III.

BIOLOGY

1. *How to Cultivate Harmless Bacteria*
2. *Growing Algae on a Window Shelf*
3. *How to Tranquilize a Rat*
4. *How to Measure the Metabolism of Animals*
5. *Chromatography*
6. *Zone Electrophoresis*

1

HOW TO CULTIVATE HARMLESS BACTERIA

The growth of the minute plants called bacteria can be selectively encouraged or retarded by the use of techniques familiar, on the large scale, to farmers. Two New York City medical students, Henry Soloway and Robert Lawrence, explain how to garden at the microscopic level and, in particular, how to experiment with the weed-killers popularly known as wonder drugs

THE SERIES OF EXPERIMENTS which follow are designed to demonstrate how certain bacteria are affected by bacteriostatic agents, drugs which retard the growth of selected bacteria. In testing such substances you first cultivate a selected bacterium in an environment which encourages its growth, subject it to the bacteriostatic agent and then measure the result. Bacteria, like other forms of life, have preferences in foods, temperature, moisture and so on. Hence no universal culture medium, in which all organisms thrive equally, has been developed. Media must be compounded according to the preferences of the bacteria under study. However, one medium in which some thousands of organisms thrive consists of beef broth, the familiar consommé of the dinner table, which has been refined and specially treated. It is used both in liquid form and, when thickened by the addition of agar, as a stiff jelly.

As in ordinary farming, "weeds" must be kept down. One is often interested in the characteristics of a single species of bacterium, and since the size of the organism makes "weeding" impractical, intruders must be prevented from gaining a foothold in the first place. This is accomplished by killing all microscopic life in the environment of the experiment except the desired organism. The

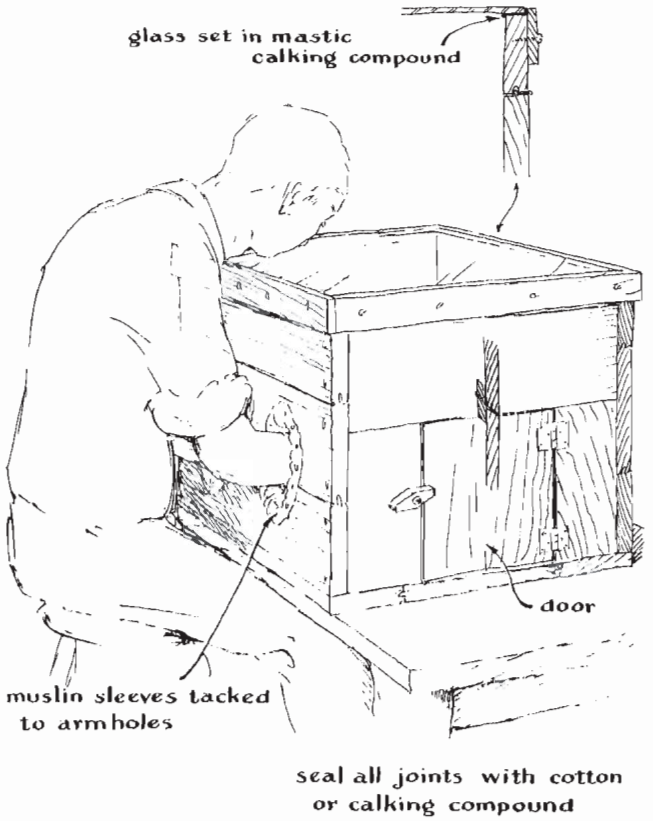
culture medium and all equipment is sterilized, exposed only to sterilized air and otherwise kept scrupulously aseptic during the experiment.

Preparing for the experiments

THESE CONDITIONS CAN BE MAINTAINED if the amateur provides himself with an aseptic transfer chamber in which all critical operations are performed. This can be a simple wooden box two feet high, two feet wide and three feet long. It is fitted with a glass top, a small door and a pair of holes in one side large enough to admit the hands and forearms comfortably as shown in Figure 41. The cracks should be calked with cotton or sealing compound. A short pair of muslin sleeves may be tacked around the holes to serve as barriers against the outside air. Inside the box one should place, among other things, an alcohol lamp or Bunsen burner, and a small atomizer of the nosespray type containing Lysol or Clorox.

Glassware should include two dozen Petri dishes, which have flat bottoms about four inches in diameter, sides about half an inch high, and are fitted with covers. The experimenter will also need three Erlenmeyer flasks of one-liter capacity and half a dozen of the quarter-liter size, a half-dozen test tubes of 20-milliliter capacity and a rack for supporting them, a dozen one-milliliter pipettes and a special rubber bulb or syringe for filling them, a 50-milliliter graduated cylinder, a wax pencil for marking the glassware, a dissecting needle fitted to a pencil-sized wooden holder, a small loop of thin wire fitted to a similar handle, a glass stirring rod about eight inches long, and a pair of tweezers. All these things should be assembled, together with the special wooden box or transfer chamber, on a substantial bench located where the materials will not be disturbed.

All the materials are then sterilized. Petri dishes and pipettes are tightly wrapped in lots of six in brown paper. Larger items are wrapped individually. Test tubes and Erlenmeyer flasks may be plugged with wads of absorbent cotton instead of being wrapped in brown paper. The glassware is then placed in an oven and heated to 325 degrees Fahrenheit for at least two hours. None of

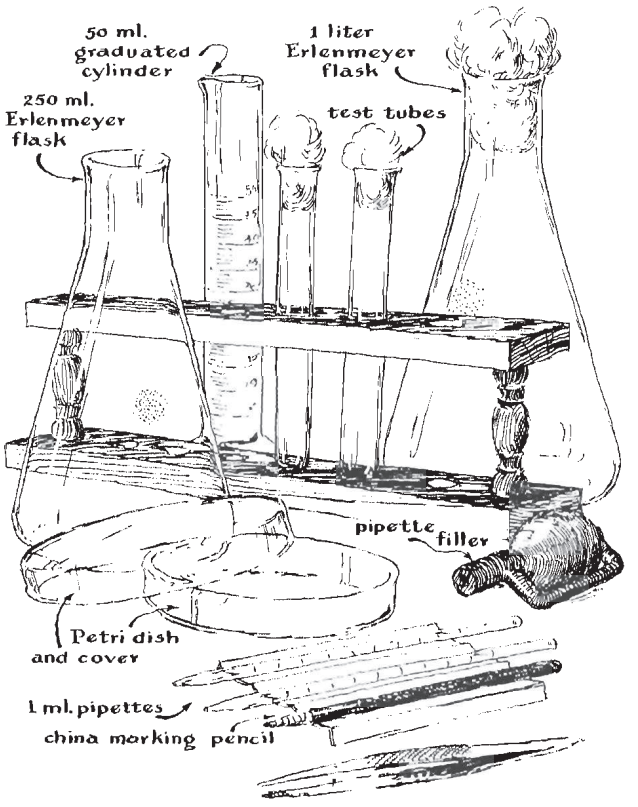


41
A sterile transfer chamber designed for amateur construction

the packages should be opened nor the cotton removed until the glassware is used.

Dehydrated culture medium, both plain and in the form of an agar infusion, may be ordered through your local druggist from the Difco Laboratories, Detroit, Mich., or from the Baltimore Biological Laboratory, Baltimore, Md. A principal object of the experiment, however, is to provide the amateur with experience in the basic techniques of culturing bacteria. The beginner is therefore urged to prepare his own culture medium.

Here is the recipe. Stir a pound of freshly ground hamburger



42
Essential equipment for experimenting with bacteria

into a liter of distilled water and put it in the icebox (at about 40 degrees F.) for 10 hours. Then skim off the fat which rises to the top and filter the remaining liquor through a single thickness of clean muslin. Add distilled water to bring the liquor back to a full liter, then add five grams of peptone and five grams of ordinary table salt and stir until the salt is dissolved. Pour 50 milliliters into a second flask and set it aside. Then add 15 grams of agar to the 950-milliliter portion.

Bacteria, like other organisms, are sensitive to the acid-base balance of the medium in which they grow. Those grown in this ex-

periment prefer a neutral medium. The two solutions just prepared will be slightly acid; they must accordingly be adjusted to neutrality (pH 7) by adding precisely enough sodium hydroxide to counteract the acid. Mix 10 grams of sodium hydroxide in a liter of distilled water. Test the beef broth with a piece of blue litmus paper. An acid broth will turn the blue paper red. The sodium-hydroxide solution will turn red litmus blue. Add a drop or two of sodium hydroxide to the liquor, stir, and with the glass rod put a drop of the solution on a piece of blue litmus. The paper in contact with the drop will probably turn pink. Add more sodium hydroxide to the liquor and test again. Continue this until the test drop causes no change in the color of either red or blue litmus.

Each container of liquor is then heated almost to 212 degrees F. for half an hour. This will precipitate the proteins in the liquor. The proteins are removed by passing the hot liquor through coarse filter paper. Each filtrate is again brought up to volume by adding distilled water.

One hundred milliliters of the hot agar medium are poured into each of six Erlenmeyer flasks, which are then stoppered with wads of absorbent cotton. Five milliliters of the liquor containing no agar are poured into each of 10 test tubes, which are similarly stoppered.

The media are now sterilized. The containers may be placed in boiling water for half an hour on each of three successive days. They may alternately be sterilized in a pressure cooker. Put the containers inside the cooker, add two inches of water and pressure-cook for 20 minutes. Be sure to cool the cooker slowly. Rushing the job by quenching the cooker with cold water will cause the internal pressure to drop suddenly and the vessels of hot medium to boil over. Stoppered tubes of tap water and other solutions may be sterilized by either of these methods.

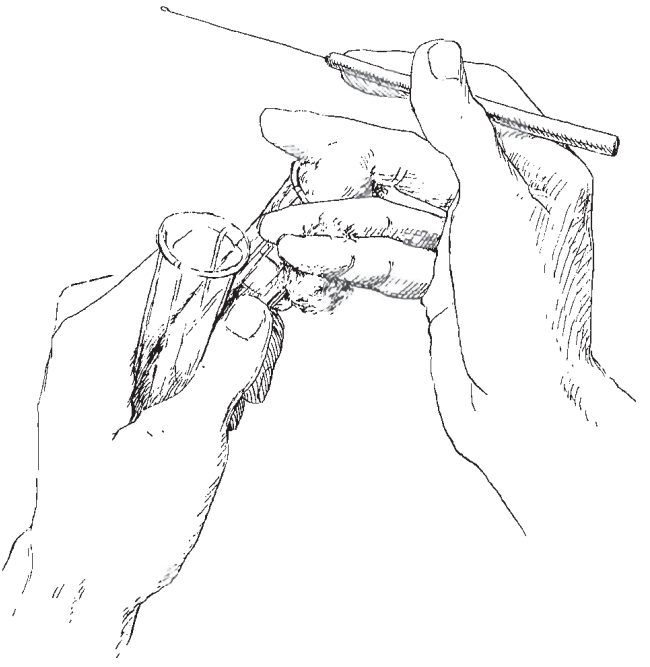
Any nonpathogenic strain of bacteria may be employed for the demonstration of bacteriostasis. *Micrococcus pyogenes* var. *albus, Proteus vulgaris* or *Alcaligenes faecalis* can be used in the experiment and may be purchased at low cost from the American Type Culture Collection, 2112 M Street, N.W., Washington 6, D.C. Amateurs may wonder why one should go to the expense of buying bacteria if they are plentiful in the air. You can, of course, grow

your own simply by exposing a quantity of the culture medium to the air and incubating it for 24 hours. Let us emphasize that this is pointless and can be dangerous. It is pointless because the average amateur has no means of identifying the microbes he has caught. It is dangerous because there is some possibility of capturing and cultivating disease organisms. Incidentally, media that have been used should be sterilized and discarded immediately. The strains recommended are inoffensive and have the further advantage of being accessible to all workers. Results of experiments may accordingly be compared. Purchased cultures can be perpetuated indefinitely by keeping them in beef broth at room temperature and inoculating a fresh tube of medium (by putting a drop of the old culture into it) every other day. If the culture can be stored at 40 degrees F., the growth of the bacteria is slowed and new media need be inoculated only once every six days.

The bacteriostasis experiment

TO PERFORM THE BACTERIOSTASIS EXPERIMENT, first place a test tube of sterile beef broth, the tube containing the flourishing culture, and the wire loop inside the transfer chamber. The chamber is sprayed thoroughly with germicide and the droplets are allowed to settle for five minutes. The alcohol lamp or Bunsen burner is lit and the wire loop heated to redness as far as the handle. Hold both test tubes obliquely in the left hand and the sterile wire loop in the right, as illustrated by Figure 43. Remove both cotton plugs from the tubes with the last two fingers of the right hand. The mouths of both tubes are passed slowly through the flame. The wire loop is then dipped into the flourishing culture for about a second, withdrawn and inserted into the tube containing the sterile broth. Both cotton plugs are replaced and the wire loop is again sterilized by heating to redness. To avoid contaminating the pure culture the beginner should run through these operations with empty tubes a few times for practice.

