang Y, Seo B, Paeng KJ. Toluene distribution of glue sniffers' biological fluid samples in Korea. J Forensic Sci 1998;43:888–890.

Chapter 49

TRICHLOROETHANE

IDENTIFYING CHARACTERISTICS

1,1,1-Trichloroethane (CAS RN: 71-55-6, methylchloroform; TCA) is a colorless, pleasant smelling liquid with a molecular weight of 1.336 g/mol and a boiling point of 71.1°C (160°F). The viscosity of this liquid at 20°C (68°F) is 0.858 mPa-sec. The vapor pressure at room temperature is 127 mm Hg; the concentration of TCA in ambient air at saturation is 160,000 ppm. The odor threshold of TCA is about 100 ppm; the smell becomes unpleasant at 1,500–2,000 ppm.[1] This highly volatile, nonflammable compound is only slightly soluble in water, but TCA is highly miscible in organic solvents (e.g., carbon tetrachloride, carbon disulfide, chloroform, acetone, methanol, benzene). Trichloroethane is a halogenated aliphatic hydrocarbon as displayed in Figure 49.1. 1,1,2-Trichloroethane (CAS RN: 79-00-5) is an isomer of TCA.

Stabilizers are added to both technical and solvent grades of TCA at concentrations of 3–8% to prevent the corrosion of aluminum and aluminum alloys. These stabilizers include nitromethane, nitroethane, butanols, isobutyl alcohol, methyl ethyl ketone, diisopropylamine, 1,4-dioxane, butylene oxide, 1,3-dioxolane, toluene, and *n*-methylpyrrole.[2] Minor impurities in 1,1,1-trichloroethane include 1,1-dichloroethane and 1,1,2-trichloroethane.

EXPOSURE

Sources

1,1,1-Trichloroethane is a volatile, chlorinated solvent that is used primarily as an industrial degreaser and consumer spot remover. Previously, typewriter correction fluid (e.g., Wite-Out™) contained trichloroethane and trichloroethylene up to about 50–60% of the product. In 1984, mustard oil was added to typewriter correction fluid in the United States to discourage the inhalation of vapors from these products. Mustard oil (allyl isothiocyanate) has an unpleasant smell and irritates the mucous membrane and gastrointestinal epithelium; however, as the abuse continued, trichloroethylene and later trichloroethane were removed from these products. Now petroleum distillates have replaced the chlorinated hydrocarbons in many of these products. Trichloroethane previously was a solvent in cosmetics.

Methods of Abuse

Similar to most volatile substances of abuse, trichloroethane is usually sniffed directly from the container, placed on a rag and inhaled as the rag is placed over the nose and mouth, or placed in a container from which the vapors are inhaled. Typically, the euphoria is compared with the ingestion of large amounts of ethanol. Repeat inhalations are necessary to maintain the desired effects because the euphoria resolves in about 15–30 minutes and the "pleasant" drowsiness by 15–60 minutes.[3]

DOSE EFFECT

Mild acute overexposure (500–1,500 ppm) produces inebriation, headache, weakness, lightheadedness, and irritability, whereas exposure to higher levels (1,500–5,000 ppm TCA) causes slurred speech, loss of coordination, giddiness, confusion, lethargy, and disequilibrium.

Abnormalities of the Romberg Test appear in susceptible individuals following exposure to about 900 ppm.[4] Table 49.1 lists the estimated physiologic response to specific TCA concentrations. The clinical effects of airborne exposures to TCA are both concentration and time dependent. Induction of anesthesia develops within 2 minutes following exposure to a concentration of 10,000–26,000 ppm, and recovery begins within 3–5 minutes after exposure ceases.[5]

TOXICOKINETICS

Absorption

In volunteers, the lungs absorb approximately 25% of the inhaled dose of 1,1,1-trichloroethane (TCA) following exposure to 300 ppm TCA for 6 hours.[6] Similarly, the lungs retain approximately 26–32% following exposure to 213 ppm TCA over 8 hours.[7] The skin is a good barrier to the uptake of TCA vapors; percutaneous absorption of TCA through undamaged, nonoccluded human skin probably is insignificant based on experimental studies and pharmacokinetic modeling.[8] Data are limited on the gastrointestinal (GI) absorption of
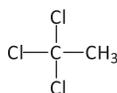
FIGURE 49.1. Chemical structure of 1,1,1-trichloroethane.

TCA. The high oral LD_{50} (i.e., approximately 10 gm/kg TCA body weight) and the absence of reported data on systemic toxicity following ingestion of TCA suggest that GI absorption of this chemical may not be substantial; however, data are insufficient to reach definitive conclusions.

Distribution

The high lipid/blood partition coefficient of TCA indicates that TCA distributes relatively more to organs with the highest fat concentrations. Postmortem concentrations document high TCA concentrations in the fat, brain, and blood. Absorbed TCA has the potential to cross both the blood–brain and placental barriers.

Biotransformation

TCA is a relatively stable molecule; humans metabolize only small amounts (i.e., <7%) of TCA, primarily via the cytochrome P450 pathway. The major metabolite is 2,2,2-trichloroethanol, which is also a product of the metabolism of tetrachloroethylene, chloral hydrate, and trichloroethylene. The glucuronide conjugate of trichloroethanol (urochloralic acid) appears in the urine following exposure to TCA. The other significant metabolite is trichloroacetic acid. The metabolism of TCA is saturable; the ratio of trichloroethanol glucuronide and trichloroacetic acid increases above 2:1 as the TCA exposure increases.[9]

TABLE 49.1. Estimated Effects of Single Exposure to the Vapors of 1,1,1-Trichloroethane. [4]

Exposure Time (min)	Air Concentration (ppm)	Expected Human Effect
5	2,000	Disturbance of equilibrium. Odor is unpleasant but tolerable (H)
	5,000	Pronounced loss of coordination (R)
	10,000	Definite incoordination (R,M)
	20,000	Complete incoordination and helplessness (R)
15	1,000	Possible beginning loss of equilibrium (H)
	2,000	Loss of equilibrium (H)
	10,000	Pronounced loss of coordination (R)
30	1,000	Mild eye and nasal discomfort; possible slight loss of equilibrium (H)
	2,000	Loss of equilibrium (H)
	5,000	Incoordination (R,M)
	10,000	Pronounced loss of coordination (R)
60	100	Apparent odor threshold (H)
	500	No detectable effect, but odor is obvious (R,H)
	1,000	Very slight loss of equilibrium (H)
	2,000	Loss of coordination (H)
	5,000	Obvious loss of coordination (R,M)
	10,000	Pronounced loss of coordination (R)
	20,000	Surgical anesthesia, possible death (R)

Note. H = Expected effects are based on human data; M = expected effects based on data from monkeys; R = expected effects based on data from rats.

Elimination

The lungs excrete >90% of an absorbed TCA dose unchanged in expired air following exposure 200 ppm TCA.[7] The relatively low blood/air partition coefficient of TCA compared with other solvents produces relatively slow distribution into tissues, resulting in the rapid elimination of TCA by the lungs.[10] The estimated elimination half-life of TCA follows a 3-compartment model: 1) 44 minutes for highly vascular tissue, 2) 5.7 hours for muscle tissue, and 3) 53 hours for poorly vascularized tissue (e.g., fat). Urinary excretion of TCA metabolites is slow (i.e., days). The kidneys excrete approximately 4.2% of an absorbed dose of TCA as trichloroethanol and approximately 1.8% of the dose as trichloroacetic acid.[7] Following exposure of human volunteers to 350 ppm TCA for 6 hours, <1% of the absorbed TCA dose remained in the body after 9 days.[6]

Tolerance

Tolerance to the effects of TCA occurs in some, but not all animal studies, depending in part on the exposure scenario.[11] These animal studies indicate that tolerance develops to some of the effects of TCA, whereas prolonged exposure to TCA causes sensitization to other effects. Following exposure of mice to TCA (0, 2,000, 6,000, 10,000, 13,300 ppm) 30 minutes daily for 15 days, tolerance developed to measures of forelimb grip strength, number of rears, and inverted screen tests; sensitization occurred to measures of locomotor activity. The development of tolerance was independent of dose.

Drug Interactions

Although pretreatment of rodents with ethanol may induce metabolic enzymes, the elimination rate in ethanol-pretreated and control rats (no ethanol) is similar following exposure to trichloroethane concentration up to 500 ppm for 6 hours.[12] Additionally, pretreatment of mice with isopropanol or acetone does not affect the serum alanine aminotransferase concentration in mice exposed to trichloroethane following injection of 1.5 mL TCA/kg or exposure to 15,000 ppm TCA for 2 hours.[13,14]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Trichloroethane affects ligand-gated ion channels, particularly the glutamate and GABA$_A$ receptor superfamilies, similar to trichloroethylene. *In vitro* animal studies indicate that trichloroethane reversibly enhances γ-aminobutyric acid (GABA)$_A$ and α$_1$ glycine receptor-mediated synaptic currents, thereby increasing the inhibitory effects of GABA similar to inhaled anesthetics.[15,16] Additionally, these studies suggest that trichloroethane also enhances serotonin-3$_A$ receptor (5-HT$_{3A}$) function.[17] Rodent studies using opioid and nicotinic antagonists/agonists suggest the lack of involvement of the μ-opioid and central nicotinic receptors in the discriminative stimulus effects of TCA misuse.[18] In these rodent studies, the effects of TCA on these receptors are similar to trichloroethylene, toluene, enflurane, and sevoflurane.[19]

Mechanism of Toxicity

Acute exposure to a high dose of 1,1,1-trichloroethane (TCA) produces CNS excitation (1,250–2,500 ppm)[20] and then CNS depression (>5,000–10,000 ppm); most deaths probably result from this depression and the subsequent hypoxia. There are multiple potential causes of death in individuals dying suddenly after inhaling vapors of products containing trichloroethane including asphyxia, aspiration of gastric contents, hypoxia, and cardiac dysrhythmias. Although strenuous exercise frequently precedes sudden death, the question of whether strenuous activity contributes to sudden death or is a consequence of central nervous stimulation or hypoxia remains unresolved.

Postmortem Examination

The autopsies of individuals dying suddenly after trichloroethane abuse demonstrate nonspecific findings including signs of asphyxia (pleural petechiae, subconjunctival hemorrhage/petechiae), irritation of the airways and conjunctiva, congestion of the viscera (brain, lungs), and aspiration of food.[21,22] Histologic examination of the heart from a 14 year old, who died shortly after sniffing Liquid Paper™ correction fluid (composition not reported), demonstrated degenerative changes including swollen and ruptured myofibrils, interfibrillary edema, and a wavy, fibrillar pattern.[23] Although congestion of the liver and kidney may be prominent, acute damage to these organs is not usually present on postmortem examination of cases of sudden death associated with TCA.[24] Rarely, autopsies of cases of sudden death demonstrate severe cerebral edema. A 15-year-old adolescent complained of diplopia and hallucinations after apparently sniffing typewriter correction fluid (Tipp-Ex®, composition not reported).[25] He collapsed and resuscitation was unsuccessful. The autopsy demonstrated severe cerebral edema with marked tonsillar herniation and uncal grooving.

CLINICAL RESPONSE

Illicit Use

The clinical effects of trichloroethane are similar to other volatile substances of abuse and include initial excitement, disinhibition, and euphoria followed by CNS depression. Nausea and vomiting are frequent complications of TCA miuse; other adverse effects include ataxia, slurred speech, hallucination, agitation, and delirium.[26] Exposure to high TCA concentrations produces a rapid onset of CNS depression. Symptoms include dizziness, headache, fatigue, and lethargy. These symptoms may progress to stupor, apnea, coma, or death.[27] The abuse of trichloroethane has not been associated with hepatic or renal damage.

Fatalities

Numerous case reports associate sudden death with TCA and trichloroethylene misuse shortly before their collapse.[28,29] Often, these individuals become agitated and exercise strenuously (e.g., run) prior to death.[30] Many of these case reports involve the misuse of typewriter correction fluid that contains both trichloroethylene and trichloroethane. Although many of the cases involve asphyxia and aspiration of stomach contents, some document the presence of ventricular fibrillation. A 12-year-old girl collapsed shortly after inhaling the vapors from a container of typewriter correction fluid thinner containing trichloroethane.[31] Bystander cardiopulmonary resuscitation was initiated immediately by a nearby physician, and an ambulance arrived within 2 minutes. The initial rhythm was coarse ventricular fibrillation, which converted to normal sinus rhythm with a single 200 J shock. She was extubated in the emergency department after awakening; recovery occurred without sequelae. Although most of these case reports are not associated with myocardial injury, rare case reports associate the development of myocardial injury with the misuse of products containing trichloroethane. A 15-year-old adolescent collapsed 15 minutes after inhaling Liquid Paper™ (composition not reported).[32] The initial rhythm 8–10 minutes after collapse was coarse ventricular fibrillation that converted to sinus rhythm after 2 countershocks. Serial electrocardiograms were consistent with anteroseptal subendocardial injury and the echocardiogram revealed a small area of hypokinesis in the distal interventricular septum. He was discharged 4 days after admission with no further complications.

Abstinence Syndrome

Animal studies suggest that TCA has the potential to produce physical dependence similar to other CNS depressant drugs. Following 4 days of continuous exposure to TCA (500–2,000 ppm), cessation of TCA exposure caused a withdrawal syndrome characterized by increased susceptibility to seizures.[33] Reexposure to TCA attenuated the severity of these seizures, as did exposure to toluene or ethanol. However, there are inadequate data in humans to conclude that an abstinence syndrome occurs in volatile substance abusers using TCA.

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantitation of trichloroethane and metabolites in biologic samples include headspace gas chromatography,[34] capillary gas chromatography with electron capture detection,[35] and dynamic headspace with gas chromatography/mass spectrometry.[36] The coefficients of variation for the latter method were below 3.5%, and the lower limit of quantitation (LLOQ) was 0.0008 mg/L. Other methods for the detection of TCA and trichloroethanol were spectrophotometric methods based on the Fujiwara reaction,[37] but these older methods have low sensitivity and specificity.

Biomarkers

1,1,1-Trichloroethane is detectable in the blood of non-occupationally exposed members of the general US population. In a convenience sample of 982 volunteers from the Third National Health and Nutrition Examination Survey (NHANES III), the median TCA concentration in whole blood samples from this group was 0.00013 mg/L (0.13 ppb) with an upper 95% confidence limit of 0.0008 mg/L (0.80 ppb).[38] Following exposure of volunteers to 600 ppm TCA for 1 hour, the TCA concentration in blood from these volunteers averaged 3–4 mg/L; the blood TCA concentration decreased to <1 mg/L within 25 minutes after cessation of exposure.[39] In blood samples, asymptomatic volunteers had TCA concentrations of 7–10 mg/L following approximately 1-hour exposure to 950 ppm TCA.[40] Thirty minutes after exposure, no TCA was detectable in the blood.

The postmortem trichloroethane concentration must be interpreted cautiously because of a variety of factors including the following: 1) potential losses during the postmortem interval and during collection and storage

of the specimen, 2) the presence of contributing factors (dysrhythmias, aspiration, preexisting disease), and 3) perimortem clearance of TCA during resuscitation. Postmortem analysis of blood samples (source not reported) from a 13-year-old girl who died shortly after inhaling vapors from Liquid Paper™, revealed trichloroethane and trichloroethylene concentrations of 2.0 mg/L and 7.5 mg/L, respectively.[41] The postmortem ethanol concentration was undetectable. Two 17-year-old adolescents were found dead with a history of abusing Liquid Paper™. The postmortem blood trichloroethane concentrations were 7 mg/L and 4 mg/L, whereas the trichloroethylene concentrations were 29 mg/L and 19.6 mg/L, respectively.[42] The trichloroethane concentrations in postmortem blood samples from 2 men found dead following autoerotic activities were 29.5 mg/L and 214 mg/L.[43]

Abnormalities

Serum electrolytes, hepatic aminotransferases, and creatinine are typically normal in patients presenting to the emergency department following the inhalation of vapors from products containing trichloroethane.

TREATMENT

The treatment of exposure to TCA is supportive and similar to the treatment of trichloroethylene exposure. Respiratory depression and dysrhythmias represent the greatest dangers to life following exposure to TCA. The adequacy of oxygenation should be immediately evaluated by pulse oximetry, and if necessary, arterial or venous blood gases. Patients with altered mental status or dyspnea should receive supplemental oxygen. Hypotension should be treated initially with fluid expansion and then vasopressor as needed, especially because TCA exposure may decrease peripheral vascular resistance.[44]

Symptomatic patients should be monitored for the development of dysrhythmias. Although sympathomimetic drugs should be administered with caution, their use in the clinical setting should not be avoided, if indicated. Myocardial irritability occurs at ambient TCA concentrations exceeding 5,000 ppm in animal studies; those concentrations are unlikely to occur in the clinical setting because of the rapid elimination of TCA through the lungs.

Potential complications of exposure to TCA are cardiac dysrhythmias, aspiration pneumonia, mild chemical hepatitis (delayed several days), and hypoxic encephalopathy. Otherwise, most patients recover from acute overexposure to TCA without sequelae. Patients exposed to high concentrations of TCA should receive a chest x-ray, electrocardiogram, and measurement of serum creatinine and hepatic aminotransferases. There are no recognized methods to enhance the elimination of TCA.

References

1. Cosmetic Ingredient Review Expert Panel. Final report on the safety assessment of trichloroethane. Int J Toxicol 2008;27(suppl 4):S107–S138.

2. International Agency for Research on Cancer. IARC monograph on the evaluation of the carcinogenic risk of chemicals to humans. 1,1,1-Trichloroethane. 1979; 20: 515–531.

3. Greer JE. Adolescent abuse of typewriter correction fluid. South Med J 1984;77:297–298, 301.

4. Stewart RD. The toxicology of 1,1,1-trichloroethane. Ann Occup Hyg 1968;11:71–79.

5. Dornette WHL, Jones JP. Clinical experiences with 1,1,1-trichloroethane; a preliminary report of 50 anesthetic administrations. Anesth Analg 1960;39:249–253.

6. Nolan RJ, Freshour NL, Rick DL, McCarty LP, Saunders JH. Kinetics and metabolism of inhaled methyl chloroform (1,1,1-trichloroethane) in male volunteers. Fundam Appl Toxicol 1984;4:654–662.

7. Humbert BE, Fernandez JG. Exposition on 1,1,1-trichloroethane; contribution a' l' e'tude de l'absorption, de l'excretion et du metabolisme pur des sujets humains. Arch Mal Prof 1977;38:415–425.

8. Poet TS, Thrall KD, Corley RA, Hui X, Edwards JA, Weitz KK, et al. Utility of real time breath analysis and physiologically based pharmacokinetic modeling to determine the percutaneous absorption of methyl chloroform in rats and humans. Toxicol Sci 2000;54:42–51.

9. International Programme on Chemical Safety. Environmental Health Criteria 136 1,1,1-trichloroethane. Geneva, World Health Organization, 1992.

10. Morgan A, Black A, Belcher DR. The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann Occup Hyg 1970;13: 219–233.

11. Kjellstrand P, Månsson L, Holmquist B, Jonsson I. Tolerance during inhalation of organic solvents. Pharmacol Toxicol 1990;66:409–414.

12. Kaneko T, Wang PY, Sato A. Enzymes induced by ethanol differently affect the pharmacokinetics of trichloroethylene and 1,1,1-trichloroethane. Occup Environ Med 1994; 51:113–119.

13. Traiger GJ, Plaa GL. Chlorinated hydrocarbon toxicity. Potentiation by isopropyl alcohol and acetone. Arch Environ Health 1974;28:276–278.

14. Cornish HH, Adefuin J. Ethanol potentiation of halogenated aliphatic solvent toxicity. Am Ind Hyg Assoc J 1966; 27:57–61.

15. MacIver MB. Abused inhalants enhance GABA-mediated synaptic inhibition. Neuropsychopharmacology 2009;34: 2296–2304.

16. Beckstead MJ, Weiner JL, Eger EI 2nd, Gong DH, Mihic SJ. Glycine and gamma-aminobutyric acid(A) receptor function is enhanced by inhaled drugs of abuse. Mol Pharmacol 2000;57:1199–1205.

17. Lopreato GF, Phelan R, Borghese CM, Beckstead MJ, Mihic SJ. Inhaled drugs of abuse enhance serotonin-3 receptor function. Drug Alcohol Depend 2003;70:11–15.

18. Shelton KL. Pharmacological characterization of the discriminative stimulus of inhaled 1,1,1-trichloroethane. J Pharmacol Exp Ther 2010;333:612–620.

19. Shelton KL. Discriminative stimulus effects of inhaled 1,1,1-trichloroethane in mice: comparison to other hydrocarbon vapors and volatile anesthetics. Psychopharmacology (Berl) 2009;203:431–440.

20. Bowen SE, Balster RL. Effects of inhaled 1,1,1-trichloroethane on locomotor activity in mice. Neurotoxicol Teratol 1996;18:77–81.

21. Hall FB, Hine CH. Trichloroethane intoxication: a report to two cases. J Forensic Sci 1966;11:404–413.

22. Fosseus CG. Danger of inhaling trichloro-ethane. S Afr Med J 1991;80:629–630.

23. Banathy LJ, Chan LT. Fatality caused by inhalation of "liquid paper" correction fluid. Med J Aust 1983;2:606.

24. Winek CL, Wahba WW, Huston R, Rozin L. Fatal inhalation of 1,1,1-trichloroethane. Forensic Sci Int 1997;87: 161–165.

25. D'Costa DF, Gunasekera NP. Fatal cerebral oedema following trichloroethane abuse. J Roy Soc Med 1990;83: 533–534.

26. Levy AB. Delirium induced by inhalation of typewriter correction fluid. Psychosomatics 1986;27:665–666.

27. Gerace RV. Near-fatal intoxication by 1,1,1-trichloroethane. Ann Emerg Med 1981;10:533–534.

28. Macdougall IC, Isles C, Oliver JS, Clark JC, Spilg WG. Fatal outcome following inhalation of Tipp-Ex. Scot Med J 1987;32:55.

29. Troutman WG. Additional deaths associated with the intentional inhalation of typewriter correction fluid. Vet Hum Toxicol 1988;30:130–132.

30. Ranson DL, Berry PJ. Death associated with the abuse of typewriter correction fluid. Med Sci Law 1986;26: 308–310.

31. Nee PA, Llewellyn T, Pritty PE. Successful out-of-hospital defibrillation for ventricular fibrillation complicating solvent abuse. Arch Emerg Med 1990;7:220–223.

32. Wodka RM, Jeong EW. Myocardial injury following the intentional inhalation of typewriter correction fluid. Mil Med 1991;156:204–205.

33. Evans EB, Balster RL. Inhaled 1,1,1-trichloroethane-produced physical dependence in mice: effects of drugs and vapors on withdrawal. J Pharmacol Exp Ther 1993;264: 726–733.

34. Ramsey JD, Flanagan RJ. Detection and identification of volatile organic compounds in blood by headspace gas chromatography as an aid to the diagnosis of solvent abuse. J Chromatogr 1982;240:423–444.

35. Schmitt TC. Determination of chloral hydrate and its metabolites in blood plasma by capillary gas chromatography with electron capture detection. J Chromatogr B Analyt Technol Biomed Life Sci 2002;780:217–224.

36. Johns DO, Dills RL, Morgan MS. Evaluation of dynamic headspace with gas chromatography/mass spectrometry for the determination of 1,1,1-trichloroethane, trichloroethanol, and trichloroacetic acid in biological samples. J Chromatogr B Analyt Technol Biomed Life Sci 2005;817: 255–261.

37. Ogata M, Takatsuka Y, Tomokuni K. A simple method for the quantitative analysis of urinary trichloroethanol and trichloroacetic acid as an index of trichloroethylene exposure. Br J Ind Med 1970;27:378–381.

38. Churchill JE, Kaye WE, Ashley DL. Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Arch Environ Health 2001;56:157–166.

39. Astrand I, Kilbom H, Wahlberg I, Ovrum P. Methylchloroform exposure. Work Environ Health 1973;10: 69–81.

40. Stewart RD, Gay HH, Erley DS, Hake CL, Schaffer AW. Human exposure to 1,1,1-trichloroethane vapor: relationship of expired air and blood concentrations to exposure and toxicity. Am Ind Hyg Assoc J 1961;22:252–262.

41. King GS, Smialek JE, Troutman WG. Sudden death in adolescents resulting from the inhalation of typewriter correction fluid. JAMA 1985;253:1604–1606.

42. Garriott J, Petty CS. Death from inhalant abuse: toxicological and pathological evaluation of 34 cases. Clin Toxicol 1980;16:305–315.

43. Gowitt GT, Hanzlick RL. Atypical autoerotic deaths. Am J Forensic Med Pathol 1992;13:115–119.

44. Savolainen H, Pfaffli P, Tangen M, Vainio H. Trichloroethylene and 1,1,1-trichloroethane: effects on brain and liver after five days intermittent inhalation. Arch Toxicol 1977;38:229–232.

Chapter 50

TRICHLOROETHYLENE

HISTORY

Fisher synthesized trichloroethylene (TCE) in 1864; this substance has a long history of use as an industrial solvent until recent times when other solvents replaced TCE. Lehman first reported the narcotic properties of TCE in 1911; Jackson first used TCE as an anesthetic in 1933. In the early 1940s, the use of impure TCE in a closed circuit with soda lime for obstetric anesthesia was associated with the development of cranial nerve palsies, primarily trigeminal hypoesthesias, as a result of the formation of a decomposition product (dichloroacetylene).[1] In 1977, the US Food and Drug Administration banned the use of TCE as a food and drug-extraction solvent because of an increased incidence of hepatocellular cancers in rodents gavaged with high TCE doses. Other prohibited uses included the administration of TCE as a general anesthetic, surgical disinfectant, pet food additive, and an extractant of caffeine.

IDENTIFYING CHARACTERISTICS

TCE is a colorless liquid at room temperature with a fruity odor reminiscent of chloroform. The odor threshold in air is approximately 20–80 ppm with clear warning properties present at TCE concentrations above 100 ppm.[2,3] This compound has a sweet, burning taste. TCE is slightly water soluble, but most common organic solvents easily dissolve TCE. Table 50.1 lists the some physical properties of TCE.

The formation of the toxic contaminant, dichloroacetylene, resulted from the use of trichloroethylene (TCE) as an anesthetic in a closed system with alkali absorbers and as a cleaning solvent in submarines and space capsules. Dichloroacetylene may also form as a result of the use of TCE solvents on moist alkaline materials (e.g., concrete).[4] Symptoms of contamination by dichloroacetylene include a sickening sweet sour smell, nasal irritation, anorexia, malaise, vomiting, headache, pruritus, and the development of cold sores.[5] The onset of trigeminal neuralgias and facial herpes has been associated with the formation of dichloroacetylene during trichloroethylene anesthesia.[6,7]

EXPOSURE

Sources

As an excellent solvent for greases, waxes, tars, and oils, TCE has been used primarily as a cleaning and extracting agent in the automotive, metal, and textile processing industries.[8] Consumer products containing TCE include spot removers, paint strippers, typewriter correction fluids, adhesives, and cleaning fluids; however, the use of TCE in these products has been declining. Previously typewriter correction fluid (e.g., Wite-Out™) contained trichloroethane and trichloroethylene at concentrations up to about 50–60% of the product. The malodorous compound, mustard oil (allyl isothiocyanate) was once added to typewriter correction fluid; however, this constituent was later removed along with TCE and trichloroethane.

Methods of Abuse

The abuse of TCE is frequently part of a pattern of polydrug use that includes the abuse of alcohol and other available volatile substances (e.g., ethyl chloride,

TABLE 50.1. Some Physical Properties of Trichloroethene (TCE).

Physical Characteristic	Value
Density	1.47 g/mL (20°C/68°F)
Solubility, Water	1.366 g/L (25°C/77°F)
Boiling Point	87.6°C (189.7°F/760 mm Hg)
Viscosity	0.58 cP (20°C/68°F)
Vapor Pressure	74 mm Hg (25°C/77°F)
	59 mm Hg (20°C/68°F)
Surface Tension	26.4 dyn/cm (20°C/68°F)
Vapor Density	4.54 (Air = 1)
Saturated Air	102,000 ppm TCE (25°C/77°F)
Density, Saturated Air	1.35 (Air = 1)

ether).[9] Addiction to TCE occurs rarely in the workplace following occupational exposure to TCE.[10] These workers may continue to abuse TCE at their home for the soporific and hallucinogenic properties.[11] Methods of abuse include sniffing and huffing as well as the ingestion of TCE.[12] Young abusers pour 20 drops to 15 mL of trichloroethylene on a cloth or handkerchief and inhale the vapors.[13] Most of the addicts describe a pleasant warm feeling, disengagement, relaxation, sleepiness, and euphoria similar to ethanol.

DOSE EFFECT

Human volunteer studies indicate that TCE exposure from 300–500 ppm for >3 hours causes decrements in manual dexterity.[14] At 1,000 ppm TCE, eye and upper respiratory tract irritation appears along with lightheadedness after 6 minutes of exposure. Drowsiness, lethargy, and nausea develop at concentrations of 2,000 ppm TCE after 5 minutes of exposure. Concentrations of 5,000 ppm TCE produce light anesthesia, while concentrations of 20,000 ppm TCE cause much deeper anesthesia in the clinical setting. The minimum alveolar concentration (MAC) of TCE necessary for the first plane of surgical anesthesia is 0.17%. Case reports indicate that chronic abusers of trichloroethylene may consume 120–180 mL trichloroethylene daily.[9]

TOXICOKINETICS

Absorption

Trichloroethylene is well-absorbed through the lungs and gastrointestinal tract, while dermal absorption appears minimal. The pulmonary retention of TCE following inhalation ranges from approximately 20–70% depending upon airborne concentration, duration of

exposure, and tidal volume. Based on sampling of blood and expired air over 44 hours, 6 healthy volunteers exhaled a mean of approximately 22% of the absorbed dose following exposure to 100 ppm TCE over 4 hours.[15] Although the absolute quantity absorbed increases with increasing ventilation, most absorption of TCE following inhalation occurs over the first few minutes as a result of the relatively high blood/air partition coefficient (i.e., about 10).

Distribution

TCE disappears from the plasma as a result of rapid distribution and metabolism.[16] Because of high lipid solubility (oil/blood partition coefficient of approximately 750), accumulation of TCE occurs in organs containing adipose tissue. Animal studies indicate that body fat, adrenals, ovaries, and blood cellular components accumulate the greatest portion of TCE.[17]

Biotransformation

There are two major pathways of TCE metabolism: cytochrome P450-dependent oxidation and conjugation with glutathione. The former pathway is the higher affinity route, whereas the latter becomes more important following exposure to high TCE concentrations. Metabolism occurs primarily in the liver in the presence of cytochrome P450 isoenzymes (e.g., CYP2E1),[18] although other potential sites of metabolism include the lung and kidney. The mixed function oxidase system converts TCE to an epoxide, which subsequently rearranges to trichloroacetaldehyde and then chloral hydrate as displayed in Figure 50.1. The other potential toxins in this pathway include dichloroacetyl chloride and dichloroacetic acid. The latter metabolite forms in animals primarily following exposure to high TCE doses. TCE can also undergo conjugation with glutathione by the catalytic action of glutathione-*S* transferase to form *S*-(1,2-dichlorovinyl)glutathione, and subsequently to the cysteine conjugate [*S*-(1,2-dichlorovinyl)-L-cysteine, DCVC] by γ-glutamyltransferase (GGT). Potentially, nephrotoxic metabolites form from the generation of reactive metabolites by the catalytic action of cysteine conjugate β-lyase on DCVC.[19] The cysteine conjugate, DCVC, also undergoes detoxification by *N*-acetylation to yield mercapturates. Although TCE metabolism in humans and animals is similar, there are substantial quantitative differences in TCE metabolism as a result of sex-, species-, and strain-dependent differences in various enzymes that metabolism TCE.[20,21] Comparatively, the metabolic rate in humans is slowest with the trichloroethanol/trichloroacetic acid ratio greatest in rabbits and smallest in humans.[22]
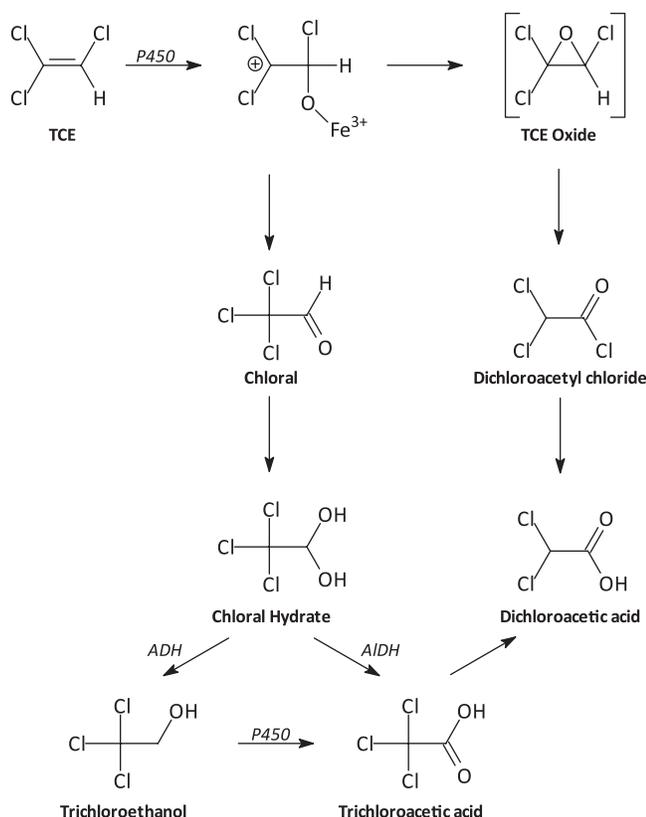
FIGURE 50.1. Oxidative metabolism of trichloroethylene. This chemical also undergoes conjugation with glutathione and subsequent biotransformation to form potentially toxic reactive metabolites.[20]

Elimination

TRICHLOROETHYLENE

Elimination of TCE involves 2 primary processes: 1) relatively rapid metabolic conversion of TCE followed by excretion of metabolites (e.g., trichloroethanol, trichloroacetic acid), and 2) the pulmonary excretion of unchanged TCE. Following exposure to low doses (<100 ppm), the liver metabolizes about 70–90% of an absorbed TCE dose, whereas the lungs excrete 10–20% of this dose unchanged.[16] Only small amounts of TCE appear in the feces.[23]

During acute TCE intoxication, the kinetics of TCE metabolism varies substantially depending on several factors including the adequacy of alveolar air exchange (i.e., pulmonary edema, pneumonitis, hyperventilation), hemodynamic status (hypovolemia, depressed cardiac output), and hepatorenal function.[24] In these situations, traditional pharmacokinetic models do not accurately describe the kinetics of trichloroethanol or trichloroace-

tic acid excretion. Michaelis-Menten kinetics (i.e., linear at low doses and dose-dependent at high doses) is probably the best model for TCE elimination; this model accounts for the differences in the half-life and elimination pattern of metabolites that occur in TCE metabolism between volunteer studies and TCE-intoxicated patients.[25] The mean half-life of TCE in the blood following an exposure sufficient to cause transient unconsciousness in 3 workers was approximately 20 hours with maximum excretion of urinary metabolites occurring 2–3 days after exposure.[26]

TRICHLOROETHANOL AND TRICHLOROACETIC ACID

The excretion of TCE metabolites is substantially longer than the elimination of TCE from the blood. During a TCE overdose, the plasma half-life of trichloroethanol increases as a result of the continued formation of trichloroethanol from TCE stored in adipose tissues. A 58-year-old worker fell into a vat of TCE, and became comatose after accidentally ingesting TCE. The serum TCE concentration increased up to 4 days postexposure; this elimination pattern followed a 2-compartment model with a half-life of 53 hours and 268 hours.[27] The serum half-life of trichloroacetic acid was similar (50 h and 277 h, respectively). The higher protein binding of trichloroacetic acid results in higher blood concentrations and a longer urinary elimination half-life than trichloroethanol. In a case report of a degreaser addicted to TCE, the renal excretion of TCE metabolites followed a 2-compartment model with the following approximate fast and slow urinary elimination half-life, respectively: 1) trichloroethanol, 6 hours and 50 hours; and 2) trichloroacetic acid, 8 hours and 73 hours.[28] Trichloroacetic acid accounted for >90% of the trichlorometabolites. In a worker comatose from TCE intoxication, the urinary half-life of the fast phase for trichloroethanol was about 26 hours compared with 52 hours for trichloroacetic acid; the urinary elimination half-life of these two metabolites for the slow phase were similar (166 h).[27]

Tolerance

There are few data on the development of tolerance in humans following volatile substance abuse of TCE. Tolerance to the effects of TCE develops in some animal studies, depending in part on the exposure scenario.[29] Rats develop tolerance to the acute behavioral effects of TCE on signal detection when they inhale TCE while performing the task. This tolerance depends more upon learning than upon changes in TCE metabolism; the effects probably result from loss of TCE-induced reinforcement.[30]

745

Drug Interactions

The interaction of ethanol and TCE produces the well-known syndrome of degreaser's flush, which is manifest by erythema of the upper torso and face. The acute ingestion of ethanol increases the TCE concentration in blood and expired air, probably by the inhibition of mixed function oxidases.[31] Exposure to other central nervous system (CNS) depressants probably enhances the CNS effects of TCE.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Toxicity

Trichloroethylene is a CNS depressant that produces anesthesia in high concentrations. Like other volatile solvents, TCE presumably causes acute CNS depression by changing membrane fluidity, and thereby altering neural transmission. Increased vagal tone during light anesthesia may cause bradycardia, nodal rhythms, or low-grade AV block. Increased myocardial contractility and ventricular dysrhythmias are associated with deeper planes of trichloroethylene-induced anesthesia.[32]

Acute centrilobular necrosis and hepatic failure may occur following chronic abuse of substances with high trichloroethylene concentrations (e.g., Carbona—44% trichloroethylene, 56% petroleum distillates).[33] Liver biopsies of these cases demonstrate acute centrilobular necrosis superimposed on chronic fibrosis.

Postmortem Examination

The postmortem examinations of individuals dying during volatile substance abuse including TCE are nonspecific (e.g., visceral congestion). A 16-year-old adolescent was last seen sniffing plastic cement; he was found dead with a plastic bag and nearly empty tube of glue beside his body.[34] The postmortem examination revealed marked pulmonary edema, severe passive congestion of the liver and spleen, cerebral edema with some herniation of unci and cerebellar tonsils, and a completely obstructed airway with aspirated gastric contents. Analysis of a steam distillate of blood and brain by gas liquid chromatography demonstrated TCE, but this compound was not a known constituent of the plastic cement. A case report documented the development of a fatal congestive cardiomyopathy and hepatorenal failure in a 24-year-old man with a 5-year history of inhaling vapors from shoe-cleaning solvent (trichloroethylene 67%, methylene chloride 18%, dipropylene glycol 10%).[35] Postmortem examination demonstrated

hypertrophied myocardial fibers, focally thickened endocardium, and thickening of the left ventricle. Although the authors attributed the cardiomyopathy to TCE abuse, the causal role of trichloroethylene is unclear because of the presence of confounding factors and the absence of laboratory confirmation of drugs of abuse.

CLINICAL RESPONSE

Illicit Use

Acute trichloroethylene intoxication causes fatigue, visual disturbances, lightheadedness, headache, slurred speech, disorientation, stupor, and altered consciousness. Recovery from the CNS depressant effects of trichloroethylene is rapid in the absence of hypoxia. A 14-year-old adolescent was found unconscious with a bottle of typewriter correction fluid in her hand.[36] Initially, she was responsive only to deep pain, but she was only slightly drowsy on arrival to the emergency department. After 10 minutes of observation, she was fully alert.

Adverse effects associated with TCE abuse include headache, nausea, vomiting, confusion, amnesia, memory disturbances, emotional lability, personality changes, delirium, insomnia, tremor, and unpleasant hallucinations.[9,37] Case reports associate chronic abuse of substances containing high TCE concentrations with hepatic failure.[33] Following cessation of use, hepatic dysfunction usually resolves without sequelae. Cranial nerve palsies may develop following exposure to TCE contaminated with the decomposition product, dichloroacetylene, as a result of demyelination of the sensory trigeminal nucleus. A 35-year-old heroin addict complained of facial numbness, diplopia, and difficulty walking after inhaling TCE continuously for 3 days to alleviate withdrawal symptoms.[38] By the sixth day of hospitalization, all symptoms resolved with the exception of facial paraesthesias and some ataxia. A case report associated the chronic abuse of plastic modeling glue (main ingredient, TCE) with a right middle cerebral artery occlusion, dense hemiparesis, and seizures.[39] Although trichloroethylene was the main ingredient of the glue, the causal role of trichloroethylene in the development of the cerebral infarct is unclear.

Fatalities

Case reports associate sudden death with trichloroethylene abuse.[40,41] Typically, these case reports involve the abuse of typewriter correction fluid, which contains both TCE and trichloroethane.[42,43] Often, these individuals become agitated and exercise (e.g., run) strenu-

ously prior to their death.[44] Although many of the cases involve asphyxia and aspiration of stomach contents, some reports document the presence of ventricular fibrillation. A 12-year-old girl collapsed shortly after inhaling the vapors from a container of typewriter correction fluid thinner containing trichloroethane.[45] A nearby physician initiated cardiopulmonary resuscitation immediately, and an ambulance arrived within 2 minutes. The initial rhythm was coarse ventricular fibrillation, which converted to normal sinus rhythm with a single 200 J shock.

Abstinence Syndrome

There are few data on withdrawal symptoms following chronic abuse of trichloroethylene. A 29-year-old woman was admitted to the hospital for trichloroethylene addiction, somnolence, and hallucination.[11] Most of her symptoms resolved by the third hospital day, but she then developed delirium and bizarre behavior (incoherent, refusal to dress and eat). These psychotic features persisted about 5 days, and her mental status returned to normal within 1 week after resolution of the delirium.

DIAGNOSTIC TESTING

Analytic Methods

Techniques to quantitate TCE and the main metabolites in biologic samples include headspace gas chromatography,[46,47] headspace gas chromatography with electrochemical detection,[48] gas chromatography/mass spectrometry (GC/MS),[49] and headspace solid-phase microextraction with GC/MS.[50] The lower limit of quantitation (LLOQ) for GC/MS is ~0.005 mg/L, whereas the LLOQ for TCE in blood following analysis with GC/MS using headspace solid-phase microextraction was 0.00025 mg/L. Gas chromatography with flame ionization or electron capture detection are the most common analytic methods for the separation of TCE from biologic matrices because of the volatility of TCE. Analysis of TCE metabolites (chloral hydrate, dichloroacetic acid, trichloroacetic acid) typically involves gas chromatography and high performance liquid chromatography (HPLC); ultraviolet detectors are frequently used with HPLC or capillary electrophoresis.[51] Extraction techniques for removing TCE and metabolites from a matrix include liquid-liquid extraction, solid-phase microextraction, protein precipitation, and solid-phase extraction depending on the specific requirements of the extraction. The TCE concentration in whole blood exceeds the plasma TCE concentration. In blood samples from 10 fasting patients scheduled for TCE anesthesia, the plasma/whole blood ratio of TCE averaged approximately 0.76 (range, 0.72–0.81).[52]

Biomarkers

BLOOD

Trichloroethylene is a contaminant of our environment. In Germany, where the estimated mean daily TCE intake ranges from 32–51 μg; 31% of whole blood samples drawn from 39 healthy volunteers demonstrated detectable levels of TCE.[53] None of the volunteers were occupationally exposed to TCE; the TCE concentration in their blood samples ranged from <0.0001–0.0013 mg/L (median <0.0001 mg/L). In a convenience sample of 982 volunteers from the Third National Health and Nutrition Examination Survey (NHANES III), the majority of the whole blood samples contained TCE concentration below the LOD (0.00001 mg/L, 0.010 ppb) with a 95th percentile of 0.000021 mg/L (0.021 ppb).[54] Admission blood from a 35-year-old heroin addict contained 119 mg trichloroethanol/L (no trichloroethylene measurement) after inhaling trichloroethylene continuously for 3 days to alleviate withdrawal symptoms.[38] Clinical effects included facial numbness, diplopia, and difficulty walking. The plasma TCE concentration ranged from 0.2–1 mg/L over the first 5 days in a case series of 4 patients with serious TCE intoxication.[55] These concentrations did not correlate well to symptoms or with the appearance of dysrhythmias.[24] Serum TCE concentrations exceeding 1.5 mg/L are usually associated with coma. Eight hours after admission for TCE intoxication, the serum TCE concentration in a deeply comatose 58-year-old man was 31.4 mg/L.[27] Two hours after exposure to TCE vapors at work, the serum from a 54-year-old man contained 9 mg TCE/L.[56] At that time, his Glasgow Coma Scale score was 7 and his vital signs were stable; he recovered without sequelae.

POSTMORTEM

The postmortem TCE concentration must be interpreted cautiously because of potential losses during the postmortem interval, collection of the specimen, and storage along with redistribution from adipose stores. Most case reports of sudden death in adolescents abusing typewriter correction fluid document the presence of both TCE and trichloroethane. Two 17-year-old adolescents were found dead with a history of abusing Liquid Paper™. The TCE concentrations in postmortem blood (site not specified) were 29 mg/L and 19.6 mg/L compared with trichloroethane concentrations of 7 mg/L and 4 mg/L.[57] A 42-year-old

construction worker was found dead at the bottom of a well that had been painted with TCE.[58] Postmortem examination confirmed the presence of asphyxia (Tardieu spots lungs and heart), and subclavian and femoral blood contained TCE concentrations of 84 mg/L and 40 mg/L, respectively. A 28-year-old man was found dead on the floor of his home with a history of glue sniffing and an empty container of commercial contact cement (95–97% trichloroethylene). Initial analysis of cardiac blood demonstrated 80 mg trichloroethanol/L.[59] Subsequent analysis of cardiac blood after 4 months of storage at 4–6°C (~39–43°F) revealed 1.1 mg TCE/L as measured by headspace gas chromatography with flame ionization detection.

Abnormalities

Conduction abnormalities are uncommon during trichloroethylene intoxication.[60] A case report associated the development of Mobitz type I A-V block with trichloroethylene intoxication secondary to volatile substance abuse.[61] Sinus rhythm returned during abstinence in the hospital. Abnormalities associated with the trichloroethylene abuse include hyperbilirubinemia, elevated serum hepatic aminotransferases, and elevated prothrombin time.[62] In patients with cranial nerve palsies and facial hypoesthesias secondary to the formation of dichloroacetylene, somatosensory evoked potentials may demonstrate impairment of the trigeminal nerve.[63]

TREATMENT

Most mild TCE intoxications respond to removal from the source of exposure and supportive care. Immediate threats to life are respiratory depression and cardiac dysrhythmias. Oxygen, intubation, intravenous lines, and cardiac monitoring should be initiated as dictated by the clinical situation. Patients who demonstrate serious toxicity should be monitored for at least 24 hours because of the potential to develop serious dysrhythmias. Profuse diarrhea may exacerbate electrolyte imbalance and predispose the patient to dysrhythmias. Consequently, generous intravenous fluids should be given to symptomatic patients and the serum electrolytes should be monitored at least daily in these patients. Liver function tests and serum creatinine should be obtained to monitor hepatorenal damage. There are inadequate clinical data regarding the use of antidysrhythmic drugs for serious dysrhythmias to recommend specific drugs other than the usual antidysrhythmic drugs (amiodarone, lidocaine).

Hypotension is unusual following the removal of the victim from exposure; the presence of hypotension necessitates an evaluation of the patient for other causes (trauma, volume depletion). Cautions against the use of sympathomimetic drugs for TCE intoxication are based on animal studies rather than therapeutic experience. Therefore, the use of sympathomimetic drugs in the clinical setting with cardiac monitoring should be based on clinical judgment. No data are available on the enhancement of elimination of TCE or associated metabolites. Sequelae are unusual following acute exposure and the patient should recover fully unless hypoxic damage occurred.

References

1. McClelland M. Some toxic effects following trilene decomposition products. Proc Roy Soc Med 1944;37:526–528.
2. May J. Odor thresholds of solvents for assessment of solvent odor in the air. Staub-Rein 1966;29:34–38.
3. Amoore JE, Hantola E. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 1983;3:272–290.
4. Greim H, Wolff T, Höfler M, Lahaniatis E. Formation of dichloroacetylene from trichloroethylene in the presence of alkaline material. Possible cause of intoxication after abundant use of chloroethylene-containing solvents. Arch Toxicol 1984;56:74–77.
5. Saunders RA. A new hazard in closed environmental atmospheres. Arch Environ Health 1967;14:380–384.
6. Boulton TB, Sweet RB. The place of trichloroethylene in modern anesthesia. J. Michigan State Med Soc 1960;59:270–273.
7. Humphrey JH, McClelland M. Cranial-nerve palsies with herpes following general anaesthesia. A report from the Central Middlesex County Hospital. Br Med J 1944;1:315–318.
8. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Trichloroethylene. Atlanta, GA: US Department of Health and Human Services, Public Health Services; 1997.
9. O'Connor WA. A case of trichloroethylene addiction. Br Med J 1954;2:451–452.
10. James WR. Fatal addiction to trichloroethylene. Br J Ind Med 1963;20:47–49.
11. Harenko A. Two peculiar instances of psychotic disturbance in trichloroethylene poisoning. Acta Neurol Scand 1967;43(suppl 31):S139–S140.
12. Wells JC. Abuse of trichloroethylene by oral self-administration. Anaesthesia 1982;37:440–441.
13. Alapin B. Trichloroethylene addiction and its effects. Br J Addict 1973;68:331–335.
14. Stopps GJ, McLaughlin M. Psychophysiological testing of human subjects exposed to solvent vapors. Am Ind Hyg Assoc J 1967;28:43–50.

15. Pleil JD, Fisher JW, Lindstrom AB. Trichloroethene levels in human blood and exhaled breath from controlled inhalation exposure. Environ Health Perspect 1998;106: 573–580.

16. Monster AC. Difference in uptake, elimination and metabolism in exposure to trichloroethylene, 1,1,1-trichloroethane and tetrachloroethylene. Int Arch Occup Environ Health 1979;42:311–317.

17. Waters EM, Gerstner HB, Huff JE. Trichloroethylene. 1. An overview. J Toxicol Environ Health 1977;2:671–707.

18. Ramdhan DH, Kamijima M, Yamada N, Ito Y, Yanagiba Y, Nakamura D, et al. Molecular mechanism of trichloroethylene-induced hepatotoxicity mediated by CYP2E1. Toxicol Appl Pharmacol 2008;231:300–307.

19. Kenna JG, Jones RM. The organ toxicity of inhaled anesthetics. Anesth Analg 1995;81:551–566.

20. Forkert PG, Baldwin RM, Millen B, Lash LH, Putt DA, Shultz MA, Collins KS. Pulmonary bioactivation of trichloroethylene to chloral hydrate: relative contributions of CYP2E1, CYP2F, and CYP2B1. Drug Metab Dispos 2005;33:1429–1437.

21. Lash LH, Fisher JW, Lipscomb JC, Parker JC. Metabolism of trichloroethylene. Environ Health Perspect 2000; 108(suppl 2):S177–S200.

22. Kimbrough RD, Mitchell FL, Houk VN. Trichloroethylene: an update. J Toxicol Environ Health 1985;15:369–383.

23. Ahlmark A, Forssman S. The effect of trichloroethylene on the organism. Acta Physiol Scand 1951;22:328–339.

24. Thomas G, Baud FJ, Galliot M, Bismuth C. Clinical and kinetic study of 4 cases of acute trichloroethylene intoxication. Vet Hum Toxicol 1987;29(suppl 2):S97–S99.

25. Davidson IWF, Beliles RP. Consideration of the target organ toxicity of trichloroethylene in terms of metabolite toxicity and pharmacokinetics. Drug Metabolism Rev 1991;23:493–599.

26. Kostrzewski P, Jakubowski M, Kolacinski Z. Kinetics of trichloroethylene elimination from venous blood after acute inhalation poisoning. Clin Toxicol 1993;31:353–363.

27. Yoshida M, Fukabori S, Hara K, Yuasa H, Nakaaki K, Yamamura Y, Yoshida K. Concentrations of trichloroethylene and its metabolites in blood and urine after acute poisoning by ingestion. Hum Exp Toxicol 1996;15: 254–258.

28. Ikeda M, Otsuji H, Kawai H, Kuniyoshi M. Excretion kinetics of urinary metabolites in a patient addicted to trichloroethylene. Br J Ind Med 1971;28:203–206.

29. Kjellstrand P, Månsson L, Holmquist B, Jonsson I. Tolerance during inhalation of organic solvents. Pharmacol Toxicol 1990;66:409–414.

30. Oshiro WM, Krantz QT, Bushnell PJ. Characterizing tolerance to trichloroethylene (TCE): effects of repeated inhalation of TCE on performance of a signal detection task in rats. Neurotoxicol Teratol 2001;23:617–628.

31. Muller G, Spassovski M, Hanschler D. Metabolism of trichloroethylene in man. III. Interaction of trichloroethylene and ethanol. Arch Toxicol 1975;33:173–189.

32. Edwards G, Morton HJ, Pask EA, Wylie WD. Deaths associated with anaesthesia; a report on 1,000 cases. Anaesthesia 1956;11:194–220.

33. Baerg RD, Kimberg DV. Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers." Ann Intern Med 1970;73:713–720.

34. Musclow CE, Awen DF. Glue sniffing: report of a fatal case. CMAJ 1971;104:315–319.

35. Mee AS, Wright PL. Congestive (dilated) cardiomyopathy in association with solvent abuse. J Roy Soc Med 1980;73: 671–672.

36. Pointer J. Typewriter correction fluid inhalation: a new substance of abuse. J Toxicol Clin Toxicol 1982;19: 493–499.

37. Miller PW, Mycyk MB, Leikin JB, Ruland SD. An unusual presentation of inhalant abuse with dissociative amnesia. Vet Hum Toxicol 2002;44:17–19.

38. Szlatenyi CS, Wang RY. Encephalopathy and cranial nerve palsies caused by intentional trichloroethylene inhalation. Am J Emerg Med 1996;14:464–466.

39. Parker MJ, Tarlow MJ, Anderson JM. Glue sniffing and cerebral infarction. Arch Dis Child 1984;59:675–677.

40. Michaux P, Delevay-Le Gueut M. Three cases of death in trichloroethylene addicts. Acta Med Leg Soc (Liege) 1980;30:89–94.

41. Kugelberg J, Skold G. [Intoxication with trichloroethylene—sniffing ending with death in spite of intensive care]. Lakartidningen 1969;66:5332–5335. [Swedish]

42. Macdougall IC, Isles C, Oliver JS, Clark JC, Spilg WG. Fatal outcome following inhalation of Tipp-Ex. Scot Med J 1987;32:55.

43. Troutman WG. Additional deaths associated with the intentional inhalation of typewriter correction fluid. Vet Hum Toxicol 1988;30:130–132.

44. Ranson DL, Berry PJ. Death associated with the abuse of typewriter correction fluid. Med Sci Law 1986;26: 308–310.

45. Nee PA, Llewellyn T, Pritty PE. Successful out-of-hospital defibrillation for ventricular fibrillation complicating solvent abuse. Arch Emerg Med 1990;7:220–223.

46. Muralidhara S, Bruckner JV. Simple method for rapid measurement of trichloroethylene and its major metabolites in biological samples. J Chromatogr B Biomed Sci Appl 1999;732:145–153.

47. Ramsey JD, Flanagan RJ. Detection and identification of volatile organic compounds in blood by headspace gas chromatography as an aid to the diagnosis of solvent abuse. J Chromatogr 1982;240:423–444.

48. Chen XM, Dallas CE, Muralidhara S, Srivatsan V, Bruckner JV. Analyses of volatile C2 haloethanes and haloethenes in tissues: sample preparation and extraction. J Chromatogr 1993;612:199–208.

49. Brown SD, Muralidhara S, Bruckner JV, Bartlett MG. Trace level determination of trichloroethylene from liver, lung and kidney tissues by gas chromatography-magnetic

sector mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003;783:319–325.

50. Liu Y, Muralidhara S, Bruckner JV, Bartlett MG. Determination of trichloroethylene in biological samples by headspace solid-phase microextraction gas chromatography/mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2008;863:26–35.

51. Delinsky AD, Bruckner JV, Bartlett MG. A review of analytical methods for the determination of trichloroethylene and its major metabolites chloral hydrate, trichloroacetic acid and dichloroacetic acid. Biomed Chromatogr 2005;19:617–639.

52. Davies DD, Douglas RB, Ling S. Distribution of trichloroethylene between plasma and erythrocytes in whole blood from fasting subjects. Br J Anaesth 1986;58:1440–1442.

53. Hajimiragha H, Ewers U, Janssen-Rosseck R, Brockhaus A. Human exposure to volatile halogenated hydrocarbons from the general environment. Int Arch Occup Environ Health 1986;58:141–150.

54. Churchill JE, Ashley DL, Kaye WE. Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Arch Environ Health 2001;56:147–166.

55. Perbellini L, Olivato D, Zedde A, Miglioranzi R. Acute trichloroethylene poisoning by ingestion: clinical and pharmacokinetic aspects. Intensive Care Med 1991;17:234–235.

56. Carrieri M, Magosso D, Piccoli P, Zanetti E, Trevisan A, Bartolucci GB. Acute, nonfatal intoxication with trichloroethylene. Arch Toxicol 2007;81:529–532.

57. Garriott J, Petty CS. Death from inhalant abuse: toxicological and pathological evaluation of 34 cases. Clin Toxicol 1980;16:305–315.

58. Coopman VA, Cordonnier JA, De Leter EA, Piette MH. Tissue distribution of trichloroethylene in a case of accidental acute intoxication by inhalation. Forensic Sci Int 2003;134:115–119.

59. Jones GR, Singer PP. An unusual trichloroethanol fatality attributed to sniffing trichloroethylene. J Anal Toxicol 2008;32:183–186.

60. Gaultier M, Efthymiou ML, Efthymiou T, Pebay-Peyroula P. [Cardiac manifestations of trichloroethylene poisoning]. Ann Cardiol Angeiol (Paris) 1971;20:185–190. [French]

61. Hantson Ph, Vandeplas O, Dive A, Mahieu P. Trichloroethylene and cardiac toxicity: report of two consecutive cases. Acta Clin Belg 1990;45:34–35.

62. Litt IF, Cohen MI. "Danger . . . vapor harmful": spot-remover sniffing. N Engl J Med 1969;281:543–544.

63. Barret L, Garrel S, Danel V, Debru JL. Chronic trichloroethylene intoxication: a new approach by trigeminal-evoked potentials? Arch Environ Health 1987;42:297–302.

D   Nitrogen Compounds

Chapter 51

AMYL and BUTYL NITRITES

HISTORY

The discovery of the vasodilatory properties of amyl nitrite occurred in 1859 when Guthrie described facial and cervical flushing after the inhalation of amyl nitrite. In 1867, Sir Thomas Lauder Bruton used amyl nitrite to treatment chest pain associated with angina pectoris.[1] He also investigated the properties of butyl nitrite in the 1880s. Although amyl and butyl nitrite had similar properties, butyl nitrite was not used clinically.[2] Generally, amyl nitrite was considered a safe drug, initially marketed as a prescription drug in 1937. In 1960, the US Food and Drug Administration (FDA) removed amyl nitrite from the list of medications requiring a prescription. However, during the 1960s, reports of the abuse of nitrites by apparently healthy young men appeared, and the FDA reinstated the requirement for a prescription of amyl nitrite in 1969. During the 1970s, amyl and butyl nitrite became a popular aphrodisiac, particularly among homosexual males.[3] Butyl nitrites are not classified as drugs by the FDA.

IDENTIFYING CHARACTERISTICS

Nitrites are highly unstable in aqueous solutions and biologic fluids as a result of volatility and hydrolysis to alcohols and nitrite ions. Alkyl nitrites (amyl, butyl) are esters of nitrous acid (HNO_2), whereas organic nitrates (e.g., nitroglycerin) are esters of nitric acid (HNO_3). The alkyl nitrites are highly flammable; fires may result from the synthesis and use of these compounds. Amyl nitrite (CAS RN: 110-46-3, $C_5H_{11}NO_2$) is a yellow liquid with a boiling point of 99.2°C (210.6°F). This compound also occurs as a mixture of isomers (CAS RN: 8017-89-8).

Figure 51.1 displays the chemical structure of amyl nitrite. There are four isomers of butyl nitrite: isobutyl, *n*-butyl, *sec*-butyl, and *tert*-butyl. *n*-Butyl nitrite (CAS RN: 544-16-1, $C_4H_9NO_2$) is a liquid with a boiling point of 78°C (172°F) and a vapor pressure of 81.3 mm Hg at 25°C (77°F). The water solubility of this compound is 1,120 mg/L at 25°C (77°F). Isobutyl nitrite (CAS RN: 542-56-3) is an isomer of *n*-butyl nitrite. These compounds are rarely available as pure substances in commercial preparations.[4] Figure 51.2 displays the chemical structure of *n*-butyl nitrite.

The rather unpleasant smell of *n*-butyl nitrite resembles a men's locker room (i.e., sweaty socks). Hence, one of the common names (Locker Room) for butyl nitrite reflects this smell. Other popular terms for butyl nitrite include Aroma of Men, Ban Apple Gas, Bang, Bolt, Bullet, Climax, Cum, Discorama, Hardware, Heart On, Jac Aroma, Lightning Bolt, Locker Room, Rush, Satan's Scent, Thrust, and Toilet Water. "Poppers" is a popular term for amyl nitrite; this term derives from the sound heard during the crushing of the pearls (glass ampules enclosed in mesh) that contain amyl nitrite. Amyl nitrite is available in pharmaceutic grade; therefore, this compound is relatively pure. Isobutyl and butyl nitrite are commercial preparations that contain a variety of other substances depending on the commercial use.

EXPOSURE

Epidemiology

Nitrites are the primary inhalant used by adults, particularly homosexual men and polysubstance drug abusers. In a study of drug abusers in the Baltimore–Washington,
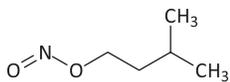
FIGURE 51.1. Chemical structure of amyl nitrite.
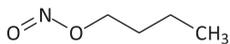
FIGURE 51.2. Chemical structure of *n*-butyl nitrite.

DC metropolitan area, the prevalence of lifetime nitrite inhalant use was approximately 11% for recreational drug users and 22% for heavy drug abusers. The mean age of first use was 25.6 years compared with 14.6 years for glue and 17.6 years for marijuana. Sixty-nine percent of a sample of homosexuals in this area used nitrite inhalants at least once. In a study of self-reported drug use by American adolescents aged 12–17 years, the prevalence of nitrite inhalant lifetime use (i.e., at least once in their life) was 1.5%.[5] The prevalence of lifetime nitrite use is higher in incarcerated youth. In a study of 723 Missouri youth confined to residential care for antisocial behavior, the lifetime prevalence of nitrite inhalant use was 1.7% (boys, 1.3%; girls, 4.3%) as determined by face-to-face interviews.[6] As a result of the AIDS (acquired immunodeficiency syndrome) epidemic, nitrite abuse has decreased among homosexual males, but the prevalence of nitrite abuse among recreational drug users remains relatively stable.[7]

Sources

Medical uses for organic nitrites include smooth muscle relaxation during difficult preterm cesarean deliveries, coronary vasodilator prior to thallium scanning, afterload reduction during heart failure, diagnostic aid for the detection of heart murmurs, and an initial antidote for the treatment of cyanide poisoning. Although the use of amyl nitrite requires a prescription in the United States, butyl and isobutyl nitrite are not regulated by the FDA because these compounds do not fit the definition of a food or a drug.

Methods of Abuse

Inhaled nitrites are used to enhance the sexual experience in homosexual men, particularly in men with a history of drug abuse and risky sexual behavior.[8] Purported effects attributed to the use of inhaled nitrites include relaxation of rectal smooth muscle and anal sphincter tone, prolongation of penile erection, prevention of premature ejaculation, and general intensifica-

tion or prolongation of the sexual experience.[7] Both sexes use inhaled nitrites reportedly to induce euphoria in social settings to enhance meditation, stimulate appreciation of music, promote dancing, and expand creativity.[9]

DOSE EFFECT

Following inhalation of nitrites, the desired effects begin within seconds and diminish rapidly within 5 minutes after inhalation ceases. Consequently, nitrites are inhaled as many as 20 or more times over several hours. Most serious cases of nitrite-induced methemoglobinemia result from the ingestion rather than inhalation of nitrites.[10]

TOXICOKINETICS

Absorption

Rodent studies indicate that the absorption of nitrites from air is rapid. In rats exposed to isobutyl nitrite up to 900 ppm for 45 minutes, the average bioavailability was 43%.[11] Following exposure to 3,855 ppm *n*-butyl nitrite for 5 minutes, pulmonary absorption of *n*-butyl nitrite during this period was 44% of the dose.[12] Volunteer studies indicate the bioavailability of sodium nitrite in solution is high (95–98%) after ingestion.[13]

Biotransformation/Elimination

Isobutyl nitrite degrades rapidly in biologic fluids as a result of enzymatic degradation and chemical hydrolysis. *In vitro* and *in vivo* studies indicate that the biotransformation of butyl nitrite in whole blood produces butanol, nitrate, and methemoglobin.[14] These studies also indicate that the nitrite distributes rapidly into total body water. In acidic water, butyl nitrite forms butanol, nitrite, and nitrate. The clearance of isobutyl nitrite is rapid following inhalation. In rats exposed to isobutyl nitrite up to 900 ppm for 45 minutes, the blood clearance was 2.9 L/min/kg (i.e., >>cardiac output) and a half-life of 1.4 minutes.[11] An *in vitro* study of isobutyl nitrite indicated that the average elimination half-life of this compound from blood is about 1 minute.[15]

Drug Interactions

In club scenes, amyl and other nitrites are used in conjunction with phosphodiesterase-5 (PDE-5) inhibitors (e.g., sildenafil).[16] Although there are few clinical data, the concomitant use of nitrites and PDE-5 inhibitors may potentially cause serious hypotension.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Besides carrying oxygen to the tissues, hemoglobin regulates vascular tone and inflammation via a redox coupling with methemoglobin. In hemoglobin, iron exists in the reduced ferrous state (Fe^{2+}), whereas methemoglobin contains iron in the oxidized ferric state (Fe^{3+}). Nitrite (NO_2) is reduced by deoxyhemoglobin to form methemoglobin and nitric oxide (NO). Nitric oxide is a gas produced by the action of nitric oxide synthetase in the endothelial cells. This compound controls systemic and coronary artery vascular tone by acting locally as an endothelial relaxing factor. The degree of oxidation of hemoglobin reflects the oxygen saturation in the blood and modulates vascular blood flow by producing nitric oxide from nitrite.[17] The action of nitrites results primarily from the formation of nitric oxide (NO). Inhalation of nitrites produces vasodilation and relaxation of smooth muscles as well as vasodilation of cerebral arteries. Although nitrites increase cerebral blood flow, neuroimaging studies of healthy volunteers receiving amyl nitrite do not demonstrate regional differences in cerebral blood flow.[18]

Mechanism of Toxicity

The binding of nitrite to oxyhemoglobin displaces oxygen; this reaction yields methemoglobin, hydrogen peroxide, and nitrogen dioxide in a free radical chain initiation step.[19] Nitrogen dioxide oxidizes ferrous hemoglobin to methemoglobin, whereas hydrogen peroxide oxidizes methemoglobin to a radical (ferryl hemoglobin). Nitrite also reacts with the ferryl hemoglobin radical to produce methemoglobin and nitrogen dioxide. Methemoglobin binds oxygen poorly; thus, oxygen delivery to the tissues decreases. Hydrolysis of alkyl nitrites may occur on contact with epithelial surfaces, and the subsequent formation of nitrous acids may cause inflammation and burns of the skin and tracheobronchial tree.

Postmortem Examination

A 34-year-old man was dead on arrival at the emergency department 1 hour after intentionally ingesting sodium nitrite.[20] The autopsy was performed 5 hours after death, and the abnormalities included dark brown ("soy sauce") blood in all organs and muscles along with hyperemia of the gastric rugal folds. The postmortem blood methemoglobin concentration was 90%. Postmortem examination of a 23-year-old man, who died after the ingestion of isobutyl nitrite in a room deodorizer, demonstrated pulmonary hyperemia with intraalveolar hemorrhage, hyperemia of the liver, and mild, diffuse hyperemia of the gastric mucosa.[21] There was no blue discoloration of the tissues, as no methylene blue was administered prior to death. The postmortem blood methemoglobin concentration was 95%.

CLINICAL RESPONSE

Illicit Use

Desired effects of the inhalation of nitrites include increased cutaneous perception, reduced sexual inhibition, heightened sexual arousal, relaxation of the anal sphincter, and prolonged orgasm.[2] Based on questionnaires, the mean duration of the euphoric effects associated with the use of inhaled nitrites is about 1 minute with a range of a few seconds to 15 minutes.[7] Adverse effects include headache, dizziness, palpitations, nausea, vomiting, weakness, incontinence, blurred vision, facial flushing, periorbital pressure, hypotension, and syncope. The inhalation of amyl nitrite may cause a reduction in systolic blood pressure along with a reflex tachycardia. Local irritant reactions may cause dermatitis, sinusitis, or irritation of the upper respiratory tract (tracheobronchitis) with cough, fever, hemoptysis, and dyspnea.[22] Although uncommon, methemoglobinemia is a potential complication of the abuse of nitrites,[23] particularly in patients with deficiencies of nicotinamide adenine dinucleotide reduced (NADH) methemoglobin reductase (NADH diaphorase).[24] A 31-year-old previously healthy man developed dyspnea and syncope after intentionally inhaling amyl nitrite aerosolized from a compressed gas blower.[25] In the emergency department, he was obtunded and hypotensive with a methemoglobin concentration of 52%; he recovered after the administration of methylene blue. A 28-year-old man was brought by ambulance to the emergency department after collapsing after sniffing amyl nitrite.[26] He was hypotensive, tachycardic, and confused with an elevated respiratory rate and navy blue discoloration of his skin. His methemoglobin concentration was 63%; he recovered after the administration of intravenous methylene blue.

Medical Complications

Yellowish, erythematous, crusted skin lesions ("poppers dermatitis") may appear around the nose, upper lips, face, penis, or scrotum.[27] Pain and swelling may occur in the nasal passages simulating sinusitis. The dermatitis typically resolves within 10 days after cessation of use.[28] Other cutaneous lesions related to the abuse of nitrites

include acrocyanosis (painless, symmetrical, gray bluish macules with edema of underlying skin on nose, ears, or dorsum of hand).[29] Acute hemolysis may occur in patients with glucose-6-phosphate dehydrogenase (G6PDase) deficiency following the abuse of amyl or butyl nitrite.[30] Case reports associate reduction in visual acuity and central differential light sensitivity with the abuse of isobutyl nitrite; however, etiology of the oph-thalmic changes is unclear.[31,32] Four patients developed prolonged visual loss as a result of damage to foveal photoreceptors after inhaling isopropyl nitrites.[33] Resolution of the visual loss occurred in several weeks in some of these patients, but 1 patient had persistent visual loss 1 month after exposure.

There is no clear evidence that the use of inhaled nitrites produces immunodeficiency or enhances the risk of acquiring AIDS; however, there are few data from controlled clinical trials to determine any causal link between the use of nitrites and AIDS.[34] In inhala-tion studies of F344 rats and B6C3F1 mice exposed up to 150 ppm 6 hours/day, 5 days/week for 103 weeks, there was an increased incidence of alveolar/bronchiolar adenoma or carcinomas combined in rats and mice.[35] However, the incidence of mononuclear cell leukemia in rats was decreased. In mice, exposure to isobutyl nitrite produces tissue-dependent alteration in the expression of cancer- and angiogenesis-related genes including hepatic mRNA and protein expression of vas-cular endothelial growth factor (VEGF).[36] The frequent use of poppers is probably a surrogate marker for fre-quent receptive anal intercourse rather than a direct risk factor for AIDS.

Fatalities

Fatalities following the use of nitrites are rare, and most fatalities involve the ingestion rather than inhalation of amyl or butyl nitrite.[37] However, the inhalation of amyl or butyl nitrite may cause severe methemoglobinemia, and potentially death.

DIAGNOSTIC TESTING

Analytic Methods

The volatility and instability of alkyl nitrites complicates the analysis of these compounds. Hydrolysis of alkyl nitrites in aqueous matrices rapidly converts these com-pounds to the corresponding alcohol and nitrite ion. Analytic methods for the analysis of nitrites in blood include gas chromatography with electron capture detection, capillary column gas chromatography/mass spectrometry,[38] solid phase microextraction with gas chromatography/flame ionization detection,[39] liquid

chromatography/mass spectrometry (LC/MS), and gas chromatography/mass spectrometry (GC/MS).[40] The lower limit of quantification (LLOQ) for these methods is approximately 0.001 mg/L. The half-life of butyl nitrites stored at 37.0°C (98.6°F) in whole blood samples from mice ranges from 2–20 minutes.[4] To reduce the formation of other volatiles, samples should be stored at a maximum temperature of 4°C (39°F) with 1–2% w/v sodium fluoride added as a preservative.

Biomarkers

A slate-blue cyanosis and chocolate brown discolor-ation of the arterial blood occurs at methemoglobin concentrations between 15–20%, but symptoms of intoxication are minimal. The administration of oxygen does not improve the cyanosis or chocolate-brown color associated with methemoglobinemia. Methemoglobin concentrations between 20–50% cause headaches, diz-ziness, lethargy, dyspnea, and syncope. Above 50% met-hemoglobinemia, signs of cardiac and cerebral hypoxia develop including confusion, metabolic acidosis, sei-zures, and dysrhythmias. Methemoglobin concentra-tions over 70% are associated with coma, pulmonary edema, and death. A 42-year-old man was deeply cya-notic with a markedly diminished level of consciousness and tachycardia after accidentally ingesting 1 gram of sodium nitrite.[41] His serum nitrite concentration on admission was 0.6 mg/dL.

In the postmortem blood from a 34-year-old man who was pronounced dead 1 hour after ingesting sodium nitrite, the nitrite and nitrate concentrations were 0.55 mg/L and 30.0 mg/L, respectively. The methemo-globin concentration in the postmortem blood was 90%, and the ratio of nitrite to nitrate in stomach contents was 2:1. Nitrite and nitrate were not detectable in vitre-ous humor. The postmortem methemoglobin concentra-tion may fall below fatal ranges as a result of perimortem administration of methylene blue or prolonged post-mortem intervals.[37]

Abnormalities

Unexplained cyanosis suggests methemoglobinemia, particularly when the clinical symptoms are mild com-pared with the degree of cyanosis and oxygen therapy does not improve the cyanosis.[42] Pulse oximetry typi-cally displays oxygen saturations in the range of 80–85% because pulse oximetry measures the light absorbance of deoxyhemoglobin (660 nm) and oxyhemoglobin (940 nm). The oximeter calculates the ratio of the absor-bance at these 2 wavelengths and displays an oxygen saturation based on the ratio of absorbance at these 2 wavelengths (e.g., 100% = absorbance ratio of 0.43).

Methemoglobin absorbs light equally at 660 nm and 940 nm; therefore, high concentrations of methemoglobin cause a spuriously high pulse oximeter reading near 80–85% (absorbance ratio = 1.0). Cooximetry accurately measures oxygen saturation by determining the differential absorbance of oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin at 4 different wavelengths. However, the absorbance of methemoglobin and sulfhemoglobin are similar (630 nm and 614 nm, respectively); therefore, cooximetry does not reliably separate these 2 types of hemoglobin. The potassium cyanide test distinguishes methemoglobin and sulfhemoglobin as a result of the conversion of methemoglobin to cyanomethemoglobin in the presence of potassium cyanide, whereas this reaction does not occur following the addition of potassium cyanide to sulfhemoglobin. The oxygen saturation reported in arterial blood gas analysis might also be misleading because the reported oxygen saturation is a calculated value based on the assumption that all hemoglobin has normal oxygen-carrying capacity. Thus, the presence of high methemoglobin concentrations causes an overestimation of the calculated oxygen saturation. A case report of a 20-year-old woman associated the self-reported sniffing of poppers with the development of radiographic changes consistent with posterior reversible encephalopathy syndrome (PRES).[43] These changes included abnormal, symmetric, high signal intensity on T2-weighted magnetic resonance images of the juxtacortical white matter of the frontal, parietal, occipital, and temporal lobes. Although she had transient loss of consciousness, confusion, paresthesias, and bilateral headache consistent with methemoglobinemia, there was no measurement of the methemoglobin concentration or discussion of how the effect of methemoglobin could be differentiated from PRES.

TREATMENT

Stabilization

Treatment of methemoglobinemia involves evaluation of the adequacy of ventilation, assessment of hemodynamic status, and the provision of supplemental oxygen. Severely affected patients may be hypotensive and hypoxic, and the rapid administration of methylene blue to these patients may be necessary.

Elimination Enhancement

Exchange transfusion is an option for patients unresponsive to maximum therapeutic doses of methylene blue, particularly in severely affected patients.[44] Several case reports document the use of hyperbaric oxygen (HBO) for patients with severe methemoglobinemia.[45] The rationale for the treatment of severe methemoglobinemia with HBO is the enhanced reduction of methemoglobin and the delivery of high oxygen concentrations to the tissues despite the limited oxygen-carrying capacity of the blood secondary to methemoglobinemia. Rodent studies indicate that the administration of HBO (4 ATA) immediately after intraperitoneal administration of sodium nitrite reduces mortality and methemoglobinemia when compared with controls (no treatment).[46]

Antidotes

Intravenous methylene blue is the initial antidote of choice for patients requiring treatment.[47] Methylene blue (toluidine blue) accelerates the enzymatic reduction of methemoglobin by NADPH-methemoglobin reductase, which normally provides a minor pathway for the reduction of methemoglobin. This enzyme reduces methylene blue to leucomethylene blue, which subsequently reduces methemoglobin to hemoglobin. This process requires an adequate supply of NADPH from glycolysis; therefore, the patient requires normal serum glucose and glucose-6-phosphate dehydrogenase concentrations to fully benefit from methylene blue. Patients with G-6-PDase deficiency develop hemolytic anemia following the administration of an oxidizing agent (i.e., methylene blue). Asymptomatic patients with methemoglobin concentrations below 30% usually require only supportive care and removal of the offending agent. The initial intravenous dose of methylene blue (1% solution by weight) is 1–2 mg/kg slowly over 3–5 minutes. Rapid improvement in cyanosis and normalization of methemoglobinemia usually occurs within 30–60 minutes.[48] The response to treatment may be difficult to assess because of the bluish skin discoloration caused by methylene blue; therefore, further treatment should be based on repeat methemoglobin concentrations (measured by cooximetry) and patient response. An initial drop (i.e., about 5%) in pulse oximeter readings may occur soon after the infusion of methylene blue because of the interference of methylene blue with pulse oximeter readings, but these readings usually return to normal within 10–30 minutes after infusion. This spurious drop in oxygen saturation results from the similar absorbance (i.e., 668 nm) of methylene blue and reduced hemoglobin. A second dose of methylene blue may be administered in 1 hour if the methemoglobin concentration does not drop significantly with a maximum total dose of 7 mg/kg. Adverse effects of high doses of methylene blue include headache, dizziness, mental confusion nausea, vomiting, abdominal pain, diaphoresis, hypertension, and possibly paradoxical

methemoglobinemia or hemolysis due to high oxidative stress. Failure to respond to sufficient doses of methylene blue may result from the following: 1) G-6-PDase deficiency, 2) NADPH methemoglobin reductase deficiency, or 3) excessive doses of methylene blue (i.e., >7 mg/kg).

References

1. Brunton TL. On the use of nitrite of amyl in angina pectoris. Lancet 1867;ii:97–98.

2. Newell GR, Spitz MR, Wilson MB. Nitrite inhalants: historical perspective. NIDA Res Monogr 1988;83:1–14.

3. Israelstam S, Lambert S, Oki G. Poppers, a new recreational drug craze. Can Psychiatr Assoc J 1978;23:493–495.

4. Maickel RP. The fate and toxicity of butyl nitrites. NIDA Res Monogr 1988;82:15–27.

5. Wu L-T, Schleger WE, Ringwalt CL. Use of nitrite inhalants ("poppers") among American youth. J Adolesc Health 2005;37:52–60.

6. Hall MT, Howard MO. Nitrite inhalant abuse in antisocial youth: prevalence, patterns, and predictors. J Psychoactive Drugs 2009;41:135–143.

7. Lange WR, Haertzen CA, Hickey JE, Snyder FR, Dax EM, Jaffe JH. Nitrite inhalants: patterns of abuse in Baltimore and Washington, D.C. Am J Drug Alcohol Abuse 1988;14:29–39.

8. Hatfield LA, Horvath KJ, Jacoby SM, Simon Rosser BR. Comparison of substance use and risky sexual behavior among a diverse sample of urban, HIV-positive men who have sex with men. J Addict Dis 2009;28:208–218.

9. Sigell LT, Kapp FT, Fusaro GA, Nelson ED, Falck RS. Popping and snorting volatile nitrites: a current fad for getting high. Am J Psychiatry 1978;135:1216–1218.

10. Osterloh J, Olson K. Toxicities of alkyl nitrites. Ann Intern Med 1986;104:727.

11. Kielbasa W, Fung H-L. Pharmacokinetics of a model organic nitrite inhalant and its alcohol metabolite in rats. Drug Metab Dispos 2000;28:386–391.

12. Osterloh JD, Goldfield D. Uptake of inhaled *n*-butyl nitrite and *in vivo* transformation in rats. J Pharm Sci 1985;74:780–782.

13. Hunault CC, van Velzen AG, Sips AJ, Schothorst RC, Meulenbelt J. Bioavailability of sodium nitrite from an aqueous solution in healthy adults. Toxicol Lett 2009;190:48–53.

14. Osterloh J, Goldfield D. Butyl nitrite transformation *in vitro*, chemical nitrosation reactions, and mutagenesis. J Anal Toxicol 1984;8:164–169.

15. Kielbasa WB, Bauer JA, Fung H-L. Analysis of isobutyl nitrite inhalant in rat and human blood: application for pharmacokinetic investigations. J Chromatogr B 1999;734:83–89.

16. Smith M, Romanelli F. Recreational use and misuse of phosphodiesterase 5 inhibitors. J Am Pharm Assoc 2005;45:63–75.

17. Umbreit J. Methemoglobin—it's not just blue: a concise review. Am J Hematol 2007;82:134–144.

18. Mathew RJ, Wilson WH, Tant SR. Regional cerebral blood flow changes associated with amyl nitrite inhalation. Br J Addict 1989;84:293–299.

19. Kohn MC, Melnick RL, Ye F, Portier CJ. Pharmacokinetics of sodium nitrite-induced methemoglobinemia in the rat. Drug Metab Dispos 2002;30:676–683.

20. Standefer JC, Jones AM, Street E, Inserra R. Death associated with nitrite ingestion: report of a case. J Forensic Sci 1979;24:768–771.

21. O'Toole JB, Robbins GB, Dixon DS. Ingestion of isobutyl nitrite, a recreational chemical of abuse, causing fatal methemoglobinemia. J Forensic Sci 1987;32:1811–1812.

22. Covalla JR, Strimlan CV, Lech JG. Severe tracheobronchitis from inhalation of an isobutyl nitrite preparation. Drug Intell Clin Pharm 1981;15:51–52.

23. Machabert R, Testud F, Descotes J. Methaemoglobinaemia due to amyl nitrite inhalation: a case report. Hum Exp Toxicol 1994;13:313–314.

24. Guss DA, Normann SA, Manoguerra AS. Clinically significant methemoglobinemia from inhalation of isobutyl nitrite. Am J Emerg Med 1985;3:46–47.

25. Lin CH, Fang CC, Lee CC, Ko PC, Chen WJ. Near-fatal methemoglobinemia after recreational inhalation of amyl nitrite aerosolized with a compressed gas blower. J Formos Med Assoc 2005;104:856–859.

26. Modarai B, Kapadia YK, Kerins M, Terris J. Methylene blue: a treatment for severe methaemoglobinaemia secondary to misuse of amyl nitrite. Emerg Med J 2002;19:270–271.

27. Fisher AA, Brancaccio RR, Jelinek JE. Facial dermatitis in men due to inhalation of butyl nitrite. Cutis 1981;27:146, 152–153.

28. Fisher AA. "Poppers" or "snappers" dermatitis in homosexual men. Cutis 1984;34:118, 120–122.

29. Hoegl L, Thoma-Greber E, Poppinger J, Rocken M. Butyl nitrite-induced acrocyanosis in an HIV-infected patient. Arch Dermatol 1999;135:90–91.

30. Stalnikowicz R, Amitai Y, Bentur Y. Aphrodisiac drug-induced hemolysis. J Toxicol Clin Toxicol 2004;42:313–316.

31. Pece A, Patelli F, Milani P, Pierro L. Transient visual loss after amyl isobutyl nitrite abuse. Semin Ophthalmol 2004;19:105–106.

32. Fledelius HC. Irreversible blindness after amyl nitrite inhalation. Acta Ophthalmol Scand 1999;77:719–721.

33. Vignal-Clermont C, Audo I, Sahel J-A, Paques M. Poppers-associated retinal toxicity. N Engl J Med 2010;363:1583–1584.

34. Romanelli F, Smith KM, Thornton AC, Pomeroy C. Poppers: epidemiology and clinical management of inhaled nitrite abuse. Pharmacotherapy 2004;24:69–78.

35. National Toxicology Program. NTP toxicology and carcinogenesis studies of isobutyl nitrite (CAS No. 542-56-3) in F344 rats and B6C3F1 mice (inhalation studies). Natl Toxicol Program Tech Rep Ser 1996;448:1–302.

36. Fung H-L, Tran DC. Effects of inhalant nitrites on VEGF expression: a feasible link to Kaposi's sarcoma? J Neuroimmune Pharmacol 2006;1:317–322.

37. Shesser R, Dixon D, Allen Y, Mitchell J, Edelstein S. Fatal methemoglobinemia from butyl nitrite ingestion. Ann Intern Med 1980;92:131–132.

38. Bal TS, Gutteridge DR, Hiscutt AA, Johnson B, Oxley I. Analysis of alkyl nitrites by capillary gas chromatography-mass spectrometry. J Forensic Sci Soc 1988;28:185–190.

39. Tytgat J, Daenens P. Solvent-free sample preparation by headspace solid-phase microextraction applied to the tracing of *n*-butyl nitrite abuse. Int J Leg Med 1996;109:150–154.

40. Kikura-Hanajiri R, Kawamura M, Uchiyama N, Ogata J, Kamakura H, Saisho K, Goda Y. Analytical data of designated substances (Shitei-Yakubutsu) controlled by the Pharmaceutical Affairs Law in Japan, part I: GC-MS and LC-MS. Yakugaku Zasshi 2008;128:971–979. [Japanese]

41. Sevier JN, Berbatis CG. Accidental sodium nitrite ingestion. Med J Aust 1976;1:847.

42. Beneteau-Burnat B, Pernet P, Vaubourdolle M, Pelloux P, Casenove L. Hypermethemoglobinemia in a substance abuser. Am J Emerg Med 2005;23:816–817.

43. Casetta I. An acutely confused young woman. Lancet 2011;378:456.

44. Jansen T, Barnung S, Mortensen CR, Jansen EC. Isobutyl-nitrite-induced methemoglobinemia; treatment with an exchange blood transfusion during hyperbaric oxygenation. Acta Anaesthesiol Scand 2003;47:1300–1301.

45. Lindenmann J, Matzi V, Kaufmann P, Krisper P, Maier A, Porubsky C, Smolle-Juettner FM. Hyperbaric oxygenation in the treatment of life-threatening isobutyl nitrite-induced methemoglobinemia—a case report. Inhal Toxicol 2006;18:1047–1049.

46. Goldstein GM, Doull J. Treatment of nitrite-induced methemoglobinemia with hyperbaric oxygen. Proc Soc Exp Biol Med 1971;138:137–139.

47. Shesser R, Mitchell J, Edelstein S. Methemoglobinemia from isobutyl nitrite preparation. Ann Emerg Med 1981;10:262–264.

48. Wilkerson RG. Getting the blues at a rock concert: a case of severe methaemoglobinaemia. Emerg Med Australas 2010;22:466–469.

PART 2

PSYCHOACTIVE PLANTS

Chapter 52

ABSINTHE

HISTORY

Since antiquity, wormwood (*Artemisia absinthium*) has been a constituent of vinous beverages with alleged medicinal properties.[1] Both the Egyptian Ebers Papyrus (1552 BC) and the Old Testament of the Bible (Jeremiah 9:15, 13:15) contain references to wormwood.[2] The Greek philosopher and mathematician, Pythagorus of Samos (569–475 BC) recommended wormwood leaves soaked in wine as a treatment for labor pains; Hippocrates (~460–377 BC) used wormwood extracts for rheumatism and menstrual pains.[3] Several of Shakespeare's plays (e.g., *Merry Wives of Windsor*) also contain references to wormwood.[4] Other *Artemisia* species have been used as herbal remedies for many years including the treatment of intestinal round worms,[5] malaria, tumors, and neonatal jaundice.[6] Artemisinin is an extract of the Chinese herb *Artemisia annua* that has been used for >2000 years as an herbal tea for treatment of fever. This compound has undergone clinical trials as an anti-malarial agent.[7]

Absinthe is historically a highly alcoholic, distilled beverage that contains flavoring extracts from wormwood as well as other herbs. In the late 1700s, a French physician in Couvet, Switzerland developed a medicinal formulation containing absinthe that was sold to Henri-Louis Pernod. The Pernod family continued to manufacture and export this drink well into the 20th century. During the 1844–1847 French military campaign in Algeria, French soldiers received wormwood-containing wine as an antipyretic. The use of absinthe became widespread among all French social classes, particularly in Paris where the summer afternoon between 5–6 PM became the "hour of absinthe." By the late 1860s, France imported almost 2 million gallons of absinthe from Switzerland.[8] Experimental work on the neurotoxic effects of absinthe by Valentin Magnan in the 1870s formed the scientific background for the campaign against absinthe.[9] He described the occurrence of generalized tonic-clonic seizures in absinthe drinkers, and he conducted animal research on the epileptic effects of absinthe preparations. In France and other parts of Europe during the last quarter of the 19th century and the first part of the 20th century, the consumption of absinthe as an ethanol-containing aperitif generated considerable controversy regarding the consequences of heavy use including noisy, aggressive behavior, post-intoxication depression and fatigue, anxiety, hallucinations, nightmares, and emaciation.[10] However, a causal link between the use of absinthe and these adverse effects has not been proven in humans apart from the chronic use of ethanol.

During the 1870s, absinthe was a popular drink of artists, musicians, financiers, and literary figures including Oscar Wilde, Edgar Alan Poe, and Charles Baudelaire. Famous paintings displaying the drinking of absinthe include Absinthe (Edgar Degas, ca. 1877), The Absinthe Drinker (Edouard Manet, ca. 1858–1859), and The Absinthe Drinker and The Poet Cornuti-Absinthe (Pablo Picasso, 1901, 1903). Toulouse Latrec depicted Vincent van Gogh drinking absinthe in a pastel portrait created in 1887. Some scholars believe that an addiction to absinthe along with brandy and turpentine exacerbated the manic depressive mood swings of van Gogh.[1,11] In 1905, the Belgian Parliament banned the manufacture and sale of absinthe; the Swiss (1908), Dutch (1910),

and US (1912) governments prohibited the sale and import of absinthe shortly thereafter. Prohibition of the use of absinthe in France began in 1922. At that time, most other European countries forbade the use of absinthe with the exception of Spain, Portugal, the Czech Republic, and the UK. During the early part of the 20th century, the intravenous administration of thujone, the active ingredient in absinthe, was a chemical model for the study of epilepsy.[12,13]

The lifting of prohibitions against the production and use of absinthe began in the latter part of the 20th century; however, the content of absinthe approved for use differed from the historical formulations of absinthe. In 1988, Council Directive 88/388/EEC reintroduced absinthe into the European Union for bitter spirit drinks with a maximum limit of 35 mg/kg α- and β-thujone. Although this regulation removed limits for the use of *Artemisia absinthium*, *Salvia officinalis*, and other thujone-containing flavoring plants in foods, synthetic thujone is prohibited as a chemical food additive. Switzerland also lifted the ban on absinthe. Importation of absinthe remains legal in Canada. In 2007, the US government approved the first authentic thujone-free (i.e., <10 ppm) absinthe product (Lucid, Viridan Spirits, Inc., Manhasset, NY).

BOTANTICAL DESCRIPTION

Wormwood refers to 2 (*Artemisia absinthium*, *Artemisia pontica*) of over 180 species of the genus *Artemisia* (sagebrush). The active ingredient in absinthe is thujone (ethanol is an additive rather than an ingredient); this compound also occurs in sand hill sage (*Artemisia filifolia* Torr.), tansy (*Tanacetum vulgare* L.), Eastern or Northern white cedar (*Thuja occidentalis* L.), and yarrow (*Achillea millefolium* L.).[14]

Common Name: Bitter wormwood, wormseed, absinth sagewort, absinth wormwood, absinthium, common sagewort, losna, pelin, assenzio, wermutkraut

Scientific Name: *Artemisia absinthium* L.

Botanical Family: Asteraceae (sunflowers, tournesols)

Physical Description: This fragrant perennial herb grows up to 1–4 feet (~30–120 cm) with silver-green leaves containing fine silk hairs. Yellow-green flowers appear between July and September. Figure 52.1 displays the bitter wormwood.

Distribution and Ecology: This introduced, perennial shrub occurs in disturbed areas of the northern United States from Oregon and Washington

FIGURE 52.1. Bitter wormwood (*Artemisia absinthium*). (Richard A. Howard Image Collection. Courtesy of Smithsonian Institution.)

to Maine and South Carolina. This plant species is native to Europe and Asia.

Common Name: Roman or petite wormwood

Scientific Name: *Artemisia pontica* L.

Botanical Family: Asteraceae (sunflowers, tournesols)

Physical Description: The height of this plant is similar to common wormwood, but leaves of Roman wormwood are smaller and more finely cut with narrower segments. The upper leaves are white with fine hairs on both sides. Numerous flowers appear in July at the tops of the branches. The flowers are darker and smaller compared with common wormwood.

Distribution and Ecology: Roman wormwood is a native plant species of temperate climates in southern Europe and Asia that naturalized in eastern Canada and the northeastern United States.

IDENTIFYING CHARACTERISTICS

Structure

α-Thujone (CAS 546-80-5) and β-thujone (CAS 471-15-8) are bicyclic monoterpenes that differ in the stereochemistry of the C4-methyl group as displayed in Figure 52.2. α-Thujone is the active ingredient in cedar leaf oil, whereas β-thujone is the major constituent of wormwood oil.[15] These compounds are structurally similar to tetrahydrocannabinol, the active ingredient in marijuana.[16] Thujone is an isomer of camphor ($C_{10}H_{16}O$).

Physiochemical Properties

In animal models, α-thujone is an epileptic agent that is about 2–3 times more potent than β-thujone.[15] Table 52.1 lists some physical properties of α-thujone.

Thujone is a relatively stable compound, except in very alkaline media. Rapid epimerization of α-thujone to β-thujone occurs at pH 11.5, but not in ethanol solutions stored at pH 2.5 or pH 6.5; rapid photodegradation of thujone to corresponding isomeric 5-methylene-6-methylhept-2-ene compounds also occurs within 6–8 hours when stored in 30% ethanol solutions.[17] Little degradation of thujone occurs at ambient temperatures up to 100°C (212°F); no deterioration of thujone occurred in absinthe samples stored at ambient temperatures for 1 year.[18] However, thujone undergoes photodegradation. Irradiating commercial absinthe with UVA doses of 16.9 mW/cm² and UVB doses of 3.9 mW/cm² for 25 hours resulted in the reduction of the β-thujone concentration from 9.7 mg/L to 1.8 mg/L.[18]
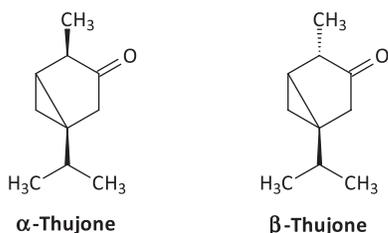
FIGURE 52.2. Chemical structures of α-thujone and β-thujone.

TABLE 52.1. Some Physical Properties of α-Thujone.

Physical Property	Value
Boiling Point	203°C (397.4°F)
log P (Octanol-Water)	2.650
Water Solubility	408 mg/L (25°C/77°F)

The taste of absinthe results primarily from the presence of absinthin (CAS RN: 1362-42-1, $C_{30}H_{40}O_6$).[15]

Form

Absinthe (German, *wermut*) is an emerald-green colored spirit that contains ethanol, essential oils of wormwood, and varying amounts of herbs including Florence or sweet fennel (*Foeniculum vulgare* P. Mill.), green anise (*Pimpinella anisum* L.), hyssop (*Hyssopus officinalis* L.), calamus, angelica (*Angelica arguta* Nutt.), lemon or common balm (*Melissa officinalis* L.), nutmeg (*Myristica fragrans* Houtt.), sweet marjoram (*Origanum majorana* L.), and Roman wormwood (*Artemisia pontica*). The green color of absinthe results from the presence of chlorophyll and/or additives.[4]

EXPOSURE

Sources

Wormwood oil is a dark green to blue-brown, bitter-tasting substance extracted from wormwood.

ORIGIN

European companies in Spain, Bulgaria, France, the Czech Republic, and Germany recently began production of absinthe; Switzerland lifted the ban on the consumption of absinthe.[2] In Canada, provincial governments control the sale of spirits, and the limits on absinthe vary between provinces. The Alcohol and Tobacco Tax and Trade Bureau in the United States lifted the ban on absinthe for products containing <10 ppm thujone. Absinthe is available over the Internet. Although the recipes for modern and vintage absinthe are similar, the origin (essential oils/herbs) of the contents of specific absinthe spirits vary by location and distillery.

COMPOSITION

HERBS/ESSENTIAL OILS. Although the whole plant contains thujone, leaves and flowers are the main source of essential oils. The concentration of the principal constituents of essential oils from *Artemisia* species depends on a variety of factors including plant part, season, growing conditions, storage conditions, species, and chemovar with the β-thujone concentration reaching up to about 60% in some cultivars. In a Serbian sample of the essential oil from aerial parts of *Artemisia absinthium*, the main ingredients were β-thujone (19.8%), *cis*-β-epoxyocimene (10.7%), *trans*-sabinyl acetate (8.8%), sabinene (8.1%), and linalyl 3-methylbutanoate (7.5%).[19] α-Thujone was a minor constituent (0.2%). In

763

contrast to the essential oil from aerial parts of wormwood plants, the two main constituents of the essential oil from wormwood roots were α-fenchene (23.3%) and linalyl butyrate (14.4%) with minor concentrations of β-thujone. Although the α-thujone/β-thujone ratio varies with the source and other variables, the concentration of β-thujone usually substantially exceeds the concentration of α-thujone.[20] Some camphor is usually detectable in samples of absinthe because thujone is an isomer of camphor.

ABSINTHE.   Traditional absinthe is an emerald spirit with a bitter taste and a high ethanol concentration. In addition to the high ethanol content, α-thujone probably is the active ingredient in absinthe.[15] Absinthe contained high and variable amounts of ethanol. The ethanol concentration varied with the following types of absinthe: absinthe *suisse* (68–72%), absinthe *demi-fine* (50–68%), and absinthe *ordinaire* (45–50%).[3,10] The estimated total thujone content of old absinthe was up to 260 mg/L (ppm) based on theoretical calculations.[21] However, there are few direct analyses of absinthe products to confirm this estimate. In 1936, Wilson detected thujone concentration from 1.8–45 mg/L in absinthe produced from various essences, whereas absinthe made from wormwood contained 2–34 mg thujone/L as measured by the semiquantitative method of Enz that did not separate thujone isomers.[22] Chemical analysis of 13 vintage samples of absinthe produced before 1915 detected total thujone concentrations (α- and β-thujone) ranging from 0.5–48.3 mg/L (mean, 25.4 ± 20.3 mg/L; median, 33.3 mg/L).[23] The concentration of β-thujone was up to 6-fold higher than α-thujone. There was no statistically significant difference in the total thujone concentrations between vintage (i.e., pre-1915) and modern absinthe. Additionally, this study did not detect toxic concentrations of methanol, fusel oils, copper, or antimony in the vintage absinthe. Theoretically, the chemical composition of vintage absinthe analyzed in this study and cheaper absinthe products mass-produced during the 19th and early 20th centuries may differ significantly. The essential oils of other herbs (e.g., oil of hyssop) used for absinthe production contain convulsant compounds (e.g., pinocamphone, fenchone), but whether these convulsants remain in toxicologically significant concentrations after distillation remains unproven.

PRODUCTION PROCESSES

The absinthe process involves 1) the ethanol-extraction of the aromatic herbs, 2) distillation of the extract, 3) the addition of color and flavors, and 4) dilution with water to reach the final ethanol content.[24] The first step in the production of absinthe with traditional recipes involves the maceration of wormwood and other dried herbs (e.g., fennel, anise) in a concentrated ethanol solution (85–90%). Distillation of the strongly bitter, green-brown macerate produces a light, aromatic, colorless fraction. The addition of *Artemisia pontica* and other herbs during the coloration step introduces the green color (i.e., chlorophyll) and a mild bitter taste. Denaturation of chlorophyll by light and heat results in the characteristic pale green color of traditional absinthe. During the final step, dilution after the coloration step results in the final ethanol concentration.

IMPURITIES

Because the addition of water changes the color of absinthe from emerald to opal, some preban manufacturers added coloring agents (e.g., aniline green, antimony chloride, copper sulfate, hyssop, indigo, nettles, tumeric).[10] These additives are not approved for use in modern absinthe.

Methods of Abuse

Because of the high ethanol content and bitter taste, traditionally absinthe was diluted with water by dripping the water over a perforated spoon (absinthe spoon) containing a lump of sugar and into a glass of absinthe. More elaborate preparation involved the use of a crystal colander (strainer) containing sugar and crushed ice mounted on a wine glass.[4] The absinthe would be poured over the colander followed by essence of anise and then water. The traditional use of absinthe continues; other, less common methods of drinking absinthe include pouring the liquor directly over the sugar cube and lighting the cube with a match and alternately drinking the liquor without dilution.

DOSE EFFECT

There are few modern data on the effect of specific doses of total thujone or specific isomers. Current European regulations limit thujone only in *Artemisia*-derived alcoholic beverage to 35 ppm.[25] Thujone may be added to food only indirectly by the addition of thujone-containing plants; the addition of thujone to food as a chemical is prohibited. The no observable effect level (NOEL) for female and male rats based on the occurrence of seizures was 5 mg/kg body weight and 10 mg/kg body weight, respectively.[26] The estimated tolerable daily intake (TDI) based on a safety factor of 500 is 10 μg/kg body weight. In the European Medicines

Agency *Artemisia absinthium* monograph, the proposed daily thujone intake for adults is 3.0 mg over a maximum of 2 weeks.[27] Using the benchmark dose (BMD) method instead of the NOEL, the estimated acceptable daily intake of thujone is 0.11 mg/kg body weight based on a BMD lower confidence limit (benchmark response of 10%) for clonic seizures in male rats.[28] The calculated amount for benchmark response of 10% was 11 mg/kg body weight/day.

The ingestion of about 10 mL of the essential oil of wormwood by a 31-year-old man was associated with seizures, myalgias, rhabdomyolysis, and acute renal failure.[29] He did not develop hyperkalemia or oliguria; all his laboratory values returned to normal ranges by 17 days after ingestion.

TOXICOKINETICS

There are few data on the toxicokinetics of thujone in humans; *in vitro* studies indicate that hydroxylation via NADPH-dependent pathways account for the biotransformation of α- and β-thujone. The major metabolites of α-thujone and β-thujone are hydroxythujone and dehydrothujone compounds.[30] In rodent studies, the major metabolite of thujone compounds is 7-hydroxy-α-thujone and minor metabolites include 4-hydroxy-α-thujone, 4-hydroxy-β-thujone, and 7,8-dehydro-α-thujone.[31] The major urinary metabolites include hydroxythujone glucuronides and 4,10-dihydrothujone. These studies indicate that there are substantial differences in the metabolism of thujone between animal species.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Absinthe has both sedative and analgesic properties that result in elevated mood and increased sense of perception. Because most of the observations of absinthe drinking resulted from historical accounts, there is limited information on the pharmacologic interactions of thujone and ethanol in absinthe as well as the contribution of each substance to the effect of the spirit. Absinthe may produce hallucination and seizures during acute intoxication, whereas withdrawal rather than acute intoxication causes these complications in chronic alcohol abusers. The cause of absinthism remains unclear with potential contributing factors including α- and/or β-thujone and contaminates [e.g., copper sulfate, antimony chloride, tansy (*Tanacetum vulgare* L.), sweet calamus (*Acorus calamus* L.)]. Analysis of α- and β-thujone in historical recipes and recently produced absinthe detected low thujone concentrations (0–4.3 mg/L) that are far below current absinthe standards (<35 mg/L). This study failed to confirm the high thujone concentrations (260 mg/L) reported in absinthe during the 19th century.[18] In animal studies, α-thujone acts as a convulsant by the noncompetitive inhibition of the γ-aminobutyric acid (GABA$_A$)-gated chloride channel.[15] *In vitro* studies suggest that α-thujone reduces 5-HT$_3$ receptor activity,[32] but the clinical significance of this inhibitory activity remains undefined as absinthe typically contains a lower concentration of this isomer than β-thujone. Although thujone is structurally similar to tetrahydrocannabinol, animal studies indicate that thujone does not bind to the CB$_1$ cannabinoid receptors in the central nervous system as determined by radioligand receptor binding assays.[33]

CLINICAL RESPONSE

Observations of heavy absinthe drinkers by historical accounts suggest that absinthe and acute ethanol intoxication produce different clinical effects. However, the physiologic effects of ethanol and absinthe are difficult to differentiate when both were consumed in the same drink. Heavy absinthe users were boisterous, impulsive, aggressive, paranoid, and violent during periods of intoxication that lasted much longer than pure ethanol intoxication. Reported adverse effects of the chronic use of absinthe included vomiting, gastritis, cachexia, restlessness, anxiety, giddiness, illusions, auditory and visual hallucinations, seizures, delirium, and coma. These effects were associated with absinthe by clinical observations during the late 1800s; therefore, the causal relationship remains unproven. There are few reported data on the adverse effects of current formulations of absinthe.

Exposure to *Artemisia* species may produce allergic manifestations (e.g., allergic rhinitis, contact dermatitis).[34] The incidental ingestion of a large dose of wormwood oil (major ingredient thujone) instead of absinthe causes seizures, rhabdomyolysis, and acute renal failure.[29] A case report associated the development of second-degree atrioventricular block (Mobitz type I) with absinthe intoxication (serum ethanol, 198 mg/dL).[35] Within a few minutes, the rhythm changed spontaneously to an accelerated junctional rhythm that converted to normal sinus rhythm within a few hours with medical treatment (specifics of treatment not reported). The patient remained hemodynamically stable throughout his clinical course. A 2-year carcinogenicity study by the US National Toxicology Program demonstrated some evidence of carcinogenicity in F344/N male rats, but none in female rats, B6C3F1 male mice, or B6C3F1 female mice.[36] There were no significant dose-response

relationships between neoplastic or nonneoplastic lesions.

DIAGNOSTIC TESTING

Methods for the quantitation of thujone, pinocamphone, and fenchone in absinthe include gas chromatography/flame ionization detection, high performance liquid chromatography, and liquid-liquid extraction with gas chromatography/mass spectrometry.[24] The limit of detection is near 80 µg/L.[18] Experimental studies of preban absinthe with UV irradiation up to 200 hours indicate that absinthe stored in green bottles does not decompose, whereas an 18% reduction in β-thujone content and concurrent discoloration occurred in absinthe stored in clear bottles for the same time.[37] Analysis of preban bottles up to 7 years did not detect significant reductions in β-thujone content.

TREATMENT

Treatment is supportive and similar to the treatment of acute ethanol intoxication. The primary focus is airway protection and prevention of withdrawal in chronic users by the liberal use of agents active at the GABA complex in the central nervous system (e.g., benzodiazepines).

References

1. Arnold WN. Vincent van Gogh and the thujone connection. JAMA 1988;260:3042–3044.
2. Haines JD. Absinthe—return of the green fairy. J Okla State Med Assoc 1998;91:405–407.
3. Padosch SA, Lachenmeier DW, Kroner LU. Absinthism: a fictitious 19th century syndrome with present impact. Subst Abuse Treat Prev Policy 2006;1:1–14.
4. Arnold WN. Absinthe. Sci Am 1989;260:112–117.
5. Quinlan MB, Quinlan RJ, Nolan JM. Ethnophysiology and herbal treatments of intestinal worms in Dominica, West Indies. J Ethnopharmacol 2002;80:75–83.
6. Fok TF. Neonatal jaundice–traditional Chinese medicine approach. J Perinatol 2001;21(Suppl 1):S98–S100.
7. van Agtmael MA, Eggelte TA, van Boxtel CJ. Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication. Trends Pharmacol Sci 1999;20:199–205.
8. Procter W Jr. Absinthe. Am J Pharm 1868;41:356–360.
9. Eadie MJ. Absinthe, epileptic seizures and Valentin Magnan. J R Coll Physicians Edinb 2009;39:73–78.
10. Vogt DD, Montagne M. Absinthe: behind the emerald mask. Int J Addict 1982;17:1015–1029.
11. Morrant JC. The wing of madness: the illness of Vincent van Gogh. Can J Psychiatry 1993;38:480–484.
12. Keith HM. The effect of various factors on experimentally produced convulsions. Am J Dis Child 1931;4:532–543.
13. Meyers IL. Cerebellar localizations: an experimental study of a new method. JAMA 1916;67:1745–1751.
14. Rohloff J, Skagen EB, Steen AH, Iversen TH. Production of yarrow (*Achillea millefolium* L.) in Norway: essential oil content and quality. J Agric Food Chem 2000;48:6205–6209.
15. Hold KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE. Alpha-thujone (the active component of absinthe): gamma-aminobutyric acid type A receptor modulation and metabolic detoxification. Proc Natl Acad Sci U S A 2000;97:3826–3831.
16. del Castillo J, Anderson M, Rubottom GM: Marijuana, absinthe and the central nervous system. Nature 1975;253:365–366.
17. Frohlich O, Shibamoto T. Stability of pulegone and thujone in ethanolic solution. J Agric Food Chem 1990;38:2057–2060.
18. Lachenmeier DW, Emmert J, Kuballa T, Sartor G. Thujone—cause of absinthism? Forensic Sci Int 2006;158:1–8.
19. Blagojevic P, Radulovic N, Palic R, Stojanovic G. Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. J Agric Food Chem 2006;54:4780–4789.
20. Rietjens IM, Martena MJ, Boersma MG, Spiegelenberg W, Alink GM. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol Nutr Food Res 2005;49:131–158.
21. Strang J, Arnold WN, Peters T. Absinthe: what's your poison? though absinthe is intriguing, it is alcohol in general we should worry about. BMJ 1999;319:1590–1592.
22. Wilson JB. Determination of thujone in absinthe-type liqueurs. JAOAC 1936;19:120–124.
23. Lachenmeier DW, Nathan-Maister D, Breaux TA, Sohnius E-M, Schoeberl K, Kuballa T. Chemical composition of vintage preban absinthe with special reference to thujone, fenchone, pinocamphone, methanol, copper, and antimony concentrations. J Agric Food Chem 2008;56:3073–3081.
24. Lachenmeier DW, Walch SG, Padosch SA, Kroner LU. Absinthe—a review. Crit Rev Food Sci Nutr 2006;46:365–377.
25. European Parliament and Council. Regulation (EC) No. 1334/2008 of the European Parliament and the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No. 1601/91, Regulations (EC) No. 2232/96 and (EC) No. 110/2008 and Directive 2000/13/EC. Off J Eur Union 2008;L354:34–50.
26. Scientific Committee on Food of the European Commission: Opinion of the Scientific Committee on food

on thujone. Available at: http://ec.europa.eu/food/fs/sc/scf/out162_en.pdf Accessed July 1, 2011.

27. European Medicines Agency. Community herbal monograph on *Artemisia absinthium* L., herba. London: European Medicines Agency;2009.

28. Lachenmeier DW, Uebelacker M. Risk assessment of thujone in foods and medicines containing sage and wormwood—evidence for a need of regulatory changes? Regul Toxicol Pharmacol 2010;58:437–443,

29. Weisbord SD, Soule JB, Kimmel PL. Poison on line—acute renal failure caused by oil of wormwood purchased through the Internet. N Engl J Med 1997;337:825–827.

30. Sirisoma NS, Hold KM, Casida JE. α- And β-thujones (herbal medicines and food additives): synthesis and analysis of hydroxy and dehydro metabolites. J Agric Food Chem 2001;49:1915–1921.

31. Hold KM, Sirisoma NS, Casida JE. Detoxification of α- and β-thujones (the active ingredients of Absinthe): site specificity and species differences in cytochrome P450 oxidation *in vitro* and *in vivo*. Chem Res Toxicol 2001;14:589–595.

32. Deiml T, Haseneder R, Zieglgansberger W, Rammes G, Eisensamer B, Rupprecht R, Hapfelmeier G. Alpha-thujone reduces 5-HT3 receptor activity by an effect on the agonist-reduced desensitization. Neuropharmacology 2004;46:192–201.

33. Meschler JP, Howlett AC. Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. Pharmacol Biochem Behav 1999;62:473–480.

34. Mitchell JC, Geissman TA, Dupuis G, Towers GH. Allergic contact dermatitis caused by *Artemisia* and *Chrysanthemum* species. The role of sesquiterpene lactones. J Invest Dermatol 1971;56:98–101.

35. Benezet-Mazuecos J, de la Fuente A. Electrocardiographic findings after acute absinthe intoxication. Int J Cardiol 2006;113:e48–e50.

36. National Toxicology Program. TR-570—alpha/beta thujone mixture: pathology tables, survival and growth curves from NTP long-term studies. The National Toxicology Program, Research Triangle Park, NC. Available at: http://ntp.niehs.nih.gov/index.cfm?objectid =BDC8D329-123F-7908-7B2AAA991BD579CE. Accessed July 3, 2011.

37. Lachenmeier DW, Nathan-Maister D, Breaux TZ, Kuballa T. Long-term stability of thujone, fenchone, and pinocamphone in vintage preban absinthe. J Agric Food Chem 2009;57:2782–2785.

Chapter 53

AYAHUASCA, HARMALA ALKALOIDS, and DIMETHYLTRYPTAMINES

AYAHUASCA, HARMALA ALKALOIDS, and *N*,*N*-DIMETHYL-TRYPTAMINE

HISTORY

Indigenous peoples of the Amazon and Orinoco River Basins of South America have used tryptamine-containing concoctions (e.g., ayahuasca) for spiritual and medicinal purposes since before the time of Christ. Ayahuasca and the vessels for drinking this beverage were sacred in these primitive cultures. An experienced healer (*ayahuascero*) conducted the ritual use of ayahuasca after many years of training and preparation (e.g., periods of isolation, dietary restrictions, sexual abstinence). These rituals included special songs (*icaros*) and the use of other plants (e.g., tobacco). Jesuit missionaries recorded the first European references to ayahuasca in the early 18th century.[1] During the middle 19th century, English botanist Richard Spruce compiled an inventory of native plant life in the Amazon.[2] Beside discovering the rubber tree (*Hevea* spp.) and cinchona bark (quinine), he identified *Banisteriopsis caapi* as one of the sources of the psychedelic brew, ayahuasca. In the 1920s, banisterine was introduced as a treatment for Parkinson disease. The source of this hallucinogenic compound was *Banisteriopsis caapi*; subsequent studies demonstrated that the β-carboline, banisterine was identical to the reversible monoamine oxidase inhibitor, harmine. Interest in using this substance as a treatment for Parkinson disease waned in the early 1930s.

During the 20th century, the ritual use of ayahuasca extended to modern syncretic religious movements that combined Christian liturgy and African spiritualism, particularly in Brazil. Raimundo Irineu Serra, a Brazilian rubber tapper founded the Santo Daime in the 1930s after discovering the use of ayahuasca by indigenous peoples in the remote forests of the Brazilian frontier state of Acre. The Barquinha split from the Santo Daime in 1945; this group formed the Uniao do Vegetal with José Gabriel da Costa in 1961. After the death of Raimundo Irineu Serra in 1971, the Santo Daime split into several factions including the Eclectic Centre of the Universal Flowing Light (CEFLURIS). The latter group expanded their church into urban Brazilian areas and also overseas while attracting international visitors to their ritual use of ayahuasca.[3] In 1987, the use of ayahuasca in religious ceremonies was legalized in Brazil. The use of ayahuasca containing *N*,*N*-dimethyltryptamine by members of the Uniao do Vegetal Church is legal in the United States based on the US Supreme Court interpretation of the 1993 Religious Freedom Restoration Act.[4]

BOTANICAL DESCRIPTION

Common Names: Ayahuasca vine, caapi, yagé, yaje, natem, oni, nishi, vine of the dead, vine of the soul

Scientific Name: *Banisteriopsis caapi* (Spruce ex Griseb.) Morton

Botanical Family: Malpighiaceae

Physical Description: This tropical vine has smooth, chocolate-brown bark and opposite, oval-shaped green leaves ranging in size 3.5–8 cm (~1.5–3 inches) wide and 8–18 cm (~3–7 inches) long. The compound inflorescence is axillary or has terminal cymose panicles (i.e., increased, irregular branching in the upper parts with each branch containing a flower). The flowers are about 1 cm (0.4 inches) in diameter with pink petals. The fruit is a samara nut that produces fan-shaped green seeds. Upon ripening, these seeds turn brown.

Distribution and Ecology: The ayahuasca vine grows indigenously in the western parts of the Amazon Basin, primarily Brazil, Colombia, and Peru. This plant is also cultivated in tropical environments.

Common Names: Amiruca panga, chacrona, chacruna, folha, reinha, sami ruca

Scientific Name: *Psychotria viridis* Ruiz & Pav.

Botanical Family: Rubiaceae (coffee)

Physical Description: *P. viridis* is a perennial bush that grows to 5 m (~15 feet) in height and has a unique combination of plant structures (foveolae, stipules) as demonstrated in Figure 53.1.[5] The elliptical, smooth leaves are opposite (i.e., produced in pairs along the stems), growing to about 5–15 cm (~2–6 inches) long and 2–6 cm (~1–2.5 inches) wide. There are 5–10 pairs of secondary veins and occasionally microscopic plant hairs appear on the lower surface. The leaves arise on leaf stalks (petioles) that are about 1–10 mm (~0.04–0.4 inches) long. The leaves are usually gray or red-brown when dried. Foveolae are small pockets found on the lower leaf surface near the junction of the side (secondary) and central veins as demonstrated in Figure 53.1. These distinctive microscopic structures have an opening near a secondary vein that provides shelter for symbiotic mites capable of destroying destructive fungi and herbivorous invertebrates. Each leaf bears at least one pair of foveolae that usually occur near the apex; vegetative stems typically contain more foveolae than reproductive stems.

Pairs of distinctive leafy structures (stipules) cover and protect the young, developing leaves. These elliptical, membranous structures are sharply angled at the apex reaching about 5–25 mm (~0.1–1 inch) long and 4–12 mm (~0.15–0.5 inch) wide. As the stipules fall off, a horizontal scar (0.3–1 mm/0.02–0.04 inch wide) develops between the insertion of 2 opposite leaves in the middle
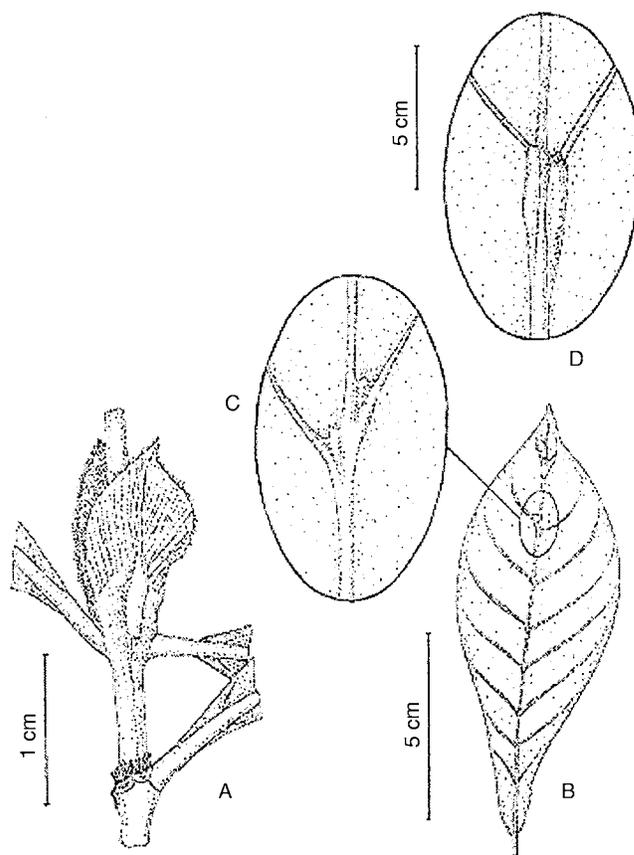
FIGURE 53.1. Distinctive vegetative features of *Psychotria viridis*. (A) upper stem with a pair of well-developed stipules (top to bottom), the bases of a pair of leaves, a stipule scar with a crown of hairs (trichomes) above the scar, the base of another leaf, and the scar from a fallen stipule. (B) Underside of leaf with an encircled pair of foveolae. (C) Enlarged view of encircled foveolae. (D) Enlarged view of foveolae from dried sample.[5]

and lower parts of the stem. A dense line of plant hairs (trichomes) appears above the scar; these trichomes are about 0.5–1 mm (~0.02–0.04 inch) long and turn red-brown when the stem is dried.

Distribution and Ecology: This plant grows in wet lowland tropical forests in Cuba and northern Central America as well as western and central South America, particularly in the Amazonian Basin near Peru and Bolivia.

Common Names: Syrian rue, harmal, African rue, harmal, peganum, harmal shrub, isband, ozallaik, peganum, steppenraute

Scientific Name: *Peganum harmala* L.

Botanical Family: Zygophyllaceae (caltrop)

Physical Description: This perennial glabrous plant grows up to 30 cm (~12 inches) in height. The

narrow, succulent leaves arise from stiff stems arranged in an alternate pattern. The small, 5-petaled flowers are white.

Distribution and Ecology: This plant grows in semiarid conditions in sandy oils in the eastern Mediterranean, Middle East, North Africa, and Central Asia.

IDENTIFYING CHARACTERISTICS

Structure

The ceremonial drink ayahuasca derived from the above plants contains the reversible monoamine oxidase A inhibitors (harmala alkaloids) and the classic hallucinogen, *N,N*-dimethyltryptamine (DMT). Collectively, harmala alkaloids are β-carboline compounds (i.e., harmine, harmaline, tetrahydroharmine). β-Carboline compounds are heterocyclic, dehydrogenated derivatives of tryptophan that occur endogenously in mammals and some plants as a result of the Pictet-Spengler condensation of indolealkylamine compounds with aldehydes.[6] Figure 53.2 demonstrates the structure of these β-carboline compounds, DMT, and serotonin. The tryptophan-derived β-carboline compounds have some structural similarity to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes irreversible parkinsonism as a result of metabolism to a neurotoxic quaternary ion, 1-methyl-4-phenyl pyridine (MPP+). Tryptamine compounds are tryptophan-derived compounds with a basic indole ring, similar to serotonin; DMT is the prototypical member of the ring-unmodified tryptamine compounds. DMT is structurally related to serotonin and similar to other psychedelic agents (e.g., lysergic acid diethylamide [LSD], mescaline).

Physiochemical Properties

DMT is a serotonergic agonist and classic hallucinogen that produces vivid visual imagery and poorly formed auditory hallucinations following inhalation or intravenous administration to experienced hallucinogen users.[7] Harmine and harmaline are reversible, competitive, type A monoamine oxidase (MAO-A) inhibitors,[8] whereas tetrahydroharmine (CAS RN: 40959-16-8) is a weak inhibitor of serotonin uptake at presynaptic sites.[9] Harmaline and harmine have pK_a values of 9.55 and 7.45, respectively.[10] Ayahuasca contains harmala alkaloids together with DMT; this combination has mild sedative and hallucinogenic properties similar to the classic hallucinogen, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM).[11] Anecdotal reports suggest that the effectiveness of ayahuasca preparations rapidly degrade after 3 days of storage.[12]

EXPOSURE

Sources

In addition to *Banisteriopsis caapi*, other plants that contain harmala alkaloids include *Peganum harmala* (Syrian rue) and *Passiflora incarnata* L. (passionflower).[13] Plants that contain appreciable quantities of *N,N*-dimethyltryptamine include *Phalaris arundinacea* L., *Phalaris aquatica* L. (canary grass, *Phalaris tuberosa* L.), *Desmanthus illinoensis* (Michx.)MacMill. (prairie bundleflower), and *Psychotria viridis* Ruiz & Pav. (chacruna). Sources of *N,N*-dimethyltryptamine for South American peoples include snuffs from seeds of *Anadenanthera peregrina* (L.) Speg. or the tree bark (*Virola* species) and the brewed tea, ayahuasca (hoasca, daime, yajé, vegetal, caapi, mihi, dapa, natema, pinde).

Harmine   **Harmaline**   **Tetrahydroharmine (THH)**

Dimethyltryptamine (DMT)   **Serotonin (5-Hydroxytryptamine, 5-HT)**
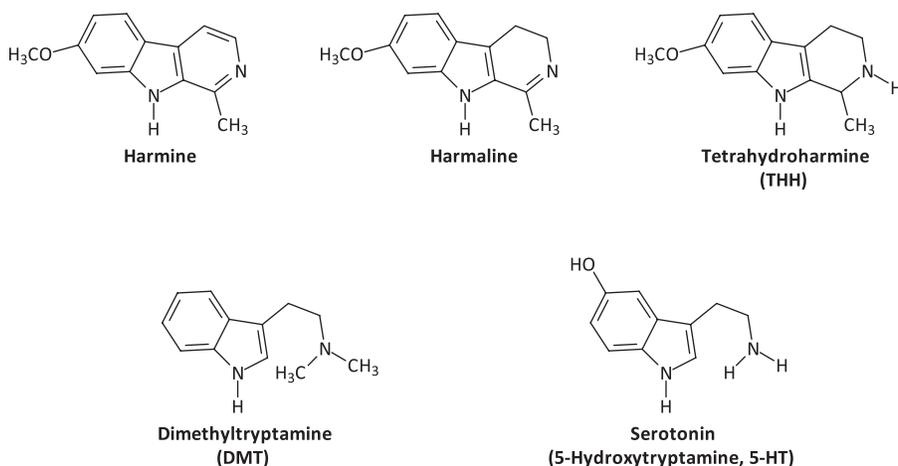
FIGURE 53.2. Chemical structures of harmala alkaloids in ayahuasca, dimethyltryptamine (DMT), and serotonin.

Hoasca is the Portuguese transliteration of ayahuasca and the accepted name of this tea in Brazil. Production of this South American tea involves the boiling or soaking of pounded stems from the ayahuasca vine (*Banisteriopsis caapi*) in combination with leaves from other plants (e.g., *Psychotria viridis*). In Ecuador and Colombia, the preparation of ayahuasca typically involves the use of leaves of *Diplopterys cabrerana* (Cuatrecasas) B. Gates [*Banisteriopsis* rusbyana (Nied.) C.V.Morton] rather than *Psychotria viridis*.[14] Traditional uses for *P. harmala* include a red dye for carpets, an abortifacient, and an emmenagogue. Specialty shops and the Internet are common sources for many psychotropic substances including dimethyltryptamine compounds and related designer drugs. A variety of flora (e.g., reed canary grass, *Phalaris arundinacea* L.) contain DMT with instruction for the extraction of smokable freebase DMT available over the Internet.[15] DMT is a schedule I drug in the United States.

Composition

The alkaloid content in the plants used for the production of ayahuasca varies substantially; consequently, the harmala alkaloid content in different ayahuasca preparations also varies. Some sampling studies suggest that the concentration of β-carboline compounds in *Banisteriopsis caapi* range from 0.05–1.95% dry weight, whereas *N,N*-dimethyltryptamine concentrations in *Psychotria viridis* range from ~0.1–0.66% dry weight. [12,14] Table 53.1 displays the mean and range of the major harmala alkaloids detected in 33 samples of *Banisteriopsis caapi* and *N,N*-dimethyltryptamine in 37 samples of *Psychotria viridis* collected from 22 sites throughout Brazil on the same day by experienced members of the União do Vegetal, and analyzed by high performance liquid chromatography.[16] The concentrations of tetrahydroharmine were highly variable in the samples of *Banisteriopsis caapi*, and these concentrations did not correlate to the concentrations of harmine or harmaline. Analysis of lyophilized aqueous extracts of the vine from *Banisteriopsis caapi* demonstrated harmine concentrations of ~20 mg/g extract.[17] Some South American shamanic snuffs derived from bark and bark-extracts of *Virola* species (Myristicaceae) contain substantial amounts of 5-methoxy-*N,N*-dimethyltryptamine in addition to smaller quantities of DMT.[18] Analysis of bark and leaf samples from *Virola* species indicated that the concentration and type of tryptamines varied greatly between samples.[19]

Analysis of an infusion of *P. harmala* associated with acute intoxication indicated that the main active ingredients were harmine, harmaline, and tetrahydroharmine.[20] Minor constituents included vasicine (CAS RN: 6159-55-3, peganine) and 3-deoxyvasicine (CAS RN: 495-59-0, deoxypeganine). In contrast to ayahuasca preparations, concentrations of DMT and 5-methoxy-DMT in the infusion of *P. harmala* were undetectable. Typically, the seeds and roots of *P. harmala* contain the highest concentrations (i.e., about 2–7%) of harmala alkaloids as well as the quinazoline derivatives, vasicinone (CAS RN: 486-64-6) and deoxyvasicinone (CAS RN: 530-53-0). In a Spanish study of various plant parts of *P. harmala* collected from May to December, the highest content of harmine (mean, 4.3% w/w) and harmaline (mean, 5.6% w/w) occurred in dry seeds.[21] Roots also contained substantial quantities of harmine (mean, 2.0% w/w) and harmol (mean, 1.4% w/w), but not harmaline. *In vitro* studies indicated that extracts of the dry seeds were stronger inhibitors of MAO-A than roots; monoamine oxidase B (MAO-B) was not inhibited by either extract.

The alkaloid content of ayahuasca varies substantially with individual recipes, preparation methods, and plant origin/composition.[22] The principal β-carboline alkaloids in this mixture are dimethyltryptamine, harmine, harmaline, and tetrahydroharmine. Analysis of a 100-mL dose of ayahuasca by high performance liquid

TABLE 53.1. Harmala Alkaloid and *N,N*-Dimethyltryptamine Content of Convenience Samples of *Banisteriopsis caapi* and *Psychotria viridis*.[16]

Substance (Source)	Sample Number	Mean ± SD (mg/g)*	Range (mg/g)*
Harmine (*B. caapi*)	33	4.83 ± 2.06	0.31–8.43
Harmaline (*B. caapi*)	33	0.46 ± 0.19	0.03–0.83
Tetrahydroharmine (*B. caapi*)	33	1.00 ± 0.79	0.05–2.94
N,N-Dimethyltryptamine (*P. viridis*)	37	7.50 ± 5.01	0.00–17.75

*Dry weight.

TABLE 53.2. Harmala Alkaloid and DMT Content of Two Samples of Ayahuasca.[1]

Compound	Sample 1 (% Total Alkaloids)	Sample 2 (% Total Alkaloids)
DMT	9.1 mg/dL (14.1%)	8.8 mg/dL (12.5%)
Harmine	47.5 mg/dL (73.1%)	9.2 mg/dL (13.2%)
Tetrahydroharmine	4.2 mg/dL (6.5%)	26.5 mg/dL (37.8%)
N-Methyltetrahydroharmine	4.1 mg/dL (6.3%)	25.5 mg/dL (36.4%)
Harmaline	Trace (<0.1%)	ND
Total Alkaloids	0.65% w/v	0.070% w/v

Abbreviation: ND = none detected.

chromatography using fluorescence detection in one study was as follows: harmine, 170 mg; harmaline, 20 mg; 1,2,3,4-tetrahydroharmine, 107 mg; and *N,N*-dimethyltryptamine, 24 mg.[23] Table 53.2 displays the harmala alkaloid and DMT concentrations in 2 preparations of ayahuasca as measured by GC/MS.

Methods of Use

Shamans in the Amazon Basin use this inebriating tea in traditional medical diagnosis and healing, divination, pathway for access to the supernatural, and rites of passage, particularly in the Brazilian-based syncretic religious groups (Santo Daime, O Centro Espirita Beneficiente União do Vegetal).[24] The latter church uses sacramental brews of ayahuasca as part of a religious ritual (*preparo*), whereas the Santo Daime church uses a decoction (santo daime) of *P. viridis* and *B. caapi* in the ritual called *feitio*.[25] During religious ceremonies, participants typically drink the tea every hour; vomiting frequently accompanies the use of the tea.[12] Piaroa shamans from southern Venezuela use the cambium from *B. caapi* alone (i.e., without DMT-containing decoctions) to heighten empathy among tribal members.[26] The Piaroa people also use *B. caapi* as a hunger suppressant, stimulant, and hunting aid to enhance visual acuity. Limited data on ayahuasca users does not suggest that the ritualistic use of ayahuasca is associated with serious deterioration in the psychosocial interactions typically caused by other drugs of abuse.[27]

Because of the poor oral bioavailability of DMT, DMT is usually smoked after adding the solid brownish material to a marijuana cigarette. Typically, the use of smokable DMT is associated with a pattern of extensive drug use.[28] The rapid intensity and resolution (i.e., about 30 minutes) of the effects associated with smoking DMT results in the eponym, businessman's lunch trip.

DOSE EFFECT

Typical doses of DMT for smoking are 40–50 mg with a range of about 20–100 mg.[13] The administration of 0.1 mg/kg intravenously causes undesirable feeling of physical tension without the euphoria present following the higher dose. In a double-blind, saline placebo-controlled, randomized study of 10 experienced hallucinogen users given DMT intravenously, the threshold for significant physical effects (e.g., increased blood pressure and heart rate, pupillary dilation, increase rectal temperature) was about 0.2 mg/kg body weight.[7] A more intense disorganizing effect, rush, and perceptual perturbations occurred after the administration of 0.4 mg/kg; some users considered these effects unpleasant. Altered perception including the appearance of rapidly moving, brightly colored visual images also occurs in volunteers administered the same intravenous doses.[29] The administration of 0.3 mg/kg followed in 1 minute by a continuous infusion of 0.02 mg/kg/min for 84 minutes produced strong alteration of visual and auditory phenomena along with paranoia and body misperceptions (e.g., body melting away, difficulty sensing the boundaries of their bodies).[44]

The typical infusion of *P. harmala* for sedative effects involves the steeping of 5–10 g crushed seeds. A 41-year-old woman boiled 100 g of seeds in water and she drank the infusion.[30] Subsequently, she developed nausea, vomiting, visual hallucination, diaphoresis, and altered consciousness followed by respiratory failure and mild hepatorenal dysfunction. She eventually recovered without sequelae. The ingestion of 50 g *P. harmala* seeds was associated with nausea, vomiting, and hallucination.[31]

TOXICOKINETICS

Absorption

There is substantial interindividual variability in the bioavailability of harmala alkaloids. Based on clinical effects, the absorption of *N,N*-dimethyltryptamine (DMT) following inhalation is rapid. Smoking DMT produces rapid onset of effects within 5 minutes, and these effects resolve within approximately 30 minutes.[13] The bioavailability of DMT after ingestion is too low to

produce clinical effects, probably as a result of the enzymatic degradation in the gastrointestinal tract to an inactive metabolite.[32] Detectable DMT concentrations following the ingestion of ayahuasca probably result from the inhibition of monoamine oxidases in the gastrointestinal tract and/or liver by the β-carboline metabolites (i.e., harmol or harmalol). These inhibitory effects limit the enzymatic degradation of the simultaneously ingested DMT in ayahuasca, resulting in enhanced absorption of DMT and allow subsequent psychedelic effects.

Volunteer studies indicate that the intense visual imagery associated with the ingestion of ayahuasca correlates to peak plasma concentrations of DMT occurring about 1.5–2 hours after ingestion.[33] Similarly, the mean peak plasma DMT concentrations was about 1.5 hours after ingestion of freeze-dried ayahuasca (0.6 and 0.85 mg DMT/kg body weight) by 18 volunteers.[34]

Biotransformation

The *O*-demethylation of harmine and harmaline to harmol and harmalol, respectively, involves a variety of cytochrome P450 isoenzymes including CYP1A1, CYP1A2, CYP2C9, CYP2C19, and CYP2D6. Kinetic studies indicate that the primary CYP subfamilies involved with the *O*-demethylation of these β-carboline compounds are CYP1A1 and CYP2D6.[35] The glucuronide and sulfate of harmol and harmalol are the main urinary metabolites of harmine and harmaline biotransformation. The metabolism of DMT following ingestion of ayahuasca involves *N*-oxidation (dimethyltryptamine-*N*-oxide) and oxidative deamination.[36] Little unchanged DMT appears in the urine.

Tolerance

Psychologic dependence on ayahuasca or withdrawal symptoms following the cessation of use are not well-documented in the medical literature. Experimental studies suggest potential for the development of some tolerance as reflected in the reduction in platelet 5-HT transporter in regular users when compared with nonusers, assuming that similar effects occur in the central nervous system (CNS).[37] However, observational studies indicate that tolerance does not develop to the psychedelic effects of DMT in contrast to many other psychedelic agents (e.g., LSD, mescaline).[45] In a double-blind randomized study of 13 experienced hallucinogen users receiving saline placebo or 0.3 mg DMT fumarate/kg 4 times at 30-minute intervals, there was no evidence of tolerance as measured by blinded clinical interviews and the Hallucinogen Rating Scale scores.[57] The DMT dose in this study produced typical psychedelic effects.

Similarly, the short-term administration of a psychedelic DMT dose (0.7 mg/kg) twice daily for 5 days did not produce pharmacokinetic or pharmacodynamic evidence of tolerance; however, 3 of the 4 users reported some variation in the response to their subjective "high."[38] Cross-tolerance to a psychedelic dose of DMT did not occur in some users with high tolerance to LSD.[39]

Drug Interactions

Potentially, the ingestion of tyrosine-containing foods along with the MAO-A inhibitors in harmala-containing plants may cause hypertensive crisis similar to monoamine oxidase inhibitor toxicity. Additionally, serotonin syndrome is a potential complication of the concomitant ingestion of selective serotonin reuptake inhibitor (SSRI) antidepressants with the monoamine oxidase-inhibiting harmala alkaloids in ayahuasca.[40]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

In addition to the monoamine oxidase inhibiting activity, *in vitro* assays suggest that β-carboline compounds may contribute to the serotonin-like effects of DMT in ayahuasca by binding with modest affinity at 5-HT_{2c} and 5-HT_{2A} receptors depending on the presence of ring substituents and ring saturation. These studies indicate that harmine and harmaline have minimal to no affinity for 5-HT_{1A} serotonin receptors, dopamine D_2 receptors, or for benzodiazepine receptors with the exception of methyl-β-carboline-3-carboxylate (β-CCM) receptors.[41] β-CCM is a benzodiazepine receptor ligand with inverse agonist properties (i.e., produces an effect opposite to that of an agonist, yet acts at the same receptor). Although tetrahydroharmine (THH) is a weak inhibitor of serotonin (5-HT) uptake,[42] the contribution of THH to the psychedelic effects of ayahuasca is not well-defined.

DMT has nearly equal affinity for the 5-HT_{1A} and 5-HT_2 receptors. In the CNS, rodent studies suggest that DMT binds to 5-HT_{1A} as an agonist and to 5-HT_2 receptors as an antagonist.[43] These relatively short-term effects cause autonomic effects (mydriasis, elevated heart rate, blood pressure, temperature) and a dramatic modification of perception, sense of self, and reality. In healthy volunteers, the administration of DMT produces a dose-dependent, schizophrenia-like state, characterized by formal thought disorder (paranoia, visual hallucinations) and inappropriate affect.[44] Apathy, withdrawal, delusions, and catatonic features are less intense than glutamate antagonists (e.g., phencyclidine). Pindolol is a potent 5-HT_{1A} antagonist, and the

administration of this drug prior to the administration of DMT enhances the psychedelic effects of DMT; these studies suggest that 5-HT$_{1A}$ agonist activity moderates the psychedelic effects associated with 5-HT$_2$ agonist activity.[45]

CLINICAL RESPONSE

The ingestion of ayahuasca containing *N,N*-dimethyltryptamine (DMT) produces mild sedative and hallucinogenic effects that peak approximately 1–1.5 hours after ingestion and resolve within 3–4 hours. The simultaneous ingestion of DMT with Syrian rue (*Peganum harmala*) may prolong the CNS effects and tachycardia up to 12 hours. The intravenous administration of DMT to volunteers was associated with psychedelic effects within 2 minutes of administration and resolution of symptoms within 30 minutes.[45] Adverse effects during ayahuasca use include myalgias, nausea, vomiting, diarrhea, eructations, uncontrollable muscle jerks, and anxiety. The most commonly reported adverse effects in a cross-sectional study of a convenience sample of 32 American members of a branch of the Santo Daime Church were nausea (34%), vomiting (28%), exhaustion 1–2 days after the ceremony (28%), and insomnia (12%).[46] Uncommon side effects included muscle spasm, reduced memory on the following day, headache, visual changes, hypoglycemia, dry mouth, and tachycardia. About 25% reported no adverse effects despite chronic use; physical examinations did not reveal any medically significant disorders. A cross-sectional study of 15 members of the syncretic church using ayahuasca for at least 10 years did not detect any personality or cognitive deterioration as measured by structured psychiatric diagnostic interviews, personality testing, and neuropsychologic testing.[47] Although the test group was compared with a matched control group, the study did not clearly indicate the extent of blinding of the interviewers and testers to the assignment of individuals to the 2 groups.

Effects associated with the ingestion of harmala alkaloids from *P. harmala* include nausea, vomiting, euphoria, visual hallucinations, diffuse tremors, elevated temperature, tachycardia, hypertension, disorientation, agitation, and seizures. An 18-year-old man presented to an emergency department with nausea, vomiting, diffuse tremors, ataxia, visual hallucinations, and agitation after drinking an infusion from *P. harmala* seeds.[20] His urine drugs of abuse screen was negative and analysis of his urine confirmed the presence of harmine and harmaline along with the corresponding metabolites, harmol and harmalol. He recovered rapidly without sequelae. Rodent studies suggest the possibility of fetal abnormalities following the ingestion of ayahuasca doses up to 10 times the typical recreational dose.[48] However, maternal toxicity (decreased maternal and fetal weight gain) at the higher doses and the presence of abnormalities in the control group (albeit lower incidence) limits conclusions regarding the etiology of these abnormalities.