The freshly inoculated tube is permitted to incubate for two hours at room temperature and is then stored at 40 degrees F. At the end of 24 hours the tube is swirled in front of a light. The pres-



43
How to hold test tubes while transferring bacterial cultures with a loop

ence of sediment indicates that the inoculation has "taken." The purchased culture may then be sterilized and discarded. If at the end of 24 hours there is no sediment, the procedure should be repeated. It is useless to wait another 24 hours.

Antibiotics for diagnostic purposes may be procured through your local druggist under the trade name Bacto-Sensitivity Disks. If they are not available in stock the druggist may order them from Difco Laboratories, Detroit 1, Mich. Each drug is shipped in a sterile vial, 50 disks to the container, and in three concentrations — low, medium and high. The following antibiotics are available in this form: aureomycin, bacitracin, chloromycetin, dihydrostreptomycin, erythromycin, magnamycin, neomycin, penicillin, poly-

myxin B, streptomycin, terramycin, tetracyline and viomycin.

The bacteriostasis test consists in exposing a series of increasingly concentrated cultures of bacteria growing on plates of agar medium to the action of the drugs. A zone of inhibition around the Bacto-Sensitivity Disks indicates that the organism is sensitive to the antibiotic. The sensitivity of an organism may be evaluated by tabulating zones of inhibition according to the table shown in Figure 44.

SENSITIVITY	CONCENTRATION		
	LOW	MEDIUM	HIGH
very sensitive	zone	zone	zone
sensitive	no zone	zone	zone
slightly sensitive	no zone	no zone	zone
resistant	no zone	no zone	no zone

44
A simple table showing the sensitivity of an organism to an antibiotic of varying concentration

A duplicate set of plates is used as a control for detecting contamination. Begin the experiment by placing the following materials in the sterile transfer chamber: (1) a liter of sterilized tap water, (2) two packages of sterile Petri dishes, (3) a dozen sterilized one-milliliter pipettes and the sterilized rubber squeeze bulb, (4) a water bath heated to 112 degrees F. in which have been placed six Erlenmeyer flasks of agar medium, (5) a test-tube rack containing six test tubes, (6) the was pencil, and (7) an open bowl of germicide.

The packaged glassware is opened and the cotton stoppers removed from the culture and test tubes. A small tuft of sterilized cotton is placed in the neck of each pipette. Nine milliliters of sterile tap water are poured into each of the six test tubes. The following operations are then carefully performed, each piece of glassware being labeled or coded as it is used. Fit the squeeze bulb to a pipette and with it transfer one milliliter of the culture to a test tube of tap water. This tube is labeled 1:10, indicating that it contains one part of culture in 10 by volume. The tube is swirled for 30 seconds to assure thorough mixing. Remove the squeeze bulb from the pipette and drop the used pipette in the bowl of germi-

cide. Select another sterile pipette and transfer one milliliter of the 1:10 mixture to a tube of tap water. Mark this tube 1:100. Again swirl the 1:100 mixture for 30 seconds, drop the used pipette into the germicide and with another sterile pipette transfer one milliliter of the 1:100 mixture to the third tube of tap water. Mark this tube 1:1,000 and proceed in the same way with the remaining tubes, labeling them 1:10,000, 1:100,000 and 1:1,000,000.

A specimen of melted agar medium is now poured from each of the six Erlenmeyer flasks into six Petri dishes, each dish being labeled to correspond with the flask from which it is poured. The dishes are then covered with their glass tops and set aside to harden. After these control plates have been poured, each batch of melted medium remaining in the flasks is inoculated with one of the dilutions in the test tubes. Pipette one milliliter of the dilution into the appropriately labeled flask. Drop the used pipette into the bowl of germicide. The flasks are stoppered with cotton and swirled gently for 30 seconds to mix their contents. The water dilutions are sterilized and discarded.

The control plates are incubated two days at 80 degrees F. The transfer box can be made to double as an incubator by fitting it with a 100-watt bulb controlled by a thermostat of the type used in tropical-fish aquariums.

Twelve Petri dishes, the Bacto-Sensitivity Disks and a pair of forceps are next introduced into the transfer chamber. The chamber is sterilized as before. Two Petri dishes are then filled from each of the six inoculated flasks, each pair being labeled to show the culture dilution. The dishes are permitted to stand for about 20 minutes until the agar solidifies. The forceps are then passed through the flame, the Bacto-Sensitivity Disks opened and the disks placed carefully on the agar medium by means of the forceps. The weaker disks are placed on one plate of the pair and the stronger on the other. (If three drug concentrations are being tested the number of cultures must be increased accordingly, of course.) One way to keep track of the disks is to draw a radius on the back of each Petri dish with the wax pencil. The disk of aureomycin is then placed on this line. All other disks are placed alphabetically, according to the name of the drug, in a clockwise circle. Crowding should be

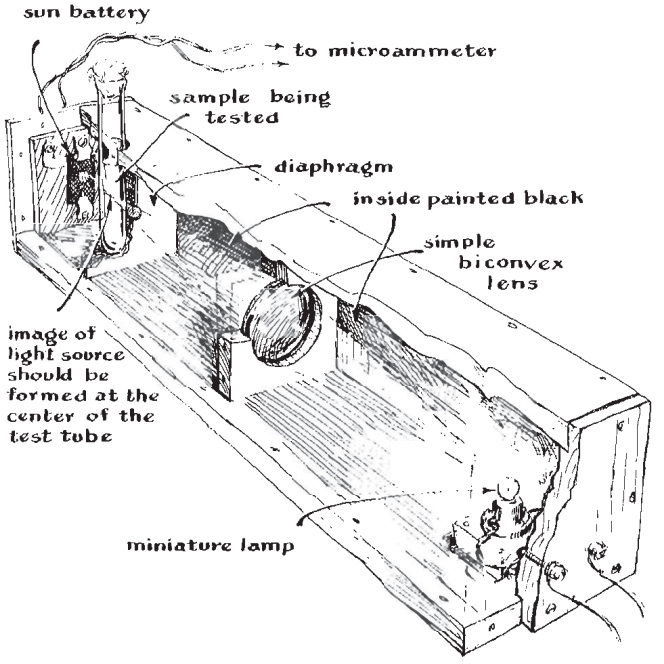
avoided; if space is limited, the last disk can be placed in the center of the plate. Covers are then placed on the dishes, and the culture is left to incubate for two days at 80 degrees F.

The effects of the several drugs and their concentrations on the various concentrations of bacteria are then evaluated by observing the growth on the plates and the rings around each drug where growth has been inhibited. The results may be tabulated by using a minus sign to indicate no growth and a plus sign for inhibition. The presence or absence of a zone of inhibition – *and not the diameter or area of the zone* – indicates the sensitivity to the antibiotics. Any growth on the control plates indicates contamination and invalidates the experiment. The test plates should be reread after four days of incubation, then sterilized and discarded.

Charting the effect of the drugs

AN INTERESTING MODIFICATION of the test permits the experimenter to chart the effect of the drugs with respect to time. Thus he can study the interval following inoculation at which each antibiotic exerts its action, and the rate, if any, at which it loses its effect. This requires the construction of a relatively simple light-meter capable of reading the relative transmission of light through a test tube. Bacterial growth in beef broth increases the turbidity of the broth and reduces its transparency. When the broth is placed in a test tube its turbidity – and hence its population of bacteria – can be measured by the light-meter. The device consists of a lamp and lens for focusing a beam on the side of a test tube, a photocell on the other side of the tube for receiving the transmitted light, and a microammeter for reading the output of the cell. The light source, lens assembly, test tube and photocell are mounted in an appropriately compartmented and light-tight box [*see Fig. 45*].

Here the operations are conducted in test tubes rather than agar. A tablet of antibiotic is dissolved in 10 milliliters of sterile tap water (in the aseptic transfer chamber). Dilutions of this solution are prepared as before, so that six dilutions span the range from 1:10 to 1:1,000,000. Observe that in this case it is the drugs, not the cultures, which are diluted. One milliliter of each of the dilutions



45
A homemade light meter for measuring the density of a bacterial culture

is added to a sterile test tube which contains four milliliters of beef broth.

The tubes are then inoculated with one loop of bacteria from a two-day-old beef-broth culture and left to incubate at 80 degrees F. At equal intervals during the incubation, say every three hours, the tubes are gently swirled and their turbidity is measured by means of the light-meter. Turbidity is then plotted against time. The result is a set of graphs showing bacteriostatic activity. The test tubes should be inspected for optical uniformity by means of the light-meter before they are used. Professional light-measuring instruments used for this test are usually calibrated in accordance with Beer's law, which states that, for solutions of a given substance in a given solvent, light will be absorbed in proportion to the thickness of the solutions.

Graphs made with instruments calibrated arbitrarily will show accurate rates of bacteriostatic effect, although the curves will not necessarily conform to those drawn with the aid of professional instruments. Tests which employ light-measuring devices can have great practical value because they show which antibiotic can be employed most effectively against a bacterium about which no data have been collected. They also disclose whether a known bacterial strain has mutated to become more resistant to a given drug.

Isolating unusual colonies and developing new strains

IN THE COURSE OF EXPERIMENTS employing plates of agar medium one may occasionally observe a small colony flourishing within the circle of inhibition. The chances are that this is a contaminant. There is always the possibility, however, that the organism is a mutant, a new strain which has been naturally selected over the original strain susceptible to the drug. All such unusual colonies should be isolated and cultured. (A portion of the colony is lifted from the plate with the tip of the dissection needle and transferred to fresh medium for incubation.) Tests can then be performed to learn if it is in fact a mutant or merely a contaminant.

Radiation is known to increase the mutation rate of all living organisms; thus it is possible to develop new strains by exposing cultures to X-rays. The homemade X-ray machine described in Section IX by Harry Simons is capable of inducing such mutations. The experimenter is cautioned, however, to avoid exposure to the X-rays. The culture should be placed in front of the tube and the machine operated by remote control from behind a shield, as suggested by Simons.

2

GROWING ALGAE ON A WINDOW SHELF

A casual stroll in the woods led the late I. C. G. Cooper, a naval architect of New York City, into the fascinating realm of algae culture. Here are his directions for isolating, identifying and growing the minute plants which may some day become an important source of man's food

ONE AFTERNOON, many years ago, I started out for a pleasant walk in our neighboring woods with William T. Davis, an amateur naturalist and one of the founders of the Staten Island Institute of Arts and Sciences. He had volunteered to teach me how to identify some of our local wildflowers. His enthusiasm was contagious, and before the afternoon was over the bug had bitten me.

During the next few months I gathered and mounted a lot of flowers and weeds. Before long it became evident that I was a little late with my discoveries; the specimens I collected were already represented in the Institute's display cases. It seemed pointless to go on duplicating work already well done. Then one evening at the end of a field trip I took a short-cut home by way of the beach and noticed a strange clump of seaweed waving back and forth in the low tide. I took off my shoes and waded in. After I had pulled up a specimen of the plant, I had an idea: Why not make a study of Staten Island's marine flora?

Although that first specimen turned out to be only a common variety of rockweed, it occupies a special place in my collection because it introduced me to the thallophytes, the grand division of the plant kingdom occupied by the algae.

You don't need a scientist's background to get fun out of collecting algae, especially the big ones. You simply float them in

whole or in part onto a sheet of paper and let them dry. The leaf-like parts of many consist of only two layers of cells coated with a clear pectinous substance. They dry on the paper without apparent thickness, like ink, and few artists paint more colorful or exotic abstractions.

Things went along nicely for a couple of years, and my original rockweed grew into quite a substantial collection. Then the job became rough. As I worked my way down the scale of algal sizes, the number of species increased all out of proportion. Identification became difficult. The reference texts, which fully describe the giant kelps and often carry colored illustrations of them, become sketchy when you get down to the species that make a pocket magnifier handy.

Without knowing it would make matters worse, I bought a microscope. The first look through it almost ended my new hobby. Here was no man's land. I could not even distinguish between plants and animals, much less identify the plants. A single drop of fluid scraped from a stalk of marsh grass would hold scores of organisms, including animals that grow in branching patterns like plants and plants that swim by means of whiplike tails and eat like animals! At this point I want to put in a good word for the patience of our museum's curators and that of my fellow members in the New York Microscopical Society. They finally succeeded in teaching me how to recognize a chloroplast when I saw one, and also to identify the cellulose walls which aid in distinguishing one biological kingdom from the other.

But learning how to tell plants from animals was only a beginning. Each drop of liquid that appears under the microscope's objective contains a unique population. Before I could complete a census, the drop would evaporate and destroy the individuals. How do you introduce order into a scramble like this, and where do you begin?

It is a good idea, the curators advised, to commence by narrowing your field. Staten Island is not large as islands go, but in terms of its algal population it is vast. In naively undertaking the collection of all our local "seaweed" I had staked out too much territory. After years of sampling the immensely various populations of algae

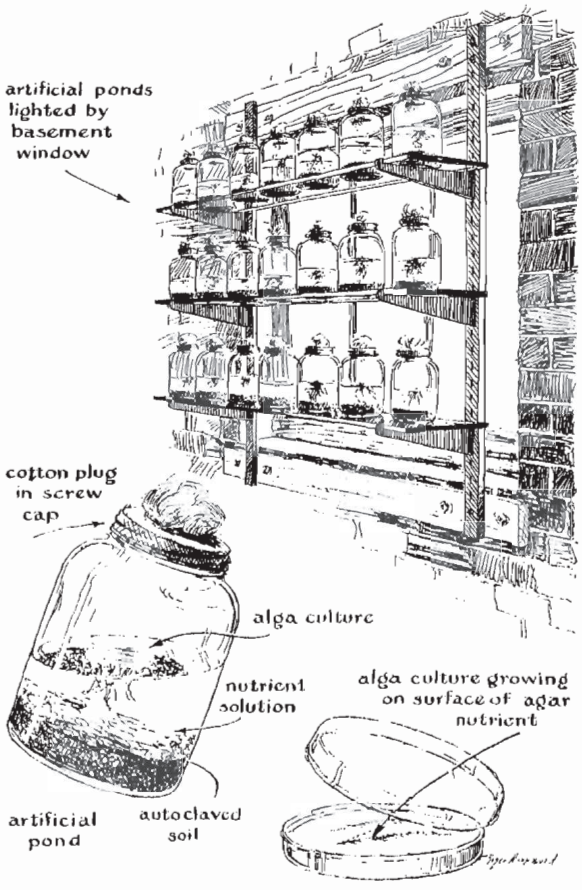
in the island's waters, I decided I would have to limit myself to the less abundant algae of the soil.

As a rule, algae are not too difficult to find in the soil once you have picked up a bit of experience in handling cultures and the microscope. But separating them into individual species and exploring their structure and behavior can get you embroiled in all sorts of puzzles and complications. Fortunately the phycologists and bacteriologists have solved the hard problems of method, and it is not difficult to adapt their techniques to an amateur's studies.

I use the so-called "soil-water" culture method advocated by E. G. Pringsheim of Cambridge University. In effect the algae grow in a miniature artificial pond — a glass jar of nutrient solution covering a bottom of mud [*see Fig. 46*]. The pond is prepared by partly filling a wide-mouthed glass container — such as a peanut-butter jar — with nutrient solution, adding a tablespoonful of soil and then sterilizing the whole in an autoclave. The pond is then inoculated with the specimen of soil to be investigated. A pinch does the job. The pond is kept at room temperature and exposed to light during incubation; a window having a northern exposure is a good light source.

After incubation is completed — when the characteristic green "scum" appears in quantity — a smear of the culture is transferred to an agar plate where it continues to grow. If the smear has been made carefully, distinct colonies of the various organisms will appear here and there on the plate. You then pick out one of these with a glass needle or a micropipette and inoculate a second sterile pond with it. What you thought was a colony of identical organisms will likely prove to be a mixture — but the second pond will be less motley than the first. You continue this cycle of operations until your species appear in splendid isolation — or your patience gives out. Sometimes I wonder if it is possible to develop a perfectly pure culture of anything.

Single-celled algae are enveloped by the same pectinous substance that causes the giant kelps to dry on paper so beautifully. This sheath is usually alive with bacteria. Just try to kill them without killing the algae! Irradiation by X-ray or ultraviolet light in measured doses tends to kill the bacteria without destroying all the



46
Cooper's setup for the cultivation of algae from the soil

algae. But even if you succeed in knocking out the bacteria without damaging the plant, you still face the job of separating the alga from the culture without contaminating it and of inducing it to grow in a fresh pond.

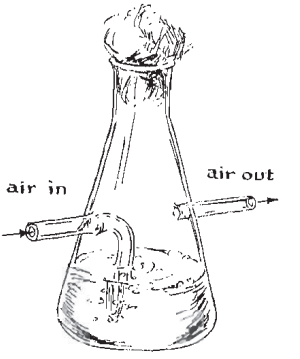
The artificial-pond technique always leaves you with a number of chemical unknowns. I hope the spectroscope will eliminate some of them. The growing culture takes part of its nourishment from elements added to the solution and part from sterilized soil. The first are under your control. If we could grow cultures by pure hydroponic methods, a lot of question marks that come with the

soil would vanish. But that would necessitate a comprehensive knowledge of the organism's nutrient requirements in advance of growing a culture of it.

Hence we combine the major elements — nitrogen, potassium, magnesium and others common to all plants — in the nutrient solution and rely on the "mud phase" to supply the minor ones plus other unknown factors such as vitamins. The mud also serves as a reservoir and a place of reduction and synthesis for keeping the heavy metals in solution. Incidentally, the proper soil for the pond's bottom must be found by trial. After a lot of sampling, I located one that works unusually well. A large quantity of it was sterilized at one time by autoclaving and stored in sealed containers for future use.

Friends sometimes ask what I do with an alga when it has been isolated and added to the collection of cultures. In a way that is like asking a philatelist what he does with his stamps. If he is a good philatelist, he preserves them carefully and tries to learn something from them. Preserving live algae is no less satisfying nor more difficult than caring for any other plant.

If you give them light, water and food, and maintain the temperature they prefer, they glow with health. In turn they challenge you to discover how they react to such things as subtle changes in diet; how, when and by means of what mechanism they reproduce; what products their metabolism yields — and the countless related secrets of their life processes. In accepting this challenge you can,



47
Aeration flask for algal culture

as they say, dive in as deeply and stay down as long as you wish. I have been at it now for some years without getting more than my feet wet.

Those who enjoy hydroponics like to develop nutrients, and I have had some success in this work. One series of experiments ended in a solution which seems to work better for me than those listed in the reference texts. You lay out a set of slightly differing ponds in a rectangular grid, with a single element in the nutrient progressively diluted more and more in each vertical row. The entire grid is inoculated and kept under observation.

A detailed record of the culture's reaction in each pond is made. The experiment can be continued by simultaneously altering the strength of two elements in each vertical row, then three elements and so on. An analysis of the accumulated record discloses the ideal concentration of each element in the nutrient for the species under study. Incidentally, a culture subjected to this study becomes a tool of great power and subtlety for investigating unknown nutrients. The alga's reactions when transferred to the unknown nutrient provide an indication of the ingredients present and, in some cases, a quantitative measure of their concentrations.

Once a culture has been standardized, that is to say, brought to a reasonable state of purity and provided with the preferred nutrient, it suggests endless other experiments. If the alga employs sexual reproduction, for example, you can attempt to mate it with a near relative and create a hybrid. It is interesting to modify a plant's diet and observe the result.

A heavy concentration of nitrogen can cause *Chlorella*, an alga which may become commercially important, to increase its production of protein from about half its weight to almost 90 per cent. In contrast, putting Chlorella on a starvation diet of nitrogen boosts fat production from something under 10 per cent to more than 70 per cent. The commercial implications are obvious.

It is easy to see how such metabolic gymnastics can fascinate the amateur. Learning to observe such changes, to take the plants apart and measure the substances of their bodies, or those that appear as by-products, will bring you into contact with as many fields of science as you have time and talent to enjoy.

3

HOW TO TRANQUILIZE A RAT

The power of certain new drugs to calm disturbed animals is demonstrated by the use of rats in an ingenious experiment designed by Sara E. Southwick, a high-school girl of Midland, Mich. She tells how to build and use the apparatus and gives the procedure for establishing experimental controls. A method for assessing the damaging effects of the drugs is described

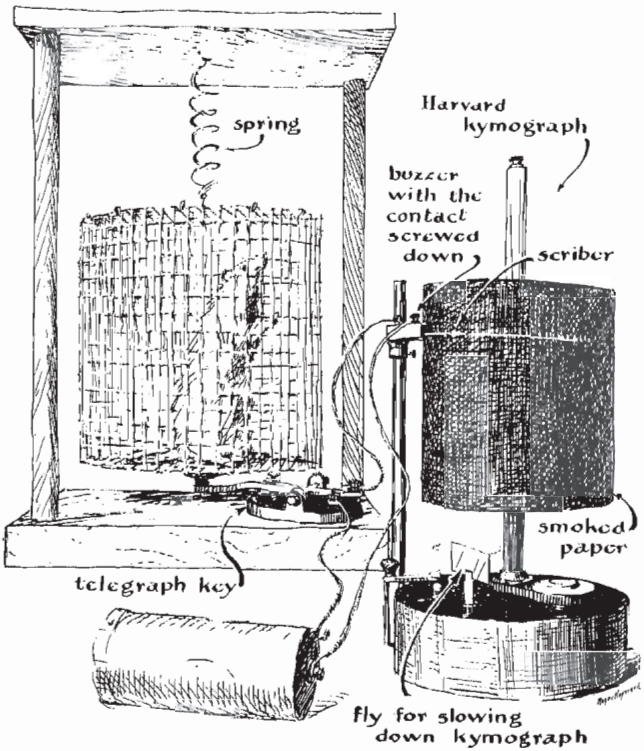
FOR MY SCIENCE FAIR PROJECT during my senior year in high school, I set up a controlled experiment to test the effect on rats of chlorpromazine, one of the new tranquilizing drugs. Tranquilizers were making news at the time, particularly in the treatment of mental disease, and this caught my interest. According to medical reports, the side effects of the tranquilizers had not been fully catalogued, and it seemed likely that a science fair project based on one of them would have a good chance of scoring high on originality.

Chlorpromazine has the property of quieting mental patients who are restless, overactive and abnormally elated. Would the drug have a similarly depressing effect on normal animals? If so, how would it affect other aspects of their functioning?

As subjects for the experiment I obtained six white rats, all males from the same litter. During the first phase of the experiment three rats were selected at random for treatment and the remaining three were reserved as controls. Each animal was tagged for identification. Midway through the experiment the treatment was switched; the controls were put on the drug, and the group previously treated became the controls. I called this the "crossover" phase. It served as a check against previous results. Otherwise all animals were maintained under identical conditions as closely as possible, and each was given a standard ration of food and water on a fixed

schedule. The experiment continued for five months as a spare-time activity.

A thorough physical examination was made of each animal at the beginning of the experiment, both to assure that the animals were in good health and to provide comparison data for subsequent use. The effects of the drug were then observed by measuring changes in activity, intelligence, blood composition, pulse rate, body temperature, weight, respiratory rate, external features, sexual behavior, internal organs and metabolism. The animals were treated by administering chlorpromazine along with their food, initially at the rate of five milligrams of the drug per kilogram of body weight.



48
Cage, telegraph key and kymograph to measure the activity of a rat

After one week the dose was increased to 15 milligrams; somewhat later, to 20 milligrams.

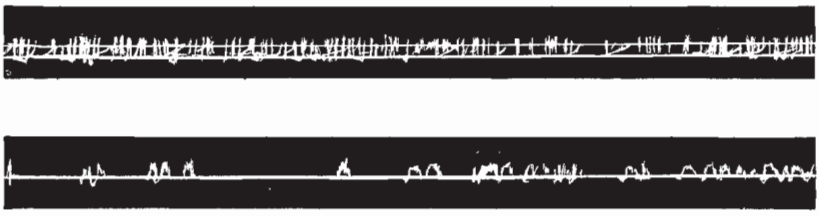
Reaction to the drug was immediate. The treated rats became quieter than the control group. Their movements were slower and more deliberate. To measure the difference I designed a "jiggle cage" consisting of a box of quarter-inch wire mesh covered at the bottom with a sheet of heavy aluminum foil of the kind used by bakers.

The cage was suspended by a weak spring from the bottom of a small table consisting of a seven-inch square of half-inch plywood fitted with legs of wooden dowel stock. The movements of the caged rat caused the cage to jiggle up and down and actuate the handle of a telegraph key in contact with the bottom of the cage. The key closed an electrical circuit between a battery and the coils of a modified buzzer. The buzzer contacts were closed; a wire stylus was attached to the armature. The stylus pressed against the smoked drum of a kymograph on which the movements of the rat registered as sharp vertical pips in an otherwise smooth trace. I borrowed the kymograph from the biology department of the Midland High School. It is not too difficult, however, to make such a drum recording-device. [One is described in Section V.] A recording speed of some three inches per second, equivalent to the speed of a six-inch drum turning at about one revolution per minute, is adequate for this experiment.

The activity of each rat was measured daily for one hour in darkness (when rats are commonly most active). Copies of two recordings are shown in the accompanying illustration [Fig. 49]. These show the activity of the same rat before and after the drug had been administered.

The effect of chlorpromazine on intelligence was tested by means of a changeable maze in which both the pattern of the paths and the obstacles (rectangular partitions) could be altered. The animal was required to crawl under a partition or jump over it, depending on whether the partition was turned so that its opening was at the top or at the bottom. The maze was covered so the animals would not be distracted during the run.

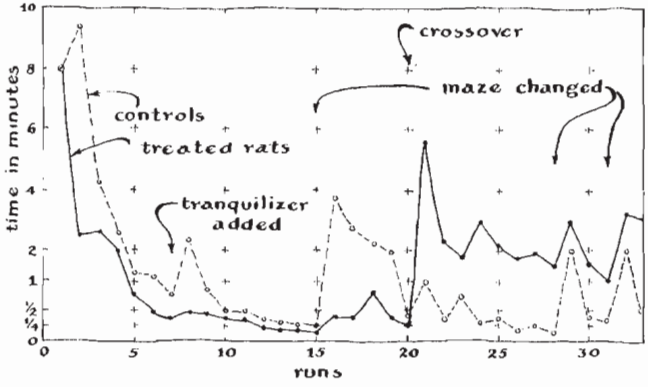
To test the adjustment of the rats to change, the maze was altered



49
Top record reflects activity of untreated rat; bottom, treated rat

four times for each group of animals during the course of the experiment. In the beginning the control rats required about seven runs to learn the maze, during which the time of the run dropped from eight minutes to 30 seconds. The treated group required substantially more practice to achieve comparable performance; at first these rats actually lost ground. The reaction of the rats was even more significant during the crossover phase of the experiment.