DIAGNOSTIC TESTING

Analytic Methods

Methods for the detection of dimethyltryptamine and β-carboline compounds in ayahuasca include high performance liquid chromatography (β-carbolines) and gas chromatography with nitrogen/phosphorus detection (dimethyltryptamine),[49] gas chromatography/mass spectrometry (GC/MS),[22,50] high performance liquid chromatography/electrospray/mass spectrometry,[51] and liquid chromatography/electrospray ionization/tandem mass spectrometry.[52] The lower limit of quantitation (LLOQ) for these compounds in plasma samples using HPLC and gas chromatography with nitrogen/phosphorus detection is ~1 ng/mL, whereas the limit of detection (LOD) of tryptamines using screening methods based on gas chromatography/mass spectrometry typically ranges from ~5–10 ng/mL.[53] The use of liquid chromatography/electrospray ionization/tandem mass spectrometry allows the detection of β-carboline and dimethyltryptamine compounds with no extraction procedures and LLOQ in the range of 5 ng/mL. The LLOQ for β-carboline and dimethyltryptamine compounds using GC/MS is approximately 20 ng/mL with a precision (relative standard deviation) of <10%.[22]

Detectable concentrations of DMT, 5-hydroxy-DMT (bufotenine), and 5-methoxy-DMT (mexamine) occur in human urine samples as a result of the normal biotransformation of tryptamine by indolethylamine *N*-methyltransferases.[54,55] Simultaneous analytic methods (e.g., gas chromatography/mass spectrometry, liquid chromatography/electrospray ionization/mass spectrometry) can quantitate a variety of tryptamines, β-carbolines, and phenethylamine hallucinogens including α-methyltryptamine, 5-methoxy-α-methyltryptamine, *N,N*-diisopropyl-5-methoxytryptamine, *N,N*-diisopropyl-4-hydroxytryptamine, *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine, bufotenine, psilocin, psilocybin, harmine, and harmaline.[51] In plasma samples, the use of high performance liquid chromatography/ion trap ion trap/mass spectrometry with heated electrospray ionization to reduce matrix effects allows the detection of major alkaloids in ayahuasca with an LOD below 1 ng/mL.[56]

Biomarkers

In a study of 13 volunteers administered 0.3 mg DMT fumarate/kg body weight intravenously at 30-minute intervals for a total of 4 doses, the mean peak DMT plasma concentration after the last dose was approximately 70 ng/mL.[57] In a study of 18 volunteers with prior experience with psychedelic drugs, the ingestion of freeze-dried ayahuasca (0.6 and 0.85 mg DMT/kg body weight) produced mean maximum plasma DMT concentrations of 12.1 ng/mL and 17.4 ng/mL, respectively; peak subjective effects correlated to peak plasma DMT concentrations.[34] A study of 15 volunteers receiving 2 mL ayahuasca/kg detected the following approximate mean peak concentrations of DMT and harmala alkaloids: DMT, 16 ng/mL (range, 11.5–25.5 ng/mL); harmine, 115 ng/mL (range, 36–222 ng/mL); harmaline, 6 ng/mL (range, <1–9 ng/mL); and tetrahydroharmine, 91 ng/mL (range, 49–135 ng/mL).[23] Figure 53.3 displays the plasma concentration-time curve for harmine, tetrahydroharmine, and DMT following the ingestion of 2 mL ayahuasca/kg body weight by 14 regular users during religious practices. All volunteers experienced intricate visual imagery and a general state of heightened awareness; the most intense visionary effects occurred ~1–2 hours after ingesting the tea. Adverse effects included vomiting, a fine tremor, and nystagmus. In a study of 4 healthy men, peak whole blood DMA concentrations of 100 ng/mL were associated with color and spatial distortions, auditory hallucinations, paranoia, and a dissociative state.[38]

A 25-year-old man ingested a preparation from a South American tree bark in the evening followed by the ingestion of 5-methoxy-dimethyltryptamine approximately 4 hours later.[58] He was found dead the following morning, and the autopsy did not demonstrate an anatomic cause of death. Table 53.3 lists the toxicologic findings in postmortem samples from this case as measured by liquid chromatography/electrospray/mass spectrometry. 5-Methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT) is typically not detected in ayahuasca, and the presence of such large amounts of 5-MeO-DMT suggests the addition of synthetic 5-MeO-DMT to this preparation.

Urine drugs of abuse screens do not typically detect the use of ayahuasca (DMT, harmala alkaloids) or other tryptamine hallucinogens. DMT and bufotenine are natural products of serotonin or tryptamine metabolism catalyzed by indolethylamine *N*-methyltransferase. Some urine specimens may contain small, but detectable quantities of DMT or bufotenine when analyzed by GC/MS.[59] Although the comparative formation of DMT in schizophrenic and normal patients was investigated in the 1970s with GC/MS, no clear difference in urinary or cerebrospinal fluid DMT concentrations was demonstrated between schizophrenic and normal populations.[60,61]

FIGURE 53.3. Averaged and standard errors of harmine, tetrahydroharmine (THH), and *N*,*N*-dimethyltryptamine (DMT) concentrations following the ingestion of 2 mL ayahuasca/kg by 14 regular users. These volunteers received the following doses of harmala alkaloids: Harmine, 252.3 ± 38.4 mg; THH, 158.8 ± 24.2 mg; and DMT, 35.5 ± 5.3 mg. Reprinted from Journal of Ethnopharmacology, Vol. 65, Issue 3, JC Callaway, DJ McKenna, CS Grob, GS Brito, LP Raymon, RE Poland, EN Andrade, EO Andrade, DC Mash, Pharmacokinetics of Hoasca alkaloids in healthy humans, p. 252, © 1999, with permission from Elsevier.

Abnormalities

The development of serotonin syndrome during intoxication by tryptamine compounds may occasionally cause leukocytosis, rhabdomyolysis (i.e., in association with muscle rigidity), or hepatorenal dysfunction (i.e., in association with hyperthermia). The effect of ayahuasca on an electroencephalogram (EEG) primarily involves a decrease in the absolute power in the classical frequency bands, particularly in the delta and theta bands. Power analysis of EEGs from 18 volunteers receiving 0.85 mg DMT/kg body weight demonstrated reductions in the power densities of the alpha-2, beta-1, delta, and theta frequency bands over the temporal-parietal-occipital junction as well as decreases in theta power over the medial temporal and medial frontal regions of the cortex.[62] These areas comprise the regions of the brain associated with emotion, memory processes,

TABLE 53.3. Toxicologic Analysis of Postmortem Samples from a 25-Year-Old Man.[58]

Compound	Heart Blood	Peripheral Blood	Urine
5-Methoxy-*N,N*-dimethyltryptamine	1,880 ng/mL	1,200 ng/mL	9,590 ng/mL
Dimethyltryptamine	20 ng/mL	10 ng/mL	890 ng/mL
Harmaline	70 ng/mL	40 ng/mL	2,260 ng/mL
Harmine	170 ng/mL	90 ng/mL	1,150 ng/mL
Tetrahydroharmine	380 ng/mL	240 ng/mL	6,020 ng/mL

and integration of sensory information. The EEG findings more closely resemble the stimulant effects associated with LSD use rather than general stimulants (e.g., amphetamine, methylphenidate).

TREATMENT

Life-threatening medical reactions following exposure to tryptamine compounds are extremely rare. All patients should be evaluated for associated diseases with vital signs, physical examination, and a complete history that includes current symptoms, prior medical problems, and previous psychiatric history. Vital signs should include an accurate core temperature that is repeated if the patient deteriorates. The sympathomimetic effects of tryptamines are usually mild and transient, except in patients with underlying cardiovascular disease. Laboratory investigations in patients with severe intoxication or signs of organic disease include complete blood count, electrolytes, hepatic aminotransferases, serum creatinine and creatine kinase, urinalysis, and urine for drugs of abuse screening as well as computed tomography (CT) of the brain if neurologic disease is suspected. The usual methods of decontamination are almost always unnecessary because of the rapid absorption of tryptamine compounds; the use of decontamination measures complicates the treatment of agitated patients. In addition to the use of benzodiazepines, reduction of sensory stimulation, and reassurance that the frightening images are not real are important aspects of the management of tryptamine-related panic attacks. The patient should be placed in a safe, quiet environment, preferably with a familiar person who can provide constant reassurance (i.e., "talkdown"). This person should reorient the patient by interpreting the anxious feelings while attempting to restore a sense of control and well-being to the patient overwhelmed with frightening visions and emotions. Continuous interpretations of sensory misperceptions and pseudo-hallucination are necessary to reduce the anxiety associated with a "bad trip."

5-METHOXY-*N,N*-DIMETHYL-TRYPTAMINE (5-MeO-DMT)

IDENTIFYING CHARACTERISTICS

5-MeO-DMT (*O*-methylbufotenine, CAS RN: 1019-45-0) is the prototypical hallucinogenic, 5-substituted tryptamine. Although 5-MeO-DMT is a natural part of ayahuasca and is approximately 10 times more potent than DMT in rodent behavioral studies,[63] 5-MeO-DMT is a minor constituent of ayahuasca and probably contributes little to the hallucinogenic effects.

EXPOSURE

5-Methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT, *O*-methylbufotenine, CAS RN: 1019-45-0) is a natural constituent of the seeds from *Anadenanthera peregrina* (L.) Speg. (yopo, cohoba tree), which is a native tree of the Caribbean and South America. The seeds from this tree are ingredients in the psychedelic yopo snuff; however, 5-MeO-DMT is a minor constituent in this snuff compared with bufotenin.[64] 5-MeO-DMT is also a minor constituent of the bark from the South American rainforest vine, *Diplopterys cabrerana* (Cuatrec.) B.Gates (chaliponga). Shamanic snuffs (e.g., epéna, nyakwana) derived from South American plants (e.g., *Virola* species) contain 5-MeO-DMT as the primary psychoactive ingredient and *N,N*-dimethyltryptamine (DMT) as the secondary ingredient.[65] As the *O*-methyl analogue of bufotenine, 5-MeO-DMT occurs in animals (e.g., Colorado River toad, *Bufo alvarius* Girard in Baird) that contain bufotenin. In most European countries and the United States, 5-MeO-DMT is a controlled sub-

stance (e.g., schedule I in the United States); however, this substance is available over the Internet.

DOSE EFFECT

Self-experimentation studies suggest that the intranasal administration of 10 mg 5-methoxy-N,N-dimethyltryptamine (0.14 mg/kg body weight) is a threshold dose for the production of psychedelic imagery.[64] Sublingual administration produced effects similar to nasal insufflation. The addition of β-carbolines (e.g., harmaline, harmine) potentiated the psychedelic effects of 5-methoxy-N,N-dimethyltryptamine. Anecdotal studies suggest that the hallucinogenic dose of 5-MeO-DMT is similar to psilocin and substantially lower than DMT.[66] In rodent studies, the intraperitoneal LD_{50} of 5-MeO-DMT is about 6-fold lower than serotonin and about 3-fold lower than bufotenine.[67]

TOXICOKINETICS

There are few data on the toxicokinetics of 5-MeO-DMT in humans. Animal studies indicate that 5-MeO-DMT is rapidly absorbed following ingestion and extensively metabolized and conjugated with glucuronide or sulfate prior to renal excretion.[68] Deamination mediated by MAO-A is the major biotransformation pathway, similar to tryptamine and DMT. Other potential pathways include N-oxygenation, N-demethylation, and O-demethylation. The latter pathway catalyzed by CYP2D6 produces the active metabolite, bufotenine in *in vitro* studies with human liver microsomes.[69] Studies in mice indicate that the CYP2D6-mediated elimination of 5-MeO-DMT is nonlinear (Michaelis-Menten kinetics) at high doses, suggesting that the risk of intoxication does not increase proportionally to the dose.[70] Inhibition of deamination by monoamine oxidase A inhibitors diverts 5-MeO-DMT biotransformation to other metabolic pathways including O-demethylation, resulting in increased production of bufotenine.

HISTOPATOLOGY AND PATHOPHYSIOLOGY

In radiolabeling studies of human 5-HT$_{2A}$ receptors, the binding affinities (K_i) of 5-MeO-DMT and DMT for the 5-HT$_{2A}$ receptor are 25 nM and 230 nM, respectively; thus, the affinity for the 5-HT$_{2A}$ receptor is substantially higher for 5-MeO-DMT than DMT. In general, the affinity of tryptamines for this receptor is lower than phenethylamine compounds (e.g., DOB, LSD).[71] There are at least 7 distinct classes of 5-HT

receptors with some (e.g., 5-HT$_2$) classes including multiple subtypes; most indoleamine and phenethylamine hallucinogens predominately stimulate 5-HT$_{2A}$ receptors. Animal studies suggest that 5-MeO-DMT differs from DOM because the psychedelic effects of the former compound rely on activation of both the 5-HT$_{1A}$ and 5-HT$_2$ receptors, whereas the psychedelic effects of DOM depends primarily on agonist action on the 5-HT$_2$ receptors.[72,73]

CLINICAL RESPONSE

Self-experimentation studies suggest that 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) produces similar or more potent psychedelic effects as DMT or bufotenine despite differences in 5-HT$_{2A}$ binding.[64] Following intranasal administration of 5-methoxy-DMT to volunteers, psychedelic effects begin within about 5 minutes, peak within about one-half hour and resolve within ~1 hour. The ingestion of an extract of Syrian rue containing harmaline and the smoking and snorting of about 25–30 mg 5-methoxy-N,N-dimethyltryptamine was associated with the development of disorientation, agitation, hyperthermia, and tachycardia.[74] The patient was extremely combative, and he later developed rhabdomyolysis that responded to fluid administration without the occurrence of renal dysfunction. The differential diagnosis included serotonin syndrome (5-methoxy-DMT) and monoamine oxidase inhibitor toxicity (harmaline).

DIAGNOSTIC TESTING

Thin layer chromatography, gas chromatography, and high performance liquid chromatography are relatively insensitive methods for detecting 5-MeO-DMT, DMT, or bufotenine concentrations compared with gas chromatography/mass spectrometry. DMT may be quantitated using gas chromatography with nitrogen phosphorus detection following liquid-liquid extraction.[23] The LOD and LLOQ for this method are 0.5 ng/mL and 5 ng/mL, respectively. The use of high performance liquid chromatography with fluorescence detection following protein precipitation allows the quantitation of harmala alkaloids with a LLOQ of ~2 ng/mL.[23] Analysis of urine samples by high performance liquid chromatography/electrospray ionization/tandem mass spectrometry allows the detection of 5-MeO-DMT and DMT with LOD of 0.1 ng/mL and a coefficient of variation of 9–11% and 4–5%, respectively.[75] Case reports associate mild hypokalemia, hyperglycemia, and mild hepatorenal dysfunction with 5-MeO-DIPT intoxication.[30]

TREATMENT

Treatment of 5-MeO-DMT intoxication is supportive, similar to 5-methoxy-diisopropyltryptamine (foxy) or *N,N*-dimethyltryptamine (DMT) intoxication.

References

1. Pomilio AB, Vitale AA, Ciprian-Ollivier J, Cetkovich-Bakmas M, Gomez R, Vazauez G. Ayahuasca: an experimental psychosis that mirrors the transmethylation hypothesis of schizophrenia. J Ethnopharmacol 1999;65: 29–51.

2. McKenna DJ. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. Pharmacol Ther 2004;102:111–129.

3. Tupper KW. The globalization of ayahuasca: harm reduction or benefit maximization? Int J Drug Policy 2008;19: 297–303.

4. Bullis RK. The "vine of the soul" vs. the Controlled Substances Act: implications of the hoasca case. J Psychoactive Drugs 2008;40:193–199.

5. Blackledge RD, Taylor CM. *Psychotria viridis*—a botanical source of dimethyltryptamine (DMT). Microgram J 2003;1:18–22.

6. Melchior C, Collins MA. The route and significance of endogenous synthesis of alkaloids in animals. Crit Rev Toxicol 1982;9:313–356.

7. Strassman RJ, Qualls CR. Dose-response study of *N,N*-dimethyltryptamine in humans. I. Neuroendocrine, autonomic, and cardiovascular effects. Arch Gen Psychiatry 1994;51:85–97.

8. Buckholtz NS, Boggan WO. Monamine oxidase inhibition in brain and liver produced by β-carbolines: structure-activity relationships and substrate specificity. Biochem Pharmacol 1977;26:1991–1996.

9. Kim H, Sablin SO, Ramsay RR. Inhibition of monoamine oxidase A by β-carboline derivatives. Arch Biochem Biophys 1997;337:137–142.

10. Douglas KT, Sharma RK, Walmsley JF, Hider RC. Ionization processes of some harmala alkaloids. Mol Pharmacol 1983;23:614–618.

11. Grella B, Dukat M, Young R, Teitler M, Herrick-Davis K, Gauthier CB, Glennon RA. Investigation of hallucinogenic and related beta-carbolines. Drug Alcohol Depend 1998;50:99–107.

12. Rivier L, Lindgren JE. Ayahuasca, the South American hallucinogenic drink: an ethnobotanical and chemical investigation. Econ Bot 1972;29:101–129.

13. Halpern JH. Hallucinogens and dissociative agents naturally growing in the United States. Pharmacol Ther 2004; 102:131–138.

14. McKenna DJ, Towers GH, Abbott F. Monoamine oxidase inhibitors in South American hallucinogenic plants: trypt-amine and beta-carboline constituents of ayahuasca. J Ethnopharmacol 1984;10:195–223.

15. Halpern JH, Pope HG Jr. Hallucinogens on the Internet: a vast new source of underground drug information. Am J Psychiatry 2001;158:481–483.

16. Callaway JC, Brito GS, Neves ES. Phytochemical analyses of *Banisteriopsis caapi* and *Psychotria viridis*. J Psychoactive Drugs 2005;37:145–150.

17. Schwarz MJ, Houghton PJ, Rose S, Jenner P, Lees AD. Activities of extract and constituents of *Banisteriopsis caapi* relevant to parkinsonism. Pharmacol Biochem Behav 2003;75:627–633.

18. Schultes RE. Fifteen years of study of psychoactive snuffs of South America: 1967-1982–a review. J Ethnopharmacol 1984;11:17–32.

19. McKenna DJ, Towers GH, Abbott FS. Monoamine oxidase inhibitors in South American hallucinogenic plants Part 2: constituents of orally-active Myristicaceous hallucinogens. J Ethnopharmacol 1984;12:179–211.

20. Frison G, Favretto D, Zancanaro F, Fazzin G, Ferrara SD. A case of β-carboline alkaloid intoxication following ingestion of *Peganum harmala* seed extract. Forensic Sci Int 2008;179:e37–e43.

21. Herraiz T, Gonzalez D, Ancin-Azpilicueta C, Aran VJ, Guillen H. β-Carboline alkaloids in *Peganum harmala* and inhibition of human monamine oxidase (MAO). Food Chem Toxicol 2010;48:839–845.

22. Pires AP, De Oliveira CD, Moura S, Dörr FA, Silva WA, Yonamine M. Gas chromatographic analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. Phytochem Anal 2009;20:149–153.

23. Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GS, Mash DC. Quantitation of *N,N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with ayahuasca. J Anal Toxicol 1996;20:492–497.

24. Dobkin de Rios M. Commentary. On "Human pharmacology of hoasca": a medical anthropology perspective. J Nerv Ment Dis 1996;184:95–98.

25. Gable RS. Risk assessment of ritual use of oral dimethyltryptamine (DMT) and harmala alkaloids. Addiction 2007; 102:24–34.

26. Rodd R. Reassessing the cultural and psychopharmacological significance of *Banisteriopsis caapi*: preparation, classification and use among the Piaroa of southern Venezuela. J Psychoactive Drugs 2008;40:301–307.

27. Fabregas JM, Gonzalez D, Fondevila S, Cutchet M, Fernandez X, Barbosa PC, et al. Assessment of addiction severity among ritual users of ayahuasca. Drug Alcohol Depend 2010;111:257–261.

28. Cakic V, Potkonyak J, Marshall. Dimethyltryptamine (DMT): subjective effects and patterns of use among Australian recreational users. Drug Alcohol Depend 2010;111:30–37.

29. Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R. Dose-response study of *N,N*-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry 1994;51:98–108.

30. Yuruktumen A, Karaduman S, Bengi F, Fowler J. Syrian rue tea: a recipe for disaster. Clin Toxicol 2008;46: 749–752.

31. Ben Salah N, Amamou M, Jerbi Z, Ben Salah F, Yacoub M. [A case of overdose with Peganum harmala L.] J Toxicol Clin Exp 1986;6:319–322. [French]

32. Barker SA, Monti JA, Christian ST. *N,N*-dimethyltryptamine: an endogenous hallucinogen. Int Rev Neurobiol 1981;22:83–110.

33. Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP, Poland RE, et al. Pharmacokinetics of hoasca alkaloids in healthy humans. J Ethnopharmacol 1999;65:243–256.

34. Riba J, Valle M, Urbano G, Yritia M, Morte A, Barbanoj MJ. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther 2003; 306:73–83.

35. Yu A-M, Idle JR, Krausz KW, Kupfer A, Gonzalez FJ. Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic β-carboline alkaloids harmaline and harmine. J Pharmacol Exp Ther 2003;305:315–322.

36. McIlhenny EH, Riba J, Barbanoj MJ, Strassman R, Barker SA. Methodology for and the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. Biomed Chromatogr 2010.

37. Callaway JC, Airaksinen MM, McKenna DJ, Brito GS, Grob CS. Platelet serotonin uptake sites increased in drinkers of ayahuasca. Psychopharmacology (Berl) 1994; 116:385–387.

38. Gillin JC, Kaplan J, Stillman R, Wyatt RJ. The psychedelic model of schizophrenia: the case of *N,N*-dimethyltryptamine. Am J Psychiatry 1976;133:203–208.

39. Rosenberg DE, Isbell H, Miner EJ, Logan CR. The effect of *N,N*-dimethyltryptamine in human subjects tolerant to lysergic acid diethylamide. Psychopharmacologia 1964;13: 217–227.

40. Callaway JC, Grob CS. Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. J Psychoactive Drugs 1998;30:367–369.

41. Glennon RA, Dukat M, Grella B, Hong S, Costantino L, Teitler M, et al. Binding of β-carbolines and related agents at serotonin (5-HT2 and 5-HT1A), dopamine D2 and benzodiazepine receptors. Drug Alcohol Depend 2000;60: 121–132.

42. Airaksinen MM, Svensk H, Tuomisto J, Komulainen H. Tetrahydro-beta-carbolines and corresponding tryptamines: *in vitro* inhibition of serotonin and dopamine uptake by human blood platelets. Acta Pharmacol Toxicol (Copenh) 1980;46:308–313.

43. Deliganis AV, Pierce PA, Peroutka SJ. Differential interactions of dimethyltryptamine (DMT) with 5-HT1A and 5-HT2 receptors. Biochem Pharmacol 1991;41: 1739–1744.

44. Gouzoulis-Mayfrank E, Heekeren K, Neukirch A, Stoll M, Stock C, Obradovic M, Kovar K-A. Psychological effects of (*S*)-ketamine and *N,N*-dimethyltryptamine (DMT): a double-blind, cross-over study in healthy volunteers. Pharmacopsychiatry 2005;38:310–311.

45. Strassman RJ. Human psychopharmacology of *N,N*-dimethyltryptamine. Behav Brain Res 1996;73:121–124.

46. Halpern JH, Sherwood AR, Passie T, Blackwell KC, Ruttenber AJ. Evidence of health and safety in American members of a religion who use a hallucinogenic sacrament. Med Sci Monit 2008;14:SR15–SR22.

47. Grob CS, McKenna DJ, Callaway JC, Brito GS, Neves ES, Oberlaender G, et al. Human psychopharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. J Nerv Ment Dis 1996;184:86–94.

48. Oliveira CD, Moreira CQ, de Sá LR, Spinosa Hde S, Yonamine M. Maternal and developmental toxicity of ayahuasca in Wistar rats. Birth Defects Res B Dev Reprod Toxicol 2010;89:207–212.

49. Yritia M, Riba J, Ortuno J, Ramirez A, Castillo A, Alfaro Y, et al. Determination of *N,N*-dimethyltryptamine and β-carboline alkaloids in human plasma following oral administration of ayahuasca. J Chromatogr B 2002;779: 271–281.

50. Forsstrom T, Tuominen J, Karkkainen J. Determination of potentially hallucinogenic *N*-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. Scand J Clin Lab Invest 2001;61:547–556.

51. Kikura-Hanajiri R, Hayashi M, Saisho K, Goda Y. Simultaneous determination of nineteen hallucinogenic tryptamines/β-carbolines and phenethylamines using gas chromatography-mass spectrometry and liquid chromatography-electrospray ionization-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 825:29–37.

52. McIlhenny EH, Pipkin KE, Standish LJ, Wechkin HA, Strassman R, Barker SA. Direct analysis of psychoactive tryptamine and harmala alkaloids in the Amazonian botanical medicine ayahuasca by liquid chromatography-electrospray ionization-tandem mass spectrometry. J Chromatogr A 2009;1216:8960–8968.

53. Vorce SP, Sklerov JH. A general screening and confirmation approach to the analysis of designer tryptamines and phenethylamines in blood and urine using GC-EI-MS and HPLC-electrospray-MS. J Anal Toxicol 2004;28:407–410.

54. Raisanen M, Karkkainen J. Mass fragmentographic quantification of urinary *N,N*-dimethyltryptamine and bufotenine. J Chromatogr 1979;162:579–584.

55. Sitaram BR, Blackman GL, McLeod WR, Vaughan GN. The ion-pair extraction, purification, and liquid chromatographic analysis of indolealkylamines in human urine. Anal Biochem 1983;128:11–20.

56. McIlhenny EH, Riba J, Barbanoj MJ, Strassman R, Barker SA. Methodology for determining major constituents of ayahuasca and their metabolites in blood. Biomed Chromatogr 2011 [Epub ahead of print]

57. Strassman RJ, Qualls CR, Berg LM. Differential tolerance to biological and subjective effects of four closely spaced doses of *N*,*N*-dimethyltryptamine in humans. Biol Psychiatry 1996;39:784–795.

58. Sklerov J, Levine B, Moore KA, King T, Fowler D. A fatal intoxication following the ingestion of 5-methoxy-*N,N*-dimethyltryptamine in an Ayahuasca preparation. J Anal Toxicol 2005;29:838–841.

59. Narasimhachari N, Baumann P, Pak HS, Carpenter WT, Zocchi AF, Hokanson L, et al. Gas chromatographic-mass spectrometric identification of urinary bufotenin and dimethyltryptamine in drug-free chronic schizophrenic patients. Biol Psychiatry 1974;8:293–305.

60. Smythies JR, Morin RD, Brown GB. Identification of dimethyltryptamine and *O*-methylbufotenine human cerebrospinal fluid by combined gas chromatography/mass spectrometry. Biol Psychiatry 1979;14:549–556.

61. Mandel LR, Prasad R, Lopez-Ramos B, Walker RW. The biosynthesis of dimethyltryptamine *in vivo*. Res Commun Chem Pathol Pharmacol 1977;16:47–58.

62. Riba J, Anderer P, Jane F, Saletu B, Barbanoj MJ. Effects of the South American psychoactive beverage Ayahuasca on regional brain electrical activity in humans: a functional neuroimaging study using low-resolution electromagnetic tomography. Neuropsychobiology 2004;50:89–101.

63. Glennon RA, Rosecrans JA. Indolealkylamine and phen-alkylamine hallucinogens: a brief overview. Neurosci Biobehav Rev 1982;6:489–497.

64. Ott J: Pharmepéna-psychonautics: human intranasal, sublingual and oral pharmacology of 5-methoxy-*N*,*N*-dimethyl-tryptamine. J Psychoactive Drugs 2001;33:403–407.

65. Ott J. Pharmahuasca: human pharmacology of oral DMT plus harmine. J Psychoactive Drugs 1999;31:171–177.

66. Shulgin A, Shulgin A. Tryptamines I have known and loved: the continuation. Berkeley, CA: Transform Press; 1997:566–568.

67. Benington F, Morin RD, Clark LC Jr. 5-Methoxy-*N*,*N*-dimethyltryptamine, a possible endogenous psychotoxin. Ala J Med Sci 1965;2:397–403.

68. Shen HW, Jiang XL, Winter JC, Yu AM. Psychedelic 5-methoxy-*N*,*N*-dimethyltryptamine: metabolism, pharmacokinetics, drug interactions, and pharmacological actions. Curr Drug Metab 2010;11:659–666.

69. Yu AM, Idle JR, Herraiz T, Küpfer A, Gonzalez FJ. Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine *O*-demethylase. Pharmacogenetics 2003;13:307–319.

70. Shen HW, Jiang XL, Yu AM. Nonlinear pharmacokinetics of 5-methoxy-*N*,*N*-dimethyltryptamine in mice. Drug Metab Dispos 2011;39:1227–1234.

71. Fantegrossi WE, Murnane AC, Reissig CJ. The behavioral pharmacology of hallucinogens. Biochem Pharmacol 2008;75:17–33.

72. Krebs-Thomson K, Ruiz EM, Masten V, Buell M, Geyer MA. The roles of 5-HT1A and 5-HT2 receptors in the effects of 5-Me)-DMT on locomotor activity and prepulse inhibition in rats. Psychopharmacology 2006;189:319–329.

73. Winter JC, Filipink RA, Timineri D, Helsley SE, Rabin RA. The paradox of 5-methoxy-*N*, *N*-dimethyltryptamine: an indoleamine hallucinogen that induces stimulus control via 5-HT1A receptors. Pharmacol Biochem Behav 2000;65:75–82.

74. Brush DE, Bird SB, Boyer EW. Monoamine oxidase inhibitor poisoning resulting from Internet misinformation on illicit substances. J Toxicol Clin Toxicol 2004;42:191–195.

75. Forsstrom T, Tuominen J, Karkkainen J. Determination of potentially hallucinogenic *N*-demethylated indoleamines in human urine by HPLC/ESI-MS-MS. Scand J Clin Lab Invest 2001;612:547–556.

Chapter 54

BETEL QUID and ARECA NUT

HISTORY

Mastication of the areca nut is an ancient custom dating back at least to Herodotus in 340 BC and the Maharmasa, a Ceylonese document from 504 BC.[1,2] References to the areca nut appeared in the Sanskrit medical literature during the early part of the First Millennium, and later in Hindu and Buddhist writings. The expansion of Buddhist practices brought the areca nut to Tibet and southern China as a stimulant, aphrodisiac, antihelminthic, and digestive agent. The areca nut was a valuable masticatory in the Wei-Chien dynasty in 421 AD,[3] and Arab physicians use it frequently in the 10th century AD. Marco Polo mentioned the use of this nut during his travels in 1298. The Dutch introduced the areca nut into Taiwan during the 18th century from Malaysia. During the 19th century in England, the areca nut was briefly a constituent of toothpaste.[4]

BOTANICAL DESCRIPTION

The areca nut is the ripe seed of the betel palm (*Areca catechu*). Betel nut is not the correct biologically name for the areca nut despite common usage of the term, betel nut. The betel pepper (*Piper betel* L.) is a climbing shrub that is native to the West Indies.

Common Name: Betel palm

Scientific Name: *Areca catechu* L.

Botanical Family: Arecaceae (Palm)

Physical Description: The betel palm grows to 100 feet (~30 m) in height with a slender trunk only 6 inches (~15 cm) in diameter. The ripe areca nut is yellow to scarlet on the surface. Throughout the year, this tropical palm bears ovoid to oblong fruit with a pointed apex 1–2 inches (~3–5 cm) in length.

Distribution and Ecology: Although the betel palm is a native of Malaysia, the betel palm is widely cultivated in Southeast Asia, East Africa, India, Taiwan, and New Guinea.

IDENTIFYING CHARACTERISTICS

Structure

The areca nut contains a variety of alkaloids including arecoline, arecaidine, guvacine, and guvacoline (norarecoline).[5] Figure 54.1 displays the chemical structures of these alkaloids.

Physiochemical Properties

The areca nut has a slightly bitter and stringent taste. The primary alkaloid, arecoline, is a muscarinic (M_1, M_2, M_3) receptor agonist and a weak ganglionic nicotinic agonist, whereas arecaidine is only a M_2 muscarinic receptor agonist.[6] Arecoline is structurally related to methacholine, which produces dose-related bronchoconstriction in asthmatic patients.[7] The bronchoconstrictive properties of arecoline are approximately 10% of the bronchoconstrictive effects of methacholine. Human studies on saliva during the mastication of betel quid (chewable mass of material, See Methods of Abuse for description) indicate that *in situ* nitrosation of compounds from the areca nut produce a variety of
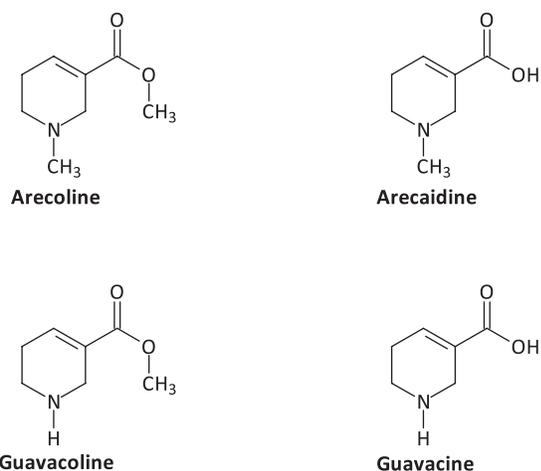
FIGURE 54.1. Chemical structures of common areca nut alkaloids.

nitrosamines including *N*-nitrosoguvacoline (CAS RN: 55557-02-3) and 3-(methylnitrosamino)propionitrile (CAS RN: 60153-49-3). When the betel quid contains tobacco, the chewing of the quid releases volatile nitrosamines into the mouth including *N*-nitrosodiethylamine (CAS RN: 55-18-5) and *N*-nitrosodimethylamine (CAS RN: 62-75-9) along with 1-nitrosoanabasine (CAS RN: 1133-64-8), *N*-nitrosonornicotine (CAS RN: 16543-55-8), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (CAS RN: 64091-50-5).[8] Autooxidation of polyphenol compounds in areca nut and catechu during chewing produces reactive oxygen species.

EXPOSURE

Epidemiology

Several hundred million people worldwide use the areca nut as a psychoactive substance, particularly in Southeast Asia and India. The practice of chewing areca nuts is a traditional psychoactive activity of native populations in the Indian subcontinent, south Asia, and Melanesia (Vanuatu, Solomon Islands, Papua New Guinea, Fiji). Migration of these populations resulted in the introduction of betel quid use into Africa, Australia, China, the United Kingdom, and the United States.[9] The use of the areca nut is one of the most commonly used psychoactive substances in the world along with alcohol, nicotine, and caffeine.[10] Betel products with and without tobacco are relatively inexpensive and available in some specialty shops (e.g., East Asian grocery stores) in parts of the United States.[11] The areca nut is a prohibited food product in the United States, but the use of betel quid is not banned. Medicinal uses of the areca nut include the treatment of tapeworms and roundworms in domestic animals as well as the herbal treatment of fever, venereal disease, headaches, stomach pain, and rheumatism in humans.

TABLE 54.1. Approximate Alkaloid Content of Fresh Australian Areca Nuts.[12]

Alkaloid	Content*
Arecoline	0.30–0.63
Arecaidine	0.31–0.66
Guvacoline	0.03–0.06
Guvacine	0.19–0.72

*Estimated % weight/weight (dry).

Sources

Table 54.1 lists the approximate alkaloid content of fresh areca nuts from Australia. The arecoline concentrations in areca nuts from India and New Guinea following steam distillation were slightly higher than the Australian nuts as measured by high performance liquid chromatography.[12] Minor alkaloids include nicotine, methyl nicotinate, ethyl nicotinate, methyl-*N*-methyl piperidine-3-carboxylate, ethyl-*N*-methyl piperidine-3-caroxylate, and ethyl-*N*-methyl-1,2,5,6-tetrahydropyridine-3-carboxyate. The arecoline content of commercial areca nuts varies from 0–1.4%.[13] Areca nut processing reduces the arecoline content, particularly following boiling in water.[14]

The betel leaf and inflorescence (flower, pod) is a frequent constituent of Taiwanese betel quid. Fresh inflorescence from *Piper betle* contains approximately 15 mg safrole/g (wet weight) along with safrole metabolites including dihydroxychavicol (about 9 mg/g) and eugenol (about 2.5 mg/g).[15] Eugenol methyl ester, isoeugenol, and quercetin are minor constituents of these inflorescence. Betel oil is a volatile extract of the betel leaf, which contains psychoactive phenols (chavicol, chavibetol or betel-phenol) eugenol, hydroxychavicol, and the stimulant, cadinene.[16]

Methods of Use

The nut is consumed either fresh or dried by sunshine, baking, or roasting. Although areca nut can be chewed alone, most use of the areca nut involves the addition of other ingredients into a chewable mass called a quid (pan, paan).[16] The betel quid typically consists of areca nut, leaves of psychoactive plants (e.g. betel pepper), and slaked lime (calcium hydroxide) paste. The quid typically is placed in the lateral gingiva for 15–20 minutes with effects occurring within 2 minutes and an average duration of 15–20 minutes.[17] The quid may be held in

the buccal vestibule over a long duration with occasional chewing; alternatively, the quid may be swallowed or expectorated. The source of the betel pepper leaves is an unrelated climbing shrub (*Piper betel*); burnt seashells, reef coral, and/or limestone are the source of the slaked lime paste. Other sources of psychoactive leaves include kratom [*Mitragyna speciosa* (Korth.) Havil.) in Southeast Asia, nutmeg (*Myristica fragrans* Houtt.) in India, and kava (*Piper methysticum* G. Forst.) in Melanesia. The slaked lime increases the pH of the oral cavity, resulting in an increased absorption of nicotine through the oral mucosa. Catechu is a reddish gum produced from the sap of the Malaysian acacia tree [*Acacia nilotica* (L.)Delile] that can be added to the quid.

The method of areca nut use depends on the region. Betel quid is a popular form of areca nut use in India, Pakistan, Bangladesh, and Sri Lanka where fresh, dried, or cured areca nut, slaked lime, catechu, and flavoring ingredients are wrapped in betel leaves. These flavoring ingredients include cloves, coconut, sugar crystals, camphor, amber, nutmeg, mace, saffron, anise, turmeric, mustard, and cardamon. Tobacco is often added to the mass, which is placed in the mouth and masticated during the day. Migrant communities from these areas retain their use of betel quid in their new countries.[18] Similar use of the betel quid occurs in southeastern Asia (e.g., Cambodia, Thailand, Myanmar, The Lao People's Republic, Philippines). In Taiwan and China, the unripe betel quid contains green areca nut, slaked lime, betel inflorescence; occasionally, betel leaves are used in the quid without tobacco. Some aboriginal groups in Asia use betel stems in the quid.[19] In Papua New Guinea, the betel quid often contains wild ginger. Betel leaf is perishable, but nonperishable forms (e.g., supari, mawa, gutka, pan masala) of betel quid are available commercially.[10] *Pan masala* is a preparation of areca nut, catechu, cardamon, lime, flavoring agents, and perfuming material. Gutkha is a variation of *pan masala* that contains tobacco.

DOSE EFFECT

Adverse effects associated with the infusion of 5 mg intravenous arecoline to Alzheimer patients included diaphoresis, flushing, nausea, and Mobitz type II second-degree atrioventricular block with a ventricular rate of 40 bpm.[20] A constant infusion of 1.5 mg/hour was not associated with adverse effects. In dog studies, the oral administration of 1 mg arecoline hydrobromide/kg produced some slowing of the heart rate and respirations, but no obvious changes in behavior, whereas there was no observable effect from the oral administration of 0.1 mg arecoline hydrobromide/kg.[21]

TOXICOKINETICS

Peak plasma concentrations of arecoline occur about 5–10 hours after dermal application of the areca nut, whereas the oral mucosal absorption of arecoline is rapid. The areca nut and tobacco contain secondary and tertiary amines that undergo nitrosation in the saliva during betel quid-chewing following reactions with available nitrites and catalysts (e.g., thiocyanate). These nitrosamines include *N*-nitrosoguvacoline and 3-(methylnitrosamino)propionitrile. The amount of nitrosation depends on several factors, including individual variation and salivary pH.[22] Volatile nitrosamines include *N*-nitrosodiethylamine and *N*-nitrosodimethylamine. Endogenous formation of nitrosamines also results from the ingestion of precursors of nitrosamines (e.g., nitrite, nitrosatable amines), particularly in the acid environment of the stomach. Formation of reactive oxygen species also develops in the oral cavity during betel-quid chewing. Hydrolysis of arecoline and guvacoline to arecaidine and guvacine, respectively, occurs in the mouth during betel-quid chewing, particularly when lime is present in the quid.[23] These latter 2 compounds are potent inhibitors of GABA uptake. Arecoline readily crosses the blood–brain barrier with brain/plasma concentration close to unity.[24] The rapid hydrolysis of arecoline by plasma esterases results in the short plasma half-life of arecoline. In a study of 15 Alzheimer patients receiving 5 mg intravenous arecoline, the mean terminal plasma half-life of arecoline was 9.3 ± 4.5 minutes with plasma arecoline concentrations returning to baseline within ~1 hour.[20] The mean volume of distribution was 2.55 ± 2.05 L/kg (range, 0.63–6.1 L/kg) with a clearance of 13.6 ± 5.8 L/min. The major urinary metabolites of areca nut and arecoline are arecaidine (urinary elimination half-life, ~4 h) and to a lesser extent, *N*-methylnipecotic acid (urinary elimination half-life, ~8 h) as measured by isotope-dilution liquid chromatography/tandem mass spectrometry.[25] The kidney excretes little arecoline unchanged.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The chewing of areca nut is associated with sympathetic activation including increased heart rate and elevated plasma epinephrine and norepinephrine concentrations.[26] Volunteer studies suggest that areca nut chewing causes transient tachycardia and elevation of blood pressure in novice users, but habituation to the pressor effects occurs with chronic use.[17] Experimental studies indicate that areca nut-chewing modulated metabolic signals regarding appetite consistent with the use of betel quid for reducing hunger and increasing stamina,

but the dose response is nonlinear.[27] The effects of betel chewing are complex and potentially involve parasympathetic, GABAergic, and sympathetic activity in the central nervous system.[23] In the presence of lime, hydrolysis of arecoline and guvacoline to arecaidine and guvacine, respectively, occurs. These hydrolyzed substances are strong inhibitors of GABA uptake. Rodent studies indicate that arecoline is strong M_1- and M_2-muscarinic receptor agonist.[28]

CLINICAL RESPONSE

Betel Quid/Areca Nut Use

Desirable effects associated with the use of the areca nut and betel quid include a sense of well-being, euphoria, increased alertness, warmth, sweating, salivation, reduced hunger, and increased stamina. Table 54.2 lists the initial and subsequent effects associated with betel quid chewing by 170 habitual users during an observational study of recreational betel quid use. Effects reported in experimental studies include tachycardia, flushing, diaphoresis, and heightened alertness.[23] Despite the widespread use of areca nut and betel quid, reports of acute toxicity associated with betel quid use are rare. In a retrospective study of 42,000 calls to the Taiwan Poison Control Center over ~10 years, there were 12

TABLE 54.2. Effects Associated with Betel-Quid Chewing based on Personal Observations of 170 Habitual Users.[23]

Time	Clinical Effects	%
Initial Effects		
	Dizziness	67
	Hot sensation	65
	Palpitation	44
	Sweating	28
	Heightened alertness	16
	Epigastric discomfort	9
Subsequent Effects		
	Heightened alertness	64
	Hot sensation	62
	Palpitation	47
	Combat of cold (increased cold tolerance)	43
	Sweating	41
	Diminished thirst	38
	Diarrhea	34
	Happy feeling	19
	Calmness	17
	Dizziness	15
	Alleviation of constipation	15
	Epigastric discomfort	15
	Prevention of hunger	11
	Increased respiration	10

adults with probable areca nut-related toxicity from intentional use.[29] Of the 7 cases involving only areca nut use, the most common effects were nausea, vomiting, abdominal colic, salivation, sweating, tremor, dizziness, palpitations, hypertension, tachycardia, and tachypnea. Most effects following the use of the areca nut or betel quid are transient and mild with effects beginning within a few minutes to 1 hour after mastication starts. Complete recovery generally occurs within 24 hours. Neophyte users may develop dizziness, vertigo, nausea, diaphoresis, and feeling of throat constriction.[1] Heavy areca nut users can develop cholinergic toxicity manifest by salivation, diaphoresis, lacrimation, diarrhea, urinary incontinence, miosis, gastrointestinal irritation, and tremor.[30,31] Case reports indicate that areca nut chewing in asthmatic patients can cause acute bronchospasm as a result of the muscarinic effects of arecoline.[32] Additionally, heavy use of areca nut has been associated with auditory hallucinations, delusions, transient psychosis, and dystonic reactions in schizophrenic patients on phenothiazine antipsychotic drugs.[33] Rare case reports temporally associate the use of betel quid with the development of acute myocardial infarction,[34] but the contribution of the betel quid use to myocardial ischemia is unclear. In a Taiwanese population-based cohort study of 4,049 participants aged 60 years or older and 2,462 participants aged 50–66 years, there was a weak association between mortality, particularly cerebrovascular death and ever-chewing betel quid.[35] The hazard ratio for the increased risk of cerebrovascular death in ever-chewers was 1.66 (95% CI: 1.19–2.30).

Nonmalignant pathologic lesions of the mouth associated with chronic betel quid use include severe erosion of the teeth, tooth discoloration, gingivitis, dark brown staining of the teeth, and deep brownish red discoloration of the oral mucosa (betel chewer's mucosa).[36] Case reports associate the habitual ingestion of betel quid with the milk-alkali syndrome (metabolic alkalosis, hypercalcemia, nephrocalcinosis, renal insufficiency) resulting from the ingestion of large amounts of slaked lime paste.[37] A retrospective hospital-based cross-sectional study of Taiwanese adults suggested an association between chronic kidney disease and betel quid use.[38] The odds ratio for the betel quid users (9.4% of study group) after adjustment for age, sex, hypertension, and diabetes was 1.812 (95% CI: 1.072–3.061, $P = .026$). However, the mechanism for this possible association remains unclear.

Abstinence Syndrome

Chronic areca nut chewing is associated with habituation, addiction, and withdrawal. These withdrawal symptoms are usually mild, similar to caffeine or nicotine

withdrawal, and include general discomfort, malaise, irritability, sore gums, and poor concentration.[39] Case reports associate chronic betel quid chewing during pregnancy with neonatal withdrawal symptoms (irritability, hypertonia) that persisted up to 5 days after birth.[30,40]

Reproductive Abnormalities

Some studies suggest that the chronic use of betel chewing during pregnancy causes adverse pregnancy outcomes. An observational study of 6 newborns from betel chewing mothers reported 1 neonate with withdrawal syndrome and 1 neonate with low birth weight, hyporeflexia, and hypotonia.[41] In a study of 62 aboriginal women in southern Taiwan with adverse pregnancy outcomes and 124 age-matched women, the prevalence of adverse pregnancy outcomes was greater in the study group.[42] After adjusting for maternal illness and number of previous pregnancies covariates, the prevalence of adverse pregnancy outcome among betel quid-chewing women was 2.8-fold higher (OR = 2.8, 95% CI:1.2–6.8) than nonbetel quid chewers.

A study of 453 pregnant women did not demonstrate a difference in birth weight or congenital abnormalities based on areca nut use.[43] A study compared the pregnancy outcomes of 400 betel chewing Papua New Guinean women with 400 nonbetel chewing controls matched for parity and province of birth.[44] There was a significant difference ($P = .05$) in the mean birth weight between the study group (2,998.5 ± 492.5 g) and the controls (3,079.5 ± 464.1 g). However, there was no statistically significant difference in Apgar scores, congenital abnormalities, or irritability (as a measure of withdrawal syndromes).

Carcinogenesis

The International Agency for Research on Cancer (IARC) lists betel quid (with or without tobacco) and areca nut as human carcinogens (Group 1) based on studies associating betel quid without tobacco with oral cancers.[45] Several case-control and cohort studies associate betel quid-containing tobacco with cancers of the oral cavity, pharynx, and esophagus as well as premalignant oral lesions. The increased risk of these cancers remains after adjustment for smoking and alcohol use. In a community-based nested case-control study of 1,029 volunteers over the age of 30 years in Sri Lanka, the adjusted odds ratio for oral potentially malignant disorders (oral erythroplakia, leukoplakia, submucous fibrosis, lichen planus) in volunteers using betel quid daily was 10.6 with a large 95% confidence interval (95% CI: 3.6–31.0).[46] The risk of these premalignant

lesions in daily betel quid chewers was dose-dependent, and the attributable risk was 90.6%. The chewing of betel quid is strongly associated with the development of oral submucous fibrosis[47] and oral leukoplakia,[48] which are precancerous lesions of the buccal mucosa. The former is a chronic disorder characterized by fibrosis of the mucosal lining of the upper gastrointestinal tract including the mouth, pharynx, and upper one-third of the esophagus. Oral leukoplakia is primarily a homogeneous (i.e., predominately white) patch or plaque on the oral mucosa most often associated with tobacco use. The appearance of the discoloration can also be speckled (red/white) or nodular. A follow-up study of a hospital-based population of 166 patients with oral leukoplakia demonstrated an annual malignant transformation rate of 2.9% over 29 months.[49] Nodular leukoplakia has the highest malignant transformation rate of the 3 types of leukoplakia.[50] In a cohort of 66 patients with oral submucous fibrosis followed an average of 8 years, the malignant transformation rate for oral cancer was approximately 4.5% during this period.[51]

DIAGNOSTIC TESTING

Analytic Methods

Methods for the detection of arecoline in biologic samples (saliva, urine) include capillary gas chromatography with nitrogen phosphorous detection,[52] gas chromatography/mass spectrometry,[53] high performance liquid chromatography/electrospray/quadrupole mass spectrometry,[54] ion-pairing high performance liquid chromatography,[55] and liquid chromatography/tandem mass spectrometry.[56] The lower limit of quantitation (LLOQ) for the latter method is 0.5 ng/mL for both arecoline and arecaidine. The LLOQ for arecoline using high performance liquid chromatography with UV detection is about 12 ng/mL compared with 5–8 ng/mL for other alkaloids (arecaidine, guvacine, guvacoline).[12]

Biomarkers

Serum or plasma arecoline or its metabolite, arecaidine are biomarkers of recent betel quid use. The placement of powdered areca nut in the oral cavity produces a rapid rise in the plasma concentration of arecoline. In a study of 15 fasted, healthy young adults receiving 10 mg arecoline via bioadhesive gels designed to mimic conventional use by allowing arecoline to bypass the portal circulation after sublingual absorption, the mean plasma arecoline concentration approximately 2½ hour after application of the gel to the buccal mucosa was approximately 0.8 ng/mL.[27] After consumption of the gel, the

mean plasma arecoline concentration increased to about 1.4 ng/mL 6 hours after the initial application of arecoline to the buccal mucosa.

Abnormalities

Neuropsychologic testing of chronic areca nut users indicates that the use of traditional amounts of areca nut does not significantly affect visual choice reaction time, digit span, or eye-hand coordination.[57]

TREATMENT

Treatment of acute toxicity associated with areca nut use is supportive, consisting primarily of fluid and electrolyte replacement along with antiemetics as needed. There are few clinical data to guide the use of atropine for obvious cholinergic symptoms (e.g., bradycardia, severe bronchorrhea) during areca nut intoxication. In general, supportive care provides adequate treatment for the transient symptoms associated with areca nut use unless the cholinergic symptoms are life-threatening. Standard treatment for bronchospasm associated with areca nut use includes inhaled β-adrenergic agonists (albuterol), corticosteroids, and ipratropium bromide.

References

1. Burton-Bradley BG. Arecaidinism: betel chewing in transcultural perspective. Can J Psychiatry 1979;24:481–488.

2. Raghavan V, Baruah HK. Areca nut, India's popular masticatory: history, chemistry, and utilization. Econ Bot 1958;12:315–345.

3. Chen KC. [The problem of betel chewing]. Sci Mon 1995;9:718–728. [Chinese]

4. Reichart PA. Toothpastes containing betel nut (*Areca catechu* L.) from England of the nineteenth century. J Hist Med Allied Sci 1984;39:65–68.

5. von Arjungi KN. Areca nut: a review. Arzneimittelforschung 1976;26:951–956.

6. Wolf-Pflugmann M, Lambrecht G, Wess J, Mutschler E. Synthesis and muscarinic activity of a series of tertiary and quaternary *N*-substituted guvacine esters structurally related to arecoline and arecaidine propargyl ester. Arzneimittelforschung 1989;39:539–544.

7. Kiyingi KS. Betel-nut chewing may aggravate asthma. P N G Med J 1991;34:117–121.

8. Nair U, Bartsch H, Hair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes *gutkha* and *pan masala*: a review of agents and causative mechanisms. Mutagenesis 2004;19:251–262.

9. Changrani J, Gany FM, Cruz G, Kerr R, Katz R. Paan and Gutkha use in the United States: a pilot study in Bangladeshi and Indian-Gujarati immigrants in New York City. J Immigr Refug Stud 2006;4:99–110.

10. Gupta PC, Ray CS. Epidemiology of betel quid usage. Ann Acad Med Singapore 2004;33(suppl):31S–36S.

11. Blank M, Deshpande L, Balster RL. Availability and characteristics of betel products in the U.S. J Psychoactive Drugs 2008;40:309–313.

12. Huang JL, McLeish MJ. High-performance liquid chromatographic determination of the alkaloids in betel nut. J Chromatogr 1989;475:447–450.

13. Canniff JP, Harvey W, Harris M. Oral submucous fibrosis: its pathogenesis and management. Br Dent J 1986;160: 429–434.

14. Awang MN. Betel quid and oral carcinogenesis. Singapore Med J 1988;29:589–593.

15. Chang MJ, Ko CY, Lin RF, Hsieh LL. Biological monitoring of environmental exposure to safrole and the Taiwanese betel quid chewing. Arch Environ Contam Toxicol 2002;43:432–437.

16. Pickwell SM, Schimelpfening S, Palinkas LA. "Betelmania". Betel quid chewing by Cambodian women in the United States and its potential health effects. West J Med 1994; 160:326–330.

17. Chu NS. Cardiovascular responses to betel chewing. J Formos Med Assoc 1993;92:835–837.

18. Warnakulasuriya S. Areca nut use following migration and its consequences. Addict Biol 2002;7:127–132.

19. Gupta PC, Warnakulasuriya S. Global epidemiology of areca nut usage. Addict Biol 2002;7:77–83.

20. Asthana S, Greig NH, Holloway HW, Raffaele KC, Berardi A, Schapiro MB, et al. Clinical pharmacokinetics of arecoline in subjects with Alzheimer's disease. Clin Pharmacol Ther 1996;60:276–282.

21. Forbes LS. The relation between method of administration, route or absorption, inhibitory actions and acute toxicity of arecoline hydrobromide in dogs. Ann Trop Med Parasitol 1964;58:119–131.

22. Nair J, Nair UJ, Ohshima H, Bhide SV, Bartsch H. Endogenous nitrosation in the oral cavity of chewers while chewing betel quid with or without tobacco. IARC Sci Publ 1987;84:465–469.

23. Chu NS. Effects of betel chewing on the central and autonomic nervous systems. J Biomed Sci 2001;8:229–236.

24. Soncrant TT, Holloway HW, Greig NH, Rapoport SI. Regional brain metabolic responsivity to the muscarinic cholinergic agonist arecoline is similar in young and aged Fischer-344 rats. Brain Res 1989;487:255–266.

25. Hu CW, Chang YZ, Wang HW, Chao MR. High-throughput simultaneous analysis of five urinary metabolites of areca nut and tobacco alkaloids by isotope-dilution liquid chromatography-tandem mass spectrometry with on-line solid-phase extraction. Cancer Epidemiol Biomarkers Prev 2010;19:2570–2581.

26. Chu N-S. Neurological aspects of areca and betel chewing. Addict Biol 2002;7:111–114.

27. Strickland SS, Veena GV, Houghton PJ, Stanford SC, Kurpad AV. Areca nut, energy metabolism and hunger in Asian men. Ann Hum Biol 2003;30:26–52.

28. Sauerberg P, Fjalland B, Larsen JJ, Bach-Lauritsen T, Falch E, Krogsgaard-Larsen P. Pharmacological profile of a novel class of muscarinic acetylcholine receptor agonists. Eur J Pharmacol 1986;130:125–131.

29. Deng J-F, Ger J, Tsai W-J, Kao W-F, Yang C-C. Acute toxicities of betel nut: rare but probably overlooked events. Clin Toxicol 2001;39:355–360.

30. Hafeman D, Ahsan H, Islam T, Louis E. Betel quid: its tremor-producing effects in residents of Araihazar, Bangladesh. Mov Disord 2006;21:567–571.

31. Nelson BS, Heischober B. Betel nut: a common drug used by naturalized citizens from India, Far East Asia, and the South Pacific Islands. Ann Emerg Med 1999;32:238–243.

32. Taylor RF, al-Jarad N, John LM, Conroy DM, Barnes NC. Betel-nut chewing and asthma. Lancet 1992;339: 1134–1136.

33. Deahl M. Betel nut-induced extrapyramidal syndrome: an unusual drug interaction. Mov Disord 1989;4:330–333.

34. Hung D-Z, Deng J-F. Acute myocardial infarction temporally related to betel nut chewing. Vet Hum Toxicol 1998;40:25–28.

35. Lan TY, Chang WC, Tsai YJ, Chuang YL, Lin HS, Tai TY. Areca nut chewing and mortality in an elderly cohort study. Am J Epidemiol 2007;165:677–683.

36. Trivedy CR, Craig G, Warnakulasuriya S. The oral health consequences of chewing areca nut. Addict Biol 2003; 7:115–125.

37. Wu K-D, Chuang R-B, Lin F-L, Hsu W-A, Jan I-S, Tsai K-S. The milk-alkali syndrome caused by betel nuts in oyster shell paste. Clin Toxicol 1996;34:741–745.

38. Kang I-M, Chou C-Y, Tseng Y-H, Huang C-C, Ho W-Y, Shih C-M, Chen W. Association between betel nut chewing and chronic kidney disease in adults. J Occup Environ Med 2007;29:776–779.

39. Wiesner DM. Betel-nut withdrawal. Med J Aust 1987; 146:453.

40. Lopez-Vilchez MA, Seidel V, Farre M, Garcia-Algar O, Pichini S, Mur A. Areca-nut abuse and neonatal withdrawal syndrome. Pediatrics 2006;117:129–131.

41. Garcia-Algar O, Vall O, Alameda F, Puig C, Pellegrini M, Pacifici R, Pichini S. Prenatal exposure to arecoline (areca nut alkaloid) and birth outcomes. Arch Dis Child Fetal Neonatal Ed 2005;90:F276–F277.

42. Yang MS, Chang FT, Chen SS, Lee CH, Ko YC. Betel quid chewing and risk of adverse pregnancy outcomes among aborigines in southern Taiwan. Public Health 1999;113: 189–192.

43. Taufa T. Betel-nut chewing and pregnancy. P N G Med J 1988;31:229–233.

44. de Costa C, Griew AR. Effects of betel chewing on pregnancy outcome. Aust N Z J Obstet Gynaecol 1982;22: 22–24.

45. International Agency for Research on Cancer. Betel-quid and areca-nut chewing and some areca-nut-derived nitrosamines. IARC Monogr 2004;85:1–295.

46. Amarasinghe HK, usgodaarachchi US, Johnson NW, Lalloo R, Warnakulasuriya S. Betel-quid chewing with or without tobacco is a major risk factor for oral potentially malignant disorders in Sri Lanka: a case-control study. Oral Oncol 2010;46:297–301.

47. Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS. Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. J Oral Pathol Med 1995;24:145–152.

48. Heidelman JF, Graham LL. Severe leukoplakia of the oral cavity induced by habitual use of betel nut quid. J Indiana Dent Assoc 1985;64:52.

49. Schepman KP, van der Meij EH, Smeele LE, van der Waal I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol 1998;34:270–275.

50. Gupta PC, Bhonsle RB, Murti PR, Daftary DK, Mehta FS, Pindborg JJ. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. Cancer 1989;63:2247–2252.

51. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucous fibrosis over a 17-year period. Community Dent Oral Epidemiol 1985;13:340–341.

52. Nair J, Ohshima H, Friesen M, Croisy A, Bhide SV, Bartsch H. Tobacco-specific and betel nut-specific N-nitroso compounds: occurrence in saliva and urine of betel quid chewers and formation in vitro by nitrosation of betel quid. Carcinogenesis 1985;6:295–303.

53. Hayes MJ, Khemani L, Bax M, Alkalay D. Quantitative determination of arecoline in plasma by gas chromatography chemical ionization mass spectrometry. Biomed Environ Mass Spectrom 1989;18:1005–1009.

54. Pichini S, Pellegrini M, Pacifici R, Marchei E, Murillo J, Puig C, et al. Quantification of arecoline (areca nut alkaloid) in neonatal biological matrices by high-performance liquid chromatography/electrospray quadrupole mass spectrometry. Rapid Commun Mass Spectrom 2003;17: 1958–1964.

55. Cox S, Piatkov I, Vickers ER, Ma G. High-performance liquid chromatographic determination of arecoline in human saliva. J Chromatogr A 2004;1032:93–95.

56. Wu IC, Chen PH, Wang CJ, Wu DC, Tsai SM, Chao MR, et al. Quantification of blood betel quid alkaloids and urinary 8-hydroxydeoxyguanosine in humans and their association with betel chewing habits. J Anal Toxicol 2010;34:325–331.

57. Wyatt TA. Betel nut chewing and selected psychophysiological variables. Psychol Rep 1996;79:451–463.

Chapter 55

CAFFEINE

HISTORY

Although the medicinal use of tea in China is commonly thought to date back to the 3rd to 1st century BC,[1] the first clear reference to the medicinal use of the tea plant in China dates back to 750 AD.[2] During the Tang Dynasty (7th–9th centuries), tea became part of ceremonies of the Chinese culture. Lu Yu wrote the classic manual on tea connoisseurship, *The Classic of Tea* during the latter 8th century, incorporating both Taoism and Confucianism. The popularity of tea increased during the Ming Dynasty (1368–1644), when tea was drunk after steeping cured loose tea leaves in boiling water. The first green tea leaves from China arrived in Amsterdam on a ship of the Dutch East India Company in the early 17th century.[2] Although coffee and tea arrived in Europe at similar times, tea became the dominant methylxanthine-containing drink in many parts of Europe.

Cultivation of coffee began in Ethiopia and later Yemen. Coffee drinking probably began around 1000 AD, but the spread of coffee in the Arab world did not begin until after the discovery of the process of roasting in the 14th century.[2] Coffee was a popular beverage in the Islamic world by the end of the 15th century. The use of coffee quickly spread to Arab-controlled territory (North Africa, Spain, Turkey, the Balkans, India). Venetian merchants introduced coffee into Europe in the early 17th century, and the use of coffee in Europe was widespread by the end of the 17th century. Colonization by the Europeans resulted in the introduction of coffee plants to their colonies in Africa and the Americas.

BOTANICAL DESCRIPTION

In contrast to most other natural psychoactive drugs (e.g., nicotine, morphine, strychnine), caffeine is widely distributed in the plant kingdom. Caffeine occurs in 13 orders of the plant kingdom with the most common sources of commercial production being coffee (*Coffea arabica* L., *Coffea canephora* Pierre ex A.Froehner) and tea [*Camellia sinensis* (L.) O. Kuntze].[3] *C. arabica* accounts for about 90% of the current coffee production.

Common Name: Coffee

Scientific Name: *Coffea arabica* L.

Botanical Family: Rubiaceae (coffee)

Physical Description: The coffee tree is an evergreen, glabrous shrub or small tree that reaches up to 5 m (~16 feet) in height. The relatively large, elliptical leaves are opposite, dark green, and glossy with acuminate tips and short petioles. The white, fragrant flowers have a tubular corolla with small, cup-shaped calyx. The fruit is a drupe about 1.5 cm (0.6 inch) long that begins as a green immature fruit and ripens to a crimson black cherry when dried after 7–9 months. There are usually 2 somewhat elliptical seeds.

Distribution and Ecology: Arabic coffee originated in Ethiopia at altitudes of about 1,400–1,800 m (4,500–6,000 feet) and was subsequently cultivated on the Indonesian island of Java. Later, the cultivation of this coffee plant spread to the West Indies, Central America, and favorable climates in

South America, India, and Sri Lanka. This plant prefers temperate to tropical environments with little or no frost and abundant rain.

Common Name: Tea

Scientific Name: *Camellia sinensis* (L.) O. Kuntze

Botanical Family: Theaceae (tea)

Physical Description: This small evergreen tree reaches up to 16 m (~50 feet) in height depending on pruning practices. On the surface, a mat of feeders arise from a strong taproot. The leaves are lanceolate to obovate, usually 4–15 cm (~1.5–6 inches) in length and 7–12 cm (~3–5 inches) wide. The white to pink flowers appear in axillary or subterminal cymes with numerous stamens; brown capsules contain 1–3 seeds per lobe.

Distribution and Ecology: The tea tree is native to Southeast Asia, as well as Sri Lanka, India, and China. Tea is widely cultivated in subtropical and tropical regions up to 2,000 m (~6,500 feet) elevation near the Equator. Production of trees requires warm, humid conditions without frost, such as occurs in the southeastern United States.

IDENTIFYING CHARACTERISTICS

Structure

Caffeine (CAS RN:58-08-2) is a methylxanthine or methylated purine derivative (1,3,7-trimethylxanthine), structurally similar to theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine).

Physiochemical Properties

Caffeine is a soft, white crystal or powder that is odorless and has a slightly bitter taste. This compound is soluble in chloroform, but only partially soluble in water or ethanol. Table 55.1 lists some physical properties of caffeine.

EXPOSURE

Epidemiology

Caffeine is the most widely used stimulant in the world—used by up to 80% of the general population.[4] In the 2004 Canadian Community Health Survey–Nutrition, coffee was the second most common beverage for most Canadians behind water, whereas tea is the most common beverage worldwide.[5] More than 20% of

TABLE 55.1. Some Physical Properties of Caffeine.

Physical Property	Value
Melting Point	238°C (~460°F)
pKa Dissociation Constant	10.4 (40°C/104°F)
log P (Octanol-Water)	−0.07
Water Solubility	2.16E+04 mg/L (25°C/77°F)

men and 15% of women aged 31 to 70 years exceeded the daily recommended maximum of 400 mg caffeine. In a survey of food intakes by a representative sample of the US population ($n = 18,081$), 87% consumed food and beverages containing caffeine.[6] The average daily caffeine intake was 193 mg (1.2 mg/kg body weight). Major sources of caffeine were coffee (71%), soft drinks (16%), and tea (12%). The age group with the highest average caffeine consumption are men and women aged 35 to 64 years. In studies from the late 1970s and early 1980s, the average daily consumption of caffeine by adults in the United States was approximately 2.4 mg/kg with consumption for the upper 95% being >7 mg/kg.[7] The consumption of caffeinated beverages varies with different populations. In a survey of Canadian and US children, 36% and 56% of these children, respectively, drank caffeinated beverages with an average daily caffeine consumption of 7 mg and 14 mg, respectively.[8] These amounts are well within the established levels of safe consumption. Caffeine is a common ergogenic aid. The average daily intake of caffeine by 270 elite Canadian athletes was 0.85 ± 13 mg/kg body weight based on self-reported 3-day food records.[9] These athletes ranged in age from 16–45 years; they competed in 38 different sports.

Sources

ORIGIN

Caffeine is the most abundant purine alkaloid belonging to a group of methylxanthines and methyl uric acids derived from purine nucleotides in the coffee plant. Plants synthesize caffeine from inosinic acid by oxidation with inosine-5-phosphate dehydrogenase to xanthylic acid and subsequent methylation with methylases and the cofactor 5-adenosyl methionine to caffeine. In some plant species, theobromine (3,7-dimethylxanthine, CAS RN:83-67-0) or methyl uric acids including theacrine (1,3,7,9-tetramethyluric acid, CAS RN: 2309-49-1) and liberine (1*H*-purine-6-dione, 7,9-dihydro-2-methoxy-1,9-dimethyl, CAS RN: 51168-26-4) are the major purine alkaloids. Table 55.2 lists the major purine alkaloids in various plant species.

Caffeine is an active ingredient of beverages (e.g., coffee, tea, cola) as well as over-the-counter cold

TABLE 55.2. Major Purine Alkaloids in Various Plant Species and Infraspecies.[3]

Plant	Common Name	Major Alkaloid
Coffea arabica L.	Arabica coffee	Caffeine
Coffea canephora Pierre ex A.Froehner	Robusta coffee	Caffeine
Coffea liberica Hiern		Theacrine, liberine
Coffea liberica var. *dewevrei* (De Wild. & T.Durand) Lebrun		Theacrine, liberine
Camellia sinensis (L.) O. Kuntze	Tea	Caffeine
Camellia sinensis var. *assamica* (J. Masters) Kitam.	Assam tea	Caffeine
	Kucha	Theacrine
Camellia taliensis (W. W. Sm.) Melch.		Caffeine
Camellia irrawadiensis Barua	Wilson's camellia	Theobromine
Camellia ptilophylla Hung T.Chang	Cocoa tea	Theobromine
Theobroma cacao L.	Cacao (cocoa)	Theobromine
Theobroma grandiflorum (Willd. ex Sprengel) Schumann	Cupu	Liberine
Paullinia cupana Kunth	Guarana	Caffeine
Cola spp.		Caffeine
Citrus spp.		Caffeine

preparations, stimulants, analgesics, and appetite suppressants.[10] Table 55.3 lists common commercial sources of caffeine with approximate caffeine content. Energy drinks are commercial beverages that typically contain variable amounts of caffeine and other ingredients (e.g., guarana, taurine, sugar derivatives) with the caffeine content ranging between 50–505 mg per container.[11] Caffeinated alcoholic beverages often contain larger amounts of ethanol per container. The 3 types of tea based on fermentation are the following: green tea (nonfermented), oolong tea (semifermented), and black or red tea (fermented). Production of green tea involves the drying and steaming of fresh tea leaves to inactivate polyphenol oxidase and eliminate oxidation, whereas black tea undergoes postharvest fermentation by polyphenol oxidase before drying and steaming. The fermentation of red tea (*pu-erh*) results from the introduction of microorganisms. Worldwide, black tea accounts for about three-fourths of tea consumption, and most of the remainder of tea consumption involves green tea. Oolong tea accounts for <2% of worldwide tea consumption. Principal areas of black tea consumption are North America, Europe, and North Africa, whereas green tea is consumed primarily in China, Japan, Korea, and Morocco.[12] Oolong tea is popular in Taiwan and China.

COMPOSITION

The caffeine content in plants and beverages varies widely. The synthesis of caffeine in plants involves the conversion of xanthosine → 7-methylxanthine → theobromine (3,7-dimethylxanthine) → caffeine (1,3,7-trimethylxanthine) via the catalytic conversion of these compounds by three *N*-methyltransferase enzymes. The caffeine content of seeds from various *Coffea* species varies from about 0.4–2.4% dry weight, whereas the caffeine content in caffeine-containing *Camellia* species ranges from <0.02% (*C. kissi* Wallich) to 2–3% [*C. sinensis* var. *sinensis* (L.) Kuntze].[3] Young leaves of the first shoots of tea plants contain relatively high concentrations of caffeine. The stems and roots contain minor amounts of caffeine. The caffeine concentration in coffee and tea beverages depends on the preparation methods, as well as the caffeine content of the bean or leaf. Similar concentrations of caffeine occur in coffee and tea beverages, when the tea percolates for at least 20 minutes.[13] In an Australian study of 17 health adults, the average caffeine content per drink over 24 hours was as follows: instant coffee, 60 ± 22 mg; brewed coffee, 80 ± 19 mg; and tea, 29 ± 14 mg.[14] Caffeine intake correlated poorly with the number of coffee and tea drinks during this 24-hour period. The dose of caffeine in analgesic tablets and cold preparations typically ranges from ~30–65 mg.[13]

The chemical composition of green tea is complex, including 15–20% proteins (enzymes) by dry weight, 1–4% aminoacids (arginine, aspartic acid, 5-*N*-ethylglutamine, glutamic acid, glycine, leucine, lysine, serine, threonine, tryptophan, tyrosine, serine), 5–7% carbohydrates (cellulose, fructose, glucose, pectins, sucrose), and 5% minerals/trace elements along with xanthic bases (caffeine, theophylline), pigments (chlorophyl, carotenoids), sterols (stigmasterol), vitamins (B, C,E), and volatile compounds (aldehydes, alcohols, esters hydrocarbons, lactones).[15] The composition of these compounds varies with geographic location, growing conditions, plant strain, and preparation methods.[16] The main polyphenols in tea are flavonoids with the concentration of these compounds

TABLE 55.3. Dietary Sources of Caffeine with Estimated Caffeine Content.[39]

Product	Source	Caffeine Content*
Coffee	Decaffeinated	<5 mg/5 oz cup (150 mL)
	Brewed (ground roasted)	60–180 mg/5 oz cup (150 mL)[†]
	Instant	50–70 mg/5 oz cup (150 mL)
Tea	Iced	65–75 mg/12 oz cup (360 mL)
	Instant	25–35 mg/5 oz (150 mL)
	Leaf or bag	30–50 mg/5 oz cup (150 mL)
Chocolate	Chocolate milk	4 mg/6 oz glass (180 mL)
	Cocoa/hot chocolate	4 mg/5 oz cup (150 mL)
	Chocolate candy	1.5–6.0 mg/L oz (5–20 mg/100 g)
	Milk chocolate	6 mg/l oz (30 mL)
	Sweet chocolate	20 mg/1 oz (30 mL)
Soft Drinks	Aspen®	36 mg/12 oz can (360 mL)
	Canada Dry Cola®	30 mg/12 oz can (360 mL)
	Coca Cola®, Diet Coke®	45 mg/12 oz can (360 mL)
	Dr. Pepper®	40 mg/12 oz can (360 mL)
	Diet Right Cola®	36 mg/12 oz can (360 mL)
	Diet Rite®	36 mg/12 oz can (360 mL)
	Mr. Pibb®	41 mg/12 oz can (360 mL)
	Mr. Pibb®, Diet	57 mg/12 oz can (360 mL)
	Mountain Dew®	54 mg/12 oz can (360 mL)
	Pepsi Cola®	38 mg/12 oz can (360 mL)
	Pepsi Light®, Diet	36 mg/12 oz can (360 mL)
	Royal Crown Cola®	36 mg/12 oz can (360 mL)
	Shasta Cola®	44 mg/12 oz can (360 mL)
	Tab®	45 mg/12 oz can (360 mL)
Energy Drinks	Red Bull®	80 mg/8.3 oz can (250 mL)
	Monster®	160 mg/16 oz can (480 mL)
	Rockstar®	160 mg/16 oz can (480 mL)
	Full Throttle®	144 mg/16 oz can (480 mL)
	No Fear®	174 mg/16 oz can (480 mL)
	Wired X505®	505 mg/24 oz can (720 mL)
	Fixx®	500 mg/20 oz can (600 mL)
	Jolt Cola®	280 mg/23.5 oz can (705 mL)
	Whoop Ass®	50 mg/8.3 oz can (250 mL)
OTC Stimulants	Caffedrine®	200 mg/capsule
	Durvitan	300 mg/capsule
	NoDoz®	200 mg/tablet
	Vivarin®	200 mg/tablet
OTC Analgesics	Anacin Analgesic®	32 mg/tablet
	Cope®	32 mg/tablet
	Dristan®	30 mg/tablet
	Excedrin®	65 mg/tablet
	Midol®	64 mg/tablet
	Vanquish®	33 mg/tablet
OTC Diuretics	Aqua Ban®	200 mg/tablet
OTC Cold Remedies	Coryban-D®	30 mg/tablet
	Neo-Synephrine®	15 mg/tablet
	Triaminicin®	30 mg/tablet
Prescription Drugs	Cafergot®	100 mg/tablet
	Darvon® Compound	32 mg/tablet
	Fiorinal®	40 mg/capsule

*Approximate caffeine content.
[†]Average about 120 mg.

TABLE 55.4. Concentrations (mg/L) of Phenolic Acids, Flavan-3-ols, Flavones, Flavonols, and Caffeine in Convenience Samples of Different Beverages.[19]

Compound	Green Tea	White Tea	Black Tea	Mate	Cola Soft-Drink	Energy Drink	Instant Coffee
Gallic acid	4.3	5.6	8.2	—	—	2.1	—
(-) Gallocatechin	29	11	0.7	—	—	—	—
Protocatechuic acid	—	—	0.4	—	—	—	—
(-) Epigallocatechin	136	45	1.6	—	—	—	—
(-) Catechin	31	28	1.3	—	—	—	—
Chlorogenic acid	7.0	2.4	1.2	134	–	–	61
Caffeine	130	140	89	40	108	314	70
Caffeic acid	—	—	—	1.8	—	—	4.6
(–) Epicatechin	12	9.6	4.2	—	—	—	—
(-) Epigallocatechin gallate	31	29	—	—	—	—	—
(-) Gallocatechin gallate	—	—	0.4	—	—	—	—
p-Coumaric acid	—	—	<0.01	—	—	—	—
(-) Epicatechin gallate	15	22	0.6	—	—	—	—
Myricetin-3-0-rhamnoside	2.5	1.9	1.7	—	—	—	—
Quercetin-3-0-rutinoside	18	12	8.6	39	—	—	—
Quercetin-3-0-glucopyranoside	5.5	2.4	1.9	3.2	—	—	—
Luteolin-7-0-glucosicle	—	—	—	—	—	—	—
Kaempferol-3-0-rutinoside	4.4	1.5	1.7	4.2	—	—	—
Kaempferol-3-0-glucoside	3.8	2.2	1.9	—	—	—	—
Apigenin-7-0-glucoside	—	—	—	—	—	—	—

Note. The absence of a number means the concentration was below the limit of detection (0.0005–0.211 mg/L).

substantially higher in green tea than black tea. The major flavonoids in green tea are catechins (flavan-3-ols) with (–)-epigallocatechin-3-gallate being the most common catechin.[17] Less common catechins in green tea include (–)-epigallocatechin, (–)-epicatechin-3-gallate, and (–)-epicatechin. Black tea contains oxidized phenolic compounds, particularly therarubigins and theaflavins, as well as relatively high concentrations of fluoride.[18] Table 55.4 compares the concentrations of phenolic compounds and caffeine in several different beverages as measured by high performance liquid chromatography with photodiode array detection.[19]

Methods of Use

Caffeine acts as a mild positive reinforcer that is not usually considered a drug of abuse. Typically, ingestion of caffeine involves the use of products similar to those listed in Table 55.3. Medically significant misuse of caffeine usually results from the overuse of caffeine-containing weight loss or energy products in pill or powder form. Caffeine is a treatment for apnea of prematurity in neonatal intensive care units.[20] The efficacy of caffeine ingestion for short-term high-intensity exercise is equivocal. Some studies demonstrate decreased performance with caffeine ingestion following repeat cycles, whereas other studies show significant improve-

ments in team sports exercise and power-based sports with caffeine ingestion, particularly in elite athletes without regular caffeine use.[21] Traditional uses of green tea in Chinese medicine include the treatment of headache, body aches, indigestion, depression, and low energy. In Chinese medicine, the use of green tea is believed to prolong life. Other uses of green tea in supplements include the treatment of hair loss and obesity. There is little evidence that the use of green tea prevents cancer.[15]

DOSE EFFECT

The response to caffeine depends on the dose, the time after consumption, and the concomitant ingestion of other substances. In general, moderate doses of caffeine (i.e., 250–300 mg/d) do not produce clinically significant alterations in cardiovascular parameters, even in most patients with coronary artery disease.[22] In some studies of healthy, adult caffeine users, ingestion of 200 mg caffeine in an energy drink was associated with an increase in the mean heart rate of 5–7 beats/min and the mean systolic blood pressure of 10 mm Hg over baseline 4 hours after ingestion.[23] The drink also contained 2,000 mg taurine, and there was no sham control without caffeine. The difference between baseline and postcaffeine heart rate was not statistically significant during

the first 3 hours of the study. Naïve caffeine users may become symptomatic (e.g., tremulous) after the ingestion of 1–2 cups of coffee (<100–200 mg caffeine). Tolerance to the effects of caffeine begins within 1–4 days based on volunteer studies.[24] Heavy caffeine consumption involves the ingestion of >500–600 mg/day (7–8 mg/kg). Consequently, heavy coffee drinkers consume >500 mg/day. Symptoms (i.e., "caffeinism") associated with these high doses include anxiety, headaches, irritability, insomnia, anorexia, lightheadedness, and tremulousness. The number of coffee or tea drinks is not a reliable measure of caffeine consumption because of the wide variation in the caffeine content of these beverages.

In a retrospective analysis of the 38 cases presenting to the Royal Infirmary of Edinburgh after the deliberate ingestion of caffeine-containing products, the median ingested amount of caffeine was 1,040 mg (25–75% interquartile range, 600–1,500 mg).[25] The most common symptoms were nausea, vomiting, and abdominal pain; no patient displayed severe caffeine toxicity. The ingestion of 80–100 mg/kg by children produces serious toxicity, whereas caffeine doses in the range of 150–200 mg/kg are potentially fatal. A one-year-old girl survived the ingestion of 200–300 mg caffeine/kg after developing seizures, coma, and ventricular fibrillation.[26] The ingestion of a caffeine dose in the range of 10 g is potentially fatal in an adult, although recovery occurred after the ingestion of 24 g caffeine following intensive supportive care.[27]

TOXICOKINETICS

Absorption

The absorption of caffeine from the gastrointestinal tract is rapid and complete including absorption through the rectal mucosa. There is no significant hepatic first-pass effect following the ingestion of caffeine. The presence of food in the stomach or ethanol does not significantly inhibit the absorption of caffeine. Peak plasma caffeine concentrations occur within about 1 hour. In a study of 18 healthy adults, the mean peak plasma concentration occurred about 60 minutes after ingesting 250 mg caffeine with a range up to 120 minutes.[24] The peak plasma caffeine ranged from 3.8–14.8 mg/L during the 7-day study with the plasma caffeine concentrations at the end of the study ~2–3 mg/L higher than the first day of caffeine consumption.

Distribution

Caffeine crosses all biologic membranes including the blood–brain barrier, resulting in wide distribution throughout the body. Caffeine distributes relatively evenly between plasma and erythrocytes, and easily crosses the placenta to enter the fetal circulation. Caffeine also enters breast milk, the bile, saliva, and semen.[39] In a study of 11 healthy adults administered an intravenous (IV) dose of 150 mg anhydrous caffeine, the mean volume of distribution was 0.53 ± 0.5 L/kg.[28] Animal studies indicate the absence of long-term accumulation of caffeine or caffeine metabolites in the body.[29]

Biotransformation

The biotransformation of caffeine is complex with at least 17 metabolites.[30] Metabolism of caffeine via demethylation produces dimethylxanthine compounds, primarily paraxanthine (1,7-dimethylxanthine) via N^3-demethylation catalyzed by CYP1A2.[31] This isoenzyme is an abundant cytochrome P450 isoenzyme, accounting for about 15% of the total CYP content. A small portion of the biotransformation reactions results from the catalytic action of CYP2E1, whereas CYP1A2 accounts for most all of isoenzymes responsible for caffeine demethylation.[32] CYP1A1 has similar affinity for caffeine as CYP1A2, but the former isoenzyme is much less abundant than the latter.[33] The dimethylxanthine metabolites are pharmacologically active, and may contribute to the effects of caffeine. There are substantial interindividual differences in the expression of CYP1A2; therefore, there are significant individual differences in the metabolism of caffeine. In humans, there are no significant differences in caffeine metabolism between genders despite the higher activity of CYP1A2 in men.[29] Subsequent pathways involve acetylation, 8-hydroxylation by CYP3A isoenzymes, and the catalytic oxidation of 1-methylxanthine to 1-methyluric acid by xanthine oxidase.

Minor metabolites include theobromine (3,7-dimethylxanthine) via N^1-demethylation and theophylline (1,3-dimethylxanthine) via N^7-demethylation. Figure 55.1 displays the initial demethylation reactions involved with the biotransformation of caffeine. In a study of 17 healthy adults ingesting tea or coffee, paraxanthine, theobromine, and theophylline accounted for about 67%, 24%, and 8% of the total dimethylxanthine compounds in plasma, respectively.[14] Major urinary metabolites of caffeine are 5-acetylamino-6-formylamino-3-methyluracil, 1-methyluric acid, 1-methylxanthine, 1,7-dimethyluric acid, and 1,7-dimethylxanthine.

Elimination

Elimination of caffeine occurs almost exclusively via biotransformation and renal excretion of metabolites
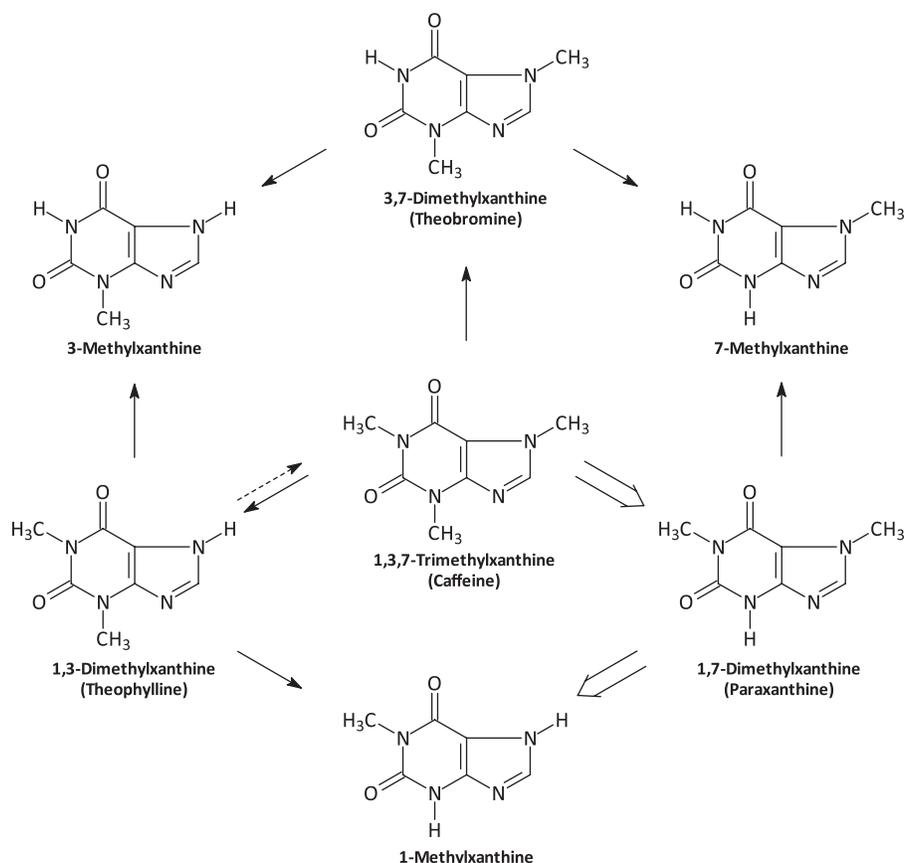
FIGURE 55.1. Initial demethylation reactions of caffeine.[7]

with little caffeine appearing in the urine unchanged. Age, renal function, and dose affect caffeine elimination. In most patients, the kidney excretes minor amounts (i.e., 1–2%) of an absorbed dose of caffeine in the urine as unchanged caffeine. The elimination process from the blood is first-order in the range of 2–10 mg/kg, whereas caffeine follows zero-order (saturation) kinetics in neonates and overdose.[29] The average serum elimination half-life of caffeine in adults ingestion typical recreational doses of caffeine is about 4–6 hours (range, 2–12 h) following typical recreational doses of caffeine. Infants up to the age of 3 months eliminate caffeine primarily by renal excretion rather than biotransformation with elimination half-life markedly prolonged at about 100 hours compared with adults.[34] By the age of 7–9 months, the renal elimination of caffeine accounts for about 2% of the elimination of caffeine, similar to adults. Liver disease and pregnancy prolong the elimination half-life of caffeine, whereas smoking and the induction of hepatic microsomal enzymes reduces the caffeine-elimination half-life. As a result of the saturation of hepatic microsomal enzymes during an overdose, the elimination half-life of caffeine during caffeine intoxication is prolonged compared with the ingestion

of <250–500 mg caffeine.[35] Based on 3 serum samples drawn within 2 days after a 6–8 g overdose of caffeine, the serum elimination half-life of caffeine was ~16 hours.[36]

Maternal and Fetal Kinetics

Methylxanthines including caffeine readily pass the placental barrier and enter fetal tissues. Caffeine elimination in nonpregnant and pregnant women in the first trimester are similar, but elimination decreases in pregnant women during the second and third trimesters with the potential for accumulation of caffeine in the mother and fetus depending on the caffeine dose.[37] The placenta and fetus lack the CYP450 isoenzymes required to metabolize caffeine; therefore, caffeine elimination by the fetus occurs by renal excretion.[38]

Drug Interactions

Various drugs induce CYP1A2 including selective serotonin reuptake inhibitors (fluvoxamine), antipsychotics (clozapine, olanzapine), psoralens, antiarrhythmics (mexiletine, diltiazem, verapamil), proton pump

inhibitors (omeprazole), quinolones (enoxacin, norfloxacin, ciprofloxacin), and theophylline.[39] Other drugs that inhibit caffeine metabolism include acute ethanol ingestion, cimetidine, disulfiram, and oral contraceptives. Smoking increases the clearance of caffeine up to 30–50%. In a study of 16 healthy adults, the ingestion of 75 mg phenylpropanolamine with 400 mg caffeine increased the mean peak plasma caffeine concentration from 2.1 ± 0.3 mg/L (caffeine alone) to 8.0 ± 2.2 mg/L (caffeine + phenylpropanolamine).[40] There are few data on the clinical relevance of these drug interactions. Existing experimental data suggest that the ingestion of caffeine will not ameliorate ethanol-induced impairment of driving skills, even in doses consistent with the use of caffeinated ethanol beverages (e.g., 69 mg caffeine/12 ounce beer with 4.8% ethanol by volume).[41]

Tolerance

Tolerance develops following chronic caffeine use to some of the central and peripheral effects (e.g., anxiety, jitteriness, blood pressure, diuresis), but not all central effects (e.g., attention, wakefulness).[42] In volunteer studies of participants without habitual caffeine use, tolerance to increases in blood pressure, heart rate, plasma epinephrine, plasma norepinephrine, plasma renin activity, and urinary catecholamines develops within 1–4 days of regular caffeine use at a daily dose of 250 mg.[24] Tolerance to the cardiovascular effects of caffeine is lost within 3 weeks after cessation of caffeine use.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mechanism of Action

Caffeine is a central nervous system stimulant that produces diverse and complex effects including increased arousal and vigilance, reduced fatigue, and improved performance on some motor tasks.[4,43] Attention is modified by caffeine use, allowing more effective processing of relevant stimulus characteristics during information processing.[44] These effects probably result from caffeine-induced adenosine receptor antagonism.[45] Ergonomic studies in endurance athletes suggest that caffeine improves performance modestly during endurance activities (i.e., 3–4%)[46] and repeated sprint ability,[47] but not during sprint activities with predetermined goals.[48,49] Volunteer studies suggest that the ingestion of coffee does not mimic the positive effects of caffeine on endurance, perhaps as a result of moderation of the caffeine-induced elevation of plasma epinephrine concentrations by other ingredients in coffee.[50] Caffeine also stimulates the medullary respiratory center by increased sensitivity to stimulation by plasma carbon dioxide concentrations. Additionally, caffeine decreases cerebral blood flow as a result of the antagonism of adenosine-induced cerebral vasodilation. Effects on sleep include increased sleep latency and reduced sleep duration.

In commonly ingested doses, the administration of caffeine alone slightly increases blood pressure, minimally reduces heart rate, and causes the release of catecholamines (epinephrine, norepinephrine) and renin in *caffeine-naïve* individuals; however, tolerance limits the effect of these changes following continued caffeine use. In a study of normotensive, caffeine-naïve volunteers, daily administration of 400 mg caffeine produced a 3 mm Hg increase in ambulatory systolic and diastolic blood pressure associated with a 3-beat/minute decrease in heart rate.[51] Both these changes were statistically significant, and these changes resolved soon after administration ceased.

The increased glomerular filtration rate and inhibition of tubular reabsorption of sodium and water results in mild diuresis with sodium and water loss. Caffeine increases minute ventilation; the ingestion of caffeine in asthmatic patients produces much less bronchodilation than theophylline.[52] Stimulation of gastric secretion of acid and pepsin occurs following the ingestion of coffee with some studies suggesting that caffeine also reduces lower esophageal sphincter tone.[4]

Mechanism of Toxicity

The toxic effects of caffeine are an extension of pharmacologic effects. Caffeine increases the synthesis and release of catecholamines as well as increasing intracellular cyclic AMP concentrations by inhibiting phosphodiesterases. These actions result in pharmacologic effects on multiple organ systems. Caffeine is a nonselective adenosine receptor (A_1, A_{2A}) antagonist, which inhibits the action of adenosine in the brain, cardiovascular system, gastrointestinal tract, lung, kidney, and adipose tissues. Adenosine A_1 receptors occur in most areas of the brain, particularly in the hippocampus, striatum, cerebral cortex, and some areas of the thalamic nuclei (e.g., medial and anterior nucleus).[53] Intermediate amounts of these receptors occur in the nucleus accumbens and the caudate-putamen as demonstrated by autoradiography in postmortem brain tissues with N^6-[^{3}H] cyclohexyladenosine. Dopamine-rich regions of the brain contain substantial amounts of adenosine A_{2A} receptors. The interaction of adenosine A_{2A} and dopamine D_2 receptors regulate the GABAergic striatopallidal neurons, whereas the interaction of adenosine A_1 and dopamine D_1 receptors are involved in the regulation of the GABAergic striatonigral and striatoentopeduncular neurons.[54] Enhancement of postsynaptic

dopamine D_2 receptors by adenosine A_{2A} antagonism probably accounts for the central effects of caffeine.[44]

There is no clear evidence that coffee increases the risk of cardiovascular diseases. Moderate ingestion of caffeine does not increase the frequency or severity of cardiac dysrhythmias in normal individuals, patients with ischemic heart disease, or those with preexisting serious ventricular ectopy.[55] Additionally, there is no clinically significant association between coffee consumption and the occurrence of coronary heart disease.[56] Caffeine reduces insulin sensitivity in skeletal muscle and may reduce glucose output from the liver.[57,58] High doses of caffeine produce vasodilation, hypotension, and marked tachycardia. Other effects at high caffeine doses (i.e., serious overdose) include mobilization of calcium from intracellular storage sites in skeletal muscle, cardiac muscle, and neurons.

CLINICAL RESPONSE

Recreational Use

For most people, the ingestion of usual doses of caffeine in beverages produces a subjective feeling of well-being, alertness, and increased energy with minimum adverse effects. Excessive doses of caffeine produce anxiety, irritability, nervousness, palpitations, insomnia, tremulousness, and nausea. A case report of a 47-year-old man associated the development of delusions and paranoid psychosis with the chronic consumption of excessive amounts of coffee (i.e., 36 cups of coffee/d).[59] The psychotic symptoms resolved after a reduction of his coffee consumption without the administration of antipsychotic medication. Behavioral studies in children suggest that acute alteration of their usual dose of caffeine results in some adverse behavior changes (e.g., volatility, inattentiveness, restlessness), but there is little evidence that caffeine produces long-term adverse behavioral effects.[60] A meta-analysis of coffee consumption and the risk of type 2 diabetes suggested that increased coffee use was associated with a dose-related decrease in type 2 diabetes.[61] The relative risk of type 2 diabetes in chronic coffee drinkers using 4–6 cups and ≥6–7 cups daily were 0.72 (95% CI: 0.62–0.83) and 0.65 (95% CI: 0.54–0.78), respectively.

Cardiovascular

There are inadequate data to associate coffee drinking with the development of hypertension. The consumption of coffee by women does not appear to increase the risk of hypertension based on a large cohort study.[62] A longitudinal study of 1,017 male former medical students demonstrated a higher incidence of hypertension in coffee drinkers during follow-up when compared with coffee nondrinkers at baseline (18.8% vs. 28.3%, respectively; $P = .03$).[63] However, the relative risk of hypertension was not dose-related after adjustment of confounding factors. The association between the risk of myocardial infarction (MI) and coffee consumption is complex, and the risk of MI may be related to the interindividual variability in the enzyme activity of CYP1A2. For younger individuals with the rapid caffeine metabolizing genotype (CYP1A2*1A allele), the consumption of 1–3 cups of coffee daily was associated with a lower risk (odds ratio = 0.69; 95% CI: 0.50–0.86) of nonfatal MI than individuals consuming <1 cup coffee daily after controlling for other risk factors.[64] Some studies suggest that individuals with the slow CYP1A2*1F allele are more susceptible to acute, nonfatal MI following chronic coffee consumption. A population case-control study of individuals with their first nonfatal MI demonstrated an increased risk of MI only in carriers of the slow CYP1A2*1F allele.[65] The odds ratio of developing a nonfatal MI in these genotypes for the consumption of ≥4 cups of coffee daily was 1.64 (95% CI: 1.14–2.34), when compared with referents (i.e., <1 cup coffee daily). For normal phenotypes, the odds ratio of acute non-fatal MI for moderate coffee consumption (1–3 cups daily) was less than unity (i.e., ~0.5).

Although typical caffeine doses mildly increase epinephrine concentrations (i.e., 6-fold less than strenuous exercise),[66] most studies do not demonstrate proarrhythmic effects following caffeine use and moderate caffeine doses are well tolerated even in patients with cardiovascular disease.[67] In a study of 18 patients with frequent ventricular premature beats (VPCs), the ingestion of 1 mg caffeine/kg was associated with increased frequency of VPCs (307 ± 350/h) compared with a caffeine-free period (307 ± 414/h, $P < .01$).[68] However, there was no significant difference in the cardiac rhythm, rate, or ventricular repolarization (QTc interval). Studies of patients with cardiovascular disease have not demonstrated an increased incidence of VPCs or other ventricular dysrhythmias during the use of caffeine including patients with histories of recent MI,[69] nonsustained ventricular tachycardia,[70] and multiple types of malignant ventricular dysrhythmias (ventricular fibrillation, recurrent ventricular tachycardia, symptomatic nonsustained ventricular tachycardia).[71] Larger epidemiologic studies have not demonstrated an association between typical caffeine use and the incidence of atrial dysrhythmias (e.g., atrial fibrillation, atrial flutter).[72]

Central Nervous System

Epidemiologic studies of coffee consumption and the risk of stroke are inconsistent, in part as a result of the

differences in study population (healthy, hypertensive patients, diabetics), outcomes (incidence, mortality), and gender. A population-based prospective cohort study of 34,670 healthy Swedish women demonstrated a statistically significant lower risk of total stroke, sub-arachnoid hemorrhage, and cerebral infarction but not intracerebral hemorrhage in coffee drinkers than seldom coffee drinkers, after adjustment for other risk factors.[73] The multivariable relative risk of total stroke for high coffee consumption (≥5 cups/d) was 0.77 (95% CI: 0.63–0.92) compared with controls (<1 cup/d). Similarly, a prospective study of 83,076 healthy women in the Nurses' Health Study demonstrated a 20% lower risk of total stroke in women consuming ≥4 cups/day than seldom coffee drinkers (<1 cup/wk).[74] However, a cohort study of 499 hypertensive, nonsmoking men detected an approximately 2-fold higher risk of isch-emic stroke in men consuming 3 cups coffee/day com-pared with noncoffee drinkers.[75] Most cohort studies of coffee consumption using stroke mortality among healthy participants and diabetics have not detected dose-related effects of coffee consumption on mortality.[76,77]

Gastrointestinal

Several case reports associate the use of green tea in herbal products with hepatotoxicity, more commonly in women. A 41-year-old woman developed jaundice after using a hair loss supplement containing 27% *Camellia sinensis* for 6 months.[78] Although her serum hepatitic aminotransferases were elevated above 1,000 IU/L and her serum bilirubin was 13.6 mg/dL (9.8 mg/dL direct), there was no evidence of liver dysfunction. Liver biopsy was consistent with a drug reaction (periportal inflam-mation with granulocytes, eosinophils, lymphocytes, plasmocytes along with intrahepatic cholestasis); her jaundice resolved with 1 month of cessation of the use of the supplement. A review of worldwide reports of adverse events (published reviews, the US Food & Drug Administration MedWatch Program, United States Pharmacopeia's MEDMARX Adverse Event Reporting System, Australian Therapeutic Goods Administration, UK Medicines and Healthcare Products Regulatory Agency, Health Canada's Canadian Adverse Drug Reaction Monitoring Program) associated with the use of green tea supplements revealed 8 cases of probable hepatotoxicity based on the Naranjo Causality Algorithm Scale.[79] Most suspected cases of hepatotoxic-ity resolve without sequelae following cessation of sup-plement use; rarely, cases reports associate acute liver failure requiring orthotopic liver transplantation with the use of green tea supplements.[80]

Overdose

Adverse effects associated with the IV administration of high doses (36–136 mg/kg) of caffeine in neonates simulate seizures including tachypnea, fine tremor, muscle fasciculations, opisthotonus, tonic-clonic move-ments, and nonpurposeful facial movements.[81] Symptoms of serious caffeine intoxication begin within several hours of ingestion, usually with the onset of vomiting. The clinical effects of severe caffeine intoxication resemble a massive catecholamine release including vomiting, abdominal cramps, weakness, miosis, nystag-mus, weakness, hypertension, tachycardia, respiratory distress, rhabdomyolysis, and cyanosis. Neurologic effects involve myoclonus, opisthotonus, agitation, hal-lucination, hyperreflexia, tremor, anxiety, delirium, and central nervous system depression ranging from leth-argy to coma.

Case reports associate excessive caffeine consump-tion with a variety of supraventricular and ventricular dysrhythmias including paroxysmal supraventricular tachycardia in a 42-year-old man with aortic insuffi-ciency from rheumatic valvular disease drinking 30 cups of coffee daily along with 3–4 cups of tea,[82] and ventricu-lar fibrillation in a 27-year-old hypotensive woman intentionally ingesting 27 g caffeine.[83] A 27-year-old motocross racer developed dull retrosternal chest pain and a cardiac arrest after a day of racing and drinking 7–8 cans of a caffeinated energy drink.[84] Although his coronary angiogram was normal, he had electrocardio-graphic and echocardiographic evidence of transmural ischemia and an elevated serum troponin I. A case report associated the ingestion of a 20-g caffeine over-dose with the development of a subendocardial MI in a 20-year-old bulimic woman.[85] However, no echocar-diography or angiography was performed to document underlying cardiac disease.

Fatalities

There are only a few case reports in the medical litera-ture documenting death following the ingestion of caf-feine alone.[86] Death from caffeine intoxication usually occurs as a result of hypotension, respiratory failure, ventricular fibrillation, and cardiorespiratory arrest.[87,88] Case reports also associate the administration of fatal doses of caffeine with child abuse.[89]

Abstinence Syndrome

Symptoms of the mild abstinence syndrome associated with chronic caffeine use include headaches, fatigue,

decreased energy, and reduced alertness. Headache is the most common withdrawal symptom with an incidence of up to 50% in some volunteer studies.[90] Other symptoms of abstinence include anxiety, depressed mood, reduced concentrating ability, irritability, nausea, vomiting, and craving for caffeine. Tremor, muscle stiffness, confusion, and rhinorrhea occur rarely during caffeine withdrawal. The withdrawal symptoms typically begin within 12–24 hours after the last caffeine dose and maximum effects occur within 20–48 hours of the last dose.[90,91] Symptoms can persist up to 1 week. A study of twins suggested genetic factors in the etiology of caffeine tolerance and withdrawal because of the increased risk of these complications in monozygotic twin pairs when compared with dizygotic twin pairs.[92]

The regular consumption of caffeine produces tolerance to many of the effects of caffeine within a few days; the severity of withdrawal symptoms depends on individual pharmacokinetics, dose, and duration of use. Volunteer studies indicate that minor caffeine withdrawal can occur following the consumption of as little as 300 mg/day for 3 days[93] The administration of 250 mg caffeine daily to 18 volunteers for 1 week did not produce withdrawal symptoms.[24] However, a study of 7 healthy volunteers suggested that mild withdrawal symptoms can occur following the consumption of caffeine doses as low as 100 mg (i.e., one cup coffee) in some, but not all individuals.[94] A volunteer study suggested that the ingestion of caffeine-containing soft drinks moderates the withdrawal symptoms associated with heavy caffeine consumption.[95] Although this abstinence syndrome can encourage the repeated use of caffeine, caffeine use is typically self-limiting because most individuals do not experience positive and pleasant behavioral effects from escalating doses of caffeine. Rodent studies suggest that the addictive potential of caffeine is relatively low based on minimal functional activation of an area (i.e., shell of the nucleus accumbens) involved with addiction and reward at usual caffeine doses.[96]

Reproductive Abnormalities

Reporting bias and multiple confounders (e.g., associated drug/nicotine use, caffeine use other than tea/coffee) complicate the determination of the effect of caffeine on the fetus. Although there are some epidemiologic evidence that caffeine in high doses adversely affects pregnancy outcome, the results are inconclusive and there is insufficient evidence to restrict caffeine intake after the first trimester. In general, most studies of moderate maternal caffeine consumption (<300 mg/d) do not associate maternal caffeine use with miscarriages or reductions in birth weight, gestational age, or fetal growth.[97,98] A meta-analysis of 15 cohort and 7 case-control studies demonstrated a combined odds ratio of 1.11 (95% CI: 0.96–1.28) for the risk of preterm births (<37 weeks gestation) comparing the highest and lowest level of caffeine consumption during the first trimester.[99] Additionally, there were no statistically significant differences in the combined odds ratios for the second and third trimesters.

Some observational studies suggest a relatively small effect from high maternal caffeine use (≥300 mg/d) during pregnancy on fetal growth restriction (number of births <10th percentile for weight, adjusted for confounders). In a prospective longitudinal observational study of 2,635 low-risk pregnant women, the odds ratios for fetal growth restriction and maternal caffeine use were as follows: 100–199 mg/day, 1.2 (95% CI: 0.9–1.6); 200–299 mg/day, 1.5 (95% CI: 1.1–2.1); and >300 mg/day, 1.4 (95% CI: 1.0–2.0).[100] The test for trend comparing maternal caffeine use of >300 mg/day and <100 mg/day was statistically significant ($P < .001$). The evaluation of self-reports of 2,291 mothers with singleton live births detected a small reduction of birthweight (−28 g/100 mg of caffeine consumed daily, 95% CI: −0.10 to −0.46, $P = .001$).[101] However, the authors believed that this reduction was probably not clinically significant unless the mother ingested at least 6 cups of coffee daily. There was no association between maternal caffeine consumption and mean gestational age. Most positive observational studies on the effect of maternal caffeine use and fetal growth restriction do not demonstrate positive associations between maternal caffeine use and other parameters of fetal well-being (e.g., still births, miscarriage, preterm labor, gestational age).[100,102]

There is no evidence that caffeine is teratogenic in humans. The association between caffeine consumption and spontaneous abortion or still-births is equivocal because of methodologic limitations including selection and recall bias, inconsistent exposure measurement, failure to account for caffeine metabolism and the timing of fetal demise, confounding by smoking and other drugs, and the interrelationship between caffeine and nausea.[103] A Danish study of 88,482 pregnant women suggested an increased risk of spontaneous abortion before 20 weeks in women consuming ≥8 cups of coffee daily (adjusted hazard ratio, 1.48; 95% CI: 1.01–2.17), but there was no statistically significant increase in the risk of stillbirth (>196 days gestation).[104] Although developmental abnormalities occur in animal studies at very high caffeine doses, consumption of these doses by pregnant mothers is highly unlikely. Based on rodent studies, the developmental no observable effect level (NOEL) is approximately 30 mg/kg/day, the teratogenic NOEL is 8,100 mg/kg/day, and the reproductive

NOEL approximately 80–120 mg/kg/day, depending on the method of administration and species.[105]

Carcinogenesis

The International Agency for Research on Cancer lists caffeine as not classifiable as to its carcinogenicity to humans (Group 3) based on inadequate evidence for the carcinogenicity of caffeine in humans and inadequate evidence for the carcinogenicity of caffeine in experimental animals.[106] The IARC lists coffee as possibly carcinogenic to humans (Group 2B) based on limited evidence in humans that coffee drinking is carcinogenic in the bladder and inadequate evidence in experimental animals for the carcinogenicity of coffee.[107] There is little scientific evidence associating female breast cancer or colon cancer with coffee drinking. There was a small, but significant increase in mortality (OR = 1.26; 95% CI: 1.05–1.51) during a 25-year study of 27,530 Seventh-Day Adventists, when comparing moderate to heavy coffee drinkers (≥3 cups/d) with non-coffee drinkers.[108] However, the causality of coffee in this increased mortality could not be determined. There is little evidence associating the use of coffee or tea with esophageal cancer; however, the drinking high-temperature mixtures is an independent risk factor for the development of esophageal cancer.[109]

DIAGNOSTIC TESTING

Analytic Methods

Methods for the quantitation of caffeine in biologic fluids include gas chromatography with nitrogen phosphorus detection,[110] capillary electrophoresis,[111] high performance liquid chromatography with UV detection,[28] and gas chromatography/mass spectrometry.[112] The limit of detection (LOD) with the latter method in biologic samples is about 0.001 mg/L with a coefficient of variation of approximately 10%.[113] Quantitation of caffeine metabolites in the subnanomolar range results from separation by high performance liquid chromatography followed by analysis by stable isotope dilution gas chromatography/mass spectrometry.[114] The use of gas chromatography/ion-trap/tandem mass spectrometry allows the detection of caffeine in environmental samples in the range of 0.000001–0.000002 mg/L.[115] High concentrations of caffeine in blood samples from overdoses can produce false-positive elevation of theophylline concentrations when analyzed by enzyme immunoassays or high performance liquid chromatography methods that do not separate paraxanthine from theophylline.[116]

Biomarkers

BLOOD

Caffeine equilibrates evenly between erythrocytes and plasma; therefore, caffeine concentrations in plasma, serum, and whole blood from the same sample are similar (i.e., variation of <5–10%).

RECREATIONAL USE.   In a convenience sample of 600 outpatients presenting to a clinical laboratory for routine blood analysis, the mean plasma caffeine concentration was 2.1 mg/L with a range of 0.2–13.1 mg/L.[117] There were no restrictions on food or drink intake including caffeine consumption. The upper 95% upper confidence limit was 5.6 mg/L. Following the ingestion of 120 mg caffeine by 13 healthy volunteers, the peak plasma caffeine concentration averaged about 3 mg/L (range, 2–4 mg/L) at 1 hour after ingestion.[118] In a study of 4 volunteers ingesting 300 mg caffeine, the mean peak plasma caffeine concentration at 1 hour after ingestion was about 8 mg/L (range, 6–9 mg/L).[119] The ingestion of 500 mg caffeine produces peak plasma caffeine concentrations up to 17 mg/L.[35]

OVERDOSE.   Plasma caffeine concentrations exceeding 30 mg/L are associated with toxicity. Tachycardia and seizures can occur when the plasma caffeine concentration is 50–60 mg/L. Serious toxicity occurs when plasma caffeine concentrations near 100 mg/L; plasma concentrations in the range of 90–180 mg/L are potentially lethal.[120,121] However, survival has occurred in patients with peak plasma caffeine concentrations in the range of 100–400 mg/L with intensive supportive care.[26,122] A 21-year-old woman became comatose, responsive only to pain with a tachydysrhythmia that deteriorated into ventricular fibrillation.[123] The initial serum caffeine concentration was 297 mg/L, but she survived with intensive cardiorespiratory support and hemoperfusion. Three hours after a large caffeine overdose, a serum caffeine concentration of 194 mg/L was associated with agitation, confusion, diaphoresis, tremulousness, hyperreflexia, tachydysrhythmia, and blood-tinged vomitus in a 58-year-old obese woman.[124]

POSTMORTEM.   Postmortem samples containing caffeine usually contain other drugs; death from caffeine alone is rare.[125] In a case report of a 31-year-old man found dead after ingesting a large dose of caffeine tablets, the femoral blood contained a caffeine concentration of 153 μg/g (~153 mg/L).[120] No other drugs were detected in postmortem blood. A 56-year-old man was found dead in his bed. His heart blood contained a caffeine concentration of 150 mg/L along with a blood ethanol concentration of 109 mg/dL and a fluoxetine concentration above usual

antemortem therapeutic concentrations.[86] A 29-year-old man suffered a fatal cardiac arrest while undergoing imaging studies in the emergency department.[126] Postmortem femoral blood contained caffeine at 192 mg/L with no other significant amounts of drugs in his postmortem blood sample. In a study of postmortem redistribution, the heart/femoral blood ratio in 3 cases ranged from 1.0–1.4 with a mean of 1.2.[127] Caffeine penetrates into the vitreous humor. In 2 fatal cases of caffeine/ephedrine intoxication, the postmortem whole blood : vitreous humor ratio was 3.59 (343.9 mg/L, 95.9 mg/L) and 1.84 (184.1 mg/L, 99.8 mg/L).[128]

Urine

The renal tubule resorbs caffeine in equilibrium with unbound caffeine in plasma. There is substantial variation in the renal excretion of caffeine between individuals. In some individuals, the daily intake of 3–6 cups of coffee can produce urine caffeine concentrations exceeding 12 mg/L.[129]

Abnormalities

The most common dysrhythmias associated with caffeine intoxication are sinus tachycardia and paroxysmal supraventricular tachycardia. Other dysrhythmias include wide-complex tachycardia, premature atrial and ventricular contractions, bigeminy, ventricular tachycardia, ventricular fibrillation, asystole, pulseless electrical activity, and asystole.[45,130] Caffeine reduces electroencephalogram power, particularly in the alpha and theta frequencies. The release of large amounts of catecholamines during severe caffeine intoxication produces lactic acidosis, hypokalemia, hyponatremia, leukocytosis, and hyperglycemia.[131,132] Rhabdomyolysis, hyponatremia, and renal dysfunction can complicate large, intentional ingestions of caffeine[133] and massive amounts (15 L/d) of tea.[134] Respiratory alkalosis can occur in association with hyperventilation.[36]

TREATMENT

Stabilization

Serious caffeine intoxication can cause intractable hypotension, seizures, coma requiring intubation, and serious dysrhythmias including ventricular fibrillation. Treatment for these complications requires careful monitoring for the development of dysrhythmias, seizures, and respiratory failure with IV access, cardiac monitoring, and pulse oximetry. Ventricular dysrhythmias may not respond to lidocaine, and supraventricular tachycardias may not respond to adenosine. Case reports suggest that some dys-

rhythmias (supraventricular tachycardias, ventricular tachycardia) may respond to esmolol (500 µg/kg bolus followed by continuous infusion of 50 µg/kg/min)[124] or procainamide.[130] Seizures usually respond to the IV administration of benzodiazepines (lorazepam, diazepam). During severe caffeine intoxication, the associated hypotension can be unresponsive to dopamine (20 µg/kg/min), norepinephrine (20 µg/min), and epinephrine (2 µg/min).[135] Case reports suggest that these patients may respond to vasopressin infusions initiated at 0.2 U/hour and titrated to 1.2 U/hour.[135] Caffeine is an adenosine receptor antagonist that may reduce the effectiveness of adenosine to convert paroxysmal supraventricular tachycardia if ingested <4 hours before treatment.[136]

Decontamination

Caffeine binds to activated charcoal, and patients should receive 50 g activated charcoal (1 g/kg) if the patient presents within 1–2 hours after ingestion of caffeine in tablet form. As with most overdoses, syrup of ipecac is contraindicated because of the potential for sudden loss of consciousness and seizures during severe caffeine intoxication. There are no data documenting improved clinical outcome following gastric lavage or any other measures of decontamination in this setting.

Enhancement of Elimination

Case reports associate clinical improvement in severe caffeine intoxication following the initiation of hemodialysis[135] or charcoal hemoperfusion.[83,137] Persistent hypotension may complicate the use of hemodialysis in severe caffeine overdose, and there are few data on the clearance rates or clinical efficacy of these methods of enhancing caffeine elimination.

Supplemental Care

During severe caffeine intoxication, patients should be monitored for dysrhythmias, electrolyte imbalance (hypokalemia, hypocalcemia), lactic acidosis, and respiratory depression. The catecholamine-induced hypokalemia is not a true potassium deficit; therefore, correction of hypokalemia should be cautious and immediate normalization of serum potassium with large doses of IV potassium avoided. There are no specific antidotes. Agitation may require the use of IV benzodiazepines as needed to calm the patient without causing respiratory depression. Treatment of the gastrointestinal symptoms include antiemetics (promethazine 25 mg IV, metoclopramide 5 mg IV, odansetron 4–8 mg IV) for vomiting and proton pump inhibitors (famotidine 20 gm IV, pantoprazole 40 mg IV) for gastritis.

References

1. Crocq M-A. Historical and cultural aspects of man's relationship with addictive drugs. Dialogues Clin Neurosci 2007;9:355–361.

2. Fredholm BB. Notes on the history of caffeine use. Handb Exp Pharmacol 2011;200:1–9.

3. Ashihara H, Suzuki T. Distribution and biosynthesis of caffeine in plants. Front Biosci 2004;9:1864–1876.

4. Benowitz NL. Clinical pharmacology of caffeine. Annu Rev Med 1990;41:277–288.

5. Garriguet D. Beverage consumption of Canadian adults. Health Rep 2008;19:23–29.

6. Frary CD, Johnson RK, Wang MQ. Food sources and intakes of caffeine in the diets of persons in the United States. J Am Diet Assoc 2005;105:110–113.

7. Stavric B. Methylxanthines: toxicity to humans. 2. Caffeine. Food Chem Toxicol 1988;26:645–662.

8. Knight CA, Knight I, Mitchell DC. Beverage caffeine intakes in young children in Canada and the US. Can J Diet Pract Res 2006;67:96–99.

9. Tunnicliffe JM, Erdman KA, Reimer RA, Lun V, Shearer J. Consumption of dietary caffeine and coffee in physically active populations: physiological interactions. Appl Physiol Nutr Metab 2008;33:1301–1310.

10. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol 1996;34:119–129.

11. Reissig CJ, Strain EC, Griffiths RR. Caffeinated energy drinks–a growing problem. Drug Alcohol Depend 2009;99:1–10.

12. Wu CD, Wei GX. Tea as a functional food for oral health. Nutrition 2002;18:443–444.

13. Galasko GR, Furman KI, Alberts E. The caffeine contents of non-alcoholic beverages. Food Chem Toxicol; 27:49–51.

14. Lelo A, Miners JO, Robson R, Birkett DJ. Assessment of caffeine exposure: caffeine content of beverages, caffeine intake, and plasma concentrations of methylxanthines. Clin Pharmacol Ther 1986;39:54–59.

15. Cabrera C, Artacho R, Gimenez R. Beneficial effects of green tea – a review. J Am Coll Nutr 2006;25:79–99.

16. Costa LM, Gouveia ST Nobrega JA. Comparison of heating extraction procedures for Al, Ca, Mg, and Mn in tea samples. Anal Sci 2002;18:313–318.

17. McKay DL, Blumberg JB. The role of tea in human health: an update. J Am Coll Nutr 2002 ;21:1–13.

18. Cao J, Zhao Y, Li Y, Deng HJ, Yi J, Liu JW. Fluoride levels in various black tea commodities: measurement and safety evaluation. Food Chem Toxicol 2006;44:1131–1137.

19. Rostagno MA, Manchón N, D'Arrigo M, Guillamón E, Villares A, García-Lafuente A, et al. Fast and simultaneous determination of phenolic compounds and caffeine in teas, mate, instant coffee, soft drink and energetic drink by high-performance liquid chromatography using a fused-core column. Anal Chim Acta 2011;685:204–211.

20. Davis PG, Schmidt B, Roberts RS, Doyle LW, Asztalos E, Haslam R, Sinha S, Tin W. Caffeine for Apnea of Prematurity Trial Group. Caffeine for Apnea of Prematurity Trial: benefits may vary in subgroups. J Pediatr 2010;156:382–387.

21. Astorino TA, Roberson DW. Efficacy of acute caffeine ingestion for short-term high-intensity exercise performance: a systematic review. J Strength Cond Res 2010;24:257–265.

22. Pelchovitz DJ, Goldberger JJ. Caffeine and cardiac arrhythmias: a review of the evidence. Am J Med 2011;124:284–289.

23. Steinke L, Lanfear DE, Dhanapal V, Kalus JS. Effect of "energy drink" consumption on hemodynamic and electrocardiographic parameters in healthy young adults. Ann Pharmacother 2009;43:596–602.

24. Robertson D, Wade D, Workman R, Woosley RL, Oates JA. Tolerance to the humoral and hemodynamic effects of caffeine in man. J Clin Invest 1981;67:1111–1117.

25. Waring WS, Laing WJ, Good AM, Malkowska AM. Acute caffeine ingestion: clinical features in patients attending the emergency department and Scottish Poison Centre enquiries between 2000 and 2008. Scott Med J 2009;54:3–6.

26. Dietrich AM, Mortensen ME. Presentation and management of an acute caffeine overdose. Pediatr Emerg Care 1990;6:296–298.

27. Benowitz NL, Osterloh J, Goldschlager N, Kaysen G, Pond S, Forhan S. Massive catecholamine release from caffeine poisoning. JAMA 1982;248:1097–1098.

28. Kennedy JS, Leduc BW, Scavone JM, Harmatz JS, Shader RI, Greenblatt DJ. Pharmacokinetics of intravenous caffeine: comparison of high-performance liquid chromatographic and gas chromatographic methods. J Chromatogr 1987;422:274–280.

29. Arnaud MJ. Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handb Exp Pharmacol 2011;200:33–91.

30. Miners JO, Birkett DJ. The use of caffeine as a metabolic probe for human drug metabolizing enzymes. Gen Pharmacol 1996;27:245–249.

31. Tassaneeyakul W, Birkett DJ, McManus ME, Tassaneeyakul W, Veronese ME, Andersson T, Tukey RH, Miners JO. Caffeine metabolism by human hepatic cytochromes P450: contributions of 1A2, 2E1 and 3A isoforms. Biochem Pharmacol 1994;47:1767–1776.

32. Kalow W, Tang BK. The use of caffeine for enzyme assays: a critical appraisal. Clin Pharmacol Ther 1993;53:503–514.

33. Ha HR, Chen J, Krahenbuhl S, Follath F. Biotransformation of caffeine by cDNA-expressed human cytochromes P-450. Eur J Clin Pharmacol 1996;49:309–315.

34. Aldridge A, Aranda JV, Neims AH. Caffeine metabolism in the newborn. Clin Pharmacol Ther 1979;25:447–453.

35. Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard JE, Cotreau MM, Harmatz JS, Shader RI. Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. J Clin Pharmacol 1997;37:693–703.

36. Leson CL, McGuigan MA, Bryson SM. Caffeine overdose in an adolescent male. Clin Toxicol 1988;26: 407–415.

37. Aldridge A, Bailey J, Neims AH. The disposition of caffeine during and after pregnancy. Semin Perinatol 1981;5:310–314.

38. Aden U. methylxanthines during pregnancy and early postnatal life. Handb Exp Pharmacol 2011;200:373–389.

39. Carrillo JA, Benitez J. Clinically significant pharmacokinetic interactions between dietary caffeine and medications. Clin Pharmacokinet 2000;39:127–153.

40. Lake CR, Rosenberg DB, Gallant S, Zaloga G, Chernow B. Phenylpropanolamine increases plasma caffeine levels. Clin Pharmacol Ther 1990;47:675–685.

41. Howland J, Rohsenow DJ, Arnedt JT, Bliss CA, Hunt SK, Calise TV, et al. The acute effects of caffeinated versus non-non-caffeinated alcoholic beverage on driving performance and attention/reaction time. Addiction 2011;106:335–341.

42. Morelli M, Simola N. Methylxanthines and drug dependence: a focus on interactions with substances of abuse. Handb Exp Pharmacol 2011;200:483–507.

43. Kerr JS, Sherwood N, Hindmarch I. Separate and combined effects of the social drugs on psychomotor performance. Psychopharmacology 1991;104:113–119.

44. Lorist MM, Tops M. Caffeine, fatigue, and cognition. Brain Cogn 2003;53:82–94.

45. Abbott PJ. Caffeine: a toxicological overview. Med J Aust 1986;145:518–521.

46. Irwin C, Desbrow B, Ellis A, O'Keeffe B, Grant G, Leveritt M. Caffeine withdrawal and high-intensity endurance cycling performance. J Sports Sci 2011;29: 509–515.

47. Pontifex KJ, Wallman KE, Dawson BT, Goodman C. Effects of caffeine on repeated sprint ability, reactive agility time, sleep and next day performance. J Sports Med Phys Fitness 2010;50:455–464.

48. Bell DG, Mclellan TM. Effect of repeated caffeine ingestion on repeated exhaustive exercise endurance. Med Sci Sports Exerc 2003;35:1348–1354.

49. Hunter AM, St. Clair Gibson A, Collins M, Lambert M, Noakes TD. Caffeine ingestion does not alter performance during a 100-km cycling time-trial performance. Int J Sport Nutr 2002;12:438–452.

50. Graham TE, Hibbert E, Sathasivam P. Metabolic and exercise endurance effects of coffee and caffeine ingestion. J Appl Physiol 1998;85:883–889.

51. Myers MG, Reeves RA. The effect of caffeine on daytime ambulatory blood pressure. Am J Hypertens 1991;4: 427–431.

52. Gong H Jr, Simmons MS, Tashkin DP, Hui KK, Lee EY. Bronchodilator effects of caffeine in coffee. A dose-response study of asthmatic subjects. Chest 1986;89: 335–342.

53. Fastbom J, Pazos A, Probst A, Palacios JM. Adenosine A1 receptors in the human brain: a quantitative autoradiographic study. Neuroscience 1987;22:827–839.

54. Ferre S, Fredholm BB, Morelli M, Popoli P, Fuxe K. Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. Trends Neurosci 1997;20:482–487.

55. Myers MG. Caffeine and cardiac arrhythmias. Ann Intern Med 1991;114:147–150.

56. Myers MG, Basinski A. Coffee and coronary heart disease. Arch Intern Med 1992;152:1767–1772.

57. Beaudoin M-S, Graham TE. Methylxanthines and human health: epidemiological and experimental evidence. Handb Exp Pharmacol 2011;200:509–548.

58. Thong FS, Derave W, Kiens B, Graham TE, Ursø B, Wojtaszewski JF, et al. Caffeine-induced impairment of insulin action but not insulin signaling in human skeletal muscle is reduced by exercise. Diabetes 2002;51: 583–590.

59. Hedges DW, Woo FL, Hoopes SP. Caffeine-induced psychosis. CNS Spectr 2009;14:127–129.

60. Rapoport JL, Berg CJ, Ismond DR, Zahn TP, Neims A. Behavioral effects of caffeine in children. Relationship between dietary choice and effects of caffeine challenge. Arch Gen Psychiatry 1984;41:1073–1079.

61. van Dam RM, Hu FB. Coffee consumption and risk of type 2 diabetes a systematic review. JAMA 2005;294: 97–104.

62. Winkelmayer WC, Stampfer MJ, Willett WC, Curhan GC. Habitual caffeine intake and the risk of hypertension in women. JAMA 2005;294:2330–2335.

63. Klag MJ, Wang NY, Meoni LA, Brancati FL, Cooper LA, Liang KY, et al. Coffee intake and risk of hypertension: the Johns Hopkins precursors study. Arch Intern Med 2002;162:657–662.

64. Panagiotakos DB, Pitsavos C, Chrysohoou C, Kokkinos P, Toutouzas P, Stefanadis C. The J-shaped effect of coffee consumption on the risk of developing acute coronary syndromes: the CARDIO2000 case-control study. J Nutr 2003;133:3228–3232.

65. Cornelis MC, El-Sohemy A, Kabagambe EK, Campos H. Coffee, CYP1A2 genotype, and risk of myocardial infarction. JAMA 2006;295:1135–1141.

66. Jackman M, Wendling P, Friars D, Graham TE. Metabolic catecholamine, and endurance responses to caffeine during intense exercise. J Appl Physiol 1996;81: 1658–1663.

67. Pelchovitz DJ, Goldberger JJ. Caffeine and cardiac arrhythmias: a review of the evidence. Am J Med 2011; 124:284–289.

68. Sutherland DJ, McPherson DD, Renton KW, Spencer CA, Montague TJ. The effect of caffeine on cardiac rate, rhythm, and ventricular repolarization. Analysis of 18

normal subjects and 18 patients with primary ventricular dysrhythmia. Chest 1985;87:319–324.

69. Myers MG, Harris L. High dose caffeine and ventricular arrhythmias. Can J Cardiol 1990;6:95–98.

70. Clee MD, Smith N, McNeill GP, Wright DS. Dysrhythmias in apparently healthy elderly subjects. Age Ageing 1979; 8:173–176.

71. Graboys TB, Blatt CM, Lown B. The effect of caffeine on ventricular ectopic activity in patients with malignant ventricular arrhythmia. Arch Intern Med 1989;149: 637–639.

72. Frost L, Vestergaard P. Caffeine and risk of atrial fibrillation or flutter: the Danish Diet, Cancer, and Health Study. Am J Clin Nutr 2005;81:578–582.

73. Larsson SC, Virtamo J, Wolk A. Coffee consumption and risk of stroke in women. Stroke 2011;42:908–912.

74. Lopez-Garcia E, Rodriguez-Artalejo F, Rexrode KM, Logroscino G, Hu FB, van Dam RM. Coffee consumption and risk of stroke in women. Circulation 2009; 119:1116–1123.

75. Hakim AA, Ross GW, Curb JD, Rodriguez BL, Burchfiel CM, Sharp DS, et al. Coffee consumption in hypertensive men in older middle-age and the risk of stroke: the Honolulu Heart Program. J Clin Epidemiol 1998;51: 487–494.

76. Mineharu Y, Koizumi A, Wada Y, Iso H, Watanabe Y, Date C, et al, and JACC Study Group. Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. J Epidemiol Community Health. 2011;65: 230–240.

77. Zhang WL, Lopez-Garcia E, Li TY, Hu FB, van Dam RM. Coffee consumption and risk of cardiovascular events and all-cause mortality among women with type 2 diabetes. Diabetologia 2009;52:810–817.

78. Verhelst X, Burvenich P, van Sassenbroeck D, Gabriel C, Lootens M, Baert D. Acute hepatitis after treatment for hair loss with oral green tea extracts (*Camellia sinensis*). Acta Gastroenterol Belg 2009;72:262–264.

79. Sarma DN, Barrett ML, Chavez ML, Gardiner P, Ko R, Mahady GB, et al. Safety of green tea extracts: a systematic review by the US Pharmacopeia. Drug Saf 2008;31: 469–484.

80. Molinari M, Watt KD, Kruszyna T, Nelson R, Walsh M, Huang WY, Nashan B, Peltekian K. Acute liver failure induced by green tea extracts: case report and review of the literature. Liver Transpl 2006;12:1892–1895.

81. Banner W Jr, Czajka PA. Acute caffeine overdose in the neonate. Am J Dis Child 1980;134:495–498.

82. Josephson GW, Stine RJ. Caffeine intoxication: a case of paroxysmal atrial tachycardia. JACEP 1976;5:776–778.

83. Zimmerman PM, Pulliam J, Schwengels J, MacDonald SE. Caffeine intoxication: a near fatality. Ann Emerg Med 1985;14:1227–1229.

84. Berger AJ, Alford K. Cardiac arrest in a young man following excess consumption of caffeinated "energy drinks." Med J Aust 2009;190:41–43.

85. Forman J, Aizer A, Young CR. Myocardial infarction resulting from caffeine overdose in an anorectic woman. Ann Emerg Med 1997;29:178–180.

86. House CJ, Palmentier JP. Two caffeine-related fatalities. Can Soc Forensic Sci J 2004;37:111–118.

87. Rudolph T, Knudsen K. A case of fatal caffeine poisoning. Acta Anaesthesiol Scand 2010;54:521–523.

88. Alstott RL, Miller AJ, Forney RB. Report of a human fatality due to caffeine. J Forensic Sci 1973;18:135–137.

89. Morrow PL. Caffeine toxicity: a case of child abuse by drug ingestion. J Forensic Sci 1987;32:1801–1805.

90. Juliano LM, Griffiths RR. A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. Psychopharmacology 2004;176:1–29.

91. Griffiths RR, Woodson PP. Caffeine physical dependence: a review of human and laboratory animal studies. Psychopharmacology 1988;94:437–451.

92. Kendler KS, Prescott CA. Caffeine intake, tolerance, and withdrawal in women: a population-based twin study. Am J Psychiatry 1999;156:223–228.

93. Evans SM, Griffiths RR. Caffeine withdrawal: a parametric analysis of caffeine dosing conditions. J Pharmacol Exp Ther 1999;289:285–294.

94. Griffiths RR, Evans SM, Heishman SJ, Preston KL, Sannerud CA, Wolf B, Woodson PP. Low-dose caffeine physical dependence in humans. J Pharmacol Exp Ther 1990;255:1123–1132.

95. Watson JM, Lunt MJ, Morris S, Weiss MJ, Hussey D, Kerr D. Reversal of caffeine withdrawal by ingestion of a soft beverage. Pharmacol Biochem Behavior 2000;66:15–18.

96. Nehlig A, Boyet S. Dose-response study of caffeine effects on cerebral functional activity with a specific focus on dependence. Brain Res 2000;858:71–77.

97. Pollack AZ, Buck Louis GM, Sundaram R, Lum KJ. Caffeine consumption and miscarriage: a prospective cohort study. Fertil Steril 2010;93:304–306.

98. Clausson B, Granath F, Ekbom A, Lundgren S, Nordmark A, Signorello LB, Cnattingius S. Effect of caffeine exposure during pregnancy on birth weight and gestational age. Am J Epidemiol 2002;155:429–436.

99. Maslova E, Bhattacharya S, Lin S-W, Michels KB. Caffeine consumption during pregnancy and risk of preterm birth: a meta-analysis. Am J Clin Nutr 2010; 92:1120–1132.

100. CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ 2008;337:a2332.

101. Bracken MB, Triche EW, Belanger K, Hellenbrand K, Leaderer BP. Association of maternal caffeine consumption with decrements in fetal growth. Am J Epidemiol 2003;157:456–466.

102. Fortier I, Marcoux S, Beaulac-Baillargeon L. Relation of caffeine intake during pregnancy to intrauterine growth retardation and preterm birth. Am J Epidemiol 1993; 137:931–940.

103. Signorello LB, McLaughlin JK. Maternal caffeine consumption and spontaneous abortion a review of the epidemiologic evidence. Epidemiology 2004;15:229–239.

104. Bech BH, Nohr EA, Vaeth M, Henriksen TB, Olsen J. Coffee and fetal death: a cohort study with prospective data. Am J Epidemiol 2005;162:983–990.

105. Christian MS, Brent RL. Teratogen update: evaluation of the reproductive and developmental risks of caffeine. Teratology 2001;64:51–78.

106. International Agency for Research on Cancer: Caffeine. IARC Monogr 1991;51:291–390.

107. International Agency for Research on Cancer: Coffee. IARC Monogr 1991;51:41–206.

108. Vandenbroucke JP, Kok FJ, van't Bosch G, van den Dungen PJ, van der Heide-Wessel C, van der Heide RM. Coffee drinking and mortality in a 25-year follow-up. Am J Epidemiol 1986;123:359–361.

109. Islami F, Boffetta P, Ren J-S, Pedoeim L, Khatib D, Kamangar F. High-temperature beverages and foods and esophageal cancer risk – a systematic review. Int J Cancer 2009;125:491–524.

110. Carregaro AB, Woods WE, Tobin T, Queiroz-Neto A. Comparison of the quantification of caffeine in human plasma by gas chromatography and ELISA. Braz J Med Biol Res 2001;34:821–824.

111. Chen CN, Liang CM, Lai JR, Tsai YJ, Tsay JS, Lin JK. Capillary electrophoretic determination of theanine, caffeine, and catechins in fresh tea leaves and oolong tea and their effects on rat neurosphere adhesion and migration. J Agric Food Chem 2003;51:7495–7503.

112. Shrivas K, Wu H-F. Rapid determination of caffeine in one drop of beverages and foods using drop-to-drop solvent microextraction with gas chromatography/mass spectrometry. J Chromatogr A 2007;1170:9–14.

113. Teeuwen HW, Elbers EL, van Rossum JM. Rapid and sensitive gas-chromatographic determination of caffeine in blood plasma, saliva, and xanthine beverages. Mol Biol Rep 1991;15:1–7.

114. Regal KA, Howald WN, Peter RM, Gartner CA, Kunze KL, Nelson SD. Subnanomolar quantification of caffeine's *in vitro* metabolites by stable isotope dilution gas chromatography-mass spectrometry. J Chromatogr B 1998;708:75–85.

115. Verenitch SS, Mazumder A. Development of a methodology utilizing gas chromatography ion-trap tandem mass spectrometry for the determination of low levels of caffeine in surface marine and freshwater samples. Anal Bioanal Chem 2008;391:2635–2646.

116. Fligner CL, Opheim KE. Caffeine and its dimethylxanthine metabolites in two cases of caffeine overdose: a cause of falsely elevated theophylline concentrations in serum. J Anal Toxicol 1988;12:339–343.

117. Smith JM, Pearson S, Marks V. Plasma caffeine concentration in outpatients. Lancet 1982;2(8305):985–986.

118. Routh JI, Shane NA, Arredondo EG, Paul WD. Determination of caffeine in serum and urine. Clin Chem 1969;15:661–668.

119. Sved S, Hossie RD, McGilveray IJ. The human metabolism of caffeine to theophylline. Res Commun Chem Pathol Pharmacol 1976;13:185–192.

120. Holmgren P, Norden-Pettersson L, Ahlner J. Caffeine fatalities – four case reports. Forensic Sci Int 2004;139:71–73.

121. Dimaio VJ, Garriott JC. Lethal caffeine poisoning in a child. Forensic Sci 1974;3:275–278.

122. Anderson BJ, Gunn TR, Holford NH, Johnson R. Caffeine overdose in a premature infant: clinical course and pharmacokinetics. Anaesth Intensive Care 1999;27:307–311.

123. Nagesh RV, Murphy KA Jr. Caffeine poisoning treated by hemoperfusion. Am J Kidney Dis 1988;12:316–318.

124. Price KR, Fligner DJ. Treatment of caffeine toxicity with esmolol. Ann Emerg Med 1990;19:44–46.

125. Mrvos RM, Reilly PE, Krenzelok EP. Massive caffeine ingestion resulting in death. Vet Hum Toxicol 1989;31:571–572.

126. Kerrigan S, Lindsey T. Fatal caffeine overdose: two case reports. Forensic Sci Int 2005;153:67–69.

127. Dalpe-Scott M, Degouffe M, Garbutt D, Drost M. A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. Can Soc Forensic Sci J 1995;28:113–121.

128. Garriott JC, Simmons LM, Poklis A, Mackell MA. Five cases of fatal overdose from caffeine-containing "look-alike" drugs. J Anal Toxicol 1985;9:141–143.

129. Birkett DJ, Miners JO. Caffeine renal clearance and urine caffeine concentrations during steady state dosing. Implications for monitoring caffeine intake during sports events. Br J Clin Pharmacol 1991;31:405–408.

130. Chopra A, Morrison L. Resolution of caffeine-induced complex dysrhythmia with procainamide therapy. J Emerg Med 1995;13:113–117.

131. Schmidt A, Karlson-Stiber C. Caffeine poisoning and lactate rise: an overlooked toxic effect? Acta Anaesthesiol Scand 2008;52:1012–1014.

132. Ergenekon E, Dalgic N, Aksoy E, Koc E, Atalay Y. Caffeine intoxication in a premature neonate. Paediatr Anaesth 2001;11:737–739.

133. Wrenn KD, Oschner I. Rhabdomyolysis induced by a caffeine overdose. Ann Emerg Med 1989;18:94–97.

134. Kamijo Y, Soma K, Asari Y, Ohwada T. Severe rhabdomyolysis following massive ingestion of oolong tea: caffeine intoxication with coexisting hyponatremia. Vet Hum Toxicol 1999;41:381–383.

135. Holstege CP, Hunter Y, Baer AB, Savory J, Bruns DE, Boyd JC. Massive caffeine overdose requiring vasopressin infusion and hemodialysis. J Toxicol Clin Toxicol 2003;41:1003–1007.

136. Cabalag MS, Taylor DM, Knott JC, Buntine P, Smit D, Meyer A. Recent caffeine ingestion reduces adenosine efficacy in the treatment of paroxysmal supraventricular tachycardia. Acad Emerg Med 2010;17:44–49.

137. Rivenes SM, Bakerman PR, Miller MB. Intentional caffeine poisoning in an infant. Pediatrics 1997;99:736–738.

Chapter 56

COCAINE

HISTORY

As one of the most potent naturally occurring central nervous system (CNS) stimulants, cocaine has a long history of use and abuse.[1] Analysis of material from a 3,000-year-old Egyptian mummy revealed detectable quantities of cocaine,[2] but there is no documentation that ancient Egyptians used cocaine for recreational purposes. Archaeological findings in Ecuador indicate that the pre-Inca inhabitants chewed cocaine long before the Spanish Conquistadors discovered the use of cocaine among the privileged groups of the Inca culture. The Moche culture flourished on the northern coast of Peru from about 100–700 AD; their ceramic sculptures from this period depict the chewing of coca leaves along with the implements to increase the extraction of cocaine from the leaves by holding alkaline powder near the leaves during mastication.[3] The Incas believed this plant was a divine gift created to alleviate hunger and thirst, and they tightly controlled coca cultivation. The Inca ruling class would chew the leaves on special occasions, whereas members of the working class were occasionally rewarded with a gift of coca leaves. In 1507, the European explorer Amerigo Vespucci mentioned the Indian practice of chewing coca with alkaline ash. Despite an early ban by Spanish Conquistadors on cocaine use, Spanish entrepreneurs used coca leaves as an incentive to employ Indians in hard labor. Although Francisco Pizarro probably brought coca leaves to the 16th-century Spanish court, Europeans did not use coca leaves during this period, in part because coca leaves would have lost their potency during the lengthy trip across the Atlantic Ocean.[4]

Sir William Hook published the first English illustration of coca in 1835 along with a translation of a book by the German naturalist, Edward Poeppig that contained warnings about the addictive properties of cocaine.[5] In 1860, Albert Niemann purified cocaine from coca leaves brought from the New World on a frigate of Archduke Ferdinand. Although the anesthetic properties of cocaine were recognized soon after purification, there was little initial interest in the medicinal properties of this compound. Cocaine-containing wines (e.g., vin Mariani), which contained 6 mg cocaine per ounce, were a popular restorative and tonic in Italy and France during the 1860s.[6]

In 1884, Sigmund Freud wrote glowing reports about the ability of cocaine to relieve depression and cure morphine addiction in his famous treatise, "Über Coca." In that same year, Carl Koller recognized the potential of cocaine to anesthetize the cornea during ophthalmologic surgery. Halstead, an American surgeon, demonstrated the peripheral nerve-blocking properties of cocaine injections. Cocaine was soon prescribed for a wide range of medical conditions from hay fever to hemorrhoid surgery. Initially, the use of cocaine was enthusiastically accepted. Robert Louis Stevenson wrote the first draft of *Dr. Jekyll and Mr. Hyde* following 3 days of cocaine administration for the treatment of tuberculosis. Sir Arthur Conan Doyle's character, Sherlock Holmes, used unnamed artificial stimulants until he became tired, wasted, and paranoid. Later, he reappeared in good health, his pipe replacing the use of stimulants.[7] Pharmacist John Styth Pemberton marketed a cocaine-caffeine mixture called Coca-Cola in 1886 after dropping a wine-cocaine product that competed unsuccessfully with vin Mariani.

The first reports of serious complications from cocaine use appeared in the mid-1880s, not long after Freud's treatise.[8] By 1891, there were over 200 reports of serious adverse reactions, including 13 deaths following the use of cocaine.[4] Unfortunately, Halstead became one of the first victims of cocaine dependence when he tried to cure his morphine addiction with cocaine. Freud was accused of unleashing the third great scourge (after ethanol and opiates) on humanity; subsequently, he removed all reference to cocaine abuse from his autobiography.

During the latter 19th century and most of the first third of the 20th century, the production and export of coca leaves were legal in most countries. The export of coca leaves from South America rose steadily during the 1880s, but the loss of potency during the long voyages led to the processing of the leaves into raw cocaine prior to shipping. During the early 1900s, a new source of coca leaves from Java undercut the South American coca trade with high-grade coca (i.e., about 1.5% compared with <1% for most coca leaves).[9] Japan also entered the coca trade with domestically manufactured cocaine and coca grown in Formosa. Coca export records from the major producing sites (Peru, Bolivia, Java, Formosa) indicate that the legal production of coca peaked around 1920; these records also indicate that cocaine consumption was first popular in North America, then in Europe and other parts of the world.