During the crossover phase the rats in the control group learned to run the maze, with practice, in five seconds. When they were under the influence of chlorpromazine, however, none of these rats could do better than one minute. In contrast, the previously treated rats, after recovering from the drug, learned to run a new pattern in five seconds. Furthermore, when the crossover phase was started,



50
Graph compares the performance of treated and untreated rats in a maze

the drug caused the controls to forget a maze pattern they had mastered. From this it would appear that the drug has a depressing effect on memory as well as on intelligence.

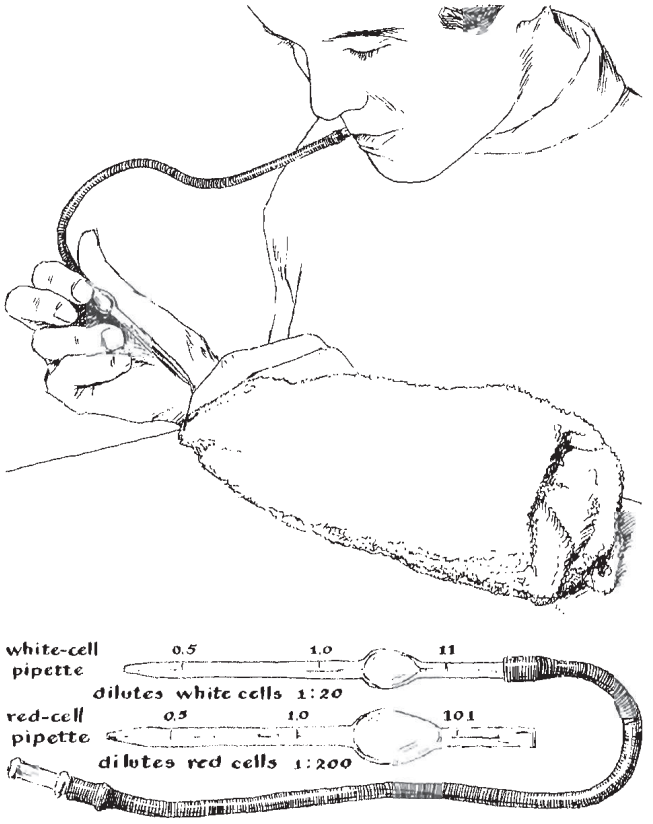
Does the drug similarly depress organic functions? This was investigated in part by examining changes in the blood of the rats. The blood was taken by clipping the tip of the rat's tail. Incidentally, I quickly learned that rats are not as cooperative in all parts of the experiment as one could wish; blood sampling is a case in point. I first tried to hold the animals in one hand while taking the specimen with the other, but soon adopted the technique of wrapping them in a towel. Later I borrowed a glass vessel especially designed for the purpose.

Standard clinical pipettes were used for withdrawing two specimens of blood from each rat: one specimen to ascertain the number of red blood cells; the other to test for the number of white blood cells. The pipettes are fitted with a short length of suction tubing; one simply places the glass tip in the fluid and withdraws enough to reach the .5 mark etched in the glass. Sufficient diluting fluid is then drawn into the pipette to reach the top mark.

When one is sampling white cells, the top mark is 11; in the case of red cells the mark is 101. The diluting fluid for white cells (which causes the red cells to disintegrate) consists of one part by volume of hydrochloric acid to 100 parts of distilled water. The red-cell specimen is diluted by a fluid consisting of .5 gram of mercuric chloride, five grams of sodium sulfate and one gram of table salt dissolved in 200 cubic centimeters of distilled water. This solution causes the white cells to disintegrate.

Blood cells are counted with the aid of a special chamber that divides the field of view into a pattern of uniform squares, somewhat like a sheet of graph paper. If one encounters difficulty in procuring a counting chamber, a rough estimate of change in the relative number of white cells and red cells can be made by comparing stained specimens. I borrowed a chamber.

The red-cell count remained constant throughout the experiment for both treated and untreated rats. But the white-cell count increased substantially during the time the animals were on the drug, averaging 22,000 cells per cubic millimeter during treatment as



51
Pipetting a specimen of rat blood

against a normal count of 14,000. Counts were taken of all rats once each week for the first three weeks after the beginning of treatment.

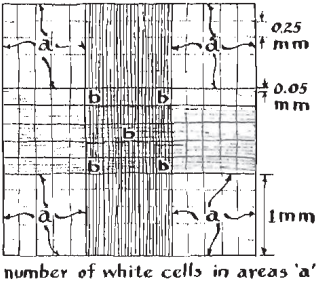
Measurements made during the crossover phase were identical with those recorded at the beginning of the experiment. In one exceptional case, however, both groups yielded identical counts. This occurred after the experiment had been running two months and suggests that the rats may have developed some tolerance for the drug.

Stained blood-specimens were examined with the aid of a micro-

scope equipped with a 10-power eyepiece, an oil-immersion objective of 97 power, and dry objectives of 43 power and 10 power. The combinations of eyepiece and objectives gave magnifying powers of 970, 430 and 100 diameters. The instrument was borrowed from the Midland Hospital.

The technique of making differential smears is not difficult if one carefully follows a standard procedure. The microscope slides must be cleaned thoroughly. Ordinary household detergents, particularly those containing a soft abrasive powder, make a satisfactory cleaning agent. A drop of blood is first placed near the end of a freshly cleaned slide. The end of a second slide is then held at an angle of about 45 degrees at a point between the drop and the center of the second slide, so that the drop wets the lower surface of the upper slide. The fluid will immediately spread by capillary attraction across the line of contact between the two slides. The second slide is then quickly pushed forward toward the far end of the first. This distributes the specimen *behind* the top slide in a film which adheres to the lower slide. Do not place the drop in front of the top slide and push it with the end of the glass; the cells will be forced to flow between the two slides and may be broken.

The smear is allowed to dry until it becomes tacky, and then is stained. I used Wright's stain, which can be procured through most drugstores. It is made into a solution consisting of .3 gram of powdered stain mixed with three grams of glycerin and 97 cubic centimeters of methyl alcohol. A drop of the solution is applied to the



52
Pattern of chamber for counting blood cells

slide and allowed to stand for three minutes. A drop of buffer solution is then added. This consists of 1.63 grames of potassium phosphate and 3.2 grams of sodium phosphate dissolved in a liter of distilled water.

The ingredients of both formulas, incidentally, may be cut in proportion if smaller quantities are desired. After the buffer has worked five minutes the slide is washed gently with distilled water, permitted to dry in open air and then placed under the microscope for examination.

The differential smears from rats under treatment showed a 20-per-cent increase in the white cells known as lymphocytes, and an equal decrease in neutrophil white cells. Again, however, one measurement made two months after the beginning of the experiment proved exceptional and suggested the development of tolerance to the drug.

As another index of reaction to the drug the pulse rate of all animals was taken twice during each phase of the experiment. This proved somewhat difficult because the pulse rate of a healthy rat is about 375 beats per minute, and, as I discovered, this rate is almost doubled by the administration of chlorpromazine. Accordingly, accurate counts could not be made by listening to the rats' heartbeats with a stethoscope.

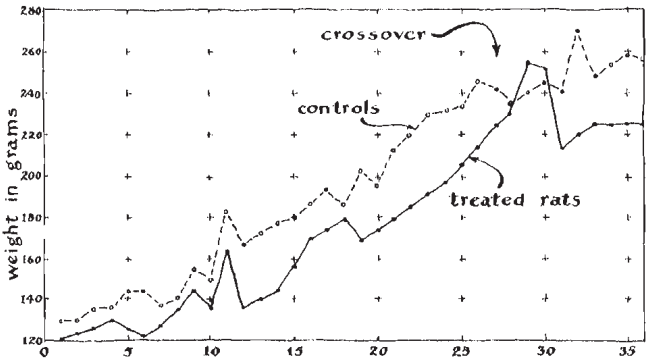
I solved the problem with the aid of an ordinary magnetic-tape recorder. The rat was held against the microphone and a recording of the heart sounds made at a tape speed of 7.5 inches per second. The record was next played back at 3.25 inches per second and the beats counted by ear against a stop watch. The count was then multiplied by two. (The absolute tape speed, which varies with the make of the machine, is not important. One is only concerned with the ratio of speeds.)

Rats tend to become excited when placed against the microphone, so they should be permitted to settle down for a few minutes before the recording is made. The pulse rate of tranquilized rats averaged 639.6 beats per minute, as against 389 beats per minute for the controls. The slightly higher than normal rate of the controls is explained by the excitement of the rats at being handled.

The body temperature of tranquilized rats was also found to be

abnormally high, averaging 100.1 degrees Fahrenheit as against an average of 99.7 degrees for the controls. The average difference is not great. But in every instance the lowest temperature observed in a treated animal was above the highest temperature among the controls. This fact, when considered together with the elevated lymphocyte and decreased neutrophil counts, suggests that the tranquilized rats had contracted an infection of some sort. This was further indicated by their behavior. Some appeared to be sick part of the time. The temperatures were taken with a conventional rectal thermometer.

A careful record of body weight was made daily, along with the weight of food and water consumed. Early in the experiment the ratio of water to total body weight changed in a direction that suggested that the drug was causing dehydration, but this was not supported by the crossover observations. Both groups made comparable gains in weight throughout the experiment [*see* Fig. 53].



53
Graph compares the weight of treated and untreated rats

Chlorpromazine lowers the respiratory rate of rats substantially. Tranquilized rats average 72 inhalations per minute as against 95 for the controls. The rates coordinate well with the relative activity of the two groups. Counts were taken three times during each phase of the experiment.

A close check was made throughout the experiment of the external appearance of the rats in both groups. I observed a number of obvious differences. Minor skin eruptions developed within a few days after each animal was put on the drug. In addition, the hair of the treated rats became rough and shed readily. In the case of the first group to receive the drug, violent muscle tremors occurred when the animals awakened from sleep. This, however, was not observed in the group treated during the crossover phase. The behavior of the groups also suggested that, at least in rats, chlorpromazine acts as a sexual stimulant.

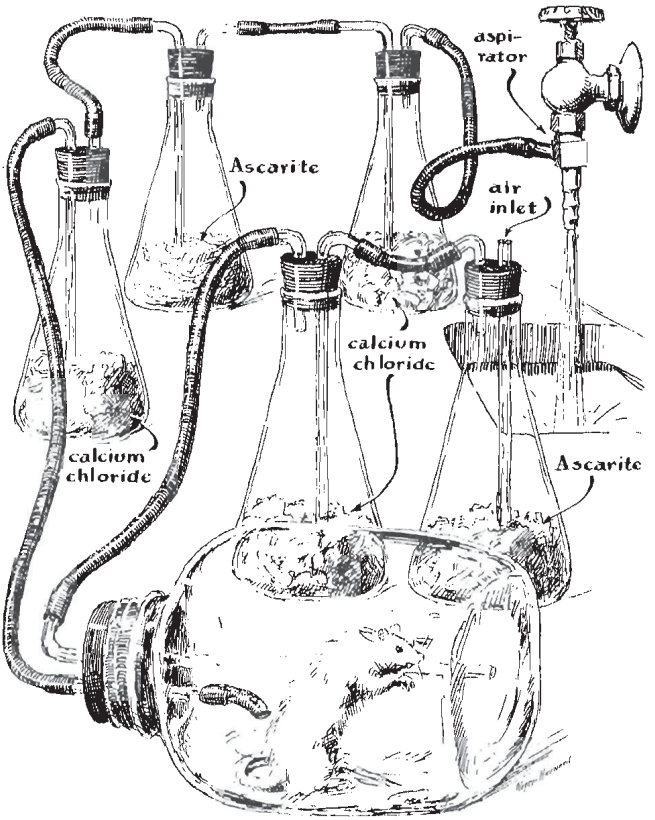
Tendencies to jaundice sometimes follow the administration of chlorpromazine, according to reports in the professional journals. This reaction appeared in both groups of rats approximately two weeks after treatment was started. Their eyes became pale and their feces lost color. To check possible damage to the liver an autopsy was performed on one control and one treated rat 17 days after the beginning of the crossover phase. Sections of liver tissue were taken from both rats and preserved in xylol. Both specimens showed abnormality. The damage appeared more extensive in the animal that was undergoing treatment at the time the autopsy was performed. This part of the experiment was interesting, but because the observations were limited to two animals the result could not be considered conclusive.

No diabetic effect was observed. The qualitative test for this reaction was made by a procedure which I learned at the Midland Hospital that requires the use of chemically treated test-strips that one must either purchase or borrow. The strip is dipped in the urine of the animal; if sugar is present, the tip of the stick turns blue within a minute.

Metabolism was measured by a variation of the method devised in 1890 by the noted British physiologist J. S. Haldane [see *How to Measure the Metabolism of Animals,* page 135]. Essentially the test consists in supplying the animal for a known interval with air containing a minimum of water vapor and carbon dioxide and then subtracting the weight lost by the animal from the weight of the water vapor and carbon dioxide exhaled during the test interval.

This gives the weight of oxygen absorbed by the animal, and

when this figure is divided into the weight of exhaled carbon dioxide (adjusted for the molecular weights of oxygen and carbon dioxide), the result is equal to the respiratory quotient of the animal. My apparatus consisted of an air pump (a water-powered aspirator) and five flasks of one liter each connected in series. The initial flask in the series contained approximately 600 cubic centimeters of Ascarite, a commercial preparation that has the property of absorbing carbon dioxide. The second flask held a comparable amount of anhydrous calcium chloride, which absorbs water vapor. The intake of the second flask was coupled to the exhaust of the third, a



54
A homemade apparatus for measuring the metabolism of small animals

wide-mouthed jar capped with a close-fitting stopper, which served as the animal chamber. The intake of the animal chamber led respectively to flasks of calcium chloride, Ascarite and calcium chloride.

All containers and the animal were weighed individually before and after a test interval of one hour. The weight (in grams) lost by the rat was then divided into the product of the weight gained by the fourth and sixth flasks multiplied by .7282 (the ratio of the molecular weights of oxygen and carbon dioxide).

Only two animals remained in each group at the time the respiratory quotient was measured, the other pair having been used for the autopsy. The respiratory quotients of the rats then under treatment were .56 and .58, whereas those of the control rats were .82 and .65. Here again the sample was too small to yield reliable figures. Differences in the individual determinations show a spread, however, which suggests that the lower activity of the tranquilized rats is accompanied by a correspondingly low respiratory rate.

From this experiment it would seem that a rat is tranquilized by the steady administration of chlorpromazine, but with at least temporary cost to its health. The drug depresses the animal's memory and intelligence, alters the composition of its blood, invites infection, increases its pulse and body temperature, lowers its metabolism, induces abnormal sexual stimulation and damages its skin, hair and liver.

4

HOW TO MEASURE THE METABOLISM OF ANIMALS

While casting about for a project to enter in a science fair, Nancy Rentschler, a high school student in Mayfield, Ohio, hit on an interesting apparatus for measuring the rate at which animals utilize food. She describes its assembly and operation, and gives the details of a typical experiment with mice as subjects

DURING MY HIGH-SCHOOL DAYS I once came across a textbook diagram of an apparatus to measure animal metabolism. It had been designed for dog-sized animals and therefore presented a space requirement considerably beyond my available facilities. The process sounded so fascinating, however, that I could not put it out of my mind. I didn't know very much about metabolism. But it seemed to me that I could learn if the apparatus could be scaled down to the size required for a mouse. I was further stimulated by the fact that the apparatus, if it could be designed, should stand up well against the 220 exhibits scheduled for entry in our science fair. I began to work on the project late in January and performed my first experiments about a month later.

The mice and the apparatus

THE MICE I USED were purchased through a pet shop. They had been inbred for three generations. At first I found it a bit difficult to handle them, but soon I learned to pick them up by the tail. After a week or so the mice became quite tame, although occasionally one would lose its temper during an experiment and try to bite the experimenter.

For the purpose of my experiments I divided 15 mice into four

groups, three in one group and four in each of the others. By placing each group on a diet or medication which differed from that of the others, I could study the effects of these differences on the metabolism of the animals. I followed the experimental method devised by the noted British physiologist J. S. Haldane in 1890. The apparatus consists mainly of an animal chamber and five flasks of chemicals interconnected by tubing so that a controlled stream of air can flow through the system [*see Fig. 51, How to Tranquilize a Rat*].

The purpose of the apparatus is to measure the amount of oxygen taken up by the animal, and the amount of carbon dioxide expelled. The ratio of oxygen inhaled to carbon dioxide exhaled by the animal during a given period indicates the rate of its metabolism, and is called the "respiratory quotient." This quotient varies with the diet of the animal. When the animal is fed a carbohydrate such as sugar, the ratio is 1. When it is fed fats, the ratio varies slightly with the composition of the fat but averages .7. The ratio for proteins also varies, but averages .8. The ratio of alcohol is .667. The respiratory quotients of normal animals under average conditions usually lie between .72 and .97.

Each flask of the apparatus is fitted with a rubber stopper and two glass tubes about half an inch in diameter. One tube reaches to within an inch of the bottom of the flask and the other just passes through the stopper. Air entering the flasks through the longer tubes is exhausted through the shorter ones. The first and fourth flasks in the series (not counting the animal chamber) are filled to a depth of about three inches with soda lime, which absorbs carbon dioxide. The second and third flasks contain the same amount of calcium chloride. The fifth flask is charged with pumice stone and sulfuric acid. These last three flasks absorb water vapor. Ideally all three should contain pumice and sulfuric acid.

I found the pumice difficult to prepare, so I made enough for one flask (to satisfy myself that I could prepare it) and "made do" with calcium chloride in the other two. The pumice is used in lumps about half an inch in diameter. Mine came from cosmetic counters, which proved to be a costly source. I learned later that chemical supply dealers list pumice at 50 cents a pound.

The stone is activated by heating it to redness with an acetylene torch and dropping it, while it is still hot, into concentrated sulfuric acid. The excess acid is then allowed to drain off. The soda lime is prepared by mixing lime with a solution of sodium hydroxide in the proportion of one ounce of sodium hydroxide (by weight) to two and a half ounces of water (by volume). Lime is added until the mixture becomes dry. The powder is then separated from the coarse particles by means of a fine sieve and discarded. Large lumps are broken down. It is the intermediate fragments — those which pass through a sieve of five meshes per inch — that are used for charging the flasks. The absorbing power of soda lime does not last long, and I had to make additional batches as the experiments progressed.

My animal chamber was a two-quart canning jar. I found it necessary to shield the exhaust tube of the chamber to keep it from pinning the mice. Before I added the shield, this happened several times, spoiling the experiment and injuring the mouse. The shield is merely a short length of rubber tubing with a slit or a few holes cut in it. It is slipped over the shorter glass tube inside the chamber. No damage is done when a mouse brushes against the end of the tube because the slit provides a second exhaust port.

The entire system must be airtight. Close-fitting stoppers should be used and all joints coated with either wax or plastic cement. The rubber tubing should be as short and straight as possible, and should be tightly fitted to the glass tubes. Air was pulled through the apparatus by means of an aspirator attached to a water faucet.

Air normally contains about 3 per cent carbon dioxide and a varying amount of water vapor. Both are removed by the first and second flasks. Thus air free of water vapor and carbon dioxide flows into the animal chamber. The animal inhales oxygen and exhales carbon dioxide and water vapor. The latter are absorbed by the remaining flasks.

The increase in weight of the third flask indicates the amount of water vapor given off by the animal. The fourth and fifth flasks measure the amount of carbon dioxide (which reacts with the soda lime in the fourth flask to form carbonic acid). The fourth and fifth flasks must be weighed together because the soda lime may give up moisture to the dry air and thus lose weight.

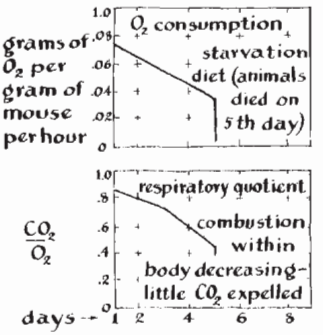
In setting up the apparatus for a test run, the last three flasks are weighed, the fourth and fifth together. The animal is then placed in the chamber, which is stoppered and weighed. The test run is timed from this moment. The chamber is now connected to the apparatus and the air pump started. I ran the mice in each group for a total time of one hour. At the end of this period the pump is stopped and the chamber removed from the apparatus, stoppered and weighed again. The third, fourth and fifth flasks are also weighed.

The respiratory quotient may now be calculated. The combined weight of the mouse and chamber at the beginning of the run minus their weight at the end of the run equals how much weight the mouse has lost. The weight of the third flask at the end of the run minus its weight at the beginning equals the amount of water absorbed by the calcium chloride and lost by the mouse. The weight of the fourth and fifth flasks at the end of the run minus their weight at the beginning equals the amount of carbonic acid formed.

The total weight of water and carbon dioxide absorbed minus the loss in weight of the mouse equals the weight of oxygen absorbed. The respiratory quotient is determined by multiplying the weight of the carbonic acid by the fraction 32/44 and dividing the result by the weight of oxygen absorbed. The quantity 32/44 is the ratio of the molecular weight of oxygen to that of carbon dioxide. Its use in the equation indicates the amount of carbon dioxide represented by the carbonic acid.

The metabolism experiment

I used two of my four groups of mice to study the effects of diet on metabolism. With the other two groups I investigated the metabolic effect of the activity of the thyroid gland. The first group of four mice was given only water. Although mice normally live about nine days without food, these died after four days. It is likely that they contracted pneumonia because their resistance was low. Their respiratory quotient dropped slightly from the beginning of the experiment but stayed within the normal limit of .7 to 1 for the first three days. It plunged sharply just before the animals died. Oxygen consumption, however, decreased at a constant



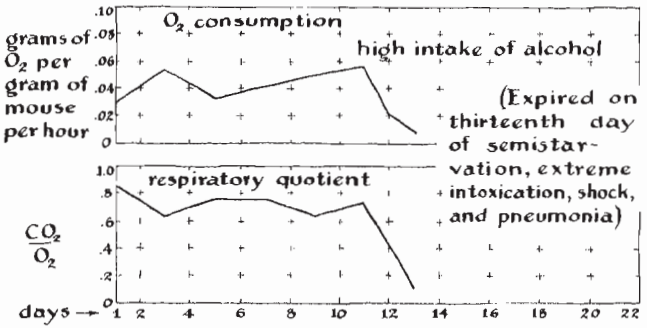
55
Metabolic graphs of starved mice

rate throughout the period of observation. At the conclusion of the experiment I plotted graphs of both oxygen consumption and respiratory quotient [*see Fig. 55*].

In the second group of mice a 17 per cent solution of ethyl alcohol was substituted for water. Each mouse also received one gram of rabbit pellets per day beginning on February 26. On March 4 I found the mice shivering and huddled together in their cage. Fearing that they might die if a test were attempted, I fed them immediately and wrapped them in warm rags. Their ration was doubled for two days and then lowered to a gram and a half on the third day. One mouse died on March 9 and another the following day. I attempted to study the remaining two in the metabolism cage but their rate of respiration was so low that no results were detectable at the end of a two-hour run.

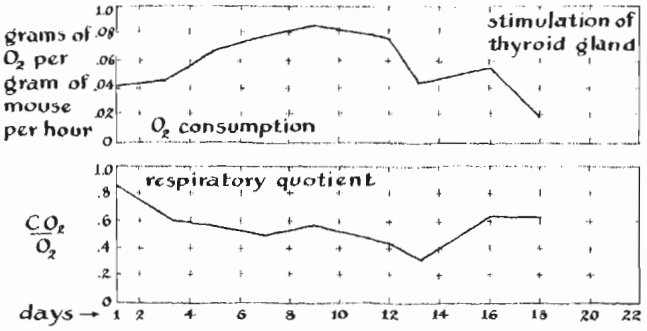
According to a doctor friend whom I consulted during the experiments, the mice in this group died of semi-starvation and extreme intoxication ending in pneumonia and shock. The oxygen consumption of the group increased sharply during the first three days of the test, dropped for two days and then climbed gradually to a peak just before the animals died. The respiratory quotient, although low, remained within normal limits almost to the end. A restricted diet with an excess of alcohol causes fat to accumulate in the liver and retard some of its functions. The results of this experiment were also plotted in graphs [*Fig. 56*].

On February 27 a group of four mice was started on a mixture of

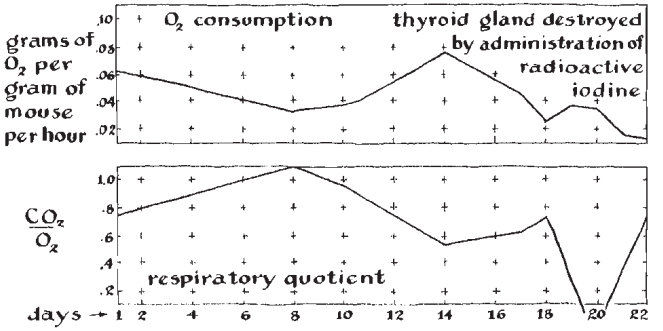


56
Graphs showing metabolic reaction of mice to alcohol

powdered rabbit pellets into which .1 per cent of desiccated thyroid gland had been mixed. Because this medication stimulates the thyroid the mice, which were permitted to eat as much as they would consume, gained weight steadily during the experiment. At one point the apparatus developed a defect and two mice suffocated. I continued with the remaining pair. Oxygen consumption appeared to drop during the final days of the experiment, but this too may have been due to a defect in the apparatus. The respiratory quotient remained below normal almost from the beginning and indicated no trend [*Fig.* 57].



57
Plot of metabolic reaction to stimulated thyroid glands in mice



58
Graph of metabolic reaction of mice to the gradual suppression of thyroid gland activity

The final group of three mice was injected with 100 microcuries of radioactive iodine (I-131) on February 26. This proved to be an overdose which destroyed the thyroid gland in about four weeks. The injections were administered in a medical laboratory, where the mice were kept for three days. Upon their return they were supplied with as much water and rabbit pellets as they would consume.