Nations that ratified the Versailles Treaty and the 3 treaties with Austria-Hungary, Bulgaria, and Turkey agreed to implement the provisions of the Hague Opium Convention of 1912.[9] This Convention required that each nation control cocaine exports so that only authorized persons could receive the drug. The United States attempted to control cocaine use through the Pure Food and Drug Act of 1906; consequently, the Coca-Cola Company removed cocaine from the coca leaves prior to formulation of their drink. To comply with the Opium Convention of 1912, the United States implemented the 1914 Harrison Narcotic Act. This legislation labeled cocaine as a narcotic with the same restrictions and penalties as heroin. Eventually, coca exports steadily declined by the 1920s. Although clandestine laboratory books on the synthesis of cocaine have been available since the 1920s,[10,11] the synthetic process is too complex and costly compared with the isolation and purification of cocaine from coca leaves.

Reports of serious cocaine toxicity were rare between the publication of Maier's classic text on cocaine abuse in 1926 and the appearance of reports on cocaine body packing in the late 1970s.[12] In 1970, the Comprehensive Drug Abuse Prevention and Control Act classified cocaine as a schedule II drug (i.e., high abuse potential with limited medical application), but again allowed the medical use of cocaine. Serious complications from cocaine use increased substantially in the 1980s when new forms (e.g., smoking freebase) of cocaine abuse dramatically increased the amount of cocaine used by addicts.[13] Cocaine-associated deaths resulted from all routes of administration, including intravenous (IV), nasal, vaginal, and oral, as well as from unintentional and deliberately inflicted trauma.[14] Beginning in 2003, the South American cocaine cartels began adding the veterinary antihelminthic, levamisole to bulk cocaine shipments as an adulterant, reportedly to potentiate the effects of cocaine; by 2008, about one-fourth of confiscated cocaine analyzed by the United States Drug Enforcement Administration (DEA) contained levamisole.[15] The intended role of levamisole in illicit cocaine remains unknown, although recent data suggest that the stimulant, aminorex is a metabolite of levamisole at least in some animal species (e.g., horses).

BOTANICAL DESCRIPTION

Common Name: Coca plant, Cuca (Peru), Epadu or Ipadu (Brazil), Hayo (Venezuela), Huánuco Coca (Bolivia), Spadic (Colombia)

Scientific Name: *Erythroxylum coca* Lam.

Botanical Family: Erythroxylaceae

Physical Description: This shrub grows to 3–6 m (~9–18 ft) in height with straight, alternate reddish branches. The pointed leaves are smooth, slightly glossy, and green to green-brown. The leaf size ranges from 1.5–3 cm in width and from 0.5–11 cm in length. These leaves contain an areolate portion with the midrib bounded by a curved, longitudinal line on each side, particularly prominent on the undersurface. Red berries form from the flowers and each berry contains only 1 seed. Following pruning of the branches, multiple new branches appear around the cut in a geometric pattern.

Distribution and Ecology: This bush requires the moist, tropical climate found in the eastern Peruvian Andes (Peru, Ecuador, Bolivia), and to a lesser extent, in Mexico, the West Indies, and Java. These plants grow best in lush high valleys of the eastern Andes. Illicit production of the coca plant occurs at elevations of 1,500–6,000 ft (~450–1,800 m) because of the lower cocaine yield at lower elevations. There are 2 recognized infraspecies of the coca plant: *Erythroxylum coca* var. *coca* Lam. and *Erythroxylum coca* var. *ipadu* Plowman. The *E. coca* var. *coca* Lam. variety is the most common source of street cocaine. The less common

source of cocaine, *E. coca* var. *ipadu* Plowman is cultivated primarily in the Amazon Valley. The coca plant should not be confused with the cocoa plant, which contains caffeine rather than cocaine.

Common Name: Columbian Coca plant

Scientific Name: *Erythroxylum novogranatense* (Morris) Hieron.

Botanical Family: Erythroxylaceae

Physical Description: This shrub or small tree grows to about 2.5 m (~8–9 ft) in height with small, obovate to narrowly elliptical, pointed leaves up to 5 cm in length. An intramarginal vein surrounds the midvein on each side. Small, solitary flowers appear throughout the year and they produce a red, oblong drupe that contains a single seed.

Distribution and Ecology: This plant grows in the drier mountainous region of Colombia, the Caribbean coast of South America, and the north coast of Peru. The 2 varieties of *Erythroxylum novogranatense* are *E. novogranatense* var. *novogranatense* (Morris) Hieron. and *E. novogranatense* var. *truxillense* (Rusby) Plowman. The leaves from a strain of *E. novogranatense* grown near Trujillo, Peru are legally exported to the Stephan Chemical Company (Maywood, NJ), which is the only legally recognized manufacturer of cocaine for pharmaceutical purposes in the United States.

IDENTIFYING CHARACTERISTICS

Structure

Cocaine (methyl benzoylecgonine) is a tertiary amine that is structurally, but not pharmacologically related to the tropane family of natural alkaloids (e.g., scopolamine, atropine) as demonstrated in Figure 56.1. Although amphetamines and cocaine share similar stimulant properties, cocaine is structurally distinct from phenylethylamine compounds. Cocaine base is methyl benzoylecgonine (CAS RN: 50-36-2, $C_{17}H_{21}NO_4$).
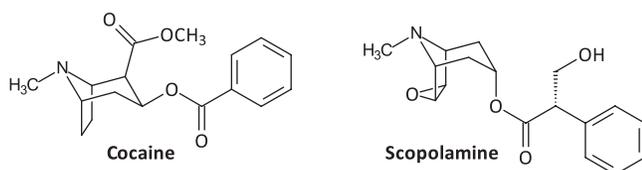
FIGURE 56.1. Chemical structures of cocaine and scopolamine.

Physiochemical Properties

Cocaine is a weak base with a pK_a of 8.6 compared with a pK_a of 8.23 for the more hydrophobic, cocaethylene. The relatively greater hydrophobicity of cocaethylene may contribute to its longer plasma half-life compared with cocaine.[16] In alkaline solutions, the non-ionized or freebase form of cocaine predominates, whereas in acidic solutions cocaine exists as the salt (e.g., hydrochloride in an HCl solution). Pure cocaine base is an odorless, colorless to white crystalline powder with a bitter taste and a melting point of 98°C (208.4°F). Cocaine base is poorly water-soluble (0.00167 g/ mL), whereas cocaine hydrochloride is very soluble in water (2.5 g/mL). The hydrochloride salt has limited volatility (i.e., very low vapor pressure) and a melting point of about 195°C (383°F). Hydrolysis of cocaine hydrochloride occurs rapidly at pH >4.

Terminology

Street names for cocaine include Coke, Snow, Flake, Gold Dust, Green Gold, Blow, Pimp's Drug, Rock, Bernice, Star-Spangled Powder, Root, C, Lady, White, White Girl or Lady, Nose Candy, Speedball (heroin and cocaine), Toot, Tick (smoked with phencyclidine), and Liquid Lady (ethanol and cocaine).

Form

Cocaine exists in illicit samples as the cocaine salt (hydrochloride) or as the cocaine base (crack). The most common form of illicit cocaine is the white crystalline, highly water soluble powder, cocaine hydrochloride as displayed in Figure 56.2. As the vapor pressure of cocaine hydrochloride is very low, vaporization of cocaine hydrochloride requires temperatures >200°C (>392°F), resulting in an unsuitable form for smoking. Unlike the hydrochloride and sulfate salts of cocaine, freebase cocaine vaporizes at 98°C (208°F); therefore, this form of cocaine is suitable for smoking. Crack is the freebase form of cocaine that has a characteristic crackling sound when smoked. Figure 56.3 displays a sample of crack cocaine. This type of cocaine should not be confused with freebasing, which is a process to purify cocaine hydrochloride by mixing aqueous cocaine with baking soda or ammonia and extracting the free form of cocaine with an organic solvent (ether). Legitimate commercial production of cocaine involves the synthesis of cocaine from (-)-ecgonine in the presence of methanol and benzoic acid after hydrolysis of the ester alkaloids extracted from the plant material.

FIGURE 56.2. Confiscated cocaine hydrochloride powder. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 56.3. Crack cocaine. (Photo courtesy of the US Drug Enforcement Agency)

EXPOSURE

Epidemiology

For many years, poverty-stricken Indians of the Andean highlands chewed coca leaves to relieve fatigue and hunger as well as to improve productivity and energy during hard labor in the mines. For 50 years after the Harrison Narcotic Act, the use of cocaine in the United States was limited to the jazz scene. In the 1960s, cocaine emerged as a substance commonly used with heroin (i.e., Speedball) by IV polydrug users,[17] but by the 1970s cocaine had become an expensive drug that was insufflated (sniffed) or smoked rather than injected. By 1980, almost 10 million people over 11 years of age reported

using cocaine during the preceding year, with almost half indicating use within the last month.[18] Members of the middle and upper class were attracted to cocaine because it promotes feelings of exhilaration and ecstasy, enhanced self-confidence, and heightened physical prowess. Concern over the illicit use of cocaine increased dramatically during the early 1980s because of the accelerated use of cocaine among all social classes and the prevalence of more hazardous forms of abuse (e.g., free-basing, crack cocaine). Overall, the number of people in the United States using cocaine doubled from the preceding 2 years. Cocaine use among American high school seniors increased from 6% in 1976 to 20% in 1982.[19]

The number of cocaine users peaked in ~1985. The estimated number of occasional cocaine users (i.e., <12 days in preceding year) decreased from 7.1 million in 1985 to about 2.4 million in 1994 and these statistics remained relatively stable during the next decade.[20,21] Between 2002 and 2009, the estimated number of individuals in the United States aged 12 years or older using cocaine for the first time in the last year declined from ~1 million to 617,000 including a reduction in the number of first time crack cocaine users from 337,000 to 94,000.[22] During this same period, the estimated number of individuals in the United States aged 12 years or older with cocaine dependence or abuse decreased from 1.5 million to 1.1 million. Introduced in the 1980s, crack cocaine was a new method of delivery rather than a new drug. Since the introduction of crack cocaine, there has been a clear trend for first-time cocaine use among polydrug users to smoke crack cocaine rather than inject cocaine.[23] Although cocaine addicts frequently begin with intranasal cocaine use, most of these addicts transition to the smoking route.[24] Patients who smoke freebase or administer IV cocaine rarely revert to insufflation (snorting).

Sources

ORIGIN/COMPOSITION

Cocaine is 1 of at least 14 alkaloids extracted from the leaves of 2 coca shrubs (*E. coca*, *E. novogranatense*) indigenous to the Andean region of South America. The relative amounts of alkaloids in coca leaves vary with age as well as with species and variety.[25] The leaves of the *ipadu* variety of *E. coca* contain much smaller amounts of cocaine compared with other varieties of the coca plant. The seeds of coca plants do not contain cocaine.[26] The lifespan of the plant is over 40 years, but production of an average coca plant declines after about 10 years.[5] The leaves contain between 0.5–2.0% (average <1%) cocaine depending on the strain and weather con-

ditions; harvest occurs in March, June, and November beginning 2 years after planting.[31] After the removal of cocaine, the residual leaf material from the Colombian coca plant (*E. novogranatense*) is a flavoring agent for the soft drink, Coca-Cola.

PRODUCTION PROCESSES

COCOA PASTE.   The processing of cocaine hydrochloride for illicit distribution involves the following 3 steps: 1) extraction of crude coca paste from the coca leaf, 2) extraction of cocaine base from coca paste, and 3) conversion of the cocaine base to cocaine hydrochloride. These steps usually occur in South America near the fields of coca plants, and the techniques are somewhat variable depending on the availability of chemicals.[27] The solvent extraction technique uses limewater and gasoline or kerosene during the maceration of coca leaves. The addition of a dilute sulfuric acid solution allows the separation of the aqueous layer containing cocaine from the gasoline or kerosene. Neutralization of the aqueous layer with alkaline material causes the precipitation of a crude coca paste containing cocaine as well as other alkaloids, basic inorganic salts, and hydrolysis products.

The acid extraction process involves the maceration of the coca leaves in a pit lined with heavy plastic. The pit contains sulfuric acid and stomping by workers causes the cocaine base to leach from the leaves in the form of an aqueous solution of cocaine sulfate. After several extractions and the filtering of the solution to remove insoluble plant materials, the excess acid is neutralized by the addition of basic lime or sodium carbonate. The cocaine sulfate solution then forms a very crude coca paste. This paste contains a variety of chemicals including *cis-* and *trans-*cinnamoylcocaine, cuscohygrine, hygrine, tropine, tropacocaine, ecgonine, benzoylecgonine, methyl ecgonine, truxilline compounds, plant waxes, and benzoic acid. The refining process may include the addition of potassium permanganate to destroy cinnamoylcocaine isomers.[5] This step allows the formation of *n*-formyl cocaine that is not normally present in the paste; this by-product can hydrolyze to norcocaine. Either acetone or ether is added to the coca paste to extract the cocaine base, and the solution is poured into a reaction vessel along with a dilute solution of hydrochloric acid in acetone. The resultant slurry is poured on a bed sheet to filter out the cocaine hydrochloride. After drying, the cocaine hydrochloride is packaged for shipping. Figure 56.4 displays confiscated cocaine bricks.

FREEBASE COCAINE.   To produce the freebase form of cocaine, sodium bicarbonate or household ammonia is

FIGURE 56.4. Confiscated cocaine bricks. (Photo courtesy of the US Drug Enforcement Agency)

mixed in a vessel containing cocaine hydrochloride and water. Diethyl ether is added to the basic aqueous solution; then, the mixture separates into a biphasic solution. The aqueous, lower layer is discarded; the upper (organic) layer containing the cocaine base is decanted and dried.[28] The processing of freebase cocaine removes water-soluble impurities (e.g., sugars) and some inert compounds (inositol, talc, cornstarch), but not basic organic compounds including local anesthetics (e.g., lidocaine, benzocaine) or stimulants (e.g., amphetamines, caffeine, ephedrine).[29] These organic bases are converted to their freebase forms in the same way that cocaine remains in the final product.

CRACK COCAINE.   *Crack* is the term for the tiny crystalline pieces (*rocks*) of freebase cocaine that produce a popping sound (cracking) when heated during smoking. Preparation of crack cocaine involves the mixing of cocaine hydrochloride with sodium bicarbonate (or household ammonia) in sterile water and the heating of the mixture in boiling water until the precipitated cocaine base forms an oily compound. Reduction of the temperature in the reaction vessel by the addition of ice allows the solidification of cocaine base on the bottom of the vessel. The cocaine base precipitates as small pellets (*rocks*) when the water bath cools. These irregularly shaped, off-white to buff-colored fragments of compressed powder are about ¼″ to ⅜″ in width. After collection of the cocaine base from the vessel, the material is dried under a heat lamp or in a microwave oven. The only distinction between processing of crack cocaine and freebase cocaine is the absence of the organic solvent extraction phase in the processing of crack cocaine. As such, incomplete drying may result in high moisture content compared with freebase cocaine.

IMPURITIES AND PROFILING

Similar to many other illicit drugs, street samples of cocaine contain impurities, contaminants, diluents, and adulterants.[30] The extraction of cocaine from the coca leaf results in the presence of other impurities (i.e., natural alkaloids: hydroxycocaine, *cis*-cinnamoylcocaine, *trans*-cinnamoylcocaine, tropacocaine, cuscohygrine, hygrine, and truxilline compounds).[27,31] Truxilline alkaloids (α-, β-, γ-, δ-, ε-truxilline) are precursors of truxillic and truxinic acids that may also occur in illicit cocaine samples.[32] Contaminants are by-products of the manufacturing process of cocaine. Degradation of cocaine during the manufacturing process produces ecgonine, benzoylecgonine, or methyl ecgonine as well as benzoic acid and methylecgonidine. Typically, illicit cocaine hydrochloride is about 30% pure as a result of additives (diluents, adulterants) added after the completion of the manufacturing process.[33] Diluents are inert substances with physical characteristics (e.g., taste, form, texture, color) similar to the illicit drug (i.e., cocaine). Common diluents of illicit cocaine include primarily sugars (mannitol, lactose, sucrose). Adulterants are pharmacologically active ingredients that enhance or antagonize the effect of the illicit drug (i.e., cocaine).

Although increased supplies of cocaine often improve the purity of street cocaine, invariably the street samples of cocaine contain many adulterants including inert compounds (inositol, lactose, mannitol), local anesthetics (benzocaine, lidocaine, procaine, tetracaine), stimulants (amphetamines, caffeine, ephedrine, theophylline, phenylpropanolamine, phendimetrazine, phenmetrazine, phentermine), and toxic compounds (quinine, strychnine).[34] Other adulterants found in cocaine samples include phenobarbital, diphenhydramine, dipyrone, aminophenazone, levamisole (veterinary antihelminthic), noraminophenazone, phenacetin, and salicylamide.[35,36] The presence of adulterants should be considered whenever adverse reactions complicate cocaine use; however, the clinical effects of these impurities are frequently difficult to separate from the effects of cocaine use. Levamisole is a known cause of agranulocytosis in veterinary medicine; analysis of clinical specimens and drug paraphernalia of some cocaine-using patients with agranulocytosis demonstrated the presence of this drug.[37,38] Cocaine use is not usually associated with agranulocytosis.

Figure 56.5 demonstrates the structures of cocaine, cocaine alkaloids, manufacturing by-products of illicit cocaine, and other impurities. Because the coca plant synthesizes only the levorotatory isomer of cocaine (*l*-cocaine), the presence of diastereoisomers or its dextroenantiomer (*d*-cocaine) indicates synthetic production. Although the synthesis of cocaine is quite

sophisticated and beyond the capabilities of most illicit laboratories, gas chromatography, nuclear magnetic resonance, and mass spectrometry methods can distinguish natural from synthetic cocaine in forensic or legal settings.[39,40]

Methods of Abuse

In modern, rural Andean society, coca chewing is a ritualistic part of poverty and hard labor that produces relatively low cocaine blood concentrations.[41] Since pre-Inca times, coca chewing has been a method of cocaine use intended to alleviate hunger, ease fatigue, and improve mood.[42,43] For cocaine users in developed countries, cocaine use is a behavior intended to add excitement to their lives, enhance self-image, or improve performance or productivity. Although these users frequently project outward signs of success, cocaine use initially suppresses feelings of depression, low self-esteem, and boredom. Compulsive cocaine use results from the need to increase the amount of cocaine to sustain the desired effects of the drug. Such drug-oriented behavior causes social, economic, and physical deterioration. Based on admissions to abuse treatment centers, 40% of addicts use cocaine nasally, 30% by freebase smoking, 20% by injection, and 10% by combined routes.[44] Chronic cocaine use does not produce the classical pattern of addiction that occurs following chronic opioid or sedative-hypnotics abuse. However, chronic use of cocaine produces acute tolerance to the euphoric effects of cocaine as well as some physical symptoms of withdrawal including insomnia, irritability, depression, and headaches. These chronic effects usually do not occur following medical or casual cocaine use.

INSUFFLATION

Nasal insufflation (sniffing or snorting) is a popular method of cocaine abuse for recreational cocaine users. These cocaine users form a line of finely crushed cocaine crystals on a hard surface (e.g., a mirror) using a sharp edge (e.g., a razor blade). Depending on the purity of the cocaine, each line represents ~10–35 mg of cocaine. After rolling a paper or using a straw, the cocaine user draws or snorts the line of powder into the nostril. Insufflation is a relatively inefficient method of cocaine use compared with smoking freebase cocaine.

SMOKING

Smoking cocaine base produces a rapid increase in plasma cocaine concentrations with peak concentrations occurring within 5 minutes and the immediate onset of euphoria.[45] As measured by VAS ratings and
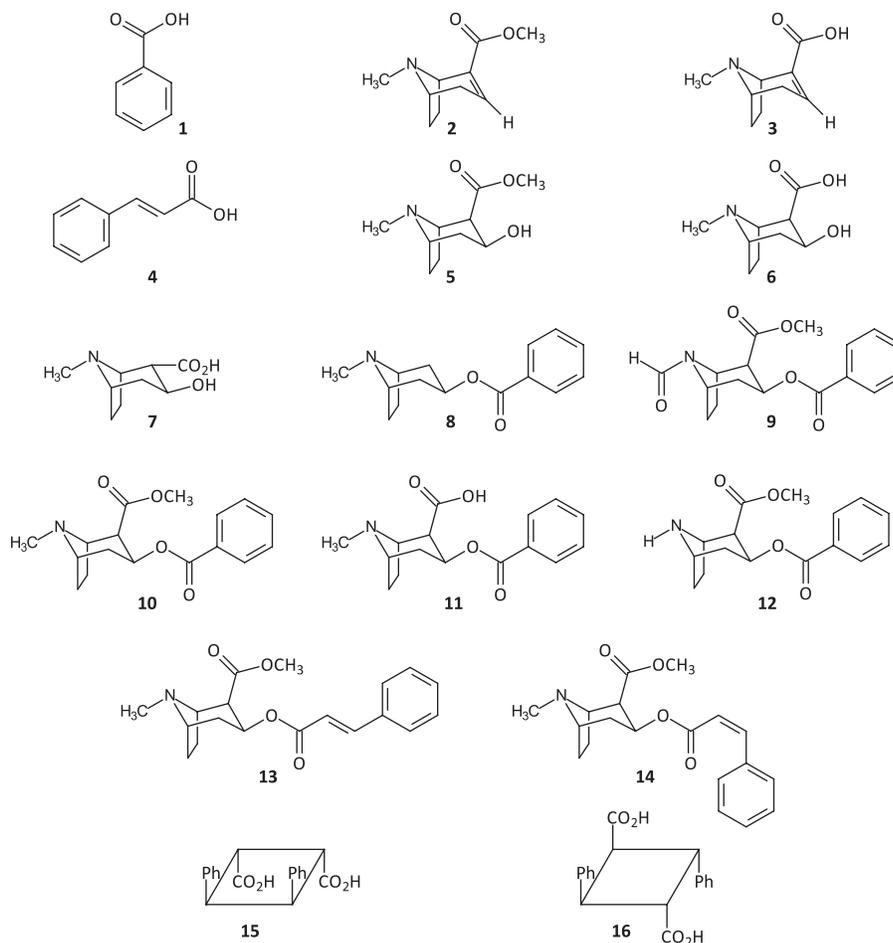
FIGURE 56.5. Chemical structures of some alkaloids in coca leaf along with alkaloidal impurities and manufacturing by-products in illicitly refined cocaine. 1 = benzoic acid; 2 = ecgonidine methyl ester; 3 = ecgonidine; 4 = trans-cinnamic acid; 5 = ecgonine methyl ester; 6 = ecgonine; 7 = pseudoecgonine; 8 = tropacocaine; 9 = *N*-formylnorcocaine; 10 = cocaine; 11 = benzoylecgonine; 12 = *N*-norcocaine; 13 = *trans*-cinnamoylcocaine; 14 = *cis*-cinnamoylcocaine; 15 = β-truxinic acid; 16 = α-truxillic acid.

vital signs in pharmacodynamic studies of 10 healthy men, the cardiovascular and subjective effects following the smoking of 50 mg cocaine were similar to the effects following the IV administration of 32 mg cocaine.[46] The exception was higher VAS ratings of "stimulated," "high," and "liking" following smoked cocaine compared with IV cocaine.

FREEBASE. Smoking of cocaine requires the conversion of the cocaine hydrochloride salt to the more volatile, freebase form. The main distinction between crack cocaine and cocaine used in freebasing is the difference in the production processes (i.e., the use of ether in an organic solvent extraction phase during processing of cocaine for freebasing). In the 1970s and 1980s, commercial drug paraphernalia shops sold extraction kits for freebasing. These processes separated the free alkali base with an ether solvent and dried the base as the benzoylmethylecgonine amino alcohol base. This process retained some adulterants (e.g., local anesthetics), but not other additives (e.g., sugars). The volatile product could be smoked in a cigarette or water pipe. However, ether is highly flammable; therefore, explo-sions potentially complicate some of these extraction processes.

An experimental study with model pipes that simulated crack smoking indicated that cocaine smoke generated by this method contains approximately 93% cocaine particles with an average particle size in the respirable range (i.e., 2.3 μm) for primates.[47] A much smaller fraction of the "crack smoke" reaches the alveolar region of rodents compared with humans. The amount of cocaine inhaled during the volatilization of freebase cocaine is highly dependent on flow rate and temperature.[48] In an experimental apparatus, heating crack cocaine to 170°C (338°F) and 225°C (437°F) reduced the amount of cocaine to 73 ± 9% and 62 ± 11%, respectively.[49] Degradation of cocaine increases with increasing temperature and lower flow rates. At a temperature of 260°C (500°F) and a flow rate of 400 mL/min, 39% of the cocaine in the glass pipe appeared in a wool trap compared with <10% when the air flow rate decreased to 100 mL/min.[50] At this temperature, 60% of the cocaine remained intact, whereas only 2% of the cocaine remained intact as the temperature increases to 650°C (1,202°F). Benzoic acid and

methylecgonidine (CAS RN: 43021-26-7, anhydroecgonine methyl ester) were the main thermal degradation products of cocaine as determined by gas chromatography/mass spectrometry (GC/MS).

Smoking freebase cocaine has a high potential for dependency and overdose compared with intranasal use because cocaine smokers are less likely to titrate the dose of cocaine.[51] The important differences between the use of intranasal and smoking freebase are the immediate onset of effects, the magnitude of the euphoria, the duration of use, and the large quantity of cocaine consumed with the latter route of administration.[52] Additionally, more concomitant drug use occurs (e.g., ethanol, sedative-hypnotics, heroin) during the use of freebase cocaine. Escalating doses of freebase cocaine results in binges or runs over 1–3 days, similar to the abuse of methamphetamine. Behavioral complications of chronic cocaine use include diminished interest socialization (family, friends, work, food) as well as physical deterioration (unkempt appearance, exhaustion, insomnia, weight loss, depression). A paranoid or depressive psychosis may develop during this period of cocaine abuse.[53] The nadir of cocaine dependency typically involves family and financial crises. Cost probably is not a factor for the selection of the route of cocaine administration because the cost of powder cocaine and freebase cocaine is similar on a per pure unit basis.[54]

Coca Paste.   Coca paste is the initial product of the cocaine extraction process that contains high concentrations of cocaine sulfate. Abuse of this product involves the smoking of tobacco or marijuana cigarettes laced with this white semisolid. Smoking these cigarettes causes an immediate euphoria similar to the smoking of freebase cocaine, but the relative cost of smoking coca paste is lower. The addiction potential of smoking coca paste and freebase cocaine is similar, and this form of cocaine use is common in South America, particularly among the poor and even among the middle-class.[55] The use of coca paste in the United States is sporadic. Chemical analysis of coca paste indicates that the paste typically contains >60% cocaine along with a variety of other substances including benzoic acid, ecgonine, ecgonine methyl ester, methyl benzoate, manganese, gasoline residues (ethylbenzene, xylene, trimethylbenzene, naphthalene), and other natural alkaloids (tropacocaine, *cis* and *trans*-cinnamoylcocaine).[56]

Intravenous Use

Most IV cocaine users are polydrug users, who use cocaine depending on availability. When cocaine is unavailable, these polydrug users return to their customary drug of abuse. Injecting cocaine alone requires frequent injections; therefore, smoking is a more popular route of abuse because of similar euphoria and the lack of complications of IV drug use. Cocaine use can produce too much hyperactivity and excitement for some addicts, who prefer the combination of heroin and cocaine (Speedballing). An IV cocaine habit is expensive, and this method of cocaine abuse produces reactions and adverse effects similar to those produced by freebase cocaine habits, as well as the other infectious and physical complications associated with IV drug abuse.

DOSE EFFECT

Illicit Use

Significant physiologic and psychologic changes develop after inhalation, injection, or insufflation of cocaine. However, predicting toxic reactions following cocaine use is complicated by a variety of factors including individual sensitivity, acute tolerance, cocaine purity, and the type of impurities and adulterants. Snort, hit, line, and dose are the street terms for individual measures of cocaine; typical doses range up to 200 mg per measure depending on purity with an average dose of cocaine salt being about 25 mg per insufflation.[33] Large quantities of cocaine are sold in grams or spoons (i.e., 0.5–1 g). A 15-mg intranasal dose of cocaine produces slight elevations in systolic pressure and a subjective feeling of euphoria.[57] A 16-mg IV dose simulates the effects of the average dose that individuals self-inject on the street.[58] Intravenous doses of 8- to 32-mg cocaine produce euphoria, stimulation, a feeling of increased vigor, enhanced psychomotor activity, and anxiety similar to the use of amphetamine.[59] Dysphoric reactions can occur following the insufflation of cocaine doses as low as 20–30 mg. A typical adult fatal dose of cocaine is approximately 1 g depending on a number of factors including individual susceptibility. Potentially sensitive individuals include those with hereditary pseudocholinesterase deficiency.

Medical Use

The major clinical indication for the use of cocaine is topical anesthesia and vasoconstriction for ear, nose, and throat surgery. The typical adult dose of intranasal cocaine is 80–200 mg (4 mL of 5% cocaine solution); however, surveys of otolaryngologists indicate that some physicians administer intranasal cocaine doses exceeding 200 mg.[60] Within 2–4 minutes of application, the administration of 20–50 mg produces adequate vasoconstriction for ear, nose, and throat surgery. In an

observational study of cocaine doses of 1.5 mg/kg for local anesthesia prior to intubation for coronary bypass surgery, the use of cocaine did not produce changes in cardiovascular parameters as measured by blood pressure, pulse rate, cardiac index, left ventricular stroke work index, total peripheral vascular resistance, and pulmonary vascular resistance.[61]

Cocaine is not currently used for topical anesthesia of the cornea because chronic cocaine use causes delayed epithelization and corneal opacification. Additionally, pupillary dilation secondary to cocaine use potentially can produce acute angle glaucoma. The Brompton cocktail was a mixture of analgesics for terminally ill patients that often contained cocaine as well as a variety of other drugs (e.g., heroin, morphine).[62] The use of the mixture has been replaced by other analgesics.

TOXICOKINETICS

Absorption

Cocaine is rapidly and efficiently absorbed via the nasal, oral, and pulmonary routes at doses commonly associated with therapeutic use. The pharmacokinetic profile of cocaine in volunteers is similar following smoking and IV use, but the intranasal administration of cocaine produces lower peak cocaine concentrations and longer intervals between intranasal administration and peak concentration compared with the other 2 routes of administration.[63] Figure 56.6 compares the time course of the mean plasma cocaine and benzoylecgonine concentrations following single cocaine doses via the IV, nasal, and pulmonary routes of exposure.

NASAL INSUFFLATION

Plasma cocaine concentration increases rapidly for the first 20–30 minutes after insufflation with peak cocaine concentrations occurring approximately 15–60 minutes after administration. Following a 1.5 mg/kg cocaine dose, the peak plasma cocaine concentration ranged from 120–474 ng/mL.[64] Maximum euphoric effects occurred before peak cocaine concentrations (i.e., 15–20 minutes after nasal insufflation), and the effects were greater on the ascending plasma cocaine curve suggesting some tachyphylaxis.[65,66] Some delay in nasal absorption can occur as a result of the vasoconstrictive properties of cocaine, and the nasal mucosa absorbs about 98% of a therapeutic dose of cocaine within 4 hours of nasal administration.[67] The subjective and physiologic effects of 25-mg IV and 100-mg intranasal cocaine doses are similar.[57]
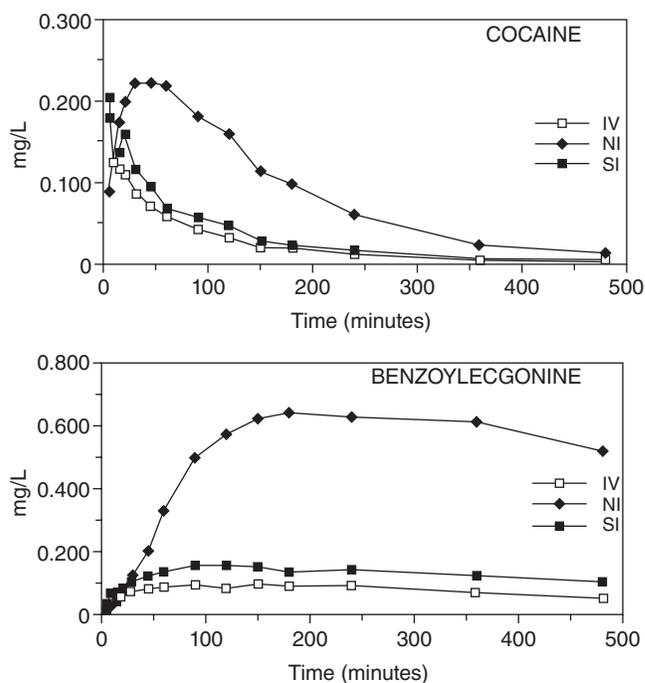
FIGURE 56.6. Time course of mean plasma cocaine and benzoylecgonine blood concentrations after the injection 20.5 mg cocaine (□, IV), nasal insufflation of 94.6 mg cocaine (◆, NI), and the inhalation of 50 mg cocaine freebase (■, SI) in adult volunteers. Reprinted with permission from AR Jeffcoat, M Perez-Reyes, JM Hill, BM Sadler, CE Cook, Cocaine disposition in humans after intravenous injection, nasal insufflation (Snorting), or smoking, Drug Metabolism and Disposition, Volume 17, p. 156, copyright 1989.

ORAL

At least one study suggests that oral and intranasal bioavailability of cocaine is similar (i.e., about 60%) at doses of 2 mg/kg.[68] At this dose, the intranasal and oral administration of cocaine produces statistically similar mean peak plasma concentrations. Oral administration of cocaine produced detectable plasma cocaine concentrations at 30 minutes with peak plasma cocaine concentration occurring between 50–90 minutes. Subsequently, the plasma cocaine concentration declined gradually over approximately 5 hours.[69] In this study, the peak subjective "high" after oral dosing was more intense than the euphoria induced by nasal insufflation, but subjective effects were delayed 45–90 minutes after administration following oral administration compared with 15–60 minutes after intranasal administration. Body stuffers ingest wrapping materials that liberate cocaine at variable rates depending on the type of container and the pH.[70] In the alkaline environment of the small intestine ($pK_a = 8.6$), the relative amount of the ionized form of cocaine decreases, whereas a relatively greater

proportion of the non-ionized form crosses the intestinal mucosa into the systemic circulation compared with the acidic environment of the stomach.

SMOKING

The absorption of freebase cocaine into the pulmonary circulation is rapid and complete with the onset of subjective response occurring soon after the initiation of smoking.[45] Consequently, the onset of action of cocaine is similar following pulmonary and IV administration. Inhalation of 15-mg freebase cocaine produces as much psychologic and physiologic effect as IV administration of 20-mg cocaine hydrochloride. Most of the freebase cocaine is delivered to the lungs within the first 4 puffs with subjective euphoria occurring within 6–11 minutes after initiation of smoking. Up to two-thirds of the cocaine may not reach the alveoli. Figure 56.7 demonstrates cocaine concentrations in volunteers after the smoking of cocaine doses of 10 mg, 20 mg, and 40 mg.

INTRAVENOUS

The IV administration and smoking of equivalent doses of cocaine produce similar cocaine concentrations in venous blood and similar times to peak effect. However, arterial concentrations of cocaine exceed venous cocaine concentrations during the first 15–20 minutes after the administration of cocaine via smoking and IV injection. In a study of 9 chronic cocaine users, the maximal arterial cocaine concentrations were at least 10-fold higher than maximal venous cocaine concentrations after both IV and smoked routes with peak arterial and venous cocaine concentrations occurring about 15 seconds and 4 minutes, respectively, after administration by either route.[71] Therefore, the pharmacokinetics of IV cocaine and freebase smoking are similar with physiologic and psychologic effects appearing several minutes after administration by either route.

DERMAL

There are limited data on the absorption of cocaine though the skin. A 5-mg dose of freebase cocaine was applied to a 20-square-inch area on the volar surface of the forearm of a single individual. The area of application was covered with a long-sleeved cotton shirt until the remaining cocaine was removed by washing 13 hours later.[72] The peak concentration of benzoylecgonine was 55 ng/mL 48 hours after application. The application of cocaine hydrochloride in a similar manner resulted in a peak urinary benzoylecgonine concentration of 15 ng/mL 24 hours after application. All urine specimens remained below the federal cutoff of 300 ng/mL for benzoylecgonine in urine drug screens.

Distribution

Cocaine has a moderate affinity for lipid tissue with an apparent volume of distribution notably smaller (2–3 L/kg) than most psychotropic drugs.[73] Following absorption, cocaine readily diffuses across the blood–brain barrier, resulting in the rapid distribution of cocaine into the brain where cocaine concentrations are several times higher in brain tissue than in the whole blood or plasma shortly after administration.[63,74] Because the serum and plasma cocaine concentrations fall more rapidly than cocaine concentrations in the brain, the cocaine brain/blood cocaine ratio increases 1–2 hours after exposure until brain cocaine concentrations become nondetectable by approximately 6–8 hours after a single cocaine exposure. *In vitro* studies with equilibrium dialysis indicate that both cocaine and cocaethylene are tightly bound to α_1-glycoprotein, whereas low-affinity sites on albumin occur for these 2 compounds.[75] In a study of serum from 12 healthy volunteers, the mean free fraction of cocaine was 0.083 ± 0.018 based on ultrafiltration.[76] The protein binding of cocaine was concentration-dependent with the most pronounced effects occurring above a cocaine concentration of 5 mg/L.

Animal studies indicate that benzoylecgonine persists in the brain at least 1 week after cessation of chronic cocaine use.[77] Animal studies also indicate that
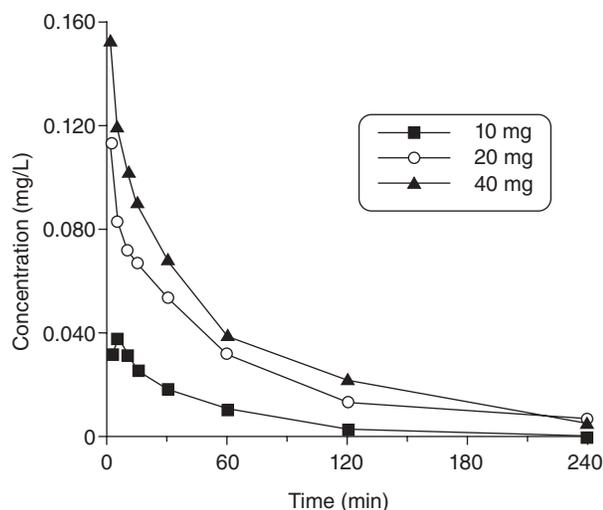
FIGURE 56.7. Mean plasma cocaine concentrations in 7 volunteers after smoking cocaine. Reprinted with permission from AJ Jenkins, RM Keenan, JE Henningfield, EJ Cone, Correlation between pharmacological effects and plasma cocaine concentrations after smoked administration, Journal of Analytical Toxicology, Vol. 26, p. 385, copyright 2002.
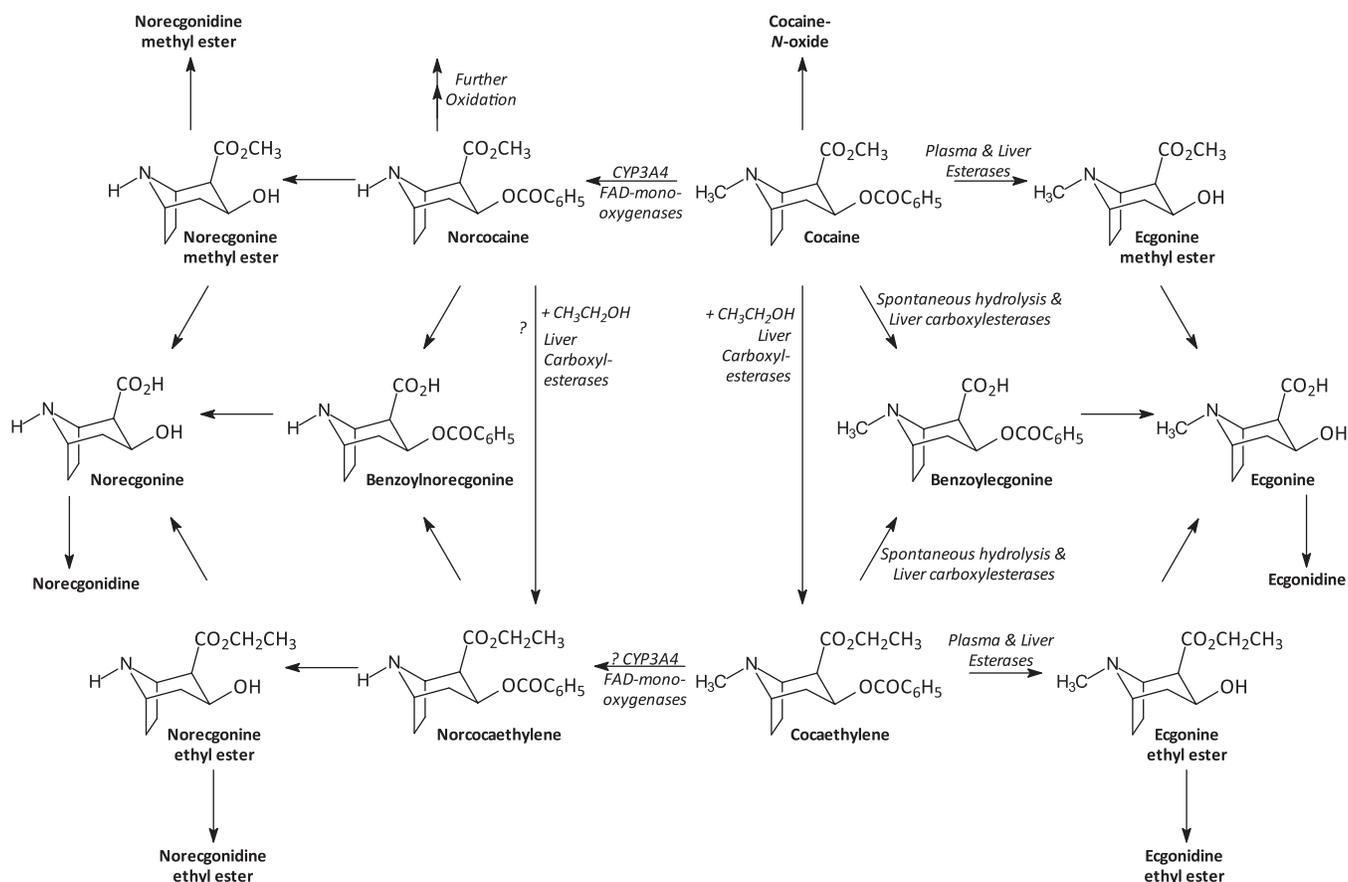
FIGURE 56.8. Cocaine metabolism.[111] FAD = flavin adenine dinucleotide.

cocaine distributes into genital organs including the epididymis and seminal vesicles. Following the administration of tritiated cocaine hydrochloride to male mice, the highest radioactivity appeared in the kidney and epididymis with sperm accounting for a substantial amount of the radioactivity associated with the epididymis.[78] Typically, the ratio of semen/plasma cocaine concentrations is less than unity.[79] Limited data from volunteer studies suggest that passive exposure to cocaine via the ejaculate of cocaine-using male partners may result from sexual activity, but the benzoylecgonine concentrations from this activity are unlikely to produce positive urine drug screens at the usual cutoff of 300 ng/mL.

Biotransformation

The major metabolic pathways of cocaine metabolism involve 1) human carboxylesterase-1 (hCE-1)-mediated and spontaneous chemical hydrolysis of cocaine to benzoylecgonine and 2) pseudocholinesterase- and human carboxylesterase-2 (hCE-2)-mediated hydrolysis of cocaine to ecgonine methyl ester.[80] These hydrolytic pathways account for the majority of the urinary metabolites of cocaine. Minor pathways include trans-

esterification of cocaine to cocaethylene by a liver cocaine carboxylesterase in the presence of ethanol, demethylation to norcocaine by cytochrome P450 isoenzymes, and the formation of several oxidative metabolites (*p*-hydroxycocaine, *p*-hydroxybenzoylecgonine, *m*-hydroxybenzoylecgonine) as demonstrated in Figure 56.8. Benzoylecgonine is the major metabolite of cocaine in the body. However, there is substantial interindividual and route-dependent variation in the biotransformation of cocaine. Enzymatic (liver methylesterase and carboxylesterase) and, to a lesser extent, spontaneous chemical hydrolysis of cocaine to benzoylecgonine accounts for ~30–45% of the total urinary cocaine metabolite in humans, depending on route of administration and interindividual variation.[81,82] In a study of 6 volunteers administered bioequivalent doses of cocaine by the IV, intranasal, and smoked routes, urinary benzoylecgonine accounted for approximately 39%, 30%, and 16%, respectively, of the administered dose.[80] In a similar pharmacokinetic study of 4 occasional cocaine users receiving IV, intranasal, and inhaled doses of tritiated cocaine, urinary benzoylecgonine represented about 36%, 39%, and 26% of the total cocaine dose, respectively.[73] The enzymatic hydrolysis of cocaine by

plasma and hepatic cholinesterases to ecgonine methyl ester accounts for ~5–40% of the total urinary metabolites of cocaine, depending on the route of exposure, species, and individual pharmacokinetics.[83]

The role of hepatic cytochrome P450 isoenzymes in the metabolism of cocaine is minor.[84] The enzyme-mediated demethylation of cocaine by the CYP3A isoenzymes to norcocaine and sequentially to *N*-hydroxynorcocaine accounts for about 2.6–6.2% of cocaine metabolism.[85,86] Other minor metabolites (<2% total) of cocaine include norcocaine, benzoylnorecgonine, ecgonidine, norecgonidine methyl ester, norecgonine methyl ester, *m*-hydroxybenzoylecgonine, *p*-hydroxybenzoylecgonine, *m*-hydroxycocaine, and *p*-hydroxycocaine.[80,87] The pyrolysis of cocaine during smoking produces benzoic acid and anhydroecgonine methyl ester (methylecgonidine), and the presence of the latter compound in the urine indicates the administration of cocaine by smoking rather than injection or insufflation.[88] Of 81 urine samples testing positive for cocaine using the enzyme-multiplied immunoassay technique, 12 (15%) were positive for anhydroecgonine methyl ester at concentrations ranging from 4–226 ng/mL as measured by GC/MS.[89] In this study, 7 hair samples were positive for anhydroecgonine methyl ester at a mean concentration of 0.6 ng/mg (range, 0.2–2.4 ng/mg).

Elimination

The elimination of cocaine from the blood is rapid with a biologic half-life of approximately 1 hour, and the plasma clearance of cocaine averages about 20–30 mL/kg/min.[59] Early studies of cocaine doses from 0.2–2 mg/kg suggest first-order (i.e., dose-dependent) kinetics,[68] whereas another study of chronic cocaine users demonstrated a 2-compartment system after a 32-mg IV dose.[92] Saturation may occur at very high cocaine dosages, resulting in changes in the elimination kinetics. Additionally, there is substantial intra- and interindividual variation in the elimination half-life of cocaine, probably because of differences in the route of exposure and plasma cholinesterase activity.[90,91] For example, the apparent plasma half-life of cocaine is slightly longer following insufflation than after oral administration, probably as a result of continued nasal adsorption. In chronic cocaine users, the plasma cocaine half-life averaged 48 minutes after a 32-mg IV injection.[92] The mean elimination half-life for cocaine following the intranasal administration of cocaine to 7 recreational users was 75 ± 5 minutes compared with 48 ± 3 minutes for the oral administration of cocaine to 4 recreational users. Cocaine plasma levels remain detectable for approximately 4–6 hours. Recent phar-macokinetic studies with more sensitive analytic methods suggest that lipid-soluble tissues may store small amounts of cocaine, resulting in an extended terminal plasma cocaine elimination half-life (4–5 h).[74]

The hydrolysis of cocaine to benzoylecgonine and ecgonine methyl ester represents ~80–90% of the urinary metabolites of cocaine with the renal excretion of unchanged cocaine accounting for <5% of the absorbed dose, depending on pH.[93] In a study of 3 cocaine users receiving an IV infusion of 253–700 mg cocaine, benzoylecgonine, and ecgonine methyl ester accounted for 14–17% and 12–21% of the cocaine dose during the first 30 hours after administration.[94] Unchanged cocaine accounted for about 2% of the absorbed dose during this period. The elimination of the 2 major cocaine metabolites is slower than the elimination of cocaine from the blood with an average urinary half-life of benzoylecgonine being slightly shorter than the urinary half-life of ecgonine methyl ester.[81] In a study of 5 IV cocaine users, the mean urinary elimination half-life of benzoylecgonine and ecgonine methyl ester was 3.1 hours and 4.5 hours, respectively.[94] Increased renal excretion of cocaine occurs in acidic urine. However, alteration of pH does not result in the accumulation of ecgonine methyl ester because the rate of the hydrolysis of this metabolite exceeds both the rate of formation and the rate of hydrolysis of cocaine to benzoylecgonine.[95]

Maternal and Fetal Kinetics

Case reports of adverse effects in infants born to cocaine-using mothers indicate that cocaine crosses the placenta and that both cocaine and benzoylecgonine enter fetal blood and brain tissue.[96,97] The cocaine concentration in fetal brain tissue exceeds plasma cocaine concentrations within several hours of administration, but the ratio of brain/plasma cocaine concentrations in the fetus is lower than in adults because of the lower lipid content of the fetal brain.[63] At autopsy, the highest cocaine concentrations are found in the urine and kidney, followed by the brain, blood, liver, and bile. A cerebral infarction occurred in the fetus of a cocaine user.[98] Some case reports suggest that cocaine diffuses into breast milk. A 2-week-old, breast-fed neonate developed tremulousness, irritability, and exaggerated startle response 1 week after her mother began using cocaine.[99] During the first day of admission to the hospital, samples of maternal milk and urine from the infant contained both cocaine and benzoylecgonine. Animal studies using ^{3}H-cocaine demonstrated a mean breast milk/maternal blood cocaine ratio of 7.8.[63] However, there are inadequate pharmacokinetic data to determine the extent of cocaine diffusion into breast

milk, particularly given the rapid biotransformation and instability of cocaine in blood.

Tolerance

Although tolerance to the therapeutic effects of cocaine does not occur, most chronic drug users develop tolerance to the euphoric and, to a lesser extent, to the cardiovascular effects of cocaine.[100] Case reports suggest that some chronic users, particularly freebase cocaine smokers, develop tolerance to large doses (>1 g) of cocaine.[101] Animal studies demonstrate that repetitive administration of cocaine produces tolerance to the convulsant and cardiorespiratory stimulatory properties of cocaine.[102]

Acute tolerance (tachyphylaxis) reduces the euphoric and chronotropic effects of cocaine within the same, but not different experimental sessions as demonstrated in volunteers administered cocaine via IV injection, insufflation, or inhalation.[103] Consequently, the cardiovascular and subjective effects of single cocaine doses dissipate more rapidly than expected from the elimination half-life. For cardiovascular effects and some subjective effects measured during these studies, the effects were greater during the ascending curve of the plasma cocaine concentration than during the descending curve (i.e., Mellanby effect).[104] During studies of volunteers maintained on a constant infusion of cocaine (i.e., plasma cocaine concentration = 0.5–0.75 mg/L), the euphoric effect of cocaine peaked at about 1 hour and then declined to baseline about 4 hours after the infusion began.[105] The chronotropic effect peaked at about 10 minutes and then declined to about one-third of the peak value with a half-life of approximately 30 minutes. Within 30 minutes after the initiation of IV cocaine, self-reports of the "rush" associated with initiation of IV cocaine decreased below statistically significant levels with or without the constant cocaine infusion.

Drug Interactions

Ethanol

Many cocaine users ingest ethanol during binges, frequently with the belief that the ingestion of ethanol with cocaine reduces unpleasant side effects of the "crash" associated with cocaine use as well as enhancing or prolonging the euphoria associated with cocaine use.[106,107] Volunteer studies suggest that the simultaneous or recent ingestion of social doses of ethanol with the intranasal administration of cocaine increases peak plasma cocaine concentrations approximately 20% compared with the exposure to cocaine alone.[108] The increased cocaine concentrations were associated with

enhanced subjective feelings of euphoria and well-being compared with the use of cocaine alone. Experimental studies suggest that the ingestion of ethanol 30 minutes after the administration of cocaine significantly reduces the subjective effects associated with the simultaneous ingestion of ethanol and exposure to cocaine.[109] Cocaine, ethanol, and cocaethylene frequently are present in biologic samples from trauma patients. In a study of 415 consecutive admissions to an urban trauma center, approximately one-third of the admission urine samples contained evidence of benzoylecgonine, and blood samples from about one-half of these patients contained cocaethylene.[110] All 114 admission blood samples from the group of trauma patients with positive urine samples for benzoylecgonine were positive for cocaine with the serum cocaine concentrations ranging from 0.04–0.70 mg/L (mean, 0.093 mg/L). About 56% of these 114 blood samples were positive for ethanol (mean, 175 ± 85 mg/dL), whereas 60% of these 114 blood samples tested positive for cocaethylene (mean, 0.041 mg/L; range, 0.003–0.213 mg/L). Plasma cocaethylene concentrations did not correlate well to blood ethanol concentrations.

PHARMACOKINETICS.   Cocaethylene, the ethyl ester of benzoylecgonine, is a marker of the simultaneous use of cocaine and ethanol, and the metabolic pathways of cocaine and cocaethylene are similar.[111] In the presence of a hepatic carboxyltransferase, the transesterification of cocaine with ethanol produces an active, but somewhat less potent metabolite, cocaethylene (ethyl cocaine).[112] Figure 56.8 demonstrates the metabolic pathways for cocaethylene and cocaine. The simultaneous use of cocaine and ethanol increases the serum cocaine concentration and reduces serum benzoylecgonine concentrations compared with the cocaine-only use.

In volunteer studies using social doses of ethanol (0.8 g/kg) and intranasal cocaine (1.25 or 1.9 mg/kg) together, the peak plasma cocaethylene concentration is usually lower (i.e., 15–20%) than the plasma cocaine concentration with the peak cocaethylene concentration occurring about 1 hour later than the peak cocaine concentration.[113] In a study of 8 volunteers receiving 100 mg intranasal cocaine and ethanol simultaneously, the mean peak plasma concentrations of cocaethylene and cocaine were 0.0485 ± 0.0147 mg/L and 0.331 ± 0.112 mg/L, respectively, whereas the mean peak plasma ethanol concentration was approximately 100 mg/dL.[108] The mean times to the peak cocaethylene and cocaine concentrations were 116.3 ± 9.9 minutes and 41.3 ± 22.3 minutes, respectively. Figure 56.9 demonstrates the time course of cocaethylene and cocaine concentrations after simultaneous ingestion of 0.8 g ethanol/kg and
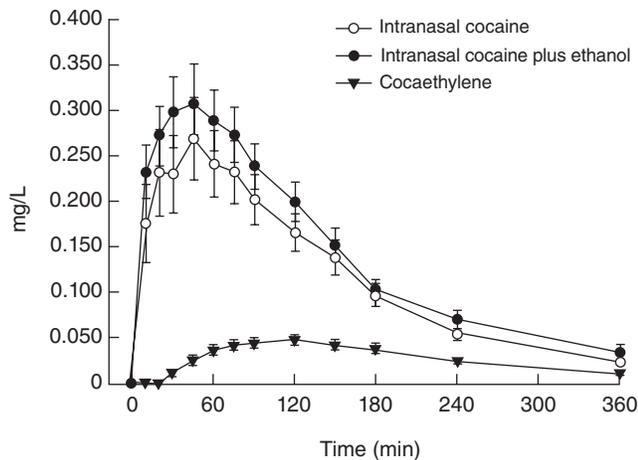
Figure 56.9. Time course comparing plasma cocaine (○) following 100 mg intranasal cocaine with plasma cocaine (●) and cocaethylene (▼) concentrations after administration of 100 mg intranasal cocaine and the simultaneous ingestion of 0.8 g ethanol/kg. Reprinted with permission from M Farré, RDL Torre, ML González, MT Terán, PN Roset, E Menoyo, J Camí, Cocaine and alcohol interactions in humans: neuroendocrine effects and cocaethylene metabolism, Journal of Pharmacology and Experimental Therapeutics, Vol. 283, p. 173, copyright 1997.

intranasal administration of 100 mg cocaine compared with cocaine concentrations following the insufflation of 100 mg cocaine without ethanol. Following intranasal administration of single or multiple doses of cocaine and ethanol, the peak concentration of cocaethylene is about 15–25% of the peak concentration of cocaine.[114] In a study of cocaethylene formation following different routes of deuterium-labeled cocaine and ethanol administration to 6 healthy volunteers, the percentage of cocaine converted to cocaethylene was as follows: oral, 34% ± 20%; intravenous, 24% ± 11%; and smoked, 18% ± 11% (excluding 2 participants with near zero delivery).[115] Only the difference in cocaethylene formation between the smoked and oral routes was statistically significant. The cocaine was administered 1 hour after the ingestion of 1 mg ethanol/kg. Although plasma clearance of cocaine decreases during the combined use of cocaine and ethanol, elimination rates of cocaine and ethanol remain unchanged.

A study of 10 volunteers receiving IV doses of cocaine up to 1.2 mg/kg 1 hour after the ingestion of ethanol (1 g/kg) demonstrated that an average of about 17% ± 6% of the cocaine was converted to cocaethylene and the urine benzoylecgonine excretion decreased by approximately 48%.[116] Although cocaethylene concentrations are relatively low compared with cocaine following single doses of cocaine and ethanol,[117] plasma cocaethylene concentrations can exceed plasma cocaine

concentrations in samples from chronic or binge cocaine abusers as a result of the relatively longer half-life of cocaethylene compared with cocaine. In a series of 41 trauma patients presenting to an urban emergency department with detectable plasma concentrations of cocaethylene, the mean plasma ratio of cocaethylene/cocaine was 1.3 with a range of 0.1–4.7.[118] The longer plasma half-life of cocaethylene compared with cocaine may account for the range of plasma cocaine/cocaethylene ratios present in trauma patients.[118] Volunteer studies indicate that the administration of single, IV, or intranasal doses of cocaethylene produces an elimination half-life for cocaethylene approximately 1.5–2 times longer than the cocaine elimination half-life.[119] Norcocaethylene is a minor *N*-demethylated metabolite of cocaethylene that occurs in relatively small concentrations compared with cocaethylene. Norcocaethylene displays a slightly longer (i.e., ~3 h vs. 2 h) plasma elimination half-life compared with norcocaine.[111] Detoxification of cocaethylene involves the formation of benzoylecgonine and ecgonine ethyl ester, similar to the biotransformation of cocaine.

PHARMACODYNAMICS.   The toxicologic profile of cocaethylene is similar to cocaine, and the effects of cocaethylene are additive to those of cocaine, although somewhat less potent intravenously than intranasally.[111,120] In volunteer studies, the intranasal administration of equivalent doses of cocaine and cocaethylene produced a similar rating of euphoria, although the euphoria tends to last longer following the administration of cocaethylene.[121] The difference in the duration of effect reflects the slower clearance, larger volume of distribution and correspondingly longer elimination half-life of cocaethylene compared with cocaine. Although experimental studies suggest that the effects of cocaine and cocaethylene are similar after intranasal administration, the IV cocaethylene is less potent than IV cocaine.[122] In a study of 6 paid volunteers, the IV administration of 0.25 mg/kg cocaethylene base produced a mean rating of "high" at ~65% of the rating for an IV injection of an equivalent dose of cocaine base.[120]

Cocaine antagonizes some of the deleterious effects of ethanol by reducing the feeling of drunkenness and ameliorating some of the impairment of psychomotor tasks (e.g., digit symbol substitution) caused by ethanol.[123] Cocaethylene binds to the dopamine transporter with the same affinity as cocaine, blocks dopamine uptake and increases extracellular concentrations of dopamine in the nucleus accumbens.[124] However, rodent studies indicate that cocaethylene acts more selectively on serotonin uptake than cocaine as a result of substantially less binding of cocaethylene to the serotonin transporter compared with cocaine.[125]

Drugs that affect catecholamine metabolism (e.g., guanethidine, dopamine, α-methyldopa, tricyclic antidepressants, monoamine oxidase inhibitors) enhance the sympathomimetic effects of cocaine. Potentially, monoamine oxidase inhibitors may augment the hypertensive effects of cocaine, but there are limited data on this interaction. The administration of selegiline, an irreversible monoamine oxidase-B (MAO-B) inhibitor did not enhance the cardiovascular effects of 40-mg IV cocaine in 5 volunteers.[126] In general, the use of tricyclic antidepressants, trazodone, and selective serotonin reuptake inhibitors (e.g., fluoxetine, paroxetine, sertraline) with cocaine does not cause any increased risk of side effects.[127] *In vitro* amitriptyline is a partial competitive inhibitor of human serum butyrylcholinesterase (K_i = 0.01 mM), but there are few clinical data to determine the clinical significance of this potential interaction.[128] Pretreatment of volunteers with haloperidol reduces the cocaine-induced increase in blood pressure, but haloperidol does not change the effect of cocaine on heart rate.[129]

Heroin often is used concomitantly with cocaine (i.e., Speedball) to enhance the cocaine-induced euphoria and reduce the overstimulation of cocaine. The interaction between opioids and the dopaminergic system is complex; opioid antagonist and agonists produce a variable effect on the euphoria associated with cocaine use.[127] Coadministration of cocaine with other stimulants is common because sympathomimetic drugs are frequently adulterants in illicit cocaine. Animal data on the simultaneous administration of cocaine and amphetamines are conflicting with some studies suggesting that cocaine may block the uptake of amphetamines.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Cocaine is a powerful CNS stimulant and sympathomimetic agent that produces effects through the potentiation of neurotransmitters, principally norepinephrine and epinephrine. Cocaine also has demonstrable effects on dopamine and serotonin.

Mechanism of Action

Cocaine has potent sympathomimetic effects in addition to stimulating norepinephrine, serotonin, and dopamine release. Cocaine is also a local anesthetic that reduces sodium ion flux during the depolarization of peripheral nerves by blockade of the voltage-gated sodium channels and thereby increases the threshold required to generate an action potential.[130] The anesthetic and local vasoconstrictive properties of cocaine make topical cocaine an excellent treatment for nasal hemorrhage. However, long-term insufflation of cocaine causes chronic inflammation of the nasal mucosa[131] as well as perforation of the nasal septum.[132] Cocaine causes mydriasis both by local and by central α-adrenergic effects on the radial muscles of the iris. Partial cycloplegia also occurs. Cocaine and amphetamine have similar effects on catecholamine reuptake inhibition, dopamine release, and serotonin release/reuptake inhibition; however, cocaine has marked local anesthetic activity compared with minimal activity for amphetamine. Additionally, cocaine has no monoamine oxidase inhibition (MAOI), whereas amphetamine has mild MAOI activity.

Mechanism of Toxicity

Cocaine exerts toxicity through 2 mechanisms that include 1) potentiation of catecholamine action (i.e., dopamine, norepinephrine) as a result of the inhibition of reuptake at the adrenergic nerve terminals, and 2) the inhibition of voltage-dependent sodium ion channels.[133] The latter effect reflects the local anesthetic properties of cocaine and evidence of blockade of the voltage-dependent sodium ion channels during animal studies occurs at cocaine concentrations 10 times higher than the effects of catecholamine potentiation.[134] Cocaine also stimulates the release of excitatory amino acids and enhances thrombosis by promoting platelet aggregation, impairing thrombolysis via increased TPA-inactivator, and accelerating atherogenesis.

Cocaine inhibits the synaptosomal uptake of catecholamines and serotonin. Blockade of the reuptake of norepinephrine at preganglionic sympathetic nerve endings in the sympathetic system produces an excess of norepinephrine in the synaptic cleft. Neuronal uptake is the primary method of eliminating catecholamines from the synaptic cleft, and cocaine intensifies the catecholamine effects at the nerve endings through blockade of catecholamine reuptake. The dopamine transporter is a transmembrane spanning protein that moves dopamine across the neuronal membrane.[135] Diffusion and binding to the dopamine transporter protein clears dopamine from the neuron after the release of dopamine from presynaptic terminals. Cocaine effectively binds dopamine transporters at low doses; at behavior-altering doses, cocaine occupies a majority of the dopamine

transporters.[135] Cocaine blocks dopamine transporter proteins, particularly in mesolimbic (nucleus accumbens) dopaminergic neurons, and this effect is important to the reinforcing properties of cocaine.[136] The rapid uptake and clearance of cocaine from the brain correlates to the euphoria associated with IV cocaine use, and the inhibition of the dopamine transporter correlates to the reinforcing properties of cocaine.[137]

In the primate brain, cocaine acts primarily on the synaptic dopamine reuptake system by binding to a specific receptor on the dopamine synaptic plasma membrane in both the nigrostriatal (caudate-putamen) and the mesolimbic dopamine neurons rather than on the dopamine synaptic vesicles.[138,139] The prolonged presence of dopamine in the synapse produces simulation of the dopamine receptors in the caudate-putamen and nucleus accumbens.[140] Self-stimulation models in animals suggest that activation of mesolimbic and nigrostriatal dopaminergic pathways may cause the euphoria and compulsive drug behavior associated with chronic cocaine abuse.[28] Chronic cocaine use causes modest elevation of dopamine transporter concentrations in the mesolimbic regions of the brain in acutely (<96 h) abstinent chronic cocaine abusers.[141] Upregulation of the cocaine binding sites in the striatum results in the need for continued cocaine use to experience the reenforcing effects of the drug.

The increase in dopamine neurotransmission from the competitive blockade of high-affinity dopamine uptake mediated by both D_1 and D_2 dopamine receptors is the primary determinant of the acute behavioral effects of cocaine.[142] Although *in vitro* studies indicate that cocaine can potentiate the D_2 receptor-mediated depolarizing effects of dopamine, the hyperpolarizing effect of dopamine acting on the postsynaptic D_1 receptors via increased outward potassium conductance probably accounts for the inhibitor effect of cocaine on postsynaptic cells in the caudate, nucleus accumbens, and medial prefrontal cortex.[143] Extracellular concentrations of serotonin in the nucleus accumbens also increase during cocaine administration and the serotonin system by modulating dopamine release during both the stimulatory and withdrawal phases.[144] In rodent studies, cocaine doses that maintain self-administration behavior inhibit the norepinephrine transporter at peripheral sympathetic nerve terminals.[145]

With increasing doses, cocaine causes a rostral-caudal stimulation beginning with the cortex. Initially, euphoria, hyperactivity, restlessness, and garrulity occur. Progressive activation of the lower brain centers results in tremor, hyperreflexia, and convulsions. Animal studies indicate that cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens,[146] but the clinical significance of these changes remains undefined. In high doses, a period of depression develops subsequent to the excitatory phase. Mechanisms for the development of seizures following cocaine exposure include direct irritation of epileptogenic foci, toxic effects of adulterants (e.g., lidocaine, amphetamine), and cerebral vasculitis.[147] Seizures may also result from the occurrence of subarachnoid hemorrhage or focal cerebral ischemia. Based on the lack of vascular pathology in cases of cocaine-associated strokes, cerebral vasospasm or vasoconstriction may contribute to the development of cocaine-associated ischemic and hemorrhagic strokes.[148]

Excited delirium shares many similarities to neuroleptic malignant syndrome and potential mechanisms of toxicity, but the pathophysiology of this syndrome is unclear. Suggested hypotheses include chronic alterations of presynaptic proteins (α-synuclein) in dopamine neurons in the midbrain, dopamine transporter, and/or dopamine D_2 receptors in the thermoregulatory centers of the brain.[149,150] Clarification of the mechanism of toxicity producing excited delirium requires further research.

Cardiovascular System

Serious complications (e.g., life-threatening arrhythmias, acute reversible myocarditis, dilated cardiomyopathy, myocardial infarction [MI], sudden death) of myocardial ischemia result from the imbalance between myocardial oxygen demand and supply during cocaine use.[151] The etiology of this imbalance is multifactorial including intracoronary thrombosis, platelet aggregation, and coronary artery vasoconstriction. Animal data and indirect human data suggests that chronic cocaine use accelerates atherosclerosis; however, the degree of atherosclerosis associated with chronic cocaine use remains somewhat controversial. A 15-year longitudinal study of 18- to 30-year-old men and women did not detect an increased prevalence of accelerated atherosclerosis in the subset of individuals with long-term, heavy cocaine use.[152] In an age- and gender-matched cohort study of 412 patients presenting to an urban emergency department with acute chest pain, 44 patients (9%) had a history of chronic cocaine use.[153] These patients had a statistically significant, sixfold higher risk for acute coronary syndrome (OR = 5.79, 95% CI: 1.24–27.02, P = .02); however, cocaine use was not associated with a higher prevalence of any plaque (P > .58), calcified plaque (P > .58), noncalcified plaque (P > .58), or the presence of significant coronary artery stenosis (P = .09).

Cocaine directly inhibits the cardiac ion channels and increases sympathetic stimulation to the heart and coronary vasculature.[154] Cocaine produces a characteristic

voltage- and frequency-dependent inhibition of the cardiac sodium current. Experimental data suggests that this inhibition occurs as a result of the blockade of the pore and the stabilization of the channels in the inactivated state.[155] A cross-sectional study of IV drug users suggests that chronic cocaine use affects some diastolic functional parameters (e.g., increased average deceleration time),[156] but the restriction of the study group to inner city IV drug users including HIV-positive patients limits conclusions regarding the effect of chronic cocaine use on other types of cocaine users. Adrenergic adaptive mechanisms mediate the chronic tolerance of experimental animals to the cardiovascular effects of cocaine.

HEMODYNAMIC CHANGES. The administration of moderate doses of cocaine (2 mg/kg intranasal) to volunteers produces mild (8–12%) coronary artery vasoconstriction and a mean reduction (~8%) in coronary sinus blood flow, even in the presence of increased myocardial oxygen demand.[157] These changes were not associated with chest pain or electrocardiographic (ECG) evidence of ischemia. Recreational doses of cocaine produce modest increases in blood pressure (20 mm Hg systolic/10 mm Hg diastolic) and heart rate (30 beats/min) that are similar to the hemodynamic changes during mild exercise.[158] Animal studies indicate that central stimulation of sympathoadrenal neural axis activity plays an important role in the development of these cardiovascular effects because inhibition of peripheral sympathetic neuronal amine uptake mechanism by cocaine is not critical for initiating hypertension, tachycardia, or release of plasma catecholamine.[159] The sympathomimetic response probably results from the blockade of norepinephrine and epinephrine uptake, which causes increased neurotransmitter concentrations at central adrenergic receptors.[45] α-Adrenergic-mediated actions probably are involved in the development of coronary artery vasoconstriction. During cocaine use, phentolamine blocks this vasoconstriction, whereas nonselective β-adrenoceptor blocking agents enhance α-adrenergic-mediated effects (vasoconstriction, increase systemic vascular resistance).[160] Other potential causes of cocaine-induced vasoconstriction include a direct effect on increased calcium influx in vascular smooth muscle and the release of vasoconstrictive factors (e.g., endothelin).[161]

DYSRHYTHMIAS. The permeability of the cardiac myocyte membrane to various cations (sodium, potassium, calcium) determines the resting conditions across the membrane (phase 4 of the action potential). The sodium-potassium exchange pump, the sodium-calcium exchanger current, and the inwardly rectifying potassium current maintain the resting membrane potential. During resting conditions, the voltage-dependent

sodium channels are closed, and the opening of the sodium channels initiates the action potential (phase 0). Phase 1 (R and S wave on the ECG) involves the inactivation of the sodium channels along with the extracellular movement of potassium and the intracellular movement of calcium. Cocaine slowly binds and disassociates from the sodium channel, resulting in the blocking of these channels in the open state and prevention of the return of the sodium channels to the inactive, resting state.[162] This action is characteristic of Vaughan-Williams Class Ic antiarrhythmic drugs (flecainide, moricizine).

In large doses, cocaine can directly depress myocardial conduction and contractility, similar to other local anesthetic agents.[163] These local anesthetic properties result from the inhibition of sodium influx into myocardial cells and the propagation of the action potential by the blockage of the myocardial fast sodium channels during phase 0 of the cardiac action potential.[164] The resulting QRS widening is similar to the effects of Vaughn-Williams type 1A and 1C antidysrhythmic agents. Cocaine also blocks potassium efflux channels in the heart resulting in QTc prolongation. In addition, increased dispersion of ventricular refractoriness and ventricular effective refractory period occurs. In overdose, the cocaine-induced blockade of cardiac sodium channels in the closed state causes prolongation of the action potential, QRS prolongation, and wide-complex tachydysrhythmias. Although *in vitro* studies indicate that the major cocaine metabolites (benzoylecgonine, ecgonine methyl ester) have little or no effect on the sodium channel, the ethanol-derived metabolite (cocaethylene) produces more potent and prolonged blockade of the sodium channel than cocaine.[165] The inhibition of impulse conduction by alterations of sodium membrane permeability may cause sudden death.[166]

Animal studies also indicate that cocaine has electrophysiologic effects of class I antiarrhythmic agents (prolongation of the effective refractory period, depression of automaticity/phase 0 depolarization).[167] The administration of a continuous 3-hour infusion of cocaine at 0.11 mg/kg/min (total dose: 20 mg/kg) to anesthetized dogs increases the ventricular fibrillation threshold about 50% compared with placebo (lactose dissolved in normal saline).[168] However, large doses of cocaine prolong the QRS complex and QTc duration that along with hypoxia, acidosis, and underlying cardiovascular disease increase the risk of developing ventricular dysrhythmias, including ventricular fibrillation.[169] Both tachycardia and acidosis increase the binding of cocaine to the sodium channels and increase the risk of serious dysrhythmias.[170] Other mechanisms for dysrhythmias include myocarditis, myocardial ischemia, intense sympathetic stimulation (contraction band necrosis), and the development of myocardial fibrosis.

The administration of high-dose cocaine (5 mg/kg) intravenously to dogs produces negative inotropic and potent type I electrophysiologic effects as manifest by decreased blood pressure, cardiac output, and coronary blood flow, as well as prolongation of cardiac conduction (increased PR, QRS, QT intervals).[171] The effects of cocaine on repolarization are biphasic resulting in 2 distinct clinical profiles based on case reports of electrocardiographically documented life-threatening dysrhythmias associated with cocaine.[172] In overdose, a monomorphic slow ventricular tachycardia or idioventricular rhythm develops, reflecting excessive sodium channel blockade. The second type of dysrhythmia reflects potassium channel blockage and is expressed as *torsade de pointes* in recreational users with underlying risk factors (e.g., congenital long QT syndrome).

MYOCARDIAL INFARCTION. The pathophysiology of cocaine-associated myocardial ischemia is multifactorial. Mechanisms of this cardiac complication include 1) increased myocardial oxygen demand in the setting of limited oxygen supply, 2) enhanced platelet aggregation and thrombus formation, and 3) coronary artery vasospasm.[173] Depending on the individual (e.g., preexisting cardiovascular disease, polydrug use, adulterants) one or several of these factors may contribute to the development of myocardial ischemia. Structural narrowing of the coronary arteries is common in patients with cocaine-related MIs.[174] Rarely, patients in substance abuse treatment programs for cocaine dependency may develop signs of silent myocardial ischemia during the first few weeks of withdrawal, but the mechanism for these changes is unclear. In a study of 21 patients with cocaine addiction undergoing Holter monitoring, 8 of these patients developed ST elevation during the first 2 weeks of withdrawal.[175] Sudden death can occur as a result of excited delirium (hyperthermia, psychotic behavior, respiratory arrest) as well as from ventricular dysrhythmias.[176]

RESPIRATORY SYSTEM

The pathologic changes of the lung associated with cocaine abuse are multifactorial, route-dependent, and in some cases idiopathic. These pulmonary changes include vascular lesions (microemboli, spasm with thrombosis, hemorrhage, edema, arterial medial hypertrophy),[177] bronchospasm in asthmatic patients, interstitial disease, hypersensitivity pulmonary disease ("crack lung"),[178] bronchiolitis obliterans,[179] barotrauma (pneumothorax, pneumomediastinum), lung cavitations, pleural empyema, and inhalation injuries (aspiration, thermal damage, foreign body deposits).[180] The etiologies of these changes are diverse and include the follow-

ing: pulmonary hemorrhage/edema from microvascular injury, thermal airway injury, hypersensitivity reactions, interstitial pulmonary fibrosis, and foreign body reactions.[181] In rodent studies, chronic crack cocaine inhalation causes extensive histologic changes in the entire respiratory tract. Following 2 months of daily exposure of 2-month-old BALB/c mice to the smoke of 5-g crack cocaine in an inhalation chamber, histologic changes in the respiratory tract included increased mucus content of the nasal epithelium, alveolar hemosiderin, and alveolar/bronchiolar macrophage cell density as well as reduced thickness of the nasal and bronchial epithelium.[182] Case reports associate crack cocaine use with diffuse histologic changes in the lungs manifest by infected bronchiectasis, interstitial desquamative pneumonia, diffuse alveolar damage, subsegmental arterial thrombosis, and foci of pulmonary infarction.[183] Clinical studies indicate that alveolar hemorrhage occurs frequently in otherwise healthy crack cocaine smokers without obvious evidence of hemoptysis or cough.[184] Histologic changes associated with crack lung include diffuse alveolar damage, alveolar hemorrhage, and eosinophilic interstitial and alveolar infiltrates with deposition of IgE.

TEMPERATURE

Large doses of cocaine produce hyperthermia by one or more of the following mechanisms: 1) increased heat production secondary to muscular hyperactivity and/or seizures, 2) direct pyogenic action on the central thermoregulatory centers in the hypothalamus, 3) reduction of heat loss by vasoconstriction, and 4) increased calorigenic activity in the liver. Volunteer studies suggest that low doses of intranasal cocaine impair thermoregulatory adjustments for the dissipation of heat. In as study of 7 cocaine-naïve volunteers subjected to progressive passive heat stress, the nasal administration of 2-mg cocaine/kg shifts the esophageal temperature threshold for cutaneous vasodilation and sweating as well as reducing the discomfort associated with heat stress.[185] These changes potentially disrupt normal autonomic responses to thermal stress as well as limit behavioral adjustments and predispose patients to hyperthermia in hot environments. However, the administration of this cocaine dose does not cause changes in core temperature in the absence of heat stress.

Rhabdomyolysis is a common complication of cocaine-induced hyperthermia, although rhabdomyolysis may develop without elevated body temperature.[186] Animal studies indicate that antagonism of dopamine receptors, particularly the DA-1 subtype, abolish cocaine-induced hyperthermia;[187] downregulation of brainstem D_1 dopamine receptors by cocaine abuse may

also contribute to the development of hyperthermia and death.[188] In fatal cases of excited delirium, marked hyperthermia usually precedes the development of status epilepticus, hypotension, rhabdomyolysis, disseminated intravascular coagulation, acute hepatorenal failure, impaired cerebral function, and death.[189] Animal models indicate that intrabrain heat production is the primary cause of functional brain hyperthermia; the cerebral circulation dissipates potentially dangerous heat from brain tissue by bringing relatively cooler blood and subsequently removing warmed blood.[190] Although cocaine induces metabolic activation of central neurons, modulations of thermal effects from both behavior and environmental variables result in relative stable brain temperatures, depending upon previous drug effect; however, the vasoconstrictive effects of cocaine during strenuous exercise in a hot, humid environment may cause serious elevation of brain temperatures and fatal hyperthermia.

GASTROINTESTINAL TRACT

Cocaine can produce nausea and vomiting via stimulation of the central vomiting centers with diarrhea and abdominal cramps developing from sympathomimetic effects on the intestinal wall. Cocaine is an intrinsic hepatotoxin in laboratory animals, but there are limited data on hepatotoxicity in humans in the absence of hyperthermia.[191] Potentially, animal models and *in vitro* studies suggest that reduced glutathione stores and the subsequent development of a reactive intermediate metabolite produce hepatic damage similar to acetaminophen toxicity.[192] However, hepatotoxicity is not a well-recognized complication of cocaine intoxication in humans except as a result of hyperthermia. Other gastrointestinal (GI) complications of cocaine abuse include GI perforation/hemorrhage, pneumoperitoneum, and mesenteric ischemia.[193]

KIDNEY

Renal complications associated with cocaine abuse include acute renal failure, renal infarction, and electrolyte imbalance.[194] Causes of acute renal failure associated with cocaine abuse include acute rhabdomyolysis,[195] hypertension,[196] interstitial nephritis,[197] thrombotic microangiopathy,[198] and antiglomerular basement membrane nephritis.[199]

Postmortem Examination

The pathologic findings on the postmortem examination of fatalities associated with recreational cocaine abuse are diverse and nonspecific because of the frequency of polydrug use and the presence of concomitant diseases.

Mechanisms of sudden death include ventricular fibrillation, respiratory arrest secondary to direct CNS effects or hyperthermia, intracerebral hemorrhage, dissecting aortic aneurysm, and disseminated intravascular coagulation with hyperthermia. Except for IV injection sites and bite marks from preterminal seizures, postmortem examination of patients dying suddenly following cocaine use demonstrate nonspecific signs of an asphyxial death (e.g., pulmonary edema, visceral congestion, petechiae). Fresh IV sites of cocaine injection characteristically present as multiple sites of ecchymosis, occasionally with central pallor. Less scarring is present in these lesions than in those of opioid addicts because cocaine abusers usually inject soluble material (e.g., mannitol) as compared with the insoluble talc and starch present in opioids. However, fatalities of cocaine users from urban cities frequently involve other drugs (e.g., heroin, ethanol).[200,201] Postmortem signs of chronic crack smoking include focal burns of varying ages on the mucosa of the lips, tips of the tongue, and opposing surfaces of the fingers (palmer surface of thumb and index finger).[202] These injuries result from burns from the surface of hot pipes or the flames from lighters. Calluses also develop on the palmer surface of the thumbs as a result of the frequent use of a lighter to heat cocaine crystals. Occasionally, singeing of the nasal hairs or eyelashes occurs. The inhalation of carbon particles during chronic crack smoking can produce black deposits in the alveoli.

COCAINE-INDUCED EXCITED DELIRIUM

Fatalities occur following all routes of cocaine administration (smoking freebase, nasal insufflation, vaginal application, ingestion). Several case reports describe the development of intense paranoia after chronic cocaine use followed by hyperthermia, bizarre, sometimes violent behavior, respiratory arrest, and death.[203] Frequently, these cases involve chronic cocaine users restrained while in police custody or during transport to a medical facility. Pathologic findings during postmortem examination of patients who die during cocaine-associated excited (agitated) delirium are usually minimal and nonspecific with or without postmortem evidence of hyperthermia. Depending on the duration of supportive care, pathologic findings during autopsies of fatalities secondary to cocaine-induced excited delirium include skeletal muscle necrosis without evidence of vasculitis, acute tubular necrosis with granular myoglobin casts in the tubules, cerebral edema, and pulmonary congestion.[204] Although these patients are often restrained, traumatic injuries are usually minor. Postmortem measurements of dopamine synaptic markers in the striatum and hypothalamus may help

differentiate deaths from excited delirium from deaths secondary to cocaine overdose or from asphyxiation.[205] However, this evaluation must be conducted by a properly equipped laboratory on adequately preserved brain tissue collected within 12 hours of death.

HISTOLOGIC ABNORMALITIES

Although normal heart tissue on autopsy examination does not exclude chronic cocaine use, endomyocardial biopsies of chronic cocaine users without significant coronary artery disease may demonstrate contraction band necrosis (coagulative myocytolysis), focal (eosinophilic) myocarditis, or dilated cardiomyopathy.[206] The frequency of these abnormalities varies between autopsy series. The presence of contraction band necrosis suggests an acute insult (e.g., elevated blood concentrations of norepinephrine), and patchy microfocal fibrosis indicates the presence of healed areas of contraction band necrosis. In the case of a 17-year-old body stuffer, who died suddenly after ingesting a large dose of cocaine (postmortem blood cocaine = 98 mg/L), histologic examination of the heart revealed contraction band necrosis characterized by rhexis of the myofibrillar apparatus and anomalous, hypereosinophilic cross bands.[207] Segments of hypercontracted sarcomeres with extremely thickened Z lines formed these cross bands. Eosinophilic and lymphocytic infiltrates are consistent with subacute injury.[208] However, neither contraction band necrosis nor lymphocytic infiltration is a specific sign of cocaine abuse.[209] In a case-control study of 30 cocaine-associated deaths, contraction band necrosis was present in 93% of the cases compared with 45% of cases dying of sedative-hypnotic intoxication.[210] A postmortem study of 40 cocaine-associated deaths and 27 victims of sudden traumatic death did not detect a statistically significant difference in the presence of contraction band necrosis between the 2 groups (25% and 41%, respectively).[211] There was a statistically significant ($P \le .05$) difference in the presence of myocarditis (mononuclear infiltrate) between these 2 groups (20% vs. 3.7%, respectively).

Interpretation of lesions in the cardiac conduction system is somewhat controversial, and these abnormalities may be incidental to the cause of death. Potentially lethal histologic changes in the cardiac conduction system that may be present in cases of sudden death associated with cocaine use include severe thickening of the atrioventricular node artery, intranodal and perinodal fibrosis, and microscopic foci of chronic inflammatory infiltration.[212] Postmortem examinations of patients dying after cocaine use usually demonstrate either significant coronary artery disease[213] or myocardial hypertrophy with or without microfocal fibrosis.[4,214]

However, postmortem examinations indicate that pathologic evidence of a MI is absent in most cases of sudden death in cocaine abusers.[209] Typically, heart weights are about 10–15% greater than predicted from nomograms on normal heart size.[215] A case report associated chronic intimal proliferation, severe coronary artery stenosis, acute platelet thrombosis, and lymphocytic myocarditis in a 21-year-old with chronic IV cocaine abuse.[216]

Autopsies of body packers dying from cocaine intoxication reveal ruptured cots, foil, and bags as well as the usual signs of asphyxial death (e.g., pulmonary edema).[217] Severe local vascular engorgement suggesting acute ischemic necrosis is occasionally seen in areas where high, localized concentrations of cocaine exist.[361]

CLINICAL RESPONSE

Illicit Use

Altered sensation during the recreational use of cocaine typically involves visual ("snow light") and tactile (i.e., "coke bugs," formication) sensations. Olfactory and auditory illusions are much less common occurrences during cocaine use.[218] Visual misperceptions most commonly involve flashing lights in the peripheral vision ("snow lights"). Cardiovascular, CNS, and pulmonary effects are the primary complications associated with cocaine abuse. In a retrospective study of 137 patients presenting to an urban emergency department with cocaine intoxication, the most common complaints included the following: altered mental status (40%), chest pain (21%), syncope (19%), suicide attempt (13%), palpitations (12%), and seizures (12%).[219] The clinical effects of cocaine develop rapidly (<30–60 min) by all routes of exposure except ingestion, and the effects usually resolve within 2 hours after the use of a single dose of cocaine. Because of the rapid elimination half-life (<1 h) of cocaine, resolution of major symptoms occurs within 1–3 hours, unless secondary damage (e.g., hypoxic encephalopathy) or complications of hyperthermia supervene.

Complications of cocaine abuse depend somewhat on the route of exposure. Epidemiologic data indicate that the medical complications of cocaine abuse have changed with the shift from IV use to smoking and insufflation.[220] Medical effects of chronic cocaine abuse include anorexia, weight loss, malnutrition, water-soluble vitamin deficiencies, dehydration, pallor, tremor, headaches, and isolated convulsions. The complications of IV drug use include localized cellulitis or abscess, bacteremia with septic arthritis, endocarditis, hepatitis, autoimmune deficiency syndrome (AIDS), malaria,

osteomyelitis, pneumonia, and metastatic abscesses as well as necrotizing fasciitis[221] and wound botulism.[222]

Behavioral Abnormalities

Cocaine produces alertness, a sense of pleasure, a reduction of social inhibitions and anxiety, and a heightened sense of energy, self-esteem, and sexuality.[223] The euphoric effects begin within minutes and last approximately 20–45 minutes. Although cocaine enhances the feeling of pleasure, the use of this drug does not usually distort reality or produce hallucinations. After the elation subsides, restlessness, hyperactivity, irritability, and depression emerge; the development of these feeling reinforce continual cocaine use. In contrast to the feelings of sadness during the sober state, memories of drug-induced euphoria promote the continued use of cocaine. Binges or runs may last several days until either physical, financial, or drug resources are exhausted. The absence of daily use does not exclude addiction to cocaine.[223] Often, cocaine addiction involves multiple drugs of abuse including the use of sedative-hypnotics, ethanol, or heroin in an effort to counteract the rebound dysphoria ("crash") associated with cocaine use. Most casual users do not become addicted to cocaine, but some users discover that higher doses intensify the pharmacologic euphoria and they focus on internal sensations to the detriment of personal relationships and career goals. Controlled use may shift to compulsive use as the dose of cocaine increases or the user switches to more potent methods of administration (IV, smoking).

Complications from cocaine use are uncommon among low-dose users, but adverse behavioral effects are common in high-dose abusers.[223] Psychologic complications from chronic cocaine use include impaired judgment, impulsiveness, compulsive behavior, psychomotor activation, and grandiosity that may result in falls, traffic accidents, homicides, suicides, atypical sexual behavior, or criminal activity.[224,225]

Adverse neuropsychiatric effects of chronic cocaine use include insomnia, irritability, agitation, paranoia, delirium, anxiety, depression, chronic fatigue, impaired memory/concentration, and panic attacks.[59] Depending on dose, chronicity, personality, genetic predisposition, and expectations, cocaine produces a continuum of psychiatric syndromes ranging from euphoria and dysphoria to a schizophreniform psychosis similar to amphetamine-induced psychosis.[53] In a series of 996 emergency department visits and 279 hospital admissions for complications of cocaine abuse, the most common psychiatric complications were dysphoria, agitation, aggressive behavior, paranoia, psychosis, and hallucinations.[285]

High-doses of cocaine or habitual cocaine use produce profound mood changes characterized by paranoid thinking, agitation, irritability, restlessness, and impulsivity. Chronic users often become depressed, suspicious, irritable, and impotent, which reinforces continual cocaine use. Distorted thought processes can cause aggressive suicidal and homicidal behavior, impaired judgment, poor job performance, and depletion of personal finances. Attentional deficits increase the chance of accidental trauma. Recreational doses of cocaine suppress rapid eye movement (REM) sleep during the cocaine binge, and volunteer studies indicate that the REM sleep patterns do not return to normal until 3 nights after the cessation of cocaine use.[226] After cocaine use, depressive symptoms (cocaine blues, crash) and occasionally suicidal ideations appear, which the user may try to alleviate by continual drug use. Such symptoms are usually transient and resolve after resumption of normal sleep patterns. Following several days of bingeing, cocaine addicts may present with a depressed level of consciousness ranging from lethargy and hypersomnolence to lighter stages of coma (washed out syndrome).[227,228] These patients typically do not have neurologic or metabolic abnormalities on testing; within 18 hours, they usually return to their baseline mental state.

Mental Disorders

Chronic stimulant use can produce a paranoid psychosis that is similar to acute paranoid schizophrenia and methamphetamine-induced psychosis. High-dose, chronic use of cocaine is associated with dysphoria and an acute paranoid psychosis marked by delusions of persecution and paranoia as well as hallucinations, particularly auditory.[229] The paranoia typically focuses on illicit activities related to drug abuse during the cocaine binge, and the paranoia resolves by the early hypersomnolent phase of abstinence.[230,231] Consequently, the paranoid ideations last hours after the pleasurable effects of cocaine cease. Following chronic cocaine abuse, the paranoia becomes more intense and occurs earlier in the cocaine binge. In a study of 55 cocaine addicts admitted for inpatient therapy, 48% of the cocaine addicts reported the development of paranoia whenever they used cocaine.[232]

Typically, the acute psychosis associated with cocaine addicts resolves after several days of abstinence, and the patient's personality returns to baseline within approximately 5 days. In addition to the schizophrenic form psychosis, a confused toxic psychosis may develop that includes disorientation, short-term memory impairment, and organic brain dysfunction. Persistence of psychiatric symptoms beyond 5–7 days suggests an

underlying psychiatric disorder; however, case reports suggest that the psychotic symptoms associated with substance-induced psychosis may persist over 1 month in a small subset of chronic abusers.[233] Over 50% of individuals with a history of schizophrenia have a coexisting substance abuse disorder.[234] Furthermore, many individuals with substance abuse disorder have comorbid psychiatric disorders, particularly phobias, major depression, and antisocial personalities. In a study of 243 unrelated, recruited, cocaine-dependent adults, 90% of these individuals met the criteria for substance use disorders other than cocaine dependence.[235] Common nonsubstance use disorders included antisocial personality disorder (77%), major depression (10%), and attention deficit-hyperactivity disorder (10%).

Medical Complications

Cocaine intoxication and chronic cocaine abuse produce a variety of medical complications primarily involving the cardiovascular, central nervous, and the pulmonary systems. Table 56.1 outlines potential medical complications associated with cocaine abuse exclusive of reproductive effects. Cocaine overdose frequently occurs in the setting of multiple-drug use, particularly ethanol, opioids, and marijuana.[236]

CARDIAC

MYOCARDIAL ISCHEMIA AND INFARCTION.   Illicit use of cocaine increases myocardial oxygen demand by increasing the pulse and blood pressure, while reducing coronary artery diameter and increasing coronary vascular resistance.[237] The imbalance between myocardial oxygen supply and demand may produce chest pain, myocardial ischemia, or MI, particularly in patients with underlying cardiovascular disease. The overall incidence of cocaine-associated MI in patients with documented cocaine use and chest pain varies between about 0.7% (Acute Cardiac Ischemia-Time Insensitive Predictive Instrument Study)[238] and 6% (Cocaine Associated Chest Pain Study),[239] depending on the study population and the criteria for MI. The latter prospective multicenter study involved only patients presenting to urban hospitals with chest pain, whereas the former study included rural hospitals and a variety of cardiovascular complaints. Most, but not all of these patients presented to the emergency department within 3 hours of the use of cocaine. In the Determinants of Myocardial Infarction Onset Study, 38 of 3946 acute MI patients admitted using cocaine within the last year and 9 patients used cocaine within 1 hour after developing their MI.[240] The risk of MI in cocaine users increased 24-fold in the first hour after use compared with nonus-

TABLE 56.1. Potential Medical Complications of Cocaine Abuse.[59]

Organ System	Complication	Clinical Effects
Cardiovascular		
	Ischemia	Angina, myocardial infarction, cardiogenic shock, renal dysfunction/infarction, intestinal infarction, limb ischemia/compartment syndrome
	Hypertension	Intracranial hemorrhage, aortic dissection
	Dysrhythmias	Sinus tachycardia, supraventricular tachycardia, ventricular tachyarrhythmias, hypotension, cardiac arrest
	Myocarditis	
Central Nervous System		
	Seizures	Trauma, hypoxia
	Stroke	Cerebral embolism/infarction, subarachnoid hemorrhage, intracranial hemorrhage
	Neurologic deficits	Transient focal neurologic effects, altered consciousness, encephalopathy, coma
Respiratory		
	Respiratory depression/arrest, pulmonary edema	Hypoxia
	Barotrauma	Pneumomediastinum, pneumothorax
Metabolic		
	Hyperthermia	Multisystem organ failure, shock
	Rhabdomyolysis	Renal dysfunction
Immune System		
	Intravenous drug use	HIV infection, hepatitis B/C, endocarditis, wound botulism, tetanus

Abbreviation: HIV = Human immunodeficiency virus.

ers; this risk decreased rapidly to 4-fold 2 hours after using cocaine.

About 31–67% of patients with cocaine-associated MIs have arteriosclerotic coronary artery disease.[241] Most of these patients present to the emergency department within 24 hours of the last cocaine use. Myocardial ischemia and infarction occur after cocaine use from all routes of exposure, and the cocaine-induced cardiovascular complications (Q-wave and non-Q-wave MIs, myocarditis, dysrhythmias, cardiomyopathy, aortic aneurysm) are not dose-related.[242,243,244] Although most patients who develop cardiac abnormalities after cocaine use have preexisting cardiac disease,[237,245] case reports indicate that acute MI may occur in patients without angiographic evidence of coronary artery disease including patients with cocaine-associated MI and coronary artery ectasia or aneurysms.[246] Inhalation of 0.25 g of cocaine immediately preceded the development of chest pain and elevated cardiac enzymes in a 21-year-old man with structurally normal coronary arteries and a prior history of angina.[247] After the inhalation of 1.5 g of cocaine over 5 hours, a 28-year-old woman developed anterior chest pain as well as ECG and enzymatic evidence substantiating an anterior MI.[248] There was no prior history suggestive of preexisting heart disease or documented cardiovascular risk factors, and coronary angiography demonstrated no fixed coronary artery disease or ergonovine-induced coronary spasm. These case histories suggest that cocaine may produce coronary artery vasospasm or alter thrombin formation even in the absence of underlying disease as documented by normal coronary angiography and normal stress ECGs.[249]

Chest Pain. Although chest pain is a common complaint following the use of cocaine, documentation of a MI in these patients is relatively rare even in the presence of ECG changes suggestive of ischemia. The rate of MIs in patients hospitalized for chest pain after cocaine use varies from 0–31% with most studies near 6%, depending on the institution, patient population, criteria for MI, and admission policies.[250,251,252] Retrospective clinical studies indicate that recent cocaine use may affect serum myoglobin concentrations and, to a much lesser extent, serum CK-MB concentrations.[253] Elevation of creatine kinase (CK) and creatine kinase-myocardial band (CK-MB) fractions may occur following cocaine use without acute myocardial ischemia. In a study of 8 cocaine users admitted to an urban hospital with elevated CK-MB fractions and chest pain in the emergency department, only 2 patients had persistent CK-MB enzyme elevations on serial testing.[254] Recent cocaine use does not affect the specificity of serum cardiac troponin I.[253] Most patients, who develop

chest pain and a MI after cocaine use, present to the emergency department with ECG evidence of a MI.[255] Myocardial perfusion imaging did not detect significant myocardial ischemia in a prospective study of 14 patients with chest pain for >15 minutes after cocaine use and no ECG evidence of myocardial ischemia.[256] Consequently, most patients with chest pain following the use of cocaine have a low risk of developing myocardial ischemia. In a study of 293 patients with cocaine-associated chest pain enrolled in a multicenter, prospective clinical trial, 10 had acute myocardial ischemia and 2 had a documented MI.[238] Furthermore, most patients presenting to the emergency department with chest pain after cocaine use have normal cardiac function (cardiac output, cardiac index, stroke volume).[257] A prospective consecutive cohort study of cocaine-associated chest pain with low to intermediate cardiac risk have a low risk (i.e., <1%) of developing an acute MI during the first year after completing a chest pain observation unit protocol.[258] Exclusion criteria during admission and observation included the development of ECG or serum cardiac markers suggestive of acute MI, history of acute MI or coronary artery bypass graft, hemodynamic instability, and unstable angina.

Dysrhythmias. Cocaine-induced dysrhythmias commonly include sinus tachycardia and premature ventricular beats. These dysrhythmias are usually transient. The presence of underlying heart disease or the administration of massive cocaine doses are not prerequisites for the development of cocaine-associated cardiovascular complications.[259] Case reports temporally associate the use of ethanol and the nasal insufflation of cocaine with supraventricular tachycardia and myocardial ischemia as well as ventricular fibrillation and *torsade de pointes*.[260,261,262] The use of cocaine may unmask electrophysiologically silent accessory pathways resulting in the onset of life-threatening cardiac dysrhythmias.[259] In addition, the use of cocaine may prolong the QT_c interval in some, but not all, patients using cocaine and consequently potentiate the risk of ventricular fibrillation in susceptible patients (e.g., idiopathic long QT syndrome).[263] In a study of 45 cocaine abusers hospitalized for cocaine toxicity, the mean QT_c interval of the cohort with chest pain was significantly longer than the QT_c interval of the cohort without chest pain (approximately 510 ± 84 msec vs. 459 ± 74 msec, respectively).[264] Three patients in the former group developed *torsade de pointes* requiring resuscitation, whereas none of the patients in the latter group required defibrillation. High doses of cocaine may produce sodium channel blockade and direct myocardial depression similar to type I antidysrhythmic drugs.[242] Brugada syndrome is a congenital electrical disorder characterized by the appearance of

distinctive QRST-T wave patterns in the right precordial leads (i.e., coved ST elevation V_1–V_3) and an increased risk of sudden death in young healthy adults. Cocaine is one of the drugs associated with the appearance of the Brugada syndrome.[265]

During severe cocaine intoxication (hyperthermia, multiple seizures, metabolic acidosis) a variety of serious arrhythmias may develop including ventricular tachycardia and unstable wide and narrow complex bradyarrhythmias.[266] However, sudden death associated with cocaine-induced excited (agitated) delirium is usually not associated with ventricular fibrillation or ventricular tachycardia, even when Tasers or pepper sprays are used during restraining procedures. In a series of 18 cases of sudden death associated with excited delirium and restraint by law enforcement personnel, paramedics determined the rhythm of 13 individuals immediately prior to cardiac arrest.[267] Ventricular dysrhythmias were the primary cardiac arrest rhythm in only 1 of 13 cases, while ventricular fibrillation was not found in any of these 13 cases. Cocaine or amphetamine was present in the postmortem blood from 10 of these 13 cases.

MYOCARDITIS.   In a study of the postmortem examination of 40 cocaine-associated and 27 sudden trauma-related deaths, there was a statistically significant increase ($P \leq .05$) in the presence of myocarditis (mononuclear infiltrate) between these 2 groups (20% vs. 3.7%, respectively).[211] Other case reports associated cocaine abuse with eosinophilic infiltration of the myocardium.

CARDIOMYOPATHY.   Long-term, high-dose cocaine use may enhance the development of cardiomyopathy in the absence of coronary artery disease based on experimental evidence in animal models.[268] Dilated cardiomyopathy is a rare complication of cocaine abuse. A few case reports and case series associate chronic cocaine use with dilated cardiomyopathy and left ventricular dysfunction in the absence of atherosclerotic coronary artery disease.[269,270] Case reports indicate that the cardiac effects of the dilated cardiomyopathy are at least partially reversible.[271,272] During 5 months of abstinence from freebase cocaine, the left ventricular ejection fraction of a cocaine addict improved from <20% to 45%.[273] A study of 40 chronic cocaine abusers enrolled in an inpatient drug rehabilitation program demonstrated a statistically significant increase in left ventricular mass index in the cocaine group compared with 30 age-matched and race-matched controls.[274] Although ECGs indicated that left ventricular cavity dimensions and wall motion were normal in all participants, more cocaine addicts than controls (13 vs. 4) had increased posterior wall thickness.

AORTIC DISSECTION.   Although acute aortic dissection is an infrequent complication of cocaine abuse, some studies indicate that crack cocaine-associated acute aortic dissections account for about one-third of the cases, particularly in an inner city population consisting of a large number of black men with untreated hypertension.[275] In a retrospective chart review of 164 cases of acute aortic dissection at an urban hospital, 16 patients (9.8%) used cocaine in the 24-hour period prior to the onset of symptoms.[276] The mean duration between cocaine use and the onset of aortic dissection was 12.8 hours (range, 4–24 h). The incidences of DeBakey dissection types I, II, IIIa, and IIIb were similar between the cocaine users and the noncocaine users, whereas the prevalence of Stanford Group B dissections was greater in cocaine users than nonusers in another case series of 46 consecutive admissions for aortic dissection to an urban hospital.[277] A previously healthy 45-year-old man with preexisting hypertension developed a fatal, acute ascending aortic dissection after smoking freebase for several hours.[278] A 45-year-old chronic cocaine abuser developed severe, sudden, crushing substernal chest pain after a 36-hour cocaine binge.[279] He underwent successful repair of a dissecting aneurysm of the ascending aorta that required replacement of the aortic valve. Although most of these case reports document the occurrence of dissecting thoracic aneurysms in patients with cardiac risk factors (i.e., hypertension),[280,281] a few of these patients did not have known cardiac risk factors for the development of aortic aneurysms.[282,283] Almost all cases of aortic dissection involve the thoracic rather than the abdominal aorta.[284]

NEUROLOGIC

Common neurologic complications of chronic cocaine abusers admitted to medical facilities include focal neurologic deficits, seizures, headaches, and transient loss of consciousness.[285] The focal neurologic abnormalities involve visual, sensory, and motor disturbances that may result from transient ischemic events or structural lesions (stroke, subarachnoid hemorrhage). With the exception of seizures during a massive overdose of cocaine, the risk of developing neurologic complications during cocaine use is not clearly related to the dose or the route of cocaine administration.[286] In the vast majority of patients with cocaine-associated neurologic complications, symptoms develop within 6 hours of the use of cocaine. In a series of 33 patients presenting to an urban hospital with neurovascular sequelae associated with cocaine use, the percentage of patients developing these complications during cocaine use and during the subsequent 6 hours was 54.5% and 33.3%, respectively.[287] Occasional case reports associate cocaine use

with movement disorders (i.e., vocal and motor ticks, generalized dystonia, acute dystonic reaction) either as an exacerbation of a preexisting movement disorder (e.g., Tourette's syndrome)[288] or *de novo* dyskinesias attributed to cocaine use.[289] A case series of 4 patients associated cocaine use with Tourette syndrome, idiopathic dystonia/essential-like tremor, and tardive dystonia.[290]

INTRACRANIAL LESIONS. All types of strokes complicate the chronic use of cocaine including subarachnoid hemorrhage, intracerebral hemorrhage, ischemic infarction, and vasculitis.[291] Additionally, these strokes occur at a younger age and in a variety of locations including unusual areas (e.g., anterior spinal artery, bilateral thalamic lesions). Hemorrhagic strokes develop after the use of cocaine hydrochloride both via insufflation and freebase cocaine, but ischemic strokes are more common following freebase cocaine use than following the use of cocaine hydrochloride.[292,293] Although most angiographic studies of cocaine-associated strokes demonstrate the presence of arteriovenous malformations or an aneurysm, cerebrovascular lesions are absent in a minority of these cases.[294] Although the clinical prognosis of cocaine users with intracranial bleeding is unclear, a case-control study of cocaine users with subarachnoid hemorrhage did not detect a difference in the score on the Glasgow Outcome Scale between cocaine users and controls.[295] Embolic strokes are a complication of cocaine users with dilated cardiomyopathies and valvular lesions. Case reports also associate cocaine use with cerebral vasculitis manifest by focal weakness, headache, dysarthria, drowsiness, and low-grade fever.[296] Clinical features of intracranial lesions include sudden onset of severe headache, nausea, vomiting, loss of consciousness, and lateralizing neurologic signs (aphasia, hemiplegia, paresthesia, dysarthria).[297]

Toxic leukoencephalopathy is a progressive, frequently fatal neurologic disease characterized by structural alteration of cerebral white matter, particularly myelin, and consciousness that results from a variety of causes including drug abuse (e.g., heroin). Although most cases of cocaine-induced neurologic disease involves intracranial hemorrhage or vasculitis, rare case reports associate toxic leukoencephalopathy with an IV cocaine overdose[298] and cocaine abuse.[299] A case report associated cocaine abuse with toxic leukoencephalopathy that was partially reversible. A 30-year-old male developed mutism, stupor, hyperreflexia, and increased muscle tone after he continue cocaine abuse after a prior episode of toxic leukoencephalopathy resolved.[300] The fluid-attenuated inversion recovery (FLAIR) images on his MRI demonstrated diffuse symmetric hyperintensity of the periventricular white matter; he

recovered after 6 months of rehabilitation with some residual cognitive deficits (impaired short-term memory, visuospatial functions).

SEIZURES. Cocaine-associated seizures occur in patients with and without preexisting seizure disorders and in patients with massive cocaine intoxication as an agonal event.[301] Seizures in cocaine abusers following cocaine use by all routes of administration are usually isolated, generalized tonic-clonic seizures that occur during or shortly after cocaine use, but occasionally seizures may be partial.[302] In adults, seizures following the insufflation of cocaine usually occur in patients with an underlying seizure disorder.[303] In series of adults presenting to the emergency department, most patients with cocaine-associated seizures have a known history of cocaine abuse.[304] Seizures in adult patients with normal neurologic examination after the seizure are usually not associated with intracranial lesions. In a retrospective study of 33 uncomplicated, cocaine-associated seizures in patients with normal neurologic examinations, diagnostic testing did not demonstrate any neurologic abnormalities.[305] The presence of seizures along with coma and hyperthermia is an ominous prognostic sign.[286] In children, focal or generalized seizures occur following accidental ingestion of cocaine.[306] The most common neurologic complications in a case series of 41 children (age 2 months to 18 years) presenting to an emergency department following exposure to cocaine were seizures followed by obtundations and delirium.[307] Seizures in these children usually, but not always, are self-limited, depending on the amount of cocaine ingested.

HEADACHES. The abrupt onset of diffuse, severe headache immediately after using cocaine suggests the possibility of a hemorrhagic or ischemic stroke. However, headache is a nonspecific sign of intracranial hemorrhage because vascular headache is the most common adverse neurologic sign present during cocaine intoxication and withdrawal.[308] Most patients presenting with the primary complaint of headache without focal neurologic signs have a benign clinical course.[285] The use of cocaine may precipitate or aggravate migraine headaches.[309]

PULMONARY

Most pulmonary complications associated with cocaine use involve the smoking of freebase cocaine.[310] Crack lung refers to an acute, inflammatory pulmonary syndrome associated with the inhalation of freebase cocaine, manifest by fever, hemoptysis, hypoxemia, diffuse alveolar infiltrates, and in rare cases, respiratory failure.[311] The etiology of most of these pulmonary

complications is not well defined; pathologic changes include both alveolar hemorrhage and interstitial edema.[312] Chronic cough and bronchitis productive of black or blood-tinged sputum frequently result from habitual freebase smoking. Case reports associate freebase cocaine with pneumomediastinum[313,314] and pneumothorax.[315] Symptoms include pleuritic chest pain and dyspnea; the clinical course of these complications is usually benign. Most patients become asymptomatic within approximately 24 hours. In a review of 42 cases of pneumomediastinum, 19% had associated pneumothorax; only 1 patient required a chest tube.[316] Pulmonary edema may develop after pure freebase cocaine use,[317] and case reports suggest that the etiology is noncardiogenic based on normal cardiac size, elevated protein levels, and increased capillary permeability detected by bronchoalveolar lavage.[318] A fatal case of pulmonary edema followed the IV injection of freebase cocaine.[319] Smoking cocaine may produce bronchospasm in cocaine abusers with[320] and without[321] a history of preexisting asthma. Other pulmonary abnormalities associated with smoking cocaine include pulmonary hemorrhage, bronchiectasis and interstitial desquamative pneumonia, bronchiolitis obliterans, and diffuse alveolar infiltrates with hemoptysis and chest pain (hemorrhagic alveolitis or crack lung).[310,322] Clinical effects associated with hemorrhagic alveolitis include dyspnea, hypoxemia, fever, hemoptysis, and cough. The effect of freebase cocaine smoking on lung function and gas exchange remains controversial.[312] Pulmonary complications from the chronic insufflation of cocaine include dyspnea on exertion, cough, pulmonary opacities, pulmonary embolism/thrombosis, and pulmonary granulomas.

HEAD AND NECK

Chronic insufflation of cocaine causes a reactive hyperemia of nasal mucosa, recurrent epistaxis, anosmia, and a persistent rhinitis.[323] Mucosal erosions and, less often, nasal perforation and sinusitis also complicate chronic cocaine insufflation.[324,325] The sinusitis associated with nasal insufflation of cocaine is not associated with eosinophils on nasal smear and fails to improve with antihistamines and nasal sprays.[326] Case reports associate cerebrospinal fluid rhinorrhea with chronic cocaine administration via the nasal route.[327] The hot vapor associated with freebasing or crack cocaine use may produce thermal injuries to the upper airway manifest by sore throat, dysphagia, odynophagia, cough, dysphonia, and stridor.[328] Additional complications documented by case reports include nasal papilloma,[329] keratitis, iritis, and maculopathy with impaired color vision after insufflation,[330,331] and crack cocaine-associated corneal ulcers.[332,333] Infection with the development of hypopyon commonly complicates the occurrence of corneal erosions, frequently by unusual organisms (e.g., *Brevibacterium casei*, *Capnocytophaga* spp., *Streptococcus mitis*, *Streptococcus sanguis*). Large central scars with neovascularization and permanent visual loss may result.

RENAL

Acute renal failure secondary to the development of rhabdomyolysis is the most common renal complication of cocaine use.[310] Risk factors for acute renal failure in patients with cocaine-induced rhabdomyolysis include hypotension, hyperthermia, and high serum creatine kinase concentrations. In a series of 39 patients with acute rhabdomyolysis secondary to cocaine use, renal failure developed in 33% of these patients.[186] Mean serum creatine kinase concentration in patients with and without renal failure were approximately 28,000 U/L and 8,000 U/L, respectively. The presence of renal failure, rhabdomyolysis, severe liver dysfunction, and disseminated intravascular coagulation in patients from this case series indicated a poor prognosis. Renal failure may occur following cocaine use in the absence of rhabdomyolysis.[334] Rare case reports associate recent cocaine use with the development of renal infarction with[335] and without acute renal failure.[336] There are inadequate data to determine the effect of chronic cocaine use on the development of acute interstitial nephritis or chronic renal disease.

GASTROINTESTINAL TRACT

Gastrointestinal complications associated with cocaine abuse include mesenteric thrombosis, perforation, and ischemia. Intestinal ischemia is a complication of cocaine abuse via all routes.[337] Clinical features of mesenteric ischemia include abdominal pain, nausea, vomiting, and bloody diarrhea. Cocaine-associated enterocolitis typically involves inflammatory or ischemic changes in the proximal colon that occur within 3 days of the last cocaine use.[338] Case reports associated gastroduodenal perforations[339] and pneumoperitoneum[340] with crack cocaine smoking. The use of cocaine is not directly related to liver dysfunction, although liver failure may occur during severe cocaine intoxications along with multiple end organ failure and severe hyperthermia.

MUSCLES

Rhabdomyolysis associated with cocaine abuse may result from a variety of etiologies; most patients with cocaine-induced rhabdomyolysis have recognizable causes of muscle toxicity, such as compartment syn-

drome, prolonged immobilization, hyperactivity, seizures, hyperthermia, or direct trauma during periods of altered behavior.[341] The presence of rhabdomyolysis following cocaine use is not usually associated with toxic effects unless severe; the clinical features of severe rhabdomyolysis include weakness, myalgias, nausea, vomiting, hypocalcemia, muscle swelling, and myalgias. In a prospective study of 34 patients presenting to a large urban hospital with complaints related to cocaine use, 24% (8 cases) of these patients had evidence of rhabdomyolysis as defined by a creatine kinase concentration 5 times normal values (>1000 U/L).[342] Muscle symptoms did not correlate to serum creatine kinase concentrations. Severe rhabdomyolysis occurs both with and without cocaine-induced excited delirium, but the nonfatal cases of severe rhabdomyolysis associated with cocaine use shares many similar risk factors with excited delirium.[343]

TEMPERATURE CONTROL

Clinically serious elevation of temperature can occur in cocaine users, particularly in association with delirium. The hyperthermia can develop along with multiorgan failure that includes renal failure, rhabdomyolysis, liver failure, and disseminated intravascular coagulation.[344] These clinical features occur following all routes of exposure including nasal insufflation,[345] IV injection,[346] and smoking freebase (crack).[347]

ANAPHYLAXIS

True anaphylactic reaction to pure cocaine is extremely rare, although street samples may contain more allergenic drugs (e.g., quinine). Severe immediate reactions to cocaine use more likely result from massive overdose or rapid IV injection. Following very large cocaine doses, cardiac collapse and respiratory arrest may occur without grand mal seizures.

SKIN

Following the probable use of levamisole-contaminated cocaine, case reports documented the development of an occlusive, necrotizing vasculitis including purpura of the face and ear, retiform purpura of the trunk,[348,349] and agranulocytosis.[350,351] A 52-year-old woman presented to an emergency department with purpura of the face, breasts, and extremities.[352] The skin biopsy demonstrated an antineutrophil cytoplasmic antibody-positive vasculitis with focal intravascular fibrin formation. Analysis of her urine 2 days after admission detected 0.92 mg levamisole/L.

Overdose

Although recreational use of cocaine may cause severe cocaine intoxication, massive cocaine intoxication usually results from body packing or, less often, body stuffing.[353] Individuals who smuggle cocaine within their bodies are called "body packers" or "mules," whereas individuals who ingest cocaine to avoid arrest are "body stuffers." Body stuffers, who ingest poorly wrapped bundles of rock (freebase) cocaine to avoid arrest, usually develop symptoms of cocaine intoxication within a few hours, whereas the appearance of symptoms in smugglers (body packers) may be delayed many hours depending on the durability of the cocaine bags during transit through their intestinal tract.[354] The amount of cocaine and the effectiveness of the wrapping in preventing leakage accounts for the different onset and severity of intoxication associated with these 2 syndromes. In the past, bags were composed of plastic bags, balloons, or condoms that easily leaked, but the process is more automated now with much stronger wrappings (i.e., latex sheath (condom, balloon) covered with several layers of latex and sealed with a hard wax coating).[355] The patient is typically a young, white man who has recently arrived from South America.[356] The clinical presentation includes hyperthermia, tachycardia, hypertension, mydriasis, nervous or agitated behavior, toxic psychosis, delirium, and seizures. Early signs of cocaine intoxication include tachycardia, hypertension, agitation, anxiety, talkativeness, and muscle hyperactivity. The presence of these signs in a body packer or a body stuffer indicates that more serious signs of cocaine toxicity may develop, although the clinical course of body stuffers is usually mild. In contrast to body packers, body stuffers who develop cocaine toxicity typically will be symptomatic on admission or shortly thereafter. In a study of 98 cocaine body stuffers brought to the emergency department by law enforcement personnel, all complications occurred within 3–4 hours of admission to the emergency department including seizures in 4 patients.[357] Rarely, delayed seizures occur in body stuffers ingesting very large amounts of freebase cocaine.[358] Terminal convulsions, ventricular dysrhythmias, or respiratory arrest usually develops within the first 30 minutes following massive cocaine exposure.[359] Abdominal x-rays may or may not demonstrate foreign bodies, and the absence of radiodense material does not exclude the presence of cocaine packets.[12] Symptoms of cocaine intoxication can recur several days after apparent recovery when remaining bags rupture,[360] leading to death over 1 week after ingestion.[361] Typically, violent seizures followed by respiratory arrest precede death secondary to massive cocaine overdose.[203]

Fatalities

Causes of cocaine-associated fatalities include sudden death, accidental trauma, accidental overdoses, suicides, and homicides. In a review of cohort studies of IV or treatment-seeking cocaine users, the standardized mortality ratios were ~4–8 times higher than age- and sex-matched peers in the general population.[362] Mortality from trauma and infection are particularly high in this population, suggesting the importance of socioeconomic factors as well as drug habits. There are few mortality data on individuals using cocaine by insufflation only.

ACCIDENTAL OVERDOSE

Deaths resulting from the pharmacologic effects of cocaine are usually sudden and occur within a few hours of cocaine use.[59] The use of cocaine can precipitate a MI, dysrhythmias, and sudden death in patients with or without cardiovascular disease. In a Spanish prospective, case-control study of 668 cases of sudden death, 3.1% of these cases were associated with cocaine use.[363] Causes of sudden death included cardiovascular (62%), cerebrovascular (14%), excited delirium (14%), respiratory complications (5%), and metabolic abnormalities (5%). Severe coronary artery disease and coronary artery thrombosis were present in 29% and 14% of the sudden death cases, respectively. Death in young cocaine abusers is frequently abrupt and idiosyncratic, presumably as a result of a lethal ventricular arrhythmia or an idiopathic response to cocaine. However, the actual documentation of life-threatening ventricular arrhythmias secondary to recreational cocaine use is uncommon, especially considering the frequency of cocaine abuse in the United States.[364] Although risk factors for ventricular dysthymias (e.g., coronary artery disease, hypertrophy, hypertension, myocarditis, ischemia) are often present in case series on cocaine-associated sudden death, MI is uncommon in postmortem examination of these patients.[365,366] In a case series of 24 autopsies that demonstrated cocaine or benzoylecgonine in postmortem samples, significant coronary arteriosclerosis (>70% occlusion) was present in over 60% of these cases.[214] Other causes of death soon after exposure to cocaine include status epilepticus, centrally mediated respiratory arrest, excited delirium, and intracerebral hemorrhage. Although respiratory collapse and death frequently occur immediately after cocaine injection, administration of cocaine by the nasal or oral routes may delay terminal seizures as long as 1 hour after exposure.[14] Retrospective studies of cardiac arrest in crack cocaine users with positive urine drug screens for cocaine suggest that these patients are younger and more likely to survive with total neurologic recovery, when compared with cardiac arrest in patients without cocaine use.[367] The exception to good neurologic outcome in these patients is the crack cocaine-using patient with excited delirium.

EXCITED DELIRIUM

Sudden death occurs in a small subset of chronic cocaine abusers following the development of agitation, bizarre behavior, and delirium during the use of cocaine. Wetli and Fishbain first described this syndrome of fatal excited delirium in 7 chronic cocaine hydrochloride abusers in 1985.[203] The inappropriate behavior includes formication, disturbed perceptions, impaired thinking, hallucinations, aggression, breaking objects, screaming, and hyperactivity. Symptoms begin with the acute onset of intense paranoia, followed by bizarre and often violent behavior that requires forcible restraint by police, health care providers, or bystanders. Cardiorespiratory arrest may develop immediately or soon after being restrained. Sudden cardiorespiratory arrest during the restraint procedures or shortly thereafter does not usually result from the restraints or from trauma associated with the restraint procedure.[368] In a retrospective study of 58 cases of cocaine-induced fatal excited delirium and 125 cases of accidental cocaine fatalities without excited delirium, the excited delirium cases were more likely to be black, male, younger, heavier, and hyperthermic compared with accidental overdoses.[369] Additionally, the excited delirium cases were more likely to die during summer months. Although the fatal excited delirium cases were more likely to occur in police custody than accidental overdoses, only about one-third of the fatal excited delirium cases died in police custody. The postmortem cocaine concentrations and heart weights were similar in both groups.

The mechanism of death in cocaine-induced excited delirium is not well defined. A case series of 18 cases of sudden death associated with excited (agitated) delirium documented ventricular dysrhythmias in only 1 of 13 cases when the primary cardiac arrest rhythm was available.[267] This case series also included 196 cases of individuals surviving excited delirium in hobble restraints without significant medical complications. Although many cases of sudden death associated with excited delirium involve restraint procedures and cocaine abuse, sudden death during excited delirium also occurs in agitated patients without the presence of stimulant drugs[370,371] and without restraint procedures.[372] Frequently, patients dying following cardiac arrest during restraint procedures are hyperthermic and profoundly acidotic. The severity of the clinical response does not correlate directly to the absorbed dose of cocaine, and postmortem blood samples from these

patients typically contain lower cocaine concentrations (e.g., <0.6–1 mg/L) than blood samples from fatalities resulting from large cocaine overdoses. Additionally, the former patients do not usually develop preterminal seizures in contrast to the typical severe cocaine overdose.

Abstinence Syndrome

Although the spectrum and time course of abstinence syndromes following chronic cocaine use remains somewhat controversial, the consensus indicates that there are 3 phases: crash, withdrawal, and extinction.[223] Typically, the clinical features of cocaine withdrawal are mild, primarily involving sleep disturbances, vivid dreams, agitation, excessive appetite, fatigue, and psychomotor retardation.[373]

CRASH

The crash is the immediate rebound that develops soon (i.e. <1 h) after the cessation of cocaine use, and this phase usually lasts a few hours to a few days. The crash represents the extreme exhaustion that occurs after a binge of cocaine use as the craving for sleep replaces cravings for stimulant use. During this period, the use of other drugs (benzodiazepines, ethanol, sedatives, opioids, marijuana) is common as a means to reduce the agitation, dysphoria, depression, insomnia, and anxiety associated with the binge use of cocaine. Prolonged hypersomnolence, irritability, and periods of hyperphagia precede the return of a more normal mood. In clinical practice, the resumption of a normal sleep pattern followed by 3 or more days of abstinence confirms the resolution of a crash.[374]

WITHDRAWAL

The severe withdrawal symptoms seen in opioid and ethanol dependence do not occur after cessation of cocaine use, but the extent of withdrawal symptoms following the habitual use of cocaine remains controversial. Intermittent cocaine users do not usually experience withdrawal symptoms. Patients, who are abstinent after heavy cocaine use, report decreased energy, listlessness, anxiety, anhedonia, and limited interest in their environment, but the symptoms fluctuate and lack the intensity associated with a major mood disorder.[223] A craving for cocaine persists through this period. Mild symptoms generally begin after the crash (i.e., 1–5 days after cessation of cocaine use) and these symptoms increase in intensity over the next 12–96 hours.[374] Symptoms may last 1–10 weeks. The dosage and chronicity of cocaine use required to produce withdrawal symptoms have not been adequately studied, but heavy users (e.g., freebase cocaine users) are at the highest risk.[101] In a study of 12

cocaine addicts, who used mostly IV methods, the mood-distress scores and cravings for cocaine were highest at admission and decreased gradually over the 28 days of the study.[375]

EXTINCTION

A third phase (i.e., extinction) ensues after abstinence is sustained throughout the first 2 phases. Brief, episodic craving recurs after withdrawal symptoms resolve and during the extinction phase, particularly when confronted with stimuli (e.g., friends, settings) that remind the cocaine addict of prior experiences with the drug. Although these cravings gradually diminish over months to years, certain conditions (white powders, pipes, syringes, people, locations, moods) associated with cocaine-induced euphoria may evoke intense cravings for cocaine.

Reproductive/Developmental Abnormalities

The effect of maternal cocaine use on reproductive abnormalities remains controversial because of recall bias, unreliable exposure data (e.g., measurement of cocaine use during pregnancy), publication bias, and multiple confounding factors (e.g., multiple drug use, poor prenatal care). The most common adverse fetal consequences of *in utero* exposure to cocaine are low birth weight (<2500 g), preterm birth, and intrauterine growth restriction.[376,377] Some longitudinal studies demonstrate reduction in growth parameters of children exposed to cocaine during the first trimester,[378] whereas other studies do not detect growth abnormalities in children exposure to cocaine prenatally.[379] Potential complications of cocaine use during pregnancy include an increased risk of *abruptio placentae*,[380] stillbirth,[381] and preterm labor.[382] Potential cocaine-associated neonatal complications include congenital malformations, decreased fetal growth, microcephaly, seizures, cerebral infarction and hemorrhage, auditory system deficits, sudden infant death syndrome, speech/language delays, and behavioral changes.[383,384] Other potential adverse consequences of cocaine use during pregnancy involve delay in cognitive development,[385] oppositional defiant disorder, aggression,[386] attention deficit–hyperactivity disorder,[387] delayed motor development (posture, tone, hand-eye coordination),[388] and poor language development.[389] Chronic use of cocaine during pregnancy probably reduces birth measurements, but longitudinal studies suggest that these cocaine-related reductions in weight, length, and head circumference disappear during early childhood development.[390,391] In a case control study of 50 cocaine-abusing mothers, reproductive abnormalities included reduced birth weight, increased

malformation rates, and higher incidences of abruptio placentae and stillbirths.[392] Another study involving 30 women admitting to the use of cocaine during the first trimester of pregnancy did not detect any adverse obstetrical or neonatal effects during the first 18 months of life.[393] Studies of children born to mothers using cocaine during pregnancy did not detect a strong association between prenatal cocaine exposure and adjustment or IQ at age 4 years, particularly in girls.[394] However, after adjustment for confounding variables, another longitudinal study of cocaine-exposed children followed to age 3 years found deficits in the scores of these children on the Bayley Mental and Psychomotor Developmental Indices when compared with controls.[390] A number of methodologic issues limit the interpretation of data on the reproductive effects of cocaine including cohorts unrepresentative of the general population, accurate quantification of cocaine use, polydrug use, and difficulty controlling confounding variables (e.g., parenting styles). These methodologic issues prevent a definitive conclusion regarding the causal relationship between cocaine and adverse fetal outcomes.[395] Studies on the teratogenicity of cocaine in animals are also conflicting.[396]

DIAGNOSTIC TESTING

Unit Conversion

Cocaine

$$1\ nmol = 3.30\ \mu g$$

$$1\ \mu g = 0.303\ nmol$$

Cocaethylene

$$1\ nmol = 3.15\ \mu g$$

$$1\ \mu g = nmol/3.15 = 0.317\ nmol$$

Analytic Methods

METHODS

SCREENING.   Most screening tests for the detection of cocaine use involve the analysis of the metabolite, benzoylecgonine, in the urine by immunoassay.[397] The rapid and extensive metabolism of cocaine in blood and the instability of cocaine produce high false-negative values, when plasma cocaine is the primary analyte for screening purposes. Most immunoassays are relatively specific for benzoylecgonine and the assays demonstrate little cross-reactivity between cocaine and benzoylecgonine.[398,399] Postmortem screening for benzoylecgonine using standard immunoassays may be negative (i.e., below the cutoff) in cases involving death soon after the ingestion of massive amounts of cocaine based on rare case reports.[400] In such cases, the heart blood (but not the femoral blood) contains very high concentrations of both cocaine and benzoylecgonine.

Extraction of benzoylecgonine from urine typically involves liquid or solid-phase extraction using an extraction column. Benzoylecgonine is a polar, amphoteric, and highly hydrophilic compound that resists extraction by styrene-divinylbenzene copolymers (e.g., XAD-2) and ion exchange resins, but is removed from the matrix by solid-phase extraction and a variety of solvents (chloroform/isopropanol,   methanol/acetonitrile).[401,402] Hydrolysis of urine samples is not necessary because benzoylecgonine in not conjugated in urine in any significant amount.

CONFIRMATION.   Most analytic procedures use solid-phase extraction and deuterated analogues as internal standards.[403] Maintaining excessively alkaline conditions (i.e., pH >9) during the extraction procedure may cause substantial hydrolysis of cocaine present in the sample to ecgonine methyl ester. Gas chromatographic techniques and capillary columns separate cocaine and metabolites from other compounds. Detection methods include nitrogen phosphorus and electron capture. The use of gas chromatographic techniques may require pre-analytic chemical derivatization for the detection of cocaine metabolites depending on the detection method (e.g., the use of nitrogen phosphorus detection allows the quantitation of cocaine without derivation, but the detection of cocaine metabolites requires derivation). The most specific technique for determining the identification of a compound (e.g., cocaine) is mass spectrometry, which records a unique "fingerprint" of mass fragments by bombarding the sample with high-energy electrons in a controlled magnetic field. Both radioimmunoassay and enzyme immunoassay are sensitive semiquantitative methods of detecting benzoylecgonine in the urine with a lower cutoff of about 0.05 mg/L.[403]

The lower limit of quantitation (LLOQ) for cocaine and benzoylecgonine in postmortem blood samples using gas chromatography/mass spectrometry (GC/MS) is ~0.05 mg/L.[404] High performance liquid chromatography is more sensitive than thin layer chromatography, but this method is less sensitive than GC/MS unless combined with tandem mass spectrometry.[405] Solid-phase extraction followed by liquid chromatography/ tandem mass spectrometry allows the detection of cocaine and benzoylecgonine in plasma at a LLOQ of 0.02 mg/L.[406] In general, liquid chromatography/tandem mass spectrometry is more sensitive than GC/MS;[407] however, liquid chromatography/tandem mass spectrometry has potentially greater variability in product ion formation using multiple reaction monitoring or

selected reaction monitoring as well as the potential for components of the sample matrix to either suppress or enhance the ionization of target analytes (matrix effect). Anhydroecgonine methyl ester is a byproduct of smoking crack cocaine, and the presence of this byproduct is a marker of freebase cocaine use.[408] The use of GC/MS limits the artifactual formation of this marker.[409] Cocaine *N*-oxide is a minor oxidative metabolite of cocaine that decomposes to cocaine at temperatures exceeding 100°C (212°F). Consequently, the analysis of cocaine samples by methods that use high-temperature injection ports may overestimate (i.e., 10–20%) the actual concentration of cocaine.[410]

STREET SAMPLE ANALYSIS

Illicit cocaine samples commonly contain a variety of impurities and contaminants including natural alkaloids present in the crude leaf extracts, products of cocaine decomposition, and byproducts of the chemical processing of illicit cocaine. Identification of these impurities may assist in the determination of the origin of cocaine street samples. Natural compounds present in the leaves of coca plants beside cocaine include *cis*- and *trans*-cinnamoylcocaine, cuscohygrine, hygrine, hydroxycocaine, tropacocaine, trimethoxycocaine, and truxilline compounds. The *cis*- and *trans*-cinnamoylcocaine are relatively stable compounds that are structurally similar to cocaine.[411] Consequently, these minor alkaloids are extracted together with cocaine during the processing of the coca plant. However, the concentration of these alkaloids in cocaine varies because the use of oxidizing agents (e.g., potassium permanganate) during the processing of cocaine may destroy some of the cinnamoylcocaine.[412] The truxilline compounds are stable alkaloids that vary substantially in concentration between coca plant varieties and chemical processing does not affect the content of these natural alkaloids.[413] Comparison of the relative ratios of tropacocaine, norcocaine, cinnamoylcocaine, and cocaine helps determine the origin of samples of illicit cocaine.[414] Furthermore, the coca plant produces only the levo-stereoisomer of cocaine, so that detection of any of the diastereoisomers of cocaine (e.g., pseudococaine, allococaine, pseudoallococaine) or its *d*-enantiomer indicates synthetic production. Manufacturing byproducts include oxidation products from permanganate bleaching (*N*-norcocaine, *N*-acetylnorcocaine, *N*-formylnorcocaine, *N*-benzoylnorecgonine methyl ester),[415] hydrolysis products (benzoylecgonine, ecgonine, ecgonine methyl ester, benzoic acid, cinnamic acids, truxinic and truxillic acids), and solvent residues (acetone, diethyl ether).[416] A variety of sophisticated profiling procedures are available to determine the origin of illicit cocaine samples. Special chromatographic and spectroscopic techniques including mass spectrometry and nuclear magnetic resonance may be necessary to determine the origin of street samples.[45] Chromatographic methods can separate cocaine from commonly encountered adulterants list under the Impurities and Profiling section.[417]

STORAGE

Both the spontaneous chemical hydrolysis of cocaine to benzoylecgonine and the conversion of cocaine to ecgonine methyl ester (EME) via acylcholine acylhydrolase (pseudocholinesterase, butyrylcholinesterase, plasma cholinesterase) occur in preserved and postmortem samples, particularly under alkaline and warm conditions.[418] In unpreserved blood, *in vitro* stability studies indicate that plasma butyrylcholinesterase hydrolyze cocaine almost exclusively to ecgonine methyl ester.[419] The loss of cocaine can be dramatic in unpreserved samples. In antemortem whole blood fortified with cocaine, the cocaine concentration decreased from 2 mg/L to 0.64 mg/L when stored at room temperature for 24 hours.[419] The addition of sodium fluoride inhibits the enzymatic hydrolysis of cocaine to ecgonine methyl ester, but not the spontaneous chemical hydrolysis of cocaine to benzoylecgonine. In unpreserved whole blood samples, benzoylecgonine is more stable than ecgonine methyl ester when stored at room temperature. Over a 35-day period, the loss of benzoylecgonine from unpreserved blood samples stored at room temperature was 25% compared with 50% for ecgonine methyl ester.[419]

Because of the spontaneous, postmortem hydrolysis of EME and benzoylecgonine to ecgonine, the molar sum of cocaine and EME approximates the *minimum* amount of cocaine present at the time of death.[420] However, unpredictable postmortem changes in cocaine concentrations after death, and the presence of ecgonine methyl ester in patients presenting with cocaine intoxication limit the usefulness of using stoichiometric relationships to interpret postmortem cocaine changes.

Cholinesterase inhibitors (e.g., physostigmine, diisopropyl fluorophosphate) and fluoride ion inhibit the conversion of cocaine to EME, but not the spontaneous hydrolysis of cocaine to benzoylecgonine. The use of 0.25% sodium fluoride in blood samples (i.e., standard gray-top Vacutainer® tubes) prevents the hydrolysis of cocaine during transport and short-term storage (<48 h), particularly when the blood sample is stored in a gray-top tube and refrigerated.[421] However, the use of 0.25% sodium fluoride does not prevent the degradation of cocaine in blood samples stored at room temperature over longer periods. In a long-term study of cocaine concentrations (<0.2 mg/L) stored in blood samples containing 100 mg sodium fluoride at ambient

temperatures, no cocaine was detectable after 3 months as measured by GC/MS.[422] During an *in vitro* study of whole blood preserved with 0.25% sodium fluoride and stored at 20°C (68°F) containing cocaine concentrations up to 1 mg/L, cocaine was not detectable after 10 days as measured by liquid chromatography/mass spectrometry.[423] A similar sample retained about 25% of the original concentration of cocaine after 14 days when stored at 4°C (39.2°F).

The average *decrease* of benzoylecgonine in these blood samples at 3 months, 6 months, and 1 year was 74%, 93%, and 99%, respectively. No benzoylecgonine was detectable after 2 years. Refrigeration retards, but does not stop, both of these degradation processes. In an experimental study cocaine samples refrigerated at16°C (60.8°F) lost 30% of the original concentration during 36 days of storage with the highest 1-day loss of 7%.[424] An *in vitro* study of cocaine in human serum incubated at body temperature demonstrate that 20% of the cocaine was metabolized during the first hour with deesterification of cocaine to ecgonine methyl ester accounting for about two-thirds of the hydrolyzed cocaine.[425] The ratio of benzoylecgonine and ecgonine to ecgonine methyl ester increased as the incubation time increased. Within 4 hours, about 67% of the cocaine was hydrolyzed with ecgonine methyl ester, benzoylecgonine, and ecgonine accounting for about 18%, 28%, and 18% of the hydrolyzed cocaine, respectively. Freezing blood samples and the use of fluoride ion significantly limits both of these degradation processes. Over a 21-day period, cocaine concentrations remain stable in refrigerated blood samples (4°C/39.2°F) maintained at pH 5 with 2% sodium fluoride.[419] Storage of blood samples at −20°C (−4°F) in 2% sodium fluoride was associated with an approximate 15% decrease in the concentration of cocaine and benzoylecgonine after about 4–5 months.[426] Cocaine concentrations may remain detectable in some decomposed bodies when tissue samples are analyzed by GC/MS.[427]

The kidney excretes very small amounts (<1–2%) of unchanged cocaine in the urine. Cholinesterases are not present in urine; therefore, conversion of cocaine to EME does not occur in urine samples. However, conversion of cocaine to benzoylecgonine and ecgonine may occur depending on temperature and pH. Optimum storage conditions for urine samples involve freezing (−15°C/5°F) the specimen at pH of 5 adjusted with ascorbic acid.[428] Under these conditions, cocaine and benzoylecgonine are stable for at least 3 months. The stability of benzoylecgonine decreases substantially in alkaline urine (>50% loss over 2–3 months with pH >8.0).[429] In contrast to the storage of blood or liver samples, the use of sodium fluoride to inhibit the effect of pseudocholinesterases on cocaine is not necessary for

urine samples that do not contain cholinesterases. Because the concentrations of EME do not increase in urine samples during storage, the presence of EME indicates the *in vivo* conversion of cocaine to EME; therefore, the use of cocaine while the individual was alive.[430] In buffered formalin (pH = 7.4), cocaine undergoes hydrolysis to benzoylecgonine with a half-life of about 1 week, whereas cocaine is relatively stable in unbuffered formalin over 30 days.[431] Within 15–30 days after preservation with formalin, the rapid extraction of cocaine into formalin and subsequent hydrolysis to benzoylecgonine limits the detection of cocaine in these tissues.

Biomarkers

Pharmacokinetic studies of volunteers and animals given radiolabeled cocaine indicate that the whole blood/plasma ratio of cocaine is approximately 1.[73,432] The most common biologic samples used for the analysis of cocaine involve the blood and urine.

BILE/LIVER

Bile may contain substantial concentrations of cocaine and cocaine metabolites as measured by high performance liquid chromatography with ultraviolet detection (235 nm) or gas chromatography with flame ionization detection; however, there are few data to interpret the significance of cocaine concentrations in bile other than to confirm exposure.[433,434] Typically, cocaine and benzoylecgonine concentrations are several fold higher in postmortem bile than in postmortem blood.[435] Case series suggest that the liver/blood ratio of cocaine does not correlate to the route of exposure.[436]

BLOOD

ANTEMORTEM.   Measurements of blood cocaine concentrations document exposure, but these values seldom are available to guide clinical management. In general, the use of cocaine via the intranasal and oral routes produces lower blood cocaine concentrations over a more prolonged time than cocaine smoking. The administration of cocaine via smoking or IV injection produces peak concentrations of cocaine that are several times higher in arterial blood than in venous blood during the first 30 minutes after use.[437] Because euphoric effects occur before peak plasma concentrations, the rate of change in plasma cocaine concentrations may be more important that the absolute plasma concentration.[3] As with lysergic acid diethylamide and ethanol, the psychologic effects of cocaine are more prominent on the upward limb of the plasma cocaine curve than the downward limb (Mellanby effect).[438] Hence, identi-

cal plasma concentrations induce different effects depending on whether the plasma cocaine concentration is rising or falling.

Typically, peak plasma concentrations following recreational doses (0.2–2 mg/kg) of cocaine average about 0.2–0.6 mg/L depending on the route of administration, whereas the peak cocaine concentrations in plasma samples from intoxicated individuals range up to several mg/L.[59,73] The average peak cocaine concentration of 3 individuals smoking six 50-mg doses of cocaine over 90 minutes was 0.89 mg/L (range 0.69–1.20 mg/L).[439] Nasal application of cocaine doses of 2 mg/kg produced a mean peak plasma concentration of 0.161 mg/L 1 hour after administration. Equivalent oral doses caused higher peak plasma concentrations (0.21 mg/L) at 1 hour after ingestion.[69] In a study of 25 habitual coca chewers, the mean plasma cocaine concentration after a 45-minute chewing ritual was approximately 0.098 mg/L (range, 0.028–0.289 mg/L).[440] In a patient with acute cocaine toxicity after ingestion of cocaine-filled condoms, the serum cocaine concentration was 2 mg/L when the patient's mental status ranged from extreme agitation to nonresponsiveness.[360] Similarly, a 48-year-old body packer developed severe cocaine intoxication 4 days after being apprehended.[441] One hour after he sustained a cardiac arrest with asystole, his plasma cocaine concentration was 1.66 mg/L.

Acute and chronic tolerance may limit the clinical effects of cocaine. In a study of plasma cocaine concentrations from patients presenting to an urban emergency departments with cocaine intoxication, a patient was minimally intoxicated with a plasma cocaine concentration of 3.9 mg/L.[442] After an observation period, the patient was released to police custody. Participants in experimental studies have attained plasma cocaine concentrations of 3.0 mg/L during cocaine infusions without significant adverse clinical effects.[94] Plasma cocaine concentrations must be interpreted cautiously because of the previously discussed degradation of cocaine during storage as well as tolerance.

POSTMORTEM. The time of cocaine administration is difficult to determine from postmortem samples unless a single dose of cocaine was administered by one route of exposure. Furthermore, the position of a single analytic result on the drug concentration versus time curve is unknown. Consequently, extrapolating antemortem kinetics to a single postmortem analytic result is not valid. Factors to consider in determining the time of ingestion and the clinical effects of the cocaine dose include storage techniques, sampling site, route of administration, tolerance, previous history of cocaine use, individual susceptibility (e.g., cardiovascular disease, pseudocholinesterase deficiencies), concomitant drug use, role of adulterants, postmortem interval, and the time between administration and death.

In general, postmortem changes in cocaine blood concentrations are inconsistent; therefore, the determination of the cause of death should be based on a careful analysis of the factors listed above.[444] Cocaine is relatively stable in the corpse when stored at 20–37°C (68°–98.6°F) during the first 24 hours, particularly when the pH of the blood specimen falls.[443] However, changes in cocaine concentrations during the postmortem interval between death and analysis are highly site-specific and variable, and there is no consistent pattern in the magnitude and direction of changes in cocaine and metabolites during the postmortem interval.[444] The postmortem changes involve a dynamic process, which depends on the balance between postmortem degradation of cocaine and the postmortem redistribution of cocaine, particularly in the left ventricle of the heart. In a case series of blood samples drawn at the scene of death and at autopsy, the cocaine concentration increased in samples from the heart and femoral vein, but the cocaine concentration decreased in samples from the subclavian vein.[445]

Postmortem cocaine blood concentrations in cocaine-associated deaths are highly variable with concentrations ranging up to 100–200 mg/L in individuals, who die after ingesting cocaine bags.[446,447] Average cocaine concentrations in postmortem samples from fatalities resulting from the recreational use of only cocaine are approximately 5–6 mg/L.[448] Postmortem cocaine concentrations are frequently found in combination with other drugs, particularly ethanol, opioid medications, and heroin. In a Texas study of 461 cocaine-related deaths from 1993–2005, ~31% resulted from the use of cocaine alone compared with 42% ethanol plus cocaine and 29% cocaine plus opioid medication.[449] Heroin was present in 13% of the case series, whereas antipsychotic/antidepressants medications were present in 12%. Table 56.2 lists the median postmortem blood cocaine and benzoylecgonine concentrations for cocaine only and cocaine plus 1 additional drug in this study.

Sudden death may occur in cocaine users with lower postmortem cocaine concentrations (<0.6–1 mg/L) when associated with hyperthermia and excited (agitated) delirium. In a retrospective review of the records from the Medical Examiner's Office in San Francisco, the presence of cocaine was considered incidental to death in 51 trauma victims.[215] The mean cocaine concentration in the postmortem blood samples was 0.487 ± 0.75 mg/L (range, 0–4.7 mg/L). The mean cocaine concentration in postmortem blood samples from 6 cases of sudden death associated with cocaine-induced excited delirium was 0.4 ± 0.3 mg/L, whereas the mean postmortem benzoylecgonine concentration

TABLE 56.2. Postmortem Blood Cocaine and Benzoylecgonine Concentrations Cocaine-Related Deaths in Bexar County, Texas 1993–2005.[449]

Cocaine Plus 1 Additional Drug (Cases)	Median Cocaine Concentration (Range)*	Median Benzoylecgonine Concentration (Range)*
Cocaine alone (143)	0.89 (0.01–78)	4.0 (0.02–90)
Ethanol (72)	0.35 (0.01–82)	1.5 (0.02–24)
Opioid medications (42)	0.08 (0.01–3.7)	1.4 (0.03–6.2)
Heroin (8)	0.12 (0.01–0.83)	1.0 (0.02–3.7)
Antipsychotics/ antidepressants (10)	0.17 (0.03–4.1)	1.1 (0.29–7.9)
Antihistamines (7)	0.33 (0.03–0.99)	2.4 (0.61–3.8)
Anxiolytics (3)	0.06 (0.01–0.11)	1.5 (0.23–2.8)

*mg/L; sampling site not reported.

was 5 ± 5 mg/L.[450] These concentrations were similar (i.e., 0.4 ± 0.4 mg/L and 2.4 ± 1.6 mg/L, respectively) to cocaine and benzoylecgonine concentrations in a case series of 21 individuals dying of inflicted trauma with cocaine in their postmortem blood. Cocaine-induced fatalities can occur at low cocaine concentrations as a result of prolonged postmortem interval, inadequate storage of postmortem blood samples, chronic cocaine-related myocardial abnormalities (myocardial hypertrophy, diffuse myocardial fibrosis, microangiopathy), coronary artery disease, and long QT syndrome.[451]

BRAIN

Cocaine diffuses easily into brain tissue, but the postmortem cocaine concentration in brain tissue varies substantially depending on the location of the sample.[452] In contrast to cocaine, the metabolite benzoylecgonine diffuses poorly into brain tissue. Fatal overdoses display a mean brain/blood benzoylecgonine ratio of 0.36, which suggests cocaine exposure within the proceeding 1–3 hours. Average ratios of brain/blood benzoylecgonine exceeding 1–1.5 suggest either chronic accumulation from prolonged use or exposure more than 8 hours previously. The cocaine concentrations in the brain are relatively stable compared with other sites. A case series suggested that the brain/blood ratios of cocaine and benzoylecgonine may help identify the time of death. A cocaine brain/blood ratio exceeding 10 commonly occurs following fatal cocaine overdoses when death occurs soon after exposure. For deaths that occurred within 1–3 hours after exposure, the average ratios were 9.6 and 0.36 for cocaine and benzoylecgonine, respectively.[453]

FREEBASE COCAINE

The major product of cocaine pyrolysis is methylecgonidine.[88] The presence of methylecgonidine (anhydroecgonine methyl ester) in urine, blood or hair samples is a biomarker of the inhalation of heated cocaine (e.g., freebase cocaine) rather than exposure via other routes.[454,455,456] Animal studies indicate that the elimination half-life of methylecgonidine is relatively short (15–20 min); therefore, the metabolite (ecgonidine) formed via esterase activity is probably a more useful biomarker because of the longer (i.e., 90–140 min) elimination half-life in blood.[457] Although both methyl ecgonidine and ecgonidine may be artifacts of GC/MS analysis,[458] the presence of these compounds in liver and urine samples suggest the use of freebase cocaine prior to death. The use of appropriate analytic techniques minimizes or avoids the formation of these artifacts. In a study of 15 fatalities associated with crack cocaine use, the median concentrations of methyl ecgonidine and ecgonidine in urine samples were 0.062 mg/L (range, 0–2.03 mg/L) and 0.456 mg/L (range, 0.109–7.452 mg/L), respectively.[459] Methyl ecgonidine was not frequently detected in postmortem liver samples, whereas ecgonidine was detectable in all liver samples (range, 0.090–3.274 μg/g).

HAIR

The use of hair analysis for cocaine has the theoretical advantage of detecting long-term cocaine use compared with urine drug screens based on the average hair growth rate of about 1 cm/month in adults, particularly when other samples are not available for analysis.[460] However, issues of external contamination,[461] variability in laboratory decontamination techniques,[462] decomposition of cocaine in alkaline media, and the variable deposition of cocaine in different types of hair complicate the analysis of hair samples as a means to document long-term cocaine use.[463,464] Consequently, the presence of cocaine and/or benzoylecgonine during hair analysis is not unambiguous proof of the use of cocaine, particularly in persons in close contact with cocaine. The studies of cocaine deposition in hair indicate that cocaine binds to the dark brown pigment (eumelanin), but not to the reddish-brown pigment (pheomelanin). There are few data on the deposition of cocaine and cocaine metabolites in hair or on the sensitivity of hair analysis for the detection of long-term cocaine use. The use of gas chromatography/tandem mass spectrometry using ion-trap detector in positive chemical ionization mode allows the simultaneous determination of cocaine, anhydroecgonine methyl ester (pyrolysis product), ecgonine methyl ester (metabolite), and cocaethylene (trans-esterification product with ethanol) in hair

samples with a LLOQ ranging from 0.005 µg/g (cocaine) to 0.05 µg/g (anhydroecgonine methyl ester).[465]

A case report suggested that at least 3 months of abstinence from cocaine use is necessary before cocaine is undetectable in hair samples as measured by GC/MS, whereas peak hair cocaine concentrations occur within 3 weeks following repeated use.[466] Extensive washing procedures (15-min isopropanol wash, followed by four 30-min phosphate buffer washes and two 60-min washes) are necessary to prevent external contamination.[467] Volunteer studies indicate that many decontamination measures do not separate external contamination from cocaine use.[468] Consequently, the occurrence of cocaine positive hair samples should be confirmed with testing other samples (e.g., urine).

Experimental studies in rodents suggest that the deposition of cocaine metabolites (e.g., benzoylecgonine, ecgonine methyl ester) in hair is low compared with the deposition of cocaine in hair.[469] Concentrations of cocaine in the hair of South American Coca chewers were much higher than concentrations of benzoylecgonine or ecgonine methyl ester (i.e., 5 and 12 times, respectively).[470] The presence of unique cocaine metabolites (norcocaine, cocaethylene) in hair samples supports the past use of cocaine.[471] A number of factors affect the sensitivity of hair analysis including sample preparation and alkaline personal hair products.

SALIVA

The passive diffusion of cocaine into saliva depends on lipid solubility, log P values, plasma protein binding, and salivary pH (cocaine is a weak base).[472] The passive diffusion of the metabolites, benzoylecgonine and ecgonine methyl ester into saliva is significantly less than cocaine. Experimental studies in human volunteers indicate that the cocaine saliva/plasma ratios generally are >1, but the screening potential of salivary cocaine in experimental studies on healthy adults is limited because the terminal half-life is shorter in saliva than in plasma.[473] However, salivary cocaine half-life is variable between individuals, in part because of differences in salivary pH, method of delivery, and duration of cocaine use. In a study of 20 chronic cocaine smokers, the mean terminal cocaine plasma half-life was 3.8 hours compared with 7.9 in saliva, whereas the mean terminal half-life of benzoylecgonine in plasma and saliva was similar (6.6 h and 9.2 h, respectively).[474] Both cocaine and benzoylecgonine were detectable in fewer participants in saliva than in plasma. All 20 participants had detectable benzoylecgonine in plasma compared with 11 positive saliva samples in the 20 participants. Contamination of saliva with cocaine occurs during exposure to intranasal or to freebase cocaine. In a volunteer study involving 2 men,

the IV administration of cocaine produced saliva/plasma concentration ratios of 0.5–2.96 during the 6 hours of sample collection after cocaine administration.[475]

URINE

Analysis of urine samples for benzoylecgonine documents exposure to cocaine, but urinary measurements of cocaine or benzoylecgonine are not clinically useful for the treatment of cocaine-intoxicated patients or for the determination of impairment.[476] Urinary drug screens are more sensitive tests for previous cocaine use than blood tests because cocaine metabolites persist longer in urine samples than in blood samples. The urine drug screen is a less sensitive test for cocaine in neonates than adults because of the reduced ability of the neonate to concentrate urine and subsequent wide fluctuations in urine osmolality. Urine concentrating ability of infants approaches adult values by 2 years of age. The cross-reactivity of immunoassays for benzoylecgonine to cocaine, ecgonine, and ecgonine methyl ester is low (i.e., <1%).[477] Several structurally similar compounds (i.e., *m*-hydroxybenzoylecgonine, *p*-hydroxybenzoylecgonine) present in meconium after maternal cocaine use produce high cross-reactivity with immunoassays for benzoylecgonine. Maximum urinary excretion of unchanged cocaine after a 1.5-mg/kg intranasal dose occurs within 2 hours and cocaine is usually detectable in the urine for 8 hours (maximum 12) after intranasal administration.[67] The urinary elimination half-life of the 2 main metabolites, benzoylecgonine and ecgonine methyl ester, substantially exceeds the elimination half-life of cocaine in the urine. Consequently, detection of cocaine use depends on the renal excretion of cocaine metabolites rather than unchanged cocaine.

The ingestion of coca leaf tea (mate de coca, Health Inca tea)[478] and the use of topical anesthetic solutions containing cocaine[479] can produce positive results in some immunoassay screens for benzoylecgonine within 24–36 hours after use. The addition of sodium chloride to urine samples may produce false-negative cocaine results on drug screening.[480] The ingestion of excessive fluid (<20 mg creatinine/dL, specific gravity <1.003) produces false-negative urine drug tests, but the urine drug tests rapidly revert to positive when the effects of dilution disappear. Consequently, the use of diuretics or herbal products for "flushing" does not change the sensitivity of the urine drug screen beyond the effect of dilution.[481]

Urinary benzoylecgonine excretion peaks from 4–6 hours postinsufflation and diminishes slowly over several days. Benzoylecgonine remains detectable in urine by chromatographic techniques or immunoassay (EMIT) for up to 48–72 hours following single doses of

cocaine depending on a variety of factors including the sensitivity of the specific assay,[482,483] whereas more sensitive (i.e., detection limits of 25 ng/mL) radioimmunoassays identify cocaine metabolites for 90–144 hours.[67] On October 1, 2010, the US Department of Health and Human Services Substance Abuse and Mental Health Services Administration (SAMHSA) issued a Final Notice of Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs that reduced the detection cutoff for illicit use of cocaine to ≥150 ng benzoylecgonine/mL urine and ≥100 ng benzoylecgonine/mL urine for confirmation using GC/MS.[484] In a study of 22 adults undergoing rhinologic procedures using intranasal 4% cocaine pledgets, the percentage of positive urine drug screens at 24, 48, and 72 hours after the procedures were 95%, 50%, and 5%, respectively.[485] The cutoff for a positive test in this study was 150 ng/mL. Studies of cocaine users admitted to medical facilities indicate that the urine drug screens for cocaine remains positive for 1–8 days after heavy cocaine use ceases.[486,487,488] During this period, urine samples from these heavy cocaine users may become positive after earlier samples contained benzoylecgonine concentrations below the established cutoff.[488] The normalization of the creatinine with a cutoff of 300 ng benzoylecgonine equivalents/mg creatinine increases detection time.[486] Case studies suggest that excretion of benzoylecgonine into the urine may continue for 1–3 weeks following heavy abuse (>8–10 g/d) of cocaine.[489] A negative urine drug screen usually indicates the absence of cocaine use within the last 2–3 days depending on the factors listed above.[93] Despite numerous references on the Internet linking false-positive urine tests for cocaine metabolites with amoxicillin use, clinical data indicate that amoxicillin is unlikely to cause false-positive immunoassays for cocaine metabolites.[490]

Determination of the time or amount of drug usage based on the urinary concentrations of cocaine or cocaine metabolites is not feasible from a single sample because of the large number of variables that affect urine concentrations of these compounds. These variables include frequency of use, dose, contaminants, route of administration, variable excretion rates, interindividual differences in cocaine kinetics, formation of condensation products (e.g., cocaethylene), drug interactions, and variation in physiologic parameters (blood flow, creatinine clearance, urine pH).[491]

Passive exposure of medical and security personnel to cocaine does not usually cause positive urine drug screen results for cocaine.[492] Some absorption of cocaine via passive inhalation may occur depending on the duration of exposure and the vaporization temperatures, but the quantity absorbed is not usually sufficient to produce a positive drug screen at the cutoff of 300 ng/mL. In a study of 6 healthy male volunteers exposed to the vapors from the smoking of 100- to 200-mg freebase cocaine in an unventilated room (12,600 L) for 1 hour, the peak benzoylecgonine concentration ranged from 0.022–0.123 mg/L and the amount of cocaine inhaled averaged 0.25 mg.[493] The minimum amount of absorbed cocaine necessary to produce a positive drug screen at the current cutoff is about 1 mg.

VITREOUS HUMOR

There are few data on postmortem changes in cocaine concentrations from samples of vitreous humor.[494] Experimental studies in animals indicate that the postmortem cocaine concentration in vitreous humor varies substantially with cocaine concentrations in antemortem blood and postmortem femoral blood.[495] In contrast to cocaine, the antemortem diffusion of benzoylecgonine into vitreous humor is limited. Because of postmortem changes including continuing metabolism, there is substantial variation between the ratio of cocaine concentrations in postmortem blood and vitreous humor samples, particularly following the use of both cocaine and ethanol. Often, the cocaine concentration is substantially higher in postmortem vitreous humor than in postmortem blood samples, and postmortem changes occur in vitreous humor as well as whole blood.[494]

Abnormalities

ILLICIT USE

PULMONARY. Pulmonary abnormalities present on chest x-ray secondary to cocaine use include pneumothorax, pneumomediastinum, pneumopericardium, hemothorax, atelectasis, and pulmonary edema.[496] Computed tomography (CT) with contrast may be necessary to detect hemorrhagic alveolitis and interstitial pneumonitis associated with crack lung as plain chest films may not demonstrate abnormalities in the presence of significant pulmonary symptoms. Abnormalities on imaging studies associated with crack lung include diffuse interstitial and alveolar opacities in the parenchyma that involve the parahilar regions.[178] Small pleural effusions may also occur. Severe cocaine intoxication may cause hypoxemia and hypercarbia as a result of respiratory depression and/or protracted seizures. Hypoxemia and reduced cardiac output contribute to the development of acid-base abnormalities. A noncardiogenic pulmonary edema may develop after IV administration of freebase cocaine and, in rare cases, contribute to hypoxemia. In severe cases, adult respiratory distress syndrome and end-stage respiratory failure results from interstitial pneumonitis and bronchiolitis obliterans with organizing pneumonia.

In a case series of 19 chronic freebase cocaine users, reduced carbon monoxide diffusing capacity (DL$_{co}$) occurred in 10 habitual users.[497] However, lung volumes, exercise testing, and forced expiratory volumes (FEV$_1$) were normal, indicating the absence of restrictive and obstructive pulmonary disease. A cross-sectional study of a convenience sample of habitual smokers of crack cocaine compared carbon monoxide diffusing capacity with matched control nonsmokers, ex-smokers, and smokers of tobacco only.[498] In contrast to tobacco smoking, neither habitual cocaine smoking nor the short-term administration of inhaled alkaloidal cocaine significantly affected DL$_{co}$.

CARDIOVASCULAR. Sinus tachycardia is the most frequent ECG change during cocaine intoxication. The initial ECG and other clinical factors are less predictive of myocardial ischemia in patients with cocaine-associated chest pain compared with patients with chest pain and no cocaine use. Additionally, there is a relatively high incidence of ECG abnormalities (e.g., left ventricular hypertrophy, early repolarization, ST elevation without ischemia) in patients with cocaine-associated chest pain, even in the absence of clinically significant coronary artery disease.[499] The sensitivity of detecting myocardial necrosis in patients with cocaine-associated chest pain is similar for serum troponin I and assays that directly measure the CK-MB protein concentration.[500] However, a study using the electrophoretic method for measuring CK-MB suggested that this method was less sensitive for detecting myocardial necrosis in these patients compared with the serum troponin I concentration.[238] The use of the relative index of CK-MB and CK increases the sensitivity of diagnosing myocardial necrosis with cardiac enzymes.[501] Myoglobin is a less-specific marker of acute myocardial infarction (AMI) than CK-MB or troponin I in patients with recent cocaine use. In a prospective study of 97 patients presenting with chest pain consistent with AMI, 20% had documented cocaine use.[253] Comparing the 2 groups, cocaine use altered the mean serum myoglobin concentration (179 vs. 74 ng/mL), but not the mean serum CK-MB or troponin I concentrations. The incidence of AMI in the two groups was comparable (cocaine use, 11%; no cocaine use, 12%).

BLOOD. Common abnormalities in blood chemistries following large cocaine doses include mild hyperglycemia and leukocytosis. Thrombocytopenia may occur following the IV use of cocaine, but the contribution of other substances (e.g., contaminates, drugs of abuse) cannot be excluded.[502] Agranulocytosis (i.e., absolute neutrophil count <0.5 × 10^9/L) may occur in chronic users of levamisole-contaminated cocaine. Morphologic features of cocaine users with agranulocytosis include plasmacytoid lymphocytes (i.e., mature lymphocyte with eccentric location of nucleus and basophilic tent to cytoplasm), bone marrow plasmacytosis, and megakaryocytic hyperplasia.[503] These patients are often HLA-B27 positive and have antineutrophil antibodies. More severe laboratory abnormalities occur in serious cocaine intoxication including profound metabolic acidosis, both with and without physical exertion. This metabolic acidosis is usually a hyperlactatemia, which is more severe and prolonged than expected from seizure activity or physical exertion. A 28-year-old woman developed coma, apnea, and generalized seizures following the unwitnessed ingestion of cocaine.[504] Her initial arterial pH was 6.39, and her rhythm deteriorated from a wide complex tachycardia to a slow idioventricular before responding to resuscitative measures. The initial arterial pH in a case series of 5 patients with cocaine-induced excited (agitated) delirium requiring restraint ranged from 6.25–6.81.[505] Two of these 5 patients survived after receiving advance cardiac life support. This severe metabolic acidosis (i.e., pH <7.0) can occur following cocaine use and exertion without physical restraint.[506] A 32-year-old chronic cocaine abuser received multiple, superficial shotgun wounds after running several blocks.[507] His oral temperature was 95°F (35°C), and his initial arterial pH was 6.91. He recovered rapidly with IV fluids. Experimental studies in healthy volunteers indicate that restraint procedures in the prone position do not contribute significantly to the profound metabolic acidosis associated with excited delirium and restraint by law enforcement personnel.[508,509,510] In patients with severe hypothermia, laboratory abnormalities include persistent hyperkalemia, transient hemoglobinuria, myoglobinuria without renal failure, elevated serum hepatic transaminases, and markedly increased serum creatine kinase concentrations.[303]

CENTRAL NERVOUS SYSTEM. The interpretation of neuropsychologic test results in chronic cocaine users is complicated by difficulty controlling confounding variables including time since last cocaine use. In a inpatient study of 8 long-term cocaine addicts without drug use for 6 months, single-photon emission computed tomography demonstrate cerebral hypoperfusion and neuropsychological testing detected deficits in attention concentration, memory, learning, word production, and visuomotor tasks based on published, age-matched normative data.[511] A meta-analysis of 15 studies on the effect of chronic cocaine use and neuropsychologic testing suggested that chronic cocaine use adversely affected attention, visual memory, design reproduction, and working memory, when compared with healthy,

normal controls.[512] Most tests of motor performance demonstrated minimal effects and the effect of cocaine use on executive functioning (planning, working memory, set-shifting) was inconsistent. However, there was substantial variability between different studies, and the effect of recent cocaine use on the neuropsychologic tests could not be excluded.

A case-control study of 62 male crack cocaine users with cerebrovascular accidents detected an increased incidence of cerebral white matter lesions on magnetic resonance imaging (MRI) when compared with age-matched controls.[513] The lesions were hyperintense on T2-weighted MR images, and they occurred primarily in areas of the middle cerebral artery with limited anastomoses. There were no lesions in the subcortical gray matter (basal ganglia, thalamus). Deficits in cerebral perfusion may occur in chronic cocaine users without evidence of cerebrovascular accidents.

BODY PACKING

Supine abdominal radiographs usually demonstrate professionally wrapped cocaine packets in the GI tract with sensitivities in case series ranging up to approximately 70–90%.[356,514,515] Radiographic signs of cocaine bags in the GI tract include multiple radiodense foreign bodies, "double-condom" or "halo" sign (air trapped between layers of condoms), and rosettes (air trapped in a knot tied on a condom).[516] The presence of thin air layers (i.e., halo sign) in the cocaine packages may result from the production process, and the occurrence of this radiographic sign does not necessarily imply rupture of the cocaine packet.[517] Contrast-enhanced plain abdominal radiography, barium enema, or an abdominal CT scan with contrast are optional imaging studies when plain abdominal radiographs do not detect cocaine packets in the GI tract of individuals suspected of body-packing.[518] There are limited clinical data on the sensitivity and specificity of ultrasound for the detection of cocaine packets in the GI tract, but a small case series suggested that ultrasound does demonstrate the presence of these packets.[519] Although urine drug of abuse screens provide adjunctive evidence for the ingestion of cocaine packets,[520] the sensitivity of these immunoassays is highly variable and the lack of a positive drug screen does not exclude the ingestion of cocaine packets.[521]

Driving

There are limited data on the effect of acute or chronic cocaine use on driving skills. Although the duration of action of cocaine is short, cocaine users frequently abuse other illicit drugs and ethanol concurrently with cocaine.

Furthermore, recreational use of freebase cocaine occurs in larger doses than administered in medical and experimental settings. Case reports associate cocaine use with serious driving impairment, but the rapid deterioration of cocaine in stored specimens limits direct correlation between cocaine concentrations and driving impairment.[522]

A number of recognized complications of cocaine use theoretically reduce the driving skills of cocaine users both during the acute phase of cocaine intoxication and the during the withdrawal phase. These potential adverse effects include light sensitivity, blurred vision with reduced glare recovery, irritability, poor judgment, anxiety with rapid steering or braking responses, ataxia, attentional deficits, risk taking, aggression, poor concentration, and fatigue.[523] In a Swedish case series of 61 drivers apprehended for suspected impaired driving with only cocaine in their blood, the mean and median cocaine concentrations in venous blood samples (time between blood draw and apprehension not reported) were 0.95 mg/L and 0.07 mg/L, respectively, compared with 1.01 mg/L and 0.70 mg/L for benzoylecgonine.[524] The maximum cocaine and benzoylecgonine concentrations were 0.5 mg/L and 3.1 mg/L, respectively; the mean benzoylecgonine/cocaine ratio was 13.8. Frequently mentioned clinical findings included conjunctival erythema, agitation, restlessness, mydriasis, unsteady gait, incoherent speech and rapid pulse.

In volunteer studies, the administration of cocaine in relatively low doses is not associated with significant deterioration of psychomotor performance. In a study of 7 volunteers, the inhalation of cocaine doses ranging from 10–40 mg did not cause statistically significant changes in responses to the digit symbol substitution test or to reaction times.[525]

Studies of postmortem blood from automobile accident victims indicate the detectable concentrations of cocaine occurs in <4–6% of the blood samples from Canadian,[526] European,[527] and Australian studies.[528] In postmortem blood from 370 fatally injured drivers in Washington State, 13 cases (3.5%) and 18 cases (4.9%) had detectable cocaine and benzoylecgonine, respectively.[529] The cocaine concentration ranged from <0.01–0.23 mg/L with a median of 0.11 mg/L. About two-thirds of these drivers also had detectable concentrations of ethanol. Difficulty determining culpability in these studies result from several variables including the deterioration of cocaine in storage, associating specific cocaine and cocaine metabolite concentrations with the phase of intoxication (i.e., acute intoxication vs. "crash"), and the frequent presence of other drugs. When cocaine is present in blood from fatal accident victims, ethanol concentrations usually indicate serious impairment.[530]

Standard field sobriety tests do not reliably detect the presence of recent cocaine use (i.e., based on positive urine drug screen) in drivers arrested for reckless driving.[531]

TREATMENT

Stabilization

Intractable seizures, respiratory arrest, hyperthermia, and dysrhythmias represent the most serious immediate risks to life. With the exception of delayed intestinal rupture of cocaine bags, major symptoms usually present within the first hour following cocaine exposure. The ingestion of cocaine base ("rock") may delay serious symptoms for several hours. Most victims of fatal cocaine intoxication die within minutes of exposure. Patients, who arrive at the hospital with vital signs, usually survive unless hyperthermia and multiorgan failure develop. The first priority is the assessment of ventilation and the establishment of an adequate airway. Nondepolarizing neuromuscular blockers (e.g., vecuronium, rocuronium, pancuronium) are preferable to depolarizing agents (e.g., succinylcholine) for rapid sequence intubation because of the theoretical potentiation of cocaine effects because plasma cholinesterase metabolize both cocaine and succinylcholine. Intravenous access should be established along with cardiac monitoring and pulse oximetry for all patients with signs of intoxication. A chest x-ray and arterial blood gas should be obtained for patients with altered consciousness or respiratory distress.[532] Invasive or non-invasive hemodynamic monitoring (echocardiography, bioimpedance techniques, Swan-Ganz catheter) may assist the clinician to maximize hemodynamic variables in patients with pulmonary edema. Naloxone (0.4–2 mg IV) in lethargic or comatose patients should be administered if the presence of heroin is suspected, beginning with low doses titrated to effect to prevent the development of opioid withdrawal.

Dysrhythmias

Sinus tachycardia is the most common dysrhythmia during cocaine intoxication, and treatment usually involves sedation with benzodiazepines (5 mg diazepam IV or 1–2 mg midazolam IV bolus repeated every 3–5 minutes as needed, titrated to effect). Sodium bicarbonate and lidocaine are the main therapeutic options for the treatment of most ventricular dysrhythmias including cocaine-induced ventricular tachycardia. Although lidocaine is a potential adulterant of cocaine, adverse reactions from the use of lidocaine during cocaine toxicity are not well documented. The administration of lido-caine in a case series of 29 patients with cocaine-associated MI was not associated with significant cardiovascular or CNS toxicity.[533] The majority of these patients received lidocaine more than 5 hours after cocaine use when the effects of cocaine would be minimal.

The use of IV sodium bicarbonate is an important option to the administration of lidocaine as a treatment for ventricular dysrhythmias, particularly when QRS prolongation (wide-complex tachyarrhythmia) occurs during obvious cocaine intoxication. Theoretically, the use of a sodium bicarbonate infusion to alkalinize the serum to a pH of 7.45–7.5 would be most beneficial for patients with a wide complex tachydysrhythmia and QRS prolongation.[169,534] However, there are few clinical data to document of the effectiveness of sodium bicarbonate therapy in this setting or to separate the effects of reversal of acidosis and hypertonic saline. Accelerated ventricular rhythm usually requires no treatment. The use of β-adrenergic antagonists, type 1A or 1C antiarrhythmic agents, and mixed α- and β-adrenergic antagonists are contraindicated in patients with cocaine intoxication.[535] There are few human data on the safety and efficacy of amiodarone for the treatment of cocaine-induce ventricular dysrhythmias. Therefore, the administration of amiodarone is not generally recommended for the treatment of cocaine-induced ventricular dysrhythmia pending further clinical data on the effect of amiodarone on QT prolongation during cocaine intoxication, particularly the interaction of amiodarone and cocaine on blockade of HERG channel currents.

Myocardial Infarction and Chest Pain

Patients with chest pain and ECG evidence of myocardial ischemia after cocaine use should be treated with the standard doses of nitrates (0.3–0.4 mg sublingual times every 5 minutes up to 3 times until relief, followed by nitroglycerin drip starting at 10–20 µg/min titrated 5–10 µg/min every 5–10 minutes to effect and blood pressure if sublingual nitroglycerin or nitroglycerine spray is ineffective), morphine (2 mg IV every 5 minutes titrated to pain relief and sedation), oxygen, aspirin (81 mg orally), and heparin. Clinical reports indicate that sublingual nitroglycerin abolishes cocaine-induced vasoconstriction both in atherosclerotic and in nonatherosclerotic segments of the coronary arteries.[536,537] Benzodiazepines (e.g., lorazepam) are safe medications for the treatment of patients with cocaine-associated chest pain,[538] and the early use of IV benzodiazepines may improve cardiac hemodynamics and relieve chest pain.[539,540] In patients with cocaine-associated chest pain and ischemic ECG changes, 20 mg diltiazem IV is a therapeutic option when the patient does not respond to benzodiazepines, nitrates, morphine, and oxygen.[541]

Intravenous phentolamine (1 mg IV every 5 minutes) titrated to relief of chest pain or hypotension also is an option for patients with chest pain unresponsive to standard treatments.[542] The elimination half-life of phentolamine is about 18 minutes. The use of β-adrenergic blockade is relatively contraindicated in the treatment of cocaine-associated chest pain because of the potentiation of cocaine-induced coronary vasoconstriction.[160] Although the early use of β-blockers in AMI is well accepted and a retrospective study suggests that the use of β-blockers reduces the risk of MI in cocaine users,[543] there are inadequate data to recommend the use of β-blockers in the treatment of patients with cardiovascular complications secondary to cocaine use. Furthermore, adverse reactions to β-blockers in this setting are variable and unpredictable; case reports and observational studies suggest that the use of β-blockers in cocaine associated MI increases the risk of myocardial necrosis and may cause catastrophic deterioration.[544,545]

Immediate coronary arteriography and angioplasty are indicated for patients with chest pain and documented ST elevation. Thrombolytic therapy should be used with caution because of the potential complication of aortic dissection and subarachnoid hemorrhage, as well as the lack of documented efficacy and poor correlation of the ECG to cocaine-associated MI.[546] When available, the use of coronary angioplasty is a safer modality than thrombolysis to determine the multifactorial etiologies of ischemic chest pain in cocaine users. However, there are few human data to guide management of cocaine-induced MI.

Hypotension

Often, fluids do not reverse the hypotension seen in the late depressive phase of cocaine intoxication. Frequently, the administration of dopamine and/or norepinephrine (Levophed) is needed to stabilize the blood pressure. Invasive or noninvasive hemodynamic monitoring may help optimize fluid resuscitation and the use of inotropic agents when pulmonary edema is present.

Hypertension

The stimulant phase of cocaine is usually short, and the treatment of hypertension and tachycardia is usually unnecessary. For cocaine-intoxicated patients requiring treatment for severe hypertension not responding to sedation, sodium nitroprusside infusion (initial rate 0.1 μg/kg/min titrated to desired end point, average therapeutic dose ranges from 0.5–8 μg/kg/min) and IV phentolamine (1–2.5 mg IV bolus, repeated in 5 minutes if no response) are the antihypertensive drugs of choice. The reduction in blood pressure must be done cautiously in patients with chronic hypertensive disease or acute cerebrovascular events with careful hemodynamic monitoring. The use of β-blockers for hypertension during cocaine intoxication is contraindicated because β-blocker drugs potentially increase cocaine-induced coronary vasoconstriction by blocking the β-receptors and by allowing unopposed α-receptor stimulation of coronary arteries.[547] A case report associated the use of IV propranolol during cocaine-induced hyperadrenergic state with a reduction in heart rate, diaphoresis, anxiety , and a significant increase in blood pressure requiring the use of sodium nitroprusside.[548] The use of calcium channel blockers offers no clear benefits in managing the hemodynamic effects of cocaine intoxication other than the second line management of cocaine-induced coronary artery vasospasm and associated myocardial ischemia.

Hyperthermia

Hyperthermia is an immediate, life-threatening complication of cocaine use. Core temperatures should be measured in all agitated patients. As measured by rectal thermocouple, a core temperature exceeding 40.5°C (105°F) indicates the need to cool the patient (e.g., ice water bath, evaporative cooling) and to monitor of vital signs every 10–15 minutes after stabilization. Ice packs and the use of hypothermic blankets are alternative measures when an ice bath is not available. The patient should be removed from ice water baths and internal cooling measures when the core rectal temperature drops to 39°C (102°F). Less-severe temperature elevations (<38–40°C/100.4–104°F) can be treated by placement in a cool room, removal of clothes, minimization of physical activity (sedation), tepid sponging, or evaporative methods (cool mist spray, fans). These patients should receive aggressive fluid resuscitation to maintain adequate urine output and to minimize the effects of rhabdomyolysis. These patients require sedation (e.g., benzodiazepines, continuous propofol infusion) and if necessary, paralysis with paralytic drugs.

Seizures

Lorazepam (2–4 mg IV in adults) and diazepam (0.1–0.3 mg/kg up to an adult dose of 10 mg) are the drugs of choice for cocaine-associated seizures. Phenobarbital (20 mg/kg loading dose with maximum infusion rate 50–100 mg/min) and propofol (3–5 mg/kg loading dose, 30–100 μg/kg/min maintenance infusion) are second-line drugs that are not well-tested in the clinical setting of cocaine-induced status epilepticus. Persistent seizures indicate the need for aggressive treatment of the hyperthermia and metabolic acidosis as well as consideration of pentobarbital-induced coma.

EXCITED DELIRIUM

There are few data to guide the treatment of excited delirium associated with cocaine intoxication. Cardiac arrest in these patients is typically associated with asystole as a terminal event (multisystem failure, rhabdomyolysis) rather than ventricular fibrillation; the prognosis of cardiac arrest in this situation is extremely poor. Hence, treatment is directed toward early recognition, sedation, detection/treatment of rhabdomyolysis, and restoration of homeostasis (hyperthermia, acidosis, fluid/electrolyte balance) prior to catastrophic events. Accurate assessment of central body temperature is essential to adequately treat patients with excited delirium.

Gut Decontamination

Because most recreational cocaine users do not ingest cocaine, gut decontamination procedures are usually unnecessary. Gut decontamination may be considered when the history suggests cocaine ingestions exceed 3 mg/kg within 1 hour of cocaine exposure. Activated charcoal is the decontamination measure of choice because of the rapid onset of action of cocaine. *In vitro* studies indicate that standard doses of activated charcoal adsorb cocaine well both in alkaline and in acidic conditions.[549] Although *in vitro* models suggest that alkalinization increases the hydrolysis of cocaine,[70] activated charcoal binds cocaine well in acidic conditions and there is little evidence that gastric alkalinization improves clinical outcome. An IV line should be started, vital signs and cardiac rhythm should be closely monitored, and medications (diazepam, lorazepam) should be immediately available for treatment.

BODY STUFFER

All cooperative body stuffers should receive 50 g activated charcoal if they present within 1–2 hours after ingestion. The administration of activated charcoal to uncooperative patients depends on clinical judgment of the risks and benefits based on the dose and on the time since ingestion. Cocaine is poorly visualized on plain abdominal radiographs. Abdominal radiographs are not necessary in body stuffers unless a large ingestion of professionally wrapped cocaine is suspected. Cocaine is radiolucent and the radiographic appearance of cocaine packages depends on the consistency of the wrapping material and the presence of air inside and outside the wrapping. CT imaging may reveal the distinctive straight line of rock cocaine surrounded by air trapped in a wrapping.[550] In a retrospective study of 98 cocaine body stuffers, none of the 17 abdominal radiographs were positive.[357]

BODY PACKER

All body packers should receive activated charcoal, 1 g/kg, mild cathartics, and a stool softener (psyllium hydrophilic mucilloid in 3- to 4-g doses several hours after cathartic) to decrease the transit time of the packets. Serial activated charcoal (25–30 g every 2–4 h until the stool is charcoal-laden) is an option for these patients, but there are few data to indicate that the administration of serial activated charcoal improves clinical outcome. The management of these patients depends on the type of bag (i.e., propensity for breakage), the location, and the presence of symptoms.[356] The presence of well-wrapped cocaine packets (McCarron type 2 or 3) indicates a good prognosis.[551] Whole bowel irrigation (polyethylene glycol electrolyte solution 1–2 L/h) is an option for these patients, particularly following large (>10 g) ingestions of cocaine. The administration of standard doses of activated charcoal should precede the use of whole bowel irrigation by 30–60 minutes because *in vitro* studies indicated that desorption of cocaine from activated charcoal occurs following the addition of polyethylene glycol electrolyte lavage solution to activated charcoal, particularly at the acidic pH present in the stomach.[552] Although there are inadequate clinical data to confirm the efficacy of metoclopramide during WBI, the administration of metoclopramide has theoretical advantages during WBI. Metoclopramide (10 mg IV) accelerates gastric emptying and enhances smooth muscle activity from the esophagus through the small bowel. The endpoint of whole bowel irrigation is the presence of clear rectal effluent and the clearance of radiopaque packets. The removal of all bags must be done cautiously because attempted removal (including endoscopy) may puncture the packet and release cocaine. The primary indication for endoscopy is the removal of cocaine packets that did not pass through the pylorus. Oil-based laxatives are not recommended because of the potential of these laxatives to reduce the tensile strength of the packets. Surgical consultation should be obtained early (i.e., during first signs of cocaine intoxication), before the development of serious complications. Confirmation of the elimination of packets by contrast-enhanced CT should be done after decontamination measures and following the passage of 3 stools without packets. Figure 56.10 displays the algorithm for the treatment of body packers based on clinical presentation; complications of cocaine toxicity are treated with standard doses of the medications as listed under Stabilization.
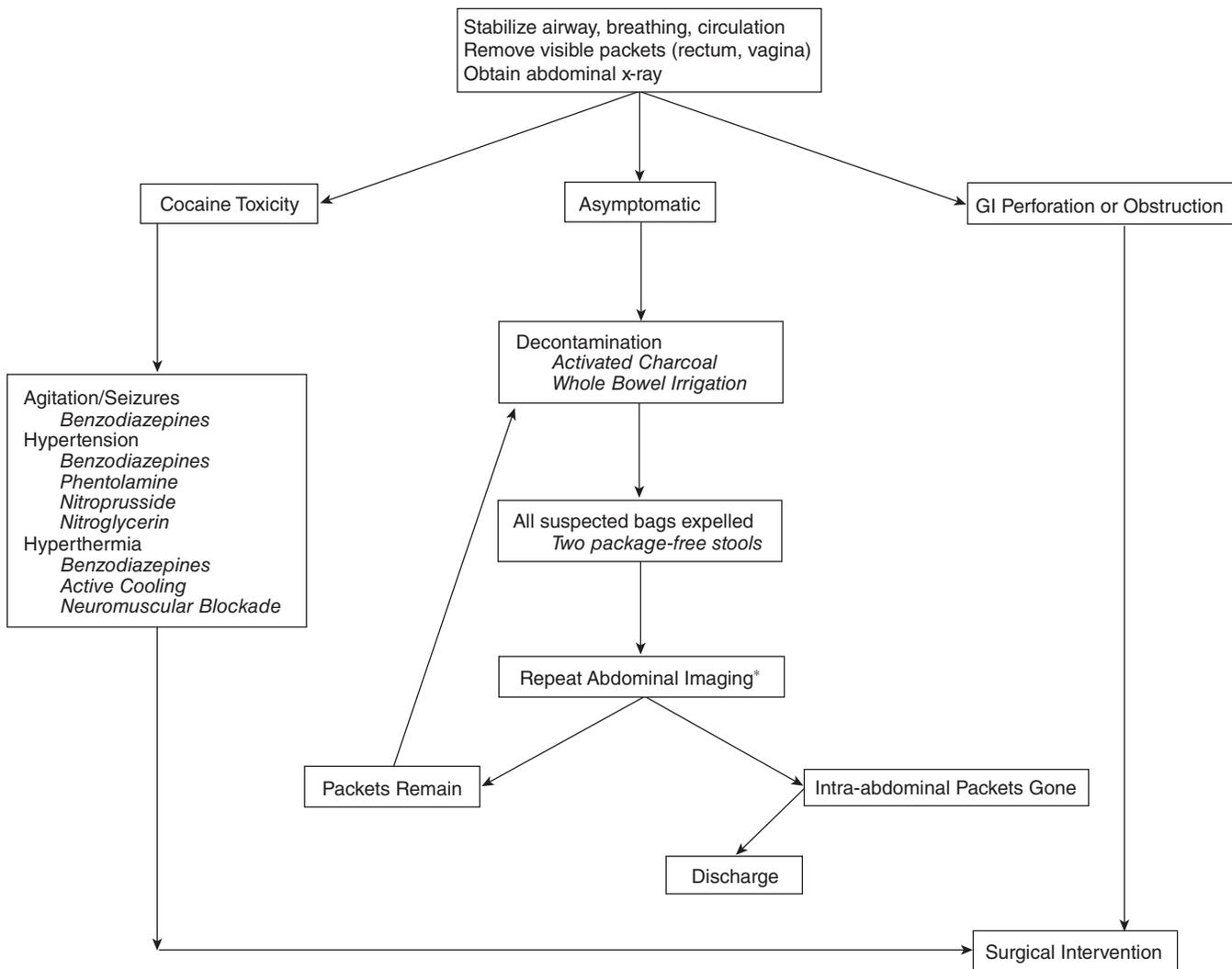
FIGURE 56.10. Algorithm for the treatment of cocaine body packers.[355] *Contrast-enhanced abdominal computed tomography or contrast-enhanced radiography

Elimination Enhancement

The short biologic half-life of cocaine, the limited urinary excretion of unchanged cocaine, and the relatively large volume of distribution of cocaine limit the effectiveness of measures to enhance cocaine elimination. Consequently, no measures to enhance the elimination of cocaine are recommended other than the use of serial doses of activated charcoal in body packers.

Antidotes

There are no antidotes for cocaine intoxication. Specifically, β-blocking drugs are not antidotes for the hyperadrenergic state induced by cocaine intoxication. Experimental antidotes for cocaine intoxication include the use of mutant human butylcholinesterase to metabolize excessive cocaine doses.[553]

Supplemental Care

CHEST PAIN

Although the risk of AMI is substantially elevated in patients using cocaine, the incidence of myocardial ischemia in patients with cocaine-associated chest pain is relatively low. Elevation of cardiac enzymes (e.g., myoglobin) without baseline ECG abnormalities may occur in the absence of cocaine-associated AMI; alternately, some patients with cocaine-associated AMI may present to the emergency department without ischemic ECG changes.[554] High-risk patients should be admitted for

exclusion of AMI. Criteria for high-risk of AMI include ischemic ECG changes (ST elevation or ST depression ≥1 mm, ischemic T-wave inversion), elevated serum troponin I or CK-MB, hemodynamic instability or ventricular dysrhythmias, or recurrent ischemic symptoms with a history of coronary artery disease. Patients with low- or intermediate-risk of MI may be observed for 12 hours in an observation area. Stress testing helps identify ischemia in patients with underlying coronary artery disease. In a study of 256 cocaine-associated chest pain patients with low-to-intermediate risk of cardiovascular disease, 4 patients developed non-fatal MIs within 30 days of discharge from the observation unit.[555] All 4 of these patients continued to use cocaine.

MILD ACUTE INTOXICATION

Sedative doses of diazepam (2.5–5 mg IV) of lorazepam (1–2 mg IV) may be administered to patients with agitation. Acceptance, reduction of stimuli (i.e., rest, quiet, cool environment), and sincere reassurance are potentially useful therapeutic interventions. When the psychosis clears, each patient should be evaluated for suicidal and homicidal ideations. Besides potential self-harm, reasons for psychiatric hospitalization include chronic freebase use or IV injection, concurrent dependence, severe psychiatric or medical problems, severe psychosocial impairment, lack of social or family support, repeated outpatient failure, and poor motivation.[101] Patients who develop serious complications of ingesting cocaine packets usually present with signs of serious intoxication (seizures, dysrhythmias).

RHABDOMYOLYSIS

The development of rhabdomyolysis is unlikely in patients with mild acute cocaine intoxication or in patients with serum creatine kinase concentrations <1000 U/L.[556] Seizures, hyperactivity, hypokalemia or hyperkalemia, severe muscle spasm, and hyperthermia contribute to the development of rhabdomyolysis, and measures to resolve these clinical features are important to prevent worsening rhabdomyolysis. The early use of high doses of benzodiazepines helps limit the progression of rhabdomyolysis and renal failure. Kidney function should be monitored daily for several days in patients with evidence of significant muscle damage (i.e., serum creatine kinase >1,000–2,000 IU/L) if they are symptomatic or if the serum CK concentrations are rising. The treatment of rhabdomyolysis includes generous fluid replacement to maintain urinary output. The efficacy of alkalinization of the urine for the treatment of rhabdomyolysis is controversial. Hemodialysis is reserved for patients with renal failure, and there is no role for the prophylactic use of hemodialysis during rhabdomyolysis. Asymptomatic patients with serum CK <1,000–2,000 IU/L should be hydrated; these stable patients may be followed closely as outpatients to confirm declining levels.

BODY STUFFING/BODY PACKING

The integrity of the cocaine packets determines the onset and likelihood of cocaine toxicity following ingestion of these packets.[557] Body stuffers usually, but not always, swallow small amounts of loosely wrapped drugs to avoid arrest by police. Although most body stuffers develop no or only mild clinical features of cocaine intoxication,[558] occasionally body stuffers develop serious or fatal cocaine toxicity.[559] Body stuffers may be discharged if the vital signs remain normal and the patient remains asymptomatic 6–8 hours after ingestion as long as the cocaine was loosely wrapped (paper, single cellophane wrapping).[357,560] Body stuffers ingesting more securely wrapped cocaine packets may require longer observation periods (12–24 h). In contrast to body stuffers, body packers (swallowers, couriers, mules, internal carriers), can carry large quantities of cocaine, and these patients may develop delayed signs of serious cocaine intoxication if the cocaine packets leak. In a retrospective study of 4,660 cocaine body packers, 64 body packers (1.4%) developed signs of serious cocaine toxicity.[561] Typically, body packers carry about 1 kg of illicit drugs divided into 50–100 packets of about 10 g each.[562] Although previous packets were loosely wrapped, current smuggling techniques probably involves an automated process that densely packs the drug into a latex sheath (condom, balloon) covered with several layers of latex and a hard wax coating.[563] These processes also use aluminum foil, carbon paper, plastic food wrap, or other material to alter the radiodensity of the packet. Frequently, the body packers ingest constipating agents (loperamide, diphenoxylate) prior to transport with unaided passage of the packets being delayed 1–2 days to 2–3 weeks.[564] Oil-based laxatives should be avoided because of the potential for these agents to reduce the tensile strength of the wrappers and increase the risk of rupture.

The decision to surgically remove cocaine packets from the GI tract requires careful consideration of the potential risks and benefits. Indications for surgical intervention include signs of esophageal or intestinal obstruction (abdominal pain, vomiting), failure of the cocaine packets to progress through the stomach and small intestine, and evidence deterioration or leakage of the cocaine packets. The halo sign by itself is not an indication for surgical intervention because some air may be introduced into the cocaine packet during

production. The presence of cocaine in the urine may result from conditions other than deterioration of the cocaine packets (e.g., contamination of the packets, the use of semipermeable membranes, the simultaneous use of cocaine by the body packer).[565] Plain abdominal radiography has a high sensitivity (i.e., about 75–90%) for the detection of cocaine packets *in* body packers.[355] However, contrast-enhanced abdominal computed tomography is more sensitive, and this imaging technique is the procedure of choice when contrast is needed to improve sensitivity. Ultrasound and MRI are not sensitive techniques for the detection of cocaine packets in the GI tract.[566]

ACUTE PSYCHOSIS

Haloperidol (2–5 mg intramuscularly) may be useful in the treatment of psychotic reactions in the absence of excited delirium; there are few human data on the effect or safety of atypical antipsychotic medications (e.g., olanzapine) on acute psychosis associated with cocaine intoxication. The course of the acute psychotic episode usually is short (24–30 h) and hospitalization is usually necessary.

ADDICTION

The treatment of cocaine addiction is multifaceted combining psychotherapy, behavior therapy, and involvement in the 12-step programs similar to the treatment of other addictions. Current studies indicate that a combination of pharmacologic and behavioral or cognitive therapies provide the best approach to treatment, but most pharmacologic agents are either poorly tolerated or the onset of action is too long.[144,567] Strategies for the use of pharmaceutic agents include reducing withdrawal, ameliorating craving, blocking euphoria, and enhancing prefrontal cortical function (i.e., modulation of glutamate, dopamine, GABA receptors).[568] However, there is no single pharmacologic agent presently that has been found to consistently reduce cocaine use or cocaine cravings in multiple clinical trials; there are also no drugs for the treatment of cocaine addiction approved by the US Food and Drug Administration (FDA). Treatment of withdrawal symptoms is supportive and symptom-directed.[569,570]

Addiction is a chronic disorder with exacerbations and remissions, and the goal of outpatient therapy is to break the cycles of recurrent bingeing. Treatment of addiction generally involves the following 3 approaches: 1) behavioral methods to help the abuser recognize the destructive effects of cocaine and the need to stop, 2) supportive environment including self-help groups and family members to disassociate the abuser from cocaine sources and situations, and 3) psychodynamic interventions to help the abuser understand the reasons for cocaine use and the methods to fulfill this need without drugs.[163] Pharmacologic therapies provide adjunctive care to minimize the discomfort of cocaine withdrawal and reduce craving for cocaine. There are limited data supporting the clinical use of carbamazepine, dopamine agonists, disulfiram, phenytoin, and lithium in the treatment of cocaine dependence.[571] Pharmacologic adjuncts to the treatment of cocaine addiction include antidepressants, bromocriptine, carbamazepine, and fluoxetine.[59] Antidepressants are frequently prescribed to cocaine addicts to relieve some of the mood swings associated with the cessation of chronic cocaine use. Tricyclic antidepressants block catecholamine uptake and stabilize adrenergic and dopaminergic receptors as well as treating the anhedonia, depression, and dysphoria associated with cocaine withdrawal. The pharmacotherapy of cocaine addiction is experimental with no one superior protocol.[572] Bromocriptine and amantadine modify the depletion of dopamine associated with cocaine craving and withdrawal. Carbamazepine potentially alters the rekindling seizures associated with the desire to use cocaine. Neuroleptic drugs potentially block the euphoria associated with cocaine use. Other experimental medications include modafinil (a mild stimulant that blocks the euphoric effects of cocaine) to initiate abstinence and vigabatrin (an irreversible inhibitor of GABA transaminase that elevates brain GABA concentrations) to prevent relapse.[573] Cocaine vaccines (e.g., succinylnorcocaine linked to recombinant cholera toxin B-subunit protein) are experimental approaches to the treatment of cocaine addiction.[574]

References

1. Ellenhorn MJ, Barceloux DG. Medical Toxicology. Diagnosis and treatment of human poisoning. New York: Elsevier; 1988.

2. Balabanova S, Parsche F, Pirsig W. First identification of drugs in Egyptian mummies. Naturwissenschaften 1992;79:358.

3. Van Dyke C, Byck R. Cocaine. Sci Am 1982;246: 128–141.

4. Karch SB. Cocaine: history, use, abuse. J Roy Soc Med 1999;92:393–397.

5. Karch SB. The pathology of drug abuse. 2nd Ed. Boca Raton, Florida, CRC Press, Inc., 1996.

6. Musto DF. Opium, cocaine and marijuana in American history. Sci Am 1991;265:40–47.

7. Musto DF. A study in cocaine. Sherlock Holmes and Sigmund Freud. JAMA 1968;204:27–30.

8. Thompson A. toxic action of cucaine. Br Med J 1886;1:67.

9. Musto DF. International traffic in coca through the early 20th century. Drug Alcohol Depend 1998;49:145–156.

10. Menzie RC, Robinson R. A synthesis of ψ-pelletierine. J Chem Soc 1924;125:2163–2168.

11. Findlay SP. The three dimensional structures of cocaine. II. Racemic allocaine and racemic allopseudocaine. J Org Chem 1958;24:1540–1550.

12. Suarez CA, Arango A, Lester JL. Cocaine condom ingestion. Surgical treatment. JAMA 1977;238:1391–1392.

13. Kleber H. Epidemic cocaine abuse: America's present, Britain's future? Br J Addiction 1988;83:1359–1371.

14. Welti W, Wright RK. Death caused by recreational cocaine use. JAMA 1979;241:2519–2522.

15. Casale JF, Corbeil EM, Hays PA. Identification of levamisole impurities found in illicit cocaine exhibits. Microgram J 2008;6:82–89.

16. Bailey DN, Bessler JB, Sawrey BA. Cocaine- and cocaethylene—creatinine clearance ratios in humans. J Anal Toxicol 1997;21:41–43.

17. Glatt MM. Reflections on heroin and cocaine addiction. Lancet 1965;2:171–172.

18. Kozel NJ, Crider RA, Adams EH. National surveillance of cocaine use and related health consequences. MMWR Morbid Mortal Wkly Rep 1982;31:265–273.

19. O'Malley PM, Bachman JG, Johnston LD. Period, age and cohort effects on substance use among American youth 1976–1982. Am J Pub Health 1984;74:682–688.

20. Banken JA. Drug abuse trends among youth in the United States. Ann N Y Acad Sci 2004;1025:465–471.

21. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Summary of findings from the 1998 National Household Survey on Drug Abuse. Rockville, MD: National Clearinghouse for Alcohol and Drug Information; 2000.

22. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Office of Applied Studies. Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of national findings. Available at http://www.oas.samhsa.gov/NSDUH/2k9NSDUH/2k9ResultsP.pdf. Accessed 2011 April 23.

23. Gossop M, Griffiths P, Powis B, Strang J. Cocaine: patterns of use, route of administration, and severity of dependence. Br J Psychiatry 1994;164:660–664.

24. Dunn J, Laranjeira RR. Transitions in the route of cocaine administration–characteristics, direction and associated variables. Addiction 1999;94:813–824.

25. Rivier L. Analysis of alkaloids in leaves of cultivated *Erythroxylum* and characterization of alkaline substances used during coca chewing. J Ethnopharmacol 1981;3:313–335.

26. Holmstedt B, Jaatmaa E, Leander K, Plowman T. Determination of cocaine in some South American species of *Erythroxylum* using mass fragmentography. Phytochemistry 1977;16:1753–1755.

27. Bono JP. Criminalistics—introduction to controlled substances. In: Karch SB (Ed.), Drug abuse handbook, Boca Raton, FL: CRC Press; 1998.

28. Loper KA. Clinical toxicology of cocaine. Med Toxicol Adverse Drug Exp 1989;4:174–185.

29. Siegel RK. Cocaine smoking. J Psychoactive Drugs 1982;14;271–359.

30. Shesser R, Jotte R, Olshaker J. The contribution of impurities to the acute morbidity of illegal drug use. Am J Emerg Med 1991;9:336–342.

31. Moore JM, Casale JF. In-depth chromatographic analyses of illicit cocaine and its precursor, coca leaves. J Chromatogr A 1994;674:165–205.

32. Moore JM, Cooper DA, Lurie IS, Kram TC, Carr S, Harper C, Yeh J. Capillary gas chromatographic-electron capture detection of coca-leaf-related impurities in illicit cocaine: 2,4-diphenylcyclobutane-1,3-dicarboxylic acids, 1,4-diphenylcyclobutane-2,3-dicarboxylic acids and their alkaloidal precursors, the truxillenes. J Chromatogr 1987;410:297–318.

33. Isenschmid DS. Cocaine—effects on human performance and behavior. Forensic Sci Rev 2002;14:62–100.

34. Shannon M. Clinical toxicity of cocaine adulterants. Ann Emerg Med 1988;17:1243–1247.

35. Cole C, Jones L, McVeigh J, Kicman A, Sed Q, Bellis M. Adulterants in illicit drugs: a review of empirical evidence. Drug Test Anal 2011;3:89–96.

36. Fucci N, De Giovanni N. Adulterants encountered in the illicit cocaine market. Forensic Sci Int 1998;95:247–252.

37. Czuchlewski DR, Brackney M, Ewers C, Manna J, Fekrazad MH, Martinez A, et al. Clinicopathologic features of agranulocytosis in the setting of levamisole-tainted cocaine. Am J Clin Pathol 2010;133:466–472.

38. Centers for Disease Control and Prevention (CDC). Agranulocytosis associated with cocaine use—four States, March 2008-November 2009. MMWR Morb Mortal Wkly Rep 2009;58:1381–1385.

39. Siegel JA, Cormier RA. The preparation of *d*-pseudococaine from *l*-cocaine. J Forensic Sci 1980;25:357–365.

40. Copper DA, Allen AC. Synthetic cocaine impurities. J Forensic Sci 1984;29:1045–1055.

41. Holmstedt B, Lindgren J-E, Rivier L. Cocaine in blood of coca chewers. J Ethnopharmacol 1979;1:69–78.

42. Buck AA, Sasaki TT, Hewitt JJ, Macrae AA. Coca chewing and health an epidemiologic study among residents of a Peruvian village. Am J Epidemiol 1968;88:159–177.

43. Zapata-Ortiz V. The chewing of coca leaves in Peru. Int J Addict 1970;5:287–294.

44. Dougherty RJ. Status of cocaine abuse 1984. J Subst Abuse Treat 1984;1:157–161.

45. Perez-Reyes M, Di Guiseppi S, Ondrusek G, Jeffcoat AR, Cook CE. Free-base cocaine smoking. Clin Pharmacol Ther 1982;32:459–465.

46. Foltin RW, Fischman MW. Smoked and intravenous cocaine in humans: acute tolerance, cardiovascular and subjective effects. J Pharmacol Exp Ther 1991;257: 247–261.

47. Snyder CA, Wood RW, Graefe JF, Bowers A, Magar K. "Crack smoke" is a respirable aerosol of cocaine base. Pharmacol Biochem Behav 1988;29:93–95.

48. Martin BR, Boni J. Pyrolysis and inhalation studies with phencyclidine and cocaine. NIDA Res Monogr 1990;99: 141–158.

49. Nakahara Y, Ishigami A. Inhalation efficiency of free-base cocaine by pyrolysis of "crack" and cocaine hydro-chloride. J Anal Toxicol 1991;15:105–109.

50. Martin BR, Lue LP, Boni JP. Pyrolysis and volatilization of cocaine. J Anal Toxicol 1989;13:158–162.

51. Jekel JF, Allen DF, Podlewski H, Clarke N, Dean-Patterson S, Cartwright P. Epidemic free-base cocaine abuse. Case study from the Bahamas. Lancet 1986; 1(8479):459–462.

52. Hatsukami DK, Fischman MW. Crack cocaine and cocaine hydrochloride. Are the differences myth or reality? JAMA 1996;276:1580–1588.

53. Post RM. Cocaine psychosis: A continuum model. Am J Psychiatry 1975;132:225–231.

54. Caulkins JP. Is crack cheaper than (powder) cocaine? Addiction 1997;92:1437–1443.

55. Montoya ID, Chilcoat HD. Epidemiology of coca deriva-tives use in the Andean region: a tale of five countries. Subst Use Misuse 1996;31:1227–1240.

56. ElSohly MA, Brenneisen R, Jones AB. Coca paste: chem-ical analysis and smoking experiments. J Forensic Sci 1991;36:93–103.

57. Resnick RB, Kestenbaum RS, Schwartz LK. Acute sys-temic effects of cocaine in man. A controlled study by intranasal and intravenous routes. Science 1977;195: 696–698.

58. Fischman MW, Schuster CR, Resnekov L, Shick JF, Krasnegor NA, Fennell W, Freedman DX. Cardiovascular and subjective effects of intravenous cocaine administra-tion in humans. Arch Gen Psychiatry 1976;33:983–989.

59. Benowitz NL. Clinical pharmacology and toxicology of cocaine. Pharmacol Toxicol 1993;72:3–12.

60. Johns ME, Henderson RL. Cocaine use by the otolaryn-gologist: a survey. Trans Sect Otolaryngol Am Acad Ophthalmol Otolaryngol 1977;84:969–973.

61. Barash PG, Kopriva CJ, Langou R, VanDyke C, Jatlow P, Stahl A, Byck R. Is cocaine a sympathetic stimulant during general anesthesia? JAMA 1980;243:1437–1439.

62. Glover DD, Lowry TF, Jacknowitz AI. Brompton's mixture in alleviating pain of terminal neoplastic disease: preliminary results. South Med J 1980;73:278–282.

63. Wiggins RC, Rolsten C, Ruiz B, Davis CM. Pharmacokinetics of cocaine: basic studies of route, dosage, pregnancy and lactation. Neurotoxicology 1989; 10:367–382.

64. van Dyke C, Barash PG, Jatlow P, Byck R. Cocaine: plasma concentrations after intranasal application in man. Science 1976;191:859–861.

65. Van Dyke C, Ungerer J, Jatlow P, Barash P, Byck R. Intranasal cocaine: dose relationships of psychological effects and plasma levels. Int J Psychiatry Med 1982; 12:1–13.

66. Javaid JL, Fischman MW, Schuster CR, Dekirmenjian H, Davis JM. Cocaine plasma concentrations. Relation of physiological subjective effects in humans. Science 1978;202:227–228.

67. Hamilton HE, Wallace JE, Shimek EL Jr, Land P, Harris SC, Christenson JG. Cocaine and benzoylecgonine excre-tion in humans. J Forensic Sci 1977;22:697–707.

68. Wilkinson P, Van Dyke C, Jatlow P, Barash P, Byck R. Intranasal and oral cocaine kinetics. Clin Pharmacol Ther 1980;27:386–394.

69. Van Dyke C, Jatlow P, Ungerer CJ, Barash PG, Byck R. Oral cocaine: plasma concentrations and central effects. Science 1978;200:211–213.

70. Aks SE, Vander Hoek TL, Hryhorczuk DO, Negrusz A, Tebbett I. Cocaine liberation from body packers in an *in vitro* model. Ann Emerg Med 1992;21:1321–1325.

71. Evans SM, Cone EJ, Henningfield JE. Arterial and venous cocaine plasma concentrations in humans: rela-tionship to route of administration, cardiovascular effects and subjective effects. J Pharmacol Exp Ther 1996; 279:1345–1356.

72. Baselt RC, Chang JY, Yoshikawa DM. On the dermal absorption of cocaine. J Anal Toxicol 1990;14:383–384.

73. Jeffcoat AR, Perez-Reyes M, Hill JM, Sadler BM, Cook CE. Cocaine disposition in humans after intravenous injection, nasal insufflation (snorting), or smoking. Drug Metab Dispos 1989;17:153–159.

74. Cone EJ. Pharmacokinetics and pharmacodynamics of cocaine. J Anal Toxicol 1995;19:459–478.

75. Bailey DN. Cocaine and cocaethylene binding in human serum. Am J Clin Pathol 1995;104:180–186.

76. Edwards DJ, Bowles SK. Protein binding of cocaine in human serum. Pharm Res 1988;5:440–442.

77. Misra AL, Patel MN, Alluri VR, Mule SJ, Nayak PK. Disposition and metabolism of [3H]cocaine in acutely and chronically treated dogs. Xenobiotica 1976;6: 537–552.

78. Yazigi RA, Polakoski KL. Distribution of tritiated cocaine in selected genital and nongenital organs follow-ing administration to male mice. Arch Pathol Lab Med 1992;116:1036–1039.

79. Cone EJ, Kato K, Hillsgrove M. Cocaine excretion in the semen of drug users. J Anal Toxicol 1996;20:139–140.

80. Cone EJ, Tsadik A, Oyler J, Darwin WD. Cocaine metab-olism and urinary excretion after different routes of administration. Ther Drug Monitor 1998;20:556–560.

81. Warner A, Norman AB. Mechanisms of cocaine hydroly-sis and metabolism *in vitro* and *in vivo*: a clarification. Ther Drug Monitor 2000;22:266–270.

82. Ambre JJ. The urinary excretion of cocaine and metabolites in humans. A kinetic analysis of published data. J Anal Toxicol 1985;9:241–245.

83. Ambre JJ, Fischman M, Ruo T-I. Urinary excretion of ecgonine methyl ester, a major metabolite of cocaine in humans. J Anal Toxicol 1984;8:23–25.

84. Roberts SM, Harbison RD, James RC. Human microsomal *N*-oxidative metabolism of cocaine. Drug Metab Dispos 1991;19:1046–1051.

85. Ladona MG, Gonzalez ML, Rane A, Peter RM, de la Torre R. Cocaine metabolism in human fetal and adult liver microsomes is related to cytochrome P450 3A expression. Life Sci 2000;68:431–443.

86. Inaba T, Stewart DJ, Kalow W. Metabolism of cocaine in man. Clin Pharmacol Ther 1978;23:547–552.

87. Zhang JY, Foltz RL. Cocaine metabolism in man: identification of four previously unreported cocaine metabolites in human urine. J Anal Toxicol 1990;14:201–205,

88. Jacob P III, Jones RT, Benowitz NL, Shulgin AT, Lewis ER, Elias-Baker BA. Cocaine smokers excrete a pyrolysis product, anhydroecgonine methyl ester (methylecgonidine). J Toxicol Clin Toxicol 1990;28:121–125.

89. Kintz P, Cirimele V, Sengler C, Mangin P. Testing human hair and urine for anhydroecgonine methyl ester, a pyrolysis product of cocaine. J Anal Toxicol 1995;19:479–482.

90. Hoffman RS, Thompson T, Henry GC, Hatsukami DK, Pentel PR. Variation in human plasma cholinesterase activity during low-dose cocaine administration. Clin Toxicol 1998;36:3–9.

91. Schwartz HJ, Johnson D. *In vitro* competitive inhibition of plasma cholinesterase by cocaine: normal and variant genotypes. Clin Toxicol 1996;34:77–81.

92. Chow MJ, Ambre JJ, Ruo Tl, Atkinson AJ Jr, Bowsher DJ, Fischman MW. Kinetics of cocaine distribution, elimination and chronotropic effects. Clin Pharmacol Ther 1985;38:318–324.

93. Jatlow P. Cocaine: analysis, pharmacokinetics, and metabolic disposition. Yale J Biol Med 1988;61:105–113.

94. Ambre J, Ruo TI, Nelson J, Belknap S. Urinary excretion of cocaine, benzoylecgonine, and ecgonine methyl ester in humans. J Anal Toxicol 1988;12:301–306.

95. Garrett ER, Seyda K. Prediction of stability in pharmaceutical preparations XX. Stability evaluation and bioanalysis of cocaine and benzoylecgonine by high-performance liquid chromatography. J Pharm Sci 1983; 72:258–271.

96. Mittleman RE, Cofino JC, Hearn WL. Tissue distribution of cocaine in a pregnant woman. J Forensic Sci 1989; 34:481–486.

97. Klein J, Greenwald M, Becker L, Koren G. Fetal distribution of cocaine: case analysis. Pediatric Pathol 1992;12: 463–468.

98. Chasnoff IJ, Bussey ME, Savich R, Stack CM. Perinatal cerebral infarction and maternal cocaine use. J Pediatr 1986;108:456–459.

99. Chasnoff IJ, Lewis DE, Squires L. Cocaine intoxication in a breast-fed infant. Pediatrics 1987;80:836–838.

100. Fischman MW, Schuster CR, Javaid J, Hatano Y, Davis J. Acute tolerance development to the cardiovascular and subjective effects of cocaine. J Pharmacol Exp Ther 1985;235:677–682.

101. Kleber HD, Gawin FH. The spectrum of cocaine abuse and its treatment. J Clin Psychiatry 1984;45:18–23.

102. Matsuzaki M, Spingler PJ, Misra AL, Mule SJ. Cocaine: tolerance to its convulsant and cardiorespiratory stimulating effects in the monkey. Life Sci 1976;19:193–203.

103. Foltin RW, Haney M. Intranasal cocaine in humans: acute tolerance, cardiovascular and subjective effects. Pharmacol Biochem Behav 2004;78:93–101.

104. Kalant H, LeBlanc AE, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 1971;23:136–191.

105. Ambre JJ, Belknap SM, Nelson J, Ruo TI, Shin SG, Atkinson AJ Jr. Acute tolerance to cocaine in humans. Clin Pharmacol Ther 1988;44:1–8.

106. Andrews P. Cocaethylene toxicity. J Addict Dis 1997;16: 75–84.

107. Pennings EJM, Leccese AP, de Wolff FA. Effects of concurrent use of alcohol and cocaine. Addiction 2002;97:773–83.

108. Farre M, de la Torre R, Gonzalez ML, Teran MT, Roset PN, Menoyo E, Cami J. Cocaine and alcohol interactions in humans: neuroendocrine effects and cocaethylene metabolism. J Pharmacol Exp Ther 1997;283:164–176.

109. Perez-Reyes M. The order of drug administration: its effects on the interaction between cocaine and ethanol. Life Sci 1994;55:541–550.

110. Brookoff D, Rotondo MF, Shaw LM, Campbell EA, Fields L. Cocaethylene levels in patients who test positive for cocaine. Ann Emerg Med 1996;27:316–320.

111. Cami J, Farre M, Gonzalez ML, Segura J, de la Torre R. Cocaine metabolism in humans after use of alcohol clinical and research implications. Recent Dev Alcohol 1998;14:437–455.

112. Hime GW, Hearn WL, Rose S, Cofino J. Analysis of cocaine and cocaethylene in blood and tissues by GC-NPD and GC-ion trap mass spectrometry. J Anal Toxicol 1991;15:241–245.

113. Perez-Reyes M, Jeffcoat AR. Ethanol/cocaine interaction: cocaine and cocaethylene plasma concentration and their relationship to subjective and cardiovascular effects. Life Sci 1992;51:553–563.

114. McCance-Katz E, Kosten TR, Jatlow P. Concurrent use of cocaine and alcohol is more potent and potentially more toxic than use of either alone—a multiple-dose study. Biol Psychiatry 1998;44:250–259.

115. Herbst ED, Harris DS, Everhart ET, Mendelson J, Jacob P, Jones RT. Cocaethylene formation following ethanol and cocaine administration by different routes. Exp Clin Psychopharmacol 2011;19:95–104.

116. Harris DS, Everhart T, Mendelson J, Jones RT. The pharmacology of cocaethylene in humans following cocaine and ethanol administration. Drug Alcohol Depend 2003; 72:169–182.

117. Farre M, De La Torre R, Llorente M, Lamas X, Ugena B, Segura J, Cami J. Alcohol and cocaine interactions in humans. J Pharmacol Exp Ther 1993;266:1364–1373.

118. Bailey DN. Comprehensive review of cocaethylene and cocaine concentrations in patients. Am J Clin Pathol 1996;106:701–704.

119. McCance-Katz EF, Price LH, McDougle CJ, Kosten TR, Black JE, Jatlow PI. Concurrent cocaine-ethanol ingestion in humans: pharmacology, physiology, behavior, and the role of cocaethylene. Psychopharmacology 1993;111: 39–46.

120. Perez-Reyes M, Jeffcoat AR, Myers M, Sihler K, Cook CE. Comparison in humans of the potency and pharmacokinetics of intravenously injected cocaethylene and cocaine. Psychopharmacology (Berl) 1994;116:428–432.

121. McCance EF, Price LH, Kosten TR, Jatlow PI. Cocaethylene: pharmacology, physiology and behavioral effects in humans. J Pharmacol Exp Ther 1995;274: 215–223.

122. Hart CL, Jatlow P, Sevarino KA, McCance-Katz EF. Comparison of intravenous cocaethylene and cocaine in humans. Psychopharmacology 2000;149:153–162.

123. Higgins ST, Rush CR, Bickel WK, Hughes JR, Lynn M, Capeless MA. Acute behavioral and cardiac effects of cocaine and alcohol combinations in humans. Psychopharmacology (Berl) 1993;111:285–294.

124. Hearn WL, Flynn DD, Hime GW, Rose S, Cofino JC, Mantero-Atienza E:, et al. Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter. J Neurochem 1991;56:698–701.

125. Bradberry CW, Nobiletti JB, Elsworth JD, Murphy B, Jatlow P, Roth RH. Cocaine and cocaethylene: microdialysis comparison of brain drug levels and effects on dopamine and serotonin. J Neurochem 1993;60: 1429–1435.

126. Haberny KA, Walsh SL, Ginn DH, Wilkins JN, Garner JE, Setoda D, Bigelow GE. Absence of acute cocaine interactions with the MAO-B inhibitor selegiline. Drug Alcohol Depend 1995;39:55–62.

127. Sands BF, Ciraulo DA. Cocaine drug-drug interactions. J Clin Psychopharmacol 1992;12:49–55.

128. Cokugras AN, Tezcan EF. Amitriptyline: a potent inhibitor of butyrylcholinesterase from human serum. Gen Pharmacol 1997;29:835–838.

129. Sherer MA, Jumor KM, Jaffe JH. Effects of intravenous cocaine are partially attenuated by haloperidol. Psychiatry Res 1989;27:117–125.

130. Mathews JC, Collins A. Interactions of cocaine and cocaine congeners with sodium channels. Biochem Pharmacol 1983;32:455–460.

131. Chow JM, Robertson AL Jr, Stein RJ. Vascular changes in the nasal submucosa of chronic cocaine addicts. Am J Forensic Med Pathol 1990;11:136–143.

132. Vilensky W. Illicit and licit drugs causing perforation of the nasal septum. J Forensic Sci 1982;27:958–962.

133. Frishman WH, Del Vecchio A, Sanal S, Ismail A. Cardiovascular manifestations of substance abuse Part 1: cocaine. Heart Dis 2003;5:187–201.

134. Jain RK, Jain MK, Bachenheimer LC, Visner MS, Hamosh P, Tracy CM, Gillis RA. Factors determining whether cocaine will potentiate the cardiac effects of neurally released norepinephrine. J Pharmacol Exp Ther 1990; 252:147–153.

135. Schenk JO. The functioning neuronal transporter for dopamine: kinetic mechanisms and effects of amphetamines, cocaine and methylphenidate. Prog Drug Res 2002;59:111–131.

136. Wang G-J, Volkow ND, Fowler J, Fischman M, Foltin R, Abumrad NN, Logan J, Pappas NR. Cocaine abusers do not show loss of dopamine transporters with age. Life Sci 1997;61:1059–1065.

137. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine self-administration appears to be mediated by dopamine uptake inhibition. Prog Neuropsychopharmacol Biol Psychiatry 1988;12:233–239.

138. Rostene W, Boja JW, Scherman D, Carroll FI, Kuhar MJ. Dopamine transport: pharmacological distinction between the synaptic membrane and the vesicular transporter in rat striatum. Eur J Pharmacol 1992;218: 175–177.

139. Madras BK, Fahey MA, Bergman J, Canfield DR, Spealman RD. Effects of cocaine and related drugs in non-human primates. I. [3H]Cocaine binding sites in caudate-putamen. J Pharmacol Exp Ther 1989;251: 131–141.

140. Pilotte NS. Neurochemistry of cocaine withdrawal. Curr Opin Neurol 1997;10:534–538.

141. Malison RT, Best SE, van Dyck CH, McCance EF, Wallace EA, Laruelle M, Baldwin RM, et al. Elevated striatal dopamine transporters during acute cocaine abstinence as measured by [123I] beta-CIT SPECT. Am J Psychiatry 1998;155:832–834.

142. Woolverton WL, Johnson KM. Neurobiology of cocaine abuse. TIPS 1992;13:193–200.

143. Uchimura N, North RA. Actions of cocaine on rat nucleus accumbens neurones in vitro. Br J Pharmacol 1990;99: 736–740.

144. Withers NW, Pulvirenti L, Koob GF, Gillin JC. Cocaine abuse and dependence. J Clin Psychopharmacol 1995; 15:63–78.

145. Tella SR, Schindler CW, Goldberg SR. Cardiovascular responses to cocaine self-administration: acute and chronic tolerance. Eur J Pharmacol 1999;383:57–68.

146. Smith JA, Mo Q, Guo H, Kunko PM, Robinson SE. Cocaine increases extra-neuronal levels of aspartate and glutamate in the nucleus accumbens. Brain Res 1995; 683:264–269.

147. Holland RW 3rd, Marx JA, Earnest MP, Ranniger S. Grand mal seizures temporally related to cocaine use:

clinical and diagnostic features. Ann Emerg Med 1992; 21:772–776.

148. Kaufman MJ, Levin JM, Ross MH, Lange N, Rose SL, Kukes TJ, et al. Cocaine-induced cerebral vasoconstriction detected in humans with magnetic resonance angiography. JAMA 1998;279:376–380.

149. Mash DC, Ouyang Q, Pablo J, Basile M, Izenwasser S, Lieberman A, Perrin RJ. Cocaine abusers have an overexpression of α-synuclein in dopamine neurons. J Neurosci 2003;23:2564–2571.

150. Staley JK, Hearn WL, Ruttenber AJ, Wetli CV, Mash DC. High affinity cocaine recognition sites on the dopamine transporter are elevated in fatal cocaine overdose victims. J Pharmacol Exp Ther 1994;271:1678–1685.

151. Isner JM, Chokshi SK. Cardiac complications of cocaine abuse. Ann Rev Med 1991;42:133–138.

152. Pletcher MJ, Kiefe CI, Sidney S, Carr JJ, Lewis CE, Hulley SB. Cocaine and coronary calcification in young adults: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am Heart J 2005:150: 921–926.

153. Bamberg F, Schlett CL, Truong QA, Rogers IS, Koenig W, Nagurney JT, et al. Presence and extent of coronary artery disease by cardiac computed tomography and risk for acute coronary syndrome in cocaine users among patients with chest pain. Am J Cardiol 2009;103: 620–625.

154. Billman GE. Cocaine: a review of its toxic actions on cardiac function. Crit Rev Toxicol 1995;25:113–132.

155. O'Leary ME, Chahine M. Cocaine binds to a common site on open and inactivated human heart (Nav 1.5) sodium channels. J Physiol 2002;541:701–716.

156. Tong W, Lima JA, Meng Q, Flynn E, Lai S. Long-term cocaine use is related to cardiac diastolic dysfunction in an African-American population in Baltimore, Maryland. Int J Cardiol 2004;97:25–28.

157. Lange RA, Cigarroa RG, Yancy DW Jr, Willard JE, Popma JJ, Sills MN, et al. Cocaine-induced coronary-artery vasoconstriction. N Engl J Med 1989;321: 1557–1562.

158. Benzaquen BS, Cohen V, Eisenberg MJ. Effects of cocaine on the coronary arteries. Am Heart J 2001;142: 402–410.

159. Tella SR, Schindler CW, Goldberg SR. Cocaine: cardiovascular effects in relation to inhibition of peripheral neuronal monoamine uptake and central stimulation of the sympathoadrenal system. J Pharmacol Exp Ther 1993;267:153–162.

160. Lange RA, Cigarroa RG, Flores ED, McBride W, Kim AS, Wells PF, et al. Potentiation of cocaine-induced coronary vasoconstriction by beta-adrenergic blockade. Ann Intern Med 1990;112:897–903.

161. Isner J, Chokshi SK. Cardiovascular complications of cocaine. Curr Probl Cardiol 1991;16:89–123.

162. Crumb WJ Jr, Clarkson CW. Characterization of the sodium channel blocking properties of the major metab-olites of cocaine in single cardiac myocytes. J Pharmacol Exp Ther 1992;261:910–917.

163. Lange RA, Hillis LD. Cardiovascular complications of cocaine use. N Engl J Med 2001;345:351–358.

164. Wilson LD, Jeromin G, Shelat C, Huettl B. Tolerance develops to the sympathomimetic but not the local anesthetic effects of cocaine. Clin Toxicol 2000;38:719–727.

165. Xu YQ, Crumb WJ Jr, Clarkson CW. Cocaethylene, a metabolite of cocaine and ethanol, is a potent blocker of cardiac sodium channels. J Pharmacol Exp Ther 1994; 271:319–325.

166. Kabas JS, Blanchard SM, Matsuyama Y, Long JD, Hoffman GW Jr, Ellinwood EH, et al. Cocaine-mediated impairment of cardiac conduction in the dog: a potential mechanism for sudden death after cocaine. J Pharmacol Exp Ther 1990;252:185–191.

167. Przywara DA, Dambach GE. Direct actions of cocaine on cardiac cellular electrical activity. Circ Res 1989;65: 185–192.

168. Tisdale JE, Shimoyama H, Sabbah HN, Webb CR. The effect of cocaine on ventricular fibrillation threshold in the normal canine heart. Pharmacotherapy 1996;16: 429–437.

169. Wang RY. pH-Dependent cocaine-induced cardiotoxicity. Am J Emerg Med 1999;17:364–369.

170. Wood DM, Dargan PI, Hoffman RS. Management of cocaine-induced cardiac arrhythmias due to cardiac ion channel dysfunction. Clin Toxicol 2009;47:14–23.

171. Beckman KJ, Parker RB, Hariman RJ, Gallastegui JL, Javaid JI, Bauman JL. Hemodynamic and electrophysiological actions of cocaine. Effects of sodium bicarbonate as an antidote in dogs. Circulation 1991;83:1799–1807.

172. Bauman JL, DiDomenico RJ. Cocaine-induced channelopathies: emerging evidence on the multiple mechanisms of sudden death. J Cardiovasc Pharmacol Ther 2002;7:195–202.

173. Pitts WR, Lange RA, Cigarroa JE, Hillis LD. Cocaine-induced myocardial ischemia and infarction: pathophysiology, recognition, and management. Prog Cardiovascular Dis 1997;40:65–76.

174. Kontos MC, Jesse RL, Tatum JL, Ornato JP. Coronary angiographic findings in patients with cocaine-associated chest pain. J Emerg Med 2003;24:9–13.

175. Nademanee K, Gorelick DA, Josephson MA, Ryan MA, Wilkins JN, Robertson HA, et al. Myocardial ischemia during cocaine withdrawal. Ann Intern Med 1989;111: 876–880.

176. Lathers CM, Tyau LSY, Spino MM, Agarwal I. Cocaine-induced seizures, arrhythmias and sudden death. J Clin Pharmacol 1988;28:584–593.

177. Murray RJ, Albin RJ, Mergner W, Criner GJ. Diffuse alveolar hemorrhage temporally related to cocaine smoking. Chest 1988;93:427–429.

178. Kissner DG, Lawrence D, Selis JE, Flint A. Crack lung: pulmonary disease caused by cocaine abuse. Am Rev Respir Dis 1987;136:1250–1252.

179. Patel RC, Dutta D, Schonfeld SA. Free-base cocaine use associated with bronchiolitis obliterans organizing pneumonia. Ann Intern Med 1987;107:186–187.

180. Laposata EA, Mayo GL. A review of pulmonary pathology and mechanisms associated with inhalation of free-base cocaine ("crack"). Am J Forensic Med Pathol 1993; 14:1–9.

181. Baldwin GC, Choi R, Roth MD, Shay AG, Kleerup EC, Simmons MS, Tashkin DP. Evidence of chronic damage to the pulmonary microcirculation in habitual users of alkaloidal ("crack") cocaine. Chest 2002;121:1231–1238.

182. Herculiani PP, Pires-Neto RC, Bueno HM, Zorzetto JC, Silva LC, Santos AB, et al. Effects of chronic exposure to crack cocaine on the respiratory tract of mice. Toxicol Pathol 2009;37:324–332.

183. Solaini L, Gourgiotis S, Salemis NS, Koukis I. Bilateral pneumothorax, lung cavitations, and pleural empyema in a cocaine addict. Gen Thorac Cardiovasc Surg 2008; 56:610–612.

184. Baldwin GC, Choi R, Roth MD, Shay AH, Kleerup EC, Simmons MS, Tashkin DP. Evidence of chronic damage to the pulmonary microcirculation in habitual users of alkaloidal ("crack") cocaine. Chest 2002;121:1231–1238.

185. Crandall CG, Vongpatanasin W, Victor RG. Mechanism of cocaine-induced hyperthermia in humans. Ann Intern Med 2002;136:785–791.

186. Roth D, Alarcon FJ, Fernandez JA, Preston RA, Bourgoignie JJ. Acute rhabdomyolysis associated with cocaine intoxication. N Engl J Med 1988;319:673–677.

187. Rockhold RW, Carver ES, Ishizuka Y, Hoskins B, Ho IK. Dopamine receptors mediate cocaine-induced temperature responses in spontaneously hypertensive and Wistar-Kyoto rats. Pharmacol Biochem Behav 1991;40:157–162.

188. Staley JK, Hearn WL, Ruttenber AJ, Wetli CV, Mash DC. High affinity cocaine recognition sites on the dopamine transporter are elevated in fatal cocaine overdose victims. J Pharmacol Exp Ther 1994;271:1678–1685.

189. Campbell BG. Cocaine abuse with hyperthermia, seizures and fatal complications. Med J Aust 1988;149: 387–389.

190. Kiyatkin EA. Brain hyperthermia during physiological and pathological conditions: causes, mechanisms, and functional implications. Curr Neurovasc Res 2004;1: 77–90.

191. Boelsterli UA, Goldlin C. Biomechanisms of cocaine-induced hepatocyte injury mediated by the formation of reactive metabolites. Arch Toxicol 1991;65:351–360.

192. Ponsoda X, Bort R, Jover R, Gomez-Lechon MJ, Castell JV. Increased toxicity of cocaine on human hepatocytes induced by ethanol: role of GSH. Biochem Pharmacol 1999;58:1579–1585.

193. Osorio J, Farreras N, Ortiz De Zárate L, Bachs E. Cocaine-induced mesenteric ischaemia. Dig Surg 2000; 17:648–651.

194. Nzeure CM, Hewan-Lowe K, Riley LJ Jr. Cocaine and the kidney: a synthesis of pathophysiologic and clinical perspectives. Am J Kidney Dis 2000;35:783–795.

195. Amand V, Siami G, Stone WJ. Cocaine-associated rhabdomyolysis and acute renal failure. South Med J 1989; 82:67–69.

196. Thakur VK, Godley C, Weed S, Cook ME, Hoffman E. Cocaine-associated accelerated hypertension and renal failure. Am J Med Sci 1996;312:295–298.

197. Alvarez D, Nzerue CM, Faruque S, Daniel JF, Hewan-Lowe K. Crack-cocaine induced acute interstitial nephritis. Nephrol Dial Transplant 1999;14:1260–1262.

198. Tumlin JA, Sands JM, Someren A. Hemolytic uremic syndrome following "crack" cocaine inhalation. Am J Med Sci 1990;299:366–371.

199. Peces R, Navascues RA, Baltar J, Seco M, Alvarez J. Anti-glomerular basement membrane antibody-mediated glomerulonephritis after intranasal cocaine. Nephron 1999;81:434–438.

200. Tardiff K, Marzuk PM, Leon AC, Hirsch CS, Stajic M, Portera L, Hartwell N. Cocaine, opiates, and ethanol in homicides in New York City: 1990 and 1991. J Forensic Sci 1995;40:387–390.

201. Tarfiff K, Gross E, Wu J, Stajic M, Millman R. Analysis of cocaine-positive fatalities. J Forensic Sci 1989;34; 53–63.

202. Stephens BG, Jentzen JM, Karch S, Mash DC, Wetli CV. Criteria for the interpretation of cocaine levels in human biological samples and their relation to the cause of death. Am J Forensic Med Pathol 2004;25:1–13.

203. Wetli CV, Fishbain DA. Cocaine-induced psychosis and sudden death in recreational cocaine users. J Forensic Sci 1985;30:873–880.

204. Nolte KB. Rhabdomyolysis associated with cocaine abuse. Human Pathol 1991;22:1141–1145.

205. Wetli CV, Mash D, Karch SB. Cocaine-associated agitated delirium and the neuroleptic malignant syndrome. Am J Emerg Med 1996;14:425–428.

206. Peng SK, French WJ, Pelikan PC. Direct cocaine cardiotoxicity demonstrated by endomyocardial biopsy. Arch Pathol Lab Med 1989;113:842–845.

207. Fineschi V, Centini F, Monciotti F, Turillazzi E. The cocaine "body stuffer" syndrome: a fatal case. Forensic Sci Int 2002;126:7–10.

208. Karch SB, Stephens BS. When is cocaine the cause of death? Am J Forensic Med Pathol 1991;12:1–2.

209. Fineschi V, Wetli CV, Di Paolo M, Baroldi G. Myocardial necrosis and cocaine a quantitative morphologic study in 26 cocaine-associated deaths. Int J Legal Med 1997;110: 193–198.

210. Tazelaar HD, Karch SB, Stephens BG, Billingham ME. Cocaine and the heart. Hum Pathol 1987;18:195–199.

211. Virmani R, Robinowitz M, Smialek JE, Smyth DF. Cardiovascular effects of cocaine: an autopsy study of 40 patients. Am Heart J 1988;115:1068–1076.

212. Michaud K, Augsburger M, Sporkert F, Bollmann M, Krompecher T, Mangin P. Interpretation of lesions of the cardiac conduction system in cocaine-related fatalities. J Forensic Leg Med 2007;14:416–422.

213. Roh LS, Hamele-Bena D. Cocaine-induced ischemic myocardial disease. Am J Forensic Med Pathol 1990;11: 130–135.

214. Mittleman RE, Welti CV. Cocaine and sudden "natural" death. J Forensic Sci 1987;32:11–19.

215. Karch S, Stephens B, Ho CH. Relating cocaine blood concentrations to toxicity—an autopsy study of 99 cases. J Forensic Sci 1998;43:41–45.

216. Simpson RW, Edwards WD. Pathogenesis of cocaine-induced ischemic heart disease. Autopsy findings in a 21-year-old man. Arch Pathol Lab Med 1986;110: 479–484.

217. Fishbain DA, Wetli CV. Cocaine intoxication, delirium and death in a body packer. Ann Emerg Med 1981;10: 531–532.

218. Siegel RK. Cocaine Hallucinations. Am J Psychiatry 1978;135:309–314.

219. Derlet RW, Albertson TE. Emergency department presentation of cocaine intoxication. Ann Emerg Med 1989;18:182–186.

220. Rubin RB, Neugarten J. Medical complications of cocaine: changes in pattern of use and spectrum of complication. Clin Toxicol 1992;30:1–12.

221. Jacobson JM, Hirschman SZ. Necrotizing fasciitis complicating intravenous drug abuse. Arch Intern Med 1982;142:634–635.

222. Brown PD, Ebright JR. Skin and soft tissue infections in injection drug users. Curr Infect Dis Rep 2002;4: 415–419.

223. Gawin FH, Ellinwood E Jr. Cocaine and other stimulants actions, abuse, and treatment. N Engl J Med 1988;318: 1173–1182.

224. Marzuk PM, Tardiff K, Leon AC, Hirsch CS, Stajic M, Portera L, Hartwell N, Iqbal I. Fatal injuries after cocaine use as a leading cause of death among young adults in New York City. N Engl J Med 1995;332:1753–1757.

225. Gold MS, Washton AM, Dackis CA. Cocaine abuse: neurochemistry, phenomenology, and treatment. Natl Inst Drug Abuse Res Monogr Ser 1985;61:130–150.

226. Watson R, Bakos L, Compton P, Gawin F. Cocaine use and withdrawal: the effect on sleep and mood. Am J Drug Alcohol Abuse 1992;18:21–28.

227. Sporer KA, Lesser SH. Cocaine washed-out syndrome. Ann Emerg Med 1992;21:112.

228. Trabulsy ME. Cocaine washed out syndrome in a patient with acute myocardial infarction. Am J Emerg Med 1995;13:538–539.

229. Mahoney JJ, Kalechstein AD, De la Garza R II, Newton TF. Persistence and persistence of psychotic symptoms in cocaine-versus methamphetamine-dependent participants. Am J Addict 2008;17:83–98.

230. Satel SL, Southwick SM, Gawin FH. Clinical features of cocaine-induced paranoia. Am J Psychiatry 1991;148: 495–498.

231. Satel SL, Edell WS. Cocaine-induced paranoia and psychosis proneness. Am J Psychiatry 1991;148:1708–1711.

232. Brady KT, Lydiard RB, Malcolm R, Ballenger JC. Cocaine-induced psychosis. J Clin Psychiatry 1991;52: 509–512.

233. Mathias S, Lubman DI, Hides L. Substance-induced psychosis: a diagnostic conundrum. J Clin Psychiatry 2008; 69:358–367.

234. Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. JAMA 1990;264:2511–2518.

235. Tang Y-L, Kranzler HR, Gelernter J, Farrer LA, Cubells JF. Comorbid psychiatric diagnoses and their association with cocaine-induced psychosis in cocaine-dependent subjects. Am J Addict 2007;16:343–351.

236. Kaye S, Darke S. Non-fatal cocaine overdose among injecting and non-injecting cocaine users in Sydney, Australia. Addiction 2004;99:1315–1322.

237. Kloner RA, Hale S, Alker K, Rezkalla S. The effects of acute and chronic cocaine use on the heart. Circulation 1992;85;407–419.

238. Feldman JA, Fish SS, Beshansky JR, Griffith JL, Woolard RH, Selker HP. Acute cardiac ischemia in patients with cocaine-associated complaints: results of a multicenter trial. Ann Emerg Med 2000;36:469–476.

239. Hollander JE, Hoffman RS, Gennis P, Fairweather P, DiSano MJ, Schumb DA, et al. Prospective multicenter evaluation of cocaine-associated chest pain. Cocaine Associated Chest Pain (COCHPA) Study Group. Acad Emerg Med 1994;1:330–339.

240. Mittleman MA, Mintzer D, Maclure M, Tofler GH, Sherwood JB, Muller JE. Triggering of myocardial infarction by cocaine. Circulation 1999;99:2737–2741.

241. Hollander JE, Henry TD. Evaluation and management of the patient who has cocaine-associated chest pain. Cardiol Clin 2006;24:103–114.

242. Ross GS, Bell J. Myocardial infarction associated with inappropriate use of topical cocaine as treatment for epistaxis. Am J Emerg Med 1992;10:219–222.

243. Hollander JE, Hoffman RS. Cocaine-induced myocardial infarction: an analysis and review of the literature. J Emerg Med 1992;10:169–177.

244. Goldfrank LR, Hoffman RS. The cardiovascular effects of cocaine. Ann Emerg Med 1991;20:165–175.

245. Cregler LL, Mark H. Special report: medical complications of cocaine abuse. N Engl J Med 1986;315: 1495–1500

246. Satran A, Bart BA, Henry CR, Murad MB, Talukdar S, Satran D, Henry TD. Increased prevalence of coronary artery aneurysms among cocaine users. Circulation 2005;111:2424–2429.

247. Schachne JS, Roberts BH, Thompson PD. Coronary artery spasm and myocardial infarction associated with cocaine use. N Engl J Med 1984;310:1665–1666.

248. Howard RE, Hueter DC, Davis GJ. Acute myocardial infarction following cocaine abuse in a young woman with normal coronary arteries. JAMA 1985;254:95–96.

249. Smith HW, Liberman HA, Brody SL, Battey LL, Donohue BC, Morris DC. Acute myocardial infarction temporally related to cocaine use: clinical, angiographic, and pathophysiologic observations. Ann Intern Med 1987;107:13–18.

250. Hollander JE, Todd KH, Green G, Heilpern KL, Karras DJ, Singer AJ, et al. Chest pain associated with cocaine: an assessment of prevalence in suburban and urban emergency departments. Ann Emerg Med 1995;26: 671–676.

251. Gitter MJ, Goldmith SR, Dunbar DN, Sharkey SW. Cocaine and chest pain: clinical features and outcome of patients hospitalized to rule out myocardial infarction. Ann Intern Med 1991;115:277–282.

252. Amin M, Gabelman G, Karpel J, Buttrick P. Acute myocardial infarction and chest pain syndromes after cocaine use. Am J Cardiol 1990;66:1434–1437.

253. Hollander JE, Levitt Ma Young GP, Briglia E, Vetli CV, Gawad Y. Effect of recent cocaine use on the specificity of cardiac markers for diagnosis of acute myocardial infarction. Am Heart J 1998;135:245–252.

254. Tokarski GF, Paganussi P, Urbanski R, Carden D, Foreback C, Tomlanovich MC. An evaluation of cocaine-induced chest pain. Ann Emerg Med 1990;19: 1088–1092.

255. Zimmerman JL, Dellinger RP, Majid PA. Cocaine-associated chest pain. Ann Emerg Med 1991;20: 611–615.

256. Feldman JA, Bui LD, Mitchell PM, Perera TB, Lee VW, Bernard SA, Fish SS. The evaluation of cocaine-induced chest pain with acute myocardial perfusion imaging. Acad Emerg Med 1999;6:103–109.

257. Baumann BM, Perrone J, Hornig SE, Shofer FS, Hollander JE. Cardiac and hemodynamic assessment of patients with cocaine-associated chest pain syndromes. Clin Toxicol 2000;38:283–290.

258. Cunningham R, Walton MA, Weber JE, O'Broin S, Tripathi SP, Maio RF, Booth BM. One-year medical outcomes and emergency department recidivism after emergency department observation for cocaine-associated chest pain. Ann Emerg Med 2009;53:310–320.

259. Isner JM, Estes NA, Thompson PD, Costanzo-Nordin MR, Subramanian R, Miller G, et al. Acute cardiac events temporally related to cocaine abuse. N Engl J Med 1986;315:1438–1443.

260. Nanji AA, Filpenko JD. Asystole and ventricular fibrillation associated with cocaine intoxication. Chest 1984; 85;132–133.

261. Boag F, Havard CW. Cardiac arrhythmia and myocardial ischemia related to cocaine and alcohol consumption. Postgrad Med J 1985;61:997–999.

262. Rollinger IM, Belzberg AS, MacDonald IL. Cocaine-induced myocardial infarction. Can Med Assoc J 1986; 135:45–46.

263. Schrem SS, Belsky P, Schwartzman D, Slater W. Cocaine induced *torsade de pointes* in a patient with idiopathic long QT syndrome. Am Heart J 1990;120:980–984.

264. Gamouras GA, Monir G, Plunkitt K, Gursoy S, Dreifus LS. Cocaine abuse: repolarization abnormalities and ventricular arrhythmias. Am J Med Sci 2000;320:9–12.

265. Bebarta VS, Summers S. Brugada electrocardiographic pattern induced by cocaine toxicity. Ann Emerg Med 2007;49:827–829.

266. Tanen DA, Graeme KA, Curry SC. Crack cocaine ingestion with prolonged toxicity requiring electrical pacing. Clin Toxicol 2000;38:653–657.

267. Stratton SJ, Rogers C, Brickett K, Gruzinski G. Factors associated with sudden death of individuals requiring restrain for excited delirium. Am J Emerg Med 2001; 19:187–191.

268. Fraker TD Jr, Temsey-Armos PN, Brewster PS, Wilerson RD. Mechanism of cocaine-induced myocardial depression in dogs. Circulation 1990;81:1012–1016.

269. Hogya PT, Wolfson AB. Chronic cocaine abuse associated with dilated cardiomyopathy. Am J Emerg Med 1990;8:203–204.

270. Bertolet BD, Freund G, Martin CA, Perchalski DL, Williams CM, Pepine DJ. Unrecognized left ventricular dysfunction in an apparently healthy cocaine abuse population. Clin Cardiol 1990;13:323–328.

271. Chokshi SK, Moore R, Pandian NG, Isner JM. Reversible cardiomyopathy associated with cocaine intoxication. Ann Intern Med 1989;111:1039–1040.

272. Henzlova MJ, Smith SH, Prchal VM, Helmcke FR. Apparent reversibility of cocaine-induced congestive cardiomyopathy. Am Heart J 1991;122:577–579.

273. Om A, Ellahham S, Ornato JP. Reversibility of cocaine-induced cardiomyopathy. Am Heart J 1992;124: 1639–1641.

274. Brickner ME, Willard JE, Eichhorn EJ, Black J, Grayburn PA. Left ventricular hypertrophy associated with chronic cocaine abuse. Circulation 1991;84:1130–1135.

275. Hsue PY, Salinas CL, Bolger AF, Benowitz NL, Waters DD. Acute aortic dissection related to crack cocaine. Circulation 2002;105:1592–1595.

276. Daniel JC, Huynh TT, Zhou W, Kougias P, El Sayed HF, Huh J, et al. Acute aortic dissection associated with use of cocaine. J Vasc Surg 2007;46:427–433.

277. Singh S, Trivedi A, Adhikari T, Molnar J, Arora R, Khosla S. Cocaine-related acute aortic dissection: patient demographics and clinical outcomes. Can J Cardiol 2007; 23:1131–1134.

278. Barth CW III, Bray M, Roberts WC. Rupture of ascending aorta during cocaine intoxication. Am J Cardiol 1986;57:496.

279. Gadaleta D, Hall MH, Nelson RL. Cocaine-induced acute aortic dissection. Chest 1989;96:1203–1205.

280. Fisher A, Holroyd BR. Cocaine-associated dissection of the thoracic aorta. J Emerg Med 1992;10:723–727.

281. Grannis FW Jr, Bryant C, Caffarantti JD, Turner AF. Acute aortic dissection associated with cocaine abuse. Clin Cardiol 1988;11:572–574.

282. Simons AJ, Arazoza E, Hare CL, Smulyan H, Lighty GW Jr, Parker FB Jr. Circumferential aortic dissection in a young woman. Am Heart J 1992;123:1077–1079.

283. Om A, Porter T, Mohanty PK. Transesophageal echocardiographic diagnosis of acute aortic dissection complicating cocaine abuse. Am Heart J 1992;123:532–534.

284. Chang RA, Rossi NF. Intermittent cocaine use associated with recurrent dissection of the thoracic and abdominal aorta. Chest 1995;108:1758–1762.

285. Lowenstein DH, Massa SM, Rowbotham MC, Collins SD, McKinney HE, Simon RP. Acute neurologic and psychiatric complications associated with cocaine abuse. Am J Med 1987;83:841–846.

286. Rowbotham MC, Lowenstein DH. Neurologic consequences of cocaine use. Annu Rev Med 1990;41:417–422.

287. Fessler RD, Esshaki CM, Stankewitz RC, Johnson RR, Diaz FG. The neurovascular complications of cocaine. Surg Neurol 1997;47:339–345.

288. Daniels J, Baker DG, Norman AB. Cocaine-induced tics in untreated Tourette's syndrome. Am J Psychiatry 1996;153:965.

289. Hegarty AM, Lipton RB, Merriam AE, Freeman K. Cocaine as a risk factor for acute dystonic reactions. Neurology 1991;41:1670–1672.

290. Cardoso FE, Jankovic J. Cocaine-related movement disorders. Mov Disord 1993;8:175–178.

291. Nalls G, Disher A, Daryabagi J, Zant Z, Eisenman J. Subcortical cerebral hemorrhages associated with cocaine abuse: CR and MR findings. J Computer Assist Tomogr 1989;13:1–5.

292. Treadwell SD, Robinson TG. Cocaine use and stroke. Postgrad Med J 2007;83:389–394.

293. Levine SR, Brust JC, Futrell N, Brass LM, Blake D, Fayad P, et al. A comparative study of the cerebrovascular complications of cocaine: alkaloidal versus hydrochloride—a review. Neurology 1991;41:1173–1177.

294. Mangiardi JR, Daras M, Geller ME, Weitzner I, Tuchman AJ. Cocaine-related intracranial hemorrhage: report of nine cases and review. Acta Neurol Scand 1988;77:177–180.

295. Conway JE, Tamargo RJ. Cocaine use is an independent risk factor for cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke 2001;32:2338–2343.

296. Kaye BR, Fainstat ML. Cerebral vasculitis associated with cocaine abuse. JAMA 1987;258:2104–2106.

297. Spivey WH, Euerle B. Neurologic complications of cocaine abuse. Ann Emerg Med 1990;19:1422–1428.

298. Kondziella D, Danielsen ER, Arlien-Soeborg P. Fatal encephalopathy after an isolated overdose of cocaine. J Neurol Neurosurg Psychiatry 2007;78:437–438.

299. Ryan A, Molloy FM, Farrell MA, Hutchinson M. Fatal toxic leukoencephalopathy: clinical, radiological, and necropsy findings in two patients. J Neurol Neurosurg Psychiatry 2005;76:1014–1016.

300. Bianco F, Iacovelli E, Tinelli E, Lepre C, Pauri F. Recurrent leukoencephalopathy in a cocaine abuser. Neurotoxicology 2011;32:410–412.

301. Kramer LD, Locke GE, Ogunyemi A, Nelson L. Cocaine-related seizures in adults. Am J Drug Alcohol Abuse 1990;16:309–317.

302. Mody CK, Miller BL, McIntyre HB, Cobb SK, Goldberg MA. Neurologic complications of cocaine abuse. Neurology 1988;38:1189–1193.

303. Myers JA, Earnest MP. Generalized seizures and cocaine abuse. Neurology 1984;34:675–676.

304. Steele MT, Westdorp EJ, Garza AG, Ma J, Roberts DK, Watson WA. Screening for stimulant use in adult emergency department seizure patients. Clin Toxicol 2000;38:609–613.

305. Holland RW III, Marx JA, Earnest MP, Ranniger S. Grand mal seizures temporally related to cocaine use: clinical and diagnostic features. Ann Emerg Med 1992;21:772–776.

306. Ernst AA. Unexpected cocaine intoxication presenting as seizures in children. Ann Emerg Med 1989;18:774–777.

307. Mott SH, Packer RJ, Soldin SJ. Neurologic manifestations of cocaine exposure in childhood. Pediatrics 1994;93:557–560.

308. Dhuna A, Pascual-Leone A, Belgrade M. Cocaine-related vascular headaches. J Neurol Neurosurg Psychiatry 1991;54:803–806.

309. Lipton RB, Choy-Kwong M, Solomon S. Headaches in hospitalized cocaine users. Headache 1989;29:224–227.

310. Warner EA. Cocaine abuse. Ann Intern Med 1993;119:226–235.

311. Forrester JM, Steele AW, Waldron JA, Parsons PE. Crack lung: an acute pulmonary syndrome with a spectrum of clinical and histopathologic findings. Am Rev Respir Dis 1990;142:462–467.

312. Ettinger NA, Albin RJ. A review of the respiratory effects of smoking cocaine. Am J Med 1989;87:664–668.

313. Bush MN, Rubenstein R, Hoffman I, Bruno MS. Spontaneous pneumomediastinum as a consequence of cocaine use. NY State J Med 1984;84:618–619.

314. Brody SL, Anderson GV Jr, Gutman JB. Pneumomediastinum as a complication of "crack" smoking. Am J Emerg Med 1988;6:241–243.

315. Shesser R, Davis C, Edelstein S. Pneumomediastinum and pneumothorax after inhaling alkaloidal cocaine. Ann Emerg Med 1981;10:213–215.

316. Alnas M, Altayeh A, Zaman M. Clinical course and outcome of cocaine-induced pneumomediastinum. Am J Med Sci 2010;339:65–67.

317. Kline JN, Hirasuna JD. Pulmonary edema after freebase cocaine smoking—not due to an adulterant. Chest 1990;97:1009–1010.

318. Cucco RA, Yoo OH, Cregler L, Chang JC. Nonfatal pulmonary edema after "freebase" cocaine smoking. Am Rev Respir Dis 1987;136:179–181.

319. Allred RJ, Erver S. Fatal pulmonary edema following intravenous "free base" cocaine use. Ann Emerg Med 1981;10:441–442.

320. Rebhun J. Association of asthma and freebase smoking. Ann Allergy 1988;60:339–342.

321. Gordon K III. Case report: freebased cocaine smoking and reactive airway disease. J Emerg Med 1989;7: 145–147.

322. Solini L, Gourgiotis S, Salemis NS, Koukis I. Bilateral pneumothorax, lung cavitations, and pleural empyema in a cocaine addict. Gen Thorac Cardiovasc Surg 2008;56: 610–612.

323. Schwartz RH, Estroff T, Fairbanks DNF, Hoffmann NG. Nasal symptoms associated with cocaine abuse during adolescence. Arch Otolaryngol Head Neck Surg 1989; 115:63–64.

324. Schweitzer VG. Osteolytic sinusitis and pneumomediastinum: deceptive otolaryngologic complications of cocaine abuse. Laryngoscope 1986;96:206–210.

325. Vilensky W. Illicit and licit drugs causing perforation of the nasal septum. J Forensic Sci 1982;27:958–962.

326. Snyder RD, Snyder LB. Intranasal cocaine abuse in an allergists office. Ann Allergy 1985;54:489–492.

327. Sawicka EH, Trosser A. Cerebrospinal fluid rhinorrhea after cocaine sniffing. Br Med J 1983;286:1476–1477.

328. Meleca RJ, Burgio DL, Carr RM, Lolachi CM. Mucosal injuries of the upper aerodigestive tract after smoking crack or freebase cocaine. Laryngoscope 1997;107: 620–625.

329. Blaise G, Vanhooteghem O, de la Brassinne M. Cocaine-sniffing lesions. J Eur Acad Dermatol Venereol 2007;21: 1262–1263.

330. Wang ES. Cocaine-induced iritis. Ann Emerg Med 1991; 20:192–193.

331. Ascaso FJ, Cruz N, Del Buey MA, Cristobal JA. An unusual case of cocaine-induced maculopathy. Eur J Ophthalmol 2009;19:880–882.

332. Sachs R, Zagelbaum BM, Hersh PS. Corneal complications associated with the use of crack cocaine. Ophthalmology 1993;100:187–191.

333. Ghosheh FR, Ehlers JP, Ayres BD, Hammersmith KM, Rapuano CJ, Cohen EJ. Corneal ulcers associated with aerosolized crack cocaine use. Cornea 2007;26:966–969.

334. Amoedo ML, Craver L, Marco MP, Fernandez E. Cocaine-induced acute renal failure without rhabdomyolysis. Nephrol Dial Transplant 1999;14:2970–2971.

335. Saleem TM, Singh M, Murtaza M, Singh A, Kasubhai M, Gnanasekaran I. Renal infarction: a rare complication of cocaine abuse. Am J Emerg Med 2001;19:528–529.

336. Hoefsloot W, de Vries RA, Bruijnen R, Bosch FH. Renal infarction after cocaine abuse: a case report and review. Clin Nephrol 2009;72:234–236.

337. Sudhakar CBS, Al-Hakeem M, MacArthur JD, Sumpio BE. Mesenteric ischemia secondary to cocaine abuse: case reports and literature review. Am J Gastroenterol 1997;92:1053–1054.

338. Ellis CN, McAlexander WW. Enterocolitis associated with cocaine use. Dis Colon Rectum 2005;48:2313–2316.

339. Cheng CL, Svesko V. Acute pyloric perforation after prolonged crack smoking. Ann Emerg Med 1994;32: 126–128.

340. Chan YC, Camprodon RA, Kane PA, Scott-Coombes DM. Abdominal complications from crack cocaine. Ann R Coll Surg Engl 2004;86:47–50.

341. Singhal P, Horowitz B, Quinones MC, Sommer M, Faulkner M, Grosser M. Acute renal failure following cocaine abuse. Nephron 1989;52:76–78.

342. Welch RD, Todd K, Krause GS. Incidence of cocaine-associated rhabdomyolysis. Ann Emerg Med 1991;20: 154–157.

343. Rutenber AJ, McAnally HB, Wetli CV. Cocaine-associated rhabdomyolysis and excited delirium: different stages of the same syndrome. Am J Forensic Med Pathol 1999;20:120–127.

344. Daras M, Kakkouras K, Tuchman AJ, Koppel BS. Rhabdomyolysis and hyperthermia after cocaine abuse: a variant of the neuroleptic malignant syndrome? Acta Neurol Scand 1995;92:161–165.

345. Merigian KS, Roberts JR. Cocaine intoxication: hyperpyrexia, rhabdomyolysis and acute renal failure. Clin Toxicol 1987;135–148.

346. Bauwens JE, Boggs JM, Hartwell PS. Fatal hyperthermia associated with cocaine use. West J Med 1989;150: 210–212.

347. Jandreski MA, Bermes EW, Leischner R, Kahn SE. Rhabdomyolysis in a case of free-base cocaine ("crack") overdose. Clin Chem 1989;35:1547–1549.

348. Chung C, Tumeh PC, Birnbaum R, Tan BH, Sharp L, McCoy E, et al. Characteristic purpura of the ears, vasculitis, and neutropenia—a potential public health epidemic associated with levamisole-adulterated cocaine. J Am Acad Dermatol 2011; [Epub ahead of print]

349. Buchanan JA, Vogel JA, Eberhardt AM. Levamisole-induced occlusive necrotizing vasculitis of the ears after use of cocaine contaminated with levamisole. J Med Toxicol 2011;7:83–84.

350. Chang A, Osterloh J, Thomas J. Levamisole: a dangerous new cocaine adulterant. Clin Pharmacol Ther 2010;88: 408–411.

351. Buchanan JA, Oyer RJ, Patel NR, Jacquet GA, Bornikova L, Thienelt C, et al. A confirmed case of agranulocytosis after use of cocaine contaminated with levamisole. J Med Toxicol 2010;6:160–164.

352. Muirhead TT, Eide MJ. Toxic effects of levamisole in a cocaine user. N Engl J Med 2011;364:352.

353. Roberts JR, Price D, Goldfrank L, Harnet L. The body stuffer syndrome: A clandestine form of drug overdose. Am J Emerg Med 1986;4:22–27.

354. Hoffman RS, Chiang WK, Weisman RS, Goldfrank LR. Prospective evaluation of "crack-vial" ingestions. Vet Hum Toxicol 1990;32:164–167.

355. Traub SJ, Hoffman RS, Nelson LS. Body packing—the internal concealment of illicit drugs. N Engl J Med 2003;349:2519–2526.

356. McCarron MM, Wood JD. The cocaine 'body packer' syndrome. JAMA 1983;250:1417–1420.

357. Sporer KA, Firestone J. Clinical course of crack cocaine body stuffers. Ann Emerg Med 1997;29:596–601.

358. Pollack CV Jr, Biggers DW, Carlton FB Jr, Achord JL, Cranston PE, Eggen JT, Griswold JA. Two crack cocaine body stuffers. Ann Emerg Med 1992;21:1370–1380.

359. Gay GR. Clinical management of acute and chronic cocaine poisoning. Ann Emerg Med 1982;11:562–572.

360. Bettinger J. Cocaine intoxication. Massive oral overdose. Ann Emerg Med 1980;9:429–430.

361. Wetli CV, Mittlemann RE. The "body packer syndrome"— toxicity following ingestion of illicit drugs packaged for transportation. J Forensic Sci 1981;26:492–500.

362. Degenhardt L, Singleton J, Calabria B, McLaren J, Kerr T, Mehta S, et al. Mortality among cocaine uses: a systematic review of cohort studies. Drug Alcohol Depend 2011;113:88–95.

363. Lucena J, Blanco M, Jurado C, Rico A, Salguero M, Vazquez R, et al. Cocaine-related sudden death: a prospective investigation in south-west Spain. Eur Heart J 2010;31:318–329.

364. Bauman JL, Grawe JJ, Winecoff AP, Hariman RJ. Cocaine-related sudden cardiac death: a hypothesis correlating basic science and clinical observations. J Clin Pharmacol 1994;34:902–911.

365. McKelway R, Vieweg V, Westerman P. Sudden death from acute cocaine intoxication in Virginia in 1988. Am J Psychiatry 1990;147:1667–1669.

366. Dressler FA, Malekzadeh S, Roberst WC. Quantitative analysis of amounts of coronary arterial narrowing in cocaine addict. Am J Cardiol 1990;65:303–308.

367. Hsue PY, McManus D, Selby V, Ren X, Pillutla P, Younes N, et al. Cardiac arrest in patients who smoke crack cocaine. Am J Cardiol 2007;99:822–824.

368. Mirchandani HG, Rorke LB, Sekula-Perlman A, Hood IC. Cocaine-induced agitated delirium, forceful struggle, and minor head injury. Am J Forensic Med Pathol 1994;15:95–99.

369. Ruttenber AJ, Lawler-Heavner J, Yin M, Wetli CV, Hearn WL, Mash DC. Fatal excited delirium following cocaine use: epidemiologic findings provide new evidence for mechanism of cocaine toxicity. J Forensic Sci 1997;42: 25–31.

370. Bunai Y, Akaza K, Jiang W-X, Nagai A. Fatal hyperthermia associated with excited delirium during an arrest. Leg Med 2008;10:306–309.

371. Ross DL. Factors associated with excited delirium deaths in police custody. Mod Pathol 1998;11:1127–1137.

372. Prahlow JA, Davis GJ. Death due to cocaine intoxication initially thought to be a homicide. South Med J 1994;87: 255–258.

373. Sofuoglu M, Dudish-Poulsen S, Brown SB, Hatsukami DK. Association of cocaine withdrawal symptoms with more severe dependence and enhanced subjective response to cocaine. Drug Alcohol Depend 2003;69: 273–282.

374. Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Arch Gen Psychiatry 1986;43:107–113.

375. Wellington WW, Brown BS, Haertzen CA, Cone EJ, Dax EM, Herning RI, Michaelson BS. Changes in mood, craving, and sleep during short-term abstinence reported by male cocaine addicts. A controlled, residential study. Arch Gen Psychiatry 1990;47;861–868.

376. Gouin K, Murphy K, Shag PS, and the Knowledge Synthesis group on Determination of Low Birth Weight and Preterm Births. Effects of cocaine use during pregnancy on low birthweight and preterm birth: systematic review and metaanalyses. Am J Obstet Gynecol 2011; 204:340e1-e12.

377. Fajemirokun-Odudeyi O, Lindow SW. Obstetric implications of cocaine use in pregnancy: a literature review. Eur J Obstet Gynecol Reprod Biol 2004;112:2–8.

378. Richardson GA, Godschmidt L, Larkby C. Effects of prenatal cocaine exposure on growth: a longitudinal analysis. Pediatrics 2007;120:e1017–e1027.

379. Brown JV, Bakeman R, Coles CD, Platzman KA, Lynch ME. Prenatal cocaine exposure: a comparison of 2-year-old children in parental and nonparental care. Child Dev 2004;75:1282–1295.

380. Macones GA, Sehdev HM, Parry S, Morgan MA, Berlin JA. The association between maternal cocaine use and placenta previa. Am J Obstet Gynecol 1997;177: 1097–1100.

381. Richardson GA, Day NL. Maternal and neonatal effects of moderate cocaine use during pregnancy. Neurotoxicol Teratol 1991;13:455–460.

382. Oro AS, Dixon SD. Perinatal cocaine and methamphetamine exposure: maternal and neonatal correlates. J Pediatr 1987;111:571–578.

383. Lewis BA, Singer LY, Short EJ, Minnes S, Arendt R, Weishampel P, et al. Four-year language outcomes of children exposed to cocaine *in utero*. Neurotoxicol Teratol 2004;26:617–627.

384. Young SL, Vosper HJ, Phillips SA. Cocaine: its effects on maternal and child health. Pharmacotherapy 1992;12: 2–17.

385. Singer LT, Arendt R, Minnes S, Farkas K, Salvator A, Kirchner HL, Kliegman R. Cognitive and motor outcomes of cocaine-exposed infants. JAMA 2002;287: 1952–1960.

386. Bendersky M, Bennett D, Lewis M. Aggression at age 5 as a function of prenatal exposure to cocaine, gender, and environmental risk. J Pediatr Psychol 2006;32:71–84.

387. Linares TJ, Singer LT, Kirchner L, Short EJ, Min MO, Hussey P, et al. Mental health outcomes of cocaine-exposed children at 6 years of age. J Pediatr Psychol 2006;31:85–97.

388. Chirigoga CA, Kuhn L, Wasserman GA. Prenatal cocaine exposures and dose-related cocaine effects on infant tone and behavior. Neurotoxicol Teratol 2007;29:323–330.

389. Beeghly M, Martin B, Rose-Jacobs R, Cabral H, Heeren T, Augustyn M, et al. Prenatal cocaine exposure and children's language functioning at 6 and 9.5 years: moderating effects of child age, birthweight, and gender. J Pediatr Psychol 2006;31:98–115.

390. Lewis MW, Misra S, Johnson HL, Rosen TS. Neurological and developmental outcomes of prenatally cocaine-exposed offspring from 12 to 36 months. Am J Drug Alcohol Abuse 2004;30;299–320.

391. Nordstrom-Klee B, Delaney-Black V, Covington C, Ager J, Slkol R. Growth from birth onwards of children prenatally exposed to drugs a literature review. Neurotoxicol Teratol 2003;24:481–488.

392. Bingol N, Fuchs M, Diaz V, Stone RK, Gromisch DS. Teratogenicity of cocaine in humans. J Pediatr 1987;110:93–96.

393. Graham K, Feigenbaum A, Pastuszak A, Nulman I, Weksberg R, Einarson T, et al. Pregnancy outcome and infant development following gestational cocaine use by social cocaine users in Toronto, Canada. Clin Invest Med 1992;15:384–394.

394. Bennett DS, Bendersky M, Lewis M. Children's intellectual and emotional-behavioral adjustment at 4 years as a function of cocaine exposure, maternal characteristics, and environmental risk. Dev Psychiatry 2002;38;648–658.

395. Frank DA, Augustyn M, Knight WG, Pell T, Zuckerman B. Growth, development, and behavior in early childhood following prenatal cocaine exposure. JAMA 2001;285;1613–1625.

396. Kain ZN, Kain TS, Scarpelli EM. Cocaine exposure *in utero*: perinatal development and neonatal manifestations—review. Clin Toxicol 1992;30:607–636.

397. Fleming SW, Dasgupta A, Garg U. Quantitation of cocaine, benzoylecgonine, ecgonine methyl ester, and cocaethylene in urine and blood using gas chromatography-mass spectrometry (GC-MS). Methods Mol Biol 2010;603:145–156.

398. Braithwaite RA, Jarvie DR, Minty PSB, Simpson D, Widdop B. Screening for drugs of abuse. I: Opiates, amphetamines and cocaine. Ann Clin Biochem 1995;32:123–153.

399. Foltz RL, Botelho C, Reuschel SA, Kuntz DJ, Moody DE, Bristow GM. Comparison of immunoassays for semi-quantitative measurement of benzoylecgonine in urine. NIDA Res Monogr 1997;175:287–302.

400. Baker JE, Jenkins AJ. Screening for cocaine metabolite fails to detect an intoxication. Am J Forensic Med Pathol 2008;29:141–144.

401. Kriger S, Gunn J, Terrell AR. Identification and quantitation of cocaine, benzoylecgonine, and cocaethylene in blood, serum, and plasma using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Methods Mol Biol 2010;603:157–164.

402. Langman LJ, Bjergum MW, Williamson CL, Crow FW. Sensitive method for detection of cocaine and associated analytes by liquid chromatography-tandem mass spectrometry in urine. J Anal Toxicol 2009;33:447–455.

403. Moeller MR, Steinmeyer S, Kraemer T. Determination of drugs of abuse in blood. J Chromatogr B 1998;713:91–109.

404. Corburt MR, Koves EM. Gas chromatography/mass spectrometry for the determination of cocaine and benzoylecgonine over a wide concentration range (<0.005-5 mg/dL) in postmortem blood. J Forensic Sci 1994;39:136–149.

405. Phillips DL, Tebbett IR, Bertholf RL. Comparison of HPLC and GC-MS for measurement of cocaine and metabolites in human urine. J Anal Toxicol 1996;20:305–308.

406. Popa D-S, Vlase L, Leucuta SE, Loghin F. Determination of cocaine and benzoylecgonine in human plasma by LC-MS/MS. Farmacia 2009;57:301–308.

407. Stout PR, Bynum ND, Mitchell JM, Baylor RM, Ropero-Miller JD. A comparison of the validity of gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry analysis of urine samples for morphine, codeine, 6-acetylmorhine, and benzoylecgonine. J Anal Toxicol 2009;33:398–408.

408. Jacob P, Lewis E, Elias-Baker B, Jones R. A pyrolysis product, anhydroecgonine methyl ester (methylecgonidine), is in the urine of cocaine smokers. J Anal Toxicol 1990;14;353–355.

409. Cone EJ, Hillsgrove M, Darwin WD. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. Clin Chem 1994;40;1299–1305.

410. Wang PP, Bartlett MG. Identification and quantitation of cocaine *N*-oxide: a thermally labile metabolite of cocaine. J Anal Toxicol 1999;23:62–66.

411. Turner CE, Ma C-Y, Elsohly MA. Constituents of *Erythroxylon coca*. II. Gas-chromatographic analysis of cocaine and other alkaloids in coca leaves. J Ethnopharmacol 1981;3:293–298.

412. Ensing JG, Racemy C, de Zeeuw RA. A rapid gas chromatographic method for the fingerprinting of illicit cocaine samples. J Forensic Sci 1992;37:446–459.

413. Moore JM, Cooper DA, Lurie IS, Kram TC, Carr S, Harper C, Yeh J. Capillary gas chromatographic-electron capture detection of coca-leaf-related impurities in illicit cocaine: 2,4-diphenylcyclobutane-1,3-dicarboxylic acids, 1,4-diphenylcyclobutane-2,3-dicarboxylic acids and their alkaloidal precursors, the truxillines. J Chromatogr 1987;410:297–318.

414. Janzen KE, Walter L, Fernando AR. Comparison analysis of illicit cocaine samples. J Forensic Sci 1992;37:436–445.

415. Ensing JG, Hummelen JC. Isolation, identification, and origin of three previously unknown congeners in illicit cocaine. J Forensic Sci 1991;36:1666–1687.

416. Chiarotti M, Fucci N. Comparative analysis of heroin and cocaine seizures. J Chromatogr B 1999;733:127–136.

417. Ensing JG, Racamy C, de Zeeuw RA. A rapid gas chromatographic method for the fingerprinting of illicit cocaine samples. J Forensic Sci 1992;37:446–459.

418. Griesemer EC, Liu Y, Budd RD, Raftogianis L, Noguchi TT. The determination of cocaine and its major metabolite, benzoylecgonine, in postmortem fluids and tissues by computerized gas chromatography/mass spectrometry. J Forensic Sci 1983;28:894–900.

419. Isenschmid DS, Levine BS, Caplan YH. A comprehensive study of the stability of cocaine and its metabolites. J Anal Toxicol 1989;13:250–256.

420. Isenschmid DS, Levine BS, Caplan YH. The role of ecgonine methyl ester in the interpretation of cocaine concentrations in postmortem blood. J Anal Toxicol 1992; 16:319–324.

421. Brogan WC, Kemp PM, Bost RO, Glamann DB, Lange RA, Hillis LD. Collection and handling of clinical blood samples to assure the accurate measurement of cocaine concentration. J Anal Toxicol 1992;16:152–154.

422. Giorgi SN, Meeker JE. A 5-year stability study of common illicit drugs in blood. J Anal Toxicol 1995;19:392–398.

423. Skopp G, Klingmann A, Pötsch L, Mattern R. *In vitro* stability of cocaine in whole blood and plasma including ecgonine as a target analyte. Ther Drug Mon 2001;23: 174–181.

424. Liu Y, Budd RD, Griesemer EC. Study of the stability of cocaine and benzoylecgonine, its major metabolite, in blood samples. J Chromatogr 1982;248:318–320.

425. Taylor D, Estevez VS, Englert LF, Ho BT. Hydrolysis of carbon-labeled cocaine in human serum. Res Commun Chem Pathol Pharmacol 1976;14:249–257.

426. Moody DE, Monti KM, Spanbauer AC. Long-term stability of abused drugs and antiabuse chemotherapeutical agents stored at −20 degrees C. J Anal Toxicol 1999;23: 535–540.

427. Manhoff DT, Hood I, Caputo F, Perry J, Rosen S, Mirchandani HG. Cocaine in decomposed human remains. J Forensic Sci 1991;36:1732–1735.

428. Hippenstiel MJ, Gerson B. Optimization of storage conditions for cocaine and benzoylecgonine in urine: a review. J Anal Toxicol 1994;18:104–109.

429. Romberg RW, Past MR. Reanalysis of forensic urine specimens containing benzoylecgonine and THC-COOH. J Forensic Sci 1994;39:479–485.

430. Levine B, Ramcharitar V, Smialek JE. Stability of ecgonine methyl ester in postmortem urine specimens. J Forensic Sci 1996;41:126–128.

431. Viel G, Nalesso A, Cecchetto G, Montisci M, Ferrara SD. Stability of cocaine in formalin solution and fixed tissues. Forensic Sci Int 2009;193:79–83.

432. Sukbuntherng J, Martin DK, Pak Y, Mayersohn M. Characterization of the properties of cocaine in blood: blood clearance, blood to plasma ratio, and plasma protein binding. J Pharm Sci 1996;85:567–571.

433. Fernández P, Aldonza M, Bouzas A, Lema M, Bermejo AM, Tabernero MJ. GC-FID determination of cocaine and its metabolites in human bile and vitreous humor. J Appl Toxicol 2006;26:253–257.

434. Fernancez P, Aldonza M, Bermejo AM, Tabernero MJ. Bile analysis for cocaine and benzoylecgonine in overdose cases. J Liquid Chromatogr Relat Technologies 2008;31:2467–2474.

435. Vanbinst R, Koenig J, Di Fazio V, Hassoun A. Bile analysis of drugs in postmortem cases. Forensic Sci Int 2002; 128:35–40.

436. Poklis A, Maginn D, Barr JL. Tissue disposition of cocaine in man; a report of five fatal poisonings. Forensic Sci Int 1987;33:83–88.

437. Chiou WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (Part I). Clin Pharmacokinet 1989;17:175–199.

438. Zahler R, Wachtel P, Jatlow P, Byck R. Kinetics of drug effect by distributed lags analysis: An application to cocaine. Clin Pharmacol Ther 1982;31:775–782.

439. Isenschmid DS, Fischman MW, Foltin RW, Caplan YH. Concentration of cocaine and metabolites in plasma of humans following intravenous administration and smoking of cocaine. J Anal Toxicol 1992;16:311–314.

440. Rerat C, Sauvain M, Rop PP, Ruiz E, Bresson M, Viala A. Liquid chromatographic analysis of cocaine and benzoylecgonine in plasma of traditional coca chewers from Bolivia during exercise. J Ethnopharmacol 1997;56: 173–178.

441. de Prost N, Megarbane B, Questel F, Bloch V, Bertaux DC, Pourriat JL, Rabbat A. Blood cocaine and metabolite pharmacokinetics after cardiac arrest in a bodypacker case. Hum Exp Toxicol 2010;29:49–53.

442. Blaho K, Logan B, Winbery S, Park L, Schwilke E. Blood cocaine and metabolite concentrations, clinical findings, and outcome of patients presenting to an ED. Am J Emerg Med 2000;18:593–598.

443. Moriya F, Hashimoto Y. Postmortem stability of cocaine and cocaethylene in blood and tissues of humans and rabbits. J Forensic Sci 1996;41:612–616.

444. Logan BK, Smirnow D, Gullberg RG. Lack of predictable site-dependent differences and time-dependent changes in postmortem concentrations of cocaine, benzoylecgonine, and cocaethylene in humans. J Anal Toxicol 1997;20:23–31.

445. Hearn WL, Keran EE, Wei HA, Hime G. Site-dependent postmortem changes in blood cocaine concentrations. J Forensic Sci 1991;36:673–684.

446. Patel F. A high fatal postmortem blood concentration of cocaine in a drug courier. Forensic Sci Int 1996;7: 167–174.

447. Amon CA, Tate LG, Wright RK, Matusiak W. Sudden death due to ingestion of cocaine. J Anal Toxicol 1986; 10:217–218.

448. Baselt RC. Disposition of Toxic Drugs and Chemicals in Man. 8th ed. Foster City, CA: Biomedical Publications; 2008.

449. Molina DK, Hargrove VM. Fatal cocaine interactions a review of cocaine-related deaths in Bexar County, Texas. Am J Forensic Med Pathol 2011;32:71–77.

450. Pollanen MS, Chiasson DA, Cairnes JT, Young JG. Unexpected death related to restraint for excited delirium: a retrospective study of deaths in police custody and in the community. CMAJ 1998;158:1603–1607.

451. Karch SB. Cocaine cardiovascular toxicity. South Med J 2005;98:794–799.

452. Browne SP, Moore CM, Scheurer J, Tebbett IR, Logan BK. A rapid method for the determination of cocaine in brain tissue. J Forensic Sci 1991;36:1662–1665.

453. Spiehler VR, Reed D. Brain concentrations of cocaine and benzoylecgonine in fatal cases. J Forensic Sci 1985; 30:1003–1011.

454. Toennes SW, Fandino AS, Hesse FJ, Kauert GF. Artifact production in the assay of anhydroecgonine methyl ester in serum using gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2003; 792:345–351.

455. Kintz P, Cirimele V, Sengler C, Mangin P. Testing human hair and urine for anhydroecgonine methyl ester, a pyrolysis product of cocaine. J Anal Toxicol 1995;19:479–482.

456. Cone EJ, Hillsgrove M, Darwin WD. Simultaneous measurement of cocaine, cocaethylene, their metabolites, and "crack" pyrolysis products by gas chromatography-mass spectrometry. Clin Chem 1994;40:1299–1305.

457. Scheidweiler KB, Plessinger MA, Shojaie J, Wood RW, Kwong TC. Pharmacokinetics and pharmacodynamics of methylecgonidine, a crack cocaine pyrolyzate. J Pharmacol Exp Ther 2003;307:1179–1187.

458. Paul BD, McWhorter LK, Smith ML. Electron ionization mass fragmentometric detection of urinary ecgonidine, a hydrolytic product of methylecgonidine, as an indicator of smoking cocaine. J Mass Spectrom 1999;34:651–660.

459. Shimomura ET, Hodge GD, Paul BD. Examination of postmortem fluids and tissues for the presence of methylecgonidine, ecgonidine, cocaine, and benzoylecgonine using solid-phase extraction and gas chromatography-mass spectrometry. Clin Chem 2001;47:1040–1047.

460. Martz R, Donnelly B, Fetterolf D, Lasswell L. The use of hair analysis to document a cocaine overdose following a sustained survival period before death. J Anal Toxicol 1991;15:279–281.

461. Blank DL, Kidwell DA. Decontamination procedures for drugs of abuse in hair: are they sufficient? Forensic Sci Int 1995;70:13–38.

462. Paulsen RB, Wilkins DG, Slawson MH, Shaw K, Rollins DE. Effect of four laboratory decontamination procedures on the quantitative determination of cocaine and metabolites in hair by HPLC-MS. J Anal Toxicol 2001; 25:490–496.

463. Hubbard DL, Wilkins DG, Rollins DE. The incorporation of cocaine and metabolites into hair: effects of dose and hair pigmentation. Drug Metab Dispos 2000;28: 1464–1469.

464. Reid RW, O'Connor FL, Deakin AG, Ivery DM, Crayton JW. Cocaine and metabolites in human graying hair: pigmentary relationship. Clin Toxicol 1996;34:685–690.

465. Cognard E, Rudaz S, Bouchonnet S, Staub C. Analysis of cocaine and three of its metabolites in hair by gas chromatography-mass spectrometry using ion-trap detection for CI/MS/MS. J Chromatogr B 2005;826: 17–25.

466. Felli M, Martello S, Marsili R, Chiarotti M. Disappearance of cocaine from human hair after abstinence. Forensic Sci Int 2005;154:96–98.

467. Schaffer MI, Wang W-L, Irving J. An evaluation of two wash procedures for the differentiation of external contamination versus ingestion in the analysis of human hair samples for cocaine. J Anal Toxicol 2002;26:485–488.

468. Romano G, Barbera N, Lombardo I. Hair testing for drugs of abuse: evaluation of external cocaine contamination and risk of false positive. Forensic Sci Int 2001; 123:119–129.

469. Nakahara Y, Kikura R. Hair analysis for drugs of abuse VII. The incorporation rates of cocaine, benzoylecgonine and ecgonine methyl ester into rat hair and hydrolysis of cocaine in rat hair. Arch Toxicol 1994;68:545–559.

470. Henderson GL, Harkey MR, Zhou C. Cocaine and metabolite concentrations in the hair of South American coca chewers. J Anal Toxicol 1992;16:199–201.

471. Cone EJ, Yousefnejad D, Darwin WD, Maguire T. Testing human hair for drugs of abuse. II. Identification of unique cocaine metabolites in hair of drug abusers and evaluation of decontamination procedures. J Anal Toxicol 1991; 15:250–255.

472. Skipp G, Potsch L. Perspiration versus saliva—basic aspects concerning their use in roadside drug testing. Int J Legal Med 1999;112;213–221.

473. Cone EJ, Oyler J, Darwin WD. Cocaine disposition in saliva following intravenous, intranasal, and smoked administration. J Anal Toxicol 1997;21:465–475.

474. Moolchan ET, Cone EJ, Wstadik A, Huestis MA, Preston KL. Cocaine and metabolite elimination patterns in chronic cocaine users during cessation: plasma and saliva analysis. J Anal Toxicol 2000;24:458–466.

475. Thompson LK, Yousefnejad D, Kumor K, Sherer M, Cone EJ. Confirmation of cocaine in human saliva after intravenous use. J Anal Toxicol 1987;11:36–38.

476. Osterloh H. Testing for drugs of abuse pharmacokinetic considerations for cocaine in urine. Clin Pharmacokinet 1993;24:355–361.

477. Oyler J, Darwin WD, Preston KL, Suess P, Cone EJ. Cocaine disposition in meconium from newborns of cocaine-abusing mothers and urine of adult drug users. J Anal Toxicol 1996;20:453–462.

478. Jenkins AJ, Llosa T, Montoya I, Cone EJ. Identification and quantitation of alkaloids in coca tea. Forensic Sci Int 1996;77:179–189.

479. Altieri M, Bogema S, Schwartz RH. TAC topical anesthesia produces positive urine tests for cocaine. Ann Emerg Med 1990;19:577–579.

480. Warner A. Interference of common household chemicals in immunoassay methods for drugs of abuse. Clin Chem 1989;35:648–651.

481. Cone EJ, Lange R, Darwin WD. *In vivo* adulteration: excess fluid ingestion causes false-negative marijuana and cocaine urine test results. J Anal Toxicol 1998;22:460–473.

482. Cone EJ, Menchen SL, Paul BD, Mell LD, Mitchel J. Validity testing of commercial urine cocaine metabolite assays: I. Assay detection times, individual excretion patterns, and kinetics after cocaine administration to humans. J Forensic Sci 1989;332:15–31.

483. Baselt RC, Chang R. Urinary excretion of cocaine and benzoylecgonine following oral ingestion in a single subject. J Anal Toxicol 1987;11:81–82.

484. Office of the Secretary, Department of Transportation. Procedures for transportation workplace drug and alcohol testing programs. Fed Regist 2010;75:49850–49864.

485. Bruns AD, Zieske LA, Jacobs AJ. Analysis of the cocaine metabolite in the urine of patients and physicians during clinical use. Otolaryngol Head Neck Surg 1994;111:722–726.

486. Preston KL, Epstein DH, Cone EJ, Wtsadik AT, Huestis MA, Moolchan ET. Urinary elimination of cocaine metabolites in chronic cocaine users during cessation. J Anal Toxicol 2002;26:393–400.

487. Cone E, Weddington W. Prolonged occurrence of cocaine in human saliva and urine after chronic use. J Anal Toxicol 1989;13:65–68.

488. Burke WM, Ravi NV, Dhopesh V, Vandegrift B, Maany I. Prolonged presence of metabolite in urine after compulsive cocaine use. J Clin Psychiatry 1990;51:145–148.

489. Weiss RD, Gawin FH. Protracted elimination of cocaine metabolites in long-term high-dose cocaine abusers. Am J Med 1988;85:879–880.

490. Reisfield GM, Haddad J, Wilson GR, Johannsen LM, Voorhees KL, Chronister CW, et al. Failure of amoxicillin to produce false-positive urine screens for cocaine metabolite. J Anal Toxicol 2008;32:315–318.

491. Wilkins JN. Quantitative urine levels of cocaine and other substances of abuse. NIDA Res Monogr 1997;175:235–252.

492. Kavanagh KT, Maijub AG, Brown JR. Passive exposure to cocaine in medical personnel and its effect on urine drug screening tests. Otolaryngol Head Neck Surg 1992;107:363–366.

493. Cone EJ, Yousefnejad D, Hillsgrove MJ, Holicky B, Darwin WD. Passive inhalation of cocaine. J Anal Toxicol 1995;19:399–411.

494. Mackey-Bojack S, Kloss J, Apple F. Cocaine, cocaine metabolite, and ethanol concentrations in postmortem blood and vitreous humor. J Anal Toxicol 2000;24:59–65.

495. McKinney PE, Phillips S, Gomez Hf, Brent J, MacIntyre M, Watson WA. Vitreous humor cocaine and metabolite concentrations: do postmortem specimens reflect blood levels at the time of death? J Forensic Sci 1995;40:102–107.

496. McCarroll KA, Roszler MH. Lung disorders due to drug abuse. J Thorac Imaging 1991;6:30–35.

497. Itkonen J, Schnoll S, Glassroth J. Pulmonary dysfunction in 'freebase' cocaine users. Arch Intern Med 1984;144:2195–2197.

498. Kleerup EC, Koyal SN, Marques-Magallanes JA, Goldman MD, Tashkin DP. Chronic and acute effects of "crack" cocaine on diffusing capacity, membrane diffusion, and pulmonary capillary blood volume in the lung. Chest 2002;122:629–638.

499. Hollander JE, Lozano M, Fairweather P, Goldstein E, Gennis P, Brogan GX, et al. "Abnormal" electrocardiograms in patients with cocaine-associated chest pain are due to "normal" variants. J Emerg Med 1994;12:199–205.

500. Kontos MC, Anderson FP, Ornato JP, Tatum JL, Jesse RL. Utility of troponin I in patients with cocaine-associated chest pain. Acad Emerg Med 2002;9:1007–1013.

501. McLaurin M, Apple FS, Henry TD, Sharkey SW. Cardiac troponin I and T concentrations in patients with cocaine-associated chest pain. Ann Clin Biochem 1996;33:183–186.

502. Burday MJ, Martin SE. Cocaine-associated thrombocytopenia. Am J Med 1991;91:656–660.

503. Czuchlewski DR, Brackney M, Ewers C, Manna J, Fekrazad MH, Martinez A, et al. Clinicopathologic features of agranulocytosis in the setting of levamisole-tainted cocaine. Am J Clin Pathol 2010;133:466–472.

504. Hassan TB, Pickett JA, Durham S, Barker P. Diagnostic indicators in the early recognition of severe cocaine intoxication. J Accid Emerg Med 1996;13:261–263.

505. Hick JL, Smith SW, Lynch MT. Metabolic acidosis in restrain-associated cardiac arrest: a case series. Acad Emerg Med 1999;6:239–243.

506. Bozzuto TM. Severe metabolic acidosis secondary to exertional hyperlactemia. Am J Emerg Med 1988;6:134–136.

507. Bethke R, Gratton M, Watson WA. Severe hyperlactemia and metabolic acidosis following cocaine use and exertion. Am J Emerg Med 1990;8:369–370.