The outward appearance of the group did not change during the period of the test. Oxygen consumption fell gradually during the first eight days and then increased to about double the minimum value on the 14th day. Thereafter it dropped more or less gradually to 10 per cent of its initial value on the 22nd day. The respiratory quotient also varied widely during the experiment but showed a gradual decrease until the final day of the run, when it shot up from a near zero value to normal [*Fig.* 58]. These changes were expected because the thyroid was slowly deteriorating and its production dropped in proportion.

5

CHROMATOGRAPHY

By washing a mixture of complex substances through a glass tube packed with a powdered absorbent such as calcium carbonate, you can separate and recover the constituents. In the following experiment this analytical technique — one of the most powerful known to biochemists — is used to separate chlorophyll from spinach leaves

WITHIN THE PAST 10 YEARS thousands of amateurs have discovered the fascination of chromatography, or "adsorption analysis," as the process is sometimes called. Despite the fact that the Russian botanist Michael Tswett described the chromatographic method in 1906, it did not come into general use even among professionals until 1930. In less than two decades chromatography has opened new avenues to knowledge, created new industries, expanded old ones and made substantial contributions to the health and well-being of millions.

No description of the chromatographic method has surpassed in clarity or conciseness that originally set down by Tswett: "If a petroleum ether solution of chlorophyll is filtered through a column of an adsorbent (I use mainly calcium carbonate which is stamped firmly into a narrow glass tube), then the pigments, according to the adsorption sequence, are resolved from top to bottom into various colored zones like light rays in the spectrum, so the different components of a pigment mixture are resolved on the calcium carbonate column according to a law and can be estimated on it qualitatively and quantitatively. Such a preparation I term a chromatogram, and the corresponding method, the chromatographic method. It is self-evident that the adsorption phenomena described are not restricted to chlorophyll pigments, and one must assume

that all kinds of colored and colorless chemical compounds are subject to the same laws."

In essence chromatography requires only three pieces of apparatus: a container for holding the sample, the chromatic column and a second container for catching the spent liquid as it drips from the bottom of the tube. After the column has been packed with adsorbing material, a portion of the sample solution is poured in at the top of the tube. This is allowed to percolate down the column for perhaps a tenth to a quarter of its length. In doing so it usually forms a solid band of color characteristic of the solution under investigation. Clear solvent is then washed down the column, and the process of separation begins. Each substance has a characteristic affinity for the solvent and for the adsorbent. Chromatographers commonly refer to this property as the adsorbent's or solvent's "activity." The activity ratio determines the position a particular substance will occupy on the column relative to others in the mixture from which it is being separated. Substances most weakly held in solution and most strongly attracted to the adsorbent will adhere to the uppermost particles of the column. Those less strongly attracted to the adsorbent will be washed down farther, the distance depending upon each substance's relative adsorption ratio, and the separated substances will form a characteristic pattern of bands in the column. Extracts prepared from some green leaves, for example, show more than 20 distinct bands, ranging from dark green through various shades of orange, pink, yellow and delicate violet to white, the colors identifying the various xanthophylls, flavoxanthins, luteins, carotenes and related pigments.

The operation of washing the column with clear solvent is known as "development." As fresh solvent flows down the column, some molecules detach themselves from the adsorbent, join the solution and move down to regions of less concentration. Here they are readsorbed. The activity of both the solvent and adsorbent appears to vary with the concentration of the substance under analysis; hence a given substance may pass out of and into solution many times in the course of its journey down the column. At first the bands are narrow and bunched near the top of the column. As development continues, all the bands progress toward the bottom and grow wider

and more distinctly separated. A fully developed chromatogram displays a series of distinct, cleanly separated bands, varying in width in proportion to the amount of each substance in the mixture.

The separated, purified substances can then be extracted in one of two ways: either by washing the successive bands out of the bottom of the column with solvent, or by pushing the cylinder of adsorbent out of the tube, separating the bands with a knife and removing the substance with a solvent. If the chromatogram is sucked to dryness, it slips readily from the tube. Some workers scoop the adsorbent out of the tube one band at a time with a slender spatula.

Thousands of different adsorbents and solvents have been tried. The selection of the most effective combination for each purpose remains largely a matter of cut and try. The following lists of adsorbents and solvents, which will resolve most of the mixtures the amateur is likely to prepare, were drawn up by William T. Beaver, a student at Princeton University and winner of a Westinghouse Science Talent Search award. The adsorbents are listed in approximate order of decreasing activity; the solvents, in the reverse order:

ADSORBENTS

1. Activated alumina
2. Charcoal
3. Magnesia
4. Silica gel
5. Lime
6. Magnesium carbonate
7. Calcium carbonate
8. Sodium carbonate
9. Talc
10. Powdered sugar

SOLVENTS

1. Petroleum ether
2. Carbon tetrachloride
3. Carbon disulfide
4. Ether
5. Acetone
6. Benzene
7. Methyl or ethyl alcohol
8. Water
9. Organic acids
10. Aqueous solutions of acids or bases

Sometimes more than one solvent may be used, either in combination or successively. For example, a small amount of benzene may be mixed with the weakly active solvent petroleum ether to speed up the development of bands. Care must be exercised, how-

ever, not to make the solvent so active that it washes the bands from the column immediately. After the bands of a cylinder of adsorbent have been cut into blocks, they may be treated with a strongly active solvent for the swift and complete extraction of the principal substances. This is called elution, and the solvent or combination of solvents used for this purpose is the "eluent." Most of the common adsorbents and solvents are inexpensive; some are found in nearly every home. Beaver advises the beginner to purchase chromatographic supplies from a chemical supply house. Those found in the home are likely to be contaminated, and a minute amount of foreign matter can confuse the result. Chromatography is an extremely sensitive technique, comparable in its field with the classic knife-edge test used by amateur telescope makers.

"The very fact," says Beaver, "that there are few fixed ground rules recommends chromatography as an avocation. Not even the most advanced professional can prescribe a hard and fast procedure for setting up and operating a chromatic column. The field is so new that it is open to all comers. The amateur has a good chance of making a worthwhile contribution to the technique."

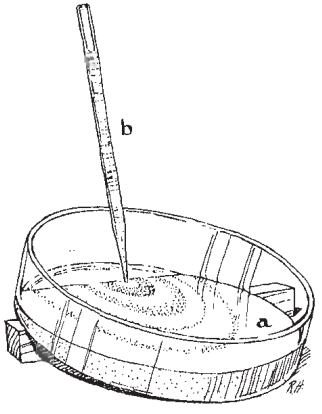
The glass column may range from a fraction of an inch to several inches in diameter, depending upon the coarseness of the adsorbent, the nature of the substance to be adsorbed, the quantity of material available and like considerations. Most workers prefer to use tubes somewhat less than an inch in diameter. Usually the column is 10 times as high as it is wide. For the separation of some isotopes, however, slender tubes 100 feet or more in length have been used. The bottom of the tube is pinched in and stoppered with a tuft of cotton or glass wool to provide support for the adsorbent. Such tubes are available through most chemical supply houses, but they may be made readily at home from glass tubing.

Most of the difficulty experienced by beginners arises from failure to pack the column uniformly. Unless the adsorbent is evenly distributed, the bands are likely to be ragged and overlap. Tswett put in dry, powdered adsorbent a little at a time, and tamped each bit firmly into place until the column reached the desired length. Subsequent experience has modified his procedure in numerous ways. After a layer is packed into place, the surface may be loos-

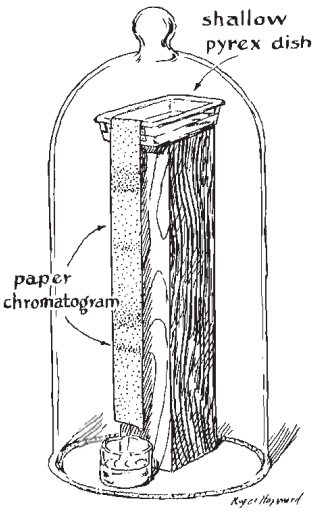
ened somewhat with a spatula so the succeeding one will join it more uniformly. Ordinary wooden dowel stock, squared at the end and slightly smaller than the inside diameter of the tubing, makes a good tamping tool. Some adsorbents settle into place satisfactorily if the tube is merely jarred while being slowly filled. Other adsorbents can be introduced in the form of a mud or paste, suction being applied simultaneously. Chromatographers agree that packing the column is an art. Like all arts, its mastery comes largely through practice.

Most workers use the standard tests that have been devised to choose appropriate solvents and adsorbents for specific jobs. One of the most popular consists in placing about a teaspoonful of adsorbent in a shallow dish, shaking it into a wedge-shaped layer on the bottom, dissolving the mixture to be tested in a weak solvent, putting a few drops of this on the thin edge of the adsorbent with a micropipette, and then trying various solvents and combinations of solvents in order of increasing activity [*see Fig. 59*].

Amateurs who get into this field will undoubtedly come sooner or later to paper chromatography, which makes the whole thing easier. The "column" in this case is a strip or sheet of paper, enclosed in a saturated atmosphere to prevent evaporation. The paper is moistened with solvent, and then a drop of the solution to be analyzed is applied to the upper edge or an upper corner of the sheet. The



59
Convenient method of testing solvents and adsorbents



60
Chromatography with paper strip
(enclosed to retard evaporation)

sheet is then bent over and dipped into a shallow dish of the solvent to be used for development. The solvent flows down the hanging sheet by capillary action, carrying the substances to be resolved with it. These adsorb as spots along the paper — the counterparts of bands in the conventional column. When development has carried the lowest spot close to the bottom, the sheet may be removed from the solvent, rotated 90 degrees and reinserted. Each spot then becomes the point of origin for a new chromatogram. If the resolved fractions are comprised of subtle mixtures, the components of each fraction will array themselves across the sheet. What you have then is a "two-dimensional" chromatogram as shown in Figure 60.

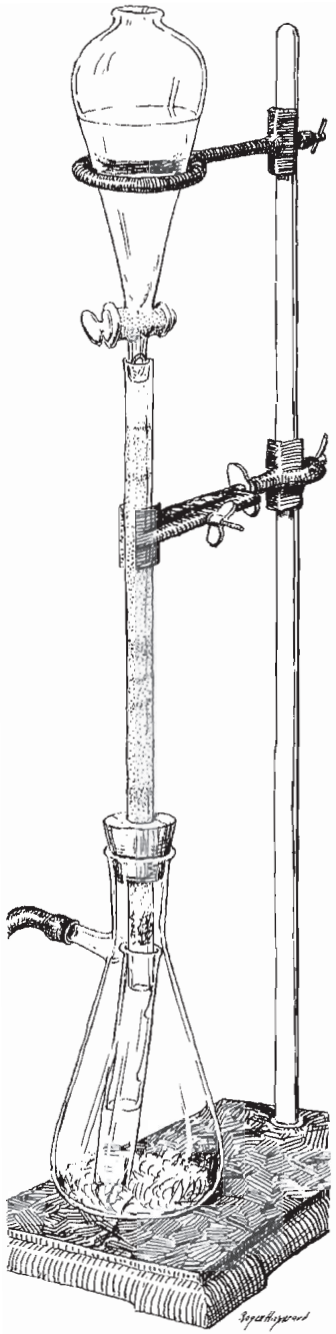
Tswett likened the bands on his column to the rays of colored light emerging from a prism in a series of colors. The two-dimensional chromatogram carries the analogy further by subjecting each "ray" to a second analysis, with increased resolution comparable with that achieved optically when physicists pass a colored light from one prism through a second. Many amateurs use the paper technique as a test method for identifying the fractions of a mixture qualitatively and follow it with a conventional column for quantitative determination.

As Tswett predicted, the chromatographic method resolves colorless fractions just as readily as colored ones. During recent years much work has been done in colorless chromatography. Many techniques have been developed to make these substances visible. The presence of amino acids, for example, is detected by spraying the extruded adsorbent, or the paper chromatogram, with ninhydrin, which turns these normally colorless substances a light purple. Other substances fluoresce under ultraviolet light. If a drop of ordinary blue-black ink is placed on a strip of chromatographic paper and developed with alcohol, several bluish bands, representing the ink's content of iron compounds and dyestuffs, form along the length of the strip. Under an ultraviolet lamp the dried paper shows many other bands, ranging in color through the reds, oranges and greens. With a second chromatogram using a known substance as a control, one may identify an unknown (but suspected) substance by comparing the positions of the respective bands on the chromatograms. In a chromatographic column colorless fractions may also be detected by their differential blending of light transmitted through the column or by polarization of the light. Recently some substances have been tagged by radioactive isotopes and detected by photographic processes, but these techniques generally lie beyond reach of the facilities commanded by the average amateur.