508. Schmidt P, Snowden T. The effects of positional restraint on heart rate and oxygen saturation. J Emerg Med 1999;17:777–782.

509. Chan TC, Vilke GM, Neuman T, Clausen JL. Restraint position and positional asphyxia. Ann Emerg Med 1997;30:578–586.

510. Chan TC, Neuman T, Clausen J, Eisele J, Vilke GM. Weight force during prone restraint and respiratory function. Am J Forensic Med Pathol 2004;25:185–189.

511. Strickland TL, Mena I, Villanueva-Meyer J, Miller BL, Cummings J, Mehringer CM, et al. Cerebral perfusion and neuropsychological consequences of chronic cocaine use. J Neuropsychiatry Clin Neurosci 1993;5:419–427.

512. Jovanovski D, Erb S, Zakzanis KK. Neurocognitive deficits in cocaine users: a quantitative review of the evidence. J Clin Exp Neuropsychol 2005;27;189–204.

513. Bartzokis G, Goldstein IB, Hance DB, Beckson M, Shapiro D, Lu PH, et al. The incidence of T2-weighted MR imaging signal abnormalities in the brain of cocaine-dependent patients is age-related and region-specific. AJNR Am J Neuroradiol 1999;20:1628–1635.

514. Beerman R, Nunez D Jr, Wetli CV. Radiographic evaluation of the cocaine smuggler. Gastrointest Radiol 1986; 11:351–354.

515. Hartoko TJ, Demey HE, De Schepper AM, Beaucourt LE, Bossaert LL. The body packer syndrome–cocaine smuggling in the gastro-intestinal tract. Klin Wochenschr 1988;66:1116–1120.

516. Traub SJ, Kohn GL, Hoffman RS, Nelson LS. Pediatric "body packing". Arch Pediatr Adolesc Med 2003;157: 174–177.

517. van Geloven AAW, van Lienden KP, Gouma DJ. Body packing—an increasing problem in the Netherlands: conservative or surgical treatment? Eur J Surg 2002;168: 404–409.

518. Gherardi R, Marc B, Alberti X, Baud F, Diamant-Berger O. A cocaine body packer with normal abdominal plain radiograms—value of drug detection in urine and contrast study of the bowel. Am J Forensic Med Pathol 1990;11:154–157.

519. Hierholzer J, Cordes M, Tantow H, Keske U, Maurer J, Felix R. Drug smuggling by ingested cocaine-filled packages: conventional x-ray and ultrasound. Abdom Imaging 1995;20:333–338.

520. Nihira M, Hayashida M, Ohno Y, Inuzuka S, Yokota H, Yamamoto Y. Urinalysis of body packers in Japan. J Anal Toxicol 1998;22:61–65.

521. Bogusz MJ, Althoff H, Erkens M, Maier RD, Hofmann R. Internally concealed cocaine: analytical and diagnostic aspects. J Forensic Sci 1995;40:811–815.

522. Dinn H. A case of impaired driving and the role of cocaine: analytical considerations and interpretation. Can Soc Forensic Sci J 2004;37:163–168.

523. Siegel RK. Cocaine use and driving behavior. Alcohol Drugs Driv 1987;3:1–8.

524. Jones AW, Holmgren A, Kugelberg FC. Concentrations of cocaine and its major metabolite benzoylecgonine in blood samples from apprehended drivers in Sweden. Forensic Sci Int 2008;177:133–139.

525. Jenkins AJ, Keenan RM, Henningfield JE, Cone EJ. Correlation between pharmacological effects and plasma cocaine concentrations after smoked administration. J Anal Toxicol 2002;26:382–392.

526. Dussault C, Brault M, Lemire AM, Bouchard J. The role of cocaine in fatal crashes: first results of the Québec drug study. Annu Proc Assoc Adv Automot Med 2001; 45:125–137.

527. Carmen del Rio M, Gomez J, Sancho M, Alvarez FJ. Alcohol, illicit drugs and medicinal drugs in fatally injured drivers in Spain between 1991 and 2000. Forensic Sci Int 2002;127:63–70.

528. Drummer OH, Gerostamoulos J, Batziris H, Chu M, Caplehorn JR, Robertson MD, Swann P. The incidence of drugs in drivers killed in Australian road traffic crashes. Forensic Sci Int 2003;134:154–162.

529. Schwilke EW, Sampaio dos Santos MI, Logan BK. Changing patterns of drug and alcohol use in fatally injured drivers in Washington State. J Forensic Sci 2006; 51:1191–1198.

530. Mercer GW, Jeffery WK. Alcohol, drugs, and impairment in fatal traffic accidents in British Columbia. Accid Anal Prev 1995;27:335–343.

531. Brookoff D, Cook CS, Williams C, Mann CS. Testing reckless drivers for cocaine and marijuana. N Engl J Med 1994;331:518–522.

532. Drake TR, Henry T, Marx J, Gabow PA. Severe acid-base abnormalities associated with cocaine abuse. J Emerg Med 1990;8:331–334.

533. Shih RD, Hollander JE, Burstein JL, Nelson LS, Hoffman RS, Quick AM. Clinical safety of lidocaine in patients with cocaine-associated myocardial infarction. Ann Emerg Med 1995;26:702–706.

534. Kerns W 2nd, Garvey L, Owens J. Cocaine-induced wide complex dysrhythmia. J Emerg Med 1997;15:321–329.

535. Hoffman S. Treatment of patients with cocaine-induced arrhythmias: bringing the bench to the bedside. Br J Clin Pharmacol 2010;69:448–457.

536. Hollander JE, Hoffman RS, Gennis P, Fairweather P, DiSano MJ, Schumb DA, et al. Nitroglycerin in the treatment of cocaine associated chest pain—clinical safety and efficacy. Clin Toxicol 1994;32:243–256.

537. Brogan WC, Lange RA, Kim AS, Moliternao DJ, Hillis LD. Alleviation of cocaine-induced coronary vasoconstriction by nitroglycerin. J Am Coll Cardiol 1991;18: 581–586.

538. Honderick T, Williams D, Seaberg D, Wears R. A prospective, randomized, controlled trial of benzodiazepines and nitroglycerine or nitroglycerine alone in the treatment of cocaine-associated acute coronary syndromes. Am J Emerg Med 2003;21:39–42.

539. Leducq N, Bono F, Sulpice T, Vin V, Janiak P, Fur GL, O'Connor SE, Herbert JM. Role of peripheral benzodiazepine receptors in mitochondrial, cellular, and cardiac damage induced by oxidative stress and ischemia-reperfusion. J Pharmacol Exp Ther 2003;306:828–837.

540. McCord J, Jneid H, Hollander JE, de Lemos JA, Cercek B, Hsue P, and the American Heart Association Acute

Cardiac Care Committee of the Council on Clinical Cardiology. Management of cocaine-associated chest pain and myocardial infarction: a scientific statement from the American Heart Association Acute Cardiac Care Committee of the Council on Clinical Cardiology. Circulation 2008;117:1897–1907.

541. Pollack CV Jr, Gibler WB. 2000 ACC/AHA Guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: a practical summary for emergency physicians. Ann Emerg Med 2001;38:229–248.

542. Chan GM, Sharma R, Price D, Hoffman RS, Nelson LS. Phentolamine therapy for cocaine-associated acute coronary syndrome (CAACS). J Med Toxicol 2006;2:108–111.

543. Dattilo PB, Hailpern SM, Fearon K, Sohal D, Nordin C. β-Blockers are associated with reduced risk of myocardial infarction after cocaine use. Ann Emerg Med 2008;51:117–125.

544. Mohamad T, Kondur A, Vaitkevicius P, Bachour K, Thatai D, Afonso L. Cocaine-induced chest pain and beta-blockade: an inner city experience. Am J Ther 2008;15:531–535.

545. Fareed FN, Chan GM, Hoffman RS. Death temporally related to the use of a beta adrenergic receptor antagonist in cocaine associated myocardial infarction. J Med Toxicol 2007;3:169–172.

546. Schwartz BG, Rezkalla S, Kloner RA. Cardiovascular effects of cocaine. Circulation 2010;122:2558–2569.

547. Boehrer JD, Moliterno DJ, Willard JE, Hillis LD, Lange RA. Influence of labetalol on cocaine-induced coronary vasoconstriction in humans. Am J Med 1993;94:608–610.

548. Ramoska E, Sacchetti AD. Propranolol-induced hypertension in treatment of cocaine intoxication. Ann Emerg Med 1985;14:1112–1113.

549. Tomaszewski C, Voorhees S, Wathen J, Brent J, Kulig K. Cocaine adsorption to activated charcoal *in vitro*. J Emerg Med 1992;10:59–62.

550. Cranston PE, Pollack CV Jr, Harrison RB. CT of crack cocaine ingestion. J Comput Assist Tomogr 1992;16:560–563.

551. Marc B, Baud FJ, Maison-Blanche P, Leporc P, Garnier M, Gherardi R. Cardiac monitoring during medical management of cocaine body packers. Clin Toxicol 1992;30:387–397.

552. Makosiej FJ, Hoffman RS, Howland MA, Goldfrank LR. An *in vitro* evaluation of cocaine hydrochloride adsorption by activated charcoal and desorption upon addition of polyethylene glycol electrolyte lavage solution. Clin Toxicol 1993;31:381–395.

553. Yang W, Xue L, Fang L, Chen X, Zhan CG. Characterization of a high-activity mutant of human butyrylcholinesterase against (-)-cocaine. Chem Biol Interact 2010;187:148–152.

554. Hollander JE, Shih RD, Hoffman RS, Harchelroad FP, Phillips S, Brent J, et al. Predictors of coronary artery disease in patients with cocaine-associated myocardial infarction. Cocaine-Associated Myocardial Infarction (CAMI) Study Group. Am J Med 1997;102:158–163.

555. Weber JE, Shofer FS, Larkin GL, Kalaria AS, Hollander JE. Validation of a brief observation period for patients with cocaine-associated chest pain. N Eng J Med 2003;348:510–517.

556. Brody SL, Wrenn KD, Wilber MM, Slovis CM. Predicting the severity of cocaine-associated rhabdomyolysis. Ann Emerg Med 1990;19:1137–1143.

557. Norfolk GA. The fatal case of a cocaine body-stuffer and a literature review—towards evidence based management. J Forensic Legal Med 2007;14:49–52.

558. June R, Aks SE, Keys N, Wahl M. Medical outcome of cocaine body stuffers. J Emerg Med 2000;18:221–224.

559. Puschel K, Stein S, Stobbe S, Heinemann A. Analysis of 683 drug packages seized from "body stuffers". Forensic Sci Int 2004;140:109–111.

560. Moreira M, Buchanan J, Heard K. Validation of a 6-hour observation period for cocaine body stuffers. Am J Emerg Med 2011;29:299–303.

561. Schaper A, Hofmann R, Bargain P, Desel H, Ebbecke M, Langer C. Surgical treatment in cocaine body packers and body pushers. Int J Colorectal Dis 2007;22:1531–1535.

562. Bulstrode N, Banks F, Shrotria S. The outcome of drug smuggling by 'body packers'–the British experience. Ann R Coll Surg Engl 2002;84:35–38.

563. Pidoto RR, Agliata AM, Bertolini R, Mainini A, Rossi G, Giani G. A new method of packaging cocaine for international traffic and implications for the management of cocaine body packers. J Emerg Med 2002;23:149–153.

564. Jones OM, Shorey BA. Body packers: grading of risk as a guide to management and intervention. Ann R Coll Surg Engl 2002;84:131–132.

565. Gherardi RK, Baud FJ, Leporc P, Marc B, Dupeyron JP, Diamant-Berger O. Detection of drugs in the urine of body-packers. Lancet 1988;1(8594):1076–1078.

566. Kofler K, Oser W. Drug smuggling by body packing: what radiologists should know about it. Eur Radiol 2004;14:736–742.

567. Kranzler HR, Amin H, Modesto-Lowe V, Oncken C. Pharmacologic treatments for drug and alcohol dependence. Psychiatr Clin North Am 1999;22:401–423.

568. Dackis CA. Recent advances in the pharmacotherapy of cocaine dependence. Curr Psychiatr Rep 2004;6:323–331.

569. Lingford-Hughes AR, Welch S, Nutt DJ. Evidence-based guidelines for the pharmacological management of substance misuse, addiction and comorbidity: recommendations from the British Association for Psychopharmacology. J Psychopharmacol 2004;18:293–335.

570. Preti A. New developments in the pharmacotherapy of cocaine abuse. Addict Biol 2007;12:133–151.

571. de Lima MS, de Oliveira Soares GB, Reisser AA, Farrell M. Pharmacological treatment of cocaine dependence: a systematic review. Addiction 2002;97;931–949.

572. Klein M. Research issues related to development of medications for treatment of cocaine addiction. Ann NY Acad Sci 1998;844:75–91.

573. Kampman KM. What's new in the treatment of cocaine addiction? Curr Psychiatry Rep 2010;12:441–447.

574. Martell BA, Orson FM, Poling J, Mitchel E, Rossen RD, Gardner T, Kosten TR. Cocaine vaccine for the treatment of cocaine dependence in methadone-maintained patients. Arch Gen Psychiatry 2009;66:1116–1123.

Chapter 57

IBOGAINE (*Tabernanthe iboga* Baill.)

HISTORY

Ibogaine is one of the psychoactive indole alkaloids found in the West Central African shrub, *Tabernanthe iboga* that has been part of tribal culture in west-central Africa (Cameroon, Gabon, Republic of the Congo) for many centuries. In the Western culture, French and Belgian explorers first described the stimulant and aphrodisiac properties of the iboga root during the 1800s.[1] Professor Henri Baillon provided the first botanical description of the iboga plant before the Linnaean Society in Paris in 1889.[2] In the first part of the 20th century, French pharmacologists isolated a crystalline compound ("ibogaine" or "ibogine") from the root of the iboga plant that displayed stimulant properties in animal studies.[3,4] The popularity of ibogaine as a stimulant was limited. Dybowski and Landrin isolated ibogaine from *Tabernathe iboga* in 1901[3]; independently in the same year, Haller and Heckel also isolated ibogaine.[5] Lambarene® was an extract of the roots from a related species in the genus *Tabernanthe* (*Tabernanthe manii*) that was marketed in France between 1939 and 1970 as a stimulant. This extract contained 8 mg ibogaine for the treatment of depression, fatigue, asthenia, and convalescence from infectious diseases.[1] The chemical structure of ibogaine was not established until 1957.[6]

The hallucinogenic effects of ibogaine were widely publicized in the United States during the 1960s, and ibogaine was used to facilitate psychotherapy. During this time, anecdotal reports suggested that the abuse of ibogaine reduced cravings for heroin and cocaine. In 1970, the US Food and Drug Administration classified ibogaine along with lysergic acid diethylamide (LSD) and mescaline as a schedule I substance with use limited only to research. The status of ibogaine was similarly restricted in Belgium, Denmark, and Switzerland. France added ibogaine to their list of controlled substances in 2007. The International Olympic Committee also banned the use of ibogaine in athletes as a potential doping agent. There was little interest in ibogaine from pharmaceutic companies, in part, because the structure of a natural substance cannot be patented. However, Howard Lotsof received a US patent for the use of ibogaine in opioid withdrawal in 1985. During the late 1980s and the 1990s, ibogaine was introduced as an unapproved, rapid treatment of addiction, primarily in alternative settings.[7] Ibogaine is administered through a variety of self-help networks in private clinics in Mexico, the Caribbean (St. Kitts), and Panama.

BOTANICAL DESCRIPTION

Common Name: Iboga

Scientific Name: *Tabernanthe iboga* Baill.

Botanical Family: Apocynaceae

Physical Description: This shrub reaches 6 feet (~1.8 m) in height with small green leaves and yellowish to pink flowers. The elongated oval fruit is orange and does not contain indole alkaloids.

Distribution and Ecology: The iboga plant is a rainforest shrub, which inhabits the undergrowth of tropical forests in central-west Africa, particularly Cameroon, Congo, and Gabon.
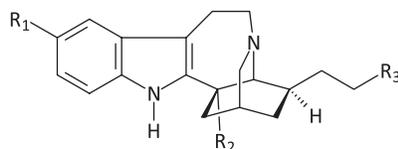
Alkaloid	R_1	R_2	R_3
Ibogaine	OCH_3	H	H
Noribogaine	OH	H	H
(±)-18-Methoxycoronaridine	H	CO_2CH_3	OCH_3

FIGURE 57.1. Chemical structures of ibogaine, noribogaine, and 18-methoxycoronaridine.

IDENTIFYING CHARACTERISTICS

Ibogaine (RN: 83-74-9, 12-methoxyibogamine) is one of several complex indole alkaloids present in root bark of the iboga plant along with tabernanthine (CAS RN: 83-94-3), ibogamine (CAS RN: 481-87-8), and ibogaline (CAS RN: 482-18-8). 18-Methoxycoronardine is a congener of ibogaine that demonstrates similar effects on drug craving in animal studies with less potential neurotoxicity.[8] Figure 57.1 displays the structure of ibogaine, noribogaine (CAS RN: 481-88-9), and 18-methoxycoronardine.

Ibogaine has a molecular weight of 310.44 g/mol and a molecular formula of $C_{20}H_{26}N_2O$. The free base is soluble in ethanol, methanol, and acetone, but insoluble in water; the hydrochloride salt is water soluble and decomposes at 299°C (~570°F). Ibogaine oxidizes spontaneously in solution, producing iboluteine and ibochine. The melting point of ibogaine is 153°C (~307°F). The pK_a of ibogaine in 80% methylcellulose is 8.1; this highly lipophilic drug has a heptane/water partition coefficient of 28.[9] In sunlight at 20°C (68°F), both ibogaine and noribogaine undergo degradation with a half-life of ~81 minutes and 11 minutes, respectively.[10]

EXPOSURE

Origin

Ibogaine is a psychoactive indole alkaloid isolated from the root bark of the tropical shrub, *Tabernanthe iboga*. Extraction of ibogaine from these roots is a complex process involving the use of alcohols and haloalkanes followed by chromatography for purification. However, the extraction of ibogaine from *T. iboga* is simpler than synthesis of ibogaine from nicotinamide. Ibogaine is also produced semisynthetically from voacangine, a component of root bark from the African tree *Voacanga africana* Stapf (Apocynaceae).[11]

Composition

Ibogaine is the most abundant alkaloid in extracts of the root bark of the iboga plant. The total alkaloid content of the dried root bark is ~5–6%.[1] The extract of *T. iboga* root extract is a complex mixture with the alkaloids in a dilute vinegar and ammonia extract of the bark divided approximately as follows: ibogaine, 80%; ibogaline, 15%; and ibogamine, up to 5%.[11] Other sources of ibogaine include *Voacanga thouarsii* var. *obtusa*,[12] *Tabernaemontana orientalis* R. Br.,[13] and *Tabernaemontana australis* (Mueell. Arg) Miers.[14]

Methods of Use

Ibogaine has a long tradition of use in West Africa as a stimulant and hallucinogen. Indigenous peoples in western Africa used extracts of the root from the iboga plant to improve endurance on hunting trips and to combat fatigue, thirst, and hunger. In higher doses, ibogaine is part of the initiation ceremonies of the Bwiti cult of Gabon, where practitioners believe that the use of ibogaine from the root of the iboga plant allows contact with ancestors in the spirit world.[15] This initiation ritual is similarly reported as near-death experiences with out-of-body feelings and a sense of floating over various landscape, encounters with deceased people, contact with divine entities, and arrival at the point of no return with subsequent return to reality.[16] To obtain psychoactive effects, the pulverized root bark of the iboga plant is swallowed with water. During these rituals, effects associated with the use of the root extract include excitation, inebriation, dramatic visual illusions, sensory synesthesias, euphoria, dysphoria, confusion, disorientation, and hallucination.[17] However, the root extract contains indole alkaloids in addition to ibogaine; therefore, the effect of ibogaine cannot be separated from other indole alkaloids in the extract.

DOSE EFFECT

Animals

In some animal studies, a single injection 100 mg ibogaine/kg to rats produces loss of Purkinje cells in the cerebellum, whereas the no observable adverse effect level (NOAEL) for neurologic damage following injection was 25 mg/kg in female rats.[18] At the higher ibo-

gaine dose, degeneration of Purkinje cells occurred as detected by the Fink-Heimer stain II and increased glial turnover detected by glial fibrillary acidic protein (astrocyte-specific protein).[19] Cerebellar degeneration appears primarily in areas 5 and 6 associated with the head and upper extremities. Clinical signs of neurologic abnormalities in rats administered doses exceeding 25 mg ibogaine/kg include ataxia, hyperexcitability, and abnormal posturing of the extremities.[20] However, there is substantial interspecies variation in the neurologic effects of ibogaine with monkeys and mice much less sensitive than rats.[21] In a study of white laboratory mice, the median lethal doses of ibogaine and noribogaine were 263 mg/kg and 630 mg/kg, respectively, with the ibogaine/noribogaine ratio being ~2.4.[22] At these higher doses, ibogaine depressed heart rate.

Humans

Based on anecdotal reports, the estimated threshold adult dose for hallucinations following ingestion of ibogaine is about 300 mg.[23] The typical dose for facilitating psychotherapeutic or spiritual insights is about 8–12 mg/kg.[7] Following oral doses of 4–25 mg/kg, open, non-blinded case reports suggested that the use of ibogaine was a rapid, easy means of suppressing the drug craving associated with heroin, cocaine, and amphetamine abuse.[24,25] The recommended oral dose for the treatment of drug addiction is 5–30 mg/kg.[8] In an open-label case series of heroin addicts receiving ibogaine for the treatment of withdrawal, the average ibogaine dose was approximately 20 mg/kg.[26] Case reports document the use of single ibogaine doses up to 1,800 mg for the treatment of heroin addiction.[27] There are inadequate data to determine the safety or efficacy of this dose for treating drug addiction.

TOXICOKINETICS

There are few toxicokinetic data on ibogaine in humans. Animal studies suggest that the oral bioavailability of ibogaine is gender- and dose-dependent. After oral administration of 5 mg/kg to rats, the bioavailability of ibogaine in male and female rats was 7% and 16%, respectively; following 50 mg/kg, the bioavailability was 43% and 71%, respectively.[28] These rodent studies also indicate that ibogaine undergoes substantial first-pass metabolism as well as large accumulation in adipose tissue as a result of the high lipophilicity.[29] The large fat reservoirs of ibogaine provide a mechanism for sustained release and prolonged action. The volume of distribution of ibogaine is large (i.e., 13 L/kg uncorrected for bioavailability).[21] Animal studies indicate that both ibogaine and noribogaine cross the blood–brain barrier.

Noribogaine (*O*-desmethylibogaine, 12-hydroxyibogamine) is the major metabolite of ibogaine, produced by CYP2D6-mediated *O*-demethylation.[30] *In vitro* studies using human liver microsomes indicate that CYP2C19 is the primary isoform involved with the biotransformation of the ibogaine analog, 18-methoxycaronaridine to its major metabolite, 18-hydroxycoronaridine.[31] In laboratory animals and volunteers, the plasma elimination half-life of ibogaine is short (i.e., 1–3 h) with >90% of the dose eliminated within 24 hours; however, pharmaceutic studies suggest that the terminal plasma half-life of ibogaine in humans (i.e., extensive CYP2D6 metabolizers) may be substantially longer (i.e., 5–8 h).[21] In rats, the gastrointestinal tract and kidneys eliminate about 60–70% of the administered dose of ibogaine; both ibogaine and noribogaine distribute into bile.[28] Limited human data suggests that the elimination half-life of noribogaine from blood is substantially longer than ibogaine.[32]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The mechanism of action of ibogaine is complex and not well-defined. At pharmacologic doses, *in vitro* studies indicate that the actions of ibogaine result from the binding of ibogaine to a variety of central nervous system (CNS) receptors and channels including the κ- and μ-opioid receptors, σ_2 receptor,[33,34] and the serotonin transporter; additionally, ibogaine is a noncompetitive blocker of the *N*-methyl-*D*-aspartate (NMDA) receptor channel.[35] Other potential sites of ibogaine and noribogaine binding include $5HT_2$ and $5HT_3$, muscarinic (M_1, M_2), and nicotinic acetylcholine receptors as well as monoamine uptake sites.[9] The active metabolite, noribogaine, displays higher affinity for serotonin transporters and opioid receptor subtypes (i.e., moderate κ- and weak μ-opioid receptor agonist) compared with ibogaine as demonstrated in Table 57.1.

TABLE 57.1. Affinity of Ibogaine and Noribogaine for Central Nervous System Receptors. [30]

Receptor	Site	Ibogaine IC_{50} (μM)	Noribogaine IC_{50} (μM)
Serotonin			
Transporter	RTI-55 DAT	00.59	0.04
Opioid Mu	DAMGO	11.00	0.16
Opioid Kappa 1	U69593	25.00	4.20
Opioid Kappa 2	IOXY	23.80	92.30
Glutamine			
NMDA	MK-801	5.2	31.40

Noribogaine has little affinity for σ_2 receptors in contrast to ibogaine,[36] whereas noribogaine is approximately 10–15 times more potent than ibogaine in binding the serotonin transporter and inhibiting serotonin reuptake.

In vitro studies indicate that ibogaine noncompetitively blocks sodium influx through ganglionic-type nicotinic receptor channels.[37] Ibogaine has moderate affinity for the σ_2 receptor, which increases intracellular calcium and induces apoptosis. This compound has low affinity for σ_1 receptors, which modulate NMDA-type glutamatergic, dopaminergic, and cholinergic receptors. Additionally, *in vitro* studies indicate that ibogaine has low affinity (i.e., K_i >100 μM) for benzodiazepine, γ-aminobutyric acid ($GABA_A$), cannabinoid, dopaminergic, metabotropic glutamatergic, and serotonergic receptors.[33] Although ibogaine is structurally similar to serotonin and harmaline as a result of the shared indole ring, ibogaine does not display significant affinity for serotonin receptors at standard pharmacologic doses. However, noribogaine and to a lesser extent, ibogaine inhibit serotonin uptake by binding to serotonin transporter sites, resulting in increased extracellular concentrations of serotonin, particularly in the nucleus accumbens.[36] Both ibogaine and noribogaine also transiently decrease tissue dopamine concentrations by stimulating dopamine metabolism.

Animal studies suggest the possibility of neurotoxicity and possibly cardiotoxicity following the administration of ibogaine. In high doses (i.e., 100 mg/kg), some animal studies indicate that ibogaine produces loss of Purkinje neurons in the cerebellar vermis, particularly lobules 5 and 6, as detected by histochemical markers including astrocytosis, microgliosis, argyrophilic degeneration, and loss of calbindin immunoreactivity.[38] Observed effects in these animals include ataxia and tremor. The cause of this damage (e.g., excessive glutamate release) is not well-defined.[39]

CLINICAL RESPONSE

Because of the illicit status of ibogaine, there are limited human data in the peer-reviewed medical literature on the clinical effects of the drug. When administered in nonhospital settings for the amelioration of opioid withdrawal, common adverse effects include vomiting, myalgias, tremor, ataxia, loss of balance, and rarely, transient hypotension.[8] The onset of action is about 1–3 hours with initial effects of a dreamlike, waking state (floating, passage along a long path) persisting 4–8 hours. This dream-like state develops with visions of transcendent beings and a "panoramic readout of long-term memory."[8] Subsequently, a reflective period begins about 4–8 hours after ingestion with attention focused on inner subjec-

tive experiences rather than external cues. This phase lasts 8–24 hours. A third phase of stimulation starts 12–24 hours after ingestion and persists 24–72 hours. During this phase, volunteers regain normal attention to the environment and experience arousal and alertness.[9] Some patients report reduced need for sleep. Deaths associated with ibogaine are not well-reported, but high doses of ibogaine may produce coma and respiratory depression.[40]

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantification of ibogaine include thin layer chromatography with UV spectrophotometry,[41] gas chromatography with flame ionization detection,[42] gas chromatography/mass spectrometry after organic extraction and derivatization with trifluoroacetic anhydride,[43] gas chromatography/electron impact/mass spectrometry in full scan mode,[44] high performance liquid chromatography (HPLC) with fluorometry,[10] liquid chromatography/electrospray ionization/mass spectrometry,[45] and liquid chromatography/tandem mass spectrometry.[46] The limit of detection (LOD) for HPLC with fluorometry is about 20 ng/mL with a coefficient of variation of 8–12%. The lower limit of quantitation (LLOQ) for both ibogaine and noribogaine using liquid chromatography/tandem mass spectrometry is 50 ng/mL with precision of 7.8% and 7.4%, respectively, and accuracy of 89.6% and 86.2%, respectively. The LOD for this method is 1 ng/mL. The LLOQ for ibogaine and noribogaine using liquid chromatography/electrospray ionization/mass spectrometry is 0.89 ng/mL and 1 ng/mL, respectively, in plasma with precision <14% and accuracy 9–10%. HPLC also allows LLOQ of ibogaine and noribogaine in the range of 1 ng/mL with precision ≤ 17%.[10] The use of solid-phase extraction along with gas chromatography/methane positive-ion/mass spectrometry allows the detection of ibogaine in plasma samples at LLOQ near 0.5 ng/mL.[47] Analysis by solid-phase extraction and HPLC with photodiode array detection allows the determination of ibogaine along with 12 other plant alkaloids (e.g., aconitine, anabasine, atropine, brucine, colchicine, cotinine, cystine, harmine, nicotine, scopolamine, strychnine, yohimbine).[48] The LLOQ of ibogaine using this screening method for analysis of serum and urine samples is 6.0 ng/mL and 3.2 ng/mL, respectively, both with coefficients of variation <5%. Aqueous solutions of ibogaine are relatively stable under refrigerated conditions. During refrigerated storage, the loss of ibogaine from a 10 mg/mL solution over 7 months was <10%.[43] There was no statistically significant difference

in ibogaine or noribogaine concentrations in plasma samples stored at $4°C$ (~$39°F$) and $-20°C$ ($-4°F$) for 1 year.[45]

Biomarkers

There are limited pharmacokinetic data on ibogaine. In heroin addicts receiving 500–800 mg ibogaine for the treatment of withdrawal, peak plasma ibogaine concentrations range between 30–1,250 ng/mL within 2–5 hours after ingestion. Plasma ibogaine concentrations decline rapidly to <10 ng/mL 24 hours after ingestion. Plasma noribogaine concentrations remain elevated substantially longer than ibogaine with peak concentrations between 700–1,200 ng/mL decreasing to 300–800 ng/mL 24 hours after the ingestion of ibogaine.[30,30] These studies indicate that there are substantial differences in the plasma ibogaine and noribogaine concentrations between poor and rapid CYP2D6 metabolizers. After the ingestion of ibogaine, poor metabolizers have much lower concentrations of noribogaine and higher concentrations of ibogaine than rapid metabolizers. A 37-year-old man was found dead on a beach after drowning.[46] The postmortem femoral blood contained 3,300 ng ibogaine/mL and 4,600 ng noribogaine/mL. The ratios of heart/femoral blood for ibogaine and noribogaine were 0.73 and 0.61, respectively. Subclavian blood drawn at the death scene from a 48-year-old drug addict found dead after ingesting the bark from *T. iboga* contained average ibogaine and noribogaine concentrations of 10,800 ± 400 ng/mL and 20,800 ± 3,000 ng/mL, respectively.[49] At autopsy 48 hours later, the femoral blood contained average ibogaine and noribogaine concentrations of 5,400 ± 1,400 ng/mL and 5,600 ± 900 ng/mL, respectively, probably as a result of oxidation.

TREATMENT

There are few clinical data on the treatment of toxicity associated with ibogaine. Treatment of adverse effects associated with ibogaine is supportive (e.g., antiemetics, rest). The hallucinogenic potential of ibogaine suggests that patients should be placed in a quiet environment with familiar friends and limited external stimuli. Although there are few clinical data, benzodiazepines are a therapeutic option for the treatment of anxiety associated with the behavioral effects of ibogaine.

References

1. Pope HG Jr. *Tabernanthe iboga*: an African narcotic plant of social importance. Econ Bot 1969;23:174–184.
2. Baillon H. Sur l'obouete du Gabon. Bull Mens Soc Linn de Paris 1889;1:782–793.
3. Dybowski J, Landrin E. Sur l'Iboga, sur ses proprietes excitants, sa composition et sur l'alcaloide nouveau qu'il renferme. CR Acad Sci 1901;133:748–750.
4. Lambert M. Sur les proprietes physiologiques de l'ibogaine. Arch Int Pharmacodyn Ther 1902;10;101–120.
5. Haller A, Heckel E. Sur l'ibogaine, principe actif d'une plante du genre *Tabernaemontana* native du Congo. CR Acad Sci 1901;133:850–853.
6. Taylor WI. Iboga alkaloids. II. The structures of ibogaine, ibogamine, and tabernanthine. J Am Chem Soc 1957; 79:3298–3299.
7. Alper KR, Beal D, Kaplan CD. A contemporary history of ibogaine in the United States and Europe. Alkaloids Chem Biol 2001;56:249–281.
8. Alper KR. Ibogaine: a review. Alkaloids Chem Biol 2001; 56:1–38.
9. Maciulaitis R, Kontrimaviciute V, Bressolle FM, Briedis V. Ibogaine, an anti-addictive drug: pharmacology and time to go further in development. A narrative review. Hum Exp Toxicol 2008;27:181–194.
10. Kontrimaviciūte V, Larroque M, Briedis V, Margout D, Bressolle F. Quantitation of ibogaine and 12-hydroxyibogamine in human plasma by liquid chromatography with fluorimetric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2005;822:285–293.
11. Jenks CW. Extraction studies of *Tabernanthe iboga* and *Voacanga africana*. Nat Prod Lett 2002;16:71–76.
12. Goldblatt A, Hootele C, Pecher J. The alkaloids of *Voacanga thouarsii* var. *obtusa*. Phytochemistry 1970;9: 1293–1298.
13. Know JR, Slobbe J. Alkaloids of *Ervatamia orientalis*. Isolation of alkaloids and structural identification of two dimers. Aust J Chem 1975;28:1813–1856.
14. Andrade MT, Lima JA, Pinto AC, Rezende CM, Carvalho MP, Epifanio RA. Indole alkaloids from *Tabernaemontana australis* (Muell. Arg) Miers that inhibit acetylcholinesterase enzyme. Bioorg Med Chem 2005;13:4092–4095.
15. Popik P, Layer RT, Skolnick P. 100 Years of ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. Pharmacol Rev 1995;47:235–253.
16. Strubelt S. The near-death experience: a cerebellar method to protect body and soul—lessons from the *Iboga* healing ceremony in Gabon. Altern Ther 2008;14:30–34.
17. Schneider JA, Sigg EB. Neuropharmacological studies on ibogaine, an indole alkaloid with central-stimulant properties. Ann N Y Acad Sci 1957;66:765–776.
18. Xu Z, Chang LW, Slikker W Jr, Ali SF, Rountree RL, Scallet AC. A dose-response study of ibogaine-induced neuropathology in the rat cerebellum. Toxicol Sci 2000; 57:95–101.
19. Molinari HH, Maisonneuve IM, Glick SD. Ibogaine neurotoxicity: a re-evaluation. Brain Res 1996;737:255–262.
20. Vocci FJ, London ED. Assessment of neurotoxicity from potential medications for drug abuse: ibogaine testing and brain imaging. Ann N Y Acad Sci 1997;820:29–40.

21. Mash DC, Kovera CA, Buck BE, Norenberg MD, Shapshak P, Hearn WL, Sanchez-Ramos J. Medication development of ibogaine as a pharmacotherapy for drug dependence. Ann N Y Acad Sci 1998;844:274–292.

22. Kubiliene A, Marksiene R, Kazlauskas S, Sadauskiene I, Razukas A, Ivanov L. Acute toxicity of ibogaine and noribogaine. Medicina (Kaunas) 2008;44:984–988.

23. Naranjo C. Psychotherapeutic possibilities of new fantasy-enhancing drugs. Clin Toxicol 1969;2:209–224.

24. Hittner JB, Quello SB. Combating substance abuse with ibogaine: pre- and posttreatment recommendations and an example of successive model fitting analyses. J Psychoactive Drug 2004;36:191–199.

25. Goutarel R. Pharmacodynamic and therapeutic applications of iboga and ibogaine. Psychedelic Monogr Essays 1993;6:71–111.

26. Alper KR, Lotsof HS, Frenken GM, Luciano DJ, Bastiaans J. Ibogaine in acute opioid withdrawal. An open label case series. Ann N Y Acad Sci 2000;909:257–259.

27. Sheppard SG. A preliminary investigation of ibogaine: case reports and recommendations for further study. J Subst Abuse Treat 1994;11:379–385.

28. Jeffcoat AR, Cook CE, Hill JM, Coleman DP, Pollack GM. Disposition of [3H] ibogaine in the rat. Natl Inst Drug Abuse Res Monogr Ser 1994;141:309.

29. Hough LB, Pearl SM, Glick SD. Tissue distribution of ibogaine after intraperitoneal and subcutaneous administration. Life Sci 1996;58:PL119–PL122.

30. Mash DC, Kovera CA, Pablo J, Tyndale RF, Ervin FD, Williams IC, et al. Ibogaine: complex pharmacokinetics, concerns for safety, and preliminary efficacy measures. Ann NY Acad Sci 2000;941:394–401.

31. Zhang W, Ramamoorthy Y, Tyndale RF, Glick SD, Maisonneuve IM, Kuehne ME, Sellers EM. Metabolism of 18-methoxycoronaridine, an ibogaine analog, to 18-hydroxycoronaridine by genetically variable CYP2C19. Drug Metab Disp 2002;30:663–669.

32. Mash DC, Staley JK, Baumann MH, Rothman RB, Hearn WL. Identification of a primary metabolite of ibogaine that targets serotonin transporters and elevates serotonin. Life Sci 1995;57:PL45–PL50.

33. Bowen WD, Vilner BJ, Williams W, Bertha CM, Kuehne ME, Jacobson AE. Ibogaine and its congeners are sigma 2 receptor-selective ligands with moderate affinity. Eur J Pharmacol 1995;279:R1–R3.

34. Bowen WD. Sigma receptors and iboga alkaloids. Alkaloids Chem Biol 2001;56:173–191.

35. Sweetnam PM, Lancaster J, Snowman A, Collins JL, Perschke S, Bauer C, Ferkany J. Receptor binding profile suggests multiple mechanisms of action are responsible for ibogaine's putative anti-addictive activity. Psychopharmacology (Berl) 1995;118:369–376.

36. Baumann MH, Pablo J, Ali SF, Rothman RB, Mash DC. Comparative neuropharmacology of ibogaine and its *O*-desmethyl metabolite, noribogaine. Alkaloids Chem Biol 2001;56:79–113.

37. Badio B, Padgett WL, Daly JW. Ibogaine: a potent non-competitive blocker of ganglionic/neuronal nicotinic receptors. Mol Pharmacol 1997;51:1–5.

38. Binienda ZK, Scallet AC, Schmued LC, Ali SF. Ibogaine neurotoxicity assessment: electrophysiological, neurochemical, and neurohistological methods. Alkaloids Chem Biol 2001;56:193–211.

39. O'Hearn E, Molliver ME. Administration of a non-NMDA antagonist, GYKI 52466, increases excitotoxic Purkinje cell degeneration caused by ibogaine. Neuroscience 2004; 127:373–383.

40. Cienki J, Mash D, Hearn W. Ibogaine fatalities. Clin Toxicol 2001;39:547.

41. Dhahir HI, Jain NC, Thornton JI. The identification of ibogaine in biological material. J Forensic Sci Soc 1972; 12:309–313.

42. Cartoni GP, Giarusso A. Gas chromatographic determination of ibogaine in biological fluids. J Chromatogr 1972; 71:154–158.

43. Gallagher CA, Hough LB, Keefner SM, Seyed-Mozaffari A, Archer S, Glick SD. Identification and quantification of the indole alkaloid ibogaine in biological samples by gas chromatography-mass spectrometry. Biochem Pharmacol 1995;49:73–79.

44. Hearn WL, Pablo J, Hime GW, Mash DC. Identification and quantitation of ibogaine and an *O*-demethylated metabolite in brain and biological fluids using gas chromatography-mass spectrometry. J Anal Toxicol 1995; 19:427–434.

45. Kontrimavičiūte V, Breton H, Mathieu O, Mathieu-Daudé JC, Bressolle FM. Liquid chromatography-electrospray mass spectrometry determination of ibogaine and noribogaine in human plasma and whole blood. Application to a poisoning involving *Tabernanthe iboga* root. J Chromatogr B Analyt Technol Biomed Life Sci 2006;843:131–141.

46. Cheze M, Lenoan A, Deveaux M, Pepin G. Determination of ibogaine and noribogaine in biological fluids and hair by LC-MS/MS after *Tabernanthe iboga* abuse iboga alkaloids distribution in a drowning death case. Forensic Sci Int 2008;176:58–66.

47. Ley FR, Jeffcoat AR, Thomas BF. Determination of ibogaine in plasma by gas chromatography-chemical ionization mass spectrometry. J Chromatogr A 1996;723: 101–109.

48. Pietsch J, Günther J, Henle T, Dreßler J. Simultaneous determination of thirteen plant alkaloids in a human specimen by SPE and HPLC. J Sep Sci 28;31:2410–2416.

49. Kontrimavičiūte V, Mathieu O, Mathieu-Daudé JC, Vainauskas P, Casper T, Baccino E, Bressolle FM. Distribution of ibogaine and noribogaine in a man following a poisoning involving root bark of the *Tabernanthe iboga* shrub. J Anal Toxicol 2006;30:434–440.

Chapter 58

KHAT (*Catha edulis* (Vahl) Forsskal Ex Endl.) and CATHINONE

HISTORY

The habit of chewing Khat for stimulant properties has a long history in eastern Africa and southern Arabia, where the plant (*Catha edulis*) is indigenous. The Arabian physician, Najeeb Al-Deen Al-Samargandi recommended the use of khat for the treatment of depressive illness in an Arabic medical book written in the 13th century.[1] Khat probably originated in Ethiopia; the use of khat as a stimulant spread to Yemen during the 13th century.[2] Swedish botanist, Peter Forskal first described the khat tree during an expedition to Egypt and Yemen in the middle 18th century. The English adventurer-writer, Sir Richard Francis Burton (1821–1890) and the French Poet, Jean Arthur Rimbaud (1854–1891) wrote about chewing khat in Harer, Ethiopia during the 19th century.[3] Until recently, the use of khat remained localized to the areas where the plant is native because the active ingredient in khat leaves decays rapidly.[4] The habit has now spread to distant ethnic communities (e.g., Somali communities in South Wales and London).

BOTANICAL DESCRIPTION

Common Name: Khat, qat (Yemen), gat, mairungi (Uganda), miraa (Kenya), muhulo (Tanzania), muraa, musitate, jaad/qaad (Somalia), tchat (Ethiopia), African salad, bushman's tea

Scientific Name: *Catha edulis* (Vahl) Forsskal ex Endl.

Botanical Family: Celastraceae (bittersweet family)

Physical Description: This leafy evergreen shrub reaches up to 6 m (~20 ft) in height with opposite or alternate leaves about 1–4 cm (~0.5–1.5 in) wide and 5–10 cm (~2–4 in) long.[5] The glossy, brownish-green, leathery, elliptical to lanceolate leaves have serrated edges that resemble withered basil; as the leaves deteriorate, they become yellow-green and leathery. The odor is slightly aromatic and the taste is slightly sweet and astringent. There are no unique anatomic features that separate khat from other similar plant species; the identification of khat requires laboratory confirmation.

Distribution and Ecology: Khat grows on moist slopes at elevations of 3,000–8,000 ft (~1,000–2,500 m) in Yemen, Ethiopia, Kenya, and east Africa. Severe frosts in winter destroy the aerial parts of the plant and limit the height of the plant. Frequently, khat is grown among other commercial crops (coffee, legumes, papayas, peaches). In Yemen, khat is not cultivated in the hot, coastal cities (e.g., Aden, Hodieda, Mokalla).

IDENTIFYING CHARACTERISTICS

Fresh khat leaves contain up to 60 different catheduline compounds (polyesters of euonyminol) and several sympathomimetic phenylalkylamine compounds including cathinone [(−)-α-aminopropiophenone, 2-aminopropiophenone, CAS RN: 71031-15-7], cathine
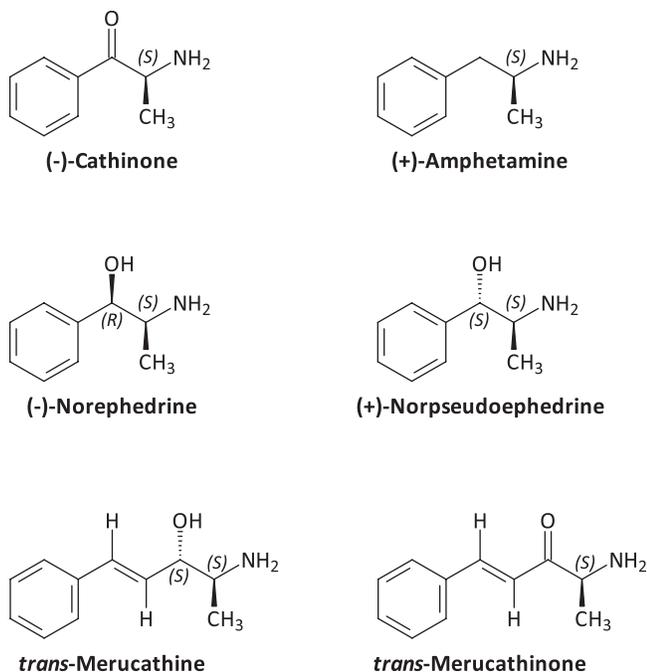
FIGURE 58.1. Chemical structure of major chemical ingredients in khat, with (*R*) and (*S*) absolute configuration indicated at the stereocenters..

[(+)-norpseudoephedrine, CAS RN: 492-39-7], and (+/−)-norephedrine [phenylpropanolamine, CAS RN: 14838-15-4]. The only structural difference between amphetamine and cathinone is the presence of a carbonyl on the α-carbon of the amphetamine side chain as displayed in Figure 58.1. The World Health Organization (WHO) includes *S*-(−)-cathinone in schedule I (i.e., most controlled substance category as a result of high abuse potential) of the United Nations Convention on Psychotropic Substances. Khat also contains phenylpentenylamine alkaloids (merucathinone, merucathine, pseudomerucathine) and catheduline compounds. Other compounds in khat include tannins, terpenoids, flavonoids, ethereal oil, sterols, amino acids (alanine, glycine, glutamic acid, tryptophan), minerals (calcium, copper, iron, zinc), vitamins (ascorbic acid, carotene, niacin, riboflavine, thiamine), and glycosides.[6,7]

Although animal studies suggest that the sympathomimetic effects of (−)-cathinone (MW: 149.2) and (+)-norpseudoephedrine (cathine) are similar, *S*-(−)-cathinone is probably the active psychoactive ingredient in khat.[8,9] Cathinone is an intermediate in the synthesis of cathine that accumulates in young, but not in adult leaves. During the wilting of khat, the reduction of cathinone to cathine occurs.[13] The sympathomimetic effects of standardized khat leaves and pure cathinone are similar in experimental studies.[10]

S-(−)-Cathinone is the ketone congener of phenylpropanolamine (norephedrine).

EXPOSURE

Epidemiology

Chewing fresh khat leaves is a habit that is endemic in regions of east Africa (Djibouti, Ethiopia, Kenya, Madagascar, Somalia, Sudan, Tanzania, Uganda) and the southwestern Arabian Peninsula, particularly in Yemen. The use of khat is also common in immigrant communities (Yemen, Ethiopia, Somalia) in the United States, United Kingdom, and Australia.[11] Commercial sources of fresh khat include Kenya, Ethiopia, and Somalia. The first khat harvest occurs about 3–5 years after planting.[12] Only the fresh leaves produce the desired stimulatory effects.[13] However, the advent of air transportation allows the rapid distribution of fresh khat leaves before the deterioration of the active ingredients. The use of khat is prohibited in Australia, Denmark, Finland, France, Germany, New Zealand, Norway, Switzerland, and the United States; possession of the unprepared plant form is legal in the United Kingdom and the Netherlands. The World Health Organization classifies khat as a drug of abuse that can produce mild to moderate psychologic dependence. Hagigat is a capsule containing 200 mg cathinone that is marketed in Israel as an natural aphrodisiac and stimulant.[14] This product is a substitute for khat. The US Drug Enforcement Agency classifies cathinone as a schedule I drug (i.e., most restrictive), whereas cathine is a schedule IV drug.

Composition

The cathinone content of khat leaves depends primarily on the growing environment; therefore, the cathinone content varies substantially between khat specimens from different regions of Yemen.[2] Young, fresh leaves contain the highest concentration of cathinone.[15] These leaves lack the (+) isomer of cathinone; consequently, the (−) isomer of cathinone is the only natural enantiomer in *Catha edulis*.[16] Mature khat leaves usually contain more (+)-norpseudoephedrine than (−)-cathinone as a result of the conversion of cathinone to cathine [(+)-norpseudoephedrine] and (−)-norephedrine during maturation.[4] (+)-Norpseudoephedrine occurs in all parts of the khat plant, whereas cathinone occurs almost exclusively in the young leaves, shoots, and branchlets.[17]

The content of specific phenylalkylamine compounds in khat leaves varies substantially both as an absolute amount and as a percentage of total phenylalkylamine

content, depending on the origin and the age of the leaves. In a study of 22 samples of fresh khat leaves from Kenya, Ethiopia, Yemen, and Madagascar, the specific phenylalkylamine content as an approximate percentage of total phenylpropyl and phenylpentenyl content ranged between the following: cathinone, 5–59%; cathine [*S*,*S*-(+)-norpseudoephedrine], 30–79%; and phenylpropanol-amine [*R*,*S* -(−)-norephedrine], 4–19%.[18] Approximate phenylalkylamine content per gram fresh khat leaves typically ranges between the following: cathinone, 0.36–1.14 mg; cathine, 0.83–1.20 mg; and norephedrine, 0.08–0.47 mg.[19] Analysis of fresh khat leaves confiscated by Swiss custom officers demonstrate the following phenylalkylamine compounds in mg/g fresh weight: (−)-cathinone, 1.02 ± 0.11; *S*,*S*-(+)-norpseudoephedrine 0.86 ± 0.06; and *R*,*S*-(−)-norephedrine, 0.47 ± 0.05.[10] In a study of khat leaves confiscated at the Frankfurt airport and stored at −20°C (−4°F), the cathinone, cathine, and norephedrine content in the leaves were 1.14 mg/g, 0.83 mg/g, and 0.44 mg/g, respectively.[20] *S*-(−)-Cathinone is relatively unstable; this active compound decomposes rapidly into (+)-norpseudoephedrine and norephedrine within a few days of harvest. The fluoride content of khat is negligible based on analyses of khat leaves and saliva from khat chewers; high fluoride content in drinking water from rural areas of khat growing countries may account for the presence of fluorosis of the tooth enamel in some khat chewers.[21]

Methods of Use

Typically, the harvest of khat begins in the early morning to reach the market by late morning as bundles of twigs, stems, and leaves wrapped in banana leaves to preserve freshness as shown in Figure 58.2.[19]

Figure 58.3 displays dried khat leaves. Khat is a euphoriant that is an integral part of the Yemeni and Somali culture, where urban khat sessions are a common social gathering after lunch in dedicated rooms (i.e.,

muffraj).[22,23] Desirable effects include the initial onset of elation, increased alertness, enhanced sociability, loquacity, and heightened self-confidence. Later in the session, irritability, emotional lability, and depressive symptoms may develop. Khat is also used during work as a means to reduce physical fatigue.[24]

DOSE EFFECT

Prediction of a dose response to khat is complicated by the variation in the alkaloids and potency of khat leaves, tolerance, and large interindividual variations in cathinone absorption from the leaves. In volunteer studies, the ingestion of 0.5 mg cathinone/kg produces stimulatory effects similar to the chewing of high-grade Kenyan khat.[10] An average khat session over 3–4 hours involves the use of 100–200 g khat leaves that contain approximately 0.5–1 mg cathinone/kg.[18] In a case series of 34

FIGURE 58.3. Dried khat leaves. (Photo courtesy of the US Drug Enforcement Agency)
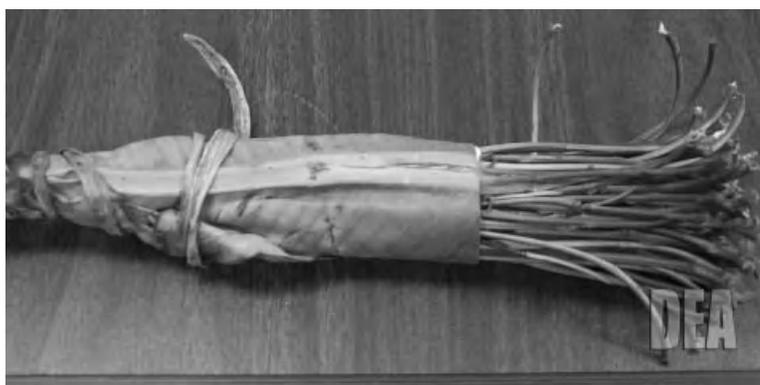
FIGURE 58.2. Bundle of khat leaves, twigs, and stems wrapped in banana leaves. (Photo courtesy of the US Drug Enforcement Agency)

calls to the Israeli Poison Information Center regarding exposure to one-half to 6 capsules of Hagigat (cathinone), the main clinical features were headache, vomiting, hypertension, nausea, tachycardia, dyspnea, chest pain, and myalgias. Clinical symptoms did not correlate to the amount of Hagigat ingested.

TOXICOKINETICS

During a khat session, absorption of cathinone occurs primarily across the mucous membranes of the mouth. In a study of 4 adults chewing 600 mg khat leaves/kg body weight (i.e., about 25% of the usual dose in a khat session), the estimated absorption of cathinone in the mouth was $59 \pm 21\%$ with a mean volume of distribution of 2.7 ± 1.6 L/kg.[25] The time to maximal cathinone concentration (t_{max}) was 2.31 ± 0.65 hours. Cathinone undergoes ketone reduction to R,R-(−)-norpseudoephedrine (cathine) and R,S-(−)-norephedrine (phenylpropanolamine).[9,26] Volunteer studies indicate that norephedrine is the major metabolite of cathinone in humans; whereas cathine is the major decomposition product of cathinone in storage. In a study of 3 volunteers, R,R-(−)-norpseudoephedrine (cathine) and R,S-(−)-norephedrine (phenylpropanolamine) accounted for 2.5–5.2% and 20.1–34.9%, respectively, of the administered dose of 24 mg S-(−)-cathinone.[27] Cathine is much less active than cathinone. During volunteer studies, mean peak S-(−)-cathinone concentrations occurred about 2 ± 1 hours after the beginning of khat chewing, and the mean elimination half-life of S(−)-cathinone was approximately 4 ± 2 hours.[10] The S(−)-cathinone content in the khat leaves used during the study were similar to the S(−)-cathinone in high-grade Kenyan khat. Although cathinone shares similar pharmacologic effects with amphetamine, the duration of action for cathinone is shorter than that of amphetamine.[13] The stimulatory effects of khat usually last about 4 hours after chewing begins. Following the chewing of one-quarter of the normal dose of khat leaves, the mean plasma elimination half-life of cathinone and cathine were 1.5 ± 0.8 hours and 5.2 ± 3.4 hours, respectively.[25] Only about 2% of the absorbed dose of cathinone appears in the urine unchanged.[27] Cathinone and methcathinone are not metabolites of phenylpropanolamine or any other over-the-counter medications. Cathinone is a minor metabolite of the anorexic agent, diethylpropion.[28] There are limited data on the development of tolerance following chronic use of khat. In animal models, repeat administration of cathinone produces tolerance to the effects of cathinone eaten with food; these animals also demonstrate cross-tolerance to d-amphetamine.[29]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Cathinone is a naturally occurring β-keto-α-methyl-phenethylamine analog of amphetamine. Cathinone produces stimulatory effects similar to amphetamine as a result of the release of neurotransmitters (dopamine, serotonin, epinephrine) from presynaptic storage sites.[13] This compound is an indirect dopamine agonist that inhibits the reuptake of dopamine as well as functioning as a presynaptic releaser of dopamine in *in vitro* models (e.g., rat synaptosomes).[30] (−)-Cathinone also releases serotonin from striatal stores similar to d-amphetamine; however, the dopaminergic and serotonergic properties of cathinone are substantially weaker (i.e., 2–10 times) than d-amphetamine.[31] In volunteer studies, the administration of cathinone increases blood pressure and heart rate as well as causing CNS stimulation and euphoria.[32] Cathine is also an active constituent of khat that has dopaminergic properties similar to cathinone; however, the potency of cathine is much lower than cathinone.[33]

CLINICAL RESPONSE

Recreational Use

The ingestion of the active ingredients in khat initially produces stimulation of the CNS. The chewing of khat in a social setting causes mild euphoria, alertness, and hyperactivity followed by loquacity and increased social interaction within about 1 hour after the onset of chewing. Like other sympathomimetic drugs, khat can produce mydriasis, transient hypertension and tachycardia, hyperthermia, anorexia, headache, dry mouth, blurred vision, garrulousness, mood elevation, and arousal. Social and medical problems associated with the recreational use of khat include depression, malnutrition, insomnia, irritability, malaise, emotional lability, gastritis, constipation, impotence, and spontaneous sperm secretion. A case series associated the chronic use of khat with the development of severe, acute hepatotoxicity in patients without other obvious causes of hepatitis.[34]

Mental Disorders

Case reports associate a toxic psychosis with khat chewing, manifest by hyperactivity, pressured speech, anorexia, insomnia, and paranoid ideations in patients both with and without a personal and/or family history of psychiatric illness.[35] These symptoms disappeared 1 week after beginning thioridazine therapy (300 mg daily) and the cessation of khat use.[36] Two reported

types of psychosis include manic psychosis with grandiose delusions and a paranoid schizophrenic-like psychosis with persecutory delusions, auditory hallucinations, fear, and anxiety; however, the contribution of khat use to the development of persistent psychosis without pre-existing psychiatric disease remains unproven.[6]

Medical Complications

In Yemen, a hospital-based case-control study of 100 cases of acute myocardial infarctions (AMI) and 100 age- and sex-matched outpatient controls compared the 2 groups based on questionnaires covering personal history of khat chewing, smoking, hypertension, diabetes, and family history.[37] The odds ratio for AMI among khat chewers was 5.0 (95% CI: 1.9–13.1) with a substantially increased risk for heavy khat users. In a prospective, multicenter study of 8,176 consecutive patients from 6 adjacent Middle Eastern countries presenting with acute coronary syndrome, 11.4% were khat chewers, mainly of Yemen origin.[38] After adjustment for covariates (age, diabetes mellitus, dyslipidemia, hypertension, sex, smoking, thrombolytic therapy), khat use was an independent risk factor for in-hospital mortality (OR, 1.9; 95% CI: 1.3–2.7; P <.001) and stroke (OR, 2.7; 95% CK: 1.3–5.9; P =.01). There was a significant delay in the presentation of khat users; 59% of the khat users presented >12 hours after onset of symptoms compared with 21.1% of nonkhat users ($P < .001$). Several case reports associate hepatotoxicity with khat chewing by Middle Eastern immigrants to European countries.[39,40] Some of these patients developed liver failure and encephalopathy. Although common etiologies of hepatotoxicity were excluded, the cause of the liver toxicity remains unclear as a proven association between liver toxicity; khat chewing has not been established as a cause of hepatotoxicity in countries where khat use is common.

There are few data on the oral carcinogenicity of khat chewing, particularly data that controls for the strong risk factors of tobacco and alcohol use.[41] There is an increased prevalence of oral mucosal keratosis (i.e., a precancerous lesion) among khat chewers; however, there is currently insufficient evidence to associate chronic khat chewing with an increased incidence of oral cancers.[42] Chronic chewing of khat can cause green discoloration of the tongue and brownish discoloration of the teeth.[43]

Abstinence Syndrome

Prolonged khat use does not produce a well-defined abstinence syndrome, although a mild depressive reaction (irritability, anorexia, insomnia, nightmares) may occur during the time immediately after the cessation of khat use.[44]

DIAGNOSTIC TESTING

Analytic Methods

Analytic techniques for the quantitation of cathinone and other phenylalkylamine compounds are similar to the methods used for the detection of amphetamine. Several methods are available to detect cathinone in biologic samples including gas chromatography/ flame ionization detection, high performance liquid chromatography with photodiode array detection,[45] cation-exchange liquid chromatography,[46] and liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry.[47] The lower limit of quantitation (LLOQ) of cathinone and methcathinone in urine samples following analysis by gas chromatography/ mass spectrometry (GC/MS) ranges between 12.5– 25 ng/mL,[48] whereas the LLOQ for cathinone and cathine in khat samples following cation-exchange liquid chromatography is 1.3 ng/mg fresh weight and 5.1 ng/mg fresh weight, respectively.[46] Detection of cathinone by immunoassay urine screens is highly variable depending on the specific reagent used and the amount of cross-reactivity between phenylpropanol-amine and cathinone. In a study of 4 volunteers chewing 0.6 g khat leaves/kg body weight, the Abbott fluorescence polarization immunoassay of subsequent urine samples did not detect the presence of amphetamine compounds.[20] Analysis of these samples by GC/MS demonstrated the presence of cathinone during the first 26 hours; the presence of cathine and norephedrine was detectable in all the samples collected during the duration of the study (i.e., 80 h). Although cathinone and methcathinone are stable for 3 days in refrigerated samples (2–4°C/~36–39°F) and in frozen samples (−18°C/~0°F) for 2 months, urine samples stored at 2–4°C for 3 months lost 79% of these compounds.[48]

Driving

Driving under the influence of khat is a suspected cause of the high road crash fatality rate in countries (e.g., Ethiopia) where khat is legal;[49] however, data and technology are not currently available in these countries to determine the effect of khat and other drugs on driving impairment and culpability for fatal crashes. In a case series of 19 individuals arrested for driving under the influence after reportedly chewing khat, the serum cathine concentration ranged from 16.5–314.7 ng/mL with a median of 128.9 ng/mL, whereas 16 serum samples had cathinone concentrations ranging from

9.2–73 ng/mL with a median of 33.2 ng/mL.[50] The serum cathinone concentration did not correlate well to impairment as detected by police observations; the driver with the worst driving performance had the lowest serum cathinone and cathine concentrations. Significant amounts of other drugs of abuse were not detected in this case series.

TREATMENT

As the intoxication that results from khat use is generally mild, patients rarely present for medical care of khat intoxication. There are no well-documented reports of severe toxicity following khat use. Treatment is supportive, similar to the treatment of amphetamine intoxication. Options for the treatment of agitation in a khat user include benzodiazepines or antipsychotic medications. Patients with hypertensive emergencies should be treated with sedation and vasodilators; patients with agitation and severe hyperthermia may require neuromuscular blockade with subsequent intubation and mechanical ventilation along with sedation and aggressive cooling.

References

1. Krikorian A. Khat and its use: a historical perspective. J Ethnopharmacol 1984;12:115–178.

2. Al-Motarreb A, Baker K, Broadley KJ. Khat: pharmacological and medical aspects and its social use in Yemen. Phytother Res 2002;16:403–413.

3. Bruce-Chwatt RM. Intoxication with Qaat, *Catha edulis* L. J Forensic Leg Med 2010;17:232–235.

4. Kalix P, Braenden O. Pharmacological aspects of the chewing of khat leaves. Pharmacologic Rev 1985;37:149–164.

5. Shadan P, Shellard E. An anatomical study of Ethiopian khat. J Pharm Pharmacol 1962;14:110–118.

6. Feyisa AM, Kelly JP. A review of the neuropharmacological properties of khat. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:1147–1166.

7. Crombie L. The cathedulin alkaloids. Bull Narc 1980;32:37–50.

8. Kalix P. Recent advances in khat research. Alcohol Alcohol 1984;19:319–323.

9. Kalix P, Geisshusler S, Brenneisen R, Koelbing U, Fisch H-U. Cathinone, a phenylpropylamine alkaloid from khat leaves that has amphetamine effects in humans. NIDA Res Monogr 1991;105:289–290.

10. Widler P, Mathys K, Brenneisen R, Kalix P, Fisch H-U. Pharmacodynamics and pharmacokinetics of khat: a controlled study. Clin Pharmacol Ther 1994;55:556–562.

11. Griffiths P, Gossop M, Wickenden S, Dunworth J, Harris K, Lloyd C. A transcultural pattern of drug use: qat (khat) in the UK. Br J Psychiatry 1997;170:281–284.

12. Nordal A. Khat: pharmacognostical aspects. Bull Narc 1980;32:51–64.

13. Kalix P. Cathinone, a natural amphetamine. Pharmacol Toxicol 1992;70:77–86.

14. Bentur Y, Bloom-Krasik A, Raikhlin-Eisenkraft B. Illicit cathinone ("hagigat") poisoning. Clin Toxicol 2008:46:206–210.

15. Guantai A, Maitai C. Relative distribution of cathinone and nor-pseudoephedrine in *Catha edulis* growing in Kenya. East Afr Med J 1982;59:394–398.

16. Schorno X, Steinegger E. CNS-active phenylpropylamines of *Catha edulis* of Kenyan origin. Experientia 1979;35:572–574.

17. Guantai AN, Maitai CK. Relative distribution of cathinone and *d*-norpseudoephedrine in *Catha edulis* (Miraa) growing in Kenya. E Afr Med J 1982;59:395–398.

18. Geisshusler S, Brenneisen R. The content of psychoactive phenylpropyl and phenylpentenyl khatamines in *Catha edulis* Forsk. of different origin. J Ethnopharmacol 1987;19:269–277.

19. Balint EE, Falkay G, Balint GA. Khat – a controversial plant. Wien Klin Wochenschr 2009;121:604–614.

20. Toennes SW, Kauert GF. Excretion and detection of cathinone, cathine, and phenylpropanolamine in urine after khat chewing. Clin Chem 2002;48:1715–1719.

21. Hattab FN, Angmar-Månsson B. Fluoride content in khat (*Catha edulis*) chewing leaves. Arch Oral Biol 2000;45:253–255.

22. Randall T. Khat abuse fuels Somali conflict, drains economy. JAMA 1993;269:12,15.

23. Lugman W, Panowski TS. The use of khat (*Catha edulis*) in Yemen. Social and medical observations. Ann Intern Med 1976;85:246–249.

24. Krikorian A, Getahun A. Khat: coffee's rival from Harar, Ethiopia. I. Botany, cultivation and use. Econ Bot 1973;27:353–377.

25. Toennes SW, Harder S, Schramm M, Niess C, Kauert GF. Pharmacokinetics of cathinone, cathine and norephedrine after the chewing of khat leaves. Br J Clin Pharmacol 2003;56:125–130.

26. Guantai AN, Maitai CK. Metabolism of cathinone to *d*-norpseudoephedrine in humans. J Pharmaceut Sci 1983;72:1217–1218.

27. Brenneisen R, Geisshusler S, Schorno X. Metabolism of cathinone to (−)-norephedrine and (−)-norpseudoephedrine. J Pharm Pharmacol 1986;38:298–300.

28. Testa B, Beckettt A. Metabolism and excretion of diethylpropion in man under acidic urine conditions. J Pharm Pharmacol 1973;25:119–124.

29. Foltin RW, Schuster CR. Behavioral tolerance and cross-tolerance to *d,l*-cathinone and *d*-amphetamine in rats. J Pharmacol Exp Ther 1982;222:126–131.

30. Wagner GC, Preston K, Ricaurte GA, Schuster CR, Seiden LS. Neurochemical similarities between *d,l*-cathinone and *d*-amphetamine. Drug Alcohol Depend 1982;9:279–284.

31. Pennings EJ, Opperhuizen A, van Amsterdam JG. Risk assessment of khat use in the Netherlands: a review based on adverse health effects, prevalence, criminal involvement and public order. Regul Toxicol Pharmacol 2008; 52:199–207.

32. Brenneisen R, Fisch H-U, Koelbing U, Geisshusler S, Kaliz P. Amphetamine-like effects in humans of the khat alkaloid cathinone. Br J Clin Pharmacol 1990;30: 825–828.

33. Woolverton WL. A review of the effects of repeated administration of selected phenylethylamines. Drug Alcohol Depend 1986;17:143–150.

34. Chapman MH, Kajihara M, Borges G, O'Beirne J, Patch D, Dhillon AP, et al. Severe, acute liver injury and khat leaves. N Engl J Med 2010;362:1642–1644.

35. Giannini AJ, Castellani S. A manic-like psychosis due to khat (*Catha edulis* Forsk.). J Toxicol Clin Toxicol 1982; 19:455–459.

36. Yousef G, Huq Z, Lambert T. Khat chewing as a cause of psychosis. Br J Hosp Med 1995;54:322–326.

37. Al-Motarreb A, Briancon S, Al-Jaber N, Al-Adhi B, AlJailani F, Salek MS, Broadley KJ. Khat chewing is a risk factor for acute myocardial infarction: a case-control study. Br J Clin Pharmacol 2005;59:574–581.

38. Ali WM, Zubaid M, Al-Motarreb A, Singh R, Al-Shereiqi SZ, Shehab A, et al. Association of khat chewing with increased risk of stroke and death in patients presenting with acute coronary syndrome. Mayo Clin Proc 2010; 85:974–980.

39. Chapman MH, Kajihara M, Borges G, O'Beirne J, Patch D, Dhillon AP, et al. Severe, acute liver injury and khat leaves. N Engl J Med 2010;362:1642–1644.

40. Peevers CG, Moorghen M, Collins PL, Gordon FH, McCune CA. Liver disease and cirrhosis because of Khat chewing in UK Somali men: a case series. Liver Int 2010;30:1242–1243.

41. Kassie F, Darroudi F, Kundi M, Schulte-Hermann R, Knasmuller S. Khat (*Catha edulis*) consumption causes genotoxic effects in humans. Int J Cancer 2001;92: 329–332.

42. Kalix P. Pharmacological properties of the stimulant khat. Pharmacol Ther 1990;48:397–416.

43. Hill CM, Gibson A. The oral and dental effects of q'at chewing. Oral Surg Oral Med Oral Pathol 1987;63: 433–436.

44. Luqman W, Danowski T. The use of khat in Yemen: social and medical observations. Ann Intern Med 1976;85: 246–249.

45. Mathys K, Brenneisen R. Determination of (S)-(−)-cathinone and its metabolites (R,S)-(−)-norephedrine and (R,R)-(−)-norpseudoephedrine in urine by high-performance liquid chromatography with photodiode-array detection. J Chromatogr 1992;593:79–85.

46. Laussmann T, Meier-Giebing S. Forensic analysis of hallucinogenic mushrooms and khat (*Catha edulis* Forsk) using cation-exchange liquid chromatography. Forensic Sci Int 2010;195:160–164.

47. Bogusz MJ, Kruger KD, Maier RD. Analysis of underivatized amphetamines and related phenethylamines with high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Anal Toxicol 2000;24:77–84.

48. Paul BD, Cole KA. Cathinone (khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. J Anal Toxicol 2001; 25:525–530.

49. Eckersley W, Salmon R, Gebru M. Khat, driver impairment and road traffic injuries: a view from Ethiopia. Bull World Health Organ 2010;88:235–236.

50. Toennes SW, Kauert GF. Driving under the influence of khat – alkaloid concentrations and observations in forensic cases. Forensic Sci Int 2004;140:85–90.

Chapter 59

KRATOM
[*Mitragyna speciosa* (Korth.) Havil.]

HISTORY

Traditionally, manual laborers in Thailand and Malaysia used the leaves of kratom as a mild narcotic to increase productivity and reduce boredom. Reported kratom use by Malaysian laborers as an opium substitute dates back to at least 1836 as described by Low.[1] In 1895, Holmes identified *Mitragyna speciosa* as the source of the kratom used by workers when opium was unavailable.[2] Ellen Field named mitragynine, when she isolated this compound from kratom in 1921.[3] Joshi et al determined the chemical structure of the main alkaloid in kratom in 1963; two years later, Zacharias et al confirmed the structure with X-ray crystallography.[4] In a search for nonopiate analgesics in the 1960s and 1970s, over 20 alkaloids were identified in kratom.[5]

Studies by Grewal in 1932 at the University of Cambridge indicated that this substance was a central nervous system stimulant similar to cocaine based on his observation of the clinical effects of the administration of kratom to 5 men.[6] In 1943, Thailand banned the use of this substance (Kratom Act 2486). Macko et al investigated the use of mitragynine in animal experiments as a substitute for the analgesic and cough suppressant activities of codeine.[7] Reported in 1972, their studies suggested that the active ingredient was a metabolite of mitragynine rather than the parent drug because the subcutaneous route was substantially less effective than oral administration. The Thailand Narcotics Act B.E. 2522 placed kratom along with marijuana in Category 5 of a five-category classification of narcotics in 1979. The use of kratom in Australia became illegal in 2005. Kratom remains a popular drug in these areas.[2]

BOTANICAL DESCRIPTION

Common Name: kratom, kakuam, ithang, or thom (Thailand); baik-biak or ketum (Malaysia); herbal speedball, krypton (mixture *O*-demethyltramadol and kratom)

Scientific Name: *Mitragyna speciosa* (Korth.) Havil.

Botanical Family: Rubiaceae (coffee)

Physical Description: This large arboreal tree grows up to 30 m (~100 ft) in height; characteristic globular flowering head contain up to 120 florets.[8] Woody shoots have 10–12 leaves in opposite and crossing (decussate) pairs; the stems of the leaves vary from light green to red. Figure 59.1 displays a young kratom tree with leaves containing reddish stems and veins. The simple inflorescence (cluster of flowers on a stem) has two secondary axes (dichasial cyme) that form the characteristic globular flowering heads. The fruiting capsule contains numerous small flat seeds. The name of the plant derives from the shape of the stigmas (i.e., the area which receives the pollen) that resembles the mitre of a bishop.

Distribution and Ecology: This tropical tree is indigenous to Thailand, Malaysia, Myanmar and other areas of Southeast Asia, primarily in swampy areas. There are 9 species from the genus, *Mitragyna* species inhabit swamp-lands in West Africa, East Africa, and India as well as Southeast Asia.

IDENTIFYING CHARACTERISITCS

Kratom contains over 25 alkaloids, primarily indole (mitragynine, paynantheine) and oxindole (mitraphylline, speciophylline, speciofoline, isospeciofoline) alkaloids.[9,10] Speciogynine and speciociliatine are diastereomers of mitragynine. Mitragynine (CAS RN: 4098-40-2, 9-methoxy-corynantheidine) is the major indole alkaloid in kratom, accounting for up to 66% of the crude base depending on location, season, growing conditions, and variety.[11] Minor alkaloids include ajmalicine (CAS RN: 483-04-5), corynantheidine (CAS RN: 23407-35-4), isocorynantheidine, 7-hydroxymitragynine, mitraversine, mitraphylline, isomitraphylline, paynantheine (CAS RN: 1346-36-7), rhynchophylline (CAS RN: 76-66-4), and stipulatine.[12,13] Mitragynine and paynantheine are unique to *M. speciosa*. Figure 59.2 displays the chemical structure of mitragynine and some minor alkaloids in kratom. Other components of kratom include flavonoids, triterpenoid saponins, monoaryl glycosides, cyclohexanone glycosides, and secoiridoid glycosides.

FIGURE 59.1. Young kratom tree with leaves containing red stems and veins. The stems of the leaves vary from light green to red. (Photo Courtesy of Darika Sai-ngam, MA, and Sawitri Assanangkornchai, MD, PhD, Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand)
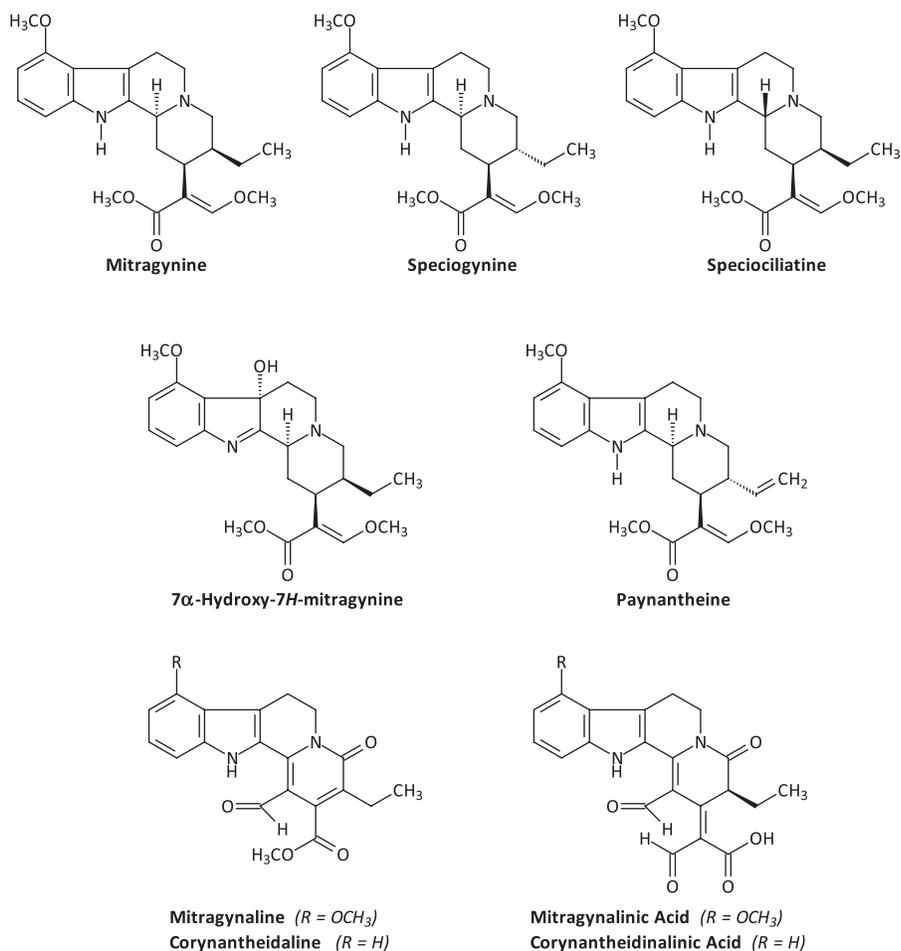
FIGURE 59.2. Chemical structures of mitragynine and minor alkaloids in kratom.[11]

Mitragynine   Speciogynine   Speciociliatine

7α-Hydroxy-7H-mitragynine   Paynantheine

Mitragynaline *(R = OCH₃)*
Corynantheidaline *(R = H)*

Mitragynalinic Acid *(R = OCH₃)*
Corynantheidinalinic Acid *(R = H)*

TABLE 59.1. Some Physical Properties of Mitragynine.

Physical Property	Value
Melting Point	104°C (~219°F)
log P (Octanol-Water)	3.330

Kratom is both a sedative and a stimulant that is structurally similar to yohimbine. *In vitro*, mitragynine (9-methoxy-corynantheidine) displays agonist activity at the μ– and δ–opioid receptors subtypes,[14] whereas corynantheidine is an opioid receptor antagonist.[15] The μ-opioid receptor is the primary receptor responsible for mediation of opioid-related respiratory depression, euphoria, and analgesia. The minor alkaloid, 7-hydroxymitragynine also demonstrates μ-opioid activity. Other alkaloids in kratom include the antinociceptive substance, 7-hydroxymitragynine, which is a minor alkaloid that is a more potent analgesic than mitragynine.[16] An oxidized derivative of mitragynine, mitragynine pseudoindoxyl, is also more potent than the parent compound.[17] Desmethyl mitragynine is a partial agonist for the μ-opioid receptor. Mitragynine is sparingly soluble in aqueous solutions at pH 4.0–5.7.[7] Table 59.1 displays some physical properties of mitragynine.

EXPOSURE

Sources

In traditional Southeast Asian medicine, kratom is used as a wound poultice, antipyretic, antidiarrheal agent, and opiate substitute during withdrawal. Natives also use the leaves of the kratom plant in cooking. Justifications for the use of kratom by rural workers in Thailand include increased endurance in the hot sun and relief of fatigue from overwork.[18] In this setting, kratom use occurs primarily among adults and the elderly. Kratom is not listed on the schedule of drugs from the US Drug Enforcement Agency, but this agency lists kratom as a drug of concern; kratom is illegal in Bhutan, Finland, Denmark, Poland, Lithuania, Thailand, Australia, Malaysia, and Myanmar (Burma). Kratom is widely available over the Internet, particularly as a dietary supplement for the amelioration of opioid withdrawal. Analysis of commercial samples of kratom indicated that the leaves of *Mitragyna speciosa* or closely related species are the source of these commercial products based on internal transcribed spacer sequence analysis of rDNA; the use of polymerase chain reaction-restriction fragment length polymorphism allows the separation of kratom from other psychoactive plants.[19] Kratom formulations include the leaf, powder, and a resin extract made from fresh kratom leaves.

The total alkaloid content and the oxindole alkaloids vary substantially between different geographic origins and seasons; however, the content of the major indole alkaloids (mitragynine, speciogynine, paynantheine) remains relatively stable in the leaves with some variation depending on season, strain, and other factors.[20] Alkaloid content also varies with the age of the plant with young plants containing higher concentrations of the mitragynine diastereomer, speciociliatine, and the paynantheine diastereomer, isopaynantheine, than older leaves. On average, green kratom leaves contain 0.2% mitragynine with the average green and dry leaf weighing ~1.7 g and~ 0.43 g, respectively.[21] Herbal mixtures of kratom may be adulterated by other substances. Krypton is the popular name of an herbal preparation containing powdered kratom leaves mixed with the synthetic opioid-receptor agonist, *O*-desmethyltramadol, which is the active metabolite of the analgesic, tramadol.[22]

Methods of Abuse

Figure 59.3 displays a man climbing a mature kratom tree to harvest the leaves for his personal use. These trees are hidden from public view to prevent theft; the wood surrounding the tree restricts others from climbing the tree to steal the leaves. The bitter leaves of kratom are chewed, smoked, or brewed as a tea; typically about 10 leaves are used daily. A popular, illicit drink for teenagers in Thailand is a kratom cocktail

FIGURE 59.3. A man climbing his mature kratom tree to harvest the leaves for his personal use. (Photo courtesy of Darika Sai-ngam, MA, and Sawitri Assanangkornchai, MD, PhD, Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand)

called 4×100 for the combination of the following 4 ingredients: cola drink, cough syrup, benzodiazepines, and kratom juice obtained by boiling kratom leaves for 1 hour. In a study of Thai kratom users, almost all were men and 90% of these users either chewed fresh leaves, ate the ground dried leaf, or drank an infusion of the dried leaves.[21] The drinking of a warm liquid (water, coffee) usually follows kratom use. Kratom users chew kratom about 3–10 times daily. In Thailand, natives sometimes combine the leaves from *Mitragyna parvifolia* (Roxb.) Korth. and the leaves of *Mitragyna speciosa*.

DOSE EFFECT

In animal models, the toxicity of mitragynine is relative low. The oral administration of 46–230 mg mitragynine/kg to rodents produced some decreased motor activity, analgesia, depressed respiratory rate, and reduced body temperature; oral mitragynine doses up to 920 mg/kg did not cause tremors, convulsions, or death in these animals.[7]

In studies of dogs, the oral administration of up to 80 mg mitragynine/kg did not produce observable effects. The subcutaneous administration of up to 69 mg mitragynine/kg to monkeys did not alter the activity of the animals. Recommended recreational doses of kratom leaves range from 2–10 g.

TOXICOKINETICS

Available data suggests that the biotransformation of mitragynine is complex. Animal studies indicate that mitragynine undergoes hydrolysis of the methylester on C16 and *O*-demethylation of the 9-methoxy and 17-methoxy groups followed by oxidation to carboxylic acids via intermediate aldehydes, reduction to alcohols, or some combination of oxidation and reduction.[23] Phase II metabolites in human urine include both glucuronide and sulfate conjugates. Following ingestion of kratom, urine compounds include mitragynine, paynantheine, and the diastereomers of mitragynine (speciogynine, speciociliatine) along with their metabolites.[24]

CLINICAL RESPONSE

The clinical effects of kratom begin soon (i.e., 5–10 min) after inhalation or mastication, and these effects typically persist for ~1 hour. The clinical effects of kratom are dose-dependent, although the dose-response curve is not well-defined. At low doses, kratom demonstrates stimulant properties, whereas at higher doses, the opioid properties of kratom predominate.[25] Desirable effects associated with the use of kratom include feelings of elation, sociability, alertness, and tranquility. Adverse effects associated with the recreational use of kratom include dry mouth, nausea, vomiting, diarrhea, nystagmus, diaphoresis, pruritus, tremor, and seizure. Complications associated with the chronic kratom use include anorexia, weight loss, hyperpigmentation of mucosal surfaces, and psychosis. In a study of 149 long-term kratom users (daily >5 years) and 168 occasional users (i.e., no more often than once weekly), the percentages of users believing they were dependent on kratom were 61% and 12%, respectively.[18] Adverse effects reported in this group of kratom users were constipation, tremulousness, headaches, fatigue, insomnia, poor concentration, impaired memory, and social withdrawal. Anecdotally, withdrawal symptoms (hostility, aggression, rhinitis, tearing, lethargy, myalgia, arthralgias, muscle jerks) associated the cessation of kratom use are usually modest.[21,26] Several case reports associate the ingestion of kratom and other drugs (modafinil during opiate withdrawal, *Datura stramonium* tea) with seizure activity, but the contribution of kratom to the development of seizures is unclear.[26,27]

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the quantitation of mitragynine in serum include high pressure liquid chromatography with UV detection (225 nm), high pressure liquid chromatography/electrospray/tandem mass spectrometry using positive-ion detection and multiple reactions monitoring,[28] liquid chromatography/linear ion trap/mass spectrometry,[23] liquid chromatography/mass spectrometry,[29] and liquid chromatography/tandem mass spectrometry with amitriptyline as the internal standard.[30] The limit of detection (LOD) and lower limit of quantitation (LLOQ) for high pressure liquid chromatography with UV detection were 0.03 mg/L and 0.1 mg/L, respectively, compared with a LLOQ of 0.0002 mg/L for liquid chromatography/tandem mass spectrometry. Analysis of urine samples with high pressure liquid chromatography/electrospray/tandem mass spectrometry using ajmalicine as the internal standard and methyl-*t*-butyl ether extraction allows the detection of mitragynine above 0.00002 mg/L.[28] The intraday precision using ajmalicine as the internal standard at 0.001 mg/L and 0.005 mg/L is 12% and 16%, respectively. After enzymatic cleavage of conjugates, solid-phase extraction, and trimethylsilylation, the use of gas chromatography/mass spectrometry allows the detection of *O*-desmethyltramadol, mitragynine, and mitragynine metabolites in urine samples following the use of krypton.[31] The limit of detection of

O-desmethyltramadol and mitragynine was 0.1 mg/L and 0.05 mg/L, respectively.

Biomarkers

In a case series of 9 fatalities involving polydrug users with detectable amounts of *O*-desmethyltramadol in their postmortem blood and nonspecific postmortem findings (lung and brain congestion), the *O*-desmethyltramadol concentrations ranged from 0.4–4.3 mg/L (µg/g).[32] The mitragynine concentrations ranged from 0.02–0.18 mg/L (µg/g). The absence of detectable tramadol and *N*-desmethyltramadol in the postmortem blood samples indicates that these individuals consumed the herbal blend, krypton. All were found in asystole, and all except one individual were found dead in their homes. All analyzed postmortem samples except one were femoral. In Thailand, the prevalence of kratom use in randomly selected drivers was similar (0.9%) to the use of marijuana (1.1%); however, there are few data on the effects of kratom on driving skills.[33]

TREATMENT

There are few clinical data on the treatment of toxicity associated with the use kratom. Potentially, naloxone is an antidote for the opioid effects of kratom, but there are few data on the efficacy of this antidote for the reversal of toxicity associated with the use of kratom.

References

1. Jansen KL, Prast CJ. Ethnopharmacology of kratom and the *Mitragyna* alkaloids. J Ethnopharmacol 1988;23:115–119.

2. Holmes EM. Some medicinal products from the Straits settlements. Pharmaceut J 1895;54:1095–1096.

3. Field E. Mitragynine and mitraversine, two new alkaloids from species of *Mitragyna*. J Chem Soc Trans 1921;119:887–890.

4. Zacharias DE, Rosenstein RD, Jeffrey GA. The structure of mitragynine hydroiodide. Acta Cryst 1965;18:1039–1043.

5. Jansen KL, Prast CJ. Psychoactive properties of mitragynine (kratom). J Psychoactive Drugs 1988;20:255–257.

6. Grewal KS. Observations on the pharmacology of mitragynine. J Pharm Exp Ther 1932;46:251–271.

7. Macko E, Weisbach JA, Douglas B. Some observations on the pharmacology of mitragynine. Arch Int Pharmacodyn Ther 1972;198:145–161.

8. Shellard EJ, Lees MD. The *Mitragya* species of Asia Part V – the anatomy of the leaves of *Mitragyna speciosa* Korth. Planta Med 1965;13:280–290.

9. Beckett AH, Shellard EJ, Phillipson JD, Lee CM. The *Mitragyna* species of Asia Part VI. Oxindole alkaloids from the leaves of *Mitragyna speciosa* Korth. Planta Med 1966;14:266–276.

10. Beckett AH, Shellard EJ, Phillipson JD, Lee CM. The *Mitragyna* species of Asia. VII. Indole alkaloids from the leaves of *Mitragyna speciosa* Korth. Planta Med 1966;14:277–288.

11. Takayama H. Chemistry and pharmacology of analgesic indole alkaloids from the Rubiaceous plant, *Mitragyna speciosa*. Chem Pharm Bull 2004;52:916–928.

12. Leon F, Habib E, Adkins JE, Furr EB, McCurdy CR, Cutler SJ. Phytochemical characterization of the leaves of *Mitragyna speciosa* grown in USA. Nat Prod Commun 2009;4:907–910.

13. Beckett AH, Shellard EJ, Phillipson JD, Lee CM. Alkaloids from *Mitragyna speciosa* (Korth.). J Pharm Pharmacol 1965;17:753–755.

14. Thongpradichote S, Matsumoto K, Tohda M, Takayama H, Aimi N, Sakai S, Watanabe H. Identification of opioid receptor subtypes in antinociceptive actions of supraspinally-administered mitragynine in mice. Life Sci 1998;62:1371–1378.

15. Matsumoto K, Takayama H, Ishikawa H, Aimi N, Ponglux D, Watanabe K, Horie S. Partial agonistic effect of 9-hydroxycorynantheidine on mu-opioid receptor in the guinea-pig ileum. Life Sci 2006;78:2265–2271.

16. Ma J, Yin W, Zhou H, Cook JM. Total synthesis of the opioid agonistic indole alkaloid mitragynine and the first total syntheses of 9-methoxygeissoschizol and 9-methoxy-Nb-methylgeissoschizol. Org Lett 2007;9:3491–3494.

17. Takayama H, Ishikawa H, Kurihara M, Kitajima M, Aimi N, Ponglux D, et al. Studies on the synthesis and opioid agonistic activities mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J Med Chem 2002;45:1949–1956.

18. Assanangkornchai S, Muekthong A, Sam-Angsri N, Pattanasattayawong U. The use of *Mitragynine speciosa* ("krathom"), an addictive plant, in Thailand. Subst Use Misuse 2006;42:2145–2157.

19. Maruyama T, Kawamura M, Kikura-Hanajiri R, Takayama H, Goda Y. The botanical origin of kratom (*Mitragyna speciosa*; Rubiaceae) available as abused drugs in the Japanese markets. J Nat Med 2009;63:340–344.

20. Shellard EJ. The alkaloids of *Mitragyna* with special reference to those of *Mitragyna speciosa*, Korth. Bull Narc 1974;26:41–55.

21. Suwanlert S. A study of kratom eaters in Thailand. Bull Narc 1975;27:21–27.

22. Arndt T, Claussen U, Güssregen B, Schröfel S, Stürzer B, Werle A, Wolf G. Kratom alkaloids and *O*-desmethyltramadol in urine of a "Krypton" herbal mixture consumer. Forensic Sci Int 2011;208:47–52.

23. Philipp AA, Wissenbach DK, Zoerntlein SW, Klein ON, Kanogsunthornrat J, Maurer HH. Studies on the

metabolism of mitragynine, the main alkaloid of the herbal drug kratom, in rat and human urine using liquid chromatography-linear ion trap mass spectrometry. J Mass Spectrom 2009;44:1249–1261.

24. Philipp AA, Wissenbach DK, Weber AA, Zapp J, Mauer HH. Metabolism studies of the kratom alkaloid speciociliatine, a diastereomer of the main alkaloid mitragynine, in rat and human urine using liquid chromatography–liner ion trap mass spectrometry. Anal Bioanal Chem 2011; 399:2747–2753.

25. Babu KM, McCurdy CR, Boyer EW. Opioid receptors and legal highs: *Salvia divinorum* and kratom. Clin Toxicol 2008;46:146–152.

26. Boyer EW, Babu KM, Adkins JE, McCurdy CR, Halpern JH. Self-treatment of opioid withdrawal using kratom (*Mitragyna speciosa* Korth). Addiction 2008;103: 1048–1050.

27. Nelsen JL, Lapoint J, Hodgman MJ, Aldous KM. Seizure and coma following kratom (*Mitragynine speciosa* Korth) exposure. J Med Toxicol 2010;6:424–426.

28. Lu S, Tran BN, Nelsen JL, Aldous KM. Quantitative analysis of mitragynine in human urine by high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B 2009;877:2499–2505.

29. Janchawee B, Keawpradub N, Chittrakarn S, Prasettho S, Wararatananurak P, Sawangjareon K. A high-performance liquid chromatographic method for determination of mitragynine in serum and its application to a pharmacokinetic study in rats. Biomed Chromatogr 2007;21: 176–183.

30. de Moraes NV, Moretti RA, Furr EB 3rd, McCurdy CR, Lanchote VL. Determination of mitragynine in rat plasma by LC-MS/MS: application to pharmacokinetics. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877: 2593–2597.

31. Philipp AA, Meyer MR, Wissenbach DK, Weber AA, Zoerntlein SW, Zweipfenning PG, Maurer HH. Monitoring of kratom or krypton intake in urine using GC-MS in clinical and forensic toxicology. Anal Bioanal Chem 2011;400:127–135.

32. Kronstrand R, Roman M, Thelander G, Eriksson A. Unintentional fatal intoxications with mitragynine and *O*-desmethyltramadol from the herbal blend krypton. J Anal Toxicol 2011;35:242–247.

33. Ingsathit A, Woratanarat P, Anukarahanonta T, Rattanasiri S, Chatchaipun P, Wattayakorn K, et al. Prevalence of psychoactive drug use among drivers in Thailand: a roadside survey. Accid Anal Prev 2009;41:474–478.

Chapter 60

MARIJUANA (*Cannabis sativa* L.) and Synthetic Cannabinoids

HISTORY

The hemp plant (*Cannabis sativa* L.) is the source of the flowers, dried leaves, and extracts (i.e., collectively called marijuana) that is a popular psychotropic drug worldwide. Cannabis was well known to the Assyrians, Egyptians, and Chinese many centuries before the beginning of Christianity;[1] however, the Old Testament does not clearly mention the use of the hemp plant (*Cannabis sativa* L.) despite prolonged trade between the Jews and Assyrians.[2] The ancient Chinese and Greeks used the plant stems in the production of rope and clothing fiber, whereas the Romans wove the cannabis fibers into rope during construction of their ships. The psychotropic and medicinal use of cannabis occurred in early India before the end of the 2nd millennium BC. The *Athara Veda* mentions cannabis as one of the five sacred plants used for "freedom from distress."[3] The medicinal use of the hemp plant dates back almost 5,000 years to the Chinese Emperor Shen-Nung, who recommended the use of this plant for several medical disorders in 2,737 BC.[4] These disorders included malaria, constipation, arthralgias, and female complaints. Between 2,000 BC and 1,400 BC, Indian physicians discovered the euphoric properties of *Cannabis sativa*; they recommended the hemp plant for sleep disorders, fever, appetite stimulant, headaches, rheumatism, and venereal diseases.[5]

Settlers at Jamestown, Virginia also cultivated this plant as a fiber source. In the mid-19th century, the Irish physician, WB O'Shaughnessy introduced the use of this plant into Western medicine as an analgesic, anticonvulsant, anxiolytic, and antiemetic.[6] During the 19th century, popular medicinal uses for the hemp plant in Western Europe included the treatment of asthma, pain, seizures, anxiety, muscle spasm, and anorexia. By the late 19th century, medicinal cannabis was being marketed by drug companies; however, more effective analgesic and sedative-hypnotic drugs replaced medicinal cannabis by 1900. Then, the Harrison Act of 1914 classified cannabis as a narcotic with cocaine and heroin, which resulted in a significant decline in the marketing and use of cannabis. The Marijuana Tax Act of 1937 banned the use of this plant in the United States.

Although the chemical structure of psychoactive cannabinoids in this plant was investigated in the 1930s and 1940s, the chemical structure of the active ingredient (Δ^9-tetrahydrocannabinaol, Δ^9-THC) was not isolated until 1964.[7] In 1985, the US Food & Drug Administration (FDA) approved the use of synthetic Δ^9-THC (dronabinol) as an antiemetic; the Belgian company, Solvay Pharmaceuticals began marketing dronabinol in the United States under the trademark, Marinol®. The existence of G-protein-coupled cannabinoid receptors was not discovered until 1988, when Devane and colleagues characterized the central CB1 receptor in rat brain.[8] In 1993, Munro and colleagues cloned the peripheral CB2 receptor from macrophages derived from the marginal zone of the spleen.[9]

BOTANICAL DESCRIPTION

There are several subspecies and varieties of *C. sativa* including *Cannabis sativa* subsp. *indica* (Lam.) E. Small & Cronq. (Oceania), *Cannabis sativa* subsp. *sativa* L. (Caribbean, North America), *Cannabis sativa* var.

sativa L., and *Cannabis sativa* var. *spontanea* Vavilov (North America).

Common Name: Hemp, marijuana, Indian hemp, Mary Jane, hashish, pot, grass, cannabis, weed

Scientific Name: *Cannabis sativa* L.

Botanical Family: Cannabaceae (Hemp)

Physical Description: *Cannabis sativa* L. is an aromatic, slightly branched, annual herb with variable numbers of palmate leaves that reach 3–10 feet (1–3 m) in height. Figure 60.1 displays a cultivated marijuana plant. The lower leaves typically are opposite and single with the leaves in the upper portion of the plant being alternate and containing 5–9 sharply serrate, smooth, dark green leaflets on long thin petioles. Usually, the small flowers are imperfect with an individual plant having either staminate (male) or pistillate (female) structures; however, some plants may contain both male and female flowers. The staminate flowers develop in loose, multibranched indeterminate inflorescence (panicle) along the erect, fibrous stems, whereas pistillate flowers appear in racemes. The male flowers have 5, almost separate, pale yellow segments, whereas the female flowers have a single, glandular, hairy, 5-veined leaf covering the ovary. The small, smooth, light brown-gray fruit contains the seed.

Distribution and Ecology: The hemp plant is a native species of Central Asia including Northeastern Iran, Afghanistan, Northern India, Southern

FIGURE 60.1. Cultivated marijuana plant. (Photo courtesy of the US Drug Enforcement Agency)

Siberia, and parts of China. However, this plant adapts well to northern temperate climates including parts of the United States and Europe; cultivation of this plant is now worldwide.

IDENTIFYING CHARACTERISTICS

Structures/Nomenclature

C. sativa contains over 400 chemicals (e.g., alcohols, steroids, sugars, fatty acids, phenols, terpenes) that include over 60 cannabinoids.[10] The main active ingredient, Δ^9-THC is a nonnitrogenous compound ($C_{21}H_{30}O_2$) that contains 2 chiral centers in *trans*-configuration. The term cannabinoids applies to the natural C_{21}-substituted monoterpenes and their carboxylic acid metabolites in marijuana, a large number of synthetic cannabinoid analogues, and the endogenous ligands of the cannabinoid receptors. Two numbering systems exist for cannabinoids that involve the monoterpenoid and dibenzopyran numbering as demonstrated in Figure 60.2.[11] In the latter numbering system for pyran compounds, the main active ingredient, tetrahydrocannabinol, is numbered Δ^9-THC. However, not all cannabinoids are pyran compounds; hence, a second nomenclature using a biogenetic basis exists. In this monoterpenoid numbering system, Δ^9-THC is Δ^1-THC. Although the biogenic numbering system has the advantages that all cannabinoids can be named and a carbon atom retains the same number in most chemical transformations, the formal dibenzopyran numbering system is more common.

There are several subclasses of cannabinoids with variable cannabinoid receptor agonist activity including cannabidiol, cannabigerol, cannabichromene, Δ^9-THC, Δ^8-THC, cannabinol, cannabicyclol, cannabielsoin, cannabinodiol, and cannabitriol compounds. Bicyclic, aminoalkylindole, and anandamide compounds are also cannabinoid receptor agonists. Quantitatively, the major cannabinoids in marijuana are cannabinoid acids of Δ^9-THC, cannabidiol, cannabichromene, and cannabigerol as demonstrated in Figure 60.3. There are 9 cannabinoids in the Δ^9-THC group consisting of compounds with 1,3,4, or 5 carbon side chains. Natural Δ^9-THC has two chiral centers at the C_{6a} and C_{10a} carbons; the term THC applies to the naturally occurring (-)-*trans*-isomer of Δ^9-THC.

Physiochemical Properties

Δ^9-THC (Dronabinol, CAS RN: 1972-08-3) is a volatile, viscous oil that is highly lipophilic and poorly water

Monoterpenoid numbering

Dibenzopyran numbering

Δ^9-Tetrahydrocannabinol

$R_1 = R_3 = H$

$R_2 = C_5H_{11}$
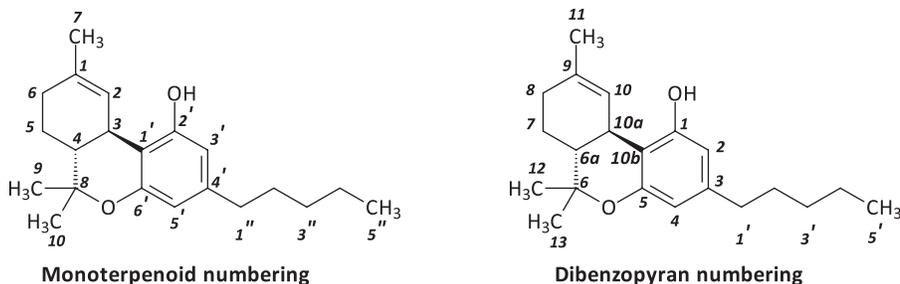
FIGURE 60.2. Monoterpenoid and dibenzopyran numbering systems for cannabinoids. According to the monoterpenoid system, the main cannabinoid is Δ^1-Tetrahydrocannabinol, whereas the same cannabinoid in the more common dibenzopyran system is Δ^9-Tetrahydrocannabinol as displayed in the figure. The common phenolic Δ^9-THC cannabinoids are 21 carbon compounds with a C_5 side chain ($R_2 = C_5H_{11}$). The corresponding carboxylic acids are A ($R_1 = COOH$) and B ($R_3 = COOH$).[11]
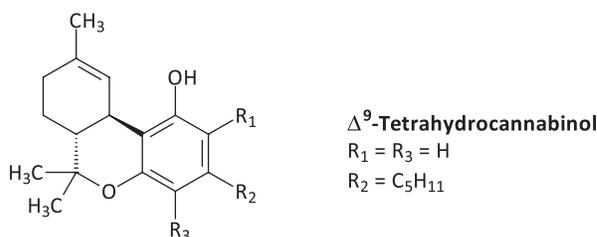
TABLE 60.1. Some Physiochemical Properties of Tetrahydrocannabinol (Dronabinol).

Physical Property	Value
Molecular Weight	314.4617 g/mol
pKa Dissociation Constant	10.6
log P (Octanol-Water)	7.600
Water Solubility	2800 mg/L (23°C/73.4°F)

Cannabigerol type

$R_1 = H$ or CO_2H

$R_2 = C_3$ or C_5 side chain

$R_3 = H$ or CH_3

Cannabichromene type

$R_1 = H$ or CO_2H

$R_2 = C_3$ or C_5 side chain

Cannabidiol type

$R_1 = H$ or CO_2H

R_2 C_1, C_3, C_4 or C_5 side chain

$R_3 = H$ or CH_3

Cannabinol type

$R_1 = H$ or C_3

$R_2 = H$ or CO_2H

$R_3 = C_1$, C_3, C_4 or C_5 side chain
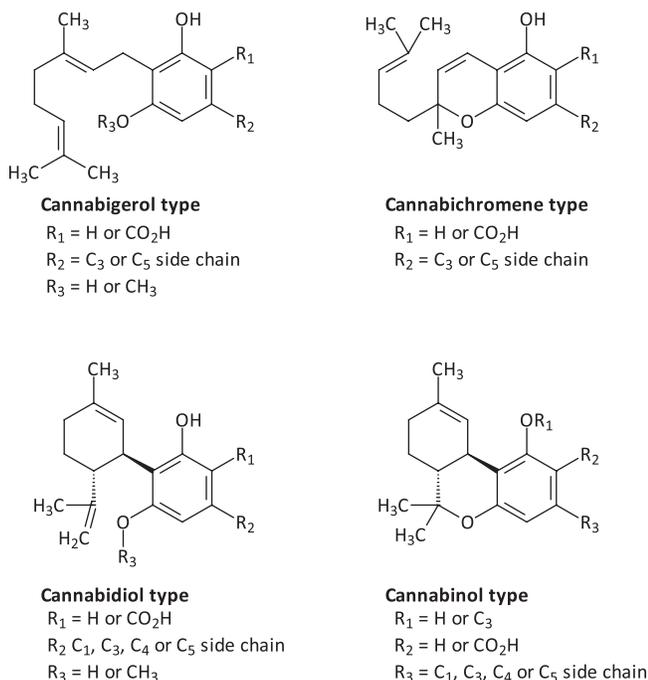
FIGURE 60.3. Four main types of cannabinoids present in *Cannabis sativa* For cannabinol, $R_1 = H$, $R_2 = H$, and $R_3 = C_5H_{11}$, whereas for cannabidiol $R_1 = H$, $R_2 = C_5H_{11}$, and $R_3 = H$.[11]

soluble.[12] The odor probably results from the volatilization of α- and β-pinene, limonene, and myrcene from the plant material.[13] Δ^9-THC readily binds to glass and plastic.[14] Most metabolites of Δ^9-THC have similar solubility. Table 60.1 lists some physiochemical properties of Δ^9-THC. Air, heat, and light degrade Δ^9-THC to cannabidiol (CBD, CAS RN: 13956-29-1),[15] and the potency of cannabis products decreases as a result of the oxidation of the major active component (Δ^9-THC) to cannabinol (CBN, CAS RN: 521-35-7), particularly in acid media.[16] Drying of the plant material does not alter the qualitative composition of the oil or the odor associated with marijuana.[17]

In experimental studies, the naturally occurring (−)-enantiomer of *trans*-Δ^9-THC is more potent (i.e., 5–100 times) than the (+)-enantiomer.[18] Δ^8-THC is a less potent, positional isomer of Δ^9-THC. The cannabinoid acids do not produce psychotropic effects; the decarboxylation of these compounds to corresponding phenols during the smoking of marijuana results in psychotropic effects.[19] The decarboxylation of cannabinoid acids of Δ^9-THC during pyrolysis of marijuana produces the main psychotomimetic compound, Δ^9-THC; however, the decarboxylation of other naturally occurring cannabinoid acids (e.g., cannabinol, cannabidiol, cannabigerol, cannabichromene, cannabidiolic acid) probably does not produce psychoactive metabolites.[20,21] Smoking does not significantly alter the ratio of cannabinoid acid isomers.[22] An isomer of Δ^9-THC [Δ^8-THC ($\Delta^{1(6)}$-THC)] and the propyl homologue of Δ^9-THC (Δ^9-tetrahydrocannabivarin, CAS RN: 28172-17-0) possess some psychoactive properties, but these compounds probably do not contribute significantly to the psychomimetic effects present during

the smoking of marijuana.[23] Dronabinol degrades rapidly in acidic solutions.

Terminology

Marijuana (marihuana) refers to plant material from the hemp plant that produces psychotomimetic or therapeutic effects, primarily the flowering tops and dried leaves. Cannabis refers to any psychoactive part or preparation of the hemp plant (*C. sativa*) including loose marijuana plant material, buds, sinsemilla, Thai sticks, Mexican kilobricks, hashish, and hash oil; cannabis is also a collective term for psychoactive compounds derived from the marijuana plant. Terms used for marijuana include Pot, Mary Jane, MJ, Weed, Grass, Puff, Hagga, and Maconha. Ganja is the resinous portion of the small leaves and tops of cultivated plants. Bhang is a beverage consisting of an extract of the dried mature leaves and flower stems along with milk and nuts. Typically, bhang contains smaller concentrations of cannabinoids compared with hashish and ganja. Hashish (charas) is the concentrated, dried resin collected from flower tops that contains substantially higher (i.e., up to 10%) concentrations of Δ^9-THC compared with ganja and bhang. This form of marijuana is particularly common in the Middle East and North Africa. Hash oil is a dark green to amber, viscous alcohol or gasoline extract of marijuana. *Sinsemilla* (without seeds) refers to unpollinated female parts of *Cannabis sativa* that mature and produce seedless flowers without contact with male plants. The seedless portion of the female cannabis plant has a relatively high concentration (i.e., 5%) of Δ^9-THC. Dronabinol is the generic name for the main psychoactive cannabinoid (Δ^9-*trans*-tetrahydrocannabinol, Δ^9-THC). Marinol™ (Unimed Pharmaceuticals, Inc., Marietta, GA) is a commercial product that contains synthetic dronabinol in doses of 2.5 mg, 5 mg, and 10 mg dissolved in sesame oil. Terms used for marijuana soaked in embalming fluid include fry, dust, wet, leak leak, tecal, dip, hemey, and illy. Spice usually refers to smokable herbal products, which contain synthetic cannabinoid receptor agonists and are purchased relatively inexpensively over the Internet. Frequently, these products contain adulterants.

Form

Preparations and extracts of marijuana occur in a variety of forms including loose marijuana, kilobricks, sinsemilla, buds, Thai sticks, hashish, and hash oil. Usually, the plant is dried, cut, and then rolled into paper used for tobacco cigarettes.

EXPOSURE

Epidemiology

Consumption of marijuana as an illicit drug is widespread throughout the world including the United States and Europe. Epidemiologic research indicates that cannabis use among individuals aged 15–64 years is greatest in developed countries, particularly the United States, Australia, and New Zealand followed by most countries in Europe.[24] The use of marijuana is particularly high among young adults. The overall prevalence of marijuana use, abuse, and dependence depends on the population studied and criteria for use. Peak prevalence of marijuana use occurred during the late 1970s and early 1980s, when approximately 20–25% of the US population used marijuana at least once, and almost one-half of high school seniors used marijuana at least once in the preceding 12 months.[25] Frequent use among US high school students declined from 1979 to 1992 with data revealing daily use in high school seniors dropping from 11% to 2% during the latter part of that period.[26]

The prevalence of marijuana use among the younger populations remains high. During the mid and late 1990s, the use of marijuana among the younger population increased as a result of a decline in the social disapproval of marijuana and a reduction in the perceived risk of using this substance. A UK questionnaire study of marijuana use by a cross-faculty sample of 3,075 second-year university students from 10 universities demonstrated that about 60% of the students had used cannabis at least once.[27] Approximately 20% of the students used cannabis at least weekly. Approximately one-third of Americans have used marijuana at least once in their lifetimes, and about 8% of the US population used marijuana within the last year based on the 2000 National Household Survey on Drug Abuse.[28] In this study, the lifetime prevalence rates of marijuana dependence was the highest (approximately 4%) of all illicit drugs. Based on an analysis of the 1991–1992 National Longitudinal Alcohol Epidemiologic Survey and the 2001–2002 National Epidemiologic Survey on Alcohol and Related Conditions, the prevalence of marijuana use in the preceding year remained stable at approximately 4% during the 1990s in the United States.[29] However, the prevalence of marijuana abuse and dependency increased to approximately 1.1% and 0.4%, respectively, during this period as defined by *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria. Based on the annual US National Surveys on Drug Use and Health by the Substance Abuse & Mental Health Services Administration, past month marijuana use among

adolescents (i.e., 12–17 years) decreased from 2002 (8.2%) to 2005 (6.8%).[30] The prevalence of past marijuana use among this group remained constant between 2005 and 2007.

Sources

ORIGIN/COMPOSITION

The hemp plant originated in Central Asia and easily adapts to temperate and tropical environments. All parts of both female and male plants contain the major psychoactive substance, Δ^9-THC, particularly the resin secreted by the female plant. The highest Δ^9-THC concentrations occur in the flowering tops (bracts), the flowers, and leaves. The stems and roots contain relatively small concentrations of Δ^9-THC, whereas the seeds contain few cannabinoids. The content of natural cannabinoids in marijuana including the relative concentration of cannabinoid acids, and the cannabinoid acid/phenol ratios varies substantially because of environmental and genetic factors (rainfall, sunshine, temperature, strain and origin of the plant, altitude, soil fertility, cultivation practices, time since harvest).[31] Marijuana and tobacco smoke are qualitatively similar with the exception of the presence of nicotine in tobacco and cannabinoids in marijuana.[32] However, cannabis-smoking techniques (breath-holding, puff duration) that maximized the delivery of Δ^9-THC may increase the delivery of carbon monoxide and tar compared with tobacco smoking.

Marijuana plants grown in the United States traditionally contain low levels of Δ^9-THC ranging from ~1–5%. Hence, an average 500-mg marijuana cigarette contains between 5 and 25 mg of Δ^9-THC. The actual Δ^9-THC varies with the type of marijuana preparation (e.g., plant part, extract) and the source. Additionally, Δ^9-THC (Δ^1-THC) concentrations in marijuana increased substantially over the last several decades.[33] During the late 1960s, the average Δ^9-THC concentration was 1.5% compared with 3.0–3.5% in the 1980s. The analysis of 35,312 marijuana preparations confiscated in the United States over 18 years indicated that the average Δ^9-THC concentration increased from 1.5% in 1980 and 3.3% in 1983 to 4.2% in 1997.[34] The range of Δ^9-THC concentrations in 16 samples of *C. sativa* confiscated in Greece was 0.08–4.41%.[35] In samples confiscated in New Zealand, the Δ^9-THC concentration of marijuana leaf averaged about 1% compared with 3.5% Δ^9-THC, in samples of the female flowering heads.[36] The Δ^9-THC concentration of marijuana leaf imported into countries (e.g., England) varies substantially, but often the Δ^9-THC concentration

exceeds 5%.[37] Analysis of 46,211 confiscated cannabis preparations by the National Institute on Drug Abuse (NIDA) Potency Monitoring program at the University of Mississippi indicates that the mean Δ^9-THC concentration of confiscated cannabis preparations increased from 3.4% in 1993 to 8.8% in 2008.[38] The increased potency resulted primarily from the confiscation of more high potency (>9% Δ^9-THC) marijuana and sinsemilla samples from nondomestic sources. Analyses of high-potency cannabis samples (>10% Δ^9-THC) reveal new cannabinoid metabolites not previously identified in older, less-potent cannabis samples.[39,40] In addition, analyses of marijuana seizures near the California–Mexico border suggest that cannabidiol concentrations are decreasing as the Δ^9-THC concentration increases. Between 1996 and 2008 in this area, the Δ^9-THC/cannabidiol ratio increased in marijuana seizures from ~26 to 188.[41]

Analysis of seeds from *C. sativa* indicates that the Δ^9-THC content is highly variable depending on the type of seed. Cannabidiol (CBD) is the major cannabinoid in fiber-type cultivars of *C. sativa* called ditchweed. Hemp (fiber) seeds from these cultivars are a common constituent of birdseed mix that contains <1% Δ^9-THC and relative high concentrations of CBD (i.e., CBD > Δ^9-THC). In contrast, the seeds from plants cultivated for marijuana use have Δ^9-THC concentrations exceeding CBD concentrations. In a study of marijuana seeds using gas chromatography/mass spectrometry (GC/MS), the Δ^9-THC content of drug-type seeds ranged from 35.6–124 μg/g compared with 0–12 μg/g for hemp seeds.[42]

Based on voluntary analysis of street drugs, few misrepresentations occur with marijuana. In marijuana samples confiscated by US authorities from 1980–1997, the Δ^9-THC concentration in sinsemilla averaged from approximately 6–11%, whereas average concentration of Δ^9-THC in hashish and hash oil ranged from about 3–20%.[34] In data from the NIDA Potency Monitoring program, the Δ^9-THC concentration in hashish was variable (16.8 ± 16.3%), but did not increase significantly between 1993 and 2008.[38] Hashish resin Δ^9-THC concentrations range from approximately 4–15%.

PRODUCTION PROCESSES

Clandestine laboratories can synthesize a number of psychotomimetic tetrahydrocannabinol compounds including Δ^{10a}-THC (Δ^3-THC), Δ^9-*cis*-tetrahydrocannabinol (Δ^9-*cis*-THC), and Δ^9-*trans*-tetrahydrocannabinol (Δ^9-*trans*-THC).[43] Modification of the dimethylheptyl side chain markedly increases the potency of tetrahydrocannabinol compounds. Marinol™ (Unimed

Pharmaceuticals, Inc., Marietta, GA) is synthetic Δ^9-THC that is structurally identical to natural Δ^9-THC.

IMPURITIES AND PROFILING

Fungal species frequently contaminate marijuana cigarettes including *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp., *Mucor* ssp., *Thermoactinomyces candidus*, and *Thermoactinomyces vulgaris*.[44] Therefore, marijuana smoking is a potential source for exposure to fungal antigens. The presence of Δ^9-tetrahydrocannabivarin, the C3 homologue of Δ^9-THC, and the carboxylic acid metabolite, Δ^9-tetrahydrocannabivarin carboxylic acid, are markers for the ingestion of natural marijuana.[45]

MEDICINAL USES

There is renewed interest in the medicinal use of cannabinoids. The two current indications for the use of the synthetic cannabinoid (dronabinol, Marinol™) are chemotherapy-induced nausea and vomiting and AIDS-related anorexia.[46] Dronabinol is an oral medication approved as a schedule II drug in the United States for the treatment of nausea and vomiting in chemotherapy patients and a stimulant for appetite in AIDS patients. Other potential uses of dronabinol in cancer patients include analgesia, mood elevation, muscle relaxation, and relief of insomnia.[47] Current areas of research for use of synthetic Δ^9-THC include bronchodilation for asthma; antiemetic for chemotherapy; reduction of intraocular pressure in glaucoma; and use as an anticonvulsant, anxiolytic, analgesic, or antiinflammatory agent. Unfortunately, unwanted side effects limit the medicinal use of Δ^9-THC, particularly in the elderly; however, investigation of synthetic derivatives of Δ^9-THC may reduce adverse reactions. Randomized, double-blind, placebo-controlled studies have not supported the use of Δ^9-THC for postoperative pain in doses of 5 mg.[48] Although cannabinoids may regulate some of the processes involved with neurodegeneration, limited clinical data currently do not support the use of cannabinoids in the treatment of neurodegenerative diseases (e.g., Alzheimer disease) or the symptoms associated with dementia.[49] Currently, over 10 US states have medical cannabis programs. These medical cannabis laws rely on postmarketing surveillance studies rather than the usual FDA approval process (i.e., small and large animal testing, human toxicity studies, dose response data, adverse effect studies). Evidence-based guidelines for the use of medical cannabis have not been formulated at the present time, and there are inadequate data to determine the efficacy and safety profile of medical cannabis.[50]

Methods of Abuse

The most common route of exposure to marijuana is inhalation by a variety of sources including cigarettes, pipes, bongs, or "buckets" (i.e., inhaling the fumes from a mass of plant or resin ignited in a plastic bottle).

Figure 60.4 displays a bong and Figure 60.5 shows a variety of pipes used to smoke cannabis. Occasionally, marijuana users add other ingredients to the marijuana (e.g., phencyclidine, embalming fluid).[51] The use of embalming fluid involves the freezing of marijuana leaves saturated with embalming fluid and the crushing of the dried leaves into a dust. This dust is then added to cigars or marijuana cigarettes. Other patterns of use include the addition of embalming fluid to mint leaves or tobacco without the concomitant use of marijuana or phencyclidine.[52] The smoking of marijuana is associated with mild euphoria, a sense of well-being, and relaxation. Among long-term marijuana users, the use and dependence on marijuana is fairly stable; some of these users fulfill the criteria for drug-seeking behavior.[53]

FIGURE 60.4. Confiscated bong for smoking cannabis. (Photo courtesy of the US Drug Enforcement Agency)

FIGURE 60.5. Assorted pipes used to smoke cannabis. (Photo courtesy of the US Drug Enforcement Agency)

Animal studies indicate that marijuana is an atypical drug of abuse that exhibits reinforcing/rewarding properties under experimental conditions different than other drugs of abuse (e.g., cocaine, heroin, alcohol, nicotine).[54]

Marijuana smokers typically inhale deeply while holding their breath to maximize absorption. The subjective effects depend on a number of factors including smoking efficiency, potency of the cannabis, pyrolysis of active ingredients, loss of Δ^9-THC in sidestream smoke, and amount of Δ^9-THC trapped in the upper respiratory tract.[55] Compared with tobacco smokers, marijuana smokers typically use fewer cigarettes, but they retain the smoke longer because of deep inhalation and breath holding during the smoking process.[56] Volunteer studies suggest that the respiratory deposition of tar and the absorption of carbon monoxide are substantially greater following the smoking of marijuana than cigarettes as a result of the differences in smoking techniques.[57] The rapid absorption of Δ^9-THC and quick onset of action after the initiation of smoking allows titration of the desired effect by adjusting the pulmonary dose of Δ^9-THC, whereas absorption of Δ^9-THC following ingestion is much slower and less predictable. Consequently, marijuana use via ingestion occurs less often, primarily as a bakery product (cookie, cake) or as an extract. Because of the limited water solubility of Δ^9-THC, water extracts of marijuana are not suitable for intravenous

(IV) administration. Although there has been a modest increase in the potency of various forms of marijuana, the major recent change in prevalence is the use of more potent forms of marijuana by young adults at an earlier age.[58]

Personality variables associated with marijuana use include heightened levels of stress, poor self-esteem, tension, guilt-prone, emotional instability, rebelliousness, impulsivity, and lack of control.[59] The issue of progression from marijuana use to other street drugs remains controversial. A cross-sectional survey of young Australian adults suggests that the early (i.e., <17 years old) use of marijuana is a significant risk factor for the later use of other drugs of abuse and the development of drug-related problems after controlling for genetic and social factors.[60] This study suggests that peers and social context affect the risks associated with early-onset cannabis use in addition to genetic and other environmental factors.

DOSE EFFECT

The individual response to recreational doses of marijuana depends on the absorbed dose, the personality, the expectation of the user, and the setting. Initial effects range from euphoria, perceptual alterations, and relaxation at low dose to depersonalization, pressured speech, paranoia, and manic psychosis at high dose. The usual form of recreational marijuana use involves the smoking of a 0.5- to 1-g cigarette. Typical low-dose marijuana cigarettes in volunteer studies contain about 16-mg Δ^9-THC, whereas high-dose cigarettes contain approximately 34-mg Δ^9-THC. The inhaled dose of Δ^9-THC associated with pharmacologic effects ranges from about 2–22 mg, depending on the variables listed above.[61] Given the bioavailable Δ^9-THC in smoke, the estimated internal dose of Δ^9-THC associated with pharmacologic effects is approximately 0.2–4.4 mg. In volunteers, the IV administration of up to 5-mg Δ^9-THC produces a variety of transient effects including euphoria, anxiety, deficits in verbal recall, altered body perception, paranoia, conceptual disorganization, illusions, depersonalization, derealization, extreme slowing of time, blunted affect, and emotional withdrawal.[62]

The use of marijuana by most adults produces mild intoxication with the exception of multisystemic failure following the IV administration of crude marijuana extract. Despite the substantially lower bioavailability of Δ^9-THC following ingestion compared with smoking, case reports indicate that children develop adverse reactions after accidental ingestion of marijuana-containing substances.[63] In experimental studies on healthy adults, the behavioral profile after smoking a marijuana cigarette (3.1% Δ^9-THC) was similar to the

effects of ingesting 20-mg Δ^9-THC with the exception of some mild abstinence symptoms (irritability, feeling miserable) after the psychotomimetic effects of smoking marijuana resolved.[64]

TOXICOKINETICS

Absorption

SMOKING

During the smoking of marijuana, the spontaneous decarboxylation of THC carboxylic acids produces the active compound, Δ^9-THC. The absorption of Δ^9-THC following inhalation is rapid. The bioavailability of Δ^9-THC via inhalation is relatively low (i.e., 10–35%) as a result of pyrolysis and the off-gassing of Δ^9-THC in sidestream smoke; consequently, the bioavailability depends on a variety of factors including the efficiency of the smoking process, puff duration, depth of inhalation, and duration of breath-holding.[65] The use of a pipe that limits sidestream smoke substantially increases (i.e., to about 45%) the bioavailability of Δ^9-THC.[22] In a small group of volunteers, the systemic bioavailability of Δ^9-THC via inhalation averaged about 27 ± 10% for the heavy users and approximately 14 ± 1% for the light users.[66] The average systemic availability of Δ^9-THC following the inhalation of a standard dose of marijuana by a group of 11 volunteers was 18 ± 6% (range, 10–50%) compared with 6 ± 3% for the ingestion of a similar dose of Δ^9-THC.[67] Plasma concentrations of Δ^9-THC increase rapidly after the initiation of smoking and peak concentrations occur about 7–8 minutes after initiation of smoking.[68] Subsequently, plasma Δ^9-THC plasma concentrations decline rapidly, and this decline in plasma Δ^9-THC occurs before maximum psychotropic effects. Subjective feelings of euphoria and maximal heart rate acceleration develop about 20–30 minutes after peak plasma Δ^9-THC concentrations; the subjective effects resolve within 3–4 hours after inhalation ceases.[69]

INGESTION

After oral administration, absorption of Δ^9-THC is slow and somewhat erratic with peak plasma Δ^9-THC concentrations occurring about 60–120 minutes after ingestion. These concentrations remain relatively constant for 4–6 hours. Following the oral administration of 5 mg and 10 mg of Δ^9-THC as Dronabinol®, peak plasma Δ^9-THC concentrations occurred about 1½ to 2 hours after administration with mean peak concentrations of approximately 1 ± 0.2 ng/mL and 3.6 ± 0.6 ng/mL, respectively.[70] A large first-pass effect reduces the

amount of Δ^9-THC entering the systemic circulation. Therefore, equivalent clinical effects following exposure to Δ^9-THC require higher oral doses compared with the pulmonary route.[67] The mean systemic bioavailability (i.e., 6% ± 3%) of Δ^9-THC after marijuana ingestion is approximately one-third of the bioavailability of Δ^9-THC after smoking.[71] Subjectively, the effects of an oral Δ^9-THC dose of 120 µg/kg are similar to those of inhalation of 50 µg/kg.[72] There are minimal differences in the absorption, distribution, and biotransformation of Δ^9-THC between sexes.[71]

Distribution

The distribution of Δ^9-THC is very rapid compared with the metabolism of Δ^9-THC. After cessation of smoking, plasma Δ^9-THC concentrations quickly decline as Δ^9-THC distributes into tissue; subsequently, a slow, terminal elimination phase begins. Δ^9-THC is highly lipid soluble, and this compound crosses the blood—brain barrier relatively easily along with the active 11-OH metabolite. Because of the extremely high lipid solubility of cannabinoids, these chemicals accumulate in fatty tissues with peak tissue concentrations of these compounds occurring about 4–5 days after exposure. Subsequently, these tissue stores release the cannabinoids slowly into the blood for elimination. As a result of the rapid disappearance of Δ^9-THC from the blood following the cessation of smoking, peripheral blood concentrations do not reflect the concentration of Δ^9-THC at the receptor sites in the brain.

The average steady state volume of distribution of Δ^9-THC after IV administration is large (i.e., approximately 9–10 L/kg body weight) compared with the relatively small, initial volume of distribution (about 0.04 L/kg) as a result of high protein binding that limits the initial disposition of Δ^9-THC.[73,74] Plasma Δ^9-THC is about 95–99% protein bound with the distribution between lipoproteins and albumin being approximately 6:4.[75] Both 11-nor-Δ^9-carboxy tetrahydrocannabinol and the glucuronide metabolite are also highly (i.e., about 97%) protein bound.

Biotransformation

Δ^9-THC

Cytochrome P450 isoenzymes metabolize almost all Δ^9-THC via microsomal hydroxylation and catalytic oxidation to a large variety of metabolites. The major metabolites of Δ^9-THC are mono-hydroxylated compounds, but the pattern of hydroxylation varies considerably between animal species as a result of the variable expression of cytochrome P450 isoenzymes.[76] Allylic

893

hydroxylation at C_8 or C_{11} converts the parent drug to hydroxylated metabolites; then, further biotransformation occurs via oxidation at the pentyl side chain. This pathway probably involves the formation of an intermediate aldehyde, followed by oxidation and the subsequent glucuronidation of the carboxy group. The conversion of Δ^9-THC to hydroxylated metabolites requires NADPH and molecular oxygen. In humans, the biotransformation of Δ^9-THC occurs primarily by hydroxylation via the CYP2C subfamily (primarily CYP2C9) and, to a lesser extent, CYP3A4. In mouse hepatic microsomes, the major isozyme responsible for the hydroxylation of cannabinoids at the 11α- and 8α-positions of Δ^9-THC was CYP2C29.[77] CYP2C9 polymorphism accounts for some variation in Δ^9-THC biotransformation. In a study of 43 healthy volunteers, median areas under the curve (AUC) of Δ^9-THC and 11-nor-9-carboxy-9-tetrahydrocannabinol were three-fold higher and 70% lower, respectively, in CYP2C9*3/*3 homozygotes than in CYP2C9*1/*1 homozygotes.[78] Figure 60.6 demonstrates the structure of the major metabolites of tetrahydrocannabinol.

The principal active metabolite, 11-OH-Δ^9-THC, appears in the plasma within 10 minutes of the IV administration of Δ^9-THC.[90] The amount of cannabinoid metabolites in the plasma depends on the route of administration, but the amount of 11-OH-Δ^9-THC is substantially less (i.e., <10%) than the concentration of Δ^9-THC after smoking or IV administration of Δ^9-THC.[71] Although the administration of 11-OH-Δ^9-THC produces psychologic symptoms in human volunteers within 3–5 minutes of administration,[79] the speed and duration of drug hydroxylation does not correlate to the clinical effects of Δ^9-THC based on evidence from volunteer studies of slow and fast drug hydroxylators.[80] Although 11-OH-Δ^9-THC contributes to the effect of marijuana after oral doses, this metabolite does not account for all actions of the drug, especially after inhalation.[23] The active metabolite undergoes further metabolism to the inactive, polar compound 8,11-dihydroxy-Δ^9-THC, which appears in the urine and feces. The primary alcohol at C-11 is metabolized to the corresponding 11-nor-acid (THC-COOH) or to the more polar acidic cannabinoids by aliphatic alcohol dehydrogenase enzymes. Ketones, aldehydes, and carboxylic acids are the end products of Δ^9-THC metabolism.[81]

Urinary excretion of acidic Δ^9-THC metabolites accounts for about 20–30% of the elimination of Δ^9-THC with a majority (about 13–16%) of the parent compound appearing in the urine within 3 days.[71] Most of the cannabinoid metabolites in urine appear in the form of acidic glucuronide conjugates.[82] Biliary excretion via the feces is the major route of elimination of
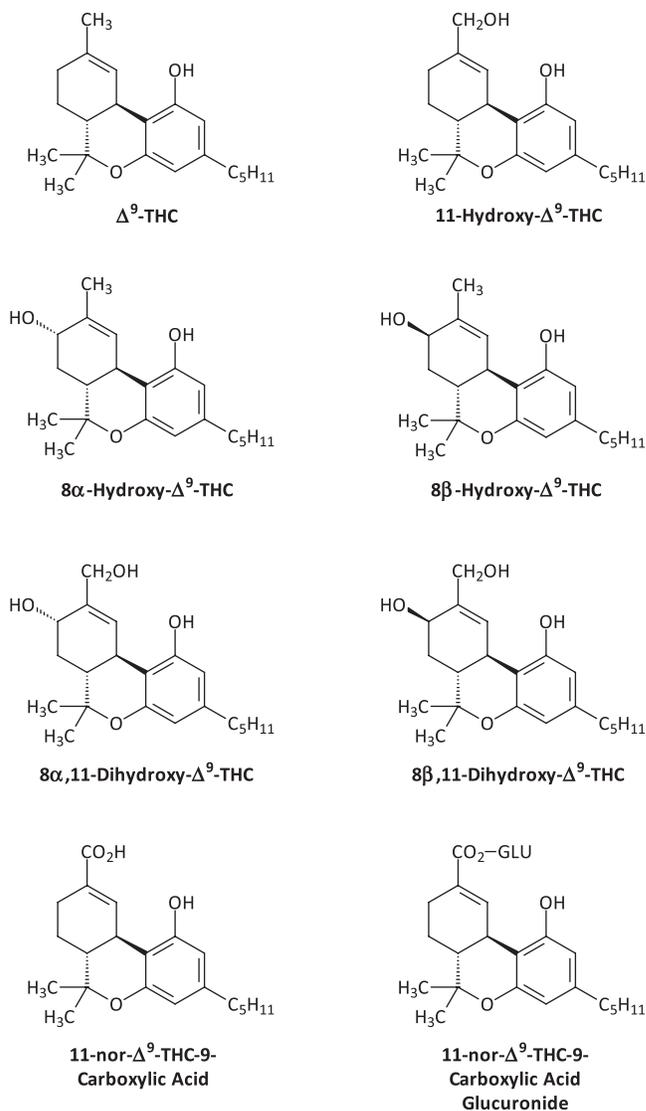
FIGURE 60.6. Major metabolites of Δ^9-tetrahydrocannabinol (Δ^9-THC) as identified in human fluids. Δ^9-THC and 11-OH-Δ^9-THC are active compounds, whereas 8β-OH-Δ^9-THC is less active and 8α-OH-Δ^9-THC is much less active. 11-nor-Δ^9-THC-9-COOH is inactive.[82]

unconjugated cannabinoid metabolites with about 30% of an IV dose and 50% of an oral dose excreted in the feces within 3 days after administration.[71] Enterohepatic recirculation contributes to prolonged excretion of metabolites.

OTHER CANNABINOIDS

Extracts of marijuana contain over 60 cannabinoids including cannabinol (CBN) and cannabidiol (CBD). These later two cannabinoids do not possess clinically significant psychoactive properties.[83,84] The metabolism

of CBD, which is a precursor of Δ^9-THC, is similar to the biotransformation of Δ^9-THC with urinary excretion of metabolites accounting for about 16% of the dose during the first 72 hours.[11] However, unchanged CBD appears in the feces in larger amounts compared with unchanged Δ^9-THC. The major route of CBD metabolism is hydroxylation and oxidation at C_7 followed by hydroxylation in the pentyl and propenyl groups to form hydroxylated derivatives of CBD-7-oic acid. At least 33 metabolites appeared in the urine of a dystonic patient treated with CBD including acids formed by β-oxidation and biotransformation of the pentyl side-chain.[85]

Elimination

Δ^9-THC

Biotransformation accounts for almost all of the elimination of Δ^9-THC with renal excretion of unchanged Δ^9-THC accounting for <1% of the absorbed dose. The time course of plasma Δ^9-THC concentrations following inhalation and IV administration is similar, and the plasma Δ^9-THC concentrations rapidly decline following the development of peak concentrations soon after the cessation of smoking. In a study of 6 volunteers smoking a single 1.75% (15.8 mg) Δ^9-THC or 3.55% (33.8 mg) cigarette, the plasma Δ^9-THC concentrations (ng/mL) at specific times after smoking began were as follows: 0.5 hour, 8–50; 1 hour, 3–20; 2 hours, 1–8; 4 hours, 0–2.5.[86] These ranges depend on the dose of marijuana (puff volume, puff number, duration and spacing of puffs, hold time, percentage active ingredient). Following the smoking of 2 marijuana cigarettes (0.15 mg/kg), the ranges of Δ^9-THC plasma concentrations at 0.5 hour and at 1 hour were 36–177 ng/mL and 14–69 ng/mL, respectively.[87] Other factors that affect these ranges in addition to Δ^9-THC dose include the route (i.e., ingestion vs. inhalation) and chronic usage (>3 times/week). Although there is substantial individual variation in plasma and serum Δ^9-THC concentrations following inhalation of cannabis, pharmacokinetic studies indicate that the dose response is relatively linear after smoking cannabis cigarettes containing up to 70 mg Δ^9-THC.[88] The ingestion of Δ^9-THC produces delayed peak plasma times and lower peak plasma concentrations compared with smoking and IV administration of Δ^9-THC. Additionally, the ingestion of Δ^9-THC produces relatively higher concentrations of 11-OH-THC compared with smoking.[71]

The mean terminal plasma elimination half-life of Δ^9-THC is relatively long because of the slow diffusion of the highly lipophilic Δ^9-THC from tissue, particularly in habitual marijuana users. The true elimination half-life is difficult to calculate because of the slow equilibrium between tissue and plasma at the low concentrations of Δ^9-THC present in the terminal phase. Earlier studies of chronic users by less-sensitive assays indicated that the terminal plasma half-life of Δ^9-THC ranged from 20–56 hours after IV administration.[89] In comparison, the mean plasma elimination half-life in naïve users was approximately 28 hours.[90] A study of 3 chronic user smoking 4 marijuana cigarettes (15 mg Δ^9-THC/cigarette) over 2 days demonstrated a plasma elimination half-life of about 4 days with a range of approximately 3–5 days.[91]

Metabolites

Excretion of Δ^9-THC metabolites after exposure to marijuana occurs over days depending on the chronicity of cannabis use as a result of the extensive enterohepatic recirculation of metabolites and the redistribution of Δ^9-THC from fatty tissues. Because of the enterohepatic recirculation and extensive protein binding of cannabinoids, excretion of these metabolites occurs predominately in the feces rather than the urine. The actual percentage of the dose excreted into the feces and urine depends of the route of exposure. Following oral administration of Δ^9-THC, excretion of acid metabolites in the feces accounts for approximately 65–80% of the dose, whereas urinary excretion accounts for about 20–35% of the dose.[71] About 5% of the dose appeared in the feces as unchanged Δ^9-THC. During the first 3 days after IV administration of Δ^9-THC to male volunteers in this same study, cumulative cannabinoid excretion was 15% ± 4% in the urine and 35% ± 11% in feces.

Blood.   The plasma elimination half-life of Δ^9-THC metabolites is substantially longer than the parent Δ^9-THC. The two most important oxidative metabolites are the active metabolite, 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and the inactive metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH). The mean peak plasma Δ^9-THC is approximately 3 times greater than the mean peak plasma THC-COOH concentration and 20 times greater than the mean 11-OH-THC concentration. Peak plasma 11-OH-THC concentrations occur immediately after the cessation of smoking. In a study of 6 healthy male volunteers smoking a single low-dose (1.75%) and high-dose (3.55%) marijuana cigarette, the mean peak plasma 11-OH-THC concentrations were 6.7 and 7.5 ng/mL, respectively.[86] Using GC/MS (limit of detection, 0.5 ng/mL), the detection times were approximately 4.5 and 11 hours, respectively. In most, but not all individuals, the

plasma Δ^9-THC concentration remained detectable longer than the plasma 11-OH-THC concentration. After about 2–3 hours after initiation of smoking, the ratio of Δ^9-THC/11-OH-THC is approximately 2:1 as demonstrated in Figure 60.7.

The mean peak plasma THC-COOH concentration occurs about 2 hours after smoking begins. Equal amounts of Δ^9-THC and THC-COOH were present in the plasma approximately 0.5 hour (range, 0.3–0.8 hour) after smoking began. A study of frequent and infrequent marijuana users administered 5 mg Δ^9-THC IV demonstrated that the ratio of Δ^9-THC/THC-COOH remained above 1 only within 45 minutes of IV administration.[92] The plasma elimination half-life of THC-COOH for frequent and infrequent cannabis users following injection of 5 mg Δ^9-THC was 5.2 ± 0.8 days and 6.2 ± 6.7 days, respectively. There were few differences in physiologic, psychologic, and pharmacokinetic data between frequent and infrequent marijuana users. Following oral administration of 15 mg Δ^9-THC plasma concentrations of Δ^9-THC are relatively low (i.e., approximately 3–6 ng/mL) compared with plasma Δ^9-THC concentrations after the inhalation of similar doses of Δ^9-THC.[93] However, the 11-OH-THC plasma concentrations are much higher compared with inhalation of similar doses of Δ^9-THC as demonstrated in Figure 60.8.

URINE. The main urinary metabolite of Δ^9-THC biotransformation is the acid glucuronide of THC-COOH. The kidneys excrete only small amounts of free THC-COOH in the urine. As a result of the high protein binding of cannabinoids and extensive enterohepatic recirculation, most of the absorbed dose of Δ^9-THC appears in the feces. Following ingestion, fecal and renal excretion accounted for about 65–80% and 20–35% of the elimination of Δ^9-THC, respectively. The terminal elimination of THC-COOH is relatively long, depending on several factors including the dose, chronicity of use, analytic method, and the duration of monitoring. In a study of 6 healthy volunteers, the urinary excretion half-life of THC-COOH ranged from 25–35 hours during a 7-day monitoring period and 44–60 hours during a 14-day monitoring period.[94] The urine from 13 heavy marijuana users was analyzed by high-performance liquid chromatography (limit of detection, 7 ng/mL) after they smoked 4 marijuana cigarettes (15 mg Δ^9-THC/cigarette) over 2 days.[95] The elimination half-life of THC-COOH ranged from about 1–10 days with a mean of approximately 3 ± 2 days. The urine of frequent marijuana users may contain small amounts of unconjugated THC-COOH for several days following the administration of Δ^9-THC; however, the urine of infrequent users usually contains only conjugated THC-COOH.

FIGURE 60.7. Mean plasma concentrations of Δ^9-Tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THC-COOH) based on 6 study participants smoking 34 mg Δ^9-THC. Reprinted with permission from MA Huestis, JE Henningfield, EJ Cone, Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana, Journal of Analytical Toxicology, Vol. 16, p. 281, copyright 1992.

FIGURE 60.8. Mean plasma concentrations of Δ^9-Tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THC-COOH) based on the ingestion of 15 mg Δ^9-THC by 6 cancer patients. Reprinted with permission from S Frytak, CG Moertel, J Rubin, Metabolic studies of delta-9-tetrahydrocannabinol in cancer patients, Cancer Treatment Reports, Vol. 68, pp. 1427–1431, copyright 1984.

TOLERANCE

Tolerance to sleep, mood, behavioral, cardiovascular, and autonomic changes associated with marijuana use develops and resolves rapidly. Tolerance to the cardiovascular and psychologic effects developed in 6 study participants following oral administration of 30 mg of Δ^9-THC every 4 hours for 10–12 days.[73] In a study of experienced marijuana users at least 5 days of steady intoxication (i.e., 20–30 mg every 3–4 hours) was necessary to produce minimal withdrawal symptoms; the degree of withdrawal was related to the dose of Δ^9-THC.[96] Tolerance to tachycardia and psychotropic effects develops after volunteers smoke marijuana cigarettes containing 40 mg Δ^9-THC (four 500-mg, 2% Δ^9-THC marijuana cigarettes) over 14–21 days.[97] Smoking lower doses of Δ^9-THC may not produce tolerance. In a study of 6 healthy volunteers smoking 1 marijuana cigarette containing about 9-mg Δ^9-THC daily for 13 days, the only significant change in the response to Δ^9-THC was a mild reduction in the tachycardia associated with smoking cannabis.[98]

The basis of tolerance associated with cannabis use probably involves pharmacodynamic events (receptor downregulation, receptor conformational changes, receptor internalization) rather than alteration of the pharmacokinetics of Δ^9-THC.[4] Tolerance to the tachycardia and psychotropic effects follows similar temporal patterns.

MATERNAL AND FETAL KINETICS

Δ^9-THC rapidly crosses the placenta in most animal species; however, animal studies suggest that the peak Δ^9-THC concentration after IV administration is substantially lower in the fetus than in the mother.[99] About 3 hours after IV administration, the Δ^9-THC concentrations in the mother and fetus are similar. The 11-OH-THC and 11-nor-9-carboxy-Δ^9-THC metabolites cross the placenta much less efficiently than Δ^9-THC.[100] Excretion of Δ^9-THC in breast milk occurs following maternal exposure, particularly following habitual marijuana use. Analysis of breast milk in a chronic heavy marijuana user demonstrated Δ^9-THC concentrations in breast milk 8.4 times higher (i.e., low ng/mL range) than maternal plasma Δ^9-THC concentrations.[101] Consequently, a nursing infant potential may ingestion daily doses of 0.01–0.1 mg Δ^9-THC from a nursing mother consuming 1–2 cannabis cigarettes daily.

DRUG INTERACTIONS

Exposure to marijuana frequently occurs along with other drugs (e.g., tobacco, ethanol, other psychoactive drugs). Potential interactions result from the presence of common metabolic pathways, competition for binding sites on plasma proteins, and functional adaption of various neural systems. In a study of 8 healthy volunteers, blood ethanol elimination rates decreased approximately 12–13% in 7 of 8 volunteers receiving 60–180 mg Δ^9-THC/day in divided doses for 14 days.[102] However, volunteer studies suggest that the effect of the simultaneous administration of ethanol and cannabis is additive pharmacodynamically with regard to subjective intoxication and behavioral impairment.[103] The administration of cannabidiol in daily oral doses of 600 mg for 5–12 days inhibited hexobarbital metabolism in 10 volunteers.[104] Chronic or concurrent use of marijuana alters the behavioral and physiologic effects of barbiturates, nicotine, ethanol, amphetamines, cocaine, and other psychoactive drugs (e.g., phencyclidine, opiates).[105] A case report associated the combined use of marijuana (i.e., CYP3A4 inhibitor) and Viagra® (Pfizer Pharmaceuticals, New York, NY) with the development of a myocardial infarction.[106]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

The behavioral effects of cannabis are unique; these complex behavioral actions probably result from the interaction of psychoactive cannabinoids with specific, G-protein-coupled cannabinoid receptors and other neurochemical systems. These G-proteins cause inhibition of adenylate cyclase and reduction in the conversion of AMP to cyclic AMP. Other G protein-coupled receptors include corticotropin and melanotropin receptors. CB_1 receptors are also coupled to ion channels, negatively to N-type and P/Q type calcium channels as well as M-type potassium channels and positively to A-type potassium channels.[11] There are at least 3 (CB_1, CB_2, CB_3) and perhaps other cannabinoid receptor subtypes.[107] The CB_1 receptors mediate psychoactive properties. The CB_2 cannabinoid receptors appear in the periphery (e.g., spleen, thymus, tonsils) primarily in immune cells (e.g., B-cell lymphocytes, natural killer cells).[108] Activation of CB_1 receptors produces psychomimetic effects, whereas activation of CB_2 receptors does not. These cannabinoid receptors affect several neurotransmitters including acetylcholine, dopamine, γ-aminobutyric acid, histamine, 5-hydroxytryptamine, norepinephrine, opioid peptides, and prostaglandins.[4] Anandamide is a fatty-acid derivative of arachidonic acid (arachidonylethanolamide) that possesses pharmacologic actions similar to Δ^9-THC. This prostaglandin-like substance is an endogenous ligand for the central and peripheral cannabinoid receptors that acts through

intracellular G-proteins and the modulation of ion transport.[4] However, anandamide has a shorter half-life and is a less potent agonist of the CB_1 and CB_2 receptors than Δ^9-THC.[109]

Central Nervous System

The CB_1 receptor is the most common cannabinoid receptor in the brain; these receptors occur in the hippocampus, basal ganglia (substantia nigra, pars reticulata, globus pallidus, entopeduncular nucleus, lateral caudate, putamen), and the molecular layer of the cerebellum. Purkinje cells and pyramidal neurons synthesize and release endocannabinoids that stimulate nearby CB_1 receptors, which directly target both GABAergic (inhibitory) and glutamatergic (excitatory) terminals. The hippocampus codes sensory information and newly acquired memory as well as influencing changes in mood and behavior. The cerebellum is involved with coordination of motor movement and motor learning, whereas the basal ganglia are involved with movement. Endogenous ligands for these cannabinoid receptors include anandamide, 2-arachidonoyl glycerol, 2-arachidonoyl glyceryl ether (noladine ether), *O*-arachidonoyl ethanolamine (virodhamine), and *N*-arachidonoyl-dopamine (NADA). These endogenous cannabinoids bind and stimulate the cannabinoid and vanilloid receptors; reuptake processes and rapid enzymatic inactivation regulate the action of these compounds. These endocannabinoids (e.g., anandamide, noladine ether, virodhamine) along with bicyclic and tricyclic analogues of Δ^9-THC are agonists, whereas 1,5-biarylpyrazoles are cannabinoid receptor antagonists.[54]

Activation of the CB_1 and CB_2 receptors inhibits adenylyl cyclase activity and reduces the intracellular concentration of cAMP, resulting in the phosphorylation and stimulation of the mitogen-activated protein kinases, extracellular signal-regulated kinase 1 (ERK1) and ERK2.[110] The CB_1 receptor is an inhibitory, G protein-coupled receptor that responds more to psychoactive cannabinoids (i.e., Δ^9-THC) than to nonpsychoactive cannabinoids. CB_1 receptors undergo downregulation and desensitization following chronic administration of THC or synthetic cannabinoid agonists.[111] Both the CB_1 and CB_2 receptors regulate the release of several central neurotransmitters including glutamate, γ-aminobutyric acid, dopamine, noradrenaline, serotonin, and acetylcholine.[112] CB_2 receptors are more prominent in the immune system than the brain. Some experimental evidence suggests the presence of non-CB_1, non-CB_2, and G protein-coupled cannabinoid receptors.[113] Synthetic CB_2 agonists are *potential* analgesics for inflammation-

based pain based on the effect of the CB_2 receptor on immune function.[114]

Several animal studies suggest that chronic exposure to Δ^9-THC alters the structure and function of the rat hippocampus, which is the portion of the brain associated with learning and memory, particularly in immature animals.[115,116] Both age and duration of exposure (i.e., >10% of the lifespan) are important variables in the responses during these animals studies.[117] Functional imaging studies of chronic cannabis users suggest the presence of subtle increases in activity within the frontal, limbic, and cerebellar regions following continued marijuana use.[118] However, the clinical significance and reversibility of these changes are unclear.

Cardiovascular System

The cardiovascular actions of cannabinoids are complex, characterized by vasodilation, increased cardiac output, and tachycardia. The CB_1 receptor agonists have complex cardiovascular actions including the inhibition of distinct voltage-gated calcium channels and activate inwardly rectifying potassium channels.[119] The vascular responses to cannabinoids potentially results from a variety of actions including stimulation of the cannabinoid, vanilloid, and novel endothelial cannabinoid receptors, the release of nitric oxide and endothelium-derived hyperpolarizing factor (EDHF), metabolism of endocannabinoids to vasoactive molecules, and interaction of the sympathetic nervous system.[120] The effect of smoking a cannabis cigarette on exercise performance is a modest reduction in maximal exercise performance with premature achievement of maximal oxygen uptake.[121] Plasma norepinephrine rises about 30 minutes after exposure and remains elevated for about 2 hours.[122] Tachycardia following cannabis use is dose-related with the typical increases averaging 20–50 beats per minute. High doses of Δ^9-THC can produce heart rates of 140 beats/minute with mild elevation of systolic blood pressure.

Pulmonary System

The inhalation or the ingestion of Δ^9-THC produces mild, dose-dependent bronchodilation in both healthy and asthmatic volunteers that persists at least 1 hour without central respiratory depressant effects,[123] however, marijuana smoke is a bronchial irritant. In some asthmatic individuals, cough and bronchoconstriction develop after inhalation of marijuana smoke or aerosolized Δ^9-THC.[124] Although studies of chronic marijuana users indicate that the chronic use of marijuana is not associated with nonspecific airway hyperresponsiveness,[125,126] the chronic smoking of cannabis

does not protect individuals smoking both marijuana and tobacco from developing increased airway hyper-reactivity.[127] Oral cannabinoids other than Δ^9-THC (e.g., cannabinol, cannabidiol) are probably not effective bronchodilators.[128]

Habitual marijuana use produces epithelial injury to the large airways similar to cigarette smoke despite the inhalation of much lower quantities of smoke. Bronchial biopsies of heavy marijuana smokers demonstrate his-tologic evidence of chronic inflammation (erythema, nonciliated basal cell hyperplasia), increased secretions (mucus-secreting goblet cell hyperplasia), and dysregu-lated growth of the bronchial epithelium.[129] A study of habitual cannabis smokers did not detect increases in alveolar epithelial permeability based on the clearance of technetium (^{99m}Tc)-labeled diethylenetriamine penta-acetate (DTPA).[130] Histologic examination of postmor-tem lung samples from heavy marijuana smokers dem-onstrated focal infiltrates of heavily pigmented macrophages surrounding bronchioles, focal ulceration of the columnar epithelium, focal early fibrosis with lymphocytic infiltration, and light to moderate infiltra-tion of pigmented monocytes within the alveoli.[131] Hyperplasia of the mucus-secreting surface epithelial (goblet) cells and replacement of normal ciliated cells by nonciliated basal cells probably accounts for the high frequency of symptoms consistent with chronic bronchi-tis.[132] Bronchoscopy of marijuana smokers indicate that pathologic bronchial mucosal changes associated with smoking tobacco and marijuana together are greater than the mucosal alterations associated with smoking either substance alone.[133]

Eye

Ophthalmic effects of exposure to Δ^9-THC include marked congestion of conjunctival vessels, slight pupil-lary constriction with preservation of the light reflex, mild reduction of tearing, and a dose-related reduction of intraocular pressure. Although the administration of Δ^9-THC can reduce intraocular pressure for several hours, the development of tolerance and significant sys-temic toxicity limits the medical application of this drug for glaucoma.[134]

Endocrine System

Animal models indicate that cannabinoid administra-tion acutely alters multiple hormonal systems, including the suppression of the gonadal steroids, growth hormone, prolactin, and thyroid hormone.[135] These effects result from the binding of the endogenous cannabinoid recep-tor to areas in the hypothalamus. Cannabinoids also activate the hypothalamic–pituitary–adrenal axis. The

human health effects of chronic cannabis use on the endocrine system remain unclear.

Immune System

Δ^9-THC is an immune modulator with predominately immunosuppressive effects on various immune cells in *in vitro* and animal testing.[136] *In vitro* studies indicate that cannabinoids modulate the immune response, pri-marily by immunosuppressive actions on various immune cells including macrophages, T-lymphocytes, and natural killer cells.[137] These studies also suggest that cannabinoids may modulate host resistance, especially the secondary immune response as well as altering the function of immune cytokines and the activity of network cells (macrophages; T helper cells, T_h1 and T_h2). Leukocytes contain cannabinoid receptors (e.g., CB_2); stimulation of these receptors causes complex actions on the release of immunostimulatory T_h1 cytokines (e.g., interleukin-2, interferon-γ) and immuno-inhibitory T_h2 cytokines (interleukin-10, interleukin-4).[138] The mecha-nisms of these actions are both cannabinoid receptor-dependent and cannabinoid receptor-independent with modulation of sensitive targets of cannabinoids (e.g., interleukin-2) being variable, depending on experimen-tal conditions.[139] Despite these *in vitro* changes, there are insufficient data to conclude that habitual use of marijuana increases the prevalence of infections or malignancies.

CLINICAL RESPONSE

Illicit Use

The individual's response to marijuana depends on the premorbid personality, existing scene, prior experience, and amount of Δ^9-THC absorbed. The subjective effects of cannabis classically involve a state of euphoria fol-lowed by drowsiness, sedation, and occasionally depres-sion that resolve within a few hours. Categories of psychologic effects after recreational marijuana use include affective (euphoria, garrulousness), somatic (feeling of floating), sensory (increased perception of external stimuli, vivid visual imagery), and cognitive (decreased short-term memory, distortion of time per-ception, reduced concentration/attention).[11] None of the clinical features of marijuana use are specific to the absorption of the active ingredient, Δ^9-THC. Group smoking of cannabis promotes social interaction, friend-liness, and laughter.

Following inhalation of Δ^9-THC, subjective effects begin within a few minutes, reach a maximum in 15–30 minutes, and resolve over 2–3 hours, whereas

psychotropic effects begin 30–90 minutes after the ingestion of cannabis, reach a maximum in about 2–3 hours, and resolve over 4–12 hours.[11] Most studies of the after-effects of smoking typical recreational doses of cannabis do not demonstrate evidence of clinically significant hangover effects common after ethanol or long-acting sedative-hypnotic drug use.[140] In a study of 12 regular marijuana smokers receiving 40 standardized puffs of marijuana smoke over 5 separate smoking periods in the late afternoon and evening, questionnaires on mood, sleep, and temporal perception were administered to determine the potential after-effects of the typical week-end use of marijuana.[141] Evaluation of performance included behavioral tasks ranging from simple reaction time to more complex tasks of psychomotor and cognitive functions. This study did not detect evidence of clinically significant, residual subjective intoxication the morning after use; most behavioral tests and mood scales returned to normal ranges the following day. Fine motor coordination and special perceptions were not tested. Case series associate blepharospasm, photophobia, and decreased accommodation with habitual marijuana use.[142] Conjunctival erythema is a more reliable sign of exposure to Δ^9-THC than tachycardia.[67]

BEHAVIORAL TOXICITY

During the late 1960s and the early 1970s, an amotivational syndrome was associated with chronic marijuana use among adolescents and young adults characterized by apathy, loss of effectiveness at completing complex tasks, easy frustration, poor concentration, diminished goal-directed behavior, and poor job performance.[143,144] The clinical features of this syndrome are similar to the clinical features associated with chronic intoxication with any sedative-type drug. In most cases, memory, alertness, concentrations, and cognitive skills return to normal within 1 month, and the contribution of the pharmacologic effects of chronic marijuana use remain unclear. Current data have not demonstrated a definite causal relationship between chronic marijuana use and psychosocial harm. Alternate explanations for the association between marijuana use and the psychosocial problems remain plausible including reverse causation (i.e., drug use is a consequence rather than a cause of psychosocial problems), bias, and confounding.[145]

The frequency of marijuana use in problem adolescent ethanol abuse and the similarity of psychosocial patterns between problem drinking and habitual marijuana use suggest that marijuana use is part of adolescent problem behavior. Such adolescents often express lower expectations of academic achievement, less compatibility with friends and parents, less religious behavior, more independence, less involvement with conventional institutions, more problem role models, greater tolerance of deviate behavior, and more involvement in problem acts (e.g., public drunkenness) compared with adolescents without drinking or cannabis problems.[146]

MENTAL DISORDERS

Rarely, a self-limited, acute psychosis develops following marijuana use with either clear consciousness or an acute confusional state.[147] When present, the acute confusional state is transient and self-limited, involving apprehension, suspiciousness, memory impairment, depersonalization, and derealization. Predisposing factors to the development of the acute confusional state include first-time use, high doses of marijuana, and possibly a predisposition to the development of psychosis.[148] The acute psychosis may present with mania-like features (e.g., grandiosity, excitement, hostility, lack of cooperation).[149] Although an acute psychosis with clear consciousness can develop following marijuana use, clinical data suggest that these clinical features usually represent the exacerbation of schizophrenia or a relapse in vulnerable patients (i.e., previously psychotic patients).[150,151] Although cannabis produces some anxiolytic effects, the heavy use of cannabis by schizophrenic patients may exacerbate some of their psychotic and manic symptoms.[152] The prevalence of cannabis abuse and dependence is relatively high among individuals with high risk of developing schizophrenia, particularly young adolescents with the early onset of dependency. In a study of 68 individuals with high risk factors for schizophrenia, 32% had a history of cannabis abuse or dependence.[153] Some population-based cohort studies suggest that the early and continued heavy cannabis use in adolescence increases the incidence of psychotic symptoms, and may increase the risk of persistent psychotic symptoms by enhancing the normal transitory developmental expression of psychotic experiences during adolescence.[154,155] These studies demonstrate a dose-dependent effect of cannabis use on the incidence of psychotic symptoms based on self-reported symptoms.

The clinical features of an acute psychosis during intoxication are not specific enough to warrant the classification of unique marijuana-related psychosis. In a study comparing heavy marijuana users and other patients admitted to psychiatric hospitals for acute psychosis, the marijuana users demonstrated more hypomania and agitation compared with the nonmarijuana group.[156] These symptoms improved substantially over 1 week. The nonmarijuana users demonstrated more affective flattening, auditory hallucinations, incoherence of speech, and hysteria compared with the marijuana

group. The etiology of the acute psychosis after chronic marijuana use remains controversial because of unresolved issues about premorbid personalities and multiple-drug use. Prospective studies suggest that continued marijuana use exacerbates psychotic symptoms and increases the risk of relapse.[157] Flashbacks are the relapse of symptoms experienced during intoxication. Although rare, flashbacks can occur during the first 3 months after cessation of cannabis use.[158]

Although cannabis intoxication can produce an acute transient psychotic episode in some individuals as well as short-term exacerbation of preexisting psychotic symptoms, controversy remains regarding the ability of chronic marijuana use to cause schizophrenia or other chronic functional psychotic states. Longitudinal studies suggest an increased risk (median odds ratio [OR], 2.3; range, 1.77–10.9) of psychosis or psychotic symptoms following the chronic use of cannabis, but concerns about reverse causality and the control of confounding variables limits definitive conclusions.[159] A review of existing medical literature indicated that marijuana use is neither a sufficient nor necessary cause for psychosis; the use of marijuana is part of a complex constellation of factors leading to psychosis.[160] However, a meta-analysis of 11 case-control and cohort studies on cannabis use and psychosis suggested a threefold increased risk of developing schizophrenia or a schizophrenia-like psychosis, particularly in individuals using cannabis during early adolescence.[161] This meta-analysis did not detect evidence of publication bias or heterogeneity.

MEDICAL COMPLICATIONS

The most common adverse effect of smoking marijuana is an unpleasant feeling of anxiety that may progress to panic. Clinical features of acute anxiety reactions include restlessness, depersonalization, fear of dying, panic, feeling of loss of control, and paranoia. High cannabis doses also produce transient mood disturbances and dysphoric reactions that include anxiety, fear, paranoia, depression, disorientation, confusion, and delusions. Lethargy occurs following the absorption of high doses of Δ^9-THC, especially several hours after cessation of smoking cannabis. Hallucinations are rare, occurring primarily at high doses. During the early 1980s, an outbreak of *Salmonella muenchen* gastroenteritis was associated with the consumption of contaminated marijuana in Ohio, Michigan, Georgia, and Alabama.[162] A case report associated jogging on a warm day after smoking a marijuana cigarette with the classic features of hyperthermia (rectal temperature 41.7°C/107.1°F, delirium, hot, dry skin).[163] Although a substantial portion of atopic individual are sensitive to marijuana pollen,[164] only rare

case reports associate marijuana exposure with the clinical manifestation of allergic symptoms.[165]

Rare case reports associate daily cannabis use (e.g., 2–4 joints) with a cyclical vomiting illness that resolves with cessation of cannabis use and recurs following resumption of cannabis use.[166,167] These patients also develop weight loss, abdominal pain, sweating, flushing, thirst, and alteration in body temperature that recur with reinstitution of cannabis use. Although periodontal disease is associated with tobacco smoking, the smoking of cannabis does not increase the risk of periodontal disease.[168] Gynecomastia is a relatively common physical finding in men as a result of the ingestion of a wide variety of drugs including calcium-channel blockers, cancer chemotherapeutic agents, ketoconazole, spironolactone, and histamine$_2$-receptor blockers. The evidence for the ability of marijuana to produce gynecomastia is contradictory.[169,170] Rare case reports associate heavy marijuana use with pancreatitis.[171]

CARDIOVASCULAR SYSTEM. In volunteer studies, elevated heart rate is a consistent finding during the acute phase of intoxication with marijuana.[172] The cardiovascular effects of Δ^9-THC do not usually cause serious health problems for most young, healthy users. However, cannabis smoking by patients with cardiovascular disease poses some health risks because of the consequences resulting from increased cardiac work, tachycardia, increased catecholamine levels, carboxyhemoglobin, and postural hypotension. Potential complications include myocardial infarction and stroke. Several case reports temporally associated the smoking of marijuana cigarettes with the development of myocardial infarctions. Elevated serum creatinine kinase concentrations without fractionation, electrocardiographic changes consistent with a subendocardial myocardial infarction, and pulmonary edema occurred in a 25-year-old man soon after smoking marijuana.[173] Subsequent coronary angiography revealed no significant occlusion of his coronary arteries and normal left ventricular dysfunction. A study of 3,882 patients with recent myocardial infarction suggested that smoking marijuana may be a rare trigger of acute myocardial infarction because of the mildly elevated risk of an acute myocardial infarction within the first hour after the initiation of smoking.[174] However, the study was based on interviews without documentation of drug use; therefore, misclassification of exposure and the presence of confounding variables complicate the interpretation of this study.

RESPIRATORY SYSTEM. Although marijuana smoke is a pulmonary irritant that contains increased concentrations of polyaromatic hydrocarbons, tars, sterols, and

terpenes compared with tobacco smoke,[175] smoking of marijuana does not usually produce clinically significant *acute* effects on the respiratory system.[132] However, important respiratory abnormalities result from the habitual use of marijuana compared with the use of tobacco alone. Controlled, population-based studies indicate that the habitual use of marijuana increases the prevalence of chronic respiratory symptoms of bronchitis including wheezing, cough, and exertional dyspnea.[176] However, not all studies of chronic marijuana users demonstrate an increased prevalence of symptoms of chronic bronchitis. A study comparing chronic marijuana use, tobacco smokers, and nonsmokers did not detect a difference in the prevalence of chronic cough, sputum production, or wheeze between marijuana and tobacco smokers.[177] Additionally, there was no was additive adverse effects of chronic marijuana use on tobacco smokers.

Studies of lung function in cannabis smokers associate cumulative cannabis use with higher lung volumes (increased forced vital capacity, functional residual capacity, residual volume, total lung capacity), suggesting hyperinflation and increased large airways resistance rather than airflow obstruction (chronic obstructive pulmonary disease) or impairment of gas transfer (emphysema).[178] Although some studies suggest an accelerated increase in airway obstruction in nontobacco smoking cannabis smokers,[179] most studies of habitual marijuana users do not support a strong association between regular marijuana smoking and chronic obstructive pulmonary disease. Evaluation of habitual marijuana smokers (daily or thrice weekly smoking for 2–5 years) suggested mild impairment of large-airway conductance, but sensitive indicators of peripheral-airway disease (e.g., midexpiratory flow rates) did not decline.[180] An 8-year longitudinal study of more than 900 young adults revealed a dose-dependent relationship between cumulative marijuana consumption and decline in FEV_1/VC. However, adjusting for confounding variables (age, weight, tobacco use) reduced the negative effect of habitual marijuana use on the FEV_1/VC to marginal significance ($p < 0.09$).[181] A study of 15 asymptomatic, healthy cannabis smokers did not detect a statistically significant reduction of the single-breath carbon monoxide diffusing capacity when compared with the nonsmoking control group.[182] This test is a sensitive physiologic indicator of emphysema; a significant reduction of this test occurred in the group of combined marijuana and tobacco smokers. Although paraquat may contaminate marijuana products from some foreign countries (e.g., Mexico), the high combustion temperatures in marijuana cigarettes destroys paraquat; therefore, there is no significant risk of paraquat-induced pulmonary fibrosis from cannabis smoking. Although the chronic histopathologic effects of inhaling cannabis smoke and tobacco smoke are similar, there are few data to clarify the carcinogenic potential of cannabis smoke on the respiratory tract.[183] Case reports suggest that multiple large bullae can occur in the lungs of marijuana smokers, particularly in the upper lobes;[184] however, macroscopic evidence of emphysema is usually absent.[185]

INTRAVENOUS USE.   In volunteer studies, the IV administration of up to 5-mg Δ^9-THC causes a variety of transient symptoms including altered perceptions, increased anxiety, euphoria, disruption of verbal recall, reduced attention, and increased distractibility.[62] General orientation is usually not impaired at these Δ^9-THC doses. Intravenous injection of boiled aqueous extract of cannabis seeds ("pot tea") produces severe, acute, multisystem illness manifest by gastrointestinal (GI) distress, fever, myalgias, rhabdomyolysis, acute renal failure, hypotension, pulmonary edema, and coagulopathy.[186,187] Severe headache, blurred vision, diaphoresis, chills, dizziness, and dyspnea begin soon after injection followed in several hours by fever, GI distress, diffuse myalgias, rhabdomyolysis, hypotension, and renal insufficiency.[188] These patients recovered within about 1 week with supportive care.

Accidental Exposure

Following accidental ingestion of marijuana cookies, case reports document the occurrence of tachycardia, conjunctival hyperemia, pallor, ataxic gait, labile affect, vertical and horizontal nystagmus, fine tremor, and altered consciousness.[63] Symptoms usually develop within a few hours of ingestion and typically resolve by 6 hours after ingestion. Alteration of systemic blood pressure is uncommon. Ingestion of marijuana-filled balloons has been associated with intestinal obstruction and GI distress.[189] Systemic symptoms other than GI complaints and lethargy are uncommon, even following rupture of the balloons.[190]

Fatalities

Few reports of marijuana-associated fatalities appear in the medical literature; the etiology of these fatalities is not well documented.[191] Although a case report of a 25-year-old man associated his death with the ingestion of bhang over the 24 hours preceding his death,[192] the contribution of Δ^9-THC to his death is unclear because of his complicated cardiac history (rheumatic heart disease, open heart surgery).

Abstinence Syndrome

Chronic marijuana use may cause psychologic dependency and a mild withdrawal reaction, but the risk of physical dependence is low compared with opioids and benzodiazepines.[193] A mild withdrawal syndrome following abstinence from chronic marijuana consumption may occur in some heavy marijuana users, particularly affective and behavioral symptoms.[194] There are few robust associations between demographic characteristics and cannabis withdrawal based on responses to questionnaires from a convenience sample of 104 nontreatment-seeking, adult cannabis smokers.[195] In contrast to opioid and alcohol withdrawal, clinically significant physical symptoms of withdrawal do not usually occur following cessation of chronic marijuana use. Withdrawal symptoms following cessation of regular cannabis use include aggression, anger, anxiety, jitteriness, irritability, restlessness, sleep disturbances, anorexia, nausea, abdominal pain, weight loss, sweating, salivation, and increased body temperature, particularly during the first 10 days after the cessation of marijuana use. In study of participants in the US National Epidemiologic Survey on Alcohol and Related Conditions, the association of withdrawal symptoms to other dependence criteria for ethanol and marijuana was similar.[196] About 44% of frequent marijuana users (≥3 times weekly) reported ≥2 withdrawal symptoms; whereas about 34% reported ≥3 withdrawal symptoms. In these structured interviews, marijuana users described 2 types of withdrawal patterns: 1) weakness, hypersomnia, and psychomotor retardation; and 2) anxiety, restlessness, depression, and insomnia.

Typically, withdrawal symptoms begin 1–3 days after cessation of regular cannabis use and reach maximum effects about 2–6 days after cessation with resolution occurring in 4–14 days.[197,198] Craving for marijuana is not usually a predominant part of the withdrawal syndrome. A 21-day inpatient study of marijuana smokers demonstrated decreased food intake and increased rating of anxiety, irritability, and abdominal pain after 4 days of smoking marijuana placebo cigarettes.[199] Not all chronic marijuana users experience withdrawal symptoms following cessation of cannabis use. In a study of current and former chronic marijuana users, 40% of the participants did not experience clinically significant symptoms following cessation of use, and the lack of craving for marijuana suggested that the physical signs in the other 60% were not as strong as other drugs of abuse.[200]

Reproductive Abnormalities

There are few data on the teratogenic and developmental issues associated with marijuana use during pregnancy. Some anecdotal evidence suggests that heavy marijuana use during pregnancy produces a fetal alcohol syndrome-like clinical presentation characterized by tremulousness, intrauterine growth retardation, and facial dysmorphogenesis.[201] Comparison of the daily use of marijuana and the absence of marijuana use during pregnancy suggests that chronic marijuana use during pregnancy reduces birth weights and increases prevalence of facial dysmorphogenesis.[202] However, the large number of confounders in women drug users limits conclusions about the teratogenic potential of marijuana.

Carcinogenesis

Cannabis and Δ^9-THC are not recognized carcinogens by the International Agency for Research on Cancer or the US National Toxicology Program. There is no definitive epidemiologic evidence that regular cannabis smoking causes cancer of the lung or lower respiratory tract similar to cigarette smoking. To date, the epidemiologic evidence is conflicting. A case series reported 34 young patients with squamous cell carcinomas that were chronic marijuana smokers,[203] and a case-control study of head and neck cancer suggested a possible causal link between chronic marijuana smoking and squamous cell cancer of the head and neck.[204] In the latter study of 173 previously untreated cases with pathologically confirmed squamous cell carcinoma of the head and neck and 176 cancer-free controls, the odds ratio (OR) for head and neck cancer among individuals who ever smoked marijuana was 2.6 (95% CI: 1.1–6.6) and 3.1 (95% CI: 1.0–9.7) for marijuana smokers under the age of 55 years. However, there are no well-controlled prospective studies of chronic marijuana users to support this evidence, and most case control studies do not report an increased risk of head and neck cancer among marijuana users.[205] In a population-based, case-control study of 407 patients with oral squamous cell carcinoma, the OR for ever-use of marijuana was 0.9 (95% CI: 0.6–1.3).[206] There were no trends in increased risk of oral cancer with increasing duration or frequency of marijuana use. There was no overall excess of cancer or of tobacco-related cancers in a study of marijuana smokers in approximately 65,000 Kaiser Permanente Medical Care Program enrollees aged 15 through 49 years based on self-administered research questionnaires.[207] However, current cannabis smokers did have an elevated risk of prostate cancer (RR = 4.7, 95% CI: 1.4–15.5). Although there is a relative increased risk of death among heavy, chronic marijuana users, most of the excess risk results from social background variables.[208] In pooled data from 4,029 head and neck cancer cases and 5,015 controls in 5 case-control studies within the INHANCE Consortium, the risk of head and neck

cancer was not elevated by ever-smoking marijuana (OR = 0.88; 95% CI: 0.67–1.16).[209] There was no increased risk associated with increasing frequency, duration, or cumulative consumption of marijuana smoking. Self-reported interview data provided the basis for the classification of marijuana smoking and known risk factors.

DIAGNOSTIC TESTING

Analytic Methods

SCREENING

Thin layer chromatography (TLC), enzyme immunoassays (EIA), cloned enzyme donor immunoassays (CEDIA), and fluorescence polarization immunoassay (FPIA) are the most common analytic techniques available for the screening of urine for the presence of marijuana metabolites.[210] Radioimmunoassay (RIA) and kinetic interaction of microparticles in solution assays (KIMS) are obsolete screen techniques that have been superseded by the above methods. Immunoassays cross-react with many cannabinoids and their metabolites; hence, these methods do not determine which cannabinoids are present in the sample. The EMIT cannabinoid assay is a reliable semiquantitative urine screening test (20–50 ng/mL THC-COOH equivalent sensitivity level) based on competitive protein binding with the inactive Δ^9-THC metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). The antiserum cross-reacts with most 9-substituted metabolites including the glucuronide conjugates; therefore, the results are expressed as THC-COOH equivalents. In contrast to the EMIT antigen, the Roche Abuscreen® (Roche Pharmaceuticals, Nutley, NJ) radioimmunoassay antigen was conjugated to the phenolic hydroxyl group of cannabinoids via a carboxymethyl derivative; thus, the Roche assay produces a different set of cross-reactivities compared with the EMIT assay. The Abbott TDx® (Abbott Laboratories, Abbott Park, IL) assay (i.e., fluorescence polarization immunoassay) binds equally to THC-COOH, its glucuronide and the corresponding isomer.[211] In general, reduced binding to the antiserum occurs in the presence of hydroxylated derivatives of Δ^9-THC; the binding to noncannabinoid constituents is small.

The accuracy, sensitivity, and specificity of immunoassays are unique to the specific product and these parameters may vary over time.[212] Depending on sensitivity, one urine sample may be positive by one immunoassay but negative by another assay. The incidence of false-positive rates from immunoassays [e.g., EMIT d.a.u. (Syva Corp., Palo Alto, CA), Roche Abuscreen ONLINE™ (Roche Diagnostics, Indianapolis, IN), Abbott TDx analyzer] is approximately 4%.[213] Typically, cross-reactivity of THC-COOH glucuronide by these immunoassays and the relatively high concentration of THC-COOH provides sufficient sensitivity to eliminate the need for hydrolysis during the screening of urine samples (GC/MS confirmation usually measures total THC-COOH after enzymatic or alkaline hydrolysis). Thin layer chromatographic procedures (e.g., TOXI-LAB, Varian, Inc., Palo Alto, CA) are sensitive compared with the EMIT-d.a.u., but the former procedure produces more false-positives near the cutoff concentration of 20 ng THC-COOH/mL.[214] With alkaline hydrolysis and Fast Blue BB staining, the limit of detection for THC-COOH in urine samples is about 5–10 ng/mL.[215] Urine specimens should be checked for pH and specific gravity whenever an unanticipated false-negative result occurs. The use of creatinine-corrected THC-COOH concentrations adjusts the effect of urinary dilution.[216]

The addition of adulterants may destroy Δ^9-THC-COOH in urine samples, depending on the pH, storage time, and urine temperature. Adding bleach to urine samples causes false-positive results in radioimmunoassay.[217] This adulterant also causes false-negative results in enzyme immunoassays and fluorescence polarization immunoassays as a result of the effect of the bleach on immunoassay reagents.[218] Many oxidizing agents can destroy Δ^9-THC-COOH in urine samples, particularly under acidic conditions. Under experimental conditions chromate, nitrite, Oxone® (potassium peroxymonosulfate), Fenton's reagent (hydrogen peroxide/ferrous ammonium sulfate), permanganate, periodate, peroxidase, and extracts from red radish, black mustard seeds, and horseradish destroyed most of the Δ^9-THC-COOH in urine samples within 48 hours of exposure.[219]

CONFIRMATION

Analytic methods for the confirmation and quantitation of Δ^9-THC and metabolites in biologic specimens include gas chromatography, high performance liquid chromatography, liquid chromatography/mass spectrometry, liquid chromatography/tandem mass spectrometry, capillary electrophoresis/mass spectrometry, and gas chromatography/mass spectrometry.[220] Gas chromatography[221] and HPLC with electrochemical detection[222] provide greater specificity compared with immunoassays, but these methods do furnish sufficient structural information. Gas chromatography/mass spectrometry provides the sensitivity and specificity necessary for the confirmation of positive results from immunoassays and for the quantitation of specific cannabinoids.[223,224] The detection limits for Δ^9-THC and the

major metabolites using GC/MS range from approximately 1–2 ng/mL. For quantitation of Δ^9-THC and metabolites in blood samples, GC/MS with electron impact ionization is a common analytic method. The use of gas chromatography/tandem mass spectrometry with negative-ion chemical ionization is a more rapid alternative to gas chromatography/electron impact/mass spectrometry and gas chromatography/positive-ion electron impact/mass spectrometry with the following lower limits of quantitation (LLOQ): Δ^9-THC, 0.5 ng/mL; 11-OH-THC, 0.5 ng/mL, and THC-COOH, 2.5 ng/mL.[225] Liquid chromatography/tandem mass spectrometry has similar LLOQ of Δ^9-THC and the major metabolites with the advantage that derivation of THC-COOH is unnecessary in contrast to GC/MS.[226,227] GC/MS methods are available for the detection of cannabinoids in hair samples with a LLOQ in the range of 0.04–0.08 ng/mg and coefficients of variation in the range of 8%.[228] High performance liquid chromatography with electrospray ionization mass spectrometry provides a limit of detection and LLOQ for Δ^9-THC of approximately 1 ng/mL and 2 ng/mL, respectively.[229] The conjugation of glucuronic acid with Δ^9-THC and its metabolites facilitates the urinary excretion of Δ^9-THC and THC-COOH.[230] During preparation of the sample, cleavage of the glucuronide bond occurs either enzymatically with β-glucuronidase or chemically with alkaline solutions. The extent of the hydrolysis of cannabinoid conjugates depends on the method and the glucuronidase activity of the particular species used.[231] Incomplete hydrolysis of the sample and diffusion of cannabinoids into glass or plastic containers can reduce the recovery of Δ^9-THC and/or metabolites in biologic samples.

STORAGE

BLOOD. The stability of Δ^9-THC and metabolites [11-hydroxy-Δ^9-tetrahydrocannabinol(11-OH-THC),11-nor-9-carboxy-Δ^9-tetrahydrocannabinol(THC-COOH)] varies with temperature, storage container, and compound. Short-term storage of Δ^9-THC in glass vials at room temperature for 4 days followed by freezing (–20°C/–4°F) for 4 weeks did not produce significant reductions in whole-blood concentrations of Δ^9-THC, but analysis of Δ^9-THC in samples stored in polystyrene containers demonstrated substantial losses.[232] Some degradation of cannabinoids occurs during prolonged storage (>1 month) at room temperature, particularly the decarboxylation of cannabinoid acids to neutral cannabinoids. After storage of blood samples 6 months at room temperature, the concentrations of Δ^9-THC and 11-OH-THC decreased by 90% and by 44%, respectively.[233] The concentration of THC-COOH was not significantly different from the control. This study detected

no statistically significant changes in the concentrations of Δ^9-THC or the two metabolites (11-OH-THC, THC-COOH) in blood samples stored at 4°C (39.2°F) or –10°C (14°F) for 4 months. During a 10-day storage period at 4°C and 20°C, the amount of 11-nor-Δ^9-carboxy THC glucuronide (THC-COOH-glucuronide) converted to 11-nor-Δ^9-carboxy THC averaged about 20% and 70%, respectively.[234] 1-Nor-Δ^9-carboxy THC glucuronide was stable at freezing temperature (–20°C/–4°F).

URINE.   In addition to the degradation of cannabinoids from the solution via oxidation, loss also occurs during the handling and storage of samples, primarily during the first few hours of storage.[235] Adsorption to material surfaces (borosilicates) probably accounts for a substantial portion of the loss of cannabinoids from solution.[236] Factors determining the loss of cannabinoids include the solvent type, the amount of exposed surface area, temperature, the type of container, sample pH, and analytic variation.[237] A study of THC-COOH losses indicated that storage losses occur both by adsorption to material surfaces and losses during pipetting and storage handling.[238] High-density polyethylene containers produced the greatest losses and untreated glass (e.g., amber sialylated glassware) caused the smallest losses. Loss of THC-COOH during storage is less from frozen urine samples than urine samples stored at room temperature. Storage of urine samples at room temperature for 10 days resulted in an average THC-COOH loss of about 22%.[239] The mean loss of this metabolite in frozen urine specimens analyzed after 40 days, 1 year, and 3 years was 8.0 ± 10.6%, 15.8 ± 4.2%, and 19.6 ± 6.7%, respectively. Storage of urine samples in plastic tubes at –16°C (3.2°F) to –18°C (–0.4°F) over 45 days resulted in a mean loss of 11% (range, 0–34%) of the THC-COOH present in the original sample.[240]

Biomarkers

11-Nor-9-carboxy-Δ^9-THC (THC-COOH) is highly lipophilic and poorly water soluble. Consequently, the highest concentrations of THC-COOH following absorption of Δ^9-THC occur in the bile and very small concentrations occur in the vitreous humor. Postmortem examination of 50 vitreous humor samples by immunoassay (cutoff 50 ng/mL) yielded no positive results for cannabinoids, whereas 30 postmortem blood specimens from the same cases tested positive for THC-COOH ranging from 4–104 ng/mL as measured by GC/MS in select ion monitoring (SIM) mode.[241] In a study of 50 traffic fatalities with positive urine drug screens for THC-COOH, the mean THC-COOH concentration in bile was 12,900 ng/mL (range, 1,030–43,700 ng/mL) compared with 81 ng/mL (range, 16–330 ng/mL) in

heart blood and 314 ng/mL (range, 44–2,330 ng/mL) in urine samples.[242] The THC-COOH concentration in 10 of the 50 samples of vitreous humor was nondetectable (<1 ng/mL), and the concentration in the rest of the vitreous samples was <10 ng/mL. Consequently, vitreous humor is a poor sampling media for the detection of marijuana use, whereas bile is a good sampling media for the detection of low postmortem concentrations of THC-COOH.

Blood

Δ^9-THC resides mostly in plasma with almost 90% of Δ^9-THC in whole blood distributed in the plasma and the remainder in the erythrocytes. Therefore, the plasma/whole blood ratio of Δ^9-THC is similar to the inverse of the hematocrit, and the whole blood concentration of Δ^9-THC is about one-half the corresponding plasma concentration.[243] In a study of 7 volunteers, the mean plasma/whole blood ratio of Δ^9-THC was 1.54 (range, 1.4–1.9).[244] The distributions of THC-COOH and 11-OH-THC in plasma and whole blood are similar to the distribution of Δ^9-THC. The mean plasma/whole blood ratio of 11-OH-THC was 1.7 (range, 1.4–2.2). In an experimental study, the mean plasma/whole blood ratios of 11-nor-Δ^9-carboxy THC (THC-COOH) and the corresponding glucuronide at a concentration of 100 ng/mL were 1.54 and 1.61, respectively.[234] At a concentration of 500 ng/mL, the mean plasma/whole ratios of both compounds were 1.47. Examination of postmortem "serum" and whole blood samples for Δ^9-THC and metabolites suggests that postmortem changes (e.g., hemolysis, protein aggregation/degradation) alter the plasma/whole blood ratio of Δ^9-THC and metabolites. In a study of 6 postmortem blood samples, the mean "serum"/whole blood ratios of Δ^9-THC and 11-OH-Δ^9-THC were 2.2 (range, 1.5–2.8) and 2.3 (range, 1.5–3.9), respectively.[244] These values demonstrated wider ranges for the ratios compared with the ratios in live humans. The postmortem variability in this ratio limits the estimation of the time of last cannabis use based on the ratio of Δ^9-THC and metabolites. Most analytic methods for the determination of Δ^9-THC and metabolites in blood measure free cannabinoids rather than conjugated or total cannabinoids.

CONCENTRATION.   Plasma concentrations of Δ^9-THC do not correlate well to physiologic and psychotropic effects based on volunteer studies; the determination of impairment requires the presence of very high concentrations of Δ^9-THC. The subjective "high" and the physiologic tachycardia following cannabis smoking lags 20–30 minutes behind peak plasma levels (i.e., ~7–8

minutes after initiation of smoking) and then declines slowly to baseline over 4 hours.[245] Effects are dose- and interval-related, and smoking higher-potency cigarettes prolongs psychotomimetic effects. Modeling of Δ^9-THC kinetics indicates that smoking one 9-mg marijuana cigarette per hour maintains the psychotropic effects of marijuana; these effects last about 2 hours after the cessation of smoking.[246] Initial peak plasma concentrations sampled 15 minutes after the initiation of smoking marijuana cigarettes (19 mg Δ^9-THC content) were between 50–70 ng/mL.[67] An average "high" (rated 4 on a scale of 10) occurred when the Δ^9-THC plasma concentration was about 9 ng/mL. Conjunctival injection consistently occurred with plasma Δ^9-THC concentrations exceeding 5 ng/mL and this sign was more reliable than tachycardia in predicting clinical effect. Maximum clinical effects do not occur at peak plasma Δ^9-THC concentrations, and substantial individual variability occurs both in the magnitude of clinical effect and in peak concentrations.[69] There are inadequate data to correlate whole blood Δ^9-THC levels below 5 ng/mL and serum or plasma concentrations <10 ng/mL to physical impairment because of the variability of individual pharmacokinetics, tolerance, and the lack of a direct relationship between blood Δ^9-THC concentrations and neuropsychologic responses. Although there is substantial interindividual variation in peak plasma Δ^9-THC concentrations following the inhalation of similar Δ^9-THC doses, pharmacokinetic studies indicate linear kinetics in Δ^9-THC doses up to at least 70 mg. In a study of 24 male occasional cannabis users, the mean maximum serum Δ^9-THC concentrations following the inhalation of marijuana cigarettes containing 49 mg and 69 mg were approximately 203 ng/mL and 231 ng/mL, respectively.[247]

The presence of THC-COOH supports the validity of Δ^9-THC measurements. Plasma Δ^9-THC concentrations do not reliably detect the smoking of 1 marijuana cigarette per day by an infrequent user, when tested in the morning following inhalation.[248] Case reports document the presence of detectable concentrations of THC in postmortem samples from patients, who die suddenly.[249,250] However, the contribution of THC to these deaths is unclear, in part because of the lack of serious cardiovascular effects in patients with acute THC intoxication.

TIME OF EXPOSURE.   Predicting the time elapsed since the initiation of smoking is complicated by factors that affect the plasma Δ^9-THC, including the chronic use of marijuana (i.e., residual plasma Δ^9-THC concentrations), the efficiency of the smoking process, and the potency of the marijuana. Peak plasma concentrations of Δ^9-THC occur within approximately 7–10 minutes

after exposure. The presence of plasma Δ^9-THC concentrations exceeding 20 ng/mL indicates smoking within the last hour and plasma concentrations >50 ng/mL indicates that smoking began within the last 20 minutes. By 1 hour after smoking, plasma Δ^9-THC concentrations are usually below 5–10 ng/mL. Plasma Δ^9-THC concentrations decline rapidly within the first hour after smoking ceases, and these concentrations are relatively low (2–3 ng/mL) within 6 hours after cessation of marijuana smoking by casual user.[87] For example, the inhalation of 1.75% and 3.55% marijuana cigarettes produced peak concentrations of 50–129 ng Δ^9-THC/mL and 76–267 ng Δ^9-THC/mL, respectively.[86] Within 2 hours of smoking these cigarettes, the plasma Δ^9-THC concentration dropped below 5 ng/mL. Chronic use of large quantities of cannabis may result in more persistent elevations of plasma Δ^9-THC and THC-COOH concentrations than occur in casual cannabis users. In a study of 18 chronic, high-dose cannabis users monitored on an inpatient research unit for 7 days, 9 (50%) of the chronic users had plasma Δ^9-THC concentrations exceeding 1 ng/mL (range, 1.2–5.5 ng/mL) on the 7th day of abstinence as measured by two-dimensional GC/MS.[251] The plasma THC-COOH concentrations in the 18 volunteers on day 7 ranged from 2.8–45.6 ng/mL. All participants had at least one daily blood specimen that contained a plasma Δ^9-THC concentration higher than the previous day.

Volunteer studies indicate that the peak concentrations of the major metabolite, THC-COOH, occur about 30 minutes after the commencement of smoking.[69] The peak effect of marijuana apparently occurs near the time after exposure when Δ^9-THC and THC-COOH are present in equivalent concentrations. Measurement of the relative amounts of these 2 compounds in whole blood or plasma helps determine when peak effects develop.[23] A THC-COOH/Δ^9-THC ratio greater than 4 suggests that pharmacologic effects are no longer present (i.e., >3–4 hours after smoking).[252] However, a study of 298 forensic blood specimens positive for cannabinoids revealed that Δ^9-THC blood concentrations >10 ng/mL were required before 70% of the samples had blood THC-COOH/Δ^9-THC ratios <4. Part of the variance probably results from the fact that chronic users reach higher blood THC-COOH concentrations more rapidly compared with infrequent users.[253] Because of interindividual variation in the pharmacokinetics of Δ^9-THC, estimation of the blood Δ^9-THC concentrations based *only* on cannabinoid metabolite concentrations is unreliable.

The pharmacokinetics of marijuana after IV administration is similar to the pharmacokinetics following smoking, but the ingestion of marijuana produces lower, delayed peak levels. After ingestion of Δ^9-THC, the onset of clinical effects develops slower and lasts longer compared with IV or pulmonary administration, but subjective effects occur at much lower plasma concentrations than after injection or inhalation. In a study of 11 healthy volunteers, the same degree of intoxication in volunteers occurred at plasma Δ^9-THC levels of 2–4 ng/mL after ingestion compared with plasma Δ^9-THC of 9 ng/mL following inhalation.[67] A small study of 4 volunteers without prior marijuana use indicated that a total plasma metabolite/Δ^9-THC ratio below 20 was consistent with recent oral cannabis consumption, but the administration of multiple Δ^9-THC doses in chronic users produced substantially higher ratios (i.e., >30) as measured by radioimmunoassay.[254]

POSTMORTEM. Most postmortem analytic tests involve whole blood samples. The concentration of Δ^9-THC in plasma is about 1.5–2 times the concentration of Δ^9-THC in whole blood because Δ^9-THC is highly bound to plasma proteins. Despite the widespread use of cannabis, there are few case reports documenting the presence of Δ^9-THC in the postmortem examination of individuals dying without an obvious cause of death.[255] During the autopsy of a suspected marijuana-induced death, the tissue concentrations of Δ^9-THC were as follows (mg/100g): kidney, 3.75; liver, 4.2; spleen, 1.2; stomach, 0.8; and intestine, 0.2.[65] Unusually high postmortem blood Δ^9-THC concentrations and low blood THC-COOH concentrations may occur in decomposed bodies, particularly when reductive anaerobic processes (e.g., during submersion) convert the metabolite to the parent compound.[256] Although case series document the presence of Δ^9-THC in the blood concentrations <5–10 ng/mL in postmortem samples from patients dying with cardiovascular disease,[250] the contribution of Δ^9-THC to the death of these patients is unclear. Despite the high lipid solubility of Δ^9-THC and the large volume of distribution, postmortem redistribution of Δ^9-THC is relatively modest (e.g., heart/femoral blood ratios ranging from 0.3–3.0); additionally, there is some variability in the distribution of THC between whole blood and serum during the postmortem period.[244]

URINE

Urine drug screens for the detection of marijuana use are particularly susceptible to false-negative results after adulteration of the urine sample (e.g., addition of bleach, glutaraldehyde, oxidants, pyridinium chlorochromate, or nitrites). Additionally, the use of nonsteroidal antiinflammatory analgesics can interfere with the derivatization of THC-COOH during GC/MS procedures, causing false-negative results for

THC-COOH.[257] Typically, urine samples are clear to pale yellow with concentrated urine in early morning samples providing the most reliable results. The temperature of urine samples recorded within 4 minutes of collection should be between 32–38ºC (89.6–100.4ºF); the pH usually ranges from 4.5–8.0. Urine creatinine concentrations from healthy individuals typically exceed 20 mg/dL. Factors suggesting alteration of the urine sample include pH <3 or >11, specific gravity <1.002 or >1.020, nitrites >500 μg/mL, and urine creatinine <5 mg/dL.[258]

DETECTION TIMES. Urinary detection times vary substantially as a result of differences in assays (limits of detection, cross-reactivity, cutoff), individuals (body mass, pharmacogenetics, chronicity of drug use), dose, urine parameters (pH, specific gravity), route of administration, and the efficacy of hydrolytic methods. The amount of cannabinoids in the urine depends on a variety of factors including the amount of drug absorbed, the time since consumption, the metabolic rate, fluid intake, individual variability, and the chronicity of use. Metabolites are detectable in plasma within minutes of the initiation of smoking and these metabolites persist after the disappearance of Δ^9-THC. Positive urine tests document the prior use of marijuana, but these urine assays do not correlate to psychomotor impairment. There is no direct correlation between blood Δ^9-THC concentrations and urine concentrations of THC-COOH.[253] Typically, the urine drug screen for cannabinoids remains positive for 1–4 days after occasional marijuana use and approximately 7–10 days following heavy cannabis abuse, depending on the factors listed above.[259]

Occasional Use. The urine cannabinoid concentration does not accurately predict the amount of Δ^9-THC absorbed because a small concentration may result either from a large dose taken a long time previously or from a small dose administered recently. The presence of total (conjugated and unconjugated) Δ^9-THC concentrations in the urine samples exceeding 2 ng/mL suggests recent use (<5–6 hours) of marijuana as measured by GC/MS following enzymatic hydrolysis.[260] In 1994, the US Department of Health and Human Services (DHHS) changed the official requirement for the immunoassay screening cutoff concentration of marijuana metabolites from 100 ng/mL to 50 ng/mL.[261] Mean detection times vary directly with the cutoff concentration of a particular assay, and there is substantial variation in detection times of various immunoassays. For a cutoff of 50 ng/mL, the mean detection times in a group of 6 healthy volunteers smoking a single low dose (1.75% Δ^9-THC) cigarette was <1 day whereas the inha-

lation of a single high-dose cigarette (3.55% Δ^9-THC) produced a positive immunoassay for <2 days (range, 57–122.3 hours).[262] The detection times using GC/MS with a 15 ng/mL cutoff were approximately twice as long as the mean detection times using an immunoassay with a 50 ng/mL cutoff. The mean detection times ranged from 1–5 days (mean, 33.7 ± 9.2 hours) after the low-dose cigarette and from 3–6 days (mean, 88.6 ± 23.2 hours) after the high-dose cigarette. Following the ingestion of marijuana brownies containing the equivalent plant material of one or two marijuana cigarettes (2.8% Δ^9-THC), the mean times to the first negative EMIT® d.a.u. Cannabinoid 20 Assay (20 ng/mL cutoff) were ~58 ± 14 hours and 82 ± 23 hours, respectively; the mean times to the last positive were 122 ± 28 hours and 143 ± 23 hours, respectively.[263] Cannabis seed oil contains Δ^9-THC concentrations ranging from 3–1,500 μg/g oil; the ingestion of the potent forms of this oil may produce positive immunoassays for cannabinoid metabolites up to about 6 days after ingestion.[264]

Chronic Use. Because Δ^9-THC can accumulate in body fat, detectable urine THC metabolites may appear several weeks after cessation of chronic marijuana use, depending on the sensitivity of the assay.[265] In patients testing positive for Δ^9-THC-COOH after previously negative urine samples collected >48 hours before the positive sample, serial evaluation of the Δ^9-THC-COOH/creatinine ratio by quantitative immunoassay results may help detect new marijuana use. A urine THC-COOH/creatinine ratio of 2 specimens collected at least 24–96 hours apart (i.e., dividing the THC-COOH/creatinine ratio of specimen 2 by the ratio in specimen 1) exceeding 0.5 suggests recent marijuana use based on the analysis by fluorescence-polarization immunoassay.[266,267] The use of a ratio of total cannabinoids/creatinine in the second specimen exceeding 150% of the first specimen as measured by EMIT®d.a.u. immunoassay is a more specific method of detecting recent marijuana use, but this method lacks the sensitivity of using the THC-COOH/creatinine ratio of 0.5.[268] However, the use of a single 0.5 ratio may generate unrealistically high reuse rates when the urine specimen contains a low THC-COOH/creatinine ratio (<50 ng/mg) and an unrealistically low rate when the urine specimen contains a high THC-COOH/creatinine ratio (200–400 ng/mg). A model development study suggests that a mono-exponential model of an initial THC-COOH/creatinine ratio and time between specimens based on the Marquardt-Levenberg algorithm provides a better data fit than the use of a single 0.5 ratio.[269]

Positive results for THC-COOH should be confirmed by GC/MS because of potential false-positive values. In a study of 148 urine specimens, the sensitivity

and specificity of the fluorescence-polarization immunoassay for detecting recent drug use was 95.3% and 44.4%, respectively, when compared with GC/MS analysis of THC-COOH.[270] These values indicate that this assay cross-reacts with cannabinoid metabolites other than THC-COOH. Marijuana research during the 1970s and 1980s used less-sensitive and less-specific assay methods compared with current immunoassays with GC/MS confirmation.[262] The excretion times of studies conducted during this period were associated with much longer excretion times compared with current analytic methods. For example, The EMIT® d.a.u. (sensitivity, 20 ng/mL) detected urine cannabinoids for an average of 27 consecutive days with an upper range of 46 consecutive days after cessation by chronic marijuana users.[271] Over 2 months may be necessary for urine cannabinoid concentrations to remain below the cutoff value for 10 consecutive days. Current methods indicate that urine samples from occasional marijuana users remain positive for cannabinoids up to ~1–4 days compared with up to 7–10 days for chronic marijuana users.

CROSS-REACTIVITY.   The major metabolic pathway of Δ^9-THC involves allylic hydroxylation at the C_{11} position and, to a lesser extent, at the C_8 position. The initial metabolite is 11-hydroxy-Δ^9-THC (11-OH-THC), which is oxidized to the major metabolite found in the blood and urine, 11-nor-9-carboxy-Δ^9-THC (THC-COOH). The glucuronidation of hydroxylated and carboxylated metabolites facilitates the excretion of these metabolites in the urine. The chief limitation of immunoassay procedures is the inherent cross-reactivity of the assay with other nonpsychoactive cannabinoids. Marijuana contains more than 400 compounds; urine immunoassays cross-react with dihydroxy-THC, polar acids, and perhaps other compounds. High concentrations of ibuprofen interfered with the methylation of THC-COOH leading to false-negative results on earlier EMIT® d.a.u. cannabinoid 100-ng assays, but later reagents were reformulated to reduce this cross-reactivity with the exception of tolmetin.[257,272] Additionally, the ingestion of the nonnucleoside reverse transcriptase inhibitor, efavirenz, may cause false-positive results for THC in some urine drug screens.[273]

PASSIVE INHALATION.   Although low concentrations of cannabinoids can occur in urine samples shortly after exposure to passive marijuana smoke in public or private settings,[274] positive cannabinoid urine immunoassays (i.e., >50 ng/mL cutoff) following passive inhalation of marijuana smoke is unlikely, except following very high, prolonged exposure (e.g., the equivalent of 16 marijuana cigarettes in a small room for 1 hour on 6 consecutive days).[275,276] In a study of naïve marijuana users sitting in a small, closed car (1,650 L air), marijuana smoking by others in the car produced urine cannabinoid concentrations near 20 ng/mL in urine samples from the nonsmoking volunteers as measured by immunoassays. These nonsmoking study participants did not develop psychotomimetic symptoms despite the presence of maximum levels of tolerable smoke.[277] Even when urine samples were collected for 24 hours after exposure in the enclosed environment, only 3 of 80 urine samples from nonsmokers were positive at the 20 ng/mL level and none were positive at the 50 ng/mL level.[278] A 1-hour exposure of 3 study participants to 4 marijuana cigarettes in a room $10 \times 10 \times 8$ feet (21,600 L air) with ventilation did not produce urine concentrations >6 ng THC-COOH/mL when tested 24 hours later by RIA.[279]

PASSIVE INGESTION.   Low concentrations (<1%) of Δ^9-THC and other cannabinoids contaminate hemp seed oil as a result of the extraction process. The Δ^9-THC content in these products depends upon the type of seed (i.e., fiber from hemp <hemp seed oil/capsules) and the presence of leaf debris. Recently, the hemp seed industry introduced new washing steps before pressing and the shelling of seeds to reduce the concentration of Δ^9-THC in hemp products. Hemp seed oil is available as a diet supplement for essential fatty acids. Given current cutoffs, the possibility of a positive urine drug screen within 48 hours after ingestion of manufacturer-recommended doses of low-Δ^9-THC containing hemp products is unlikely. However, positive drug screens for marijuana may occur rarely depending on the concentration of the product and the time since ingestion. The ingestion of large amounts (e.g., 135 mL over 4½ days) of older hemp seed oil may produce positive immunoassays (50 ng/mL cannabinoid metabolites by immunoassay screening, 15 ng/mL of THC-COOH by GC/MS confirmation) up to 2 days after ingestion.[280] However, volunteer studies indicate that hemp oil contains sufficiently low concentrations of Δ^9-THC to prevent the occurrence of confirmed false-positive drug of abuse screens after the ingestion of usual quantities of hemp oil. The daily ingestion of 0.6 mg Δ^9-THC for 10 days did not produce urine THC-COOH concentrations above the federal confirmation cutoff (i.e., 15 ng/mL).[281] This dose is equivalent to the consumption of approximately 125 mL of hemp oil containing Δ^9-THC concentrations of 5 µg/g or 300 g of hulled seeds containing 2 µg THC/g. Although the ingestion of several cookies and candy bars containing hemp seed oil may produce positive immunoassays at the 50 ng/mL and the 20 ng/mL cutoff levels, the urinary THC-COOH concentrations do not usually exceed the criteria for GC/MS of 15 ng

THC-COOH/mL).[282] Trace amounts of THC-COOH also may be present in the urine of individuals ingesting hemp seed tea, but these trace concentrations do not usually exceed GC/MS confirmatory concentrations (15 ng/mL).[283] The ingestion of Marinol® in daily doses of 7.5–14.8 mg commonly produces positive cannabinoid immunoassays for marijuana use within 4 days after ingestion based on volunteer studies.[284] Δ^9-tetrahydrocannabivarin is a C3 homologue of Δ^9-THC that occurs in urine specimens following exposure to Δ^9-THC, but not following exposure to Marinol®. Consequently, the presence of Δ^9-tetrahydrocannabivarin-9-carboxylic acid in the urine indicates the use of marijuana rather than Marinol®,[45] but detection of this metabolite is not readily available from commercial sources. Validation of claims of positive urine tests resulting from the ingestion of hemp products requires analysis of the ingested products for Δ^9-THC content and evaluation of urine Δ^9-THC metabolites for consistency with the dose ingested.

HAIR

The incorporation of Δ^9- THC and THC-COOH into hair is very low compared with cocaine. The latter drug of abuse has the highest incorporation rate in hair with a cocaine/THC-COOH incorporation ratio of 3,600.[285] The recent use of marijuana is difficult to detect by analysis of hair samples for marijuana metabolites, but several analytic methods are available to screen hair for the presence of cannabinoids as a result of chronic cannabis use including GC/MS with headspace solid-phase microextraction[286] and gas chromatography/negative ion chemical ionization/mass spectrometry.[287,288] Major problems include the potential contamination of hair by external sources of marijuana, the difficulty detecting the low concentrations of THC-COOH present in the hair after exposure to cannabis, and the photodegradation of Δ^9-THC by sunlight.[289,290] Basic drugs (amphetamines, cocaine, opiates) are incorporated into hair in preference to acidic drugs (e.g., THC-COOH). In contrast to these basic drugs, THC-COOH has a low affinity for melanin; therefore, hair color is not an important determinant of the THC-COOH content in hair.[291] In a study of 12 regular marijuana users and 10 controls (no marijuana use), the concentration of Δ^9-THC in hair was not significantly related to self-reports of amount of marijuana use.[292] However, the sum of major cannabinoids (THC, CBD, CBN) in the hair of regular users did correlate to the amount of marijuana use. Gas chromatography/mass spectrometry is the analytic method of choice for the quantitation of cannabinoids in hair because of the low concentration of these substances in the hair of marijuana users. Studies on the long-term stability of Δ^9-THC and its metabolites in hair are necessary before the significance of cannabinoids in hair can be determined.

SWEAT

Passive diffusion of an unbound drug across a concentration gradient and through lipid membranes results in the transfer of the drug from plasma to sweat. Basic drugs accumulate more easily in sweat than acidic drugs because the mean pH of sweat is relatively acidic (i.e., pH = 6.3). Patches applied to the skin allow oxygen, carbon dioxide, and water vapor to escape while the drug remains adsorbed in the patch. Δ^9- THC is a relatively neutral drug and the accumulation of Δ^9- THC in sweat is lower (low ng/patch) compared with basic drugs (cocaine, amphetamines, opiates). Δ^9-THC also adheres to glass and plastic surfaces as well as constituents of the patch. The principal acidic urinary metabolite of Δ^9-THC (11-nor-9-carboxy-Δ^9-THC) is not usually detected in sweat patches.[293] The US Substance Abuse and Mental Health Services Administration (SAMHSA) established a cutoff of 1.5 ng Δ^9-THC/2.5 mL of eluate with a confirmatory cutoff of 0.5 ng Δ^9-THC/2.5 mL of eluate. Analytic methods (e.g., gas chromatography/ negative ion chemical ionization/mass spectrometry) are available to measure Δ^9-THC concentrations in this range.[294] The potential advantage of sweat patch testing is the use of one patch during a week. However, issues that complicate the interpretation of Δ^9-THC in sweat include the inability of washing procedures to remove all drug deposited on the skin and resorption/volatilization of Δ^9-THC from the patches.

SALIVA

The smoking of marijuana exposes the oral mucosa to high concentrations of Δ^9-THC, resulting in direct deposition of Δ^9-THC into oral fluid; only minor amounts of Δ^9-THC and metabolites diffuse into saliva from the blood. Advantages of testing saliva for cannabis use include ease of collection, direct supervision of samples, and closer correlation between cannabis exposure and testing; limitations compared with urine testing include the relatively smaller sample size and the lower concentration of Δ^9-THC and metabolites in saliva. In general, detection time for cannabis is shorter in saliva than urine.

In a study of 22 injured drivers with detectable THC-COOH in their urine samples as measured by GC/MS, 14 saliva samples collected with Sarstedt Salivettes (Etten Leur, Netherlands) were positive for cannabis use.[295] In a study of 10 current cannabis users smoking a single marijuana cigarette with an average Δ^9-THC

content of 20–25 mg, oral fluid specimens collected with the Intercept™ DOA Oral Specimen Collection Device (OraSure Technologies, Inc., Bethlehem, PA) was consistently positive for cannabis over a mean time of 13 ± 2 hours (range, 1–24 hours) after smoking as measured by GC/MS/MS (15 ng/mL cutoff), whereas the average time to the last positive oral specimen was 34 ± 11 hours (range, 1–72 hours).[296] The urine samples tested positive consecutively for cannabis for an average of 33 ± 10 hours (range, 4–72 hours); the average time to the last positive urine specimen was 58 ± 6 hours (range, 16–72 hours) using the same method.

Abnormalities

BLOOD

Laboratory abnormalities during marijuana intoxication typically are minor. However, following the IV administration of boiled marijuana extract, blood abnormalities include transient leukocytosis and thrombocytopenia, mild increase in serum hepatic aminotransferases, moderate rhabdomyolysis (increased serum creatine kinase, myoglobinuria), mild to moderate renal dysfunction (increased serum creatinine), and electrolyte imbalance.[297,298]

CARDIOVASCULAR

Rare case reports temporally associate marijuana smoking with the development of asymptomatic second-degree (Mobitz type I) heart block,[299] right bundle-branch-type ventricular tachycardia with near syncope,[300] and atrial fibrillation.[301] A case report associated the use of high doses of marijuana with the development of syncope and the Brugada syndrome.[302]

NEUROLOGIC

IMAGING STUDIES.   There is no conclusive evidence that the chronic use of marijuana causes structural abnormalities in the brain. Two cross-sectional studies of long-term marijuana users did not detect structural differences in the brains of long-term marijuana users and controls.[303,304] In particular, these imaging studies do not demonstrate significant changes in tissue volumes of the gray and white matter, cerebrospinal fluid or the hippocampus.[305] There was no direct evidence of cerebral atrophy or abnormalities of the ventricles in the brain computerized axial tomography images of 19 habitual marijuana users.[306] A functional MRI study of nine chronic cannabis smokers demonstrated decreased activation of cortical areas (Brodmann areas 6, 24, and 26) associated with complex information processing and

attention related to motor function during the first 4–36 hours after cessation of marijuana use.[307] These changes are consistent with deficits in attention during the early withdrawal phase.

NEUROPSYCHOLOGIC TESTING

Acute.   During the state of intoxication, alteration of motor control (e.g., body sway, sustained attention), and sensory function (e.g., overestimation of time) occurs. Long-term memory, reaction times, and hand–eye coordination are relatively resistant to the acute effects of typical recreational marijuana doses. After controlling for premorbid intelligence in a group of college students, almost daily marijuana use was associated with significantly greater impairment in attentional and executive functions when compared with monthly marijuana smokers.[308] This study did not attempt to determine neuropsychologic function during a period of abstinence. Selective attentional deficits[309] and short-term memory impairment[310] may persist during the first month of abstinence, but most neuropsychologic test scores return to normal within one month of the beginning of abstinence despite a history of heavy cannabis use.[311]

The acute smoking of marijuana may impair acquisition and working (i.e., short-term) memory, but not the retrieval processes during the period of intoxication.[4] Some researchers report impairment of multiple cognitive domains (short-term memory,[312] verbal episodic memory,[313] sustained attention, risk taking,[314] executive function[315]) during acute cannabis intoxication, whereas other studies did not detect impairment of cognitive flexibility,[316] mental calculation, reasoning, risk taking,[315] and executive function.[317] In general, acute cannabis intoxication slows responses during task performance, while overall performance accuracy is less affected. The performance effects of frequent cannabis users appear more subtle than occasional users.[313]

Chronic.   There is limited evidence that chronic marijuana use causes permanent gross cognitive impairment other than the effects of acute drug use, particularly following abstinence of 1–3 months.[318] The areas of concern following chronic marijuana use involve mild deficits in executive cognitive functioning, working memory, abstract reasoning, processing speed, overall IQ, and selective attention. The issue of the chronic effects of heavy marijuana use on the central nervous system remains unresolved, partly because of the difficulty determining the effect of confounders (e.g., anxiety disorders, major depression), establishing control groups with similar baseline psychologic testing scores, and evaluating the persistence of Δ^9-THC beyond the testing period. Experimental studies suggest that

some neurocognitive deficits remain in heavy cannabis users within 7 days after exposure ceases, but there are limited data to suggest that heavy cannabis use causes permanent reduction in neuropsychologic test scores.[319] In a study of college students abstinent for 1 day, heavy marijuana users (i.e., marijuana use 29 days in last month) displayed significantly greater impairment on attention/executive functions, as evidenced by greater perseverations on card sorting and reduced learning of word lists, compared with light marijuana users (i.e., marijuana use 1 day within the last month).[320] In a study of adolescent marijuana users, verbal working memory improved after 3 weeks of abstinence, but some attention deficits remained when compared with testing at the time of last cannabis use.[321]

A prospective, longitudinal study of 113 young adults assessed since infancy separated the cohort into light (<5 joints) and heavy (>5 joints/week) current users, former users (i.e., at least 3 months of abstinence from marijuana use), and nonusers.[322] After controlling for confounding factors and predrug performance on baseline neuropsychologic testing, the current regular heavy users did significantly worse than nonusers in scores on overall IQ, immediate and delayed memory, and processing speed. The adjusted scores on sustained attention and abstract reasoning were not statistically different between these two groups. Former marijuana smokers did not demonstrate any significant cognitive impairment, and the impairment of current heavy users resolved after 3 months of abstinence. A small case series of 10 volunteers using ganja chronically for religious purposes did not detect neuropsychologic abnormalities as measured by standardized neuropsychologic testing.[323]

Some studies suggest that the effects of heavy marijuana use may extend beyond the short-term period of intoxication. A retrospective, cross-sectional neuropsychologic study of nearly daily, long-term marijuana use indicate that heavy marijuana users scored significantly lower on tests of memory and attention compared with shorter-term (i.e., 10 year) marijuana use and nonuser controls.[324] A battery of neurocognitive tests administered to heavy marijuana users after a 28-day period of abstinence demonstrated persistent decrements in tests measuring memory, executive function, psychomotor speed, and manual dexterity when compared to a light marijuana user group.[325] However, the sample size was small, and there was a statistically significant difference in education level between the two groups. Additionally, the decrements in performance were not clinically abnormal, except for a few tests. Comparison of neuropsychologic test scores from current heavy marijuana users (>5,000 times during lifetime), former heavy users <50 times last 3 months), and controls (<50 times during lifetime) demonstrated no significant differences among

the group test scores nor any correlation between cumulative marijuana use and test scores after 7 days of abstinence.[326] There were significant differences between current chronic marijuana users and test scores on the recall of word lists for the first 7days of abstinence, but after that time the test scores between the groups were similar. Areas tested included general intellectual function, abstraction ability, sustained attention, verbal fluency, and ability to learn and recall new verbal and visuospatial information.

Driving

Although laboratory data including driving simulator studies indicate that smoking cannabis impairs some psychomotor skills and cognition (e.g., free recall of previously learned items) in a dose-dependent relationship, current culpability and case-control data is inconsistent with regard to the association of smoking typical doses of cannabis with either serious driving impairment or increased risk of traffic accidents.[327,328] Epidemiology data indicate that cannabis is the most common *illicit* drug detected in impaired drivers and motor vehicle injury accidents; however, interpretation of current epidemiology studies is limited by the lack of adequate control data (e.g., similar of risky behavior between 2 groups) and the rapid decline of Δ^9-THC between the incident and blood sampling. In general, the effect of marijuana on the learning of new tasks appears greater compared with the execution of well-trained tasks; however, the effect of Δ^9-THC is greater on highly automated tasks (e.g., road tracking control) compared with cognitive driving tasks requiring conscious control.[329] The greatest performance impairment from cannabis occurs during the first 20–40 minutes after smoking cannabis with impairment declining to baseline 2.5–4 hours after cannabis use.[330] Culpability analysis of 44 accidents involving injured drivers using only Δ^9-THC and no other drugs did not detect an increased risk of accidents involving only Δ^9-THC, but there was insufficient power in the study to determine an increased risk for whole blood Δ^9-THC concentrations exceeding 2 ng/mL.[331] Often, the use of cannabis occurs with other drugs, particularly ethanol; existing data suggest that the use of ethanol and cannabis together increases the risk of injury in motor vehicle accidents.[329]

Sensory, Motor, and Cognitive Tasks

Although laboratory studies suggest that Δ^9-THC impairs some psychomotor and cognitive functions including tracking, coordination, vigilance, divided attention, memory information processing, and perception, these decrements in performance affect primarily

highly automatic driving function rather than more complex tasks that require conscious control. In contrast, ethanol affects complex driving tasks more than highly automated driving tasks.[329] The issue of whether these decrements in driving performance are sufficiently large to increase the risk of accidents is inconclusive, in part because cannabis users tend to overestimate their impairment and may employ compensatory strategies depending on the degree of impairment.[336] The effect of Δ^9-THC on cognitive and psychomotor tasks associated with driving varies between individuals during the first few hours after inhalation, depending on the Δ^9-THC dose (smoking technique), tolerance, and the subject's ability to compensate for impaired skills and perception.[332] Furthermore, most subjective measures of cannabis exposure are more sensitive to Δ^9-THC effects than performance measures following doses that produce peak plasma Δ^9-THC concentrations below 170 ng/mL.[333] Overall, the impairment of driving skills is substantially less following the smoking of typical doses of marijuana than during the period of time associated with blood ethanol concentrations of 80–100 mg/dL.[334] In experimental studies, behavioral and physiologic effects begin concurrently or within minutes after the appearance of Δ^9-THC in the blood despite the substantial time delay between peak plasma Δ^9-THC concentrations and drug-induced effects.[335]

Modest impairment of human performance primarily involves divided attention, vigilance, perceptual speed, and complex tracking tasks (e.g., increased lateral position variability, headway control or distance between driver's vehicle and vehicle in front, critical tracking). The peak effects of Δ^9-THC on memory and divided attention occur approximately 15–30 minutes after smoking stops.[336,337] Testing of sensory functions in the laboratory setting is usually less affected by cannabis use than perceptual performance. Static visual acuity and visual search behavior are not significantly altered directly by the use of marijuana under either low or high contrast conditions based on volunteer studies using typical recreational marijuana doses. [338,339] Reported alterations of color vision associated with marijuana use include dyschromatopsia, yellow vision, increased color perception, and colored, flashing lights.[340] For at least 2 hours after ingesting Δ^9-THC, a dose-related decrease in glare recovery occurs as a result of alterations in light adaptation.[341] In a study of 10 cannabis users smoking cigarettes containing low dose (1.77% Δ^9-THC) and high dose (3.95% Δ^9-THC), the high dose increased body sway and marginally increased brake latency compared with placebo.[342] There was no significant difference between scores after placebo, low dose, or high dose on choice reaction time, critical flicker fusion, or a rapid judgment task. There are minimal effects on simple reaction times following smoking typical doses (peak plasma Δ^9-THC concentrations 63–188 ng/mL) of marijuana based on volunteer studies.[334] Experimental studies suggest tolerance to the impairment of some driving skills occurs in regular cannabis smokers. In a study of chronic cannabis users and nonusers, cannabis users were less impaired in peripheral signal detection than nonusers.[343]

The concurrent consumption of ethanol and marijuana may produce additive effects on some, but not all psychomotor functions associated with driving. In a controlled study of human volunteers ingesting 320 µg Δ^9-THC/kg, modest decrements in cognition, standing steadiness, psychomotor coordination, and reaction speed occurred. The addition of 0.54 g ethanol/kg (i.e., average peak ethanol concentration = 80 mg/dL) resulted in additional decrements in standing steadiness and psychomotor coordination, but no reduction in scores on cognition or simple reaction times.[344] Potentially, some cross-tolerance may occur in chronic marijuana users between Δ^9-THC and ethanol based on the attenuation of ethanol-induced impairment on computerized tracking skills of chronic marijuana users (i.e., daily for at least 3 years) compared with infrequent marijuana users.[345]

DRIVING COURSES AND SIMULATORS

The degree of impairment associated with cannabis use in driving simulators and driving courses is somewhat inconsistent and relatively minor. Although laboratory studies demonstrate some impairment of individual psychomotor skills immediately following Δ^9-THC exposure, there is minimal to modest impairment of overall performance in city and highway driving tests, in part because of the adjustment of drivers to their perception of impairment.[346,347] For example, the tendency to increase the distance between their car and the car in front of them as well as slower driving in these studies compensate for impairment of lane control and other driving skills. In some driving studies, experienced cannabis smokers demonstrate minimal impairment while driving a closed course under the influence of cannabis, except when combined with ethanol.[348] A high school driver education instructor, safety manager (American Automobile Association) and off-duty patrol officer evaluated performance on the closed-driving course based on their interpretation of impairment. A study of performance on a closed-course driving after cannabis use did not detect significant impairment. Volunteer studies using driving simulators suggest that drivers tested within 1 hour of smoking a marijuana cigarette (13 mg, 17 mg) drive slower than under control conditions.[349] In contrast, the average driving speed during a simulated driving test of the same drivers after ingesting

ethanol (estimated ethanol concentration = 0.05%) was higher compared with control conditions.

In general, driving impairment as evaluated by roadside sobriety tests does not correlate well with Δ^9-THC concentrations during the first 2 hours after exposure, especially compared with the dose-related impairment associated with ethanol consumption.[350] Scores on most psychomotor and cognitive tasks including simulated car-driving were normal the morning after marijuana use, despite the presence of subjective and behavioral effects the previous evening.[351] An uncontrolled study of 9 pilots using flight simulators suggested that some impairment of very complex human performance (i.e., flying skills) may occur up to 24 hours after the use of recreational doses (e.g., 20 mg Δ^9-THC) of marijuana.[352]

CULPABILITY AND CASE-CONTROL STUDIES

The results of culpability and case control studies that compare the actual risk of causing an accident between a sober driver and a driver under the influence of cannabis are inconsistent. Some,[353] but not all,[354] culpability studies of drivers involved in accidents while using illicit drugs suggest that the use of marijuana can increase the risk of an accident, particularly when the whole blood Δ^9-THC concentration exceeds 5 ng/mL. A review of culpability studies suggested that the presence of Δ^9-THC in the blood of crash victims increases the risk of culpability, particularly at higher doses as compared with drivers without recent drugs or alcohol use.[355] The concomitant use of Δ^9-THC and alcohol sharply increases the crash risk in epidemiologic analyses compared with either drug alone, even at relatively low concentrations. Although smoking marijuana does impair some driving skills, the drivers are usually aware of the impairment and compensate by focusing their attention on the perceived task.[327] However, they may not adequately compensate for unexpected events or for those tasks that require continuous attention.[356] Part of the difficultly in interpreting the effect of marijuana in some studies is the use of markers of recent exposure (11-hydroxy-Δ^9-THC) rather than Δ^9-THC concentrations as well as the high prevalence of concomitant ethanol and marijuana use. The rapid biotransformation of the active ingredient (Δ^9-THC) in marijuana complicates the use of blood as a biomarker of driving impairment because of the typical delay (i.e., 30–90 minutes) between apprehension and withdrawal of the blood sample. A 10-year Swedish study of individuals apprehended for suspected driving under the influence of drugs included 1,276 individuals with only Δ^9-THC in their blood samples.[357] The mean and median Δ^9-THC whole blood concentrations were 3.6 ng/mL and 2.0 ng/mL, respectively, with a range up

to 10 ng/mL. The study did not report the time between the initial stop for suspected driving under the influence and the sampling of venous blood. Over 85% of the 8,794 individuals arrested with Δ^9-THC in their blood also used other drugs.

BLOOD Δ^9-THC CONCENTRATIONS

There are limited data on the impairment associated with specific Δ^9-THC concentrations in plasma/serum and whole blood; consequently, there are difficulties deriving a risk-based *per se* limit for driving under the influence of cannabis, particularly given the rapid decline of Δ^9-THC concentrations between the time of the incident and sampling. Interpretation of the effect of a blood Δ^9-THC requires analysis of a variety of factors including the driving scenario (type of crash, road conditions, vehicle defects, cause of crash, appropriateness of driving response, compensatory actions, fatigue), magnitude of Δ^9-THC concentration, time interval between incident and withdrawal of the blood sample, concomitant presence of other drugs, and the time of the incident in relation to the last use of cannabis. In a study of 440 drivers apprehended for suspected driving under the influence of drugs, 53% of these drivers tested positive for Δ^9-THC with a median plasma concentration of 3 ng/mL (range, 1–35 ng/mL).[358] However, the study did not separate drivers with multiple drugs from drivers with only Δ^9-THC in their blood samples. Although the degree of impairment increases with increasing plasma/serum Δ^9-THC, the magnitude of the impairment is not linearly correlated to low plasma/serum Δ^9-THC concentrations as a result of individual sensitivities. Experimental studies suggest that the plasma/serum Δ^9-THC correlates better to the proportion of observations indicative of significant impairment rather than the magnitude of the impairment. In a study using critical tracking task (measure of on-the-road tracking performance), stop signal task (motor impulsivity), and Tower of London task (executive function/planning), significant impairment of all tasks in all 20 recreational cannabis users occurred at serum Δ^9-THC concentrations exceeding 30 ng/mL.[359] Limited epidemiology data suggest that serum Δ^9-THC concentrations of 12–16 ng/mL correlate to the odds ratio (i.e., 1.5–2) of an accident associated with a BAC of 50 mg/dL.[360]

TREATMENT

Stabilization

The most common presentation to the emergency department following marijuana exposure involves anxiety or dysphoria. These patients usually respond to

reassurance, reduction of external stimuli, and rest. They usually require no laboratory studies to aid management other than urine drug screens to confirm cannabis use and other tests as needed to exclude concurrent illnesses. Mild tranquilizers (e.g., lorazepam, 1–2 mg IV or intramuscularly [IM]) may be necessary if supportive measures do not calm the patient. Patients who do not respond rapidly to supportive care should be carefully evaluated for trauma and concurrent illnesses including rapid evaluation for hypoglycemia. Although the cardiovascular effects of Δ^9-THC do not usually cause serious health problems for most young, healthy users, rarely patients with significant cardiovascular disease may develop angina, dysrhythmias, myocardial infarction, or pulmonary edema. These patients should receive an IV line, cardiac monitoring, an electrocardiogram, cardiac enzymes, and other measures (aspirin, beta blockers, anticoagulants, antiplatelet drugs, coronary angiography) as dictated by the extent of coronary artery occlusion and myocardial damage.

Serious alterations of vital signs after cannabis use is extremely rare, except following the IV administration of marijuana extract. Hypovolemia from severe GI fluid loss may require the administration of fluids. These patients require hospitalization because of the potential development of multisystem failure including adult respiratory distress syndrome and acute renal failure within several days after injection. Consequently, careful management of both fluid and electrolyte balance is necessary. Laboratory analysis includes complete blood count, electrolytes, calcium, serum glucose, creatinine, blood urea nitrogen (BUN), hepatic aminotransferase, and muscle enzymes, complete coagulation profiles, electrocardiogram, chest x-ray, and urinalysis for myoglobin and protein.

Gut Decontamination

Decontamination measures are usually unnecessary following exposure to marijuana. Theoretically, the administration of activated charcoal to children, who present to the emergency department within 1 hour after ingesting ≥1 whole marijuana cigarette or cookie, is appropriate, but there are few data to guide management. Consumption of <1 whole marijuana cigarette does not require decontamination measures. Whenever patients ingest other substances (e.g., phencyclidine [PCP]) with the marijuana cigarette, decontamination measures are determined by treatment for that substance.

Acute Psychosis

Most psychotic symptoms that develop after marijuana use resolve within 6 hours, but rarely the acute psychosis may persist up to 1 week following heavy, habitual marijuana use. Most patients respond to reduction of stimuli in a normally lit room with a few familiar people and reassurance by medical personnel. Diazepam (5 mg IV) or lorazepam (1–2 mg IV) may be necessary to sedate these patients. Continued aggressive behavior despite the use of these measures may necessitate the use of physical restraints and neuroleptic drugs (e.g., haloperidol 5 mg IM).

Withdrawal/Dependence

Withdrawal symptoms may develop within 1–2 days after the cessation of heavy, chronic marijuana use (i.e., repeated daily smoking) manifest by GI distress, anorexia, anxiety, insomnia, irritability, and aggression.[199] Typically, these symptoms resolve within about 10–14 days. There are few data on the pharmacologic treatment of these withdrawal symptoms; potential therapies beside cognitive and behavioral therapies include oral tetrahydrocannabinol, mirtazapine, rimonabant, and buspirone.[361] Compared with placebo, nefazodone maintenance therapy (i.e., 450 mg/day) decreases some (anxiety, muscle pain), but not all (e.g., irritability, insomnia, malaise) withdrawal symptoms.[362] In 2 placebo-controlled, within-subject studies, the mood stabilizer, divalproex (500 mg/day increased every 2 days until a maintenance dose of 1,500 mg/day), reduced the craving for marijuana, but this dosage increased ratings of anxiety, irritability, and malaise while decreasing performance on psychomotor tasks.[363] Oral Δ^9-THC reduced both marijuana craving and withdrawal symptoms at a dose (10 mg/daily) that did not produce subjective effects. Withdrawal symptoms (marijuana craving, decreased appetite, sleep disturbances) during abstinence phases may contribute to cannabis dependence.[364] Treatment of cannabis dependence involves referral of the patient to sources for cognitive behavioral, motivation enhancement, and contingency management therapies.[28] Oral Δ^9-THC therapy typically is short-term in contrast to the use of methadone for opiate dependence.

SYNTHETIC CANNABINOIDS

HISTORY

In the late 1970s and the 1980s, chemists at Pfizer, Inc. investigated the analgesic properties of synthetic

FIGURE 60.9. Packages of spice. (Photo courtesy of the *Drug Identification Bible*)

nonclassical CP series of cannabinoids (e.g., CP-47,497); these compounds lacked the classical dibenzopyran structure of Δ^9-THC.[365,366] This investigation included the synthesis of the potent cannabinoid analog, cannabicyclohexanol. Research into the pharmacologic properties of synthetic nonclassical cannabinoids expanded in the 1990s and early 2000s with the investigations of JW Huffman and colleagues into the structure-activity relationships of indene-, indole-, and pyrrole-derived cannabinoids (JWH series).[367] Research at the Hebrew University of Jerusalem produced the HU series of cannabinoid receptor agonists (e.g., HU-210). In 2006, hits appeared in the World Wide Web promoting the smoking of herbal incense ("spice") that contained legal synthetic cannabinoids. These compounds were derivatives of aminoalkylindole class of ligands that bind and activate the CB_1 receptors. These commercial herbal products were distributed widely in brightly colored packages throughout Europe, Japan, and the United States by 2008.

Figure 60.9 displays some brightly colored packages of spice. In January 2009, the German Health Authorities banned the use of herbal incense containing CP-47,497-C8 and JWH-018; however, other synthetic cannabinoids (e.g., JWH-073, JWH-250, JWH-398) soon appeared on the market as suppliers responded to prohibitions and the prosecution of sellers.[368,369] The US

Drug Enforcement Agency (DEA) added JWH-018, JWH-073, JWH-200, CP-47,497, and cannabicyclohexanol to the list of schedule I substances in late 2010.

IDENTIFYING CHARACTERISTICS

Synthetic cannabinoids are a diverse group of compounds that are derived from indole, indene, and pyrrole structures and bind to one or both cannabinoid receptors with different affinities. Most synthetic cannabinoids (e.g., HU-210, CP-55,950) are potent cannabinoid receptor agonists, whereas Δ^9-THC is a weak partial agonist at both cannabinoid receptors. There are the following 4 structural classes of synthetic cannabinoids: 1) classical cannabinoids, 2) nonclassical cannabinoids, 3) aminoalkylindoles, and 4) eicosanoids as displayed in Figure 60.10.[370] Classical cannabinoids are dibenzopyran compounds that include Δ^9-THC, other plant cannabinoids (cannabinol, cannabidiol), and the synthetic compound, HU-210. The nonclassical cannabinoids lack a pyran ring structure and include the cyclohexylphenols, CP-47,497 and cannabicyclohexanol. The aminoalkylindoles include the well-studied naphthoylindole compounds in the JWH series (see Figure 60.10 and Figure 60.11). Most synthetic cannabinoids in spice (K2) belong to the JWH series that primarily activate CB_1 receptors (e.g., JWH-018) rather than CB_2 receptors (e.g., JWH-015). The eicosanoid group includes natural endocannabinoids (e.g., anandamide), synthetic derivatives, and noladine ether.

In vitro testing (i.e., cannabinoid receptor binding studies) indicates that the synthetic cannabinoids in herbal incense products are more potent than Δ^9-THC.[371] For example, JWH-018 has an approximately 4-fold higher affinity to the CB_1 receptor and about a 10-fold higher affinity for the CB_2 receptor than Δ^9-THC.[367] The affinity of the synthetic cannabinoid compounds for the cannabinoid receptors varies. CP-47,497-C8, and JWH-073 have higher affinity for the CB_1 receptor compared with the CB_2 receptor,[365] whereas JWH-018 and JWH-250 have similar affinity for both cannabinoid receptors. JWH-015 binds primarily to CB_2 receptors.[372] Street names for synthetic cannabinoids include Spice, Spice Gold, K2, Dream, Aroma, Scence, Skunk, Smoke, and Yucatan Fire.

EXPOSURE

Spice refers to herbal products that are marketed as incense and legal alternatives to cannabis; often these products contain synthetic cannabinoids. In a study of herbal products sold in Japan for cannabis-like effects, the concentrations of detected synthetic cannabinoids

Classical Cannabinoids
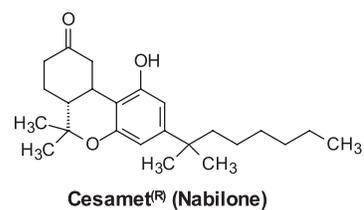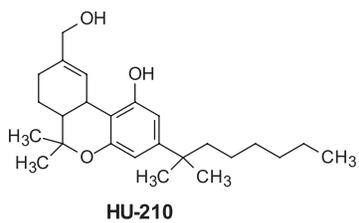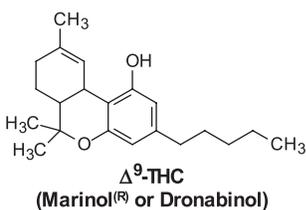
Non-Classical Cannabinoids
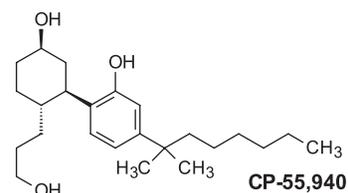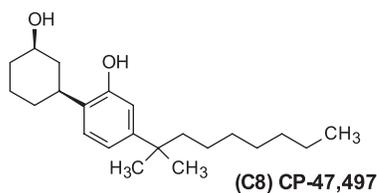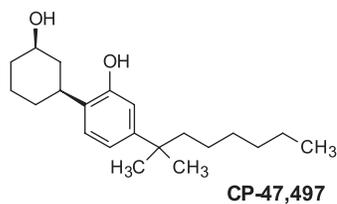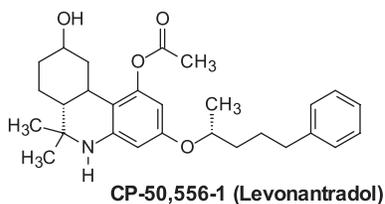
Aminoalkylindoles
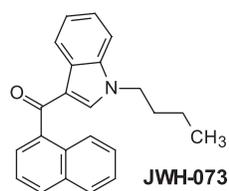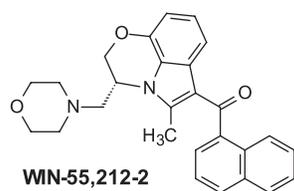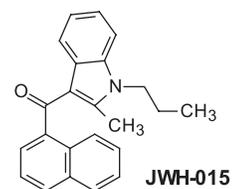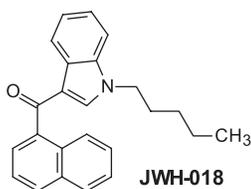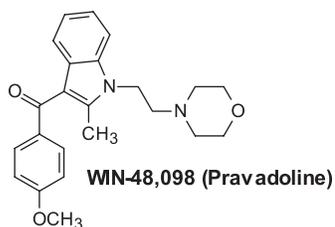
Eicosanoids
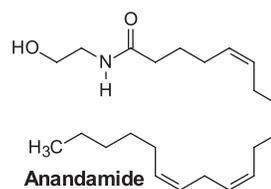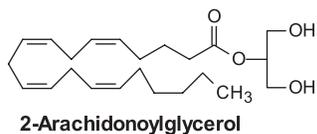
FIGURE 60.10. Classification of synthetic cannabinoids based on the four structural types of cannabinoid ligands that bind to cannabinoid receptors.[370]

Naphthoylindoles (R = H, unless otherwise specified)
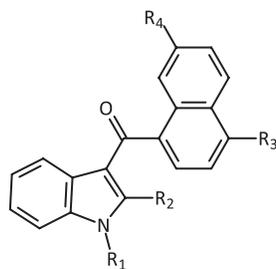
JWH-018 (342.1852)	R_1 = pentyl
JWH-073 (328.1696)	R_1 = butyl
JWH-398 (376.1463)	R_1 = pentyl, R_3 = Cl
JWH-081 (372.1958)	R_1 = pentyl, R_3 = OCH_3
JWH-007 (356.2009)	R_1 = pentyl, R2 = CH_3
JWH-019 (356.2009)	R_1 = hexyl
JWH-047 (356.2009)	R_1 = butyl, R2 = CH_3, R4 = CH_3
JWH-122 (356.2009)	R_1 = pentyl, R_3 = CH_3
JWH-180 (356.2009)	R_1 = propyl, R_3 = propyl
JWH-049 (384.2322)	R_1 = hexyl, R_2 = CH_3, R_4 = CH_3
JWH-182 (384.2322)	R_1 = pentyl, R_3 = propyl
JWH-213 (384.2322)	R_1 = pentyl, R_2 = CH_3, R_3 = ethyl

Cyclohexylphenols (R = H, unless otherwise specified)
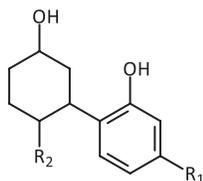
CP-47,497 (319.2632)	R_1 = 1,1-dimethylheptyl
Analog V (305.2475)	R_1 = 1,1-dimethylhexyl
Analog VII (333.2788)	R_1 = 1,1-dimethyloctyl
Analog VIII (347.2945)	R_1 = 1,1-dimethylnonyl

FIGURE 60.11. Chemical structures of some naphthoylindole (JWH) and cyclohexylphenol (CP) type synthetic cannabinoid compounds. Specific compounds are listed by identification number followed by isotopic mass in parentheses. Adapted from Reference 381.

was as follows: cannabicyclohexanol, 1.1–16.9 mg/g; JWH-018, 2.0–35.9 mg/g, and oleamide, 7.6–210.9 mg/g.[373]

TOXICOKINETICS

There are few human data on the kinetics of synthetic cannabinoids; similar to Δ^9-THC, the biotransformation of JWH-018 is complex. *In vitro* studies indicate that JWH-018 metabolites include a variety of phase I metabolites including monohydroxylated, dihydroxylated, trihydroxylated, *N*-dealkylated, carboxylated, and dehydrated compounds, as well as metabolites containing a dihydrodiol structure.[374] Limited data in humans suggests that the kidney excretes little JWH-018 unchanged in the urine with hydroxylation of the indole ring and the *N*-alkyl chain forming most metabolites that are conjugated with glucuronic acid.[375,376]

CLINICAL RESPONSE

There are few data on the adverse effects associated with smoking herbal products containing synthetic cannabinoids. Potential toxic effects include nausea, vomiting, blurred vision, tremors, ataxia, altered consciousness, confusion, irritability, paranoia, hallucinations, mydriasis, hypertension, and tachycardia. Typically, patients admitted to the emergency department after smoking products containing synthetic cannabinoid compounds complain of anxiety, disorientation, agitation, and palpitations; abnormal physical findings include conjunctival erythema, lateral gaze nystagmus, tachycardia, and altered mood (e.g., inappropriate laughter).[377,378] The clinical features usually resolve soon after admission to the emergency department. Several case reports associate the sudden reemergence of florid psychosis in previously stable patients with histories of prior psychotic episodes precipitated by cannabis.[379] Manifestations of these episodes involve primarily agitation and disorganization with grandiose and paranoid delusions. Case reports associate the chronic use with the development of withdrawal symptoms. A 20-year-old man with a history of using spice gold daily for 8 months developed withdrawal symptoms 3 days after cessation of use.[380] Clinical features of the withdrawal syndrome included drug craving, anxiety, nightmares, diaphoresis nausea, headache, tremor, hypertension, and sinus tachycardia. The symptoms resolved by the 7th day of hospitalization.

DIAGNOSTIC TESTING

Synthetic cannabinoids typically are not detected by urine drug screens. Methods for the quantitation of synthetic cannabinoids in confiscated samples include gas

chromatography/mass spectrometry in electron impact mode (70eV), liquid chromatography/high resolution accurate mass spectrometry,[381] and ultra high performance liquid chromatography/electrospray ionization/mass spectrometry.[373] Accuracy for the latter method ranged from −6.9–4.4%. Metabolites of synthetic cannabinoids are detectable with liquid chromatography/tandem mass spectrometry.[382] The lower limit of detection with this method is about 0.1 ng/mL. The use of spice products does not usually produce positive urine drug screens, and a negative urine drug of abuse screen does not exclude exposure to synthetic designer cannabinoid compounds. Laboratory abnormalities associated with the use of these drugs include leukocytosis, lactic acidosis, and elevated serum creatine kinase.[377]

TREATMENT

Treatment is supportive, similar to the treatment of cannabis.

References

1. Touw M. The religious and medicinal uses of cannabis in China, India and Tibet. J Psychoactive Drugs 1981;13:23–34.

2. Mechoulam R, Devane WA, Breuer A, Zahalka J. A random walk through a cannabis field. Pharmacol Biochem Behav 1991;40:461–464.

3. Aldrich MR. Tantric cannabis use in India. J Psychedelic Drugs 1977;9:227–233.

4. Adams IB, Martin BR. Cannabis: pharmacology and toxicology in animals and humans. Addiction 1996;91:1585–1614.

5. Mechoulam R, Feigenbaum JJ. Towards cannabinoid drugs. Prog Medicinal Chem 1987;24:159–207.

6. Snyder SH. Uses of marijuana. New York: Oxford University Press, 1971.

7. Gaoni Y, Machoulam R. Isolation, structure and partial synthesis of the active constituent of hashish. J Am Chem Soc 1964;86:1646–1647.

8. Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 1988;34:605–613.

9. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61–65.

10. Turner CE, Elsohly MA, Boeren EG. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J Nat Prod 1980;43:169–234.

11. Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 2003;42:327–360.

12. Garrett ER, Hunt CA. Physiochemical properties, solubility, and protein binding of delta 9-tetrahydrocannabinol. J Pharm Sci 1974;63:1056–1064.

13. Hood LV, Dames ME, Barry GT. Headspace volatiles of marijuana. Nature 1973;242:402–403.

14. Bond GD 2nd, Chand P, Walia AS, Liu RH. Observation of reduced concentration of delta 9-THC-carboxylic acid in urine specimen containers using internal barcode labels. J Anal Toxicol 1990;14:389–390.

15. Fairbairn JW, Liebmann JA, Rowan MG. The stability of cannabis and its preparations on storage. J Pharm Pharmacol 1976;28:1–7.

16. Harvey DJ. Stability of cannabinoids in dried samples of cannabis dating from around 1896–1905. J Ethnopharmacol 1990;28:117–128.

17. Ross SA, ElSohly MA. The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. J Nat Prod 1996;59:49–51.

18. Martin BR, Balster RL, Razdan RK, Harris LS, Dewey WL. Behavioral comparisons of the stereoisomers of tetrahydrocannabinols. Life Sci 1981;29:565–574.

19. Grotenhermen F. Pharmacology of cannabinoids. Neuro Endocrinol Lett 2004;25:14–23.

20. Mechoulam R, Hanus L. Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. Chem Phys Lipids 2002;121:35–43.

21. Mechoulam R. Marihuana chemistry. Science 1970;168:1159–1166.

22. Agurell S, Leander K. Stability, transfer and absorption of cannabinoid constituents of cannabis (hashish) during smoking. Acta Pharm Suec 1971;8:391–402.

23. Mason AP, McBay AJ. Cannabis: pharmacology and interpretation of effects. J Forensic Sci 1985;30:615–631.

24. Hall W, Degenhardt L. Adverse health effects of nonmedical cannabis use. Lancet 2009;374:1383–1391.

25. Relman AS. Marijuana and health. N Engl J Med 1982;306:603–605.

26. Johnston LD, O'Malley PM, Bachman JG. National Survey results on drug use from monitoring the future study, 1975–1994, vol. I. Washington, DC: US Department of Health and Human Services, 1995.

27. Webb E, Ashton CH, Kelly P, Kamali F. Alcohol and drug use in UK university students. Lancet 1996;348:922–925.

28. McRae AL, Budney AJ, Brady KT. Treatment of marijuana dependence: a review of the literature. J Subst Abuse Treat 2003;24:369–376.

29. Compton WM, Grant BF, Colliver JD, Glantz MD, Stinson FS. Prevalence of marijuana use disorders in the United States. 1991–1992 and 2001–2002. JAMA 2004;291:2114–2121.

30. US Department of Health and Human Services. Marijuana use and perceived risk of use among adolescents: 2002–2007. Available at http://oas.samhsa.gov/2k9/MJrisks/MJrisks.pdf. Accessed 2011 March 15.

31. Pitts JE, Neal JD, Gough TA. Some features of cannabis plants grown in the United Kingdom from seeds of known origin. J Pharm Pharmacol 1992;44:947–951.

32. Huber GL, First MW, Grubner O. Marijuana and tobacco smoke gas-phase cytotoxins. Pharmacol Biochem Behav 1991;40:629–636.

33. Wachtel SR, ElSohly MA, Ross SA, Ambre J, de Wit H. Comparison of the subjective effects of delta(9)-tetrahydrocannabinol and marijuana in humans. Psychopharmacology (Berl) 2002;161:331–339.

34. ElSohly MA, Ross SA, Mehmedic Z, Arafat R, Yi B, Banahan BF 3rd. Potency trends of delta-9-THC and other cannabinoids in confiscated marijuana from 1980–1997. J Forensic Sci 2000;45:24–30.

35. Stefanidou M, Athanaselis S, Alevisopoulos G, Papoutsis J, Koutselinis A. Δ^9-Tetrahydrocannabinol content in cannabis plants of Greek origin. Chem Pharm Bull 2000;48:743–745.

36. Poulsen HA, Sutherland GJ. The potency of cannabis in New Zealand from 1976–1996. Sci Justice 2000;40:171–176.

37. Pitts JE, O'Neil PJ, Leggo KP. Variation in the THC content of illicitly imported *Cannabis* products—1984–1989. J Pharm Pharmacol 1990;42:817–820.

38. Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, et al. Potency trends of Δ^9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. J Forensic Sci 2010;55:1209–1217.

39. Radwan MM, Ross SA, Slade D, Ahmed SA, Zulfiqar F, ElSohly MA. Isolation and characterization of new cannabis constituents from a high potency variety. Planta Med 208;74:267–272.

40. Radwan MM, ElSohly MA, Slade D, Ahmed SA, Khan IA, Ross SA. Biologically active cannabinoids from high-potency *Cannabis sativa*. J Nat Prod 2009;72:906–911.

41. Burgdorf JR, Kilmer B, Pacula RL. Heterogeneity in the composition of marijuana seized in California. Drug Alcohol Depend 2011;117:59–61.

42. Ross SA, Mehmedic Z, Murphy TP, El Sohly MA. CC-MS analysis of the total Δ^9-THC content of both drug- and fiber-type cannabis seeds. J Anal Toxicol 2000;24:715–717.

43. Churchill KT. Synthetic tetrahydrocannabinol. J Forensic Sci 1983;28:762–772.

44. Kurup VP, Resnick A, Kagen SL, Cohen SH, Fink JN. Allergenic fungi and actinomycetes in smoking materials and their health implications. Mycopathologia 1983;82:61–64.

45. ElSohly MA, deWit H, Wachtel SR, Feng S, Murphy TP. Δ^9-tetrahydrocannabivarin as a marker for the ingestion of marijuana versus Marinol®: results of a clinical study. J Anal Toxicol 2001;25:565–571.

46. Walsh D, Nelson KA, Mahmoud FA. Established and potential therapeutic applications of cannabinoids in oncology. Support Care Cancer 2003;11:137–43.

47. Kumar RN, Chambers WA, Pertwee RG. Pharmacological actions and therapeutic uses of cannabis and cannabinoids. Anaesthesia 2001;56:1059–1068.

48. Buggy DJ, Toogood L, Maric S, Sharpe P, Lambert DG, Rowbotham DJ. Lack of analgesic efficacy of oral δ-9-tetrahydrocannabinol in postoperative pain. Pain 2003;106:169–172.

49. Krishnan S, Cairns R, Howard R. Cannabinoids for the treatment of dementia. Cochrane Database Syst Rev 2009;(3):CD007204.

50. Fanciullo GJ. Medical cannabis. J Opioid Manag 2009;5:245–246.

51. Singer M, Clair S, Schensul J, Huebner C, Eiserman J, Pino R, Garcia J. Dust in the wind: the growing use of embalming fluid among youth in Hartford, CT. Subst Use Misuse 2005;40:1035–1050.

52. Peters RJ, Tortolero SR, Addy RC, Markiham C, Yacoubian GS, Escobar-Chaves SL. Drug use among Texas alternative school students: findings from Houston's Safer Choices 2 Program. J Psychoactive Drugs 2003;35:383–387.

53. Swift W, Hall W, Copeland J. One year follow-up of cannabis dependence among long-term users in Sydney, Australia. Drug Alcohol Depend 2000;59:309–318.

54. Panagis G, Vlachou S, Nomikos GG. Behavioral pharmacology of cannabinoids with a focus on preclinical models for studying reinforcing and dependence-producing properties. Curr Drug Abuse Rev 2008;1:350–374.

55. Perez-Reyes M. Marijuana smoking: factors that influence the bioavailability of tetrahydrocannabinol. NIDA Res Monogr 1990;99:42–61.

56. Polen MR, Sidney S, Tekawa IS, Sadler M, Friedman GD. Health care use by frequent marijuana smokers who do not smoke tobacco. West J Med 1993;158:596–601.

57. Wu TC, Tashkin DP, Djahed B, Rose JE. Pulmonary hazards of smoking marijuana as compared with tobacco. N Engl J Med 1988;318:347–351.

58. Hall W, Swift W. The THC content of cannabis in Australia: evidence and implications. Aust N Z J Pub Health 2000;24:503–508.

59. Labouvie EW. Personality and alcohol and marijuana use: patterns of convergence in young adulthood. Int J Addict 1990;25:237–252.

60. Lynskey MT, Heath AC, Bucholz KK, Slutske WS, Madden PAF, Nelson EC, et al. Escalation of drug use in early-onset cannabis users vs co-twin controls. JAMA 2003;289:427–433.

61. Martin BR. Cellular effects of cannabinoids. Pharmacol Rev 1986;38:45–74.

62. D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu Y-T, et al. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacology 2004;29:1558–1572.

63. Weinberg D, Lande A, Hilton N, Kerns DL. Intoxication from accidental marijuana ingestion. Pediatrics 1983;71:848–849.

64. Hart CL, Ward AS, Haney M, Comer SD, Foltin RW, Fischman MW. Comparison of smoked marijuana and oral Δ^9-tetrahdrocannabinol in humans. Psychopharmacology 2002;164:407–415.

65. Azorlosa JL, Greenwald MK, Stitzer ML. Marijuana smoking: effects of varying puff volume and breathhold duration. J Pharmacol Exp Ther 1995;272:560–569.

66. Ohlsson A, Lindgren JE, Wahlen A, Agurell S, Hollister LE, Gillespie HK. Single dose kinetics of deuterium labelled delta 1-tetrahydrocannabinol in heavy and light cannabis users. Biomed Mass Spectrom 1982;9:6–10.

67. Ohlsson A, Lindgren J-E, Wahlen A, Agurell S, Hollister LE, Gillespie HK. Plasma Δ^9-tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. Clin Pharmacol Ther 1980;28:409–416.

68. Moeller MR, Doerr G, Warth S. Simultaneous quantitation of delta-9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) in serum by GC /MS using deuterated internal standards and its application to a smoking study and forensic cases. J Forensic Sci 1992;37:969–983.

69. Perez-Reyes M, Di Guiseppi S, Davis KH, Schindler VH, Cook CE. Comparison of effects of marihuana cigarettes of three different potencies. Clin Pharmacol Ther 1982;31:617–624.

70. Thompson JP, Lam E, Thomas AM, Harry F, Hutchings AD, Marshall RW, Routledge PA. The pharmacokinetics and CNS effects of 5 and 10 mg of oral Δ^9-tetrahydrocannabinol in man. Br J Clin Pharmacol 2000;50:385–386.

71. Wall ME, Sadler BM, Brine P, Taylor H, Perez-Reyes M. Metabolism, disposition and kinetics of Δ^9-tetrahydrocannabinol in men and women. Clin Pharmacol Ther 1983;34:352–363.

72. Isbell H, Gorodetzsky CW, Jasinski D, Claussen U, von Spulak F, Korte F. Effects of (–(-Δ^9-trans-tetrahydrocannabinol in man. Psychopharmacologia 1967;11:184–188.

73. Lemberger L, Silberstein SD, Axelrod J, Kopin IJ. Marihuana: Studies on the deposition and metabolism of Δ^9-tetrahydrocannabinol in man. Science 1970;170: 1320–1322.

74. Hunt CA, Jones RT. Tolerance and deposition of tetrahydrocannabinol in man. J Pharmacol Exp Ther 1980;215:35–44.

75. Wahlqvist M, Nilsson IM, Sandberg F, Agurell S. Binding of delta-1-tetrahydrocannabinol to human plasma proteins. Biochem Pharmacol 1970;19:2579–2584.

76. Harvey DJ, Brown NK. comparative *in vitro* metabolism of the cannabinoids. Pharmacol Biochem Behavior 1991;40:533–540.

77. Yamamoto I, Watanabe K, Narimatsu S, Yoshimura H. Recent advances in the metabolism of cannabinoids. Int J Biochem Cell Biol 1995;27:741–746.

78. Sachse-Seeboth C, Pfeil J, Sehrt D, Meineke I, Tzvetkov M, Bruns E, et al. Interindividual variation in the pharmacokinetics of Δ^9-tetrahydrocannabinol as related to genetic polymorphisms in CYP2C9. Clin Pharmacol Ther 2009;85:273–276.

79. Lemberger L, Martz R, Rodda B, Forney R, Rowe H. Comparative pharmacology of Δ^9-tetrahydrocannabinol and its metabolite. 11-OH-Δ^9-Tetrahydrocannabinol. J Clin Invest 1973;52:2411–2417.

80. Hollister LE, Gillespie HK. Action of Δ^9-tetrahydrocannabinol. An approach to the active metabolite hypothesis. Clin Pharmacol Ther 1975;18:714–719.

81. Agurell S, Edward C, Halldin M, Leander K, Levy S, Lindgren J-E, Mechoulam R, Nordqvist M, Ohlsson A. Chemical synthesis and biological occurrence of carboxylic acid metabolites of $\Delta1(6)$-tetrahydrocannabinol. Drug Metab Dispos 1979;7:155–161.

82. Hawks RL. The constituents of cannabis and the disposition of cannabinoids. NIDA Res Monogr 1982;43: 125–137.

83. Chiang CN, Rapaka RS. Pharmacokinetics and disposition of cannabinoids. NIDA Res Monogr 1987;79: 173–188.

84. Hollister LE. Cannabidiol and cannabinol in man. Experientia 1973;29:825–826.

85. Harvey DJ, Mechoulam R. Metabolites of cannabidiol identified in human urine. Xenobiotica 1990;20:303–320.

86. Huestis MA, Henningfield JE, Cone EJ. Cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol 1992;16:276–282.

87. McBurney LJ, Bobbie BA, Sepp LA. GC/MS and EMIT analyses for Δ^9-tetrahydrocannabinol metabolites in plasma and urine of human subjects. J Anal Toxicol 1986;10:56–64.

88. Hunault CC, van Eijkeren JC, Mensinga TT, de Vries I, Leenders ME, Meulenbelt J. Disposition of smoked cannabis with high Δ^9-tetrahydrocannabinol content: a kinetic model. Toxicol Appl Pharmacol 2010;

89. Lemberger L. Tetrahydrocannabinol metabolism in man. Drug Metab Dispos 1973;1:461–468.

90. Lemberger L, Tamarkin NR, Axelrod J, Kopin IJ. Δ^9-Tetrahydrocannabinol: metabolism and disposition in long term marijuana smokers. Science 1971;178:72–74.

91. Johansson E, Halldin MM, Agurell S, Hollister LE, Gillespie HK. Terminal elimination plasma half-life of delta 1-tetrahydrocannabinol (delta 1-THC) in heavy users of marijuana. Eur J Clin Pharmacol 1989; 37:273–277.

92. Kelly P, Jones RT. Metabolism of tetrahydrocannabinol in frequent and infrequent marijuana users. J Anal Toxicol 1992;16:228–235.

93. Frytak S, Moertel CG, Rubin J. Metabolic studies of delta-9-tetrahydrocannabinol in cancer patients. Cancer Treat Rep 1984;68:1427–1431.

94. Huestis MA, Cone EJ. Urinary excretion half-life of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol in humans. Ther Drug Monit 1998;20:570–576.

95. Johansson EK, Hollister LE, Halldin MM. Urinary elimination half-life of delta-1-tetrahydrocannabinol-7-oic acid in heavy marijuana users after smoking. NIDA Res Monogr 1989;95:457–458.

96. Jones RT, Benowitz NL, Herning RI. Clinical relevance of cannabis tolerance and dependence. J Clin Pharmacol 1981;21(suppl 8–9):143S-152S.

97. Nowlan R, Cohen S. Tolerance to marijuana: Heart rate and subjective "high." Clin Pharmacol Ther 1977;22:550–556.

98. Perez-Reyes M, White WR, McDonald SA, Hicks RE, Jeffcoat AR, Cook CE. The pharmacologic effects of daily marijuana smoking in humans. Pharmacol Biochem Behavior 1991;40:691–694.

99. Bailey JR, Cunny HC, Paule MG, Slikker W Jr. Fetal disposition of delta 9-tetrahydrocannabinol (THC) during late pregnancy in the rhesus monkey. Toxicol Appl Pharmacol 1987;90:315–321.

100. Tennes K, Avitable N, Blackard C, Boyles C, Hassoun B, Holmes L, Kreye M. Marijuana: prenatal and postnatal exposure in the human. NIDA Res Monogr 1985;59:48–60.

101. Perez-Reyes M, Wall ME. Presence of Δ^9-tetrahydrocannabinol in human milk. N Engl J Med 1982;307:819–820.

102. Benowitz NL, Jones RT. Effects of Δ^9-tetrahydrocannabinol on drug distribution and metabolism. Antipyrine, pentobarbital and ethanol. Clin Pharmacol Ther 1977;22:259–268.

103. Chait LD, Perry JL. Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. Psychopharmacology 1994;115:340–349.

104. Benowitz NL, Nguyen TL, Jones RT, Herning RI, Bachman J. Metabolic and psychophysiologic studies of cannabidiol-hexobarbital interaction. Clin Pharmacol Ther 1980;28:115–120.

105. Foltin RW, Fischman MW, Pedroso JJ, Pearlson GD. Marijuana and cocaine interactions in humans: cardiovascular consequences. Pharmacol Biochem Behav 1987;28:459–464.

106. McLeod AL, McKenna CJ, Northridge DB. Myocardial infarction following the combined recreational use of Viagra and cannabis. Clin Cardiol 2002;25:133–134.

107. Wiley JL, Martin BR. Cannabinoid pharmacology: implications for additional cannabinoid receptor subtypes. Chem Phys Lipids 2002;121:57–63.

108. Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 1995;232:54–61.

109. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology.

110. Howlett AC, Blume LC, Dalton GD. CB(1) cannabinoid receptors and their associated proteins. Curr Med Chem 2010;17:1382–1393.

111. Sim-Selley LJ. Regulation of cannabinoid CB1 receptors in the central nervous system by chronic cannabinoids. Crit Rev Neurobiol 2003;15:91–119.

112. Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology 2004;47(Suppl 1):345S-358S.

113. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev 2010;62:588–631.

114. Ibrahim MM, Rude ML, Stagg NJ, Mata HP, Lai J, Vanderah TW, et al. CB2 cannabinoid receptor mediation of antinociception. Pain 2006;122:36–42.

115. Stiglick A, Kalant H. Behavioral effects of prolonged administration of delta 9-tetrahydrocannabinol in the rat. Psychopharmacology (Berl) 1983;80:325–330.

116. Stiglick A, Kalant H. Residual effects of chronic cannabis treatment on behavior in mature rats. Psychopharmacology (Berl) 1985;85:436–439.

117. Scallet AC. Neurotoxicology of cannabis and THC: a review of chronic exposure studies in animals. Pharmacol Biochem Behavior 1991;40:671–676.

118. Quickfall J, Crockford D. Brain neuroimaging in cannabis use: a review. J Neuropsychiatry Clin Neurosci 2006;18:318–332.

119. Randall MD, Kendall DA, O'Sullivan S. The complexities of the cardiovascular actions of cannabinoids. Br J Pharmacol 2004;142:20–26.

120. Randall MD, Kendall DA, O'Sullivan S. The complexities of the cardiovascular actions of cannabinoids. Br J Pharmacol 2004;142:20–26.

121. Renaud AM, Cormier Y. Acute effects of marihuana smoking on maximal exercise performance. Med Sci Sports Exerc 1986;18:685–689.

122. Gash A, Karliner JS, Janowsky D, Lake CR. Effects of smoking marihuana on left ventricular performance and plasma norepinephrine. Studies in normal men. Ann Intern Med 1978;89:448–452.

123. Vachon L, Fitzgerald MX, Solliday NH, Gould IA, Gaensler EA. Single dose effect of marihuana smoke. Bronchial dynamics and respiratory center sensitivity in normal subjects. N Engl J Med 1973;288:985–989.

124. Tashkin DP, Reiss S, Shapiro BJ, Calvarese B, Olsen JL, Lodge JW. Bronchial effects of aerosolized Δ^9-tetrahydrocannabinol in healthy and asthmatic subjects. Am Rev Respir Dis 1977;115:57–65.

125. Tashkin DP, Simmons MS, Chang P, Liu H, Coulson AH. Effects of smoked substance abuse on nonspecific airway

XXVII. Classification of cannabinoid receptors. Pharmacol Rev 2002;54:161–202.

hyperresponsiveness. Am Rev Respir Dis 1993;147: 97–103.

126. Hernandez MJ, Martinez F, Blair Ht, Miller WC. Airway response to inhaled histamine in asymptomatic long-term marijuana smokers. J Allergy Clin Immunol 1981;67:153–155.

127. Tashkin DP, Simmons M, Clark V. Effect of habitual smoking of marijuana alone and with tobacco on nonspecific airways hyperreactivity. J Psychoactive Drugs 1988;20:21–25.

128. Gong H Jr, Tashkin DP, Simmons MS, Calvarese B, Shapiro BJ. Acute and subacute bronchial effects of oral cannabinoids. Clin Pharmacol Ther 1984;35:26–32.

129. Barsky SH, Roth MD, Kleerup EC, Simmons M, Tashkin DP. Histopathologic and molecular alterations in bronchial epithelium in habitual smokers of marijuana, cocaine, and/or tobacco. J Natl Cancer Inst 1998;90: 1198–1205.

130. Gil E, Chen B, Kleerup E, Webber M, Tashkin DP. Acute and chronic effects of marijuana smoking on pulmonary alveolar permeability. Life Sci 1995;56:2193–2199.

131. Morris RR. Human pulmonary histopathological changes from marijuana smoking. J Forensic Sci 1985;30: 345–349.

132. Tashkin DP, Baldwin GC, Sarafian T, Dubinett S, Roth MD. Respiratory and immunologic consequences of marijuana smoking. J Clin Pharmacol 2002;42(suppl 11): 71S–81S.

133. Fligiel SE, Venkat H, Gong H Jr, Tashkin DP. Bronchial pathology in chronic marijuana smokers: a light and electron microscopic study. J Psychoactive Drugs 1988; 20:33–42.

134. Flach AJ. Delta-9-tetrahydrocannabinol (THC) in the treatment of end-stage open-angle glaucoma. Trans Am Ophthalmol Soc 2002;100:215–222.

135. Brown TT, Dobs AS. Endocrine effects of marijuana. J Clin Pharmacol 2002;42(Suppl 11):90S–96S.

136. Klein TW, Friedman H, Specter S. Marijuana, immunity and infection. J Neuroimmunol 1998;83:102–115.

137. Berdyshev EV. Cannabinoid receptors and the regulation of immune response. Chem Phys Lipids 2000;108: 169–190.

138. Friedman H, Newton C, Klein TW. Microbial infections, immunomodulation, and drugs of abuse. Clin Microbiol Rev 2003;16:209–219.

139. Kaplan BL, Rockwell CE, Kaminski NE. Evidence for cannabinoid receptor-dependent and -independent mechanisms of action in leukocytes. J Pharmacol Exp Ther 2003;306:1077–1085.

140. Chait LD, Fischman MW, Schuster CR. "Hangover" effects the morning after marijuana smoking. Drug Alcohol Depend 1985;15:229–238.

141. Chait LD. Subjective and behavioral effects of marijuana the morning after smoking. Psychopharmacology 1990;100:328–333.

142. Shapiro D. The ocular manifestations of the cannabinols. Ophthalmologica 1974;168:366–369.

143. Tennant FS Jr, Preble MR, Groesbeck CJ, Banks NI. Drug abuse among American soldiers in West Germany. Mil Med 1972;137:381–383.

144. McGlothlin WH, West LJ. The marihuana problem: an overview. Am J Psychiatry 1968;125:126–134.

145. Macleod J, Oakes R, Copello A, Crome I, Egger M, Hickman M, et al. Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. Lancet 2004;363:1579–1588.

146. Jessor R, Chase JA, Donovan JE. Psychosocial correlates of marijuana use and problem drinking in a national sample of adolescents. Am J Public Health 1980;70: 604–613.

147. Thomas H. Psychiatric symptoms in cannabis users. Br J Psychiatry 1993;163:141–149.

148. Linszen D, van Amelsvoort T. Cannabis and psychosis: an update on course and biological plausible mechanisms. Curr Opin Psychiatry 2007;20:116–120.

149. Chaudry HR, Moss HB, Bashir A, Suliman T. Cannabis psychosis following bhang ingestion. Br J Addict 1991; 86:1075–1081.

150. Hall W, Degenhardt L. Cannabis use and psychotic disorders: an update. Drug Alcohol Rev 2004;23:433–443.

151. Turner WM, Tsuang MT. Impact of substance abuse on the course and outcome of schizophrenia. Schizophr Bull 1990;16:87–95.

152. Katz G, Durst R, Shufman E, Bar-Hamburger R, Grunhaus L. Cannabis abuse and severity of psychotic and affective disorders in Israeli psychiatric inpatients. Compr Psychiatry 2010;51:37–41.

153. Dragt S, Nieman DH, Becker HE, van de Fliert R, Dingemans PM, de Haan L, et al. Age of onset of cannabis use is associated with age of onset of high-risk symptoms for psychosis. Can J Psychiatry 2010;55:165–171.

154. Conlon SR, McDonald GD. Cannabis use and non-clinical dimensions of psychosis in university students presenting to primary care. Acta Psychiatr Scand 2011;123:21–27.

155. Kuepper R, van Os J, Lieb R, Wittchen H-U, Hofler M, Henquet C. Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. BMJ 2011;342:d738.

156. Rottanburg D, Robins AH, Ben-Arie O, Teggin A, Elk R. Cannabis-associated psychosis with hypomanic features. Lancet 1982;2:1364–1366.

157. Hides L, Dawe S, Kavanagh DJ, Young RM. Psychotic symptom and cannabis relapse in recent-onset psychosis. Prospective study. Br J Psychiatry 2006;189:137–143.

158. Ghodse AH. Cannabis psychosis. Br J Addict 1986;81: 473–478.

159. Fergusson DM, Poulton R, Smith PF, Boden JM. Cannabis and psychosis. BMJ 2006;332:172–175.

160. Arseneault L, Cannon M, Witton J, Murray RM. Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry 2004;184:110–117.

161. Semple DM, McIntosh AM, Lawrie SM. Cannabis as a risk factor for psychosis: systematic review. J Psychopharmacology 2005;19:187–194.

162. Taylor DN, Wachsmuth IK, Shangkuan YH, Schmidt EV, Barrett TJ, Schrader JS, et al. Salmonellosis associated with marijuana: a multistate outbreak traced by plasmid fingerprinting. N Engl J Med 1982;306:1249–1253.

163. Walter FG, Bey TA, Ruschke DS, Benowitz NL. Marijuana and hyperthermia. Clin Toxicol 1996;34:217–221.

164. Freeman GL. Allergic skin test reactivity to marijuana in the Southwest. West J Med 1983;138:829–831.

165. Anibarro B, Fontela JL. Allergy to marihuana. Allergy 1996;51:200–201.

166. Donnino MW, Cocchi MN, Miller J, Fisher J. Cannabinoid hyperemesis: a case series. J Emerg Med 2011;40: e63–e66.

167. Allen JH, de Moore GM, Heddle R, Twartz JC. Cannabinoid hyperemesis: cyclical hyperemesis in association with chronic cannabis abuse. Gut 2004;53: 1566–1570.

168. Thomson WM, Poulton R, Broadbent JM, Moffitt TE, Caspi A, Beck JD, et al. Cannabis smoking and periodontal disease among young adults. JAMA 2008;299: 525–531.

169. Thompson DF, Carter JR. Drug-induced gynecomastia. Pharmacotherapy 1993;13:37–45.

170. Schydlower M. Breast masses in adolescents. Am Fam Physician 1982;25:141–145.

171. Wargo KA, Geveden BN, McConnell VJ. Cannabinoid-induced pancreatitis: a case series. JOP 2007;8:579–583.

172. Zuurman L, Ippel AE, Moin E, van Gerven JM. Biomarkers for the effects of cannabis and THC in healthy volunteers. Br J Clin Pharmacol 2008;67:5–21.

173. Charles R, Holt S, Kirkham N. Myocardial infarction and marijuana. Clin Toxicol 1979;14:433–438.

174. Mittleman MA, Lewis RA, Maclure M, Sherwood JB, Muller JE. Triggering myocardial infarction by marijuana. Circulation 2001;103:2805–2809.

175. Fehr KO, Kalant A. Analysis of cannabis smoke obtained under different combustion conditions. Can J Physiol Pharmacol 1972;50:761–767.

176. Sherrill DL, Krzyzanowski M, Bloom JW, Lebowitz MD. Respiratory effects of non-tobacco cigarettes: a longitudinal study in general population. Int J Epidemiol 1991;20:132–137.

177. Tashkin DP, Coulson AH, Clark VA, Simmons M, Bourque LB, Duann S, et al. Respiratory symptoms and lung function in habitual heavy smokers of marijuana alone, smokers of marijuana and tobacco, smokers of tobacco alone, and nonsmokers. Am Rev Respir Dis 1987;135:209–216.

178. Hancox RJ, Poulton R, Ely M, Welch D, Taylor DR, McLachlan CR, et al. Effects of cannabis on lung function: a population-based cohort study. Eur Respir J 2010;35:42–47.

179. Bloom JW, Kaltenborn WT, Paoletti P, Camilli A, Lebowitz MD. Respiratory effects of non-tobacco cigarettes. Br Med J 1987;295:1516–1518.

180. Tashkin DP, Calvarese BM, Simmons MS, Shapiro BJ. Respiratory status of seventy-four habitual marijuana smokers. Chest 1980;78:699–706.

181. Taylor DR, Fergusson DM, Milne BJ, Horwood LJ, Moffitt TE, Sears MR, Poulton R. A longitudinal study of the effects of tobacco and cannabis exposure on lung function in young adults. Addiction 2002;97:1055–1061.

182. Tilles DS, Goldenheim PD, Johnson DC, Mendelson JH, Mello NK, Hales CA. Marijuana smoking as cause of reduction in single-breath carbon monoxide diffusing capacity. Am J Med 1986;80:601–606.

183. Taylor DR, Hall W. Respiratory health effects of cannabis: position statement of the Thoracic Society of Australia and New Zealand. Intern Med J 2003;33:310–313.

184. Johnson MK, Smith RP, Morrison D, Laszlo G, White RJ. Large lung bullae in marijuana smokers. Thorax 2000;55:340–342.

185. Aldington S, Williams M, Nowitz M, Weatherall M, Pritchard A, McNaughton A, et al. Effects of cannabis on pulmonary structure, function and symptoms. Thorax 2007;62:1058–1063.

186. Mims RB, Lee JH. Adverse effects of intravenous cannabis tea. J Natl Med Assoc 1977;69:491–495.

187. Vaziri ND, Thomas R, Sterling M, Seiff K, Pahl MV, Davila J, Wilson A. Toxicity with intravenous injection of crude marijuana extract. Clin Toxicol 1981;18:353–366.

188. Farber SJ, Huertas VE. Intravenously injected marihuana syndrome. Arch Intern Med 1976;136:337–339.

189. Dassel PM, Punjabi E. Ingested marihuana-filled balloons. Gastroenterology 1979;76:166–169.

190. Vowels M, Harvey PM. Ingestion of hashish oil-filled condoms. Med J Aust 1980;2:509–510.

191. Tewari SN, Sharma JD. Detection of Δ^9-tetrahydrocannabinol in the organs of a suspected case of cannabis poisoning. Toxicol Lett 1980;5:279–281.

192. Gupta BD, Jani CB, Shah PH. Fatal "bhang" poisoning. Med Sci Law 2001;41:349–352.

193. Anthony JC, Warner LA, Kessler RC. Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: basic findings from the National Comorbidity Survey. Exp Clin Psychopharmacol 1994;2:244–268.

194. Vandrey R, Budney AJ, Kamon JL, Stanger C. Cannabis withdrawal in adolescent treatment seekers. Drug Alcohol Depend 2005:78:205–210.

195. Copersino ML, Boyd SJ, Tashkin DP, Huestis MA, Heishman SJ, Dermand JC, et al. Sociodemographic characteristics of cannabis smokers and the experience of cannabis withdrawal. Am J Drug Alcohol Abuse 2010;36:311–319.

196. Hasin DS, Keyes KM, Alderson D, Wang S, Aharonovich E, Grant BF. Cannabis withdrawal in the United States: results from NESARC. J Clin Psychiatry 2008;69: 1354–1363.

197. Budney AJ, Moore BA, Vandrey RG, Hughes JR. The time course and significance of cannabis withdrawal. J Abnormal Psychol 2003;112:393–402.

198. Budney AJ, Hughes JR, Moore BA, Vandrey R. Review of the validity and significance of cannabis withdrawal syndrome. Am J Psychiatry 2004;161:1967–1977.

199. Haney M, Ward AS, Comer SD, Foltin RW, Fischman MW. Abstinence symptoms following smoked marijuana in humans. Psychopharmacology (Berl) 1999;141: 395–404.

200. Kouri EM, Pope HG Jr. Abstinence symptoms during withdrawal from chronic marijuana use. Exp Clin Psychopharmacol 2000;8:483–492.

201. Qazi QH, Mariano E, Milman DH, Beller E, Crombleholme W. Abnormalities in offspring associated with prenatal marihuana exposure. Dev Pharmacol Ther 1985;8:141–148.

202. Hingson R, Alpert JJ, Day N, Dooling E, Kayne H, Morelock S, Oppenheimer E, Zuckerman B. Effects of maternal drinking and marijuana use on fetal growth and development. Pediatrics 1982;70:539–546.

203. Wengen DF. [Marijuana and malignant tumors of the upper aerodigestive tract in young patients. On the risk assessment of marijuana]. Laryngorhinootologie 1993;72:264–267. [German]

204. Zhang ZF, Morgenstern H, Spitz MR, Tashkin DP, Yu GP, Marshall JR, Hsu TC, Schantz SP. Marijuana use and increased risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol Biomarkers Prev 1999;8: 1071–1078.

205. Aldington S, Harwood M, Cox B, Weatherall M, Beckert L, Hansell A, et al. Cannabis use and cancer of the head and neck: case-control study. Otolaryngol Head Neck Surg 2008;138:374–380.

206. Rosenblatt KA, Daling JR, Chen C, Sherman KJ, Schwartz SM. Marijuana use and risk of oral squamous cell carcinoma. Cancer Res 2004;64:4049–4054.

207. Sidney S, Beck JE, Tekawa IS, Quesenberry CP, Friedman GD. Marijuana use and mortality. Am J Public Health 1997;87:585–590.

208. Andreasson S, Allebeck P. Cannabis and mortality among young men: a longitudinal study of Swedish conscripts. Scand J Soc Med 1990;18:9–15.

209. Berthiller J, Lee YC, Boffetta P, Wei Q, Sturgis EM, Greenland S, Morgenstern H, et al. Marijuana smoking and the risk of head and neck cancer: pooled analysis in the INHANCE consortium. Cancer Epidemiol Biomarkers Prev 2009;18:1544–1551.

210. Goodall CR, Basteyns BJ. A reliable method for the detection, confirmation, and quantitation of cannabinoids in blood. J Anal Toxicol 1995;19:419–426.

211. Elsohly MA, Jones AB, Elsohly HN. Cross-reactivity of selected compounds in the Abbott TDx® cannabinoid assay. J Anal Toxicol 1990;14:277–279.

212. Weaver ML, Gan BK, Allen E, Baugh LD, Liao FY, Liu RH, et al. Correlations on radioimmunoassay, fluorescence polarization immunoassay, and enzyme immunoassay of cannabis metabolites with gas chromatography/mass spectrometry analysis of 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid in urine specimens. Forensic Sci Int 1991;29:43–56.

213. Kintz P, Machart D, Jamey C, Mangin P. Comparison between GC-MS and the EMIT II, Abbott TDx, and Roche Online immunoassays for the determination of THCCOOH. J Anal Toxicol 1995;19:304–306.

214. Foltz RL, Sunshine I. Comparison of a TLC method with EMIT and GC/MS for detection of cannabinoids in urine. J Anal Toxicol 1990;14:275–278.

215. King DL, Gabor MJ, Martel PA, O'Donnell CM. A rapid sample-preparation technique for thin-layer chromatographic analysis for 11-nor-delta 9-tetrahydrocannabinol-9-carboxylic acid in human urine. Clin Chem 1989; 35:163–166.

216. Bell R, Taylor EH, Ackerman B, Pappas AA. Interpretation of urine quantitative 11-nor-delta-9 tetrahydrocannabinol-9-carboxylic acid to determine abstinence from marijuana smoking. Clin Toxicol 1989;27:109–115.

217. Bronner W, Nyman P, von Minden D. Detectability of phencyclidine and 11-nor-delta 9-tetrahydrocannabinol-9-carboxylic acid in adulterated urine by radioimmunoassay and fluorescence polarization immunoassay. J Anal Toxicol 1990;14:368–371.

218. Schwarzhoff R, Cody JT. The effects of adulterating agents on FPIA analysis of urine for drugs of abuse. J Anal Toxicol 1993;17:14–17.

219. Paul BD, Jacobs A. Effects of oxidizing adulterants on detection of 11-nor-Δ^9-THC-carboxylic acid in urine. J Anal Toxicol 2002;26:460–463.

220. Frazee CC 3rd, Kiscoan M, Garg U. Quantitation of total 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in urine and blood using gas chromatography-mass spectrometry (GC-MS). Methods Mol Biol 2010;603:137–144.

221. Garrett ER, Hunt CA. Separation and analysis of Δ^9-tetrahydrocannabinol in biological fluids by high pressure liquid chromatography and GLC. J Pharm Sci 1977;66:20–26.

222. Nakahara Y, Sekine H. Studies on confirmation of cannabis use. I. Determination of the cannabinoid contents in marijuana cigarettes, tar and ash using high performance liquid chromatography with electrochemical detection. J Anal Toxicol 1985;9:121–124.

223. Kemp PM, Abukhalaf IK, Manno JE, Manno BR, Alford DD, Abusada GA. Cannabinoids in humans. I. Analysis of Δ^9-tetrahydrocannabinol and six metabolites in plasma and urine using GC-MS. J Anal Toxicol 1995; 19:285–291.

224. Altunkaya D, Clatworthy AJ, Smith RN, Start IJ. Urinary cannabinoid analysis: comparison of four immunoassays

with gas chromatography-mass spectrometry. Forensic Sci Int 1991;50:15–22.

225. Thomas A, Widmer C, Hopfgartner G, Staub C. Fast gas chromatography and negative-ion chemical ionization tandem mass spectrometry for forensic analysis of cannabinoids in whole blood. J Pharm Biomed Anal 2007; 45:496–503.

226. Coulter C, Miller E, Crompton K, Moore C. Tetrahydrocannabinol and two of its metabolites in whole blood using liquid chromatography-tandem mass spectrometry. J Anal Toxicol 2008;32:653–658.

227. Del Mar Ramirez Fernandez M, De Boeck G, Wood M, Lopez-Rivadulla M, Samyn N. Simultaneous analysis of THC and its metabolites in blood using liquid chromatography-tandem mass spectrometry. J Chromatogr B 2008;875:465–470.

228. Villamor JL, Bermejo AM, Tabernero MJ, Fernandez P. Determination of cannabinoids in human hair by GC/MS. Anal Lett 2004;37:517–528.

229. Teixeira H, Proenca P, Castanheira A, Santos S, Lopez-Rivadulla M, Corte-Real F, et al: Cannabis and driving: the use of LC-MS to detect Δ^9-tetrahydrocannabinol (Δ^9-THC) in oral fluid samples. Forensic Sci Int 2004;146 (suppl): 61S–63S.

230. Wall ME, Perez-Reyes M. The metabolism of delta-9-tetrahydrocannabinol and related cannabinoids in man. J Clin Pharmacol 1981:21(suppl 8–9):178S–189S.

231. Kemp PM, Abukhalaf IK, Manno JE, Manno BR, Alford DD, McWilliams ME, et al. Cannabinoids in humans. II. The influence of three methods of hydrolysis on the concentration of THC and two metabolites in urine. J Anal Toxicol 1995;19:292–298.

232. Christophersen AS. Tetrahydrocannabinol stability in whole blood: Plastic versus glass containers. J Anal Toxicol 1986;10:129–131.

233. Johnson JR, Jennison TA, Peat MA, Foltz RL. Stability of delta 9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC in blood and plasma. J Anal Toxicol 1984;8:202–204.

234. Skopp G, Potsch L, Mauden M, Richter B. Partition coefficient, blood to plasma ratio, protein binding and short-term stability of 11-nor-Δ^9-carboxy tetrahydrocannabinol glucuronide. Forensic Sci Int 2002;126:17–23.

235. Garrett ER, Hunt CA. Physicochemical properties, solubility, and protein binding of Δ^9-tetrahydrocannabinol. J Pharm Sci 1974;63:1056–1064.

236. Joern WA. Surface adsorption of the urinary marijuana carboxy metabolite: the problem and a partial solution. J Anal Toxicol 1992;16:401.

237. Dugan S, Bogema S, Schwartz RW, Lappas NT. Stability of drugs of abuse in urine samples stored at –20ºC. J Anal Toxicol 1994;18:391–396.

238. Roth KDW, Siegel NA, Johnson RW Jr, Litauszki L, Salvati L, Harrington CA, Wray LK. Investigation of the effects of solution composition and container material type on the loss of 11-nor-Δ^9-THC-9-carboxylic acid. J Anal Toxicol 1996;20:291–301.

239. Fraga SG, Diaz-flores Estevez JF, Diaz Romero C. Stability of cannabinoids in urine in three storage temperatures. Ann Clin Lab Sci 1998;29:160–162.

240. Paul BD, McKinley RM, Walsh JK Jr, Jamir TS, Past MR. Effect of freezing on the concentration of drugs of abuse in urine. J Anal Toxicol 1993;17:378–380.

241. Jenkins AJ, Oblock J. Phencyclidine and cannabinoids in vitreous humor. Leg Med 2008;10:201–203.

242. Lin D-L, Lin R-L. Distribution of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol in traffic fatality cases. J Anal Toxicol 2005;29:58–61.

243. Widman M, Agurell S, Ehrnebo M, Jones G. Binding of (+)- and (minus(-Δ^1-tetrahydrocannabinols and (1)-7-hydroxy-Δ^1-tetrahydrocannabinol to blood cells and proteins in man. J Pharm Pharmacol 1974;26: 914–916.

244. Giroud C, Menetrey A, Augsburger M, Buclin T, Sanchez-Mazas P, Mangin P. Δ^9-THC, 11-OH- Δ^9 –THC and Δ^9-THC-COOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people. Forensic Sci Int 2001;123: 159–164.

245. Cocchetto DM, Owens SM, Perez-Reyes M, DiGuiseppi S, Miller LL. Relationship between plasma delta-9-tetrahydrocannabinol concentration and pharmacologic effects in man. Psychopharmacology 1981;75:158–164.

246. Harder S, Rietbrock S. Concentration-effect relationship of Δ-9-tetrahydrocannabiol and prediction of psychotropic effects after smoking marijuana. Int J Clin Pharmacol Toxicol 1997;35:155–159.

247. Hunault CC, Mensinga TT, de Vries I, Kelholt-Dijkman HH, Hoek J, Kruidenier M, Leenders ME, Meulenbelt J. Delta-9-tetrahydrocannabinol (THC) serum concentrations and pharmacological effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg THC. Psychopharmacology (Berl) 2008;201: 171–181.

248. Mendelson JH, Mello NK, Teoh SK, Lex BW, Lukas SE, Ellingboe J. Plasma delta-9-THC levels as a predictive measure of marijuana use by women. NIDA Res Monogr 1989;95:152–158.

249. MacInnes DC, Miller KM. Fatal coronary artery thrombosis associated with cannabis smoking. J R Coll Gen Pract 1984;34:575–576.

250. Bachs L, Morland H. Acute cardiovascular fatalities following cannabis use. Forensic Sci Int 2001;124:200–203.

251. Karschner EL, Schwilke EW, Lowe RH, Darwin WD, Herning RI, Cadet JL, Huestis MA. Implications of plasma Δ^9-tetrahydrocannabinol, 11-hydroxy-THC, and 11-nor-9-carboxy-THC concentrations in chronic cannabis smokers. J Anal Toxicol 2009;33:469–477.