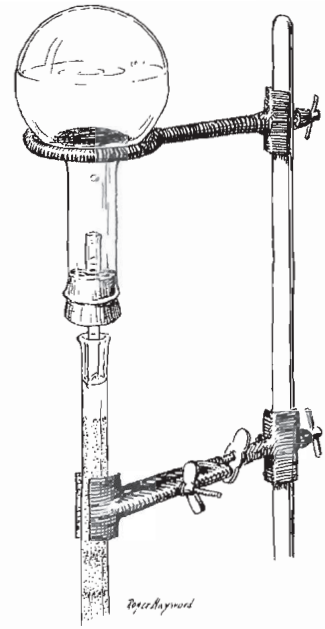
The extraction of the chlorophylls, carotenes and xanthophylls from spinach leaves can serve as a highly colorful introduction to the chromatographic technique. The following experiment, suggested by Beaver, should be made in a well ventilated room because the solvents are highly volatile and inflammable. Moreover, fumes from one, wood alcohol, are poisonous.

Into columns made of 10-millimeter glass tubing about a foot long, fire-polished at one end and flared at the other to facilitate filling, is packed the adsorbent (Merck's alumina standardized according to Brockmann, of 80 to 200 mesh). It is packed in successive small portions while jarring the tube. Suction materially reduces the development time; Roger Hayward's drawing [*Fig. 61*] shows how to set up the column for use with a vacuum flask. An alternative, omitting the vacuum provision, is shown in Figure 62.

Ten grams of dried spinach leaves are steeped in 100 milliliters



61
A vacuum chromatograph column



62
A chromatograph column arranged for gravity feed

of wood alcohol for 24 hours. The material is then filtered and the residue is washed with an additional 50 milliliters of wood alcohol. This extract is shaken with 50 milliliters of petroleum ether; 100 milliliters of water are added, and the solution is placed in a separatory funnel. After a distinct separation has taken place, the lower alcohol-water layer is discarded, and the upper petroleum ether layer, containing the extract, is filtered.

You run about half of this extract into the column of alumina and then develop the column with benzene. The first fraction to pass down the column is a fairly narrow yellow-orange band of carotene. It is followed by much wider pink and yellow bands of xanthophylls. These are familiar pigments that cause wooded countrysides to take on the colors of fall after frost has killed the chlorophyll. Fractions of these pigments may be collected as they emerge from the bottom of the column and evaporated to dryness. The two groups (carotenes and xanthophylls) may then be further resolved into their components by dissolving them in a few milliliters of petroleum ether, passing them through fresh columns and developing with benzene-petroleum ether or, for greater eluent activity, with pure benzene.

In the column the chlorophylls form a dark green band. The band is scooped from the column; the pigments are washed out with five milliliters of wood alcohol, and the solution is filtered. The filtrate is put in a separatory funnel with five cubic centimeters of petroleum ether, and five milliliters of water is added. The petroleum ether extracts the chlorophylls, and the water and alcohol form a separate layer which can be poured off. Then the petroleum ether extract is washed several times with water and run through a column packed with powdered sugar (sucrose) in the form of a slurry with petroleum ether. Now you develop the column with petroleum ether. The chlorophylls separate into two components — a yellow-dark-green band of beta-chlorophyll near the top of the column and a bluish-green band of alpha-chlorophyll farther down.

Because of its vital role in photosynthesis, chlorophyll has become the glamor plant-pigment in popular imagination. But many amateur chromatographers find the carotenes just as interesting. Unlike the chlorophylls, which act as catalysts, the carotenes play a

direct chemical role, both in animals and plants. They appear to be essential to the body's manufacture of vitamin A, and they play a part in the mechanisms of vision and sex. As the name implies they may be extracted from carrots.

To extract carotene you grind five grams of dried carrot root to dust in a mortar and then add 50 milliliters of a mixture of equal parts of wood alcohol and petroleum ether. Shake the mixture thoroughly, add five milliliters of water and pour into a separatory funnel. The carotenes, plus xanthophyll esters, are concentrated in the petroleum ether layer that forms at the top. Separate this layer and concentrate it by evaporating some of the fluid, leaving 20 milliliters. Then add three milliliters of a solution of 5 per cent sodium hydroxide in wood alcohol, which saponifies the xanthophyll esters so they can be removed by washing. Wash the mixture several times with 85 per cent wood alcohol in water; then wash several times with pure water to remove traces of wood alcohol.

Now let the petroleum ether separate from the water and then filter it. The yellow-orange solution that remains bears the complex of carotenes. To separate them, pass about half of the solution into a column of alumina and develop the column with a mixture of benzene and petroleum ether in the ratio of 1 to 3. You will get three well-defined bands, containing, from the top down, gamma carotene, beta carotene and alpha carotene. You can recover the pigments either by washing them successively out of the column or by extracting them from the separate bands of adsorbent with wood alcohol.

Chromatography is a far more subtle method of separation than the traditional chemical techniques of distillation, precipitation with reagents, and crystallization. Fortunately for amateurs, it is also a method of beautiful simplicity.

6

ZONE ELECTROPHORESIS

When a solution of chemical compounds is applied to an electrified sheet of porous paper, each compound migrates in the electrical field at a rate and in a direction determined by its own characteristic charge. Amateurs can use this phenomenon for analyzing chemical mixtures of extreme subtlety. The construction of a simple apparatus and its use are described

WITHIN RECENT YEARS biochemists and others have acquired a powerful new tool for separating intimately related compounds: the electrophoresis apparatus. The word *electrophoresis* means "borne by electricity." In a broad sense the movement of charged pith balls in an electrostatic field is an electrophoretic effect. So is the Cottrell process for eliminating smoke particles from flue gases by passing them between electrodes of high potential difference. After picking up a charge from one electrode, the particles are attracted to the other, where they clump and fall into a collecting bin. The electrode position of colloidal rubber suspensions on electrodes of special shape — a process widely used in the manufacture of rubber gloves and other common articles — is another example.

But electrophoresis is the special name given to the technique of separating molecular mixtures into fractions. Most suspensions of molecules in water are charged and hence can conduct an electric current. Even molecules which normally do not carry a charge tend to adsorb ions from the water. Some molecules pick up more charge than others, depending upon their chemical nature and the concentration of ions in the solution.

If the ionic concentration (pH) is properly adjusted all closely related molecules, such as those of the proteins, appear to adsorb charges of the same sign. Consequently when they are subjected to

an electric field they migrate in the same direction, although at rates which vary with the amount of charge on each member of the family. Many amateur microscopists have observed such migration on a gross scale with objects such as blood cells or protozoa. If a voltage is applied across a drop containing cells in suspension, the cells will migrate. Alexander Reuss first described the experiment 148 years ago, and it was a favorite of Michael Faraday.

To analyze molecular mixtures, the Swedish chemist Arne Tiselius hit upon a radically different scheme. He poured the material to be studied into the bottom of a U-shaped tube and carefully laid a buffered solution on top in each arm of the U so that sharp boundaries formed between the mixture and buffer. When a current was passed through the three-part solution, the material under analysis migrated down one arm of the U and up the other. Each of its fractions moved at a characteristic rate. The boundaries of each fraction were made visible by an elaboration of the schlieren optical technique devised by Léon Foucault for testing the figure of parabolic mirrors and lenses. Like the ruling engine for making diffraction gratings, the Tiselius technique of "free" electrophoresis is simple in principle. Like the ruling engine, too, the method appears easy until you set up the apparatus and try to make it work! In this domain the gifted professional appears safe from amateur challenge.

A less precise yet powerful method of zone electrophoresis has found wide application during the past ten years and the following description of it has been made possible through the cooperation of H. G. Kunkle of the Rockefeller Institute. In the zone method, particles move in liquid that fills the spaces of a finely divided solid instead of a U-tube. Molecules of like kind migrate as distinct zones which can easily be identified and recovered as purified products. Porous solids of many kinds can serve as the medium. One medium frequently used is filter paper. Zone electrophoresis thus bears a superficial resemblance to partition chromatography. The electrophoretic separation, however, depends not upon the properties of solubility and adsorption, as in the case of chromatography, but upon the electrical charge carried by the molecules of the substance that is being analyzed.

The amount and sign of the charge picked up by compounds in

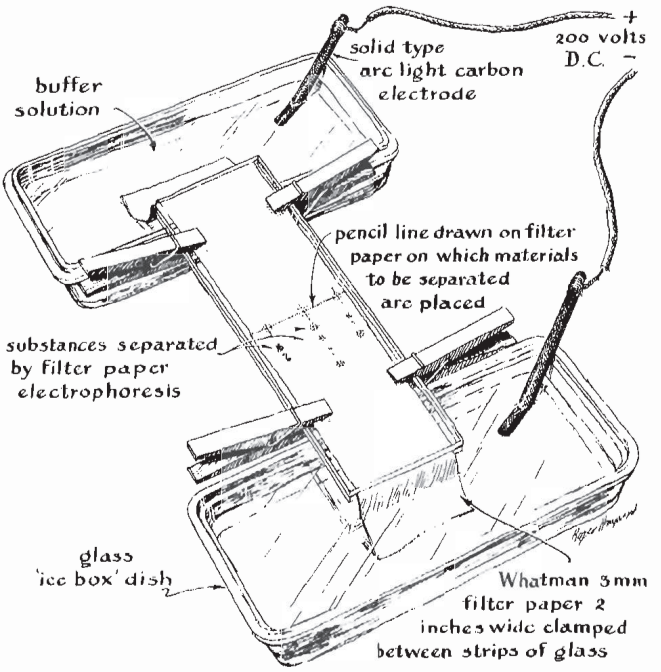
solution depend both upon the chemical nature of the compound and upon the pH, or acid-alkaline balance, of the solvent. Molecules which normally carry a weak charge, such as the slightly alkaline proteins, are highly sensitive to changes in pH. A small shift in acidity or alkalinity can cause a substantial change in the rate at which such particles migrate and may even reverse the direction of their movement. One therefore selects for the solution electrolytes (sources of charge) which have a "buffering" action: that is, which tend to supply positive and negative ions to the solution at a rate precisely offsetting that at which ions are removed or dissipated. Many common salts have a strong buffering action, although table salt (sodium chloride) is not one of them.

If you take up electrophoresis you will have to find your own way through woods where few trees are blazed. Except for protein chemistry, you must develop your own electrolytes, buffers and solid media, and must find out by experiment just what voltages and current densities work best for the substance under analysis. The field of electrophoresis has barely been scratched. If you enjoy original work, you can dig in almost anywhere, certain that you are breaking fresh ground.

To give you a start, the essentials of an apparatus which uses filter paper as the solid medium are illustrated in Figure 63. You can set it up and put it into operation in a single evening.

The ends of the paper dip into two vessels containing an electrolytic solution connected through carbon electrodes to a source of direct current. To retard evaporation of the solution the paper is sandwiched between a pair of glass plates. The plates, about two inches wide and eight inches long, are cut from quarter-inch plate glass. As a safety precaution it is a good idea to round the edges and corners of the glass on either a whetstone (using water as a lubricant) or on a sheet of glass smeared with a slurry of carborundum.

It is desirable to maintain an even pressure of the glass on the filter paper, so that migration proceeds in a symmetrical and reproducible pattern. Pressure improves the sharpness of the zones, because it reduces the amount of fluid in the paper. However, if the pressure is too high, it will bend the glass and distort the zones. Some workers have attempted to solve the problem by using plates



63
A simple setup for experimenting with zone electrophoresis

an inch or more thick. The bottom plate is supported by a flat base and the top one rests on the paper as a weight. Others suspend the paper from glass rods laid across the buffer vessels. The apparatus is then covered by a bell jar and operated in a buffer-saturated atmosphere. The latter method has the disadvantage that the buffer tends to gravitate toward the low point of the strip with consequent distortion of the pattern of separation.

Capillary effects between the glass plates and the paper also introduce some distortion. This is minimized by coating the plates with a thin film of grease. Vaseline will work, but not so well as silicone grease of the type used for lubricating the stopcocks of chemical glassware.

Glass containers of any convenient shape can be used as buffer

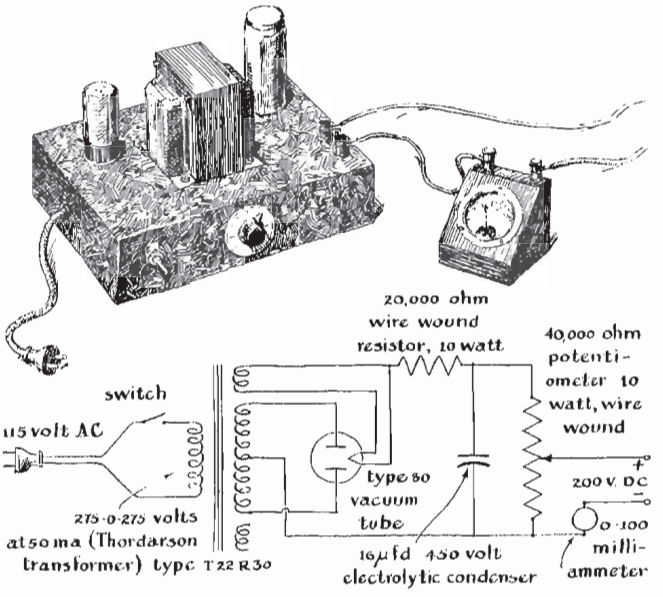
vessels. Heavy Pyrex icebox dishes, available from hardware dealers, work as well as specially made glassware. The principal considerations in the selection of containers are chemical inertness and enough weight so the empty vessels will support the plates, paper strip and clamps without upsetting.