252. Garriott JC, Di Maio VJ, Rodriguez RG. Detection of cannabinoids in homicide victims and motor vehicle fatalities. J Forensic Sci 1986;31:1274–1282.

253. Moody DE, Monti KM, Crouch DJ. Analysis of forensic specimens for cannabinoids. II. Relationship between blood Δ^9-tetraydrocannabinol and blood and urine 11-nor-Δ^9-tetrahydrocannabiol-9-carboxylic acid concentrations. J Anal Toxicol 1992;16;302–306.

254. Law B, Mason PA, Moffat AC, Gleadle RI, King LJ. Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of cannabis resin. J Pharm Pharmacol 1984;36:289–294.

255. Teware SN, Sharma JD. Detection of delta-9-tetrahydrocannabinol in the organs of a suspected case of cannabis poisoning. Toxicol Lett 1980;5:279–281.

256. Collins M, Easson J, Hansen G, Hodda A, Lewis K. GC-MS-MS confirmation of unusually high Δ^9-tetrahydrocannabinol levels in two postmortem blood samples. J Anal Toxicol 1997;21:538–542.

257. Brunk SD. False negative GC/MS assay for carboxy THC due to ibuprofen interference. J Anal Toxicol 1988;12:290–291.

258. Jaffee WB, Trucco E, Levy S, Weiss RD. Is this urine really negative? A systematic review of tampering methods in urine drug screening and testing. J Subst Abuse Treat 2007;33:33–42.

259. Vandevenne M, Vandenbussche H, Verstraete A. Detection time of drugs of abuse in urine. Acta Clinica Belg 2000;55:323–333.

260. Manno JE, Manno BR, Kemp PM, Alford DD, Abukhalaf IK, McWilliams ME, et al. Temporal indication of marijuana use can be estimated from plasma and urine concentrations of delta9-tetrahydrocannabinol, 11-hydroxy-delta-9-tetrahydrocannabinol, and 11-nor-delta9-tetrahydrocannabinol-9-carboxylic acid. J Anal Toxicol 2001; 25:538–549.

261. Mandatory guidelines for federal workplace drug testing programs. Fed Regist 1994;59:29908–29931.

262. Huestis MA, Mitchell JM, Cone EJ. Detection times of marijuana metabolites in urine by immunoassay and GC-MS. J Anal Toxicol 1995;19:443–449.

263. Cone EJ, Johnson RE, Paul BD, Mell LD, Mitchell J. Marijuana-laced brownies: behavioral effects, physiologic effects, and urinalysis in humans following ingestion. J Anal Toxicol 1988;12:169–175.

264. Lehmann T, Sager F, Brenneisen R. Excretion of cannabinoids in urine after ingestion of cannabis seed oil. J Anal Toxicol 1997;21:373–375.

265. Johansson E, Halldin MM. Urinary excretion half-life of Δ^1-tetrahydrocannabinol-7-oic acid in heavy marijuana users after smoking. J Anal Toxicol 1989;13:218–223.

266. Fraser AD, Worth D. Urinary excretion profiles of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol study III. A Δ^9-THC-COOH to creatinine ratio study. Forensic Sci Int 2003; 137:196–202.

267. Huestis MA, Cone EJ. Differentiating new marijuana use from residual drug excretion in occasional marijuana users. J Anal Toxicol 1998;22:445–454.

268. Fraser AD, Worth D. Urinary excretion profiles of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol: a Δ^9-THC-COOH to creatinine ratio study #2. Forensic Sci Int 2003;133: 26–31.

269. Schwilke EW, Gullberg RG, Darwin WD, Chiang CN, Cadet JL, Gorelick DA, et al. Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users. Addiction 2011;106: 499–506.

270. Fraser AD, Worth D. Monitoring urinary excretion of cannabinoids by fluorescence-polarization immunoassay: a cannabinoid-to-creatinine ratio study. Ther Drug Monit 2002;24:746–750.

271. Ellis BM Jr, Mann MA, Judson BA, Schramm NT, Tashchian A. Excretion patterns of cannabinoid metabolites after last use in a group of chronic users. Clin Pharmacol Ther 1985;38:572–578.

272. Joseph R, Dickerson S, Willis R, Frankenfield D, Cone EJ, Smith DR. Interference by nonsteroidal anti-inflammatory drugs in EMIT and TDx assays for drugs of abuse. J Anal Toxicol 1995;19:13–17.

273. Rossi S, Yaksh T, Bentley H, van den Brande G, Grant I, Ellis R. Characterization of interference with 6 commercial delta9-tetrahydrocannabinol immunoassays by efavirenz (glucuronide) in urine. Clin Chem 2006;52: 896–897.

274. Röhrich J, Schimmel I, Zörntlein S, Becker J, Drobnik S, Kaufmann T, et al. Concentrations of delta9-tetrahydrocannabinol and 11-nor-9-carboxytetrahydrocannabinol in blood and urine after passive exposure to cannabis smoke in a coffee shop. J Anal Toxicol 2010;34:196–203.

275. Cone EJ, Johnson RE. Contact highs and urinary cannabinoid excretion after passive exposure to marijuana smoke. Clin Pharmacol Ther 1986;40:247–256.

276. Hayden JW. Passive inhalation of marijuana smoke: a critical review. J Subst Abuse 1991;3:85–90.

277. Morland J, Bugge A, Shuterud B, Steen A, Wethe GH, Kjeldsen T. Cannabinoids in blood and urine after passive inhalation of cannabis smoke. J Forensic Sci 1985;30: 997–1002.

278. Perez-Reyes M, Di Guiseppi S, Mason AP, Davis KH. Passive inhalation of marijuana smoke and urinary excretion of cannabinoids. Clin Pharmacol Ther 1983; 34:36–41.

279. Mule SJ, Lomax P, Gross SJ. Active and realistic passive marijuana exposure tested by three immunoassays and GC/MS in urine. J Anal Toxicol 1988;12:113–116.

280. Struempler RE, Nelson G, Urry FM. A positive cannabinoids workplace drug test following the ingestion of commercially available hemp seed oil. J Anal Toxicol 1997;21:283–285.

281. Leson G, Pless P, Grotenhermen F, Kalant H, El Sohly MA. Evaluating the impact of hemp food consumption on workplace drug tests. J Anal Toxicol 2001;25: 691–698.

282. Fortner N, Fogerson R, Lindman D, Iversen T, Armbruster D. Marijuana-positive urine test results from consumption of hemp seeds in food products. J Anal Toxicol 1997;21:476–481.

283. Steinagle CG, Upfal M. Concentration of marijuana metabolites in the urine after ingestion of hemp seed tea. J Occup Environ Med 1999;41:510–513.

284. Gustafson RA, Levine B, Stout PR, Klette KL, George MP, Moolchan ET, Huestis MA. Urinary cannabinoid detection times after controlled oral administration of Δ^9-tetrahydrocannabinol to humans. Clin Chem 2003;49:1114–1124.

285. Nakahara Y, Takahashi K, Kikura R. Hair analysis for drugs of abuse. X. Effect of physicochemical properties of drugs on the incorporation rates into hair. Biol Pharm Bull 1995;18:1223–1227.

286. Musshoff F, Junker HP, Lachenmeier DW, Kroener L, Madea B. Fully automated determination of cannabinoids in hair samples using headspace solid-phase microextraction and gas chromatography-mass spectrometry. J Anal Toxicol 2002;26:554–560.

287. Kintz P, Ciremele V, Mangin P. Testing human hair for cannabis II. Identification of THC-COOH by GC-MS-NCI as a unique proof. J Forensic Sci 1995;40:619–622.

288. Wilkins D, Haughey H, Cone E, Huestis M, Foltz R, Rollins D. Quantitative analysis of THC, 11-OH-THC, and THC-COOH in human hair by negative ion chemical ionization mass spectrometry. J Anal Toxicol 1996;19:483–491.

289. Thorspecken J, Skopp G, Pötsch L. In vitro contamination of hair by marijuana smoke. Clin Chem 2004;50:596–602.

290. Skopp G, Pötsch L, Mauden M. Stability of cannabinoids in hair samples exposed to sunlight. Clin Chem 2000;46:1846–1848.

291. Mieczkowski T. Assessing the potential of a "color effect" for hair analysis of 11-nor-9-carboxy-delta(9)-tetrahydrocannabinol: analysis of a large sample of hair specimens. Life Sci 2003;74:463–469.

292. Skopp G, Strohbeck-Kuehner P, Mann K, Hermann D. Deposition of cannabinoids in hair after long-term use of cannabis. Forensic Sci Int 2007;170:46–50.

293. Staub C. Chromatographic procedures for determination of cannabinoids in biological samples, with special attention to blood and alternative matrices like hair, saliva, sweat and meconium. J Chromatogr B Biomed Sci Appl 1999;733:119–126.

294. Saito T, Wtsadik A, Scheidweiler KB, Fortner N, Takeichi S, Huestis MA. Validated gas chromatographic-negative ion chemical ionization mass spectrometric method for delta(9)-tetrahydrocannabinol in sweat patches. Clin Chem 2004;50:2083–2090.

295. Kintz P, Cirimele V, Ludes B. Detection of cannabis in oral fluid (saliva) and forehead wipes (sweat) from impaired drivers. J Anal Toxicol 2000;24:557–561.

296. Niedbala RS, Kardos KW, Fritch DF, Kardos S, Fries T, Waga J, et al. Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. J Anal Toxicol 2001;25:289–303.

297. Payne RJ, Brand SN. The toxicity of intravenously used marihuana. JAMA 1975;233:351–354.

298. Brandenburg D, Wernick R. Intravenous marijuana syndrome. West J Med 1986;145:94–96.

299. Akins D, Awdeh MR. Marijuana and second degree A-V block. South Med J 1981;74:371–373.

300. Rezkalla SH, Sharma P, Kloner RA. Coronary no-flow and ventricular tachycardia associated with habitual marijuana use. Ann Emerg Med 2003;42:365–369.

301. Fisher BA, Ghran A, Vadamalai V, Antonios TF. Cardiovascular complications induced by cannabis smoking: a case report and review of the literature. Emerg Med J 2005;22:679–680.

302. Daccarett M, Freih M, Machado C. Acute cannabis intoxication mimicking Brugada-like ST segment abnormalities. Int J Cardiol 2007;119:235–236.

303. Tzilos GK, Cintron CB, Wood JB, Simpson NS, Young AD, Pope HG Jr, et al. Lack of hippocampal volume changes in long-term heavy cannabis users. Am J Addict 2005;14:64–72.

304. Block RI, O'Leary DS, Ehrhardt JC, Augustinack JC, Ghoneim MM, Arndt S, Hall JA. Effects of frequent marijuana use on brain tissue volume and composition. Neuroreport 2000;11:491–496.

305. Hannerz J, Hindmarsh T. Neurological and neuroradiological examination of chronic cannabis smokers. Ann Neurol 1983;13:207–210.

306. Kuehnle J, Mendelson JH, Davis KR, New PF. Computed tomographic examination of heavy marijuana smokers. JAMA 1977;237:1231–1232

307. Pillay SS, Rogowska J, Kanayama G, Jon D-I, Gruber S, Simpson N, et al. Neurophysiology of motor function following cannabis discontinuation in chronic cannabis smokers: an fMRI study. Drug Alcohol Depend 2004;76:261–271.

308. Pope HG Jr, Yurgelun-Todd D. The residual cognitive effects of heavy marijuana use in college students. JAMA 1996;275:521–527.

309. Solowij N, Michie PT, Fox AM. Differential impairments of selective attention due to frequency and duration of cannabis use. Biol Psychiatry 1995;37:731–739.

310. Schwartz RH, Gruenewald PJ, Klitzner M, Fedio P. Short-term memory impairment in cannabis-dependent adolescents. Am J Dis Child 1989;143:1214–1219.

311. Pope HG Jr. Gruber AJ, Hudson JI, Huestis MA, Yurgelun-Todd D. Neuropsychological performance in long-term cannabis users. Arch Gen Psychiatry 2001;58:909–915.

312. Hunault CC, Mensinga TT, Böcker KB, Schipper CM, Kruidenier M, Leenders ME, et al. Cognitive and psychomotor effects in males after smoking a combination of

tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). Psychopharmacology (Berl) 2009;204:85–94.

313. Hart CL, Ilan AB, Gevins A, Gunderson EW, Role K, Colley J, Foltin RW. Neurophysiological and cognitive effects of smoked marijuana in frequent users. Pharmacol Biochem Behav 2010;333–341.

314. Lane SD, Cherek DR, Tcheremissine OV, Lieving LM, Pietras CJ. Acute marijuana effects on human risk taking. Neuropsychopharmacology 2005;30:800–809.

315. Ramaekers JG, Kauert G, van Ruitenbeek P, Theunissen EL, Schneider E, Moeller MR. High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology 2006;31:2296–2303.

316. Hart CL, van Gorp W, Haney M, Foltin RW, Fischman MW. Effects of acute smoked marijuana on complex cognitive performance. Neuropsychopharmacology 2001;25:757–765.

317. Ramaekers JG, Kauert G, Theunissen EL, Toennes SW, Moeller MR. Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. J Psychopharmacol 2009;23:266–277.

318. Iversen L. Long-term effects of exposure to cannabis. Curr Opin Pharmacol 2005;5:69–72.

319. Jager G, Kahn RS, van den Brink W, van Ree JM, Ramsey NF. Long-term effects of frequent cannabis use on working memory and attention: an fMRI study. Psychopharmacology 2006;185:358–368.

320. Pope HG Jr, Yurgelun-Todd D. The residual cognitive effects of heavy marijuana use in college students. JAMA 1996;275:521–527.

321. Hanson KL, Winward JL, Schweinsburg AD, Medina KL, Brown SA, Tapert SF. Longitudinal study of cognition among adolescent marijuana users over three weeks of abstinence. Addict Behav 2010;35:970–976.

322. Fried PA, Watkinson B, Gray R. Neurocognitive consequences of marihuana—a comparison with pre-drug performance. Neurotoxicol Teratol 2005;27:321–239.

323. Schaeffer J, Andrysiak T, Ungerleider JT. Cognition and long-term use of ganja (cannabis). Science 1981;213:465–466.

324. Solowij N, Stephens RS, Roffman RA, Babor T, Kadden R, Miller M, et al. Marijuana Treatment Project Research Group: Cognitive functioning of long-term heavy cannabis users seeking treatment. JAMA 2002;287:1123–1131.

325. Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL. Dose-related neurocognitive effects of marijuana use. Neurology 2002;59:1337–1343.

326. Pope GH Jr. Gruber AJ, Hudson JI, Huestis MA, Yurgelun-Tood D. Neuropsychological performance in long-term cannabis users. Arch Gen Psychiatry 2001;58:909–915.

327. Gieringer DH. Marijuana, driving, and accident safety. J Psychoactive Drugs 1988;20:93–101.

328. Bates MN, Blakely TA. Role of cannabis in motor vehicle crashes. Epidemiol Rev 1999;21:222–232.

329. Sewell RA, Poling J, Sofuoglu M. The effect of cannabis compared with alcohol on driving. Am J Addict 2009;18:185–193.

330. Hollister LE, Gillespie HK, Ohlsson A, Lindgren JE, Wahlen A, Agurell S. Do plasma concentrations of delta-9-THC reflect the degree of intoxication? J Clin Pharmacol 1981;21(Suppl 8–9):171S–177S.

331. Longo MC, Hunter CE, Lokan RJ, White MJ, White MA. The prevalence of alcohol, cannabinoids, benzodiazepines and stimulants amongst injured drivers and their role in driver culpability. Part II: The relationship between drug prevalence and drug concentrations, and driver culpability. Accid Anal Prev 2000;32:623–632.

332. Klonoff H. Marijuana and driving in real life situations. Science 1974;186:317–324.

333. Azorlosa JL, Heishman SJ, Stitzer ML, Mahaffey JM. Marijuana smoking: effect of varying Δ^9-tetrahydrocannabinol content and number of puffs. J Pharmacol Exp Ther 1992;261:114–122.

334. Heishman SJ, Arasteh K, Stitzer ML. Comparative effects of alcohol and marijuana on mood, memory, and performance. Pharmacol Biochem Behav 1997;58:93–101.

335. Huestis MA, Sampson AH, Holicky BJ, Henningfield JE, Cone EJ. Characterization of the absorption phase of marijuana smoking. Clin Pharmacol Ther 1992;52:31–41.

336. Huestis MA. Cannabis (marijuana)—effects on human behavior and performance. Forensic Sci Rev 2002;14:18–60.

337. Barnett G, Licko V, Thompson T. Behavioral pharmacokinetics of marijuana. Psychopharmacology 1985;85:51–56.

338. Adams AJ, Brown B, Flom MC, Jones RT, Jampolsky A. Alcohol and marijuana effects on static visual acuity. Am J Optom Physiol Opt 1975;52:729–735.

339. Moskowitz H, Ziedman K, Sharma S. Visual search behavior while viewing driving scenes under the influence of alcohol and marihuana. Hum Factors 1976;18:417–432.

340. Green K. Marijuana and the eye—a review. J Toxicol Cutaneous Ocul Toxicol 1982;1:3–32.

341. Adams AJ, Brown B, Haegerstrom-Portnoy G, Flom MC, Jones RT. Marijuana, alcohol, and combined drug effects on the time course of glare recovery. Psychopharmacology 1978;56:81–86.

342. Liguori A, Gatto CP, Robinson JH. Effects of marijuana on equilibrium, psychomotor performance, and simulated driving. Behav Pharmacol 1998;9:599–609.

343. Marks DF, MacAvoy MG. Divided attention performance in cannabis users and non-users following alcohol and cannabis separately and in combination. Psychopharmacology (Berl) 1989;99:397–401.

344. Belgrave BE, Brid KD, Chesher GB, Jackson DM, Lubbe KE, Starmer GA, Teo RK. The effect of (-) *trans*-Δ^9-tetrahydrocannabinol, alone and in combination with

ethanol, on human performance. Psychopharmacology 1979;62:53–60.

345. Wright KA, Terry P. Modulation of the effects of alcohol on driving-related psychomotor skills by chronic exposure to cannabis. Psychopharmacology 2002;160: 213–219.

346. Kelly E, Darke S, Ross J. A review of drug use and driving: epidemiology, impairment, risk factors and risk perceptions. Drug Alcohol Rev 2004;23:319–344.

347. Robbe H. Marijuana's impairing effects on driving are moderate when taken alone but severe when combined with alcohol. Hum Psychopharmacol Clin Exp 1998;13 (Suppl 2): 70S–78S.

348. Sutton LR. The effects of alcohol, marihuana and their combination on driving ability. J Stud Alcohol 1983;44: 438–445.

349. Ronen A, Gershon P, Drobiner H, Rabinovich A, Bar-Hamburger R, Mechoulam R, et al. Effects of THC on driving performance, physiological state and subjective feelings relative to alcohol. Accid Anal Prev 2008;40: 926–934.

350. Reeve VC, Grant JD, Robertson W, Gillespie HK, Hollister LE. Plasma concentrations of Δ^9-tetrahydrocannabinol and impaired motor function. Drug Alcohol Depend 1983;11:167–175.

351. Rafaelsen OJ, Bech P, Christiansen J, Christrup H, Nyboe J, Rafaelsen L. Cannabis and alcohol: effects on stimulated car driving. Science 1973;179:920–923.

352. Leirer VO, Yesavage JA, Morrow DG. Marijuana carry-over effects on aircraft pilot performance. Aviat Space Environ Med 1991;62:221–227.

353. Drummer OH, Gerostamoulos J, Batrziris H, Chu M, Caplehorn J, Robertson MD, Swann P. The involvement of drugs in drivers of motor vehicles killed in Australian road traffic crashes. Accid Analysis Prev 2004;36: 239–248.

354. Lowenstein SR, Koziol-McLain J. Drugs and traffic crash responsibility: a study of injured motorists in Colorado. J Trauma 2001;50:313–320.

355. Ramaekers JG, Berghaus G, van Laar M, Drummer OH. Dose related risk of motor vehicle crashes after cannabis use. Drug Alcohol Depend 2004;73:109–119.

356. Smiley AM. Marijuana: on-road and driving simulator studies. Alcohol Drugs Driv 1986;2:121–134.

357. Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of cannabis: a 10-year study of age and gender differences in the concentrations of tetrahydrocannabinol in blood. Addiction 2008;103:452–461.

358. Augsburger M, Donzé N, Ménétrey A, Brossard C, Sporkert F, Giroud C, Mangin P. Concentration of drugs in blood of suspected impaired drivers. Forensic Sci Int 2005;153:11–15.

359. Ramaekers JG, Moeller MR, van Ruitenbeek P, Theunissen EL, Schneider E, Kauert G. Cognition and motor control as a function of Δ^9-THC concentration in serum and oral fluid: limits of impairment. Drug Alcohol Depend 2006;85:114–122.

360. Grotenhermen F, Leson G, Berghaus G, Drummer OH, Kruger H-P, Longo M, et al. Developing limits for driving under cannabis. Addiction 2007;102:1910–1917.

361. Benyamina A, Lecacheux M, Blecha L, Reynaud M, Lukasiewcz M. Pharmacotherapy and psychotherapy in cannabis withdrawal and dependence. Expert Rev Neurother 2008;8:479–491.

362. Haney M, Hart CL, Ward AS, Foltin RW. Nefazodone decreases anxiety during marijuana withdrawal in humans. Psychopharmacology (Berl) 2003;165:157–165.

363. Haney M, Hart CL, Vosburg SK, Nasser J, Bennett A, Zubaran C, Foltin RW. Marijuana withdrawal in humans: effects of oral THC or divalproex. Neuropsychopharmacology 2004;29:158–170.

364. Budney AJ, Hughes JR, Moore BA, Novy PL. Marijuana abstinence effects in marijuana smokers maintained in their home environment. Arch Gen Psychiatry 2001;58: 917–924.

365. Weissman A, Milne GM, Melvin LS Jr. Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. J Pharmacol Exp Ther 1982;223:516–523.

366. Johnson MR, Melvin LS, Althuis TH, Bindra JS, Harbert CA, Milne GM, Weissman A. Selective and potent analgetics derived from cannabinoids. J Clin Pharmacol 1981;21(Suppl 8–9):271S–282S.

367. Wiley JL, Compton DR, Dai D, Lainton JA, Phillips M, Huffman JW, Martin BR. Structure-activity relationships of indole- and pyrrole-derived cannabinoids. J Pharmacol Exp Ther 1998;285:995–1004.

368. Dresen S, Ferreiros N, Putz M, Westphal F, Zimmermann R, Auwarter V. Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. J Mass Spectrom 2010;45:1186–1194.

369. Lindigkeit R, Boehme A, Eiserloh I, Luebbecke M, Wiggermann M, Ernst L, Beuerle T. Spice: a never ending story? Forensic Sci Int 2009;191:58–63.

370. Seely KA, Prather PL, James LP, Moran JH. Marijuana-based drugs: innovative therapeutics or designer drugs of abuse? Mol Interv 2011;11:36–51.

371. Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS, Johnson MR, Martin BR. Cannabinoid structure-activity relationships: correlation of receptor binding and *in vivo* activities. J Pharmacol Exp Ther 1993; 265:218–226.

372. Huffman JW, Szklennik PV, Almond A, Bushell K, Selley DE, He H, et al. 1-Pentyl-3-phenylacetylindoles, a new class of cannabimimetic indoles. Bioorg Med Chem Lett 2005;15:4110–4113.

373. Uchiyama N, Kikura-Hanajiri R, Ogata J, Goda Y. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. Forensic Sci Int 2010;198: 31–38.

374. Wintermeyer A, Moller I, Thevis M, Jubner M, Beike J, Rothschild MA, Bender K. *In vitro* phase I metabolism

of the synthetic cannabimimetic JWH-018. Anal Bioanal Chem 2010;398:2141–2153.

375. Sobolevsky T, Prasolov I, Rodchenkov G. Detection of JWH-018 metabolites in smoking mixtures post-administration urine. Forensic Sci Int 2010;200:141–147.

376. Wintermeyer A, Möller I, Thevis M, Jübner M, Beike J, Rothschild MA, Bender K. *In vitro* phase I metabolism of the synthetic cannabimimetic JWH-018. Anal Bioanal Chem 2010;398:2141–2153.

377. Simmons J, Cookman L, Kang C, Skinner C. Three cases of "spice" exposure. Clin Toxicol 2011;49:431–433.

378. Schneir AB, Cullen J, Ly BT. "Spice" girls: synthetic cannabinoid intoxication. J Emerg Med 2011;40: 296–299.

379. Müller H, Sperling W, Köhrmann M, Huttner HB, Kornhuber J, Maler JM. The synthetic cannabinoid Spice as a trigger for an acute exacerbation of cannabis induced recurrent psychotic episodes. Schizophr Res 2010;118: 309–310.

380. Zimmermann US, Winkelmann PR, Pihatsch M, Nees JA, Spanagel R, Schulz K. Withdrawal phenomena and dependence syndrome after the consumption of "spice gold." Dtsch Arztebl Int 2009;106:464–467.

381. Hudson S, Ramsey J, King L, Timbers S, Maynard S, Dargan PI, Wood DM. Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products. J Anal Toxicol 2010;34:252–260.

382. Moller I, Winterneyer A, Bender K, Jubner M, Thomas A, Krug O, et al. Screening for the synthetic cannabinoid JWH-018 and its major metabolites in human doping controls. Drug Test Anal 2011;3:609–620.

Chapter 61

Mate Tea (*Ilex paraguariensis* A. St. Hil.)

HISTORY

Indigenous tribes (e.g., Guarani Indians) from South America traditionally used tea from the *Ilex paraguariensis* A. St. Hil. as a mild stimulant.[1] In the 16th century, cultivation of this plant began in Jesuit settlements in South America, leading to the designation of the beverage as Jesuitic tea. This tea was introduced into Europe by the Jesuits. Mate is a derivative of the Quechua word ("matí") for the cup or vessel used to drink the tea. In Brazil, the word for this vessel is *chimarrão*.

BOTANICAL DESCRIPTION

Ilex species are medium to large trees distributed in tropical and subtropical regions, mostly in South America and East Asia; many *Ilex* species appear similar to the mate tree. *I. brevicuspis* Reissek and *I. theezans* Reissek grow in similar habitats in northeastern Argentina, southern Brazil, and eastern Paraguay as *I. paraguariensis* A. St. Hil. *I. argentina* Lillo is a native tree in northwestern Argentina.

Common Name: Mate (tea), chimarrão (tea or vessel), yerba-buena (tea)

Scientific Name: *Ilex paraguariensis* A. St. Hil.

Botanical Family: Aquifoliaceae (holly)

Physical Description: The perennial, evergreen tree reaches 18 m (~60 feet) in height. The alternate, obovate leaves have a serrated margin and obtuse apex. The inflorescences are a close group of flat-topped flowers with the outer flowers opening first (corymboid fascicles). These small and simple flowers appear from October through November with a whitish corolla. The fruit has 4–5 single seeds that develop from March to June.

Distribution and Ecology: This subtropical species prefers climates with relatively even rainfall throughout the year and mean temperatures 21–22°C (~70°F); however, this tree does tolerate temperatures below freezing in the plateaus and mountain regions of southern Brazil. The mate tree is a native species of parts of southern Brazil, Uruguay, Paraguay, and Argentina.

IDENTIFYING CHARACTERISTICS

Mate tea contains caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine, CAS RN: 83-67-0) in significantly higher concentrations than coffee. The tea does not contain significant amounts of theophylline (1,3-dimethylxanthine). Figure 61.1 displays the chemical structures of the major xanthines and caffeoyl derivatives in mate tea. The physiochemical properties of caffeine are discussed in the caffeine chapter. Table 61.1 displays some physiochemical properties of theobromine.

EXPOSURE

Epidemiology

Mate tea is a popular drink in southern South America including Argentina, Bolivia, Brazil, Chile, Ecuador, Paraguay, and Uruguay. Argentina is the major mate tea producer, followed by Brazil and Paraguay.[4] In the
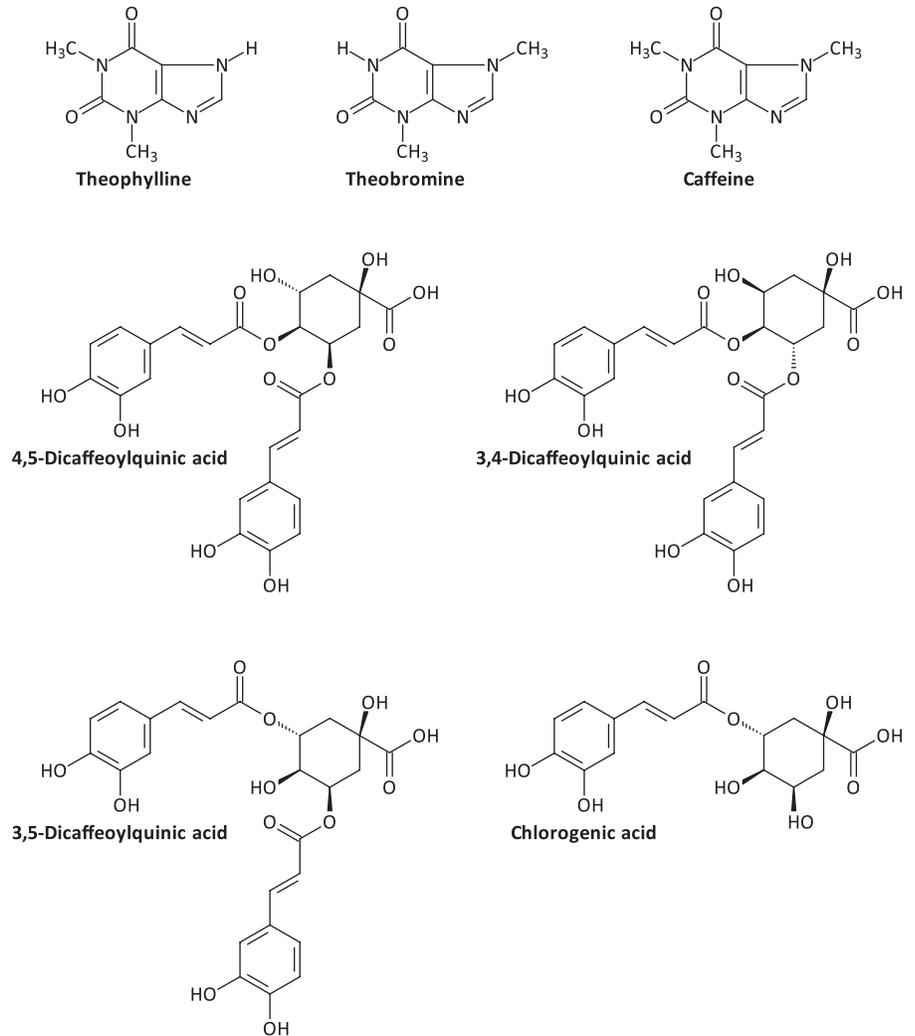
FIGURE 61.1. Chemical structures of major xanthines and caffeoyl derivatives in mate tea.

TABLE 61.1. Some Physiochemical Properties of Theobromine.

Physical Property	Value
Melting Point	357°C (~675°F)
pKa Dissociation Constant	9.9
log P (Octanol-Water)	−0.78
Water Solubility	330 mg/L (25°C/77°F)
Vapor Pressure	8.95E-10 mm Hg (25°C/77°F)

Middle East, the Druze of Lebanon, Syria, and the Golan Heights mate tea is also consumed. Currently, the gauchos of South America, primarily in Argentina, Southern Brazil, Paraguay, and Uruguay, consume mate tea as a traditional beverage. Guarani indigenous tribes from South America use an infusion (erva-maté, yerba maté) from the dried leaves and twigs of *Ilex paraguariensis* as a mildly stimulating beverage. In folk medicine, this tea is a central nervous system stimulant, diuretic,

and a treatment for rheumatoid arthritis. Other folk uses for yerba maté include the treatment of headaches, obesity, fatigue, hemorrhoids, constipation, hypertension, and hepatic disorders.[2]

Sources

ORIGIN

Mate tea is an aqueous infusion prepared from dried leaves of *Ilex paraguariensis*. Precursors for the biosynthesis of caffeine in *Ilex paraguariensis* are purine nucleotides (e.g., adenine, guanosine, hypoxanthine). This biosynthesis occurs primarily in young leaves via salvage enzymes during the early stages of development.[3]

COMPOSITION

Mate tea contains a variety of substances including xanthines (caffeine, theobromine), chlorogenic acids

933

(caffeoylquinic, dicaffeoylquinic, feruloylquinic, diferuloylquinic, *p*-coumaroylquinic, caffeoyl-*p*-coumaroylquinic, caffeoyl-feruloylquinic, caffeoyl-sinapoylquinic, tricaffeoylquinic, dicaffeoyl-feruloylquinic), shikimic acid derivatives (caffeoylshikimates, dicaffeoylshikimates, tricaffeoylshikimates, feruloylshikimates), flavonoids (kaempferol, quercetin, rutin), amino acids, minerals (calcium, iron, phosphorus), vitamins (B_1 B_2, C), and saponins (mate saponin 1–5), depending on brewing condition, the age of the leaves, and the *Ilex* species.[4,5] Caffeine is the most common purine alkaloid in mate leaves; theophylline is usually not found in significant amounts in mate leaves. Typical concentrations of caffeine and theobromine in these leaves are 1–2% and 0.3–0.9% dry weight, respectively. Older mate leaves contain less caffeine and theobromine than younger leaves. Analysis of leaves of different ages suggest that the concentration of these 2 compounds may decrease in older leaves up to approximately 50% and 20%, respectively.[6] The concentration of caffeine and theobromine is highest in *I. paraguariensis* compared with other *Ilex* species including *Ilex argentina* Lillo*, I. brevicuspis* Reissek, and *I. theezans* Reissek. In 14 samples of commercial mate tea prepared from leaves and stems of *I. paraguariensis*, the caffeine and theobromine content varied from 0.30–1.72% and 0.08–0.66%, respectively, as measured by high performance capillary electrophoresis.[7] The caffeine and theobromine content varied somewhat with temperature and filter size. Theophylline was not quantifiable in these species including *Ilex paraguariensis* with the exception of *I. pseudobuxus* (0.6 ± 0.2 mg/100 g dried herb). Caffeine and theobromine occur in the leaf epicuticular waxes of *Ilex paraguariensis* at average concentrations of about 0.5% dry leaf weight.[8]

The leaves from *Ilex paraguariensis* contain approximately 5–10% saponins along with caffeoyl derivatives (caffeic acid, chlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid) and flavonoids (kaempferol, quercetin, rutin).[9,10] This fraction contains at least 10 glycosides of ursolic or oleanolic acids including mate saponins 1-5, $J1_{a/b}$, $J2_{a/b}$, $J3_{a/b}$, and J4.[11]

PRODUCTION PROCESSES

Commercial production of mate tea involves both harvesting from rainforests and cultivation on mate plantations. Sun-exposed leaves of mate trees grown on plantations usually have higher polyphenol concentrations than leaves harvested from more-shaded areas in rainforests.[12] After harvest, blanching of the leaves inactivates degradation enzymes (e.g., polyphenol oxidase) and fermentation is halted by placing the leaves in a rotating cylinder (*sapeco*) near an open flame. In contrast to the flash heating of mate leaves over an open flame, green tea undergoes steaming or pan-frying. Black teas are not blanched before drying; thus, substantial fermentation occurs during the processing of black teas. Further drying of the mate leaves results from the use of continuous rotary/belt driers or the traditional discontinuous bed drier (*barbaqua*) with a wood fire. This wood-fired process is slow compared with the rapid, high-temperature air drying of green tea. As a result of the differences in processing procedures, mate tea contains high concentrations of the chlorogenic acid and caffeoyl derivatives compared with green and black teas.[13] Additionally, mate tea contains no catechin in contrast to the other two types of tea.

IMPURITIES

As analyzed by high performance liquid chromatography, other *Ilex* species contain nondetectable to minimal amounts of caffeine and theobromine including *I. argentina*, *I. brevicuspis*, *I. dumosa* Reissek var. *dumosa*, *I. microdonta* Reissek, *I. pseudobuxus* Reissek, and *I. theezans*.[14] These species may be substituted for purported *I. paraguariensis* as adulterants of mate tea include the addition *I. integerrima* (Vell.) Reissek and *I. taubertiana* Loes. These plants also contain triterpene saponins, flavonoids, phenolic acids, and xanthines, and the concentrations of these compounds vary compared with *Ilex paraguariensis*. Multivariate analysis of the metabolite fingerprints of plant material extracts by ^{1}H NMR spectroscopy allows the identification of various *Ilex* species.[15] Consumption of tea from *Ilex paraguariensis* contaminated with belladonna alkaloids (hyoscyamine, scopolamine) resulted in anticholinergic poisoning manifest by dry skin, tachycardia, mydriasis, dry skin, agitation, and hallucination.[16]

Methods of Use

Preparation of mate tea (chimarrão) involves placing dried, minced leaves inside a mate receptacle (i.e., dried porongo gourd) and pouring hot water over the leaves.[17] This method is a partial infusion as some of the mate leaves remain dry. The aqueous extract is usually imbibed through a metal straw (*bombilla*), which has a metal strainer on the lower end. Hot water may be added to the gourd several times during the day with some users drinking more than 1 L of the tea daily. An alternate method for drinking this tea (*terere*) involves the successive addition of cold water to the mate leaves or branches along with the addition of sugar, other plant species, and/or other flavorings. Some differences in the methylxanthine and phenolic content

result from different types of infusions with coarse-ground mate extracted by hot water (chimarrão) containing the highest methylxanthine content.[18] The vessel typically contains about 50 g mate. *Ilex paraguariensis* is the primary ingredient of the commercial product, yerba maté, and most mate teas. Commercial products often are distributed as individual tea bags (1–2 g). The addition of boiling water to the infusion results in the ingestion of very hot liquid in most locations with the exception of Paraguay and southwestern Brazil, where the beverage is consumed cold.

DOSE EFFECT

One cup (150 mL) of mate tea contains about 80 mg caffeine, which is a similar caffeine concentration as a cup of coffee; however, the ingestion of higher amounts (i.e., up to 500 mL) of mate tea results in higher total caffeine intake for the average individual. Analysis of Brazilian samples of commercial mate tea prepared by traditional gaucho methods indicates that a typical infusion of mate contains approximately 260 mg caffeine, 240 mg chlorogenic acids, and 170 mg caffeic acid.[19]

TOXICOKINETICS

There are few data on the toxicokinetics of caffeine in humans after the ingestion of mate tea; however, there are no data to indicate that the absorption and biotransformation of caffeine is significantly different following the ingestion of mate tea than after the ingestion of coffee.

CLINICAL RESPONSE

The International Agency for Research on Cancer (IARC) lists mate tea and hot mate tea as unclassified (Group 3) and probable (Group 2A) human carcinogens, respectively, based on limited case-control studies indicating an increased risk primarily of squamous cell esophageal cancer.[20] There are few animal data on the carcinogenicity of the active constituents of mate tea, and most epidemiologic data does not separate the drinking of hot and cold mate tea. Epidemiologic studies suggest that the chronic ingestion of hot mate tea is associated with a moderate risk of malignant neoplasms of the upper aerodigestive tract. The excess risk as determined by the relative risk (RR) of upper aerodigestive cancer in a case-control study of mate tea drinkers from southern Brazil was 1.6 (95% CI: 1.2–2.2) after adjustment for confounding factors (tobacco use, alcohol drinking).[21] Most of the increase risk involves oral cancer (RR = 1.9, 95% CI: 1.1–3.3) and laryngeal cancer (RR = 2.2, 95% CI 1.1–4.5). Limited *in vitro* studies suggest that mate tea contains genotoxic and mutagenic compounds as measured by lysogenic induction in *Escherichia coli* and the induction of mutagenesis in *Salmonella typhimurium* (Ames test).[22]

Limited studies on mate tea drinkers and esophageal cancer suggest the risk of esophageal cancer increases independently with both the amount of mate tea consumed (i.e., about ~3-fold for highest categories of use) and the temperature of the drink.[23] In a retrospective, hospital-based case-control study, the relative risk of esophageal cancer in mate tea drinkers was 2.84 (95% CI: 1.41–5.73).[24] After adjusting for cumulative mate tea consumption, the reported drinking of very hot mate tea almost doubled the risk of esophageal cancer when compared with the drinking of warm to hot mate tea, whereas patients with high cumulative mate tea exposure with little tobacco or alcohol use had a statistically insignificant increase (OR = 1.52, 95% CI: 0.88–2.62) in the occurrence of esophageal cancer. In a series of 5 case-control South American studies, the cumulative effects of mate amount and mate temperature were more than multiplicative.[25] The odds ratio of esophageal cancer in heavy drinkers (>1.50 L/d) of very hot mate tea was 4.14 (95% CI: 2.24–7.67) compared with light mate tea drinkers (<0.50 L/d) of cold/warm/hot mate tea. Other cancers of the aerodigestive tract associated with mate tea drinking include the larynx, tongue, and oropharynx.[26,27] These risks persist after adjustment for the strong risk factors of tobacco and alcohol use. A case-control study of 107 cases of laryngeal cancer and 290 controls without tobacco or alcohol use demonstrated an approximate 3-fold increase in the risk of laryngeal cancer, after controlling for the effects of age, tobacco and alcohol consumption.[28] The drinking of hot mate tea also increases the risk of chronic esophagitis.[29] A case-control study of mate tea drinking suggested a small increase (OR = 1.6, 95% CI: 1.1–2.4) in the risk of lung cancer in heavy mate tea drinkers (≥2 L/d) after adjustment for smoking, primarily for small cell lung cancer.[30] Pulmonary adenocarcinoma was not associated with mate tea drinking. The very high maternal consumption (1 L daily) of mate tea has been associated with a neonatal withdrawal syndrome (increased jitteriness, irritability, hypertonia, brisk tendon reflexes, and high-pitched cry).[31] Symptoms of withdrawal began about 17 hours after birth and resolved by 84 hours after birth. The estimated maternal intake of caffeine during pregnancy was 930 mg daily.

DIAGNOSTIC TESTING

Analytic methods for the identification and quantitation of phenolic compounds in mate tea include high performance liquid chromatography with photodiode array

detection,[12] conventional gas chromatography/mass spectrometry (GC/MS),[32,33] and solid-phase microextraction with 2-D GC/MS.[34] There are few data on caffeine or theobromine concentrations after the ingestion of mate tea. Abnormalities following the ingestion of mate tea and coffee probably are similar because caffeine is the main active ingredient.

TREATMENT

Mate tea contains substantial amounts of caffeine; the treatment of intoxication with this substance is identical to the treatment of caffeine toxicity (e.g., coffee). However, serious caffeine intoxication is unlikely because of the volume of mate tea necessary to produce blood caffeine concentrations similar to those reported in serious caffeine overdose.

References

1. Fredholm BB. Notes on the history of caffeine use. Handb Exp Pharmacol 2011;200:1–9.

2. VanderJagt TJ, Ghattas R, VanderJagt DJ, Crossey M, Glew RH. Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. Life Sci 2002;70:1035–1040.

3. Ashihara H. Purine metabolism and the biosynthesis of caffeine in mate leaves. Phytochemistry 1993;33:1427–1430.

4. Jaiswal R, Sovdat T, Vivan F, Kuhnert N. Profiling and characterization by LC-MSn of the chlorogenic acids and hydroxycinnamoylshikimate esters in mate (*Ilex paraguariensis*). J Agric Food Chem 2010;58:5471–5484.

5. Heck CI, de Mejia EG. Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. J Food Sci 2007;72:R138–R151.

6. Reginatto F, Athayde ML, Gosmann G, Schenkel EP. Methylxanthines accumulation in *Ilex* species—caffeine and theobromine in erva-mate (*Ilex paraguariensis*) and other *Ilex* species. J Braz Chem Soc 1999;10:443–446.

7. Pomilio AB, Trajtemberg S, Vitale AA. High-performance capillary electrophoresis analysis of mate infusions prepared from stems and leaves of *Ilex paraguariensis* using automated micellar electrokinetic capillary chromatography. Phytochem Anal 2002;13:235–241.

8. Athayde ML, Coelho GC, Schenkel EP. Caffeine and theobromine in epicuticular wax of *Ilex paraguariensis* A. St. Hil. Phytochemistry 2000;55:853–857.

9. Filip R, Lopez P, Giberti G, Coussio J, Ferrraro G. Phenolic compounds in seven South American *Ilex* species. Fitoterapia 2001;72:774–778.

10. Schenkel EP, Montanha JA, Gosmann G. Triterpene saponins from maté. Adv Exp Med Biol 1996;405:47–56.

11. Gosmann G, Guillaume D, Taketa AT, Schenkel EP. Triterpenoid saponins from *Ilex paraguariensis*. J Nat Prod 1995;58:438–441.

12. Heck CI, Schmalko M, Gonzalez d Mejia E. Effect of growing and drying conditions on the phenolic composition of mate teas (*Ilex paraguariensis*). J Agric Food Chem 2008;56:8394–8403.

13. Chandra S, De Mejia Gonzalez E. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. J Agric Food Chem 2004;52:3583–3589.

14. Filip R, Lopez P, Coussio J, Ferraro G. Mate substitutes or adulterants: study of xanthine content. Phytother Res 1998;12:129–131.

15. Choi YH, Sertic S, Kim HK, Wilson EG, Michopoulos F, Lefeber AW, et al. Classification of *Ilex* species based on metabolomic fingerprinting using nuclear magnetic resonance and multivariate data analysis. J Agric Food Chem 2005;53:1237–1245.

16. Hsu CK, Leo P, Shastry D, Meggs W, Weisman R, Hoffman RS. Anticholinergic poisoning associated with herbal tea. Arch Intern Med 1995;155:2245–2248.

17. Bracesco N, Sanchez AG, Contreras V, Menini T, Gugliucci A. Recent advances on *Ilex paraguariensis* research: mini-review. J Ethnopharmacol 2011;136:378–384.

18. Meinhart AD, Bizzotto CS, Ballus CA, Rybka AC, Sobrinho MR, Cerro-Quintana RS, et al. Methylxanthines and phenolics content extracted during the consumption of mate (*Ilex paraguariensis* St. Hil) beverages. J Agric Food Chem 2010;58:2188–2193.

19. Mazzafera P. Mate drinking: caffeine and phenolic acid intake. Food Chem 1997;60:67–71.

20. International Agency for Research on Cancer: Mate. IARC Monogr 1991;51:273–290.

21. Pintos J, Franco EL, Oliveira BV, Kowalski LP, Curado MP, Dewar R. Maté, coffee, and tea consumption and risk of cancers of the upper aerodigestive tract in southern Brazil. Epidemiol 1994;5:53–590.

22. Fonseca CA, Otto SS, Paumgartten FJ, Leitao AC. Nontoxic, mutagenic, and clastogenic activities of Mate-Chimarrão (*Ilex paraguariensis*). J Environ Pathol Toxicol Oncol 2000;19:333–346.

23. Islami F, Boffetta P, Ren J-S, Pedoeim L, Khatib D, Kamangar F. High-temperature beverages and foods and esophageal cancer risk—a systematic review. Int J Cancer 2009;125:491–524.

24. Sewram V, de Stefani E, Brennan P, Boffetta P. Maté consumption and the risk of squamous cell esophageal cancer in Uruguay. Cancer Epidemiol Biomarkers Prev 2003;12:508–513.

25. Castellsague X, Munoz N, De Stefani E, Victora CG, Castelletto R, Rolon PA. Influence of mate drinking, hot beverages and diet on esophageal cancer risk in South America. Int J Cancer 2000;88:658–664.

26. Goldenberg D, Golz A, Joachims HZ. The beverage maté: a risk factor for cancer of the head and neck. Head Neck 2003;25:595–601.

27. Franco EL, Kowalski LP, Oliveira BV, Curado MP, Pereira RN, Silva ME, et al. Risk factors for oral cancer in Brazil: a case-control study. Int J Cancer 1989;43:992–1000.

28. De Stefani E, Correa P, Oreggia F, Leiva J, Rivero S, Fernandez G, et al. Risk factors for laryngeal cancer. Cancer 1987;60:3087–3091.

29. Munoz N, Victora CG, Crespi M, Saul C, Braga NM, Correa P. Hot mate drinking and precancerous lesions of the oesophagus: an endoscopic survey in southern Brazil. Int J Cancer 1987;39:708–709.

30. De Stefani E, Fierro L, Correa P, Fontham E, Ronco A, Larrinaga M, et al. Mate drinking and risk of lung cancer in males: a case-control study from Uruguay. Cancer Epidemiol Biomarkers Prev 1996;5:515–519.

31. Martin I, Lopez-Vilchez MA, Mur A, Garcia-Algar O, Rossi S, Marchei E, Pichini S. Neonatal withdrawal syndrome after chronic maternal drinking of mate. Ther Drug Monit 2007;29:127–129.

32. Araújo HC, Lacerda ME, Lopes D, Bizzo HR, Kaplan MA. Studies on the aroma of maté (*Ilex paraguariensis St.* Hil.) using headspace solid-phase microextraction. Phytochem Anal 2007;18:469–474.

33. Jacques RA, dos Santos Freitas L, Flores Peres V, Dariva C, de Oliveira JV, Bastos Caramão E. Chemical composition of mate tea leaves (*Ilex paraguariensis*): a study of extraction methods. J Sep Sci 2006;29:2780–2784.

34. Purcaro G, Tranchida PQ, Jacques RA, Caramao EB, Moret S, Conte L, et al. Characterization of the yerba mate (*Ilex paraguariensis*) volatile fraction using solid-phase microextraction-comprehensive 2-D GC-MS. J Sep Sci 2009;32:3755–3763.

Chapter 62

MORNING GLORY FAMILY (CONVOLVULACEAE)

HISTORY

In Mexico, native Indians have used seeds from *Ipomoea violacea* L. (*tlitliltzin*) and *Turbina corymbosa* (L.) Raf. (seeds = *ololiuqui*; plant = *coaxihuitl* or snake plant) in religious rites since the time of the Aztecs. Their use of ololiuqui for divinatory and medicinal purposes were well-guarded secrets from the Spanish Conquistadors that remained shrouded in mystery until a specimen *T. corymbosa* was discovered in a Zapotec indian garden in the Mexican state of Oaxaca.[1] Samuel Richardson, an officer of the Anglo-Mexican Mining Association, transported morning glory seeds from Mexico to England in the 1830s; now the morning glory in a common cultivar in England and North America.

During the 1950s and 1960s, Albert Hoffman and his associates investigated extracts of ololiuqui and *Ipomoea violacea* with the subsequent isolation of various ergot alkaloids including lysergic acid amides.[2] In 1960, Aldous Huxley identified the hallucinogen in ololiuqui as ergine (*d*-lysergic acid amide), which was structurally similar to ergot alkaloids in the fungus, *Claviceps purpurea*.[3] Further research indicated that the plant tissue in the embryo of the seed of *T. corymbosa* produced this ergot alkaloid rather than a fungal infection of the seed coat or seed membrane.[4] Following the discovery of ergine in ololiuqui, research with thin layer chromatography in the 1960s revealed ergot alkaloids in other members of the Convolvulaceae (morning glory) family including *Ipomoea violacea*.[5] In the 1960s, the publication of the hallucinogenic properties of these seeds in the popular press led to an upsurge of morning glory seed consumption among teenagers and young adults.

BOTANICAL DESCRIPTION

Common Name: Heavenly-blue morning glory, beach moonflower, badoh negro

Scientific Name: *Ipomoea violacea* L. [*Calonyction tuba* (Schlecht.) Colla, *Ipomoea macrantha* Roemer & J.A. Schultes, *Ipomoea tuba* (Schlecht.) G. Don]

Botanical Family: Convolvulaceae (morning glory)

Physical Description: This glabrous (hairless), perennial plant has smooth, twining, round, or angular stems. The ovate leaves narrow at the apex (acuminate or cuspidate) and are 5–16 cm long by 5–14 cm wide with petioles 3.5–6 cm long. The solitary flowers are narrowly funnel-shaped with the corolla opening at night. The flowers are hermaphrodite (both male and female) and they appear July through September. The fruit is obtuse at the apex with the capsule being globose and containing black seeds. Figure 62.1 displays the seeds of beach moonflower (*Ipomoea violacea* L.).

Distribution and Ecology: The distribution of this plant extends to North America, the Caribbean, and Oceania, particularly in tropical coastal regions (e.g., Texas, Florida, Hawaii, Puerto Rico). Cultivars of morning glory are common in North America and England. Popular cultivars of morning glories are Heavenly Blue (blue flowers) and Pearly Gates (white flowers), Wedding Bells (lavender flowers), Flying Saucers (variegated blue and white flowers), Summer Skies (pale blue flowers), and Blue Star (pale blue flowers).

FIGURE 62.1. Seeds (ololiuqui) of a beach moonflower (*Ipomoea violacea* L). (Photo courtesy of Steve Hurst and the USDA-NRCS PLANTS Database).

FIGURE 62.2. Leaves and flowers of the Christmas vine (*T. corymbosa*). (Richard A. Howard Image Collection. Photo courtesy of Smithsonian Institution).

Common Name: Christmas vine, Christmas wreath, aguinaldo blanco, corona de novia, badoh

Scientific Name: *Turbina corymbosa* (L.) Raf. [*Rivea corymbosa* (L.), *Ipomoea corymbosa* (L.) Roth ex Roemer & J.A. Schultes, *Convolvulus corymbosus* L.]

Botanical Family: Convolvulaceae (morning glory)

Physical Description: This woody vine reaches up to 5 m (~16 feet) laterally with gray stems up to 2.5 cm (1 inch) in diameter. The leaves have cordate blades 5–8 cm (~2–3 inches) long with elongated tips. The inflorescences are corymbose (flat-topped) cymes that arise from the leaf axils. The trumpet-shaped corolla is white with a red or purple throat and green or greenish gray radiating stripes. Each capsule contains one brown, pubescent seed. Seeds mature about 60 days after fertilization. Figure 62.2 displays the leaves and flowers of the Christmas vine.

Distribution and Ecology: The Christmas vine is indigenous to the West Indies, Mexico, Central America, and the tropical portion of South America. This plant has naturalized to areas in Florida, Hawaii and other Pacific Islands, Australia, and some some parts of Europe, Africa, and Asia. The Christmas vine grows as a single plant or a patch of vines on open sunny, undisturbed, well-drained areas of roadsides, pastures, streams, and secondary forests

Common Name: Elephant creeper, woodrose, baby woodrose, Hawaiian woodrose, Hawaiian baby woodrose

Scientific Name: *Argyreia nervosa* (Burm. f.) Bojer [*Rivea nervosa* (Burm. f.) Hallier f., *Argyreia speciosa* (L. f.) Sweet]

Botanical Family: Convolvulaceae (morning glory)

Physical Description: This woody, climbing tropical plant contains large, heart-shaped leaves with dense, white silky hairs protruding from the underside of the leaf. The shallow lobed, purple-pink pedals are about 6 cm (~2 ½ inches) long. The yellow-brown fruit is spherical and about 2 cm (~¾ inch) in diameter.

Distribution and Ecology: *Argyreia nervosa* grows in the subtropical environment of Hawaii, California, and Florida. This plant is native to India, but cultivation of this plant occurs throughout Southeast Asia and other tropical countries. The elephant creeper prefers brushland and margins of secondary forests up to 1500 m (~5,000 feet) altitude.

IDENTIFYING CHARACTERISTICS

The seeds of certain members of the morning glory family contain psychotomimetic indole alkaloids that are structurally related to lysergic acid diethylamide (LSD). The mind-altering compounds of the morning glory family are similar to the ergot alkaloids (ergoline) found in the ergot fungus (*Claviceps purpurea*), which was associated with epidemic outbreaks of St. Anthony's Fire (ergotism) during the Middle Ages. Ergoline (lysergic acid)-like alkaloids in the morning glory family (Convolvulaceae) include *d*-lysergic acid amide (ergine), isolysergic acid amide (isoergine), ergonovine (ergometrine), lysergol, ergometrinine, penniclavine,

939
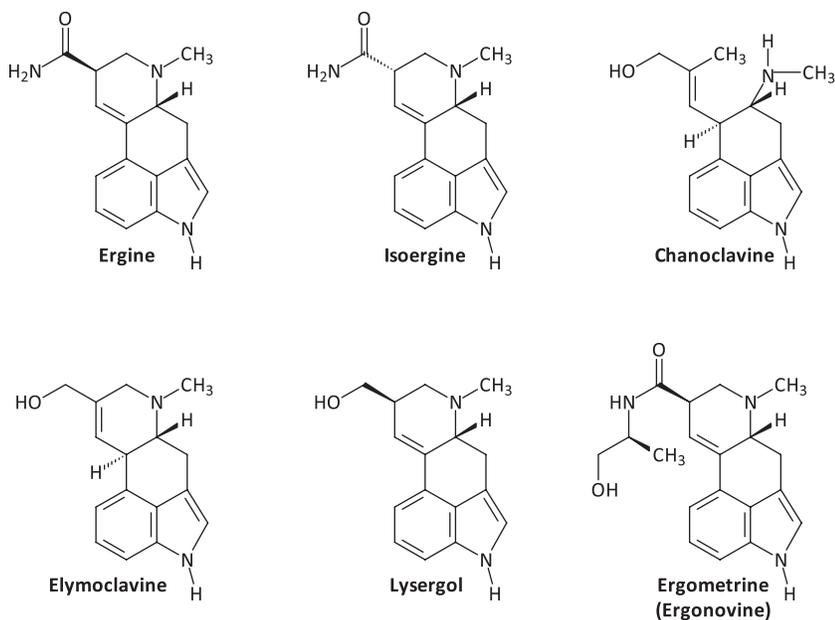
FIGURE 62.3. The chemical structures of ergoline alkaloids present in *Ipomoea violacea* L.

chanoclavine, elymoclavine, and lysergic acid α-hydroxyethylamide.[6,7] Ergoline alkaloids detected in the Hawaiian woodrose (*Argyreia nervosa*) include ergine, isoergine, and penniclavine.[8] Ergine (lysergamide, CAS RN: 478-94-4), isoergine (*d*-isolysergic acid amide, CAS RN: 2889-26-1), chanoclavine, and elymoclavine are the major constituents of morning glory seeds. The psychomimetic properties of ergine are substantially less (i.e., ~10%) than the psychomimetic potency of LSD,[3] whereas chanoclavine and elymoclavine demonstrate no psychomimetic properties.[9] Figure 62.3 displays the chemical structures of the ergoline alkaloids from *I. violacea*.

EXPOSURE

Composition

The indole alkaloids (ergine, isoergine) are the active constituents of morning glory seeds. Analytic data suggest that biosynthesis of the ergoline alkaloids also occurs in clavicipitaceous fungi; translocation then can transfer these substances to the infected plant.[10] Most varieties of morning glory plants contain seeds with similar indole alkaloid content.[11] These alkaloids represent up to about 0.1% of the dry weight of the seeds from *I. violacea* with the highest alkaloid content occurring during the early stages of seed development.[12,13] As measured by spectrophotofluorimetry, the total alkaloid content of a convenience sample of *I. violacea* leaves ranged from 0.027–0.04%.[14] The psychomimetically inactive compound, chanoclavine is the most abundant

alkaloid in the immature seed. Common varieties of morning glories without evidence of hallucinogenic alkaloids in the seeds include common morning glory [*I. purpurea* (L.) Roth], white edge morning glory [*I. nil* (L.) Roth], tropical white morning glory (*I. alba* L.), cardinal climber morning glory [*I. multifida* (Raf.) Shinners], cypress vine morning glory (*I. quamoclit* L.), and Mexican or red morning glory (*I. coccinea* L.).[11] The seeds from *Turbina corymbosa* contain about 40% of the isoergine concentration and <20% the ergine content of seeds from *I. violacea*. Ergine content of one seed from *A. nervosa* is similar to the ergine content of 75 to 100 *I. violacea* seeds.[15] Other genera (e.g., *Argyreia*) in the Convolvulaceae family also contain large amounts of these potentially mind-altering indole compounds. Typically, the ergoline alkaloid content in the seeds from the Hawaiian woodrose (elephant creeper, *Argyreia nervosa*) is higher than *I. violacea*. Analysis of seeds from the Hawaiian woodrose demonstrate total indole alkaloid content of 0.5–0.9% with ergine and isoergine accounting for the largest fraction (0.136% and 0.188%, respectively).[16]

Methods of Use

The abuse of morning glory seeds is limited by adverse effects and the relatively low potency of the hallucinogenic components of the seeds. Most of the case reports about the misuse of morning glory seeds were published in the 1960s after the publication of the ergoline alkaloid content of the Hawaiian woodrose by Hylin and Watson.[8] The black seeds of the baby woodrose

(*Argyreia nervosa*) are an inexpensive hallucinogen for Hawaiians, but adverse side effects (e.g., constipation, lethargy, nausea, blurred vision) and mild psychomimetic effects limit use.[17] The morning glory plant is a traditional Chinese medicine (QianNiuZi, *Ipomoea purpurea* (L.) Roth) used for the treatment of edema, ascites, obesity, induction of labor, and fever.[18]

DOSE EFFECT

Large numbers of well-pulverized seeds from *Ipomoea violacea* are necessary to produce psychomimetic effects.[11] The content of psychoactive compounds in these seeds varies with individual plants, time of harvest, environmental conditions, and cultural practices. Reportedly, Indian ololiuqui users would ingest 13 seeds; however, the ingestion of this amount of ololiuqui seeds did not produce effects other than apathy in self-experimenters.[19] In general, the ingestion of 20–50 seeds is a low dose that may produce increased or decreased social interaction, restlessness, and heightened awareness for several hours. Doses of 60–100 seeds were associated with irritable apathy, passivity, some increased awareness, and heightened visual perceptions.[19] The ingestion of 100–150 seeds causes similar effects to 75–150 µg LSD with spacial distortions, visual illusions, elation, and enhanced imagery lasting up to 4 hours. The ingestion of 200–500 seeds is a large dose that produces intense psychomimetic effects along with unpleasant adverse reactions (nausea, vomiting, abdominal pain, paresthesias, cold extremities, and lethargy).[20]

The ingestion of 15 seeds from the Hawaiian woodrose (*Argyreia nervosa*) along with other drugs (marijuana, ether, dextromethorphan) was associated with visual hallucinations, suicidal and homicidal ideations, sinus tachycardia, hypertension, and hyperreflexia in an 18-year-old male.[21] This patient was being treated for depression with paroxetine at the time of the ingestion. The ingestion of 12 Hawaiian woodrose seeds was associated with auditory hallucination, blurred vision, diaphoresis, lightheadedness, nausea, and vomiting.[22]

TOXICOKINETICS

The absorption of indole alkaloids from morning glory seeds requires the pulverization or thorough mastication of the seeds to produce psychomimetic effects. There are few data on the toxicokinetics of the active compounds, ergine and isoergine. In rodent studies, the plasma elimination of isoergine is rapid with an estimated plasma elimination half-life of 30 minutes.[23] Within about 30 minutes after intraperitoneal administration, the brain and plasma concentrations are similar.

CLINICAL RESPONSE

Clinical features of morning glory intoxication are somewhat similar to the effects caused by the sympathomimetic and hallucinogenic properties of LSD. Ingestion of seeds from *I. violacea* produces dilated pupils, hyperreflexia, facial erythema, a dissociative state, and emotional lability within 3 hours followed by irritability and anxiety.[24] Adverse effects include nausea, vomiting, diarrhea, polyuria, numbness, cool extremities, muscle stiffness, lethargy, and uterine stimulation. Hypotension occurred following the intravenous injection of the boiled extract of morning glory seeds.[25] Abnormalities of behavior and perceptions usually resolve in 24–36 hours. However, hallucinogen persisting perception disorder (flashbacks) and recurrent dissociative feelings may recur after symptoms initially resolve.[20] Like LSD, the ingestion of well-masticated morning glory seeds can cause panic reactions, marked paranoia, and violent behavior. Panic states, prolonged dissociative reactions, and psychotic behavior have been associated with trauma and suicide.[26] Memory, intellect, and orientation are less impaired than behavior. Case reports associate the ingestion of seeds from the Hawaiian woodrose (*Argyreia nervosa*) with hallucinations, disorientation, agitation, lightheadedness, blurred vision, nausea, and vomiting.[22,27] Physical signs included tachycardia, hypertension, nystagmus, and mydriasis. Flashbacks characterized by auditory hallucinations recurred at least 1 month after ingestion.[22] Case reports suggest that the clinical features of Hawaiian woodrose intoxication are more similar to the sedative effects and autonomic disturbances associated with scopolamine than the gastrointestinal and psychomimetic effects of LSD.[28]

DIAGNOSTIC TESTING

Chromatographic methods can distinguish the indole alkaloids present in morning glory seeds.[29] Methods for the detection of indole alkaloids from the morning glory family include spectrophotofluorimetry[14] and thin layer chromatography.[30] However, gas chromatography/mass spectrometry allows the confirmation and quantitation of ergine, isoergine, and other indole alkaloids from the morning glory family.[31] Alternate methods of quantitation include liquid chromatography/electrospray ionization/tandem mass spectrometry in positive selected reaction monitoring mode[32] and mixed-mode cation exchange solid-phase extraction followed by ultra performance liquid chromatography/time of flight/mass spectrometry (LLOQ: 1.3 ng/mL, blood; 5.0 ng/mL, urine).[33] Using the latter method, analysis of post-mortem blood from a 29-year-old man, who died 3

hours after ingesting Hawaiian baby woodrose seeds when he attempted to fly out of the 4th floor of a building, demonstrated ergine (lysergamide) concentrations in postmortem blood and urine of 4.9 ng/mL and 1,000 ng/mL, respectively. A surviving witness ingested 6 seeds at the same time; his blood ergine concentration 9 hours after ingestion was 1.8 ng/mL. The seeds were soaked for about 3 hours prior to ingestion, and both men smoked cannabis soon after ingesting the seeds. Leukocytosis may occur during intoxication with morning glory seeds.[34]

TREATMENT

The management of morning glory seed intoxication is supportive, similar to the treatment of LSD intoxication. Decontamination measures are usually unnecessary unless indicated by the concomitant ingestion of other substances. Management involves reassurance to calm the patient and a protective environment to prevent accidental trauma and intentional self-harm. Benzodiazepines (e.g., diazepam, lorazepam) are the sedatives of choice; the use of benzodiazepines may improve symptoms of muscle stiffness. Patients with severe muscle stiffness should be evaluated for rhabdomyolysis and renal dysfunction. Behavioral symptoms usually resolve within 8–12 hours. Persistence of symptoms necessitates thorough psychiatric evaluation.

References

1. Huxtable J. Cover illustration—regional ethnopharmacology: *Turbina corymbosa* (L.) Raf. Proc West Pharmacol Soc 1994;37:1–2.

2. Stauffacher D, Tscherter H, Hofmann A. [Isolation of ergosine and ergosinine as well as agroclavine from seeds of *Ipomoea argyrophylla* Vatke (Convolvulaceae). 64. On ergot alkaloids (1)]. Helv Chim Acta 1965;48:1379–1380. [German]

3. Hofman A, Tscherter H. [Isolation of lysergic acid alkaloids from the Mexican drug ololiuqui (*Rivea corymbosa* (L.) Hall.f.)]. Experientia 1960;46:414. [German]

4. Taber WA, Heacock RA. Location of ergot alkaloid and fungi in the seed of *Rivea corymbosa* (L.) Hall. f., "ololiuqui". Can J Microbiol 1962;8:137–143.

5. Niwaguchi T, Inoue T. Chromatographic separation of lysergic acid amide and isolysergic acid amide in morning glory seeds. J Chromatogr 1969;43:510–512.

6. Der Marderosian A. Psychotomimetics and their abuse. Am J Pharm Sci Support Public Health 1968;140:83–96.

7. Der Marderosian A, Cho E, Chao J-M. The isolation and identification of the ergoline alkaloids from *Ipomoea muelleri*. Planta Med 1974;25:6–16.

8. Hylin JW, Watson DP. Ergoline alkaloids in tropical wood roses. Science 1965;148:499–500.

9. Farnsworth NR. Hallucinogenic plants. Science 1968;162:1086–1092.

10. Markert A, Steffan N, Ploss K, Hellwig S, Steiner U, Drewke C, et al. Biosynthesis and accumulation of ergoline alkaloids in a mutualistic association between *Ipomoea asarifolia* (Convolvulaceae) and a clavicipitalean fungus. Plant Physiol 2008;147:296–305.

11. Der Marderosian A. Psychotomimetic indoles in the Convolvulaceae. Am J Pharm 1967;139:19–26.

12. Genest K. Changes in ergoline alkaloids in seeds during ontogeny of *Ipomoea violacea*. J Pharm Sci 1966;55:1284–1288.

13. Genest K. The effect of gibberellic acid treatment on the alkaloid content of mature *Ipomoea violacea* L. seeds. Experientia 1966;22:681–682.

14. Weber JM, Ma TS. Microchemical investigation of medicinal plants. XV. Quantitation of total alkaloid content in the leaves of *Ipomoea violacea* (Morning Glory) via spectrophotofluorimetry. Mikrochim Acta 1976;(6 Pt 1):581–588.

15. Brown JK, Malone MH. "Legal highs"-Constituents, activity, toxicology, and herbal folklore. Clin Toxicol 1978;12:1–31.

16. Chao J-M, Der Marderosian AH. Ergoline alkaloidal constituents of Hawaiian baby wood rose, *Argyreia nervosa* (Burm. f.) Bojer. J Pharm Sci 1973;62:588–591.

17. Shawcross WE. Recreational use of ergoline alkaloids from *Argyreia nervosa*. J Psychoactive Drugs 1983;15:251–259.

18. Ma C, Bi K, Zhang M, Su D, Fan X, Ji W, Wang C, Chen X. Toxicology effects of morning glory seed in rat: a metabonomic method for profiling of urine metabolic changes. J Ethnopharmacol 2010;130:134–142.

19. Osmond H. Ololiuqui: the ancient Aztec narcotic remarks on the effects of *Rivea corymbosa* (Ololiuqui). J Ment Sci 1955;101:526–537.

20. Whelan FJ, Bennett FW, Moeller WS. Morning glory seed intoxication: a case report. J Iowa Med Soc 1968;57:946–948.

21. Gertsch JH, Wood C. Case report: an ingestion of Hawaiian baby woodrose seeds associated with acute psychosis. Hawaii Med J 2003;62:127, 129.

22. Al-Assmar SE. The seeds of the Hawaiian baby woodrose are a powerful hallucinogen. Arch Intern Med 1999;159:2090.

23. Vogel WH, Carapellotti RA, Evans BD, Der Marderosian A. Physiological disposition of isoergine [from *Argyreia nervosa* (Burm. F.) Bojer Convolvulaceae] and its effects on the conditioned avoidance response in rats. Psychopharmacologia (Berl.) 1972;24:238–242.

24. Ingram AL. Morning glory seed reaction. JAMA 1964;190:107–108.

25. Fink PJ, Goldman MJ, Lyons I. Morning glory seed psychosis. Arch Gen Psychiatry 1966;15:209–213.

26. Cohen S. Suicide following morning glory seed ingestion. Am J Psychiatry 1964;120:1024–1025.

27. Göpel C, Maras A, Schmidt MH. [Hawaiian baby rose wood: case report of an *Argyreia nervosa* induced toxic psychosis]. Psychiatr Prax 2003;30:223–224. [German]

28. Borsutzky M, Passie T, Paetzold W, Emrich HM, Schneider U. [Hawaiian baby wood rose: (Psycho-) Pharmacological effects of the seeds of *Argyreia nervosa*. A case-orientated demonstration]. Nervenarzt 2002; 73:892–896. [German]

29. Brackett JW Jr, Carter WA, Harding DM, Dougherty PM. Identification of seeds of *Ipomea purpurea* (morning glory family reported to have psychotomimetic properties) by paper chromatography. J Forensic Sci 1966;6:90–96.

30. Weber JM, Ma TS. Microchemical investigations of medicinal plants. XIV. Identification of the alkaloids in the leaves of *Ipomoea violacea* using preparative thin layer chromatography and solid probe mass spectrometry. Mikrochim Acta 1976;(2–3 Pt 1):227–242.

31. Weber JM, Ma TS. Microchemical investigations of medicinal plants. XIII. Separation of the alkaloids in the leaves of *Ipomoea violacea* using thin layer chromatography. Mikrochim Acta 1976;(2–3 Pt 1):193–225.

32. Björnstad K, Beck O, Helander A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci 2009;877:1162–1168.

33. Klinke HB, Muller IB, Steffenrud S, Dahl-Sorensen R. Two cases of lysergamide intoxication by ingestion of seeds from Hawaiian baby woodrose. Forensic Sci Int 2010;194:e1–e5.

34. Björnstad K, Hultén P, Beck O, Helander A. Bioanalytical and clinical evaluation of 103 suspected cases of intoxications with psychoactive plant materials. Clin Toxicol (Phila) 2009;47:566–572.

Chapter 63

PEYOTE [*Lophophora williamsii* (Lem. Ex Salm-Dyck) Coult.] and MESCALINE

HISTORY

The use of peyote for hallucinogenic purposes dates back many centuries, particularly in the southwestern United States and Mexico. Peyote buttons containing psychoactive alkaloids (mescaline) were found in prehistoric Native American caves in Texas dating from 3,780–3,660 BC[1] and in Mexican burial caves dating back to 1,070–810 BC.[2] The Franciscan missionary, Bernardino de Sahagun documented the use of peyote by local native Indians during the Spanish conquest of Mexico.[3] The Mescalero Apaches of the Great Plains originated a peyote rite during the 19th century; the use of peyote spread to the Kiowa and Comanche tribes. They believed that God was the Great Spirit, who infused part of his being into peyote; Christ was the man who provided peyote to them when needed.[4] In 1888, the German toxicologist Lewin discovered the ritual use of the peyote cactus by Indian tribes in Mexico.[5] Heffter isolated mescaline from peyote buttons along with three other tetrahydroisoquinoline compounds by 1898,[6] and Spath synthesized mescaline in 1919.[7] During the end of the 19th century, interest in mescaline as a mind-altering drug increased briefly. In 1918, the Native American Church formed; part of the ritual of this church includes the legal use of peyote. Reports on the hallucinogenic effects of peyote appeared in the medical literature during the 1920s.[8] In 1964, the California Supreme Court ruled in People v. Woody that individuals could not be denied the sacramental use of peyote even though state law prohibits the use of the plant. Although federal statutes (American Indian Religious Freedom Act) exempt Native American Church members from federal prosecution of the sacramental use of peyote, litigation continues on issues of membership, equal protection, and due process.[9] Recent US Supreme Court decisions allow states to prohibit the use of peyote for religious purposes.

BOTANICAL DESCRIPTION

Common Name: Peyote, mescal button, mescal

Scientific Name: *Lophophora williamsii* (Lem. ex Salm-Dyck) Coult.

Botanical Family: Cactaceae (cactus)

Physical Description: This small spineless blue-green cactus has a diameter of about 3–10 cm (~1–4 inches) with well-defined ribs and furrows, pinkish white flowers, and a large, cylindrical perennial rootstock. In the center of the spherical portion of each button, tufts of light yellow hairs develop where the flowers later form. The small dome-shaped heads are removed and dried as mescal or peyote buttons. Figure 63.1 displays a specimen of *L. williamsii* with the flower.

Distribution and Ecology: Peyote is indigenous to the dry slopes and rocky cliffs of the Rio Grande Valley in Texas as well as the Mexican plateau in northern and central Mexico.

IDENTIFYING CHARACTERISTICS

The 2 major classes of psychedelic hallucinogens are phenethylamine compounds (e.g., mescaline) and indoleamine compounds (e.g., *d*-lysergic acid diethylamide or LSD). Mescaline (3,4,5-trimethoxy-β-

phenylethylamine, CAS RN: 54-04-6) shares the phenethylamine nucleus with LSD, whereas serotonin and psilocin share the indolethylamine nucleus with LSD as displayed in Figure 63.2.

Of the more than 60 alkaloids in peyote, mescaline is the major hallucinogenic compound. Other potentially active β-phenylethylamine and isoquinoline alkaloids in peyote include anhalamine, anhalidine, anhalinine, anhalonidine, anhalonine, *N*-methylmescaline, pellotine, and hordenine (anhaline).[4] However, these compounds do not share the hallucinogenic properties of mescaline. Mescaline shares hallucinogenic properties with other phenylethylamine compounds including methamphetamine designer drugs (e.g., 3,4-methylenedioxymethamphetamine, ecstasy). The intensely bitter, acrid taste of mescaline distinguishes peyote from the tasteless hallucinogen, LSD. Table 63.1 lists some physiochemical properties of mescaline. Mescaline is moderately soluble in water and soluble in alcohol and chloroform, but not in ether.

FIGURE 63.1. Flowering peyote cactus (*Lophophora williamsii*). (Richard A. Howard Image Collection. Photo courtesy of Smithsonian Institution.)

Form

Peyote buttons are round fleshy tops from the cactus that are sliced and dried for prolonged storage. The dried mescal buttons used in religious ceremonies are transverse slices of the crown of the cactus. Forms of peyote included peeled fresh buttons, dried or ground powder, steeped tea, and dried whole buttons. Crystalized mescaline is distributed illegally in the form of a capsule or pill.

EXPOSURE

Sources

ORIGIN

In addition to peyote, several South American cactus species contain mescaline including the large columnar San Pedro cactus of Andean slopes in Ecuador, Peru, northern Chile, and Bolivia [*Echinopsis pachanoi* (Britton & Rose) Friedrich & G. D. Rowley, formerly known as *Trichocereus pachanoi* Britton & Rose], Dona Ana or nipple beehive cactus [*Coryphantha macromeris* (Engelm.) Lem.], and the candelabra-like Peruvian cactus (*Trichocereus peruvianus* Britton & Rose, Peruvian torch). The false peyote (*Ariocarpus retusis* Scheidw.) of Mexico contains few or no hallucinogenic compounds.[10] Several other members of the cactus family contain structurally related phenylethylamine derivatives. Although the San Pedro cactus and

TABLE 63.1. Physiochemical Properties of Mescaline.

Physical Property	Value
Melting Point	35.5°C (~96°F)
pKa Dissociation Constant	9.56
log P (Octanol-Water)	0.78
Water Solubility	8.41E + 04 mg/L (25°C/77°F)
Vapor Pressure	3.22E-04 mm Hg (25°C/77°F)

FIGURE 63.2. Chemical structures of serotonin (5-HT), psilocin, *d*-lysergic acid diethylamide (LSD), and mescaline. The A, B, and C rings represent the ring structures in LSD that are shared at least in part by serotonin, mescaline, and psilocin. Mescaline shares the phenethylamine nucleus with LSD.[28]

peyote both contain mescaline, the latter contains other mescaline-like and tetrahydroisoquinoline alkaloids (anhalonidine, anhalonine, lophophine, lophophorine, 3,4-methylenedioxyphenethylamine or MDPEA, *N,N*-dimethyl-3,4-methylenedioxyphenethylamine or lobivine, pellotine).[11] Although some of these alkaloids (e.g., lophophine) may be psychoactive, the contribution of these minor alkaloids to psychomimetic effects is unclear because of their relatively low concentrations. The Dona Ana cactus also produces a variety of methylated catecholamine derivatives of which the phenylethylamine normacromerine (CAS RN: 5653-66-7, *N*-demethylmacromerine) is the most abundant. Other β-hydroxyphenethylamine compounds include macromerine (CAS RN: 2970-95-8, *N,N*-dimethyl-3,4-dimethoxy -β-hydroxyphenethylamine), *N*-formylnormacromerine, metanephrine, *N*-methylmetanephrine, synephrine, and *N*-methyltyramine.[12] Although animal studies suggest that some of these phenethylamine compounds are psychoactive, mescaline remains the most potent hallucinogen isolated from the cactus family to date.[13]

COMPOSITION

The estimated total alkaloid content of dried peyote buttons is about 3.7% compared with 0.41% for fresh peyote buttons with mescaline representing ~6% of the alkaloid fraction.[7] Each button contains ~50–100 mg of mescaline.[30] In a study of 13 mescaline-containing specimens of *L. williamsii*, the mescaline concentration ranged from 12.7–48.3 mg/g.[14] Other *Lophophora* specimens did not contain detectable concentrations of mescaline. Specimens of *E. pachanoi* usually contain higher concentrations of mescaline than other *Echinopsis* species. The mescaline content of 7 specimens of *E. pachanoi* ranged from 0.40–4.7% dry weight as measured by high performance liquid chromatography with mass-selective detection in electron ionization mode.[15] The mescaline content of 6 specimens of the San Pedro cactus (*Echinopsis pachanoi*) from Swiss flower shops, shopping centers, and private collections varied from 0.11–2.37% dry weight as measured by high performance liquid chromatography with photodiode array detection.[16]

IMPURITIES

Purported samples of peyote or mescaline may contain *d*-lysergic acid diethylamide (LSD) as a surreptitious additive. Analysis of 50 California samples reported to contain mescaline indicated that mescaline was present in only 17% of the samples.[17]

946

Methods of Use

The primary route of exposure to peyote and mescaline is oral, rarely mescaline powder is insufflated. In a 12-year retrospective review of the California Poison Control System data, 31 cases of peyote or mescaline exposure were retrieved with 97% oral and 3% nasal exposures.[18] The Native American Church (peyote religion) is a Navajo revitalization movement that uses peyote as a sacrament and medicinal herb to treat a variety of physical and psychologic conditions.[19] Consumption of peyote usually occurs during an all-night communal healing ritual called a peyote meeting. A celebrant (*Road Man*) typically leads the participants during these rituals that often involve personal or family crises. Beginning at nightfall, the participants consume peyote in the form of a powder or a tea. The ritual includes singing, playing of musical instruments, and praying until dawn. Addiction to peyote is unusual. Preliminary data from studies of illicit (i.e., nonceremonial) use of peyote suggests that illicit peyote use among American Indian adolescents with serious substance abuse disorders is uncommon.[20]

DOSE EFFECT

The clinical pattern following the administration of 5 mg mescaline/kg, 1 μg LSD, and 150 μg psilocybin/kg is similar, although the intensity and duration of the psychomimetic effects of mescaline is somewhat greater at these doses.[21] Mescaline has the lowest potency among orally active naturally occurring hallucinogens.[22] A typical adult mescaline dose is about 200 mg (5–7 mg/kg) with average doses ranging up to 500 mg. This dose correlates to about 3–8 peyote buttons and initially produces nausea, tremor, and diaphoresis followed in 1–2 hours by a dream-like state with kaleidoscopic visual illusions before sleep occurs.[4] In animal studies, mescaline doses exceeding 20–60 mg/kg produce hypotension, bradycardia, and respiratory depression.[23] In humans, fatalities usually result from trauma secondary to distorted sensory perception rather than intoxication.[24]

TOXICOKINETICS

There are limited data on mescaline toxicokinetics. The rapid onset of symptoms following the ingestion of peyote indicates that gastrointestinal absorption of mescaline begins soon after swallowing.[21] Following the oral administration of 5–6 mg mescaline/kg to adult volunteers, the biologic half-life of mescaline was about 6 hours with 87% of the dose of mescaline eliminated in the urine within 24 hours.[25,26] During this study, the

renal excretion of unchanged mescaline accounted for 55–60% of the administered dose, whereas the major metabolite (3,4,5-trimethoxyphenylacetic acid) accounted for about 27–30%. *N*-Acetyl-*β*-(3,4,dimethoxy-5-hydroxyphenyl) ethylamine and *N*-acetyl mescaline were minor metabolites. Unlike some other psychoactive amphetamine derivatives (e.g., *p*-methoxyamphetamine, PMA), mescaline does not interact significantly with CYP2D6.[27] Substrates for this isoenzyme include tricyclic antidepressants, lipophilic β-adrenoceptor blockers, and amphetamine.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Mescaline belongs to the phenethylamine class of hallucinogens that includes the methamphetamine-related designer drugs. Along with the other major class of psychedelic hallucinogens (i.e., indoleamine compounds including LSD), mescaline shares an affinity for the 5-HT$_{2A}$ serotonin receptor subtype, where experimental studies in rats suggest that mescaline acts as a partial agonist.[28] Although these receptor subtypes occur in multiple regions of the brain (olfactory tubercle and bulb, facial nucleus, nucleus accumbens), most of these receptors reside in the cerebral cortex.[29] Stimulation of these receptor subtypes may alter glutamatergic excitatory postsynaptic potentials in the pyramidal cells of the cerebral cortex, but the exact mechanism of action remains undefined.

CLINICAL RESPONSE

The ingestion of peyote (mescaline) produces the rapid onset (<1 h) of mild gastrointestinal distress (nausea, rarely vomiting and diarrhea).[3] Autonomic signs (mydriasis, mild tachycardia, elevated blood pressure, diaphoresis, tremor) and alteration of perception, thought, and feeling follow the gastrointestinal phase.[18] The bitter, acrid taste of peyote is often associated with nausea and vomiting in the novice user, particularly with large doses.[30] Nystagmus, headache, ataxia, and hyperreflexia may also occur. After the gastrointestinal phase subsides, a sensory phase begins, manifested by vivid visual hallucinations that reach a peak about 3–4 hours after ingestion. Other stimulatory effects include euphoria, a general feeling of well-being, and feelings of physical power.[31] Auditory hallucinations are uncommon with mescaline intoxication. Although the sensorium remains clear, emotional lability, anxiety, agitation, and panic reactions predispose intoxicated patients to self-inflicted or accidental trauma. Rare complications associated with the use of peyote include the development of severe gastrointestinal bleeding from Mallory-Weiss

tear[32] and psychosis with sleep deprivation.[33] The latter effect was reported in a 54-year-old Native American man, who became convinced he was hunted by animal spirits within a few hours of ingesting peyote juice during a healing ceremony. These psychotic symptoms persisted for 2 weeks with complete sleep deprivation, but resolved following trazodone therapy and sleep.

Medically related fatalities secondary to the ingestion of peyote are rare; death during peyote intoxication usually results from trauma secondary to altered perceptions.[24] Symptoms of acute peyote intoxication typically resolve ~6–12 hours after ingestion. Peak psychologic effects occur about 1.5–2 hours after injection of mescaline, and most of the effects resolved within 9 hours. A case report associated development of botulism with the ingestion of peyote contaminated with botulism B toxin.[34] The ceremonial tea was prepared from dried alkaline-ground peyote buttons stored in a closed jar for 2 month under refrigeration.

There are limited data on the reproductive effects of chronic peyote use. A controlled study of members of the Navajo Native American Church, where peyote use is legal, did not detect residual psychologic or cognitive effects with long-term peyote use.[35] Controlled studies of habitual peyote users among Mexican Indians with a long cultural tradition of religious peyote consumption detected no increased incidence of chromosomal aberration in the peripheral blood lymphocytes.[36] Daily use of mescaline produces tolerance to the effects of mescaline as well as cross-tolerance to LSD.[3] However, the tolerance regresses rapidly and disappears about 3–4 days after cessation of use.

DIAGNOSTIC TESTING

Analytic Methods

Older immunoassays do not usually detect the presence of mescaline in urine drug of abuse screens.[37] Methods for the quantitation of mescaline in biologic samples include high performance liquid chromatography with photodiode array detection,[16] gas chromatography with nitrogen phosphorus detection after liquid-liquid extraction using butyl chloride,[38] liquid chromatography/tandem mass spectrometry,[39] cation-exchange liquid chromatography,[40] and gas chromatography/mass spectrometry in selected ion-monitoring (SIM) mode after solid phase extraction with C8 and cation exchange cartridges.[41] The precision (i.e., coefficient of variation) for the latter method in plasma was ≤20%. The limit of detection for mescaline in urine using liquid chromatography/tandem mass spectrometry was 3–5 ng/mL with an accuracy <3%. The use of gas chromatography/mass spectrometry in SIM mode allows the quantitation of

mescaline in hair in the range of 0.08 ng/mg with inter-day accuracy between -12.7% and 11.6%.[42]

Biomarkers

The mean concentration of mescaline in whole blood samples from 12 volunteers given 500 mg mescaline orally was 3,800 ng/mL at 2 hours and 1,500 ng/mL at 7 hours after dosing as measured by total radioactivity.[25] In a study of 11 volunteers receiving 5 mg mescaline/kg intravenously, the mean mescaline concentration in blood samples drawn during peak effects 2 hours after dosing was 2,100 ng/mL.[43] The postmortem mescaline concentration in blood samples from an individual who deliberately jumped from a 600 ft cliff while under the influence of mescaline was 9,700 ng/mL.[24] The mescaline concentration in a postmortem femoral blood sample from a 53-year-old man who was murdered while participating in a peyote ceremony was 2,950 ng/mL.[38]

Abnormalities

In a study of 12 healthy volunteers administered 0.5 g mescaline, functional brain imaging with 99mTc-HMPAO single photon emission computed tomography (SPECT) demonstrated increase regional blood flow in the anterior cerebrum, particularly in the right anterior cortical regions.[44] There was no increased blood flow in the subcortical (limbic) structures. In a study of long-term peyote users from the Navajo Native American Church, total lifetime peyote use did not correlate to neuropsychologic performance on tests of memory, attention, or executive functions.[35]

TREATMENT

Management of peyote intoxication is similar to the treatment for LSD intoxication. Placing the patient in a quiet, dark environment with calm reassurance provides the best setting. Because of the rapid absorption of mescaline, gastrointestinal decontamination is usually unnecessary unless indicated by the concomitant ingestion of another drug within 1 hour prior to presentation. Benzodiazepines (diazepam, lorazepam) are the treatment of choice if the patient does not respond to a calm, reassuring environment.

References

1. El-Seedi HR, De Smet PA, Beck O, Possnert G, Bruhn JG. Prehistoric peyote use: alkaloid analysis and radiocarbon dating of archaeological specimens of *Lophophora* from Texas. J Ethnopharmacol 2005;101:238–242.
2. Bruhn JG, Lindgren JE, Holmstedt B. Peyote alkaloids: Identification in a prehistoric specimen of *Lophophora* from Coahuila, Mexico. Science 1978;199:1437–1438.
3. Kapadia GJ, Fayez M. Peyote constituents: chemistry, biogenesis, and biological effects. J Pharm Sci 1970;59: 1699–1727.
4. Der Marderosian A. Current status of hallucinogens in the Cactaceae. Am J Pharm Sci Support Publ Health 1966;138:204–212.
5. Gouzoulis-Mayfrank E, Hermle L, Thelen B, Sass H. History, rationale and potential of human experimental hallucinogenic drug research in psychiatry. Pharmacopsychiatry 1988;31(suppl):63S–68S.
6. Ellis H. Mescal: a new artificial paradise. Contemp Rev 1898;73:130–141.
7. Kapadia GJ, Fayez MB. The chemistry of peyote alkaloids. Lloydia 1973;36:9–35.
8. [No authors listed]. Mescal. Br J Ophthalmol 1929;13: 69–70.
9. Bullis RK. Swallowing the scroll: legal implications of the recent Supreme Court peyote cases. J Psychoactive Drugs 1990;22:325–332.
10. Pardanani HH, McLaughlin JL, Kondrat RW, Cooks RG. Cactus alkaloids. XXXVI. Mescaline and related compounds from *Trichocereus peruvianus*. Lloydia 1977;40: 585–590.
11. Bruhn JG, El-seedi HR, Stephanson N, Beck O, Shulgin AT. Ecstasy analogues found in cacti. J Psychoactive Drugs 2008;40:219–222.
12. Keller WJ, McLaughlin JL, Brady LR. Cactus alkaloids. XV. β-phenethylamine derivatives from *Coryphantha macromeris* var. *runyonii*. J Pharm Sci 1978;62:408–411.
13. Keller WJ. Catecholamine metabolism in a psychoactive cactus. Clin Toxicol 1980;10:233–243.
14. Argane M, Sasaki Y, Nakajima J, Fukumori N, Yoshizawa M, Suzuki Y, et al. Peyote identification on the basis of differences in morphology, mescaline content, and trnL/trnF sequence between *Lophophora williamsii* and *L. diffusa*. J Nat Med 2011;65:103–110.
15. Ogunbodede O, McCombs D, Trout K, Daley P, Terry M. New mescaline concentrations from 14 taxa/cultivars of *Echinopsis* spp. (Cactaceae) ("San Pedro") and their relevance to shamanic practice. J Ethnopharmacol 2010;131:356–362.
16. Helmlin H-J, Brenneisen R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection. J Chromatogr 1992;593:87–94.
17. Renfroe CL, Messinger TA. Street drug analysis: an eleven year perspective on illicit drug alteration. Semin Adolesc Med 1985;1:247–257.
18. Carstairs SD, Cantrell FL. Peyote and mescaline exposures: a 12-year review of a statewide poison center database. Clin Toxicol (Phila) 2010;48:350–353.

19. Calabrese JD. Spiritual healing and human development in the Native American Church: toward a cultural psychiatry of peyote. Psychoanal Rev 1997;84:237–255.

20. Fickenscher A, Novins DK, Manson SM. Illicit peyote use among American Indian adolescents in substance abuse treatment: a preliminary investigation. Subst Use Misuse 2006;41:1139–1154.

21. Hollister LE, Hartman AM. Mescaline, lysergic acid diethylamide and psilocybin: comparison of clinical syndromes, effects on color perception and biochemical measures. Compr Psychiatry 1962;3:235–241.

22. Nichols DE. Hallucinogens. Pharmacol Ther 2004;101: 131–181.

23. Patel AR. Mescaline and related compounds. Fortschr Arzneimittelforsch 1968;11:11–47.

24. Reynolds PC, Jindrich EJ. A mescaline associated fatality. J Anal Toxicol 1985;9:183–184.

25. Charalampous KD. Comparison of metabolism of mescaline and 3,4-dimetoxyphenylethylamine in humans. Behav Neuropsychiatry 1971;2:26–29.

26. Charalampous KD, Walker KE, Kinross-Wright JK. Metabolic fate of mescaline in man. Psychopharmacologia 1966;9:48–63.

27. Wu D, Otton V, Inaba T, Kalow W, Sellers EM. Interactions of amphetamine analogs with human liver CYP2D6. Biochem Pharmacol 1997;53:1606–1612.

28. Aghajanian GK, Marek GJ. Serotonin and hallucinogens. Neuropsychopharmacology 1999;21(suppl 2):16S–23S.

29. Monte AP, Waldman SR, Marona-Lewicka D, Wainscott DB, Nelson DL, Sanders-Bush E, Nichols DE. Dihydrobenzofuran analogues of hallucinogens. 4. Mescaline derivatives. J Med Chem 1997;40:2997–3008.

30. Schwartz RH. Mescaline: a survey. Am Fam Physician 1988;37:122–124.

31. Leikin JB, Krantz AJ, Zell-Kanter M, Barkin RL, Hryhorczuk DO. Clinical features and management of intoxication due to hallucinogenic drugs. Med Toxicol Adverse Drug Exp 1989;4:324–350.

32. Nolte KB, Zumwalt RE. Fatal peyote ingestion associated with Mallory-Weiss lacerations. West J Med 1999;170:328.

33. Lu BY, Woofter C, Escalona R. A case of prolonged peyote-induced psychosis resolved by sleep. J Clin Psychiatry 2004;65:1433–1434.

34. Hashimoto H, Clyde VJ, Parko KL. Botulism from peyote. N Engl J Med 1998;339:203–204.

35. Halpern JH, Sherwood AR, Hudson JI, Yurgelun-Todd D, Pope HG Jr. Psychological and cognitive effects of long-term peyote use among Native Americans. Biol Psychiatry 2005;58:624–631.

36. Dorrance DL, Janiger O, Teplitz RL. Effect of peyote on human chromosomes. Cytogenic study of the Huichol Indians of northern Mexico. JAMA 1975;234:299–302.

37. Cody JT. Cross-reactivity of amphetamine analogues with Roche Abuscreen radioimmunoassay reagents. J Anal Toxicol 1990;14:50–53.

38. Henry JL, Epley J, Rohrig TP. The analysis and distribution of mescaline in postmortem tissues. J Anal Toxicol 2003;27:381–382.

39. Björnstad K, Helander A, Beck O. Development and clinical application of an LC-MS-MS method for mescaline in urine. J Anal Toxicol 2008;32:227–231.

40. Laussmann T, Meier-Giebing S. Forensic analysis of hallucinogenic mushrooms and khat (*Catha edulis* Forsk) using cation-exchange liquid chromatography. Forensic Sci Int 2010;195:160–164.

41. Habrdova V, Peters FT, Theobald DS, Maurer HH. Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. J Mass Spectrom 2005;40:785–795.

42. Kim JY, Jung KS, Kim MK, Lee JI, In MK. Simultaneous determination of psychotropic phenylalkylamine derivatives in human hair by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 2007;21: 1705–1720.

43. Mokrasch LC, Stevenson I. The metabolism of mescaline with a note on correlations between metabolism and psychological effects. J Nerv Ment Dis 1959;129:177–183.

44. Hermle L, Funfgeld M, Oepen G, Botsch H, Borchardt D, Gouzoulis E, Fehrenbach RA, Spitzer M. Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. Biol Psychiatry 1992;32:976–991.

Chapter 64

PSILOCYBIN and HALLUCINOGENIC MUSHROOMS

HISTORY

The Aztecs and neighboring tribes used ceremonial mushrooms called teonanácatl (God's flesh) in religious rites before the arrival of the Spaniards in the New World;[1] medieval Spanish books from the 16th and 17th centuries mentioned the use of small gill fungi ("teonanácatl") by North American Indians for ritual and medicinal purposes.[2] Small stone statues of fungi from ancient Mayan ruins in Guatemala suggest the use of hallucinogenic mushrooms before the time of Christ.[3,4] The use of mushrooms by North American Indians to produce mystical revelations during religious ceremonies continued into the 20th century. In the late 1950s, Wasson rediscovered the mushroom cult in Southern Mexico. *Psilocybe mexicana* R. Heim was identified as the active component of teonanácatl;[5] subsequently, Heim et al provided botanical descriptions of several species of hallucinogenic *Psilocybe* fungi.[6] In 1958, Hofmann et al isolated 2 hallucinogenic components (i.e., psilocybin, psilocin) from mushrooms used in Mazatec Indian ceremonies in the state of Oaxaca, Mexico.[7] These indole compounds have LSD-like properties and produce alterations of autonomic function, motor reflexes, behavior, and perception. Hoffer and Osmond elucidated the chemical structures of psilocybin and psilocin in 1967. In the 1960s, recreational ingestion of psilocybin-containing mushrooms was popularized in the western United States by Aldous Huxley and Carlos Castaneda. Later, recreational use of these substances spread to Australia followed by the United Kingdom and then to the rest of Europe.[8] In the late 1990s, hallucinogen use (mescaline, psilocybin) increased significantly in high school students.[9]

BOTANICAL DESCRIPTION

The three most important genera of mushrooms containing psilocybin are *Psilocybe*, *Panaeolus*, and *Gymnopilus* as listed in Table 64.1.[10] The best-known European psychoactive fungi is *Psilocybe semilanceata* (Fries) Kummer, whereas the most common hallucinogenic mushroom in the United States is *Psilocybe cubensis* (Earle) Singer and, to a lesser extent, *Psilocybe stuntzii* Guzman and Ott and *Panaeolus subbalteatus* (Berkley et Broome) Sacc.[11] The principal hallucinogenic mushrooms in North America grow primarily in the Pacific Northwest, Hawaii, Texas, and Florida, usually on animal manure in pastures and grain fields. Psychoactive mushrooms in the Hawaiian Islands that contain psilocybin include *Copelandia anomala* (Murrill) Singer*, Copelandia bispora* (Malençon & Bertault) Singer & R.A. Weeks, *Copelandia cambodginiensis* (Ola'h & R. Heim) Singer & R.A. Weeks, *Copelandia cyanescens* (Berk. & Broome) Singer, *Copelandia tropicalis* (Ola'h) Singer & R.A. Weeks (*Panaeolus tropicalis*), and *Panaeolus subbalteatus* (Berkley et Broome) Sacc.[12] The use of fungi as a psychoactive agent is uncommon in South America and Asia. Wavy caps refers to *Psilocybe cyanescens* Wakef. (*Psilocybe subaeruginosa* Cleland) as displayed in Figure 64.1.

Common Name: Gold cap, cubes, blue legs, golden tops, common large psilocybe

Scientific Name: *Psilocybe cubensis* (Earle) Singer

Botanical Family: Strophariaceae

Physical Description: These white to pale yellow mushroom have broad (1.5-8 cm/~0.5–1.5 inches)

FIGURE 64.1. Wavy caps of the genus *Psilocybe*. (Photo courtesy of *Drug Identification Bible*)

TABLE 64.1. Major Psilocybin-Containing Mushrooms.

Genus	Species
Psilocybe	*argentipes, bohemica, cubensis, cyanescens, fimetaria, liniformans, mexicana, pelliculosa, pseudobullacea, semilanceata, serbica, strictipes, sylvatica, subcubensis*
Panaeolus	*ater, cyanescens, papilionaceus, semilanceatus, subbalteatus*
Panaeolina	*foenisecii*
Gymnopilus	*junonius (spectabilis)*
Pluteus	*nigroviridis, salicinus*
Inocybe	*aeruginascens, coelestium, corydalina, haemacta, tricolor*

convex caps and smooth stems measuring up to 1.5 cm (~0.5 inch) in height. The elliptical spores are dark purple brown to violet brown and about 8–10 μm × 11–17 μm in size. Characteristically, the flesh turns blue with handling.

Distribution and Ecology: The distribution of this mushroom occurs across Central and South America, India, Southeast Asia, Western Australia, and Oceania.

Common Name: Liberty cap

Scientific Name: *Psilocybe semilanceata* (Fries) Kummer

Botanical Family: Strophariaceae

Physical Description: This small, dark chestnut brown to pale yellow mushroom has a small (0.5–2.5 cm), broad cap with a sharply conical top and an indented (striate) margin. The relatively thin stem is smooth with slight thickening at the base.

The gills are violet-grey to deep purple-brown with elliptical spores (10–14 μm × 6–8 μm) and a dark purple-brown spore mass.

Distribution and Ecology: This popular recreational hallucinogenic mushroom appears in pastures, meadows, and occasionally in lawns in the US Pacific Northwest.[13,14] In Europe, *Psilocybe semilanceata* is the most widely distribute psilocybin-containing fungus growing areas in countries bordering the Atlantic Ocean. This fungus also grows in Central Europe in pastures and grassy fields that contain waste products.

Common Name: Blue-staining Panaeolus, blue meanies, gold caps, gold tops

Scientific Name: *Panaeolus cyanescens* (Berkley et Broome) Sacc.

Botanical Family: *Incertae sedis* (uncertain placement), Order: Agaricales

Physical Description: This widely distributed mushroom has a thin, whitish to dark brown stalk 50–70 mm × 2.5–5 mm and a smooth surface covered with white powder. The broad (1–4 cm) cap is convex with dark purple to black spores.

Distribution and Ecology: This mushroom is widely distributed in tropical and subtropical regions (e.g. Gulf coast, Hawaii, Mexico, India, Australia, Thailand, Philippines), particularly in or near dung in pastures.

Common Name: Belted Panaeolus

Scientific Name: *Panaeolus subbalteatus* (Berkley et Broome) Sacc.

Botanical Family: *Incertae sedis* (uncertain placement), Order: Agaricales

Physical Description: The brown to reddish brown pileus is 13–50 mm in length, and the stalk is rather stout measuring 35–90 mm × 3–8 mm. The gills are moderately broad and lanceolate. The bluing of flesh with trauma distinguishes *P. subbalteatus* from other species in this genus.[15]

Distribution and Ecology: *Panaeolus subbalteatus* grows throughout North and South America, Africa, and Europe, particularly in manure, compost, and fertilized lawns during warm weather.

Common Name: Giant Gymnopilus, big laughing mushroom

Scientific Name: *Gymnopilus junonius* (Fr.) P.D. Orton (*G. spectabilis*)

Botanical Family: Strophariaceae

Physical Description: The large, broad, convex cap reaches 40 cm with a smooth to silky, dry surface and small scales. The cap is bright yellow-orange initially, but changes to a rust-orange with age. The gills are notched to adnate. The stalk is thick and somewhat expanded in the middle. The veil is pale yellow to rusty in color and usually forms a superior ring on the stalk. The spores are elliptical and slightly wrinkled.

Distribution and Ecology: This mushroom appears around stumps and trees, particularly in conifer and hardwood forests in the fall, winter, and early spring. This mushroom is distributed worldwide over the United States, Europe, Japan, Australia, India, North Africa, and South America.

IDENTIFYING CHARACTERISTICS

Structure

The active constituents of hallucinogenic mushrooms are probably indole compounds belonging to a small, unique group of natural products that contain hydroxy or phosphate groups in position 4 of the indole ring and which are derived from tryptamine and the precursor, tryptophan.[11] Psilocybin (CAS RN: 520-52-5, 4-phosphoryloxy-*N*,*N*-dimethyltryptamine) and the active, dephosphorylated metabolite, psilocin (CAS RN: 520-53-6, 4-hydroxy-*N*,*N*-dimethyltryptamine) are the main active ingredients, but the exact role of these compounds and other constituents (phenylethylamine, baeocystin) is not well-defined. Psilocybin and psilocin are structurally similar to tryptamines (bufotenine, harmine, LSD). Figure 64.2 displays the chemical structures of psilocybin, psilocin, and the demethylated psilocybin compound, baeocystin. In addition to psilocybin and baeocystin, the hallucinogenic Central European mushroom, *Inocybe aeruginascens* contains a quaternary ammonium compound, *N,N, N*-trimethyl-4-phosphoryloxytryptamine (CAS RN: 114264-95-8, aeruginascin) that is structurally related to bufotenidine (CAS RN: 487-91-2).[16]

Physiochemical Properties

Psilocybin and psilocin are soluble in methanol and aqueous ethanol, but relatively insoluble in petroleum ether and chloroform. The rapid dephosphorylation of psilocybin to psilocin suggests that psilocybin may be a prodrug.[17] In equimolar amounts, psilocybin and psilocin produce similar hallucinogenic effects, but psilocin is approximately 1½ times more potent hallucinogen than psilocybin.[11] The demethylated psilocybin compounds (baeocystin, norbaeocystin) are serotonin analogues that occur in some *Psilocybe* species.

Psilocybin is colorless, and the incubation of this compound with ceruloplasmin does not cause the uptake of oxygen or the formation of a blue color. Psilocin lacks the stabilizing phosphate group. The oxidation of this compound or the addition of ceruloplasmin or copper oxidase from the gill plates of *Mytilus edulis* (blue mussel) produces strongly blue-colored products.[18]

EXPOSURE

Origin

Psilocybe baeocystis and *Psilocybe semilanceata* contain the demethylated psilocybin compounds, baeocystin and norbaeocystin. Specimens from other *Psilocybe* species [*P. cyanescens, P. cubensis, P. pelliculosa* (A.H. Sm.) Singer & A.H. Sm.*, P. sylvatica* (Peck) Singer & A.H. Sm.*, P. stuntzii)* and genera (*Conocybe smithii* Watling, *Panaeolus subbalteatus*] usually contain smaller concentrations of baeocystin.[19,20] Psilocybin-containing mushrooms are widely available from Internet sources.[21]

PSILOCYBE

In studies of the psilocybin content in mushroom samples from the genus *Psilocybe*, *P. aztecorum* var. bonetii (*P. bonetii* Guzmán), *P. zapotecorum* R. Heim (P. *candidipes*), and *P. stuntzii* (Guzmán and Ott) contained detectable concentrations of psilocybin whereas *P. bolivarii* did not.[22,23] Psilocin concentrations in these

Psilocybin   **Baeocystin**   **Psilocin**

FIGURE 64.2. Chemical structures of psilocybin, psilocin, and baeocystin.

TABLE 64.2. Indole Alkaloid Content (% dry weight) of Cultivated and Natural Mushrooms Following Extraction in Pure Methanol.[31]

Species	Psilocybin	Psilocin	Baeocystin
*Psilocybe semilanceata**	0.98	—	0.34
Psilocybe bohemica	0.85	0.02	0.04
*Psilocybe bohemica**	0.93	0.04	0.02
*Psilocybe cubensis**	0.63	0.11	0.02
*Gymnopilus purpuratus**	0.34	0.29	0.05
Inocybe aeruginascens	0.40	—	0.21
Panaeolus cyanescens	0.32	0.51	0.02

*Cultivated.

samples were not detectable. Other species that contain significant concentrations of psilocybin include *P. semilanceata* (Fries) Kummer, *P. bohemica* Sebek, *P. cubensis* (Earle) Singer, *Gymnopilus purpuratus* (Cooke et Messe) Sing., *Panaeolus cyanescens* (Berkley et Broome) Sacc., and *Inocybe aeruginascens* Babos.[11]

PANAEOLUS

In a study of the psilocybin content in mushroom samples from the genus *Panaeolus*, *P. cambodginiensis* and *P. subbalteatus* (Berkley et Broome) Sacc. contained detectable concentrations of psilocybin whereas *P. foenisecii* (Pers.) J. Schröt. (*Psathyrella foenisecii*) and *Panaeolus papilionaceus* (Bull.) Quél (*P. sphinctrinus*) did not.[22] Psilocin concentrations in these samples were not detectable.

GYMNOPILUS

Species from *Gymnopilus* that contain psilocybin include *G. luteus* (Peck) Hesler, *G. purpuratus*, *Gymnopilus junonius* (*G. spectabilis*), *G. subspectabilis*, *G. validipes* (Peck) Hesler, *G. aeruginosus* (Peck) Singer, and *G. viridans* Murrill.[24] The psilocybin content of some of the species (e.g., *G. junonius*) is highly variable and do not contain detectable amounts of psilocybin as analyzed by high performance liquid chromatography (HPLC; LOD not reported).[25]

Composition

The content of psilocybin and psilocin in hallucinogenic mushrooms depends on species, season, environmental conditions, storage, extraction method, and soil conditions (phosphorus, nitrogen). Experimental studies indicate that psilocybin appears in the initial fruiting phase (mycelium knots); the vegetative portion (mycelium) of the fungus does not contain psychoactive indole alkaloids.[26] Even under rigidly controlled growing conditions, the psilocybin content of individual samples of

Psilocybe cubensis varied by a factor of 4.[27] The alkaloid content of psilocybin-containing mushrooms averages about 1–2% dry weight (water content approximately 89 ± 2%), depending on the species, drying method, analytic procedure, and season.[27] In a study of 74 samples of *P. cubensis* obtained from retail sources, the mean percentage (dry weight) of psilocybin and psilocin were 1.151 ± 0.228 % and 0.126 ± 0.066 %, respectively, as measured by cation-exchange liquid chromatography.[28] The average highest alkaloid content by dry weight (3.00 ± 0.24 %) in this study of several psilocybin-containing mushrooms occurred in specimens of *Panaeolus cyanescens* Berkeley and Broome. In a study of 20 species of mushrooms from 7 genera in the Pacific Northwest United States, the total psilocybin and psilocin concentration by dry weight ranged from 0.1% to about 2% as measured by HPLC.[29]

Table 64.2 lists the indole alkaloid content of psilocybin, psilocin, and baeocystin in samples both from cultivated and naturally grown mushroom species. Generally, the content of psilocybin is substantially higher than psilocin; mushrooms from *Psilocybe semilanceata* and *Psilocybe bohemica* usually contain the highest concentrations of psilocybin.[30,31] The psilocybin and psilocin content in fresh and confiscated samples of *P. semilanceata* were approximately 1.8% and 0.2%, respectively.[32] The baeocystin content was about 0.85%. Typically, the psilocybin content of *Psilocybe cubensis* is about 10–12 mg/g dried mushroom.[33] The average psilocybin concentration in another study of samples of *Psilocybe cubensis* was about 7 mg/10 g fresh weight (7 mg/g dry weight).[34] In 2 series, the psilocybin and psilocin content of samples from *Psilocybe cubensis* varied from approximately 0.17–1.07% dry weight and 0.11–0.42% dry weight, respectively, as measured by HPLC and gas chromatography/mass spectrometry (GC/MS).[31,35] Analysis of specimens of *Psilocybe semilanceata* demonstrated the presence of phenylethylamine at lower concentrations than psilocybin,[36] but the role of phenylethylamine in producing the psychomimetic effects of hallucinogenic mushrooms remains unclear.

Production Processes

The chemical synthesis of psilocybin and psilocin is difficult compared with 5-hydroxy substituted indole derivatives (e.g., bufotenin, serotonin). A reported synthetic route to psilocybin requires 10 steps and has a relatively low yield.[37] Hence, most sources of psilocybin are natural.

Impurities and Profiling

In samples of hallucinogenic mushrooms confiscated by German authorities, the most common mushroom was *Psilocybe cubensis* followed by *Psilocybe semilanceata*, *Panaeolus cyanescens*, and *Psilocybe tampanensis* Guzmán & S.H. Pollock.[33] Table 64.3 outlines the psilocybin and psilocin content of the confiscated mushrooms. Psilocybin and psilocin are illegal drugs classified by the US Drug Enforcement Agency as schedule I (no recognized medical use with high abuse potential).

Psilocybe subcubensis is macroscopically similar to *Psilocybe cubensis*; analysis of a confiscated sample from *P. subcubensis* demonstrated psilocybin concentrations in the cap and stem of 0.86 mg/g dry weight and 0.80 mg/g dry weight, respectively.[38] The content of psilocin was relatively low (0.02 mg/g dry weight and 0.03 mg/g dry weight, respectively).

Methods of Use

Desirable effects of hallucinogenic (magic) mushroom use include feelings of a different perspective, hallucination, joviality, and a sense of being part of the natural surroundings. In a Swedish study of 103 suspected cases of intoxication from psychoactive plants, analysis of urine samples indicated that psilocin was the most frequently detected drug, accounting for 54% of the cases.[39] The source of psilocin for a majority of these individuals was the purchase of hallucinogenic mushrooms over the Internet. Use of these mushrooms is relatively infrequent, but the experience is often intense. In a self-selected convenience sample of hallucinogenic

Table 64.3. Psilocybin and Psilocin Content of Hallucinogenic Mushrooms Confiscated in Germany.[33]

Species	Psilocybin Range*	Psilocin Range*	Sample Size
Psilocybe cubensis	N.D.–1.07	0.01–0.23	18
Psilocybe semilanceata	0.01–0.91	0.01–0.90	9
Psilocybe tampanensis	N.D.–0.19	0.01–0.03	4
Panaeolus cyanescens	0.02–1.15	0.09–0.90	6

Abbreviation: N.D. = nondetectable.
*Reported as % dry weight

mushroom users in Edinburgh and Bristol, 47% of the users reported the ingestion of hallucinogenic mushrooms 4–12 times yearly.[40] Reasons for the infrequent use included the intensity of the experience, anxiety, and paranoia. This study was completed prior to the reclassification of hallucinogenic mushrooms to UK Class A drug (most harm, greatest penalty) in 2005. A cross-sectional survey of dance drug users in the UK prior to the reclassification of psilocybin indicated a lifetime (i.e., ever) and current use (i.e., within last month) prevalence of 48% and 13.7%, respectively.[41]

DOSE EFFECT

The typical recreational dose of psilocybin required to produce desired effects is approximately 5–15 mg with a threshold of about 0.040 mg psilocybin/kg body weight and a range up to approximately 50 mg or 3.5–5 g dry weight (35–50 g, wet weight), depending on the species and psilocybin content. [42,43] In volunteer studies, the ingestion of 0.25 mg psilocybin/kg produced euphoria, sense of grandiosity, visual illusions, difficulty thinking, and impaired functioning.[44] In an double-blind experimental study of 12 volunteers receiving up to 0.25 mg/kg psilocybin (high dose condition), reductions occurred in the speed of voluntary movements and working memory along with feelings of depersonalization and derealization.[45] In a clinical trial of the efficacy of psilocybin in the treatment of 9 patients with obsessive-compulsive disorder, psilocybin doses ranged up to 0.30 mg/kg.[46] Other than transient mild hypertension in one subject, no adverse effects were reported.

The ingestion of psilocybin causes wide variation in the clinical response of different individuals ingesting the same dose. Agitation and hallucination developed after the ingestion of 10 mushrooms by one patient, whereas the ingestion of 200 mushrooms by an unrelated patient produced only abdominal pain.[47] The low concentration of active ingredients in these species limits symptoms after the ingestion of 1–2 mushrooms; relatively large numbers of mushrooms (10–100) are necessary to produce psychomimetic effects depending on the mood, tolerance, setting, and personality of the patient. A case report associated the development of coma, convulsions, and hyperthermia with the ingestion of cooked mushrooms by young children, but there was no laboratory confirmation or quantitation of psilocybin;[48] the clinical course was poorly documented.

TOXICOKINETICS

In a study of 3 fasted volunteers receiving 0.224 mg psilocybin/kg body weight (i.e., about 10–20 mg), the bioavailability of psilocybin was 52.7% ± 20%.[17]
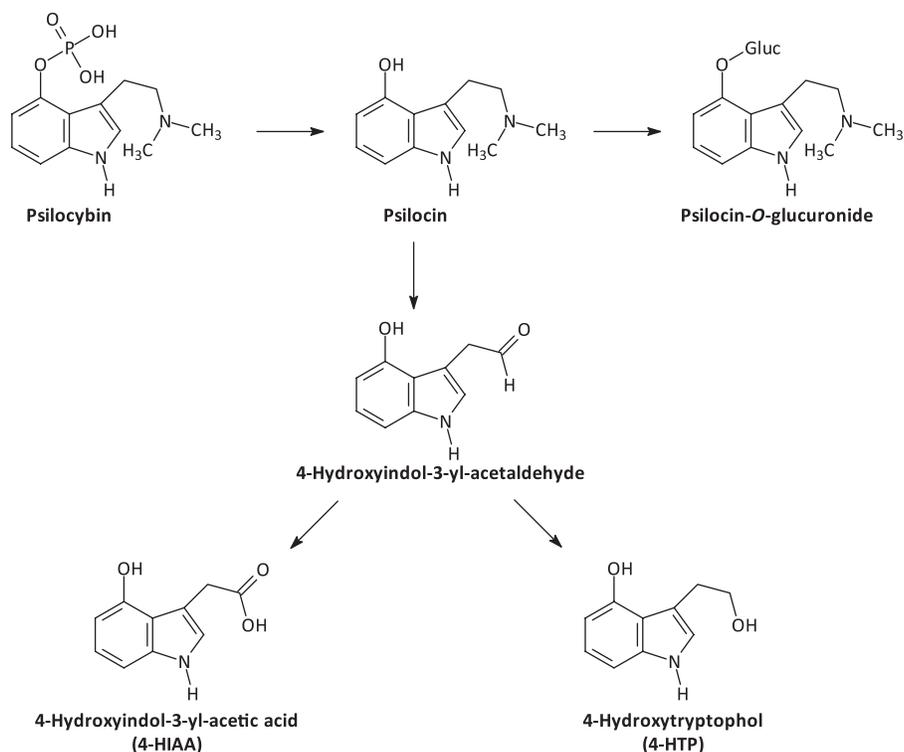
FIGURE 64.3. Psilocybin metabolism.[50]

Psilocybin underwent rapid dephosphorylation to psilocin followed by glucuronidation and renal excretion as the glucuronide conjugate. In this study, peak plasma psilocin concentration occurred at a mean of ~105 ± 37 minutes after psilocybin ingestion. The plasma elimination half-life of psilocin following oral dosing was approximately 2.5 ± 1 hours. Figure 64.3 outlines the metabolism of psilocybin. An alternative metabolic pathway is inactivation of psilocin by monoamine oxidase to 4-hydroxy-tryptopole and 4-hydroxyindole-3-acetic acid. Excretion of psilocin in the urine occurs primarily by psilocybin glucuronidation, catalyzed by UGT1A10 in the small intestine and by UGT1A9 in the liver.[49] Within the first 24 hours after ingesting 0.212 ± 0.025 mg psilocybin/kg body weight, the average amount of unchanged psilocin excreted in urine samples from 8 volunteers was 3.4% ± 0.9% of the applied dose.[50] In a case report involving the ingestion of psilocybin-containing mushrooms, the free and total psilocin concentrations in a urine sample were 230 ng/mL and 1,760 ng/mL, respectively, when analyzed by GC/MS after enzymatic hydrolysis.[51]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Animal studies demonstrate that psilocybin produces mild sympathomimetic stimulation and relatively low toxicity with an intravenous LD_{50} about 2.5 times less than the phenethylamine hallucinogen, mescaline.[52,53] Because of the structural similarity to tryptamines, both psilocybin and psilocin demonstrate high affinity for serotonin receptors, particularly 5-HT$_{2A}$ and to a lesser extent 5-HT$_{1A}$ receptors. Receptor binding studies in rats demonstrate psilocin binds primarily to 5-HT$_{2A}$ receptors (K_i = 6 nM) with lower affinity for the 5-HT$_{1A}$ receptors (K_i = 190 nM).[54] In contrast to another indoleamine hallucinogen, LSD, psilocybin does not directly stimulate dopamine D_2 receptors and most, but not all of the hallucinogenic effects of psilocybin are mediated by the 5-HT$_{2A}$ receptor.[55] Administration of the 5-HT$_{2A}$ antagonist, ketanserin, blocks most of the subjective effects of psilocybin,[56] whereas the intravenous administration of the dopamine D_2 receptor antagonists produces little alteration of the hallucinogenic effects of psilocybin.[57]

Positron emission tomography (PET) studies indicate that psilocybin decreases receptor binding in the caudate nucleus and putamen consistent with an increase in endogenous dopamine concentrations in the striatum.[44] Hallucinations are illusions that the afflicted person believes are real, and these distorted perceptions cause changes in emotions and behavior. Unregulated agonist activity of neurons associated with the processing of visual and emotional information produces hallucination without alteration of state of consciousness.

Hallucinogenic drugs (e.g., psilocybin) induce states of consciousness similar to acute schizophrenic disorders along with deficits in sustained and spatial attention.[58] The sense of the passage of time is slowed, resulting in a subjective overestimation of time intervals.

CLINICAL RESPONSE

The ingestion of hallucinogenic mushrooms containing psilocybin produces clinical features qualitatively similar to LSD and mescaline, but the dose of psilocybin required to produce these symptoms is much higher.[59] Desired subjective effects include giddiness, laughter, euphoria, elation, and visual enhancement and alterations of perceptions (e.g., waves, moving surfaces). A short latent period of ~30–60 minutes precedes the onset of somatic symptoms (facial erythema, mydriasis, headache, tremor, diaphoresis, agitation, euphoria). The onset of clinical effects after the ingestion of water extracts (soup, tea) is more rapid compared with the whole mushroom (i.e., about 10 minutes vs. ~20–40 minutes, respectively). Common clinical features associated with the ingestion of hallucinogenic mushrooms include disturbances of affect, body image (depersonalization), and perception; mydriasis; blurred vision; hyperreflexia; and tachycardia. Clinical signs (e.g., tachycardia, hypertension, hyperreflexia) are usually mild.[60] Compared with LSD, the ingestion of psilocybin frequently produces less intensive depersonalization, milder somatic symptoms, and more intense spatial illusions.[11] Alterations of perception (i.e., distortion of shapes and colors) occur in most patients, but true visual hallucinations are uncommon. Auditory hallucinations occur very rarely, although a heightened awareness of sound and paresthesias do develop during psilocybin intoxication. Drowsiness and a dream-lacking sleep often follow the perceptual alterations. The potential for self-inflicted or accidental trauma during periods of altered behavior represents the greatest health hazard.[61]

Emergency department visits are uncommon after the ingestion of psilocybin-containing mushrooms. In those who do seek medical assistance, typical presentation occurs 2–4 hours after ingestion and includes symptoms of nausea, vomiting, headache, lightheadedness, weakness, anxiety, and confusion.[62] Other adverse clinical effects of hallucinogenic mushrooms include fever, mydriasis, tachycardia, lightheadedness, ataxia, anxiety, fear, apprehension, hallucinations, muscle weakness, compulsive movements, and stupor.[63,64] Adverse psychologic reactions during psilocybin intoxication include depersonalization, derealization, dysphoria, disorientation, agitation, delirium, and aggressive behavior. Most symptoms resolve within 4–6 hours with some sleep disturbances lasting up to 24 hours; the persistence of dysphoric symptoms beyond 12 hours is rare.[65] Adverse psychiatric symptoms do not usually persist, although some case series report the recurrence of flashbacks and panic attacks within the first 4 months after ingestion.[47,61]

Several case reports associated suspected intravenous administration of psilocybin extract with vomiting, myalgias, hyperpyrexia, hypoxemia, and mild methemoglobinemia; however, the case reports did not include the documentation of psilocybin in serum or urine samples.[66] Rare case reports associate recurrent perceptual distortions with the use of psilocybin-containing mushrooms.[67] These perceptual disturbances include abnormal colored images, intensified trailing colors, distorted geometrical forms, micropsia, and macropsia. Fatalities during intoxication from hallucinogenic mushrooms usually result from altered perception and trauma rather than intoxication, frequently in association with other drugs.[42]

DIAGNOSTIC TESTING

Analytic Methods

Analytic methods for the detection of psilocybin and the less stable metabolite, psilocin, include infrared spectroscopy,[68] ultraviolet spectrophotometry,[68] thin layer chromatography,[69] capillary electrophoresis, GC/MS,[35,70] liquid chromatography/tandem mass spectrometry,[71] and high performance liquid chromatography (HPLC).[72] Limitations of gas chromatographic methods result from the poor volatility of psilocybin and decomposition of psilocybin (i.e., heat-induced loss of phosphate group) during the injection phase; therefore, liquid chromatographic methods are preferred to separation by gas chromatography. The presence of a phosphate ester in psilocybin limits the detection of this compound, but not psilocin by GC/MS. HPLC is the most common analytic technique with detection modes including ultraviolet,[73] fluorescence,[74] electrochemical,[75] voltametric,[76] mass spectrometry, and chemiluminescence.[77] The limit of detection (LOD) for HPLC ranges from 1.2×10^{-8} mol/L (chemiluminescence) to 9.7×10^{-6} mol/L (fluorescence). Other tests for damaged or decomposed samples containing psilocybin include DNA-based testing using amplified fragment length polymorphism.[78] The one-step methanol extraction is a simple, efficient method for removing psilocybin and related compounds from a fungal mass.[30]

Enzymatic hydrolysis of glucuronide conjugates in urine samples and derivation with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide reduces the lower limit of quantitation for psilocin from 200 ng/mL to 10 ng/mL, as measured by GC/MS after solid phase

extraction.[79] Enzymatic hydrolysis of glucuronide conjugates increases the yield of psilocin when compared with acid and alkaline hydrolysis.[71] Psilocin is thermolabile, and degradation of psilocin occurs with storage. In a urine sample stored at 4ºC (~39ºF) for 26 days without preservatives, the psilocin concentration decreased ~44%.[80]

Biomarkers

About 5–6 hours after the ingestion of an estimated dose of 4–5 g hallucinogenic mushrooms, the serum-free and total psilocybin concentrations were 18 ng/mL and 52 ng/mL, respectively.[51] At the time the blood was drawn, the patient had ataxia, slurred speech, mydriasis, disorientation, and lethargy. In a study of 6 volunteers receiving 0.224 mg/ psilocybin/kg body weight, the mean peak plasma psilocin concentration about 1½ hours after ingestion was approximately 8 ± 3 ng/mL.[17] The active metabolite, psilocin is detectable in urine for about 24 hours after ingestion of a medium dose of psilocybin. Enzymatic hydrolysis of urine samples with β-glucuronidase extends the detection time of psilocin in the urine beyond 24 hours after ingestion.[50]

Although urine drugs of abuse screens do not specifically analyze specimens for psilocybin, occasionally urine drug tests are positive for amphetamine following the use of hallucinogenic mushrooms. Anecdotal reports suggest that some polyclonal antibody assay kits detect the consumption of some hallucinogenic mushrooms as positive for the presence of amphetamine. Analysis of urine from the false-positive tests for amphetamine suggested that the presence of phenethylamine account for the false-positive tests.[36] A urine fluorescence polarization immunoassay for amphetamine/methamphetamine was positive despite the presence of only psilocin; the calculated cross-reactivity with the immunoassay was 1.3% at 50,000 ng/mL.[80] Foods (chocolate, cheese, wine) are also endogenous sources for phenethylamine.

Abnormalities

Rare case reports document mild elevations of lactate dehydrogenase (LDH), serum aspartate aminotransferase, and alkaline phosphatase following the ingestion and intravenous administration of psilocybin-containing mushrooms, but liver function is usually normal.[81] Following the ingestion of psilocybin, cerebral metabolic patterns as determined by fluorodeoxyglucose-PET scanning demonstrated hypermetabolism in the prefrontal and inferior temporal regions of the right hemisphere (e.g., anterior cingulate) and relatively hypometabolism in the subcortical regions (e.g., thalamus) when compared with placebo.[82] These findings are similar to the hypermetabolism in the frontal regions associated with the ingestion of mescaline.[83]

TREATMENT

Most patients ingest psilocybin-containing mushrooms without developing symptoms requiring emergency medical care. Decontamination measures are usually unnecessary; the use of decontamination measures should be limited to cases where other toxic drugs or compounds have been ingested within 1–2 hours of presentation. There are no specific data on the efficacy of decontamination measures (e.g., activated charcoal) after the ingestion of hallucinogenic mushrooms. Rarely, the ingestion of hallucinogenic compounds is associated with hyperthermia that requires undressing the patient and rapid cooling measures.

Dysphoria is the major complaint for most patients presenting to the emergency department after ingesting hallucinogenic mushrooms; a quiet, supportive environment (darkened room with familiar faces) along with calm reassurance usually provides sufficient treatment for the dysphoria. The patient should be observed until the dysphoria resolves (i.e., usually within 4–6 h). The treatment room should contain minimal visual and auditory stimuli, and the presence of relatives or friends to give reassurance is helpful. Benzodiazepines (e.g., diazepam, lorazepam, midazolam) may be necessary for sedation if dysphoria persists. Antipsychotics (e.g., haloperidol) should be reserved for frank hallucinations. Severely agitated patients should be evaluated for rhabdomyolysis if the agitation persists, and the possibility of *Amanita phalloides* poisoning should be considered in patients presenting with the late onset (e.g., >6 h postingestion) of gastrointestinal symptoms.

References

1. Pollock SH. Psilocybian mycetismus with special reference to *Panaeolus*. J Psychedelic Drugs 1976;8:43–57.
2. Wasson RG. Traditional use of North America of *Amanita muscaria* for divinatory purposes. J Psychedelic Drugs 1979;11:25–28.
3. McDonald A. Mushrooms and madness hallucinogenic mushrooms and some psychopharmacological implications. Can J Psychiatry 1980;25:586–594.
4. Lowy B. New records of mushroom stones from Guatemala. Mycologia 1971;63:983–992.
5. Wasson RG. Seeking the magic mushroom. Life 1957;42:100–120.
6. Heim R. [Hallucinogenic agarics of the genus *Psilocybe* collected during our recent mission to southern and

central Mexico in the company of Mr. Gordon Wasson.] C R Hebd Seances Acad Sci 1957;244:695–700. [French]

7. Hofmann A, Frey A, Ott H, Petrzilka T, Troxler F. Konstitutionsaufklärung und synthese von psilocybin. Experienta 1958;15:397–399.

8. Lassen JF, Lassen NF, Skov J. [Consumption of psilocybin containing hallucinogenic mushrooms by young people.] Ugeskr Laeger 1992;154:2678–2681. [Danish]

9. Adlaf EM, Ivis JF. Recent findings from the Ontario student drug use survey. CMAJ 1998;159:451–454.

10. Schwartz RH, Smith DE. Hallucinogenic mushrooms. Clin Pediatr 1988;27:70–73.

11. Wurst M, Kysilka R, Flieger M. Psychoactive tryptamines from Basidiomycetes. Folia Microbiol 2002;47:3–27.

12. Merlin MD, Allen JW. Species identification and chemical analysis of psychoactive fungi in the Hawaiian Islands. J Ethnopharmacol 1993;40:21–40.

13. Guzman G, Ott J, Boydston J, Pollock SH. Psychotropic mycoflora of Washington, Idaho, Oregon, California and British Columbia. Mycologia 1976;68:1267–1272.

14. Pollock SH. Liberty caps: recreational hallucinogenic mushrooms. Drug Alcohol Depend 1976;76;1:445–447.

15. Watling R. Panaeolus poisoning in Scotland. Myco-pathologia 1977;61:187–190.

16. Jensen N, Gartz J, Laatsch H. Aeruginascin, a trimethylam-monium analogue of psilocybin from the hallucinogenic mushroom *Inocybe aeruginascens*. Planta Med 2006;72: 665–666.

17. Hasler F, Bourquin D, Brenneisen R, Bär T, Vollenweider FX. Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. Pharm Acta Helv 1997;72:175–184.

18. Levine WG. Formation of blue oxidation product from psilocybin. Nature 1967;215:1292–1293.

19. Repke DB, Leslie DT, Guzmán G. Baeocystin in *Psilocybe*, *Conocybe* and *Panaeolus*. Lloydia 1977;40:566–578.

20. Leung AY, Paul AG. Baeocystin and norbaeocystin: New analogs of psilocybin from *Psilocybe baeocystis*. J Pharm Sci 1968;57:1667–1671.

21. Lott JP, Marlowe DB, Forman RF. Availability of websites offering to sell psilocybin spores and psilocybin. J Psychoactive Drugs 2009;41:305–307.

22. Guzman G, Ott J. Description and chemical analysis of a new species of hallucinogenic *Psilocybe* from the Pacific Northwest. Mycologia 1976;68:1261–1267.

23. Ott J, Guzman G. Detection of psilocybin in species of *Psilocybe*, *Panaeolus* and *Psathyrella*. Lloydia 1976;39: 258–260.

24. Hatfield GM, Valdes LJ, Smith AH. The occurrence of psilocybin in *Gymnopilus* species. Lloydia 1978;41: 140–144.

25. Kusano G, Koike Y, Inoue H, Nozoe S. The constituents of *Gymnopilus spectabilis*. Chem Pharm Bull 1986;34: 3465–3470.

26. Gross ST. Detecting psychoactive drugs in the develop-mental stages of mushrooms. J Forensic Sci 2000;45: 527–537.

27. Bigwood J, Beug MW. Variation of psilocybin and psilocin levels with repeated flushes (harvests) of mature sporo-carps of *Psilocybe cubensis* (Earle) Singer. J Ethnopharmacol 1982;5:287–291.

28. Laussmann T, Meier-Giebing S. Forensic analysis of hal-lucinogenic mushrooms and khat (*Catha edulis* Forsk) using cation-exchange liquid chromatography. Forensic Sci Int 2010;195:160–164.

29. Beug MW, Bigwood J. Psilocybin and psilocin levels in twenty species from seven genera of wild mushrooms in the Pacific Northwest, U.S.A. J Ethnopharmacol 1982;5:271–285.

30. Wurst M, Kysilka R, Koza T. analysis and isolation of indole alkaloids of fungi by high-performance liquid chro-matography. J Chromatogr 1992;593:201–208.

31. Gartz J. Extraction and analysis of indole derivatives from fungal biomass. J Basic Microbiol 1994;34:17–22.

32. Borner S, Brenneisen R. Determination of tryptamine derivatives in hallucinogenic mushrooms using high-performance liquid chromatography with photodiode array detection. J Chromatogr 1987;408:402–408.

33. Musshoff F, Madea B, Beike J. Hallucinogenic mushrooms on the German market—simple instructions for examina-tion and identification. Forensic Sci Int 2000;113: 389–395.

34. Badham ER. Ethnobotany of psilocybin mushrooms, especially *Psilocybe cubensis*. J Ethnopharmacol 1984;10: 249–254.

35. Repke DB, Leslie DT, Mandell DM, Kish NG. GLC-mass spectral analysis of psilocin and psilocybin. J Pharm Sci 1977;66:743–744.

36. Beck O, Helander A, Karlson-Stiber C, Stephansson N. Presence of phenylethylamine in hallucinogenic Psilocybe mushroom: possible role in adverse reactions. J Anal Toxicol 1998;22:45–49.

37. Hofmann A, Frey A, Ott H, Petr Zilka T, Troxler F. [Elucidation of the structure and the synthesis of psilocy-bin.] Experientia 1958;14:397–399. [German]

38. Keller T, Schneider A, Regenscheit P, Dirnhofer R, Rucker T, Jaspers J, Kisser W. Analysis of psilocybin and psilocin in *Psilocybe subcubensis* Guzman by ion mobility spec-trometry and gas chromatography-mass spectrometry. Forensic Sci Int 1999;99:93–105.

39. Bjornstad K, Hulten P, Beck O, Helander A. Bioanalytical and clinical evaluation of 103 suspected cases of intoxica-tions with psychoactive plant materials. Clin Toxicol 2009;47:566–572.

40. Riley SC, Blackman G. Between prohibitions: patterns and meanings of magic mushroom use in the UK. Subst Use Misuse 2008;43:55–71.

41. McCambridge J, Winstock A, Hunt N, Mitcheson L. 5-Year trends in use of hallucinogens and other adjunct drugs

among UK dance drug users. Eur Addict Res 2007;13:57–64.

42. Halpern JH. Hallucinogens and dissociative agents naturally growing in the United States. Pharmacol Ther 2004;102:131–138.

43. van Amsterdam J, Opperhuizen A, van den Brink W. Harm potential of magic mushroom use: a review. Regul Toxicol Pharmacol 2011;59:423–429.

44. Vollenweider FX, Vontobel P, Hell D, Leenders KL. 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man—a PET study with [11C]raclopride. Neuropsychopharmacology 1999;20: 424–433.

45. Wittmann M, Carter O, Hasler F, Cahn BR, Grimberg U, Spring P, et al. Effects of psilocybin on time perception and temporal control of behaviour in humans. J Psychopharmacol 2007;21:50–64.

46. Moreno FA, Wiegand CB, Taitano EK, Delgado PL. Safety, tolerability, and efficacy of psilocybin in 9 patients with obsessive-compulsive disorder. J Clin Psychiatry 2006;67: 1735–1740.

47. Francis J, Murray VSG. Review of enquiries made to the NPIS concerning Psilocybe mushroom ingestion, 1978-1981. Hum Toxicol 1983;2:349–352.

48. McCawley EL, Brummett RE, Dana GW. Convulsions from Psilocybe mushroom poisoning. Proc West Pharmacol Soc 1962;5:27–33.

49. Manevski N, Kurkela M, Höglund C, Mauriala T, Court MH, Yli-Kauhaluoma J, Finel M. Glucuronidation of psilocin and 4-hydroxyindole by the human UDP-glucuronosyltransferases. Drug Metab Dispos 2010;38: 386–395.

50. Hasler F, Bourquin D, Brenneisen R, Vollenweider FX. Renal excretion profiles of psilocin following oral administration of psilocybin: a controlled study in man. J Pharmaceut Biomed Anal 2002;30:331–339.

51. Sticht G, Kaferstein H. Detection of psilocin in body fluids. Forensic Sci Int 2000;113:403–407.

52. Aboul-Enein HY. Psilocybin: a pharmacological profile. Am J Pharm Sci Support Publ Health 1974;146:91–95.

53. Weidmann H, Taeschler M, Konzett H. Zur pharmakologie von psilocybin, einem wirkstoff aus Psilocybe mexicana Heim. Experientia 1958;24:378–379.

54. McKenna DJ, Repke DB, Peroutka SJ. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptors subtypes. Neuropharmacology 1990;29: 193–198.

55. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. Neuropharmacology 2011;61:364–381.

56. Carter OL, Burr DC, Pettigrew JD, Wallis GM, Hasler F, Vollenweider FX. Using psilocybin to investigate the relationship between attention, working memory, and the serotonin 1A and 2A receptors. J Cogn Neurosci 2005;17: 1497–1508.

57. Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Bäbler A, Vogel H, Hell D. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 1998;9:3897–3902.

58. Gouzoulis-Mayfrank E, Thelen B, Maier S, Heekeren K, Kovar KA, Sass H, Spitzer M. Effects of the hallucinogen psilocybin on covert orienting of visual attention in humans. Neuropsychobiology 2002;45:205–212.

59. Hollister LE, Hartman AM. Mescaline, lysergic acid diethylamide and psilocybin: comparison of clinical syndromes, effects on color perception and biochemical measures. Compr Psychiatry 1962;3:235–241.

60. Peden NR, Bissett AF, McCauley KEC, Crooks J, Pelosi AJ. Clinical toxicology of "magic mushroom ingestion." Postgrad Med J 1981;57:543–545.

61. Benjamin C. Persistent psychiatric symptoms after eating psilocybin mushrooms. Br Med J 1979;1:1319–1320.

62. Peden NR, Pringle SD, Crooks J. The problem of psilocybin mushroom abuse. Hum Toxicol 1982;1:417–424.

63. Malitz S, Esecover H, Wilkens B, Hoch PH. Some observations on psilocybin, a new hallucinogen, in volunteer subjects. Compr Psychiatry 1960;1:8–17.

64. Musha M, Ishii A, Tanaka F, Kusano G. Poisoning by hallucinogenic mushroom hikageshibiretake (Psilocybe argentipes K. Yokoyama) indigenous to Japan. Tohoku J Exp Med 1986;148:73–78.

65. Satora L, Goszcz H, Ciszowski K. Poisonings resulting from the ingestion of magic mushrooms in Krakow. Przegl Lek 2005;62:394–396.

66. Curry SC, Rose MC. Intravenous mushroom poisoning. Ann Emerg Med 1985;14:900–902.

67. Espiard M-L, Lecardeur L, Abadie P, Halbecq I, Dollfus S. Hallucinogen persisting perception disorder after psilocybin consumption: a case study. Eur Psychiatry 2005;20:458–460.

68. Lee RE. A technique for the rapid isolation and identification of psilocin from psilocin/psilocybin-containing mushrooms. J Forensic Sci 1985;30:931–941.

69. Beug MW, Bigwood J. Quantitative analysis of psilocybin and psilocin in Psilocybe baeocystis Singer and Smith by high-performance liquid chromatography and by thin layer chromatography. J Chromatogr 1981;207:379–385.

70. Lurie IS, Cooper Da, Krull IS. High performance liquid chromatography using continuous on-line post-elution photoirradiation with subsequent diode-array UV or thermospray mass spectrometry detection. J Chromatogr 1993;629:143–151.

71. Kamata T, Nishikawa M, Katagi M, Tsuchihashi H. Optimized glucuronide hydrolysis for the detection of psilocin in human urine samples. J Chromatogr B Analyt Technol Biomed Life Sci 2003;796:421–427.

72. Kysilka R, Wurst M. High-performance liquid chromatographic determination of some psychotropic indole derivatives. J Chromatogr 1989;464:434–437.

73. Tsujikawa K, Kanamori T, Iwata Y, Ohmae Y, Sugita R, Inoue H, Kishi T. Morphological and chemical analysis

of magic mushrooms in Japan. Forensic Sci Int 2003;138:85–90.

74. Saito K, Toyo'oka T, Fukushima T, Kato M, Shirota O, Goda Y. Determination of psilocin in magic mushrooms and rat plasma by liquid chromatography with fluorometry and electrospray ionization mass spectrometry. Anal Chim Acta 2004;527:149–156.

75. Christiansen AL, Rasmussen KE. Screening of hallucinogenic mushrooms with high-performance liquid chromatography and multiple detection. J Chromatogr 1983;270:293–299.

76. Kysilka R, Wurst M, Pacakova V, Stulik K, Haskovec L. High-performance liquid chromatographic determination of hallucinogenic indoleamines with simultaneous UV photometric and voltametric detection. J Chromatogr 1985;320:414–420.

77. Anastos Lewis SW, Barnett NW, Sims DN. The determination of psilocin and psilocybin in hallucinogenic mushrooms by HPLC utilizing a dual reagent acidic potassium permanganate and tris(2,2'-bipyridyl)ruthenium(II) chemiluminescence detection system. J Forensic Sci 2006;51:45–51.

78. Lee JC, Cole M, Linacre A. Identification of hallucinogenic fungi from the genera *Psilocybe* and *Panaeolus* by

amplified fragment length polymorphism. Electrophoresis 2000;21:1484–1487.