Chemical inertness is a major consideration in the choice of electrodes. Most professionals use platinum, but carbon rods will work. Avoid the cored carbons used in sun lamps. These cores are charged with finely divided metal (to enrich the emission of ultraviolet rays) and will contaminate the solution. Carbon electrodes from dry cell batteries are ruled out for the same reason. Solid carbons designed for low-intensity motion-picture projectors are good and can be procured from theater-supply dealers.

The amount of electric current needed varies with the substance under analysis. A rectifier capable of operating between 50 and 300 volts at an output of 20 milliamperes will be ample for most work. You may get a good rectifier from a junked radio receiver. Just connect a 40,000-ohm wire-wound resistor (of the type fitted with an adjustable tap) across the filter condenser. The resistor should be of at least the 10-watt size. Take the output from across the ground side of the resistor and the tap. If no old radio set is at hand, you can get the parts specified in the drawing [*Fig. 64*] from radio-supply dealers.

It is frequently desirable, particularly during the experimental phase of analyzing unknown substances, to maintain either a constant voltage across the paper strip or a constant current through it. Power supplies with automatic regulating features can be constructed, but they are costly and complex. Good results can be achieved with a manual control. Substituting a continuously variable potentiometer for the tapped resistor makes adjustment easy, and the knob will protect your fingers from the hot resistance element. If the current is limited to 15 milliamperes or less the rating of the potentiometer need not exceed 10 watts.

Almost any soft paper will demonstrate zone electrophoresis. You can use strips cut from white blotters, paper towels, cleansing tissues, even the unprinted parts of old newspapers. Clear, reproducible patterns, however, require a specially made paper of uniform



64
Power supply for zone electrophoresis apparatus

texture and free of contamination. A good paper is Whatman 3MM, supplied in 600-foot rolls by the Fisher Scientific Company of New York City, which also has most of the chemicals used in electrophoresis experiments. You can order the Fisher materials at drug stores.

The separation of the artificial coloring used in a cheap wine is a nice electrophoretic project for a beginner. You can make your own mixture for analysis by adding a few drops of food coloring to grape juice. For an electrolyte you can use a weak solution of common salt buffered with a small amount of baking soda (sodium bicarbonate). Later you can investigate electrolytes made with other salts, many of which provide their own buffering action.

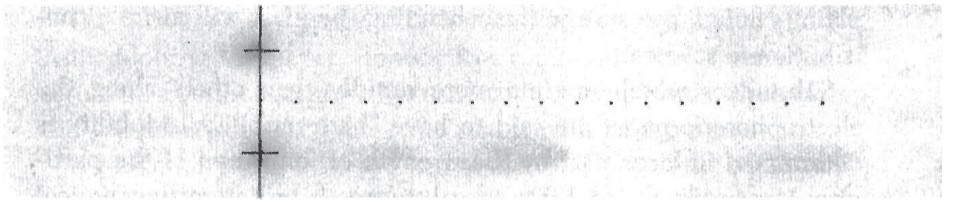
Food coloring migrates nicely in an electric field of 25 volts per inch at a current of 10 milliamperes. This means that the buffer-moistened filter paper should have a resistance of about 2,500 ohms per inch. To obtain this value of resistance you will have to experi-

ment with various dilutions of the electrolyte. Begin by drawing enough tap water to fill the icebox dishes to within half an inch of the top. Put all this water in one container and add a level teaspoon of salt. After it dissolves, immerse the paper strip in the solution. Remove the strip, blot it thoroughly and clamp it between the glass plates. Then pour the solution into the icebox dishes, suspend the ends of the paper in it, connect the power supply to the solution through the carbon electrodes and adjust the potentiometer or tapped resistor to the prescribed potential of 200 volts.

If the resulting current is less than 10 milliamperes, turn off the power, remove the strip, return the solution to the common container, add more salt and try again. Usually a level teaspoon of salt for each 12 ounces of water produces the desired conductivity, but the amount needed varies with the purity of the tap water. Finally add a quarter teaspoon of baking soda for each 12 ounces of solution. (It will affect the resistance only slightly.)

After you have an electrolyte with the proper resistance, draw a light pencil line across the middle of a fresh strip of filter paper, dip the strip into the buffered electrolyte, blot it and then apply a very small drop of wine to the pencil line with the blunt end of a toothpick. The wine should first be concentrated by letting it evaporate at room temperature to half or less of its normal volume. Now spread a film of grease on the inner faces of the glass plates. Clamp the paper between them, seal the edges of the plates with grease, immerse the protruding ends of the paper into the buffer and switch on the power.

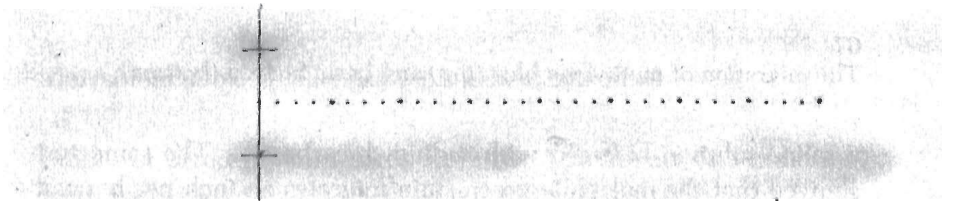
If the wine sample contains artificial coloring, in about five minutes the edge of the wine spot nearest the anode should become sharper and the edge toward the cathode should grow fuzzier. Within an hour a blotch of dye, probably comet-shaped, will have migrated a substantial distance from the point of origin. As the process continues, comets of other colors, each a constituent of the dye, will trail the first one down the length of the paper as shown in Figures 65 and 66. The dye fractions in the wine should be fully resolved in about six hours. (The blotch made by the wine itself will move little, if at all.) By spacing drops along the pencil line you



65
Electrophoresis separations of imported Chianti wine *(top)* and an inexpensive domestic Chianti to which coloring has been added *(bottom)*

can analyze several samples of fluid simultaneously on the same strip of paper.

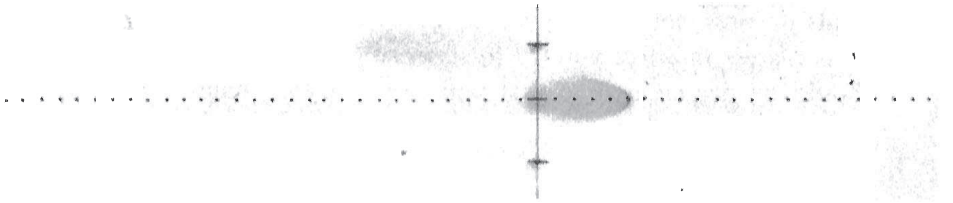
The tendency of zones to smear, trail, assume comet shapes and otherwise depart from sharpness is one of the undesirable features of zone electrophoresis on filter paper. It represents a challenge to the experimenter. In general the drier you can run the filter paper (or other solid medium), the sharper the zones will be. Within limits dryness can be achieved by applying heavy pressure on the glass plates: in effect you try to squeeze out the buffer. The spots should be dry enough so that you can rub your hand across the



66
Electrophoretic analysis of pure grape juice *(top)* and grape juice colored with vegetable dyes *(bottom)*. Note migration of dyes

paper as it comes from the apparatus at the end of a run without smearing the pattern. The amateur who resolves the dilemma of applying enough pressure without bending the glass will make a contribution to science.

Substances which migrate more rapidly than others along the electrophoretic paper are said to have "high mobility." Mobility is determined in large part by the strength of ionization of the particles. Measuring the mobility of substances is an interesting project for beginners. You simply time the rate of migration of each substance along a scale ruled on the strip of paper, using a control buffer of a certain pH and concentration. Stains used for coloring organisms to show them under the microscope make nice test specimens. A particularly good series is eosin Y, methylene blue, basic fuchsin, malachite green, Bismarck brown, safranine and gentian violet. The chemical properties of these stains are listed in reference texts. Each migrates in a saline solution at a characteristic rate. Figure 67 shows the relative migration rates of positively ionized methylene blue (*top*) and basic fuchsin (*bottom*), and negatively ionized eosin Y (*middle*). These were resolved on filter paper with



67
The migration of methylene blue *(top)* and basic fuchsin *(bottom)*

a saline solution buffered with sodium bicarbonate. The same test showed that the malachite green stain migrates an inch per hour at 70 degrees Fahrenheit under 200 volts and 10 milliamperes.

Amateurs who wish to have a go at something more sophisticated may enjoy trying to separate blood proteins. This entails the sacrifice of a few drops of blood. You will also need access to a centri-

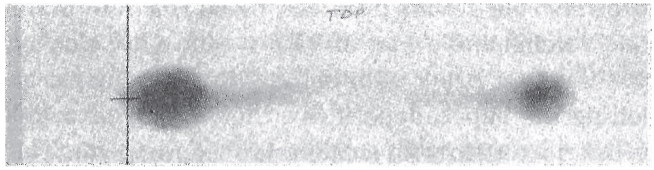
fuge (to extract the serum from the blood), a few grams of the barbiturate veronal and a liter of 95 per cent ethyl alcohol.

Dampen the filter paper with barbital buffer adjusted to pH 8.6. After blotting the paper, deposit five thousandths of a milliliter of serum on the ruled strip with a calibrated micropipette. Then clamp the paper between the plates and seal it with silicone grease.

A potential of 15 volts per centimeter and a current of 15 milliamperes will resolve a specimen in five or six hours; however, the pattern may show traces of smearing. Four volts per centimeter and four milliamperes increases the time to 12 hours but yields sharper patterns. Blood-serum fractions are difficult to see. The albumin can be made more strikingly visible by labeling it with a few crystals of bromphenol blue. After the albumin has migrated an arbitrary distance, say seven centimeters, the paper is removed and dipped for two minutes into a solution of 95 per cent ethyl alcohol saturated with mercuric chloride, to which 1 per cent bromphenol blue is added. The strip will emerge from the stain a deep yellow. It is then washed repeatedly in water containing a thousandth part of acetic acid. On contact with water, the yellow changes to a deep blue. The color gradually disappears from the paper during washing but is retained by the protein fractions. Figures 68 and 69 show typical sepa-

68
The pattern of blood proteins from a normal individual

rations of blood proteins taken from two individuals, one in normal health and the other diseased. The density of the spots in each pattern indicates the amount of protein in each fraction. From right to left Figure 68 (normal serum) shows albumin, alpha-one globulin, alpha-two globulin, beta globulin and gamma globulin. The dense spot at the left in Figure 69 is characteristic of the bone disease



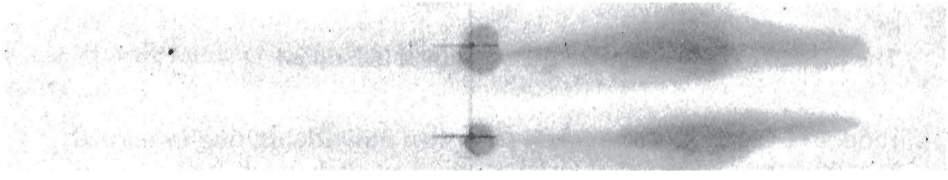
69
The pattern of blood proteins from an individual suffering from myeloma

myeloma. Other diseases produce characteristic patterns which serve as valuable aids in diagnosis.

The result of an amateur's first attempt to fractionate albumin (the white of chicken egg) is shown in Figure 70. The smeared pattern explains this experimenter's passion for anonymity. Here the buffer was salt, baking soda and water.

A number of techniques have been devised for making quantitative measurements of protein patterns. In one the strip is sectioned into eighth-inch segments. The dye in each is then quantitatively eluted in a two milliliter solution of 1 per cent N-sodium hydroxide and read, after an hour or so, on a colorimeter. The resulting values are plotted as points. The smooth curve drawn through them is equivalent to the curve derived by free electrophoresis.

As mentioned earlier, zone electrophoresis is not limited to filter paper. It is interesting to compare the behavior of a given test substance and buffer in media compounded of starch grains, silica gel,



70
An amateur's first attempt to fractionate the white of an egg

activated alumina and similar materials, as well as the reaction of various buffers with respect to a given medium. A slab of starch, for example, is easy to prepare. Put a pound of potato starch into a sieve lined with filter paper. Wash the starch for 30 minutes and pour it as a batter into a rectangular mold. The slab (about ⅜ inch thick) is then thoroughly blotted, and with suitable carbon electrodes it can be used in principle just like filter paper. The only limit to variations in the physical arrangement of the apparatus is set by the ingenuity of the worker. It is possible, for example, to adapt electrophoresis for the continuous separation of material in gross amounts. At least one amateur telescope maker prepares colloidal rouge by means of continuous electrophoretic separation. Buffer is allowed to flow down a wide strip of filter paper by capillary attraction. It drips from the bottom edge of the paper into a container below. The rouge mixture is fed onto the paper from a continuously flowing micropipette near the top. Electrical contact is made with the edges of the strip through wicks saturated by buffer. Fractions not ionized flow down the strip vertically. Ionized fractions take a diagonal course, the steepness of which depends upon the strength of the ionization. A scallop is cut into the bottom of the strip in line with each fraction, and collecting vessels are placed beneath the points.