79. Grieshaber AF, Moore KA, Levine B. The detection of psilocin in human urine. J Forensic Sci 2001;46:627–630.

80. Tiscione NB, Miller MI. Psilocin identified in a DUID investigation. J Anal Toxicol 2006;30:342–345.

81. Sivyer G, Dorrington L. Intravenous injection of mushrooms. Med J Aust 1984;140:182.

82. Gouzoulis-Mayfrank E, Schreckenberger M, Sabri O, Arning C, Thelen B, Spitzer M, et al. Neurometabolic effects of psilocybin, 3,4-methylenedioxyethylamphetamine (MDE) and *d*-methamphetamine in healthy volunteers. A double-blind, placebo-controlled PET study with [18F] FDG. Neuropsychopharmacology 1999;20:565–581.

83. Hermle L, Funfgeld M, Oepen G, Botsch H, Borchardt D, Gouzoulis E, Fehrenbach RA, Spitzer M. Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. Biol Psychiatry 1992;32:976–91.

Chapter 65

Salvia divinorum Epling & Jativa and Salvinorin A

HISTORY

For many centuries, Mazateca shamans from the Sierra Mazateca region of the Mexican state of Oaxaca used the psychotropic effects of the hallucinogenic mint, *Salvia divinorum*, as a substitute for psilocybin-containing mushrooms in ceremonial healing and divination rituals.[1] The shamans considered the hallucinogenic effects of *Salvia divinorum* weak; this plant was substituted for hallucinogenic mushrooms in ceremonies only when the supply of these mushrooms was inadequate. Jean Basset Johnston first described the use of *Salvia divinorum* in 1939 while studying the use of psilocybin mushrooms by the Mazateca people. This plant was not characterized botanically until 1962.[2] Ortega et al isolated and identified the main psychoactive ingredients, salvinorin A and B in 1982.[3] Traditionally, use of *Salvia divinorum* involved the ingestion of a water infusion or mastication of the leaves; however, as the use of this plant spread to other parts of North America and Europe, smoking the leaves became a more popular route of administration. Most countries do not regulate the distribution of *Salvia divinorum*; additionally, *Salvia divinorum* products are available over the Internet.[4]

BOTANICAL DESCRIPTION

Common Name: Diviner's sage, Mexican mint, leaves of Mary the Shepherdess, magic mint, Sally-D, Sage of the Seers, ska Pastora, ska Maria, hierba de Maria, hojas de la Pastora, hojas de Maria

Scientific Name: *Salvia divinorum* Epling & Jativa

Botanical Family: Lamiaceae (mint)

Physical Description: This perennial herb has large green leaves and hollow, square, flowering stems that reach over 1 meter in height. White flowers with purple calyces appear from May thorough September. This plant is primarily cultivated, and produces fertile seeds. One plant has sufficient cuttings to provide a user with a constant supply of the leaves.

Distribution and Ecology: *S. divinorum* is a native plant of a relatively small Sierra Mazateca region in the mountainous northeastern Mexican state of Oaxaca. *S. divinorum* grows in forested ravines and other humid areas of the Sierra Mazateca at altitudes between 750–1500 m (~2,500–5,000 ft).

IDENTIFYING CHARACTERISTICS

The main active ingredient in this plant is a water-insoluble, trans-neoclerodane diterpene called salvinorin A (CAS RN: 83729-01-5, $C_{23}H_{28}O_8$).[5] Closely related compounds present in lower concentrations include salvinorin B, salvinorin C, salvinorin D, salvinorin E, salvinorin F, divinatorin A, divinatorin B, divinatorin C, and (-)-hardwickiic acid. Other compounds in *S. divinorum* include alkanes, tocopherol, stigmasterol, fatty acids, and neophytadiene.[6] Salvinorin A is a structurally unique furanolactone neoclerodane diterpene that is distinct from other naturally occurring hallucinogens (e.g., psilocybin, mescaline, *N,N*-dimethyltryptamine), synthetic hallucinogens (e.g., LSD, 4-bromo-2,5-dimethoxyphylisopropylamine or DOB,

	R
Salvinorin A	Ac
Salvinorin B	H

	R₁	R₂
Salvinorin C	OAc	OAc
Salvinorin D	OAc	OH
Salvinorin E	OH	OAc
Salvinorin F	OH	H
Salvinorin G	O	OAc

	R₁	R₂	R₃
Divinatorin A	OH	H	H
Divinatorin B	OH	OH	CH₃
Divinatorin C	H	OAc	H
Divinatorin D	OH	OAc	CH₃
Divinatorin E	OH	O	CH₃

Salvinicin A

Salvinicin B
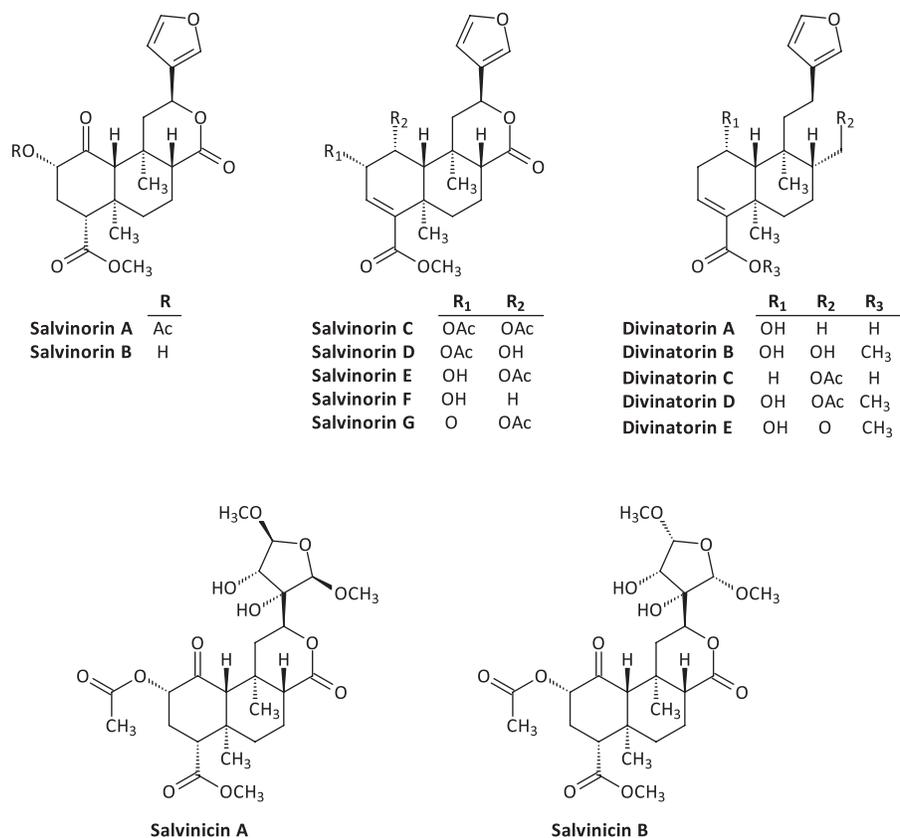
FIGURE 65.1. Chemical structures of salvinorin compounds.

ketamine) and opiates; salvinorin A lacks the amino and phenolic moieties common to all opiate-based ligands. This compound is the first reported nonnitrogenous (non-alkaloidal) opioid receptor agonist and hallucinogen.[7] In contrast to most hallucinogens, salvinorin A has κ-opioid agonist activity (analgesia, dysphoria, perceptual distortions, sedation) rather than affinity for 5-HT$_{2A}$ receptors.[8] Figure 65.1 demonstrates the structurally related salvinorin compounds. Under normal conditions, drying the leaves does not affect potency.[9]

EXPOSURE

Sources

Indians from the Sierra Mazateca region of Mexico used this plant for divination. Mazateca shamans (curanderos or folk healers) utilize the dream-like states induced by this indigenous plant to venture into supernatural realms and contact divine entities to remedy the complaints of their clients. Additionally, this plant has been part of healing ceremonies of the Mazateca Indians of Oaxaca for headaches, anemia, diarrhea, and rheumatism.[1] In Western drug cultures, visionary illu-

sions are the desired effects from *S. divinorum*. Case reports describe the use of a quid of *Salvia divinorum* leaves as a treatment for depression,[10] but the lack of clinical trials limits any conclusions regarding the effectiveness of *Salvia divinorum* as an antidepressant.

The plant is available over the Internet and in smart shops (vendors specializing in natural psychoactive drugs) as dried plant material, fortified plant material, and liquid extracts.[11] Figure 65.2 displays confiscated salvia leaves, and Figure 65.3 displays the liquid extract of *S. divinorum*. Most countries do not regulate the use of this plant, except Australia, Belgium, Denmark, Estonia, Finland, Italy, South Korea, Spain, and Sweden. In Europe, *S. divinorum* is cultivated as a substitute for marijuana. *S. divinorum* is not listed as a controlled substance under the US Controlled Substances Act, but some individual states (Delaware, Louisiana, Maine, Missouri, North Dakota, Oklahoma, Tennessee) control the use of this plant. *Salvia divinorum* is commonly smoked in home environments, although a significant minority use *Salvia divinorum* in more social settings (parks, bars, parties).[12] Use of *Salvia divinorum* is particularly common among polydrug users (e.g., lysergic acid diethylamide, ecstasy, phencyclidine, cocaine, heroin).[13]

FIGURE 65.2. Dried salvia leaves. (Photo courtesy of *Drug Identification Bible*)

FIGURE 65.3. Liquid extract of *Salvia divinorum*. (Photo courtesy of *Drug Identification Bible*)

Composition

Extracts of *S. divinorum* contain at least 15 neoclerodane diterpene compounds (salvinorin A-I, divinatorin A-F, salvidivin A-D).[14] Salvinorin A and related compounds are components of a complex resin that accumulates in the subcuticular space of peltate glandular trichomes.[15] In a study of 20 dry leaf samples, the salvinorin A content ranged between 0.0895–0.37% dry

TABLE 65.1. Salvinorin Concentrations in Samples from Sierra Mazateca and Hawaii.[18]

Compound	Sierra Mazateca (% w/w)	Hawaii (% w/w)
Salvinorin A	0.76	0.78
Salvinorin B	0.42	1.04
Salvinorin C	0.59	0.62
Salvinorin D	0.05	0.05
Salvinorin E	0.02	0.03
Salvinorin F	0.16	0.15

weight with an average of about 0.245%.[16] The stems contain much smaller (e.g., 4%) salvinorin A concentrations compared with the dried leaves. In 3 samples of dried *S. divinorum* leaves obtained over the Internet in Japan, the salvinorin A and salvinorin B (deacetylated metabolite) content of the dried leaves ranged between 0.32–0.50% and 0.01–0.017%, respectively.[17] The concentrated extracts of *S. divinorum* from this study contained much higher concentrations of salvinorin A (0.41–3.89%) and salvinorin B (0.026–0.242%) than the dried leaves. Some geographic variation occurs in the content of salvinorin A and other salvinorin compounds. Table 65.1 lists the salvinorin content of *S. divinorum* leaves grown in Sierra Mazateca and in Hawaii. This analysis indicates that salvinorin A may not be the most abundant salvinorin compound in certain strains of *S. divinorum* leaves.[18]

The salvinorin A content in *Salvia divinorum* products available over the Internet and in drug paraphernalia shops is highly variable. Analysis of a convenience sample of 4 *S. divinorum* leaves and one *S. divinorum* extract marketed as herbal products (Internet, drug paraphernalia shops) suggested that these products contain much less (i.e., 1–16%) salvinorin A than the label claims.[19] The content of salvinorin A in the leaves ranged between 0.126–1.137 mg/g, whereas the extract contained 0.952 mg/g.

Salvinorin C and other salvinorin compounds are minor constituents of *S. divinorum* compared with salvinorin A, and the former compound has weaker affinity for the κ-opioid receptor than salvinorin A.[20] The ratio of salvinorin C/salvinorin A is greater in mature leaves and stems than in young leaves and bracts. Woody vascular tissue and the pith of the stems do not contain salvinorin compounds.

Methods of Abuse

Traditionally, leaves from *S. divinorum* are masticated as a quid or crushed and the extract ingested as a beverage. The main route of administration for the

963

recreational use of *S. divinorum* is smoking the dry leaves.[21] The large number of leaves required for hallucinogenic effects and bitterness of the prepared aqueous extracts of this plant limit abuse. The effects of salvinorin A appeal more to individual experimentalists seeking philosophical insights rather than to party-goers seeking pleasurable experiences. Preliminary data suggests that the chronic use of *S. divinorum* is not prevalent among college students.[11]

DOSE EFFECT

The potency of salvinorin A is similar to synthetic hallucinogens (e.g., lysergic acid diethylamide [LSD], 2,5-dimethoxy-4-bromoamphetamine [DOB]). The effective oral dose of salvinorin A in humans ranges from about 0.2–1 mg. In an experimental study of healthy, hallucinogen-experienced participants, the inhalation of salvinorin A doses up to 0.021 mg/kg bodyweight did not alter heart rate or blood pressure despite the presence of subjective effects.[22] Typically, dried leaves from *S. divinorum* contain 0.9–3.8 mg salvinorin A, and the inhalation of vapors from <500 mg of these leaves are required to produce a hallucinogenic experience.[23] By convention, counting of leaves is done in pairs rather than individual leaves. Traditionally, small doses (4–5 pairs of fresh or dried leaves) are used for a tonic, whereas large doses (20–60 pairs of leaves) produce vivid hallucinations. The chewing of less than 20 pair of leaves reportedly does not produce altered states of consciousness.[9]

TOXICOKINETICS

Salvinorin A is well-absorbed through the oral mucosa and the lungs; however, the gastrointestinal tract deactivates this compound. The administration of up to 4 mg salvinorin A sublingually for 5 minutes did not result in physiologic or subjective effects in 8 volunteers with experience using *S. divinorum*.[24] Consequently, use of this compound for hallucinogenic purposes typically involves smoking or prolonged mastication (>10 min) rather than ingestion.[25] The mean plasma elimination half-life of salvinorin A in primates following intravenous administration was 56.6 ± 24.8 minutes.[26] The C2 hydroxyl derivative, salvinorin B, is the major metabolite of salvinorin A as a result of the hydrolysis of the 2-acetoxy group of salvinorin A; salvinorin B is an inactive metabolite.[27,28] *In vitro* studies suggest the involvement of UGT2B7 and several cytochrome P450 isoenzymes in the biotransformation of salvinorin A including CYP2D6, CYP1A1, CYP2E1, and CYP2C18.[29] Other enzymes (e.g., lactonases, UGT2B7) may catalyze

the production of increasingly hydrophilic metabolites before excretion by the kidney.[7]

The distribution and elimination of salvinorin A in the brain is consistent with the rapid action and brief (i.e., 10 minutes) duration of action. In a positron emission tomography (PET) study of baboons using [^{11}C]-salvinorin A, 3.3% of the injected dose appeared in the brain within 40 seconds; the mean elimination half-life of this radiolabeled compound from the brain was approximately 8 minutes.[30] In a rodent study, the mean plasma elimination half-life was about 75 minutes.[29]

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Salvinorin A is a potent κ-opioid receptor agonist that is structurally distinct from classical hallucinogens and opioid agonists.[27] *In vitro* studies indicate that salvinorin A binds to G protein-coupled κ-opioid receptors, but not to cloned μ-opioid or δ-opioid receptors.[31] Salvinorin A demonstrates no affinity for the 5-HT$_{2A}$ serotonin receptor, which is the principal molecular target of classical hallucinogens.[32] These *in vitro* studies indicate that salvinorin A has little affinity for norepinephrine, dopamine, glutamine, or GABA-transporters. Salvinorin B, salvinorin D, and salvinorin E are inactive at the κ-opioid receptor, but salvinorin C may possess weak κ-opioid agonist activity.[27]

CLINICAL RESPONSE

Depending on the dose and individual sensitivity, the clinical response ranges from subtle changes in perception to profound dream-like visions and bizarre feelings of depersonalization. Pulmonary absorption of high doses of salvinorin A from *S. divinorum* produces auditory and visual hallucinations, loss of body control, alterations in perception, sense of motion, perception of being in more than one location at a time, reliving childhood experiences, amnesia, hysterical laughter, and loss of consciousness.[25] Typically, the hallucinations involve synesthesias (i.e., hearing colors, smelling sounds) that are accompanied by a feeling of separation of the mind from the body (spatiotemporal dislocation).[33] The intensity of the hallucinogenic experience associated with smoking *S. divinorum* leaves varies substantially depending on the individual and the setting based on observations of videos of *S. divinorum* users.[34] The most frequently endorsed responses of recent *S. divinorum* users in an online survey were "unexpected effects" and "excessively intense experience"; these responses suggest that the hallucinogenic and dissociative effects of using this compound are greater than other hallucinogens (LSD, psilocybin) used by this group.[35] Some

users compare S. *divinorum* with ketamine except the effects of ketamine are more consistent and not associated with uncontrolled laughter.[36] Other interviews of *S. divinorum* users suggest the effects of smoking leaves from this plant are similar to LSD, cannabis, or represent an entirely unique experience.[37]

The onset of effects is rapid (i.e., 1 minute) following smoking of *S. divinorum* leaves with resolution of effects occurring within 10–20 minutes. The onset following the use of a quid (i.e., a portion suitable for chewing but not swallowing) is about 5–15 minutes with cessation of effects ~2 hours after swallowing the quid.[25] The effects of ingesting the extract lasts ~1 hour.[25] Shivering, exhaustion, and poor concentration may occur for several hours following disappearance of psychomimetic effects of *S. divinorum*. Other potential adverse reactions following the use of *S. divinorum* include panic attacks, terror, fear, diaphoresis, and psychosis, particularly in individuals unintentionally inhaling smoke from the leaves.[38] In a retrospective study of reports of *S. divinorum* use to the California Poison Control System, adverse effects associated with *S. divinorum* exposure included confusion, disorientation, hallucination, giddiness, dizziness, a flushed sensation, and tachycardia.[39] Of the individuals in this study with intentional exposure to only *S. divinorum*, 72% had psychotomimetic or neuromotor disturbances.

Although case reports associate persistent psychosis (paranoia, echolalia, flight of ideas, psychomotor agitation) with the use of *S. divinorum*,[40] the causal role of this plant in the etiology of psychosis is not well-defined. Case reports suggest that an increasing number of leaves are necessary to maintain the initial psychotropic effects from the ingestion of a quid.[23] Based on information from questionnaires reported by users of *Salvia divinorum*, some users experience a state of mental and physical malaise after use similar to a hangover from heavy ethanol ingestion.[41]

DIAGNOSTIC TESTING

Methods for the quantitation of salvinorin compounds in biologic samples include liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry,[42] gas chromatography/mass spectrometry (GC/MS),[43] gas chromatography/mass spectrometry after acetyl-derivation,[6] and liquid chromatography/electrospray ionization/mass spectrometry in selected ion monitoring (SIM) mode.[44] The limits of detection (LOD) and lower limit of quantitation (LLOQ) for the latter method in biologic specimens are 2.5 ng/mL and 5.0 ng/mL, respectively, with a coefficient of variation <8.5%. For liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry, the LLOQ

for salvinorin A in plasma is 2 ng/mL. For analysis of these compounds in plant material, analytical methods include liquid chromatography/electrospray ionization/multistage ion trap/mass spectrometry,[18] high performance liquid chromatography with UV detection,[16] and thin layer chromatography/gas chromatography/mass spectroscopy.[45] Routine urine drug screens do not usually detect the use of *S. divinorum*.[46] Following the smoking of 75 mg *Salvia divinorum* leaves, urine samples collected from 2 volunteers about 1.5 hours after smoking contained salvinorin A concentrations of 2.4 ng/mL and 10.9 ng/mL, as measured by GC/MS (LOD, 5 ng/mL).[43] Blood samples could not be collected from these 2 volunteers because they experienced intense hallucinations immediately after smoking began. Salvinorin A was not detectable in urine samples collected 9.5 hours after smoking began when measured by GC/MS (LLOQ, 15 ng/mL).

TREATMENT

Although agitation, delirium, or confusion may occur after the use of *Salvia divinorum*, the physiologic and psychologic effects of this hallucinogen typically are mild.[47] These patients usually respond to a quiet, supportive environment. Benzodiazepines are rarely required for sedation. The major risk following the use of *Salvia divinorum* is trauma secondary to altered sensorium. Naloxone is a potential antidote for the clinical effects of *Salvia divinorum* based theoretically on the opioid agonist activity of salvinorin A, but there are inadequate data to determine the effectiveness of naloxone on the reversal of clinical effects of this plant. In mice, treatment with the κ–opioid receptor antagonist, norbinaltorphimine (CAS RN: 105618-26-6) prior to the intravenous administration of salvinorin A completely prevents the analgesic effects of salvinorin A.[48]

References

1. Valdes LJ 3rd, Diaz JL, Paul AG. Ethnopharmacology of ska Maria Pastora (*Salvia divinorum*, Epling and Jativa-M.). J Ethnopharmacol 1983;7:287–312.

2. Epling C, Játiva-M CD. A new species of *Salvia* from Mexico. Botanical Museum Leaflets (Harvard University) 1962:20:75–76.

3. Ortega A, Blount JF, Machand PS. Salvinorin a new trans-neoclerodane diterpene from *Salvia divinorum* (Labiatae). J Chem Soc Perkin Trans 1982;1:2505–2508.

4. Dennehy CE, Tsourounis C, Miller AE. Evaluation of herbal dietary supplements marketed on the Internet for recreational use. Ann Pharmacother 2005; 39:1634–1639.

5. Prisinzano TE. Psychopharmacology of the hallucinogenic sage *Salvia divinorum*. Life Sci 2005;78:527–531.

6. Giroud C, Felber F, Augsburger M, Horisberger B, Rivier L, Mangin P. *Salvia divinorum*: an hallucinogenic mint which might become a new recreational drug in Switzerland. Forensic Sci Int 2000;112:143–150.

7. Cunningham CW, Rothman RB, Prisinzano TE. Neuropharmacology of the naturally occurring κ-opioid hallucinogen salvinorin A. Pharmacol Rev 2011;63: 316–347.

8. Yan F, Roth BL. Salvinorin A: a novel and highly selective kappa-opioid receptor agonist. Life Sci 2004;75: 2615–2619.

9. Valdes LJ 3rd. *Salvia divinorum* and the unique diterpene hallucinogen, salvinorin (divinorin) A. J Psychoactive Drugs 1994;26:277–283.

10. Hanes KR. Antidepressant effects of the herb *Salvia divinorum*: a case report. J Clin Psychopharmacol 2001;21: 634–635.

11. Khey DN, Miller BL, Griffin OH. *Salvia divinorum* use among a college student sample. J Drug Educ 2008;38: 297–306.

12. Kelly BC. Legally tripping: a qualitative profile of *Salvia divinorum* use among young adults. J Psychoactive Drugs 2011;43:46–54.

13. Wu LT, Woody GE, Yang C, Li JH, Blazer DG. Recent national trends in *Salvia divinorum* use and substance-use disorders among recent and former *Salvia divinorum* users compared with nonusers. Subst Abuse Rehabil 2011(2):53–68.

14. Shirota O, Nagamatsu K, Sekita S. Neoclerodane diterpenes from the hallucinogenic sage *Salvia divinorum*. J Nat Prod 2006;69:1782–1786.

15. Siebert DJ. Localization of salvinorin A, and related compounds in glandular trichomes of the psychoactive sage, *Salvia divinorum*. Ann Bot 2004;93:763–771.

16. Gruber JW, Siebert DJ, Der Marderosian AH, Hock RS. High performance liquid chromatographic quantification of salvinorin A from tissues of *Salvia divinorum* Epling and Jativa-M. Phytochem Anal 1999;10:22–25.

17. Tsujikawa K, Kuwayama K, Miyaguchi H, Kanamori T, Iwata YT, Yoshida T, Inoue H. Determination of salvinorin A and salvinorin B in *Salvia divinorum*-related products circulated in Japan. Forensic Sci Int 2008;180:105–109.

18. Medana C, Massolino C, Pazzi M, Baiocchi C. Determination of salvinorins and divinatorins in *Salvia divinorum* leaves by liquid chromatography/multistage mass spectrometry. Rapid Commun Mass Spectrom 2006;20: 131–136.

19. Wolowich WR, Perkins AM, Cienki JJ. Analysis of the psychoactive terpenoid salvinorin A content in five *Salvia divinorum* herbal products. Pharmacotherapy 2006;26: 1268–1272.

20. Valdes LJ, Chang H-M, Visger DC, Koreeda M. Salvinorin C, a new neoclerodane diterpene from a bioactive fraction of the hallucinogenic Mexican mint *Salvia divinorum*. Org Lett 2001;3:3935–3937.

21. Grundmann O, Phipps SM, Zadezensky I, Butterweck V. *Salvia divinorum* and salvinorin A: an update on pharmacology and analytical methodology. Planta Med 2007;73: 1039–1046.

22. Johnson MW, Maclean KA, Reissig CJ, Prisinzano TE, Griffiths RR. Human psychopharmacology and dose-effects of salvinorin A, a kappa opioid agonist hallucinogen present in the plant *Salvia divinorum*. Drug Alcohol Depend 2011;115:150–155.

23. Bucheler R, Gleiter CH, Schwoerer P, Gaertner I. Use of nonprohibited hallucinogenic plants: increasing relevance for public health? A case report and literature review on the consumption of *Salvia divinorum* (Diviner's sage). Pharmacopsychiatry 2005;38:1–5.

24. Mendelson JE, Coyle JR, Lopez JC, Baggott MJ, Flower K, Everhart ET, et al. Lack of effect of sublingual salvinorin A, a naturally occurring kappa opioid, in humans: a placebo-controlled trial. Psychopharmacology 2011;214: 993–999.

25. Siebert DJ. *Salvia divinorum* and salvinorin A: new pharmacologic findings. J Ethnopharmacol 1994;43:53–56.

26. Schmidt MD, Schmidt MS, Butelman ER, Harding WW, Tidgewell K, Murry DJ, et al. Pharmacokinetics of the plant-derived kappa-opioid hallucinogen salvinorin A in nonhuman primates. Synapse 2005;58:208–210.

27. Chavkin C, Sud S, Jin W, Stewat J, Zjawiony JK, Siebert DJ, et al. Salvinorin A, an active component of the hallucinogenic sage *Salvia divinorum* is a highly efficacious κ-opioid receptor agonist: structural and functional considerations. J Pharmacol Exp Ther 2004;308: 1197–1203.

28. Roth BL, Lopez E, Beischel S, Westkaemper RB, Evans JM. Screening the receptorome to discover the molecular targets for plant-derived psychoactive compounds: a novel approach for CNS drug discovery. Pharmacol Ther 2004; 102:99–110.

29. Teksin ZS, Lee IJ, Nemieboka NN, Othman AA, Upreti VV, Hassan HE, et al. Evaluation of the transport, *in vitro* metabolism and pharmacokinetics of salvinorin A, a potent hallucinogen. Eur J Pharm Biopharm 2009; 72:471–477.

30. Hooker JM, Xu Y, Schiffer W, Shea C, Carter P, Fowler JS. Pharmacokinetics of the potent hallucinogen, salvinorin A in primates parallels the rapid onset and short duration of effects in humans. Neuroimage 2008;41:1044–1050.

31. Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg SA, et al: Salvinorin A. A potent naturally occurring nonnitrogenous κ opioid selective agonist. Proc Natl Acad Sci U S A 2002;99:11934–11939.

32. Nichols DE. Hallucinogens. Pharmacol Ther 2004;101: 131–181.

33. Vortherms TA, Roth BL. Salvinorin A from natural product to human therapeutics. Mol Interv 2006;6: 257–265.

34. Lange JE, Daniel J, Homer K, Reed MB, Clapp JD. *Salvia divinorum*: effects and use among YouTube users. Drug Alcohol Depend 2010;108:138–140.

35. Sumnall HR, Measham F, Brandt SD, Cole JC. *Salvia divinorum* use and phenomenology: results from an online survey. J Psychopharmacol 2011;25:1496–1507.

36. Dalgarno P. Subjective effects of *Salvia divinorum*. J Psychoactive Drugs 2007;37:143–149.

37. Albertson DN, Grubbs LE. Subjective effects of *Salvia divinorum*: LSD- or marijuana-like? J Psychoactive Drugs 2009;41:213–218.

38. Paulzen M, Grunder G. Toxic psychosis after intake of the hallucinogen salvinorin A. J Clin Psychiatry 2008;69:1501–1502.

39. Vohra R, Seefeld A, Cantrell FL, Clark RF. *Salvia divinorum*: exposures reported to a statewide poison control system over 10 years. J Emerg Med 2011;40:643–650.

40. Przekop P, Lee T. Persistent psychosis associated with *Salvia divinorum* use. Am J Psychiatry 2009;166:832.

41. Gonzalez D, Riba J, Bouso JC, Gomez-Jarabo G, Barbanoj MJ. Pattern of use and subjective effects of *Salvia divinorum* among recreational users. Drug Alcohol Depend 2006;85:157–162.

42. Schmidt MS, Prisinzano TE, Tidgewell K, Harding W, Butelman ER, Kreek MJ, Murry DJ. Determination of salvinorin A in body fluids by high performance liquid chromatography-atmospheric pressure chemical ionization. J Chromatogr B Analyt Technol Biomed Life Sci 2005;818:221–225.

43. Pichini S, Abanades S, Farré M, Pellegrini M, Marchei E, Pacifici R, et al. Quantification of the plant-derived hallucinogen Salvinorin A in conventional and non-conventional biological fluids by gas chromatography/mass spectrometry after *Salvia divinorum* smoking. Rapid Commun Mass Spectrom 2005;19:1649–1656.

44. McDonough PC, Holler JM, Vorce SP, Bosy TZ, Magluilo J Jr, Past MR. The detection and quantitative analysis of the psychoactive component of *Salvia divinorum*, salvinorin A, in human biological fluids using liquid chromatography-mass spectrometry. J Anal Toxicol 2008;32:417–421.

45. Wolowich WR, Perkins AM, Cienki JJ. Analysis of the psychoactive terpenoid salvinorin A content in five *Salvia divinorum* herbal products. Pharmacotherapy 2006;26:1268–1272.

46. Tidgewell K, Harding WW, Schmidt M, Holden KG, Murry DJ, Prisinzano TE. A facile method for the preparation of deuterium labeled salvinorin A: synthesis of [2,2,2-2H3]-salvinorin A. Bioorg Med Chem Lett 2004;14:5099–5102.

47. Babu KM, McCurdy CR, Boyer EW. Opioid receptors and legal highs: *Salvia divinorum* and kratom. Clin Toxicol 2008;46:146–152.

48. McCurdy CR, Sufka KJ, Smith GH, Warnick JE, Nieto MJ. Antinociceptive profile of salvinorin A, a structurally unique kappa opioid receptor agonist. Pharmacol Biochem Behav 2006;83:109–113.

Chapter 66

Tobacco, Nicotine, and Pituri

COMMON TOBACCO (*Nicotiana tabacum* L.)

HISTORY

The tobacco plant was a common commercial plant long before Christopher Columbus reached the Americas. The major active ingredient of tobacco, nicotine, was named after Jean Nicot, who popularized the use of tobacco in the 16th century as an analgesic for headaches.[1] The indigenous North American tobacco plant is *Nicotiana rustica*. Although the South American variety, *N. tabacum,* was cultivated in Virginia by European settlers for commercial purposes, Native Americans smoked *N. rustica* (Aztec tobacco) leaves for many centuries prior to the voyages of Columbus. On October 11, 1492, Christopher Columbus was given dried tobacco leaves at the House of the Arawaks. The Indians taught tobacco smoking to European sailors; subsequently, the tobacco plant was introduced along all major trade routes in Europe, Asia, Africa, and Australia. The tobacco plant (*N. tabacum*) first grown in France and Spain originated in Brazil and Mexico, whereas in Portugal and England, the tobacco plants (*N. rustica*) first originated in Virginia and Florida.[2] Nicotine was isolated from *Nicotiana* species in 1828, but the pharmacologic properties of nicotine were not identified until 1898.[1]

The modern history of tobacco smoking started with the design of the cigarette vending machine patented by James Bonsack in 1880, encouraged in part by concern over the spread of tuberculosis by the spitting of chewing tobacco. Since the 1920s, cigarettes accounted for most of the consumption of tobacco with cigars, chewing tobacco, and pipes declining to a relatively small portion of tobacco consumption.[2] Although the association between smoking and pulmonary diseases was first recognized in the 1870s, the association of smoking with various diseases was ignored until the 1950s. Several case-control studies during the early 1950s related smoking to the development of lung cancer; by the end of the 1950s, cohort studies suggested a causal relationship between smoking and lung cancer as well as other diseases.[3] The US Surgeon General first suggested a link between tobacco smoking, emphysema, and cancer in 1964.[4] By 1984, sufficient data accumulated that allowed the US Surgeon General to state that cigarette smoking was a major cause of chronic obstructive lung disease.[5] As a result of increased awareness and antitobacco sentiment in the United States, tobacco smoking rates decreased from 42% in 1965 to 25% in 1990. Since that time, US smoking rates have declined slowly with the US tobacco smoking rate being about 21.6% in 2003,[6] 20.9% in 2004,[7] and 19.8% in 2007.[8] Within the last 5 years, there has been little change in U.S. smoking rates with an estimated 20.6% of U.S. adults over 18 years of age being current cigarette smokers in 2009.[9]

BOTANICAL DESCRIPTION

The genus *Nicotiana* consists of about 60 plant species in North and South America, Australia, and the South Pacific.[1] *N. rustica* L. is native to Virginia, but the com-

FIGURE 66.2. Chemical structure of nicotine.

TABLE 66.1. Physiochemical Properties of Nicotine.

Physical Property	Value
Melting Point	−79°C (−110°F)
Boiling Point	247°C (~477°F)
pKa Dissociation Constant	3.1
log P (Octanol-Water)	1.17
Water Solubility	1.00E + 06 mg/L

IDENTIFYING CHARACTERISTICS

Nicotine [(*S*)-3-(1-methyl-2-pyrrolidinyl)pyridine, CAS RN: 54-11-5] is a tertiary amine consisting of a pyridine and a pyrrolidine ring (Figure 66.2). Tobacco products contain the citrate and malate salts of nicotine, whereas insecticides contain the free alkaline base or sulfate salt.[10] Minor alkaloids in tobacco leaves with nicotine-like pharmacologic activity include nornicotine (CAS RN: 494-97-3), metanicotine (CAS RN: 538-79-4), and anabasine (CAS RN: 494-52-0); however, these minor alkaloids are less potent pharmacologically than nicotine.[11] Nicotine is a water soluble, weak base with an alkaline pK_a of 8.0–8.5. At pH 8.9, about 90% of nicotine is unbound. Table 66.1 lists some of the physiochemical properties of nicotine.

EXPOSURE

Origin

Cultivation of tobacco occurs in over 100 countries with the top five producers in order (China, Brazil, India, United States, Malawi) accounting for two-thirds of the worldwide production. Nicotine is the major addictive constituent of tobacco products. Cigarettes are the most common method of smoking tobacco, both as manufactured and hand-rolled products. These products used fine-cut tobaccos wrapped in paper or maize leaves. Cigars contain cut tobacco surrounded by a binder leaf and a wrapper leaf rolled on the outside of the tobacco contents, whereas bidis contain shredded tobacco wrapped in nontobacco leaves (e.g., dried tendu leaves, *Diospyros melanoxylon* Roxb.). Table 66.2 describes the

FIGURE 66.1. Leaves and flowers of *Nicotiana tabacum* L. (common tobacco). (Photo courtesy of J. S. Peterson and USDA-NRCS PLANTS Database.)

mercial value of this plant is substantially less than the South American native, *N. tabacum*.

Common Name: Burley tobacco, common tobacco, cultivated tobacco

Scientific Name: *Nicotiana tabacum* L.

Botanical Family: Solanaceae (nightshade)

Physical Description: A stout annual herb with few leafy branches and a height of about 2–3 m (~6–10 feet). The long leaves are ovate or lanceolate, measuring up to 60 cm (~2 feet) in length. Pale, cream-colored to pink flowers are about 5 cm (2 inches) long and clustered at the ends of the branches. The globular seeds are brown and somewhat kidney-shaped. Figure 66.1 displays the leaves and flowers of the common tobacco plant.

Distribution and Ecology: This plant is a native species of South America that is now cultivated worldwide. Common tobacco prefers humid environments (80–85% humidity) and warm temperatures (20–30°C/68–86°F). This plant does not survive well in the wild.

TABLE 66.2. Common Types of Tobacco Smoking Products. Adapted from Reference 2.

Type	Description
Bidi	Hand-rolled Indian cigarettes that contain 0.2–0.3 g sun-dried tendu (temburni) leaf rolled into a conical shape together with flaked tobacco and secured with a thread
Chuttaa	Hand-rolled, home-made small cigar prepared by rolling local tobacco inside a sun-dried tobacco leaf. Reverse smoking (burning end inside mouth) of chuttaa is prevalent in women from rural India.
Cigar	Any roll of tobacco wrapped in leaf tobacco or in any other substance containing tobacco. Varieties include regular cigars, premium cigars, little cigars, and small cigars ("cigarillos") with filters and shaped like cigarettes. Regular cigars are up to 17 mm (2/3 inch) in diameter and 110–150 mm (4¼–6 inches) in length.
Cigarette	Any roll of tobacco wrapped in paper or other nontobacco material with or without filter. Dimensions: approximately 8 mm (1/3 inch) in diameter and 70–120 mm (2¾–4 ¾)in length
Hookah	Waterpipe using sweetened and flavored tobacco (maassel) along with charcoal to maintain combustion of the moist tobacco.
Kretek	Small cigar containing tobacco (approximately 60%), cloves, and cocoa. The burning blend gives a characteristic flavor and honey-like taste to the smoke.

common tobacco smoking products used in the world. Iqmik is a mixture of tobacco leaves and alkaline fungus ash (*Phellinus igniarius* L.) used by native peoples in Western Alaska as a recreational smokeless tobacco product.[12] The use of narghile waterpipes (hookah, shisha) is an increasing popular method of smoking tobacco.[13] The highly aromatic and flavored tobacco paste (maassel) is placed in the head of the waterpipe as demonstrated in Figure 66.3. The maassel may include a variety of flavorings (e.g., apple, cappuccino, mint). The prepackaged paste contains glycerol and ~25% w/w tobacco along with the flavoring.[14] Because of the high water content of maassel, the use of charcoal is necessary to maintain combustion of the maassel. A typical hookah session lasts about 1 hour. The prevalence of the use of waterpipes to smoke tobacco is increasing in the Middle East and worldwide including the United States, particularly among adolescents and young adults who use alcohol, tobacco, and cannabis.[15,16] In a cross-sectional survey of 689 high school students in southern California, ever-use and current use of hookah was ~26% and ~10%, respectively.[17]

Composition

TOBACCO PRODUCTS

Nicotine is the major toxic alkaloid in *N. tabacum* (cultivated tobacco) leaves averaging between 0.2–4.75% in cured tobacco by weight, depending on growing conditions, maturity, use of fertilizers, position of leaf on the stalk, and plant genetics.[2] In a study of 15 popular US brands of cigarettes, the cigarette tobacco contained an average nicotine content of 1.57% ± 0.33% weight, and
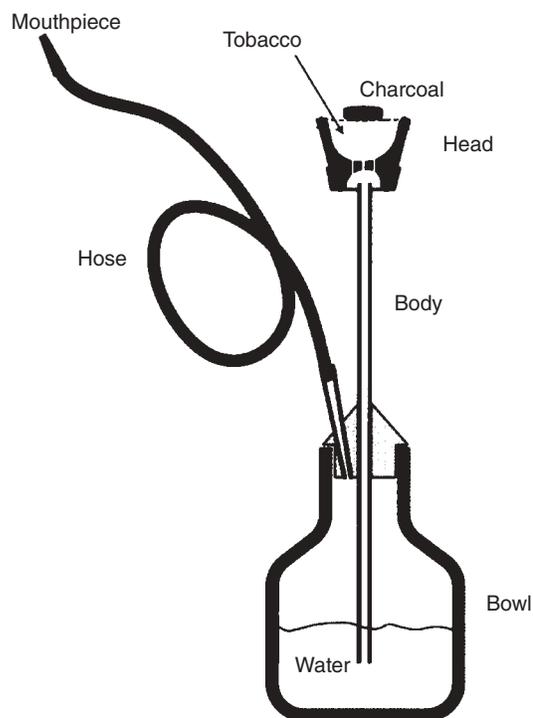
FIGURE 66.3. Schematic representation of a narghile (hookah) waterpipe.[14] Sweetened tobacco (maassel) is placed in the head with burning charcoal on top of the tobacco to maintain combustion. Perforated aluminum foil separates the burning charcoal and maassel. Smoke exits the head when inhalation at the mouthpiece increases the temperature of the charcoal; the smoke then flows through the water and into the hose. Reprinted from Food and Toxicology, Vol. 48, Issue 11, E Sepetdjian, N Saliba, A Shihadeh, Carcinogenic PAH in waterpipe charcoal products, p. 3243, copyright 2010, with permission from Elsevier.

nicotine represents approximately 95% of the total alkaloid content.[18] The classification of tobacco is based primarily on curing methods and tobacco types. For example, US tobacco types used in cigarettes include flue-cured, fire-cured, light air-cured, and dark air-cured. These tobacco products are blended to achieve a specific pH, taste, nicotine content, and burning characteristics. The nicotine content of oral snuff and pipe tobacco are similar to cigarette tobacco, whereas cigars and chewing tobacco contain about half the nicotine content of cigarette tobacco.[19] In general, a typical commercial tobacco cigarette (rod) contains about 8–14 mg nicotine.[20] In a study of the nicotine content of Japanese cigarettes, mean percentage of nicotine in the tobacco in 32 filtered cigarettes was $1.77 \pm 0.30\%$.[21] The amount of tobacco in these cigarettes ranged between 800–1,000 mg and the nicotine content between 13–30 mg. The usual absorbed dose of nicotine from smoking a cigarette is about 1–1.5 mg with a range of 0.3–2 mg depending on nicotine content and smoking techniques. In a volunteer study, estimates of the absorbed nicotine dose from average exposure to tobacco products was as follows: cigarette, 1.8 mg; snuff, 3.6 mg; chewing tobacco, 4.5 mg; and gum, 1.9 mg.[22] The nicotine in tobacco is primarily the (S)-isomer with the (R)-isomer representing only about 0.1–0.6% of the total nicotine content. However, racemization during the combustion process increases the (R)-isomer content to about 10% of the nicotine content in smoke.[23] The most abundant minor alkaloids are nornicotine and anatabine (CAS RN: 581-49-7) followed by anabasine and metanicotine. Unburned tobacco contains fewer carcinogens than tobacco smoke. The most potent carcinogens in smokeless tobacco products are nitrosamines including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK] and N'-nitrosonornicotine [NNN].[24] Ammonium hydroxide and diammonium phosphate are added to reconstituted tobacco leaf as a flavoring agent and binder. In rodent studies, the addition of these compounds up to 5% of tobacco leaf increases per cigarette yields of nicotine, tar, and total particulate matter; however, 90-day inhalation studies demonstrate a decrease in formaldehyde content in smoke and minimal histopathologic changes when compared with tobacco leaf without these 2 compounds.[25]

TOBACCO SMOKE

MAINSTREAM SMOKE. Mainstream smoke is the smoke released from the mouth-end of the tobacco smoking product during puffing that is an aerosol containing gases (i.e., primarily nitrogen, oxygen, carbon dioxide) and about 10^{10} particles/mL (tar). The distribution of chemicals between the two phases depends on environ-

mental characteristics and the physiochemical properties (e.g., volatility, stability) of the individual chemicals. Table 66.3 lists the yields from 40 chemicals in mainstream smoke from 26 US cigarette brands using the Massachusetts method of machine-smoking. Sidestream smoke is released from the burning end of the tobacco or through the surrounding material mostly between puffs, whereas secondhand (environmental) smoke is an

TABLE 66.3. Selected Yields of Chemicals in Mainstream Cigarette-Machine Smoke from 26 US Brands. Adapted from Reference 2.

Constituent	Median Yield	Range
Tar	25.8 mg	6.1–48.7 mg
Carbon monoxide	22.5 mg	11.0–40.7 mg
Nicotine	1.70 mg	0.50–3.32 mg
Acetaldehyde	1618.1 µg	596.2–2133.4 µg
Isoprene	713.2 µg	288.1–1192.8 µg
Acetone	627.9 µg	258.5–828.9 µg
Nitric oxide	457.3 µg	202.8–607.1 µg
Hydrogen cyanide	380.8 µg	98.7–567.5 µg
Methyl ethyl ketone	170.3 µg	72.5–230.2 µg
Acrolein	162.9 µg	51.2–223.4 µg
Toluene	124.2 µg	48.3–173.7 µg
Propionaldehyde	110.2 µg	46.8–144.7 µg
Hydroquinone	103.9 µg	27.7–203.4 µg
Catechol	92.1 µg	28.1–222.8 µg
Benzene	75.9 µg	28.0–105.9 µg
1,3-Butadiene	75.2 µg	23.6–122.5 µg
Butyraldehyde	70.0 µg	28.8–95.6 µg
Formaldehyde	49.5 µg	12.2–105.8 µg
Crotonaldehyde	44.1 µg	11.6–66.2 µg
Ammonia	36.6 µg	9.8–87.7 µg
Phenol	25.1 µg	7.0–142.2 µg
Acrylonitrile	23.2 µg	7.8–39.1 µg
m-Cresol + p-cresol	19.4 µg	7.3–77.3 µg
Pyridine	14.9 µg	2.8–27.7 µg
Styrene	11.7 µg	4.5–19.3 µg
o-Cresol	8.0 µg	ND–33.9 ug
Quinoline	1.0 µg	0.3–2.7 µg
NNN	199.1 ng	99.9–317.3 ng
N-Acetyltransferase	186.3 ng	95.2–298.6 ng
NNK	147.3 ng	53.5–220.7 ng
Cadmium	131.8 ng	31.0–221.8 ng
Lead	52.1 ng	11.0–92.1 ng
1-Aminonaphthalene	30.7 ng	13.4–64.5 ng
N-Nitrosoanabasine	26.2 ng	14.2–45.3 ng
Benzo[a]pyrene	22.5 ng	5.6–41.5 ng
2-Aminonaphthalene	15.5 ng	5.7–28.6 ng
Arsenic	10.7 ng	1.6–24.9 ng
Mercury	4.8 ng	2.5–14.2 ng
4-Aminobiphenyl	4.5 ng	18.–7.8 ng
3-Aminobiphenyl	2.9 ng	1.3–4.8 ng

Abbreviations: NNN = N'-nitrosonornicotine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

air-diluted mixture of sidestream smoke and exhaled mainstream smoke. About 15–25% of the nicotine in tobacco appears in mainstream smoke, while sidestream smoke contains about 75% of the nicotine in cigarette tobacco.[26]

The particulate phase of tobacco smoke contains at least 3,500 chemicals and over 60 carcinogens for which there is sufficient evidence of carcinogenicity in either laboratory animals or humans as evaluated by the International Agency for Research on Cancer (IARC). These carcinogens include polycyclic aromatic hydrocarbons (e.g., benzo[a]pyrene), nitrosamines, and aromatic amines), aromatic amines, benzene, N-nitrosamines, and low-molecular-weight organic compounds as well as other lung carcinogens, tumor promoters, and cocarcinogens.[27] Many of the compounds are carcinogenic in some, but not all, animal models with the exception of the tobacco-specific N-nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). This compound is a potent carcinogen in all three rodent models (hamster, mouse, rat) tested. In general, the concentrations of strong carcinogens (e.g., polycyclic aromatic hydrocarbons, nitrosamines, aromatic amines) occur in smaller amounts than weak carcinogens (e.g., acetaldehyde).[28] Cigarette smoke also contains metals including cadmium, lead, chromium, nickel, mercury, and arsenic. The carcinogenicity of metals in tobacco smoke is not well defined because of the lack of data on valence states and anions that strongly influence the carcinogenic properties of these metals. Table 66.4 lists the most important carcinogens in mainstream tobacco smoke. In addition to being a tumor promoter, cigarette smoke contains cocarcinogens (e.g., catechol, methylcatechol, pyrogallol, pyrene, benzo[e]pyrene, fluoranthene, decane, undecane).[27] Cigarette smoke contains free radicals in the particulate phase that can induce oxidative damage.[29]

Most of these carcinogens form during combustion; consequently tobacco smoke contains more carcinogens than tobacco leaves.[30] The chemical composition of tobacco smoke depends primarily on the type of tobacco and smoking method rather than stated tar yields. Newer curing technologies can reduce the concentrations of tobacco-specific nitrosamines (e.g., NNK). Other factors determining the composition of tobacco smoke include the presence of filters, type of additives, amount of ventilation, and the paper porosity. Almost three-quarters of the nicotine in a cigarette appears in the sidestream smoke, primarily in the gaseous or vapor phase. In most developed countries, the yield of tar, nicotine, and carbon monoxide in cigarette smoke has decreased over recent decades based on standard machine-smoking tests. The sales-weighted average yield of nicotine in US cigarette smoke in 1998 was

0.9 mg compared with 1.4 mg in 1968.[31] The tar yield, nicotine content, and carbon monoxide concentrations in tobacco smoke do not correlate directly to the amounts of various carcinogens present in tobacco smoke. Narghile waterpipe smoke (hookah, shisha) contains substantial amounts of nicotine and polycyclic aromatic hydrocarbons associated with cancer, at least in part as a result of the combustion of charcoal during the heating of tobacco; however, the exact composition of waterpipe smoke differs from cigarette smoke.[32,33]

SECONDHAND (ENVIRONMENTAL) SMOKE. Secondhand (environmental) tobacco smoke is a mixture of exhaled mainstream smoke and sidestream smoke from the tobacco smoking source that is diluted with ambient air. Secondhand tobacco smoke is qualitatively different than mainstream smoke, consisting of a gas phase and a particulate phase that change with time and distribution in the environment. Table 66.4 lists the ratio of the concentration of some carcinogens in sidestream smoke to the ratio of these compounds in mainstream smoke. There is substantial variability in the chemical composition of inhaled secondhand smoke depending on the smoking source and smoking patterns. Secondhand smoke contains nicotine as well as carcinogens and other toxins. The dilution of sidestream smoke in ambient air causes substantially less intake of carcinogens than mainstream smoke; therefore, the carcinogenic risk of lung cancer is proportionally reduced for passive smoking compared with active smoking.[34] The typical nicotine concentration in the ambient air from homes of tobacco smokers ranges from about 2–10 µg/m^3.

DOSE EFFECT

Tobacco Products

The average manufactured cigarette in the United States contains 8–14 mg of nicotine; during usual smoking conditions, the cigarette smoker absorbs approximately 1–2 mg nicotine/cigarette.[35,36] Nicotine extraction from 5–10 cigarettes in hot water and the subsequent administration of the fluid as an enema resulted in moderately severe poisoning.[37] Although most cases of cigarette ingestion by children result in no or mild symptoms, severe poisonings (depressed respiration, dysrhythmias, convulsions) occurred after the reported ingestion of 2 European cigarettes (nicotine content not reported) by 9- and 10-month-old infants.[38] A retrospective review of 700 US children below the age of 6 years indicated that serious toxicity is unlikely following the ingestion of <2 whole cigarettes or <6 cigarette butts.[39] This study also indicated that the inges-

TABLE 66.4. Recognized Pulmonary Carcinogens in Mainstream Tobacco Smoke. Adapted from Reference 27.

Carcinogen Class	Compound	Amount (ng/cigarette)*	Ratio†
Polycyclic aromatic hydrocarbons	Benzo[a]pyrene	20–40	2.5–3.5
	Benzo[b]fluoranthane	4–22	
	Benzo[f]fluoranthane	6–21	
	Benzo[k]fluoranthane	6–12	
	Dibenzo[a, i]pyrene	1.7–3.2	
	Indeno[1,2,3-cd]pyrene	4–20	
	Dibenz[a,h]anthracene	4	
	5-Methylchrysene	0.6	
Asaarenes	Dibenz[a,h]acridine	0.1	
	7H-Dibenzo[c,g]carbazole	0.7	
N-Nitrosamines	N-Nitrosodiethylamine	ND–2.8	<40
	4-(Methylnitrosamino)-1- (3-pyridyl)-1- butanone (NNK)	80–770	1–4
Miscellaneous organic compounds	1,3-Butadiene	$20–70 \times 10^3$	
Metals	Ethyl carbamate	20–38	
	Nickel	0–510	13–30
	Chromium	0.2–500	
	Cadmium	0–6670	7.2
	Polonium-210	0.03–1.0 pCi	1.0–4.0
	Arsenic	0–1400	
Inorganic compounds	Hydrazine	24–43	

Abbreviation: ND = Not detected

*Mainstream smoke.

†Ratio of amount in sidestream smoke to amount in mainstream smoke. Although most of these ratios exceed 1, dilution with ambient air substantially reduces passive uptake of these carcinogens by nonsmokers.

tion of 7.5–15 g of smokeless tobacco by young children was associated with vomiting, agitation, and altered consciousness. Symptoms of nicotine toxicity may develop in patients following the ingestion of smaller quantities of tobacco products from other countries because these products may contain more nicotine than corresponding US tobacco products.

Overdose

Although the commonly quoted estimated lethal adult dose of nicotine is approximately 60 mg,[40] there are few data correlating serious nicotine toxicity to dose. A 17-year-old man developed vomiting and sudden cardiopulmonary arrest a few minutes after ingesting a solution containing an estimated dose of 5 g nicotine.[10]

Risk Assessment

Tobacco smoking causes an estimated 90% of male lung cancer deaths and 75–80% of female lung cancer deaths in the United States. Fewer than 20% of smokers will develop lung cancer, depending on the balance between the activation and detoxification of carcinogens in tobacco smoke and their smoking techniques. Smoking

cessation reduces the risk of developing lung cancer after 5 years of abstinence, but the relative risk of lung cancer in a smoker never returns to the lung cancer risks of lifelong nonsmokers.[27] Reduction of smoking by 50% in heavy smokers (≥15 cigarettes/d) also reduces the risk of lung cancer.[41]

TOXICOKINETICS

Absorption

Case reports of nicotine toxicity document the absorption of clinically significant amounts of nicotine from the skin,[42] lungs, rectum,[43] and gastrointestinal tract. [44] The absorption of nicotine is highly pH-dependent and the amount of nicotine dose absorbed depends on product pH, formulation, mastication, and nicotine content. The oral bioavailability of nicotine is approximately 20–45% as a result of a large first-pass effect.[45] As a result of the alkaline pK_a of nicotine, the acid pH of the buccal cavity and the stomach limit the absorption of nicotine from these locations. Nicotine absorption is much higher in the alkaline environment of the duodenum than in the acid milieu of the stomach. Nicotine chewing gum is formulated with a buffered ion

exchange resin base of pH 8.5 that releases nicotine slowly for absorption through the buccal mucosa and small intestine. Experimental studies demonstrated that absorption of nicotine from a solution buffered to a pH of 1, 7.4 and 9.8 was 3.3%, 8.2%, and 18.6%, respectively.[46] Case reports indicate that serious toxicity may result from the dermal absorption of nicotine. Dermal absorption of a 95% nicotine solution resulted in coma and respiratory failure.[47]

The absorption of nicotine in smoke delivered to the alveoli and small airways is rapid as a result of the easy diffusion of nicotine across pulmonary membranes in the physiological pH (i.e., 7.4) of the lungs. Peak plasma concentrations of nicotine during smoking occur about 10 minutes after inhalation.[48]

Distribution

Nicotine distributes extensively and rapidly into tissues with a volume of distribution (V_d) ranging from about 2.2–3.3L/kg.[49] The average V_d following the intravenous administration of a nicotine dose equivalent to that present in commercial cigarettes was approximately 1.7 L/kg.[50] The V_d of cotinine is smaller (i.e., about 0.7–0.9 L/kg) than nicotine. Although there is substantial interindividual variation, nicotine concentrations are generally higher in arterial blood than venous blood with a mean ratio of 2.3–2.8 (range up to 10) during active smoking.[51] Plasma concentrations of nicotine decline rapidly over 20 minutes following the cessation of smoking with a distribution half-life of ~8 minutes.[23]

Plasma protein binding of both nicotine and cotinine is low (i.e., <5%). Cotinine distributes into semen at concentrations similar to those in the blood.[52]

Biotransformation

The liver extensively metabolizes nicotine to at least 6 primary metabolites as displayed in Figure 66.4. Hepatic cytochrome P450 enzymes catalyze the metabolism of about 70–80% of administered dose of nicotine to inactive metabolites (e.g., cotinine, nicotine-1′-N-oxide) via C-oxidation. In vitro and in vivo studies indicate that CYP2A6 is the primary enzyme for the oxidation of nicotine and cotinine. CYP2A6 genetic variants contribute to the interindividual and interethnic variation in nicotine and cotinine metabolism.[53] Experimental studies of poor and extensive metabolizers of dextromethorphan (i.e., a probe for CYP2D6 activity) indicate that CYP2D6 is not the major isoenzyme for the metabolism of nicotine or cotinine.[54] CYP2B6 is the second most active cytochrome P450 isoenzyme in nicotine C-oxidation, and CYP2E1 has some activity in vitro at high nicotine concentrations.[55] Nornicotine is a minor metabolite of nicotine as well as a constituent of tobacco. Cotinine has 6 primary metabolites including 3′-hydroxycotinine, 5′-hydroxycotinine, cotinine N-oxide, cotinine methonium ion, cotinine glucuronide, and norcotinine.

The initial step in the bioactivation of carcinogens in smoke involves the cytochrome P450 isoenzymes, whereas monoamine oxidases, lipoxygenases, cyclooxygenases, and myeloperoxidases are less commonly
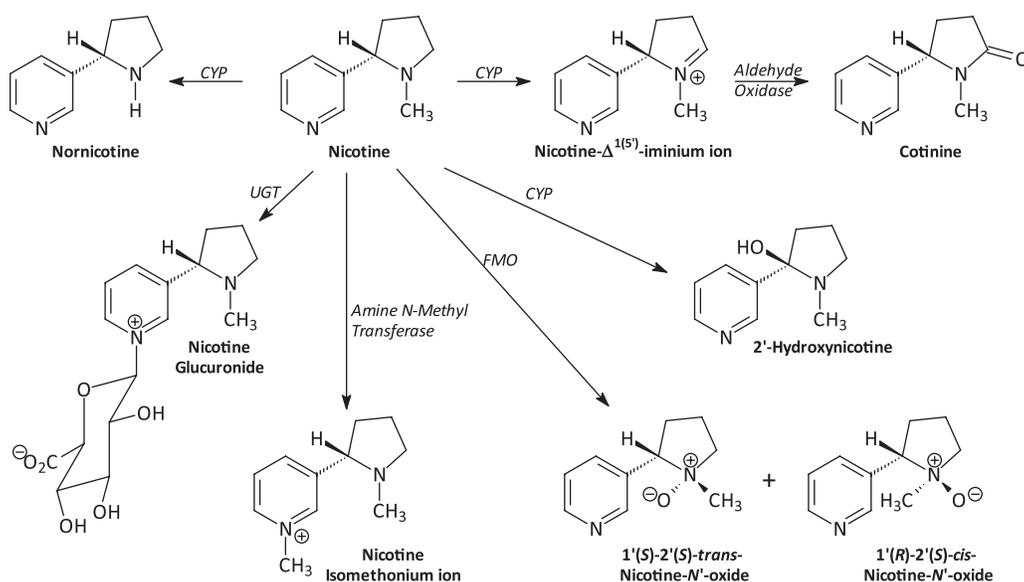
FIGURE 66.4. Primary routes of nicotine metabolism.[23] CYP = cytochrome P450 isoenzymes; FMO = Flavin-containing monooxygenase 3; UGT = uridine diphosphate-glucuronosyl transferases.

involved with nicotine metabolism. Metabolic activation of the carcinogens typically results in electrophilic, oxygenated intermediates that covalently bind to DNA or other macromolecules to form adducts. Detoxification of these electrophilic intermediates involves further transformation catalyzed by glutathione *S*-transferases, epoxide hydrolase, *N*-acetyl transferases, uridine-5′-diphosphate-glucuronosyltransferases, sulfotransferases, and other enzymes. The metabolic activation of benzo[a]pyrene results in the formation of the highly electrophilic 7,8-diol-9,10-epoxide, which forms adducts with exocyclic N^2 of guanine. α-Hydroxylation is the major metabolic activation pathway for both NNK and the main metabolite [4-(*N*-methylnitrosamino)-1(3-pyridyl)-1-butanol, NNAL]. This pathway produces diazonium ions and the subsequent formation of methyl adducts (7-methylguanine, O^6-methylguanine) as well as pyridyloxobutyl adducts. *In vivo* studies indicate that tobacco smoke induces several phase I and phase II enzymes including CYP1A1, CYP1A2, CYP2E1, and some isoforms of uridine-5′-diphosphate-glucuronosyltransferase.[56]

Elimination

Although nicotine has high affinity for CYP2A6, the clearance of nicotine from the blood depends more on hepatic blood flow than intrinsic metabolic activity. The excretion of nicotine occurs by glomerular filtration and tubular secretion depending on urinary pH. In acidic urine, nicotine appears primarily in the ionized form and tubular reabsorption is minimal. The kidneys excrete nicotine metabolites as 3′-hydroxycotinine, unchanged compounds or glucuronide conjugates with 3′-hydroxycotinine being the main urinary metabolite of nicotine and cotinine in all mammalian species studied.[23,66] Figure 66.5 displays urinary nicotine and metabolites in humans as a percentage of an absorbed dose of nicotine. About 5–10% of the absorbed dose of nicotine from cigarette smoking appears in the urine as unchanged nicotine, depending on urinary pH.[57] The kidneys excrete about 10–15% of an absorbed dose of nicotine as unconjugated cotinine in the urine, whereas the remainder of this metabolite is converted to cotinine glucuronide, *trans*-3′-hydroxycotinine and *trans*-3′-hydroxycotinine glucuronide. The average plasma elimination half-life of nicotine following intravenous administration is about 2–3 hours, whereas the plasma half-life of the inactive metabolite cotinine is about 16–20 hours. Elimination of nicotine and cotinine is similar in smokers and nonsmokers, but there is substantial individual variation in the clearance rates of both of these compounds.[58] After 3–4 days, blood and urine cotinine concentrations decline to values associated with nonsmokers.[59] Table 66.5 lists the approximate urinary elimination half-life of tobacco alkaloids determined in a study of 34 habitual tobacco users.[60]

Maternal and Fetal Kinetics

Nicotine crosses the placenta easily without evidence of biotransformation to cotinine by the placenta.[61]
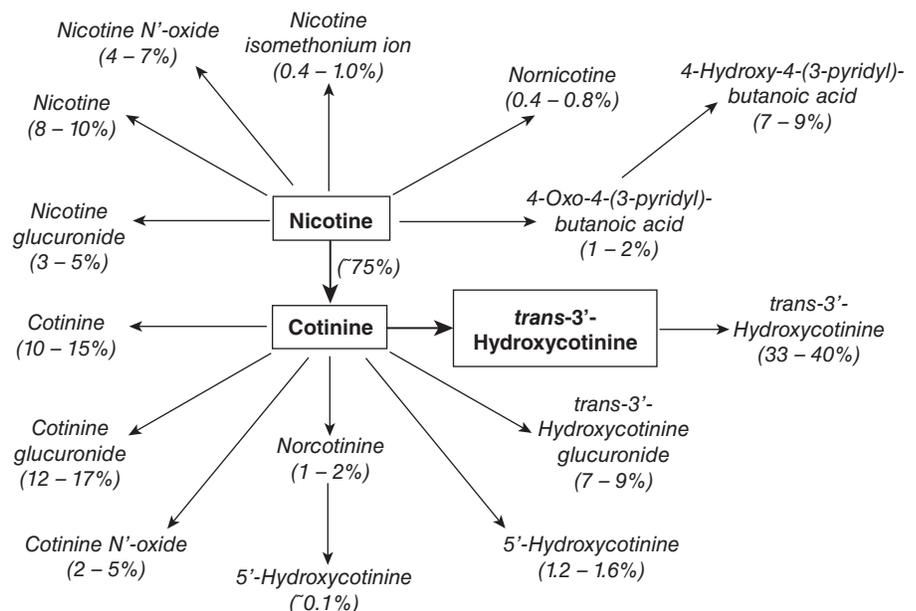
FIGURE 66.5. Urinary elimination of an absorbed dose of nicotine.[23]

TABLE 66.5. Approximate Urinary Elimination Half-Life of Tobacco Alkaloids.[60]

Alkaloid	Mean Urinary t$_{1/2}$*	Range*
Anabasine	16	10–27
Anatabine	10	6–15
Nornicotine	12	6–27
Nicotine	11	4–31
Cotinine	19	10–37

*Rounded to nearest hour; t$_{1/2}$ = half-life in hours.

Potentially, the nicotine concentration is slightly higher in fetal serum than in maternal serum. Secretion of nicotine in breast milk also occurs with a milk/maternal plasma nicotine ratio of approximately 3.[23] In a study of 18 smoking (i.e., 2–20 cigarettes/d) and 2 snuff-taking mothers, the mean daily intake of nicotine by breast-feed infants was about 7 μg/kg.[62]

Drug Interactions

Potential drug interactions involved with the induction of cytochrome P450 isoenzymes involved with nicotine metabolism heparin, theophylline, caffeine, tacrine, imipramine, haloperidol, pentazocine, propranolol, flecainide, and estradiol; however, there are limited human data on the clinical relevance of these potential drug interactions.

HISTOPATHOLOGY AND PATHOPHYSIOLOGY

Pathologic examinations of the bronchial mucosa in smokers reveal a variety of histologic changes including epithelial and goblet cell hyperplasia, submucosal glandular hypertrophy, subepithelial mononuclear cells (e.g., macrophages, CD8$^+$ T-cells), and ciliary denudation. Although these bronchial changes occur in the vast majority of smokers, these changes are not specific to tobacco smoking.[63] Characteristics of emphysema-type destruction of the distal bronchiole are almost universally present in heavy smokers (i.e., 2 packs/d).[64] Typically, centrilobular emphysema occurs in the upper lobes in tobacco smokers with chronic obstructive pulmonary disease (COPD).

Nicotine

Nicotine is the classic agonist for nicotinic cholinergic receptors. Nicotinic cholinergic receptors are ligand-gated ion channels consisting of 5 subunits; these receptors occur in the brain, autonomic ganglia, and the neuromuscular junction.[65] Although nicotinic receptors appear throughout the brain including the amygdala, septum, locus ceruleus, and brainstem motor nuclei, the highest concentrations of binding sites are located in the cortex, interpeduncular nucleus, and the thalamus.[66] Initially, nicotine stimulates the nicotinic cholinergic receptors stereoselectively in the sympathetic and parasympathetic ganglia of the autonomic nervous system as well as in the neuromuscular junction.[67] (S)-Nicotine is the active stereoisomer whereas (R)-nicotine is a weak agonist at the nicotinic cholinergic receptors. Depending on the dose of nicotine, the stimulatory phase may be transient with prolonged ganglionic blockade resulting from persistent membrane depolarization. Nicotine is not likely a direct carcinogen. Studies on the cocarcinogenicity and tumor promoting property via activation of protein kinase A/B are inconsistent.[28]

Tobacco Smoke

Tobacco smoke acts on multiple stages of carcinogenesis as well as causing chronic inflammation and disruption of natural protective barriers. In tobacco smoke, there are numerous carcinogens capable of initiating and promoting tumors in addition to acting as cocarcinogens. Figure 66.6 demonstrates the effect of carcinogens in tobacco smoke on multiple mutations and cellular processes in the development of lung cancer. Hallmarks of cancer include self-sufficiency in growth signals, insensitivity to antigrowth signals, sustained angiogenesis, tissue invasion and metastasis, evasion of apoptosis, and limitless replicative potential.[68] The stem cell and its early progenitor cell are the target cells for the initiation event during carcinogenesis.[69] Most carcinogens require metabolic activation, primarily by the cytochrome P450 isoenzymes. Cigarette smoking induces CYP1A1 and CYP2D6; the former helps metabolize polycyclic aromatic hydrocarbons in tobacco smoke, whereas the latter isoenzyme activates several nitrosamines including NNK.[70] The oxygenated intermediates formed by cytochrome P450 isoenzymes typically undergo further transformation by phase II enzymes (e.g., glutathione S-transferases, uridine-5′-diphosphate-glucuronosyltransferases, sulfotransferases). The GSTM1 gene codes for the mu class of glutathione S-transferases that acts upon various carcinogens including polycyclic aromatic hydrocarbon diol epoxide.[71] Biotransformation of carcinogens by the cytochrome P450 system detoxifies most of these compounds, but some of these electrophilic compounds form DNA adducts. Cellular repair mechanisms eliminate most DNA adducts before mutations occur by direct repair, base excision repair, and nucleotide excision repair. Apoptosis (i.e., programmed cell death) also removes cells with damaged DNA. Some DNA adducts
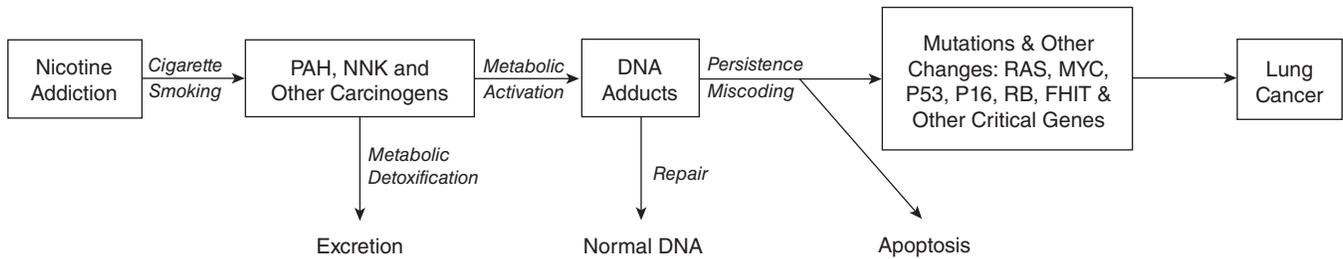
FIGURE 66.6. The effect of carcinogens in tobacco smoke on the multiple mutations and cellular processes in the development of lung cancer.[27]

evade normal repair mechanisms, leading to miscoding and permanent critical point mutations in oncogenes (e.g., Kirsten-ras) and tumor suppressor genes (e.g., p53). These mutations disrupt cell-cycle checkpoints and ultimately result in loss of normal growth control and the clinical appearance of a tumor.

Reinke's edema involves the swelling of the superficial lamina propria of the vocal cords, primarily in middle-aged female smokers.[72] The degree of edema and the associated voice abnormalities (hoarse, raspy) are directly related to the daily cigarette consumption and duration of exposure to smoke. These lesions are not precancerous.

CLINICAL RESPONSE

Acute Nicotine Poisoning

The onset of symptoms following the ingestion of toxic doses of nicotine is rapid (15–30 minutes) beginning with gastrointestinal distress (nausea, vomiting, abdominal pain, diarrhea, salivation). Case reports from the older medical literature indicate that death can occur within 5–10 minutes of the ingestion of liquid pesticide containing 40% nicotine.[73,74] Vomiting is almost always present following the ingestion of tobacco products, but the absence of vomiting does not necessarily exclude the development of potentially serious nicotine toxicity.[75] Symptoms in mild nicotine poisoning resolve in several hours. Severe nicotine poisoning involves a stimulatory phase followed by an inhibitory phase that occurs over 24 hours. Transiently, hypertension and tachycardia can develop followed by hypotension, bradycardia, decreased deep tendon reflexes, fasciculations, paralysis, and cardiovascular collapse. Neurologic symptoms include headache, diaphoresis, ataxia, lightheadedness, weakness, confusion, and in severe poisonings, convulsions, coma, and respiratory failure.[44] Nicotine acts unpredictably as an agonist of the muscarinic receptors; therefore, the presence of cholinergic symptoms (miosis, salivation, lacrimation, bronchorrhea, diarrhea, bradycardia) is variable.[76]

Green Tobacco Sickness

Case series associate the harvesting of green tobacco in a moist environment with an occupational disease called green tobacco sickness, particularly in nonsmokers.[77,78] This disease affects up to one-quarter of tobacco farm workers. Predisposing factors include working conditions and location,[79] lack of protective clothing (e.g., rain suits),[80] working with burley- or flue-cured tobacco,[81] working in humid environments with wet tobacco leaves, and workers with disruption of their skin integrity (e.g., superficial wounds, rash).[82,83] Cigarette smokers rarely display symptoms of green tobacco sickness, suggesting that a tolerance to nicotine can develop as a result of chronic tobacco smoking.[84] This self-limited illness is characterized by nonspecific effects including headache, pallor, nausea, vomiting, diarrhea, abdominal cramps, weakness, lightheadedness, diplopia, and difficulty breathing.[85] Symptoms typically begin during the afternoon or evening after a full day of work with moist, green tobacco. The disease typically resolves by the following day. Occasionally, hospitalization may be required for bradycardia, hypotension, and dehydration.[86]

Tobacco Smoking

Tobacco smoking increases overall morbidity and mortality as a result of the increased incidence of various cancers, chronic obstructive pulmonary disease, cardiovascular disease, cerebrovascular disease, and atherosclerotic peripheral vascular disease. These increased overall mortality ratios for smokers are approximately 2.2 and 2.4 for men smoking <1 pack cigarettes per day and >1 pack cigarettes per day, respectively, compared with nonsmokers. The mortality ratios for women

smoking similar amounts of cigarettes are about 1.6 and 2.1, respectively.[87]

Cancer

The International Agency for Research on Cancer lists tobacco smoking and tobacco smoke as human carcinogens (Group 1).[2] Although tobacco smoke is carcinogenic, there are few data supporting nicotine as a carcinogen. Tobacco smoke is the most extreme example of a systemic carcinogen with tobacco smoking identified as the cause of cancer at more organ sites than any other carcinogen. Smoking causes cancers of the lungs, oral cavity, pharynx, larynx, esophagus (squamous cell carcinoma), pancreas, bladder, and renal pelvis. The increased risks of developing these cancers in tobacco smokers range from a 3-fold increase in pancreatic cancer to a 20-fold increased risk of lung cancer. Additionally, there is a causal association between tobacco smoking and cancers of the nasal cavity and sinuses, adenocarcinoma of the esophagus, stomach, liver, renal cell cancer of the kidney, cervical cancer of the uterus, and myeloid leukemia. In general, there is an approximate 2- to 3-fold increased risk of developing these types of cancers in tobacco smokers when compared with nonsmokers. Although there is some evidence of an association between tobacco smoking and colorectal cancers, the difficulty controlling potential confounders in these studies limits conclusions regarding a causal link between tobacco smoking and colorectal cancer. Tobacco smoking probably does not cause an increased risk of developing endometrial, skin, ovary, central nervous system, thyroid, prostate, or female breast cancers.[88] There is also no clear association between tobacco smoking and multiple myeloma, lymphomas, lymphocytic leukemia, or soft tissue sarcoma. Tobacco smoke and in particular, the base/neutral fractions of cigarette smoke condensate are genotoxic in humans and in experimental animals, producing numerous mutagens in the urine.[89] In humans, tobacco smoking produces gene mutations and chromosomal abnormalities with lung tumors of smokers containing higher frequencies of *TP53* and *KRAS* mutations relative to nonsmokers.

Lung. Lung cancer is the most common cause of cancer death in the world; however, only about 20% of regular smokers develop lung cancer and the cause of this susceptibility is unclear.[90] Tobacco smoking accounts for up to 90% of lungs cancer deaths in populations with a high prevalence of tobacco smoking. The risk of lung cancer increases with the number of cigarettes smoked, the age of onset of tobacco smoking, and the duration of tobacco smoking with the latter risk

factor being the strongest determinant of lung cancer in these smokers. The use of filters and low tar cigarettes reduces mortality rates from tobacco smoking-induced lung cancer only slightly.[91] The risk of dying from lung cancer in a person smoking >2 packs cigarettes per day is up to 23-fold higher than that of nonsmokers.[92] Cessation of smoking eliminates the further increase in the risk of lung cancer. Tobacco smoking increases the risk of all histologic types of lung cancer, particularly squamous cell and small cell carcinoma.[93] In a meta-analysis of 48 studies from the last decades of the 20th century, the combined estimate of relative risk for heavy smokers (i.e., $\geq$ 30 cigarettes/d) ranged from 4.10 (95% CI: 3.16–5.31) for adenocarcinoma to 11.3 (95% CI: 8.10–15.9) for squamous cell carcinoma, and 18.3 (95% CI: 9.26–36.4) for small cell carcinoma.[94] There was a similar dose response curve for smoking duration with the highest risk for squamous cell and small cell carcinomas. Family history of lung cancer and concomitant exposure to radon or asbestos substantially increases the risk of lung cancer in tobacco smokers.[95]

Upper Aerodigestive Tract. Tobacco smoking is causally associated with cancers of the oral cavity (lip, tongue, mucosa), nasal cavity and paranasal sinuses, nasopharynx, oropharynx, hypopharynx, and larynx. The relative risk of cancers of the oral cavity and pharynx associated with smoking ranges from about 2- to 10-fold depending on associated risk factors.[88] The use of smokeless tobacco or ethanol in combination with tobacco smoking substantially increases the risk of cancer in the oral cavity and larynx. Smoking over 30 cigarettes daily and drinking 20 ounces of ethanol weekly increases the risk of developing upper airway cancer ~9-fold compared with nonsmokers.[96] Squamous cell carcinoma of nasal cavity and paranasal sinuses is more closely associated with tobacco smoking than adenocarcinomas in this area. Nasopharyngeal cancer is endemic in regions of Southeast Asia and North Africa as a result of Epstein-Barr virus infection. The relative risk of these cancers is about 2–5 times greater in smokers than nonsmokers.[97]

Upper Digestive Tract. In most epidemiologic studies, the risk of all types of esophageal cancers increases with the duration of smoking and numbers of cigarettes smoked daily, particularly with squamous cell carcinoma. Over 90% of esophageal cancers are either squamous cell carcinomas or adenocarcinomas. Tobacco smoking in combination with ethanol consumption greatly increases the risk of squamous cell cancer of the esophagus. There is a dose-related increase in pancreatic cancer related to the number of cigarettes smoked daily and the duration of smoking. Both cohort and

case-control studies of tobacco smoking and stomach cancer demonstrate a consistent increased risk associated with tobacco smoking in both men and women, even after control of confounders (e.g., ethanol consumption, dietary factors, *Helicobacter pylori* infection). A meta-analysis of 40 epidemiologic studies on the risk of stomach cancer in tobacco smokers indicated a relative risk of about 1.5–1.6 when compared with non-smokers.[98] There is sufficient evidence to associate a moderate increased risk of liver cancer in tobacco smokers after control for ethanol consumption and hepatitis B and hepatitis C infections. Most studies indicate that the risk of liver cancer increases with higher daily cigarette consumption and duration of tobacco smoking.

URINARY TRACT. Tobacco smoking is an important, dose-related risk factor in developing transitional cell carcinomas of the bladder, ureter, and renal pelvis. Several cohort and case-control studies indicate that tobacco smoking increases the risk of renal cell carcinoma in both men and women. A meta-analysis of 19 case-control studies and 5 cohort studies demonstrates a relative risk of renal cell carcinoma of 1.54 (95% CI: 1.42–1.68) and 1.22 (95% CI: 1.09–1.36) for male and female smokers, respectively.[99] There was a strong dose-dependent increase in the relative risk associated with intensity of smoking (i.e., daily cigarette consumption).

CERVIX. Several studies associate invasive cervical squamous cell carcinoma with tobacco smoking, even after adjustment for human papillomavirus infection. In a case-control study of 180 women with adenocarcinoma of the cervix, 391 women with squamous cell carcinoma of the cervix, and 923 population controls, long duration of smoking (≥20 years) was associated with a 2-fold increase in the risk of squamous cell carcinoma of the cervix.[100] Tobacco smoking was not associated with the risk of adenocarcinoma in the cervix. Limited studies of adenocarcinomas and adenosquamous carcinomas have not demonstrated a consistent relationship between tobacco smoking and these cervical cancers. In a meta-analysis of 6 case-control studies, current smoking was associated with a significantly increased risk of squamous cell carcinoma (summary odds ratio [OR] = 1.47, 95% CI: 1.15–1.88), but not with an increased risk of adenocarcinoma (summary OR = 0.82, 95% CI: 0.60–1.11).[101]

LEUKEMIA. There is a causal relationship between tobacco smoking and myeloid leukemia that increases with the amount of tobacco smoking. Two prospective studies demonstrated an approximately 50% increase in myeloid leukemia in tobacco smokers.[102] Other types of leukemia and lymphomas have not been directly linked to tobacco smoking.

CARDIOVASCULAR DISEASE

Prospective studies suggest that tobacco smoking increases the incidence of coronary artery disease 2- to 4-fold with a similar increased risk of sudden death as a result of the prothrombotic properties of tobacco smoke, after adjustment for dietary factors, blood pressure, and serum lipoproteins.[103,104] Tobacco smoking also increases the risk of coronary artery disease in individuals with other risk factors (e.g., hypercholesterolemia, diabetes mellitus). Cessation of smoking significantly reduces the increased relative risk of myocardial infarction in patients under the age of 55 years to near zero after 2 years.[105] Tobacco smoking accelerates aortic atherosclerosis and increases mortality from aortic aneurysm when compared with nonsmokers.[91] Cigarette smoking is a strong risk factor for peripheral arterial disease; smoking increases the risk of peripheral vascular disease severalfold in a dose-dependent manner.[103] Analysis of data from the Framingham Offspring Study indicated that the OR of peripheral vascular disease associated with smoking was 2.0 (95% CI: 1.1–3.4).[106] A hospital-based case-control study compared 291 smokers with newly diagnosed peripheral vascular disease and 828 age- and sex-matched smokers without peripheral vascular disease.[107] Smokers in the highest quartile had an increased risk of developing peripheral vascular disease compared with smokers in the lowest quartile The odds ratio of peripheral vascular disease for the highest quartile (>48 pack-years) of smokers was 1.63 (95% CI: 1.11–2.39; *P* = .011) compared with the lowest quartile (<31 pack-years). Epidemiologic studies indicate that exposure to secondhand tobacco smoke also increases the risk of coronary artery disease. Available meta-analyses suggest that involuntary smoking increases the risk of an acute coronary event by about 25–35%; however, studies on the risk of cardiovascular disease and the use of snuff are inconsistent. A Swedish case-control study compared the use of snuff in 1,760 men, age 45–70 years, with a history of myocardial infarction in 1992–1994 based on mailed questionnaires. After adjustment for age, hospital catchment area, and smoking, the relative risk of first acute myocardial infarction in former and current snuff users was 1.1 (95% CI: 0.8–1.5) and 1.0 (95% CI: 0.8–1.3), respectively. A Swedish mortality study of 6,297 smokeless tobacco users and 32,546 nontobacco users demonstrated an age-adjusted increased risk of death from all cardiovascular diseases (RR = 1.4, 95% CI: 1.2–1.6) during a 12-year follow-up.[108] There was no increased risk of cancer-related deaths in smokeless tobacco users.

However, a Swedish population-based cohort study of 16,754 women and 10,473 men over 10 years did not detect any statistically significant increased risk of myocardial infarction or stroke in men or women.[109] The relative risk (RR) of myocardial infarction in male snuff users compared with nontobacco users was 1.05 (95% CI: 0.8–1.4, $P = .74$) after adjustment for age and potential confounders.

PULMONARY DISEASE

Tobacco smoking is the most important risk factor for the development of COPD. Chronic obstructive pulmonary disease includes chronic bronchitis and emphysema; the smoking-attributable disease burden (mortality, morbidity, cost) is greater for COPD than for smoking-attributable disease associated with coronary artery disease or lung cancer.[110] Chronic bronchitis is defined by the presence of chronic productive cough for at least 3 months in 2 consecutive years after the exclusion of other causes of chronic cough. Emphysema is characterized by abnormal, permanent expansion of airspaces distal to the terminal bronchiole along with destruction of the walls and the absence of obvious fibrosis.

The unique inflammatory process associated with asthma separates this disease from COPD, although some airway hyperresponsiveness occurs in some cases of COPD. About 50% of tobacco smokers develop chronic bronchitis, manifest by inflammation of the large airways and chronic, productive cough. Approximately 30% of tobacco smokers do not develop chronic respiratory symptoms or clinically significant abnormalities of lung function despite the presence of pulmonary inflammation and subtle changes in lung function.[111] Objectively, COPD is manifest by irreversible decline of forced expiratory volume in one second (FEV_1) exceeding 50 mL/year, increasing dyspnea and other respiratory symptoms (cough, sputum production, wheezing), and progressive deterioration of health. Chronic obstructive pulmonary disease results in a reduction of the ratio of FEV_1 to forced vital capacity (FVC) below 88% of predicted in males and 89% of predicted in females.[112] The reversibility of tobacco-induced decline in FEV_1 differs between smokers with COPD, smokers with nonobstructive bronchitis, and smokers without chronic respiratory symptoms. Cessation of tobacco smoking reduces bronchial hyperresponsiveness and respiratory symptoms, and also prevents the excessive decline in lung function in all three of these groups. Cross-sectional studies indicate that FEV_1 is lowest in tobacco smokers, intermediate in ex-smokers, and highest in nonsmokers. The exception is the group of ex-smokers over the age of 70 years. The FEV_1 of these ex-smokers is similar to smokers of the same age, probably as a result of the cessation of smoking by symptomatic smokers (i.e., "healthy smoker effect").[113]

The initial diagnosis of COPD is usually established in individuals over the age of 45–50 years with a history of smoking for several decades. Tobacco smokers develop more respiratory symptoms (cough, sputum production, wheezing, dyspnea), increased respiratory infections, and an accelerated decline in the FEV_1 when compared with nonsmokers and former tobacco smokers.[114] The suboptimal development of lung function during childhood and early adolescence along with accelerated decline of FEV_1 as a result of smoking substantially increase the risk of clinically significant COPD in later years.[115] The development of COPD in tobacco smokers depends on genetic susceptibility as only 10–20% of tobacco smokers develop clinically significant airway obstruction, but to date only α-1-antitrypsin deficiency has been identified as a cause of the increased susceptibility.[116] Consequently, the dose-response of active tobacco smoking on the severity of airflow obstruction (i.e., FEV_1) is relatively weak. The impact of environmental smoke on adult lung function is relatively small compared with active tobacco smoking. Although studies indicate that exposure to environmental smoke causes a statistically significant accelerated decline in FEV_1, the magnitude of the decreases is relatively small (i.e., about 3–4 mL/y).[117]

Cigarette smoking is associated with several diffuse lung diseases that result from bronchiolar and interstitial lung inflammation secondary to chronic tobacco smoke inhalation. These diseases occur primarily in relatively young adult smokers. Smoking-related diffuse lung diseases include desquamative interstitial pneumonia, respiratory bronchiolitis-associated interstitial lung disease, pulmonary Langerhans cell histiocytosis, and acute eosinophilic pneumonia; smoking also increases the risk of developing fibrotic interstitial lung diseases (e.g., idiopathic pulmonary fibrosis).[118]

CEREBROVASCULAR DISEASE

After controlling for hypertension and coronary artery disease, the risk of stroke in tobacco smokers is about 2-fold greater than in nonsmokers.[103] In general, the relative risk of a tobacco smoker developing cerebrovascular disease is lower than coronary artery disease or aortic aneurysm. Pooled estimates using death as the endpoint in 9 epidemiologic studies indicated that the association of ever smoking with aortic aneurysm is 2.5 times (95% CI: 2.2–2.8) greater than the association of ever smoking with coronary artery disease and 3.5 times (95% CI: 2.4–5.3) greater than the association of ever smoking with cerebrovascular disease.[119]

Prenatal maternal smoking is associated with placental abruption, placenta previa, premature rupture of the membranes, fetal growth restriction, and preterm delivery.[120] Maternal smoking is also associated with an increased incidence of complications during pregnancy, particularly intrauterine growth retardation resulting in lower neonatal birth weights, when compared with nonsmokers.[121] There is a dose-related effect of maternal smoking on birthweight as a result of intrauterine growth retardation rather than preterm delivery.[122] The estimated effect is a 5% reduction in relative weight/ daily pack of cigarettes smoked by the mother.[123] Cessation of smoking by the 16th week of pregnancy results in normal neonatal birth weights, when compared with infants from nonsmoking mothers.[124] However, there is no definite evidence that maternal smoke causes long-term adverse effects on growth or cognitive function.[125] A large cross-section study suggested that maternal smoking causes a borderline increase (OR = 1.5, 95% Cl: 1.0–2.2) in the risk of abruptio placentae,[126] whereas the risk of placenta previa is mildly increased (OR = 1.9, 95% CI: 1.2–3.0) in maternal smokers.[127] In general, studies demonstrate that the risk of hypertensive disease during pregnancy including preeclampsia is reduced in maternal smokers when compared with nonsmokers.[128]

Abstinence Syndrome

Mild symptoms of withdrawal develop in some smokers following cessation of tobacco use. Symptoms include anxiety, depression, difficulty concentrating, irritability, anger, restlessness, and sleep difficulties.[129] Distinguishing features of nicotine withdrawal include mild slowing of heart rate and weight gain. Abstinence symptoms begin about 6–12 hours after the cessation of smoking and peak in 1–3 days. Resolution of symptoms occurs over 3–4 weeks.[130] With the exception of nicotine craving, these symptoms are not protracted. Neonatal withdrawal from maternal use of tobacco probably does not occur.[131]

Involuntary Smoking

The International Agency for Research on Cancer lists involuntary (passive) tobacco smoking as a human carcinogen (Group 1).[2] Similarly, the US Toxicology Program lists environmental tobacco smoke as a human carcinogen and the US EPA lists environmental smoke as a class A carcinogen. There is substantial evidence to document the genotoxicity of secondhand smoke.

Review of human biomarker studies of nonsmokers with involuntary exposure to tobacco smoke demonstrate the presence of DNA adducts, urinary mutagenicity, urinary metabolites of carcinogens, and sister chromatid exchanges (SCEs).[132] Meta-analysis of studies of lung cancer risks in nontobacco smokers with smoking partners demonstrate a dose-related increase in the risk of lung cancer in the nontobacco smoking partner compared with nonsmokers without a smoking partner. After controlling for confounders, these relative risks of lung cancer in the nonsmoker are ~20% in women and 30% in men.[133] The excess risk of lung cancer increases with increasing exposure to secondhand smoke. Meta-analyses of secondhand smoke in the workplace demonstrate smaller excess risk of lung cancer in nonsmokers in the range of 12–19%. Analysis of data from the Alcohol-Related Cancers and Genetic Susceptibility in Europe included 2,103 upper aerodigestive tract squamous cell carcinoma cases (oral cavity, oropharynx, hypolarynx, larynx) and 2,221 controls in the project.[134] Among never smokers, ever exposure to involuntary smoking was associated with an increased risk of these cancers (OR = 1.60; 95% CI: 1.04–2.46). The evidence of an increased risk of breast cancer or childhood cancers as a result of secondhand smoke is inconsistent. The absence of a strong dose-response relationship and the lack of a causal link between breast cancer and active smoking strongly argue against a causal association between breast cancer and secondhand smoke. Similarly, the association between exposure to environmental tobacco smoke (ETS) as a child and adult cancers is controversial. A study of 112,430 never smokers within the European Prospective Investigation into Cancer and Nutrition cohort did not detect a substantial risk of developing adult cancers after childhood exposure to ETS.[135] After stratifying by age, sex, and study center as well as adjusting for eduction, alcohol consumption, body mass index, physical activity, nutrition, and adult ETS exposure, there was no association between childhood ETS exposure in never smokers and overall cancer risks (hazard ratio = 0.97; 95% CI: 0.92–1.02) based on self-reports of childhood ETS. The only exception was pancreatic cancer; after adjustment for diabetes, the hazard ratio for pancreatic cancer in never smokers was 2.09 (95% CI: 1.14–3.84) as estimated by Cox proportional hazards models.

DIAGNOSTIC TESTING

Analytic Methods

Techniques for the determination of nicotine and cotinine concentrations in biologic samples include enzyme

immunoassay, chemiluminescent immunoassay, gas chromatography, high performance liquid chromatography, and gas or liquid chromatography/mass spectrometry. The limit of detection for the chemiluminescent immunoassay (Diagnostic Products Corp., Los Angeles, CA) was 2 ng/mL in 20 μL serum compared with 5 ng/mL in 50 μL urine for double antibody radioimmunoassay (Diagnostic Products Corp.).[136] The cross-reactivity of 3-hydroxycotinine for the chemiluminescent and cotinine enzyme immunoassay (Diagnostic Reagents, Sunnyvale, CA) was about 28% and 50%, respectively, with little cross-reactivity to other substances. Cotinine is stable in urine stored up to 30 hours at room temperature;[137] this compound is relatively stable in frozen urine samples stored at –20ºC (–4ºF) for at least several years.[138]

Biomarkers

Potential biomarkers for exposure to tobacco smoke include nicotine, cotinine, thiocyanate, carboxyhemoglobin, hydroxyproline, *N*-nitrosoproline, 1- and 2-naphthol, hydroxyphenanthrene compounds, phenanthrene dihydrodiols, 1-hydroxypyrene, benzene metabolites (*trans*, *trans*-muconic acid, *S*-phenylmercapturic acid), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL] and glucuronides (NNAL-Gluc), tobacco-specific adducts (protein, DNA), 8-oxodeoxyguanosine, 3-ethyladenine, and aromatic amines.[139] Active smoking increases the concentration of benzene, volatile organic compounds, and carbon monoxide in exhaled air. The concentrations of urinary metabolites of known carcinogens, including benzene metabolites, in smoke are higher in the urine of tobacco smokers than nonsmokers.

The most widely used biomarker of exposure to active or passive tobacco smoke is cotinine, which is a highly specific internal dose marker of both mainstream smoke and environmental tobacco smoke. Cotinine concentrations reflect exposure to the particulate-phase constituents (tar), but there are no direct biomarkers for tar uptake. NNAL and NNAL-Gluc are useful biomarkers because these two compounds are metabolites of the tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Other sensitive biomarkers for tobacco products include *trans*, *trans*-muconic acid, *S*-phenylmercapturic acid, naphthalene metabolites (1-, 2-napthol), 1-hydroxypyrene, 8-oxodeoxyguanosine, and 3-ethyladenine.[139] However, these biomarkers lack specificity as only NNAL and NNAL-Gluc are specific for exposure to tobacco products. Serum thiocyanate concentrations increase during moderate to heavy smoking, but determination of serum thiocyanate concentrations does not distinguish between nonsmokers, light smokers, or passive smokers.[2]

NICOTINE

The mean plasma nicotine concentration following smoking of one cigarette ranges from about 5–30 ng/mL as a result of the absorption of ~1 mg nicotine/cigarette smoked.[140] After smoking a cigarette, the average plasma nicotine concentration of regular smokers increases to ~10–11 ng/mL.[141] Typical plasma nicotine concentrations of smokers during the afternoon range from about 10–50 ng/mL.[142] The short plasma elimination half-life of nicotine limits the use of plasma nicotine as a biomarker of tobacco smoke exposure. In a study of volunteers undergoing treatment for nicotine addiction, the steady-state serum nicotine concentrations 2 weeks after the daily administration of 22 mg nicotine via transdermal patches ranged between 10–50 ng/mL.[143] Blood samples were drawn immediately before the daily nicotine patches were applied.

During occupational exposure to tobacco leaves at harvest time, the mean plasma nicotine concentrations from 10 healthy, nonsmoking women drawn during the morning work shift ranged between 0.79 ± 0.12 and 2.16 ± 0.84 ng/mL.[144] Peak serum nicotine concentrations exceeding 2,000 ng/mL indicate serious toxicity. A case report of a woman, who died of asphyxia from a plastic bag tied over her head following the placement of multiple nicotine patches on her body, suggests that some postmortem redistribution of nicotine may occur, although the distribution of the nicotine patches on the body may also alter the heart/femoral blood nicotine concentrations.[145]

COTININE

Cotinine is a sensitive, specific biomarker of exposure to tobacco smoke for up to about 2–3 days after exposure, depending on the intensity of tobacco smoking.[146] Estimates of serum and urine cotinine concentrations for children and adults are available based on pharmacokinetic equations using respiratory rate, duration of daily exposure, and concentration of nicotine in ambient air as displayed for adults in Equation 66.1 (for urine) and Equation 66.2 (for serum).[147]

$$U = 0.039\, \rho HN \qquad (66.1)$$

$$S = 0.006\, \rho HN \qquad (66.2)$$

where U = urine cotinine concentration in adults; ρ = respiration rate (sedentary = 0.5 m³/h, light activity = 1.0 m³/h); H = daily exposure duration (hours); and N = atmospheric nicotine concentration (μg/m³); and S = serum.

BLOOD. During occupational exposure to tobacco leaves at harvest time, the mean plasma cotinine concentrations from 10 healthy, nonsmoking women drawn during the morning work shift ranged between 8.74–20.54 ng/mL.[144] Typical plasma cotinine concentrations of smokers during the afternoon ranges from about 250–300 ng/mL.[142] The steady-state serum cotinine concentration in a group of active smokers ranged from 94–444 ng/mL.[143] In a study of volunteers undergoing treatment for nicotine addiction, the steady-state serum cotinine concentrations 2 weeks after the daily administration of 22 mg nicotine via transdermal patches ranged between 35–249 ng/mL.[143] In a study of 88 adult nonsmokers, light to moderate smokers (1–19 cigarettes daily), and heavy smokers (≥20 cigarettes daily), the median cotinine concentrations in blood were 0.8 ng/mL, 137 ng/mL, and 467 ng/mL, respectively, as measured by radioimmunoassay.[148] Serum cotinine concentrations below 10 ng/mL are highly suggestive of the lack of tobacco smoking in the last few days to a week.[136]

URINE. Although nicotine occurs in some foods (e.g., tomatoes, potatoes, bell pepper, cauliflower, black tea), the dose is relatively small compared with the nicotine in environmental tobacco smoke. The estimated average daily intake of nicotine from food sources was about 9 μg with range up to 100 μg, primarily as the result of ingesting black and instant teas.[149] The maximum daily urinary cotinine concentration in this study was approximately 6 ng/mL. Nicotine replacement therapy causes increased urinary cotinine concentrations; therefore, the analysis of minor alkaloids (e.g., anatabine, anabasine) from tobacco smoke is necessary to separate tobacco smoking from nicotine replacement therapy.[150]

Cotinine is a sensitive and specific biomarker for discriminating the source of tobacco smoke exposure. The concentration of cotinine in urine samples from smokers is about one magnitude greater than serum. In a study of 88 adult nonsmokers, light to moderate smokers (1–19 cigarettes daily), and heavy smokers (≥20 cigarettes daily), the median cotinine concentrations in urine samples from the 3 groups were 17 ng/mL, 3,416 ng/mL, and 7,179 ng/mL, respectively.[148] Urine cotinine concentrations below 200 ng/mL are highly suggestive of the lack of tobacco smoking in the last few days to a week.[136] In a study of involuntary and active smokers, receiver operating characteristics analysis determined an optimal urine cotinine cutoff of 31.5 ng/mL for the separation of these 2 groups with a sensitivity of 97.1 % and specificity of 93.9%.[151] Urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is also a sensitive and specific urinary marker of involuntary and active smoking that is present up to about 1 month after exposure.

DNA/PROTEIN ADDUCTS

DNA adducts directly measure the extent of carcinogen reactions with DNA. However, DNA and protein adducts are nonspecific markers of the early biologic effects of tobacco smoke. Protein adducts (hemoglobin, albumin) are surrogates for DNA adducts that are easier to obtain than DNA adducts. Covalent binding of carcinogens in tobacco smoke to blood proteins results in an increased incidence of these adducts in tobacco smokers compared with nonsmokers, primarily at guanine or adenine. These adducts result from a variety of chemicals in tobacco smoke including benzene, aromatic amines, polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone], acrylonitrile, and acrylamide. Protein adducts reflect the integrated exposure to tobacco smoke over the preceding 4 months as a result of their long elimination half-life. For example, N-(2-hydroxyethyl)valine results from the reaction between ethylene oxide in tobacco smoke and N-terminal valine in hemoglobin. This protein adduct is a nonspecific biologic effective dose marker of exposure to tobacco smoke.[152]

Abnormalities

FEV_1

The classical functional deficits associated with COPD and emphysema include airflow obstruction (i.e., decreased FEV_1), increased lung compliance, reduced diffusion capacity, and progressive air trapping with hyperinflation. In healthy subjects, maximal lung function occurs near the age of 20–25 years followed by a slow decline beginning about 35 years of age. The decline in FEV_1 in cross-sectional studies averages about 20–30 mL/year in nonsmokers compared with 25–80 mL/year in current smokers. The largest declines in FEV_1 occur in the approximate 15% of the smoking population susceptible to developing COPD and results in annual rates of decline up to 150 mL.[153] After adjustment for age and height, tobacco smokers overall had an average accelerated decline in the FEV_1 of ~13–14 mL/year during longitudinal studies lasting 5–10 years.[154,155] In these longitudinal studies, declines in FVC are not as closely associated with tobacco smoking as FEV_1.[156] Most studies demonstrate that after about 2 years of smoking cessation, the annual decline in FEV_1 of ex-smokers is similar to nonsmokers, but the overall FEV_1 of ex-smokers is not similar to nonsmokers because of the persistence of previous lung damage in ex-smokers.[157] The improvement in FEV_1 depends on several factors including airway responsiveness to beta-agonist, baseline FEV_1, methacholine reactivity, age, sex,

race, and baseline smoking rate. In a 5-year, longitudinal study of ex-smokers and nonsmokers, the mean annual rate of decline in FEV_1 after the first year of the study among the participants with sustained cessation of smoking was half the rate among those continuing to smoke (i.e., 31 ± 48 mL and 62 ± 55 mL, respectively).[158] The severity of the decline in FEV_1 is strongly related to cumulative cigarette consumption and preexisting airway hyperresponsiveness. The impact of tar content and filters on the severity of airflow obstruction measured by FEV_1 is unclear because many smokers alter inhalation patterns as an adaption to the lower nicotine content. In a study of 1,492 smokers of plain cigarettes and 1,936 smokers of filter cigarettes, there was no significant difference in the decline of FEV_1 between smokers of filter and nonfiltered cigarettes over a 5-year period.[159] A meta-analysis of 6 cross-sectional studies comparing water-pipe smokers, cigarette smokers, and nonsmokers did not detect a statistically significant difference in the decline in FEV_1 between water-pipe smokers and cigarette smokers.[160] The pooled standardized mean difference for FEV_1 was –0.43 (95% CI: –0.58–0.29) for water-pipe smokers compared with nonsmokers. The difference was equivalent to a 4.04% reduction in FEV_1. There was low homogeneity between the studies, and none of the studies reported using a standardized exposure assessment tool.

CARBOXYHEMOGLOBIN

Tobacco smoking is a known source of carboxyhemoglobin in smokers. There is a moderate correlation between cigarette smoking and carboxyhemoglobin depending on a variety of factors related to smoking topography (i.e., frequency of cigarette consumption, duration of inhalation, cigarette puff volume, cigarette length, the time since last smoking) as well as respiratory rate, ambient ventilation, and ambient carbon monoxide concentrations.[161,162,163] Under normal atmospheric conditions, the terminal elimination half-life of carboxyhemoglobin is approximately 4–6 hours. Most non-smoking urban dwellers have carboxyhemoglobin concentrations below 3% compared with 3–8% for smokers. In a study of 14 adults smoking at least one pack per day, the venous carboxyhemoglobin concentration averaged 8.2% (range. 1.4–14.5%).[164] A study of 856 Italian smokers and nonsmokers detected mean arterial capillary (ear lobe) carboxyhemoglobin concentrations of $4.17\% \pm 2.19\%$ and $1.82\% \pm 0.75\%$, respectively.[165] Smokers were defined as smoking ≥5 cigarettes daily with the last cigarette within the preceding 48 hours, whereas nonsmokers were defined as responding

"no" to the question, "Have you ever smoked cigarettes daily?" Carboxyhemoglobin concentrations exceeding 3% are very unusual in nonsmokers without an external source of carbon monoxide; the presence of carboxyhemoglobin concentrations exceeding these values suggests recent tobacco smoking absent an external source of carbon monoxide.[166] Blood carboxyhemoglobin concentrations exceeding 10% in smokers suggests an external source of carbon monoxide, unless the exposure resulted from inhaled smoke from a waterpipe (narghile, hookah) in hookah cafes and bars;[167] typically patrons in these establishments have higher exhaled carbon monoxide concentrations than patrons of traditional bars.[168] The burning of charcoal during the use of a waterpipe may result in substantial elevations of carboxyhemoglobin (i.e., 25–30%) in the user.

TREATMENT

Stabilization

Respiratory failure represents the greatest risk to life following serious nicotine intoxication; therefore, patients should be evaluated carefully for evidence of respiratory insufficiency. Patients with altered sensorium should receive intravenous fluids, cardiac monitoring, pulse oximetry, and oxygen as needed in addition to rapid evaluation of blood glucose and intravenous naloxone if opiate intoxication is suspected. The initial hypertension and tachycardia associated with nicotine intoxication should not be aggressively treated unless there is evidence of end-organ failure because a depressive phase of bradycardia and hypotension often follows the initial period of stimulation.

Decontamination

Because nicotine stimulates the emetic center in the medulla oblongata, spontaneous vomiting occurs in most nicotine ingestions; therefore, most nicotine ingestions require no decontamination measures. Most children only taste tobacco products, and they do not usually develop symptoms. The administration of activated charcoal to alert children is usually unnecessary; there are no clinical data to confirm the clinical efficacy of activated charcoal following the ingestion of cigarettes or plant parts from *Nicotiana* species. Additionally, the rapid onset of changes in consciousness and the potential for seizures during serious tobacco intoxications increases the risk of using decontamination measures. All skin contaminated with nicotine solutions should be washed thoroughly with a nonalkaline soap and cool water.

Elimination Enhancement

Continuous nasogastric suction and serial activated charcoal are theoretically advantageous, but there are inadequate clinical data to recommend these procedures routinely. Acidification of the urine also has theoretical pharmacokinetic advantages, but the use of acid diuresis is discouraged because of the lack of data to support the efficacy of this method, the short duration of nicotine action, and complications (e.g., rhabdomyolysis) associated with acid diuresis.

Antidotes

Atropine may improve bradycardia and hypotension, but this drug does not reverse neuromuscular weakness during nicotine intoxication.

Supplemental Care

The treatment of nicotine poisoning is primarily supportive. Serious poisoning must be followed closely in an intensive care setting for the development of respiratory failure within the first 12 hours. Seizures can be treated with benzodiazepines (diazepam, lorazepam); the use of large benzodiazepine doses may impair the ability of the patient to protect their airway, requiring intubation.

CORKWOOD TREE and PITURI [*Duboisia hopwoodii* (F. MUELL.) F. MUELL.]

HISTORY

For many centuries in Central Australia, Aboriginal people habitually chewed wild nicotine-containing plants from the Solanaceae family, primarily *Nicotiana* spp. [e.g., *N. rosulata* subs. *ingulba* (J.M.Black) P.Horton] and *Duboisia hopwoodii* (F. Muell.) F. Muell. There are a variety of names for the substances extracted from these plants as a result of the many languages and dialects including bedgery, pedgery, pitchery, and most commonly pituri.[169] In the middle 1800s, European explorers on the eastern margin of Australia's central desert observed the use of the dried leaves and terminal stems of the desert plant, *Duboisia hopwoodii* as a stimulant and narcotic for long marches similar to cocoa leaves in Bolivia.[170,171] Tribal elders used the plant to induce a state of consciousness that allowed the inculcation of values, beliefs, and religious tenets of the tribe as well as the performance of painful initiation rites.[172] Plant species from specific locations in western Australia and the Mulligan-Georgina Rivers area were highly prized in the Australian Aboriginal culture as valuable trade item until the 1930s.[173] Experimental studies in the later 1800s indicate that nicotine was the main active ingredient.[174]

BOTANICAL DESCRIPTION

There are 3 species of *Duboisia* in Australia including *D. hopwoodii*, *D. myoporoides* R. Br., and *D. leichhardtii* (F. Muell.) F. Muell. *D. hopwoodii* is the only *Duboisia* species that contains substantial amounts of nicotine and the only *Duboisia* species used by Australian Aborigines to make pituri. *D. myoporoides* (corkwood) grows in eastern coastal Australia from Cooktown to Nowra and New Caledonia, whereas *D. leichhardtii* grows in the higher elevations of inland Queensland from Monto to Yarraman.[175]

Common Name: Corkwood tree
Scientific Name: *Duboisia hopwoodii* (F. Muell.) F. Muell.
Botanical Family: Solanaceae (nightshade)
Physical Description: An erect shrub 3–4 m (~10–13 feet) tall with attractive white flowers that produce black berries. The bark is light with the consistency of cork; the leaves are alternate, smooth, and narrowly elliptical.
Distribution and Ecology: This shrub is a native species of Australia including New South Wales, Northern Territory, Queensland, South Australia, and Western Australia. *D. hopwoodii* occurs primarily in the arid regions of central Australia. Figure 66.7 displays the flowers, leaves, and stems of this plant.

EXPOSURE

Origin

In contrast to other *Duboisia* species, *D. hopwoodii* contains primarily nicotine and nornicotine rather than tropane alkaloids present in *D. myoporoides* and *D. leichhardtii*. The latter two *Duboisia* species are

FIGURE 66.7. *Duboisia hopwoodii* (F. Muell.) F. Muell. (Photography by G. Byrne, C.P. Campbell, and G Cocktern. Image used with the permission of the Western Australian Herbarium, Department of Environment and Conservation; http://florabase.dec.wa.gov.au/)

commercial sources of hyoscine, scopolamine, and hyoscyamine (*l*-atropine); plantations of *D. myoporoides* in Queensland supply the bulk of the world's raw scopolamine.[176]

Composition

Analysis of leaves from *D. hopwoodii* demonstrated the presence of nicotine, nornicotine, myosmine [3-(1-pyrroline-2-yl)pyridine], *N*-formyl nornicotine, cotinine, N-acetyl nornicotine, anabasine, anatabine, anatalline, and bipyridyl.[173] Nicotine was the major alkaloid in the West Australian collection, whereas nornicotine was predominant in the Northern Territory sample. The amounts of nicotine and nornicotine in pituri range up to about 5% and 4%, respectively, with the highest nicotine content in the young shoots. In an analysis of 70 samples of pituri, the nicotine and nornicotine concentrations ranged between 0–5.3% and 0.1–4.1%, respectively.[177] Analytic data from the early 1900s suggest that the nicotine content of pituri was approximately 2.5%.[178]

Methods of Use

Aboriginal people of Australia traditionally chewed the leaves of nicotine-containing desert plants, *Duboisia hopwoodii* and other *Nicotiana* species, as a recreational and ritual drug called pituri. About 1 tablespoon of cured leaf and stem from specific strains of *D. hopwoodii* are ground with alkali plant ash to form a brownish-gray paste or pituri quid ready for mastication. The range of wood burned for ash included *Acacia* spp., *Eucalyptus* spp., and *Grevillea* spp.[169] The masticated leaves (quid) is retained in the lower lip or buccal mucosa to continuously extract the nicotine. Ceremonially, the quid was passed from mouth to mouth beginning with the head man. When not retained in the mouth, the quid may be stored behind the ear, under the breast, or held against the skin by a headband or armband. These people carried the leaves and stems in a specially woven bag containing approximately ~1.5 kg of material. Older men of the tribe kept the details of the drying process as part of the knowledge of the sacred ritual. The addition of alkali ash to the quid increases the release of the weakly basic nicotine from the plant material and facilitates the diffusion of nicotine across the oral mucosa. With the deterioration of traditional Aboriginal lifestyles, the plant material for pituri was sun dried by women; much of the knowledge of the use of psychoactive drugs was lost after European immigration into Australia.[172] Aborigines in Central Australia used *D. hopwoodii* primarily as a poison to capture fish and emu.[173]

DOSE EFFECT

There are few data on dose-related effects of pituri. The concentrations of nicotine and nornicotine in pituri are similar to common tobacco; hence, the dose-effect of these substances are expected to be similar.

CLINICAL RESPONSE

There are few data on the clinical effects of pituri. Nicotine is the main active ingredient in pituri; the clinical features of tobacco and pituri intoxication are expected to be similar as noted in the nicotine section. The onset of symptoms following the ingestion of toxic doses of nicotine is rapid (15–30 minutes) beginning with gastrointestinal distress (nausea, vomiting, abdominal pain, diarrhea, salivation). Other species of Duboisia (*D. myoporoides*, *D. leichhardtii*) produce an atropine-like, anticholinergic poisoning similar to henbane (*Hyoscyamus niger*) rather than nicotine poisoning.

TREATMENT

The treatment of pituri intoxication is supportive and similar to nicotine and common tobacco poisoning.

References

1. Panter KE, James LF, Gardner DR. Lupines, poison-hemlock and *Nicotiana* spp: toxicity and teratogenicity in livestock. J Nat Toxins 1999;8:117–134.

2. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risk Hum 2004;83:1–1438.

3. Doll R. Uncovering the effects of smoking: historical perspective. Stat Methods Med Res 1998;7:87–117.

4. Surgeon General: Smoking and health. Report of the Advisory Committee to the Surgeon General of the Public Health Service. Washington, DC: US Government Printing Office; 1964.

5. Surgeon General. The health consequences of smoking: chronic obstructive lung disease. Washington, DC: US Government Printing Office; 1984.

6. Trosclair A, Caraballo R, Malarcher A, Husten C, Pechacek T, Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, CDC. Cigarette smoking among adults—United States, 2003. MMWR Morb Mortal Wkly Rep 2005;54:509–513.

7. Maurice E, Trosclair A, Merritt R, Caraballo R, Malarcher A, Husten C, et al. Cigarette smoking among adults—United States, 2004. MMWR Morb Mortal Wkly Rep 2005;54:1121–1124.

8. Thorne SL, Malarcher A, Maurice E, Caraballo R, Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, CDC. Cigarette smoking among adults—United States, 2007. MMWR Morb Mortal Wkly Rep 2008;57:1221–1226.

9. Dube SR, McClave A, James C, Caraballo R, Kaufmann R, Pechacek T, Office on Smoking and Health, National Center for Chronic Disease Prevention and Health Promotion, CDC. Vital signs: current cigarette smoking among adults aged ≥18 years—United States, 2009. MMWR Morb Mortal Wkly Rep 2010;59:1135–1140.

10. Lavoie FW, Harris TM. Fatal nicotine ingestion. J Emerg Med 1991;9:133–136.

11. Clark MS, Rand MJ, Vanov S. Comparison of pharmacological activity of nicotine and related alkaloids occurring in cigarette smoke. Arch Int Pharmacodyn Ther 1965; 156:363–379.

12. Thomas JL, Renner CC, Patten CA, Decker PA, Utermohle CJ, Ebbert JO. Prevalence and correlates of tobacco use among middle and high school students in western Alaska. Int J Circumpolar Health 2010;69: 168–180.

13. Maziak W, Ward KD, Afifi Soweid RA, Eissenberg T. Tobacco smoking using a waterpipe: a re-emerging strain in the global epidemic. Tob Control 2004;13:327–333.

14. Sepetdjian E, Saliba N, Shihadeh A. Carcinogenic PAH in watepipe charcoal products. Food Chem Toxicol 2010;48:3242–3245.

15. Chan WC, Leatherdale ST, Burkhalter R, Ahmed R. Bidi and hookah use among Canadian youth: an examination of data from the 2006 Canadian youth smoking survey. J Adolesc Health 2011;49:102–104.

16. Nakkash RT, Khalil J, Afifi RA. The rise in narghile (shisha, hookah) waterpipe tobacco smoking: a qualitative study of perceptions of smokers and non-smokers. BMC Public Health 2011;11:315.

17. Smith JR, Novotny TE, Edland SD, Hofstetter CR, Lindsay SP, Al-Delaimy WK. Determinants of hookah use among high school students. Nicotine Tob Res 2011;13:565–572.

18. Benowitz NL, Hall SM, Herning RI, Jacob P III, Jones RT, Osman A-L. Smokers of low-yield cigarettes do not consume less nicotine. N Engl J Med 1983;309:139–142.

19. Lu GH, Ralapati S. Application of high-performance capillary electrophoresis to the quantitative analysis of nicotine and profiling of other alkaloids in ATF-regulated tobacco products. Electrophoresis 1998;19:19–26.

20. Kozlowski LT, Mehta NY, Sweeney CT, Schwartz SS, Vogler GP, Jarvis MJ, West RJ. Filter ventilation and nicotine content of tobacco in cigarettes from Canada, the United Kingdom, and the United States. Tob Control 1998;7:369–375.

21. Fukumoto M, Kubo H, Ogamo A. Determination of nicotine content of popular cigarettes. Vet Hum Toxicol 1997; 39:225–227.

22. Benowitz JL, Porchet H, Sheiner L, Jacob P III. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clin Pharmacol Ther 1988;44:23–28.

23. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacol Rev 2005; 79–115.

24. Spiegelhalder B, Bartsch H. Tobacco-specific nitrosamines. Eur J Cancer Prev 1996;5(Suppl 1):33S –38S.

25. Coggins CR, Sena EJ, Oldham MJ. A comprehensive evaluation of the toxicology of cigarette ingredients: inorganic compounds. Inhal Toxicol 2011;23:157–171.

26. Benowitz NL. Biomarkers of environmental tobacco smoke exposure. Environ Health Perspect 1999;107(Suppl 2):349S –355S.

27. Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999;91:1194–1210.

28. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer 2003;3: 733–744.

29. Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ Health Perspect 1997;105(Suppl 4): 875S –882S.

30. Brunnemann KD, Prokopczyk B, Djordjevic MV, Hoffmann D. Formation and analysis of tobacco-specific N-nitrosamines. Crit Rev Toxicol 1996;26:121–137.

31. Federal Trade Commission (FTC). “Tar,” nicotine, and carbon monoxide of the smoke of 1294 varieties of domestic cigarettes for the year 1998. Washington, DC: Federal Trade Commission; 2000.

32. Sepetdjian E, Saliba N, Shihadeh A. Carcinogenic PAH in waterpipe charcoal products. Food Chem Toxicol 2010;48:3242–3245.

33. Sepetdjian E, Shihadeh A, Saliba NA. Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke. Food Chem Toxicol 2008;46: 1582–1590.

34. Blot WJ, McLaughlin JK. Passive smoking and lung cancer risk: what is the story now? J Natl Cancer Inst 1998;90:1416–1417.

35. US Department of Health and Human Services. The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General (DHHS Publication No. (CDC) 88-8406). Rockville, MD, US Department of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health Promotion and Education, Office on Smoking and Health; 1988.

36. Benowitz NL, Henningfield JE. Establishing a nicotine threshold for addiction: the implications for tobacco regulation. N Eng J Med 1994;331:123–125.

37. Garcia-Estrada H, Fischman CM. An unusual case of nicotine poisoning. Clin Toxicol 1977;10:391–393.

38. Malizia E, Andreucci G, Alfani F, Smeriglio M, Nicholai P. Acute intoxication with nicotine alkaloids and cannabinoids in children from ingestion of cigarettes. Hum Toxicol 1983;2:315–316.

39. McGee D, Brabson T, McCarthy J, Picciotti M. Four-year review of cigarette ingestions in children. Pediatr Emerg Care 1995;11:13–16.

40. Larson PS, Haag HB, Silvette H. Tobacco—experimental and clinical studies. Baltimore: Williams & Wilkins; 1961.

41. Godtfredsen NS, Prescott E, Osler M. Effect of smoking reduction of lung cancer risk. JAMA 2005;294: 1505–1510.

42. Woolf A, Burkhart K, Caraccio T, Litovitz T. Self-poisoning among adults using multiple transdermal nicotine patches. Clin Toxicol 1996;34:691–698.

43. Knudsen K, Strinnholm M. A case of life-threatening rectal administration of moist snuff. Clin Toxicol 2010;48: 572–573.

44. Oberst BB, McIntyre RA. Acute nicotine poisoning. Pediatrics 1953;11:338–340.

45. Zins BJ, Sandborn WJ, Mays DC, Lawson GM, McKinney JA, Tremaine WJ, et al. Pharmacokinetics of nicotine tartrate after single-dose liquid enema, oral, and intravenous administration. J Clin Pharmacol 1997;37: 426–436.

46. Ivy KJ, Triggs EJ. Absorption of nicotine by the human stomach and its effect on gastric ion fluxes and potential difference. Dig Dis 1978;23:809–814.

47. Lockhart LP. Nicotine poisoning. Br Med J 1933;1: 246–247.

48. Armitage AK, Dollery CT, George CF, Houseman TH, Lewis PJ, Turner DM. Absorption and metabolism of nicotine from cigarettes. Br Med J 1975;4:313–316.

49. Svensson CK. Clinical pharmacokinetics of nicotine. Clin Pharmacokinet 1987;12:30–40.

50. Rosenberg J, Benowitz NL, Jacob P, Wilson KM: Disposition kinetics and effects of intravenous nicotine. Clin Pharmacol Ther 1980;28:517–522.

51. Henningfield JE, Stapleton JM, Benowitz NL, Grayson RF, London ED. Higher levels of nicotine in arterial than in venous blood after cigarette smoking. Drug Alcohol Depend 1993;33:23–29.

52. Vine MF, Hulka BS, Margolin BH, Truong YK, Hu P-C, Schramm MM, et al. Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. Am J Public Health 1993;83:1335–1338.

53. Mwenifumbo JC, Lessov-Schlaggar CN, Zhou Q, Krasnow RE, Swan GE, Benowitz NL, Tyndale RF. Identification of novel CYP2A6*1B variants: the CYP2A6*1B allele is associated with faster in vivo nicotine metabolism. Clin Pharmacol Ther 2008;83:115–121.

54. Benowitz NL, Jacob P III, Perez-Stable E 3rd. CYP2D6 phenotype and the metabolism of nicotine and cotinine. Pharmacogenetics 1996;6:239–242.

55. Le Gal A, Dreano Y, Lucas D, Berthou F. Diversity of selective environmental substrates for human cytochrome P450 2A6: alkoxyethers, nicotine, coumarin, N-nitrosodiethylamine, and N-nitrosobenzylmethylamine. Toxicol Lett 2003;144:77–91.

56. Zevin S, Benowitz NL. Drug interactions with tobacco smoking. An update. Clin Pharmacokinet 1999;36: 425–438.

57. Benowitz NL, Jacob P III, Fong I, Gupta S. Nicotine metabolic profile in man: comparison of cigarette

smoking and transdermal nicotine. J Pharmacol Exp Ther 1994;268:296–303.

58. Benowitz NL, Jacob P III. Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. Clin Pharmacol Ther 1993;53;316–323.

59. Jarvis MJ, Russell MA, Benowitz NL, Feyerabend C. Elimination of cotinine from body fluids: implications for noninvasive measurement of tobacco smoke exposure. Am J Public Health 1988;78:696–698.

60. Jacob P III, Yu L, Shulgin AT, Benowitz NL. Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes. Am J Public Health 1999;89:731–736.

61. Pastrakuljic A, Schwartz R, Simone C, Derewlany LO, Knie B, Koren G. Transplacental transfer and biotransformation studies of nicotine in the human placental cotyledon perfused *in vitro*. Life Sci 1998;63: 2333–2342.

62. Dahlstrom A, Ebersjo C, Lundell B. Nicotine exposure in breastfed infants. Acta Paediatr 2004;93:810–816.

63. Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med 1961;265:253–267.

64. Auerbach O, Hammond EC, Garfinkel L, Benante C. Relation of smoking and age to emphysema. Whole-lung section study. N Engl J Med 1972;286:853–857.

65. Changeux JP, Galzi JL, Devillers-Thiery A, Betrand D. The functional architecture of the acetylcholine nicotinic receptor explored by affinity labeling and site-directed mutagenesis. Q Rev Biophys 1992;25:395–432.

66. Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol 1996;36: 597–613.

67. Grenhoff J, Svensson TH. Pharmacology of nicotine. Br J Addict 1989;84:477–492.

68. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.

69. Trosko JE, Chang CC, Upham BL, Tai MH. Ignored hallmarks of carcinogenesis: stem cells and cell-cell communication. Ann N Y Acad Sci 2004;1028:192–201.

70. Nishikawa A, Mori Y, Lee I-S, Tanaka T, Hirose M. Cigarette smoking, metabolic activation and carcinogenesis. Curr Drug Metab 2004;5:363–373.

71. Rebbeck TR. Molecular epidemiology of the human glutathione *S*-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1997;6:733–743.

72. Marcotullio D, Magliulo G, Pezone T. Reinke's edema and risk factors: clinical and histopathologic aspects. Am J Otolaryngol 2002;23:81–84.

73. Moore HW. Poison case report of the month. Acute nicotine poisoning. SC Med Assoc J 1962;58:445.

74. McNally WD. A report of seven cases of nicotine poisoning. J Lab Clin Med 1922;8:83–85.

75. Smolinske SC, Spoerke DG, Spiller SK, Wruk KM, Kulig K, Rumack BH. Cigarette and nicotine chewing gum toxicity in children. Hum Toxicol 1988;7:27–31.

76. Davies P, Levy S, Pahari A, Martinez D. Acute nicotine poisoning associated with a traditional remedy for eczema. Arch Dis Child 2001;85:500–502.

77. Trape-Cardoso M, Bracker A, Grey M, Kaliszewski M, Oncken C, Ohannessian C, et al. Shade tobacco and green tobacco sickness in Connecticut. J Occup Environ Med 2003;45:656–661.

78. Arcury TA, Quandt SA, Preisser JS, Norton D. The incidence of green tobacco sickness among Latino farmworkers. J Occup Environ Med 2001;43:601–609.

79. Parikh JR, Gokani VN, Doctor PB, Kulkarni PK, Shah AR, Saiyed HN. Acute and chronic health effects due to green tobacco exposure in agricultural workers. Am J Ind Med 2005;47:494–499.

80. Arcury TA, Quandt SA, Garcia DI, Preisser JS Jr, Norton D, Rao P. A clinic-based, case-control comparison of green tobacco sickness among minority farmworkers: clues for prevention. South Med J 2002;95:1008–1011.

81. Trapé-Cardoso M, Bracker A, Dauser D, Oncken C, Barrera LV, Gould B, Grey MR. Cotinine levels and green tobacco sickness among shade-tobacco workers. J Agromed 2005;10:27–37.

82. McKnight RH, Spiller HA. Green tobacco sickness in children and adolescents. Public Health Rep 2005;120: 602–605.

83. Arcury TA, Vallejos QM, Schulz MR, Feldman SR, Fleischer AB Jr, Verma A, Quandt SA. Green tobacco sickness and skin integrity among migrant Latino farmworkers. Am J Ind Med 2008;51:195–203.

84. Gehlbach SH, Williams WA, Perry LD, Woodall JS. Green tobacco sickness. An illness of tobacco harvesters. JAMA 1974;229:1880–1883.

85. Satora L, Goszcz H, Gomolka E, Biedron W. Diplopia in green tobacco sickness. J Agromed 2009;14:66–69.

86. Boylan B, Brandt V, Muehlbauer J, Auslander M, Spurlock C, Finger R, et al. Green tobacco sickness in tobacco harvesters—Kentucky, 1992. MMWR Morb Mortal Rep 1993;42:237–240.

87. Sherman CB. Health effects of cigarette smoking. Clin Chest Med 1991;12:643–658.

88. Levitz JS, Bradley TP, Golden AL. Overview of smoking and all cancers. Med Clin N Am 2004;88:1655–1675.

89. DeMarini DM. Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. Mutat Res 2004; 567:447–474.

90. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Tobacco smoking. IARC Monogr Eval Carcinog Risk Hum 1986;38:127–135.

91. US Department of Health and Human Services. The health consequences of smoking: the changing cigarette: a report of the Surgeon General (DHHS publication No. 82-50179). Bethesda, MD: US Department of Health and Human Services, Office of Smoking and Health; 1983.

92. Kahn HA. The Dorn study of smoking and mortality among U.S. veterans: report on eight and one-half years of observation. Natl Cancer Inst Monogr 1966;19: 1–125.

93. De Stefani E, Boffetta P, Ronco AL, Brennan P, Correa P, Deneo-Pellegrini H, et al. Squamous and small cell carcinomas of the lung: similarities and differences concerning the role of tobacco smoking. Lung Cancer 2005; 47:1–8.

94. Khuder SA. Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis. Lung Cancer 2001;31:139–148.

95. Saracci R. The interactions of tobacco smoking and other agents in cancer etiology. Epidemiol Rev 1987;9: 175–193.

96. Schottenfeld D. Epidemiology of cancer of the esophagus. Semin Oncol 1984;11:92–100.

97. Sasco AJ, Secretan MB, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. Lung Cancer 2004;45(suppl 2):3S –9S.

98. Tredaniel J, Boffetta P, Buiatti E, Saracci R, Hirsch A. Tobacco smoking and gastric cancer: review and meta-analysis. Int J Cancer 1997;72:565–573.

99. Hunt JD, van der Hel OL, McMillan GP, Boffetta P, Brennan P. Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. Int J Cancer 2005; 114:101–108.

100. Green J, Berrington de Gonzalez A, Sweetland S, Beral V, Chilvers C, Crossley B, et al. Risk factors for adenocarcinoma and squamous cell carcinoma of the cervix in women aged 20–44 years: the UK National Case-Control Study of Cervical Cancer. Br J Cancer 2003;89: 2078–2086.

101. Berrington de Gonzalez A, Sweetland S, Green J. Comparison of risk factors for squamous cell and adenocarcinomas of the cervix: a meta-analysis. Br J Cancer 2004;90:1787–1791.

102. Garfinkel L, Boffetta P. Association between smoking and leukemia in two American Cancer Society prospective studies. Cancer 1990;65:2356–2360.

103. Price JF, Mowbray PI, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Relationship between smoking and cardiovascular risk factors in the development of peripheral arterial disease and coronary artery disease: Edinburgh Artery Study. Eur Heart J 1999;20:344–353.

104. US Department of Health and Human Services. Reducing the health consequences of smoking: 25 years of progress: a report of the Surgeon General (DHHS publication No. 89-8411). Bethesda, MD: US Department of Health and Human Services, Center for Disease Control, Center for Chronic Disease Prevention and Health Promotion; 1989.

105. Rosenberg L, Kaufman DW, Helmrich SP, Shapiro S. The risk of myocardial infarction after quitting smoking in men under 55 years of age. N Engl J Med 1985;313: 1511–1514.

106. Murabito JM, Evans JC, Nieto K, Larson MG, Levy D, Wilson PW. Prevalence and clinical correlates of peripheral arterial disease in the Framingham Offspring Study. Am Heart J 2002;143:961–965.

107. Powell JT, Edwards RJ, Worrell PC, Franks PJ, Greenhalgh RM, Poulter NR. Risk factors associated with the development of peripheral arterial disease in smokers: a case-control study. Atherosclerosis 1997;129:41–48.

108. Bolinder G, Alfredsson L, Englund A, de Faire U. Smokeless tobacco use and increased cardiovascular mortality among Swedish construction workers. Am J Public Health 1994;84:399–404.

109. Janzon E, Hekblad B. Swedish snuff and incidence of cardiovascular disease. A population-based cohort study. BMC Cardiovasc Disord 2009;9:21.

110. Zaher C, Halert R, Dubois R, George D, Nonikov D. Smoking-related diseases: the importance of COPD. Int J Tuberc Lung Dis 2004;8:1423–1428.

111. Willemse BW, Postma DS, Timens W, ten Hacken NH. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. Eur Respir J 2004;23:464–476.

112. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, et al. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force. Eur Respir J 1995;8:1398–1420.

113. Burr ML, Phillips KM, Hurst DN. Lung function in the elderly. Thorax 1985;40:54–59.

114. Higgins MW, Enright PL, Kronmal RA, Schenker MB, Anton-Culver H, Lyles M. Smoking and lung function in elderly men and women. The Cardiovascular Health Study. JAMA 1993;269:2741–2748.

115. Kerstjens HA, Rijcken B, Schouten JP, Postma DS. Decline of FEV1 by age and smoking status: facts, figures, and fallacies. Thorax 1997;52:820–827.

116. Anto JM, Verveire P, Vestbo J, Sunyer J. Epidemiology of chronic obstructive pulmonary disease. Eur Respir J 2001;17:982–994.

117. Gold DR, Wang X, Wypij D, Speizer FE, Ware JH, Dockery DW. Effects of cigarette smoking on lung function in adolescent boys and girls. N Engl J Med 1996;335: 931–937.

118. Vassallo R, Ryu JH. Tobacco smoke-related diffuse lung diseases. Semin Respir Crit Care Med 2008;29:643–650.

119. Lederle FA, Nelson DB, Joseph AM. Smokers' relative risk for aortic aneurysm compared with other smoking-related diseases: a systematic review. J Vasc Surg 2003;38: 329–334.

120. Dietz PM, England LJ, Shapiro-Mendoza CK, Tong VT, Farr SL, Callaghan WM. Infant morbidity and mortality attributable to prenatal smoking in the U.S. Am J Prev Med 2010;39:45–52.

121. Haworth JC, Ellestad-Sayed JJ, King J, Dilling LA. Relation of maternal cigarette smoking, obesity, and

energy consumption to infant size. Am J Obstet Gynecol 1980;138:1185–1189.

122. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987;65:663–737.

123. Kramer MS, Olivier M, McLean FH, Willis DM, Usher RH. Impact of intrauterine growth retardation and body proportionality on fetal and neonatal outcome. Pediatrics 1990;86:707–713.

124. MacArthur C, Knox EG. Smoking in pregnancy: effects of stopping at different stages. Br J Obstet Gynaecol 1988;95:551–555.

125. MacArthur C, Knox EG, Lancashire RJ. Effects at age nine of maternal smoking in pregnancy: experimental and observational findings. BJOG 2001;108:67–73.

126. Williams MA, Lieberman E, Mittendorf R, Monson RR, Schoenbaum SC. Risk factors for abruptio placentae. Am J Epidemiol 1991;134:965–972.

127. Williams MA, Mittendorf R, Lieberman E, Monson RR, Schoenbaum SC, Genest DR. Cigarette smoking during pregnancy in relation to placenta previa. Am J Obstet Gynecol 1991;165:28–32.

128. Beste LA, England LJ, Schisterman EF, Qian C, Yu KF, Levine RJ. Pregnancy outcomes in smokers who develop pre-eclampsia. Paediatr Perinat Epidemiol 2005;19:12–18.

129. Hughes JR. Tobacco withdrawal in self-quitters. J Consult Clin Psychol 1992;60:689–697.

130. Gross J, Stitzer ML. Nicotine replacement: ten-week effects on tobacco withdrawal symptoms. Psychopharmacology (Berl) 1989;98:334–341.

131. Hughes JR, Higgins ST, Bickel WK. Nicotine withdrawal versus other drug withdrawal syndromes: similarities and dissimilarities. Addiction 1994;89:1461–1470.

132. Husgafvel-Pursiainen K. Genotoxicity of environmental tobacco smoke: a review. Mutat Res 2004;457:427–445.

133. Vineis P, Airoldi L, Veglia P, Olgiati L, Pastorelli R, Autrup H, et al. Environmental tobacco smoke and risk of respiratory cancer and chronic obstructive pulmonary disease in former smokers and never smokers in the EPIC prospective study. BMJ 2005;330(7486):277.

134. Lee YC, Marron M, Benhamou S, Bouchardy C, Ahrens W, Pohlabeln H, et al. Active and involuntary tobacco smoking and upper aerodigestive tract cancer risks in a multicenter case-control study. Cancer Epidemiol Biomarkers Prev 2009;18:3353–3361.

135. Chuang SC, Gallo V, Michaud D, Overvad K, Tjønneland A, Clavel-Chapelon F, Romieu I, et al. Exposure to environmental tobacco smoke in childhood and incidence of cancer in adulthood in never smokers in the European Prospective Investigation into Cancer and Nutrition. Cancer Causes Control 2011;22:487–494.

136. Bramer SL, Kallungal BA. Clinical considerations in study designs that use cotinine as a biomarker. Biomarkers 2003;8:187–203.

137. Lequang NT, Roussel G, Roche D, Migueres ML, Chretien J, Ekindjian OG. [Urine collection for nicotine and cotinine measurement in studies on nicotine addicts]. Pathol Biol (Paris) 1994;42:191–196. [French]

138. Riboli E, Haley NJ, De Waard F, Saracci R. Validity of urinary biomarkers of exposure to tobacco smoke following prolonged storage. Int J Epidemiol 1995;24:354–358.

139. Hecht SS. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. Carcinogenesis 2002;23:907–922.

140. Benowitz NL. Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addition. N Engl J Med 1988;319:1318–1330.

141. Patterson F, Benowitz N, Shields P, Kaufmann V, Jepson C, Wileyto P, et al. Individual differences in nicotine intake per cigarette. Cancer Epidemiol Biomarkers Prev 2003;12:468–471.

142. Gori GB, Lynch CJ: Analytical cigarette yields as predictors of smoke bioavailability. Regul Toxicol Pharmacol 1985;5:314–326.

143. Hurt RD, Dale LC, Offord KP, Lauger GG, Basking LB, Lawson GM, Jiang NS, Hauri PJ. Serum nicotine and cotinine levels during nicotine-patch therapy. Clin Pharmacol Ther 1993;54:98–106.

144. D'Alessandro A, Benowitz NL, Muzi G, Eisner MD, Filiberto S, Montanari L, Abbriti G. Systemic nicotine exposure in tobacco harvesters. Arch Environ Health 2001;56:257–263.

145. Kemp PM, Sneed GS, George CE, Distefano RF. Postmortem distribution of nicotine and cotinine from a case involving the simultaneous administration of multiple nicotine transdermal systems. J Anal Toxicol 1997;21:310–313.

146. Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev 1996;18:188–204.

147. Repace J, Al-Delaimy WK, Bernert JT. Correlating atmospheric and biological markers in studies of secondhand tobacco smoke exposure and dose in children and adults. J Occup Environ Med 2006;48:181–194.

148. Vine MF, Hulka BS, Margolin BH, Truong YK, Hu PC, Schramm MM, et al. Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. Am J Public Health 1993;83:1335–1338.

149. Davis RA, Stiles MF, deBethizy JD, Reynolds JH. Dietary nicotine: a source of urinary cotinine. Food Chem Toxicol 1991;29:821–827.

150. Jacob P 3rd, Yu L, Shulgin AT, Benowitz NL. Minor tobacco alkaloids as biomarkers for tobacco use: comparison of users of cigarettes, smokeless tobacco, cigars, and pipes. Am J Public Health 1999;89:731–736.

151. Goniewicz ML, Eisner MD, Lazcano-Ponce E, Zielinska-Danch W, Koszowski B, Sobczak A, et al. Comparison of urine cotinine and the tobacco-specific nitrosamine metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-

butanol (NNAL) and their ratio to discriminate active from passive smoking. Nicotine Tob Res 2011;13: 202–208.

152. Bono R, Vincenti M, Schiliro T, Traversi D, Pignata C, Scursatone E, et al. Cotinine and *N*-(2-hydroxyethyl) valine as markers of passive exposure to tobacco smoke in children. J Expo Anal Environ Epidemiol 2005;15: 66–73.

153. Sherrill DL, Lebowitz MD, Knudson RJ, Burrows B. Longitudinal methods for describing the relationship between pulmonary function, respiratory symptoms and smoking in elderly subjects: the Tucson Study. Eur Respir J 1993;6:342–348.

154. Camilli AE, Burrows B, Knudson RJ, Lyle SK, Lebowitz MD. Longitudinal changes in forced expiratory volume in one second in adults. Effects of smoking and smoking cessation. Am Rev Respir Dis 1987;135:794–799.

155. Tashkin DP, Clark VA, Coulson AH, Simmons M, Bourque LB, Reems C, et al. The UCLA population studies of chronic obstructive respiratory disease. VIII. Effects of smoking cessation on lung function: a prospective study of a free-living population. Am Rev Respir Dis 1984;130:707–715.

156. Bosse R, Sparrow D, Garvey AJ, Costa PT, Weiss ST. Cigarette smoking, aging, and decline in pulmonary function: a longitudinal study. Arch Environ Health 1980;35: 247–252.

157. Postma DS, de Vries K, Koeter GH, Sluiter HJ. Independent influence of reversibility of air-flow obstruction and nonspecific hyperreactivity on the long-term course of lung function in chronic air-flow obstruction. Am Rev Respir Dis 1986;134:276–280.

158. Scanlon PD, Connett JE, Waller LA, Altose MD, Bailey WC, Buist AS. Smoking cessation and lung function in mild-to-moderate chronic obstructive pulmonary disease. The Lung Health Study. Am J Respir Crit Care Med 2000;161:381–390.

159. Lange P, Groth S, Nyboe J, Mortensen J, Appleyard M, Jensen G, Schnohr P. Decline of the lung function related to the type of tobacco smoked and inhalation. Thorax 1990;45:22–26.

160. Raad D, Gaddam S, Schunemann HJ, Irani J, Jaoude A, Honbeine R, Akl EA. Effects of water-pipe smoking on lung function a systematic review and meta-analysis. Chest 2011;139:764–774.

161. Hatsukami D, Morgan SF, Pickens RW, Hughes JR. Smoking topography in a non-laboratory environment. Int J Addict 1987;22:719–725.

162. Klesges RC, Debon M, Ray JW. Are self-reports of smoking rate biased? Evidence from the second National Health and Nutrition Examination Survey. J Clin Epidemiol 1995;48:1225–1233.

163. Rieben FW. Smoking behaviour and increase in nicotine and carboxyhaemoglobin in venous blood. Clin Investig 1992;70:335–342.

164. Smith CJ, Guy TD, Stiles MF, Morton MJ, Collie BB, Ingebrethsen BJ, Robinson JH. A repeatable method for determination of carboxyhemoglobin levels in smokers. Hum Exp Toxicol 1998;17:29–34.

165. Imbriani M, Melotti A, Ghittori S. Methemoglobin and carboxyhemoglobin levels in smokers and non-smokers. G Ital Med Lav 1987;9:11–14.

166. Klesges LM, Klesges RC, Cigrang JA. Discrepancies between self-reported smoking and carboxyhemoglobin: an analysis of the Second National Health and Nutrition Survey. Am J Public Health 1992;82:1026–1029.

167. Marshall MD, Kales SN, Christiani DC, Goldman RH. Are reference intervals for carboxyhemoglobin appropriate? A survey of Boston area laboratories. Clin Chem 1995;41:1434–1438.

168. Barnett TE, Curbow BA, Soule EK, Tomar SL, Thombs DL. Carbon monoxide levels among patrons of hookah cafes. Am J Prev Med 2011;40:324–328.

169. Ratsch A, Steadman Kj, Bogossian F. The pituri story: a review of the historical literature surrounding traditional Australian Aboriginal use of nicotine in Central Australia. J Ethnobiol Ethnomed 2010;6:26.

170. Ringer S, Murrell W. On Pituri. J Physiol 1878;1: 377–383.

171. Cawte J. Psychoactive substances of the South Seas: betel, kava and pituri. Aust N Z J Psychiatry 1985;19: 83–87.

172. de Rios MD, Stachalek R. The *Duboisia* genus, Australian Aborigines and suggestibility. J Psychoactive Drugs 1999;31:155–161.

173. Watson PL, Luanratana O, Griffin WJ. The ethnopharmacology of pituri. J Ethnopharmacol 1983;8:303–311.

174. Langley JN, Dickinson WL. Pituri and Nicotin. J Physiol 1890;11:265–306.

175. Pearn J. Corked up clinical hyoscine poisoning with alkaloids of the native corkwood, *Duboisia*. Med J Aust 1981;2:422–423.

176. Griffin WJ, Brand HP, Dare JG. Analysis of *Duboisia myoporoides* R. Br. and *Duboisia leichhardtii* F. Muell. J Pharm Sci 1975;64:1821–1825.

177. Everist SL. Poisonous plants of Australia. Sydney: Angus & Robertson; 1981.

178. Rothera AC. The alkaloid of pituri obtained from *Duboisia hopwoodii*. Biochem J 1911;5:193–206.

INDEX

Please note that *f* indicates figures and *t* indicates tables.