Biosynthesis of Human Growth Hormone and Prolactin

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ABSTRACT. Human pituitary glands were incubated with ^{3}H-1,4,5-leucine in Krebs-Ringer bicarbonate buffer to study the biosynthesis and secretion of human pituitary proteins generally and growth hormone specifically. After 4 and 24 hr, anti-HGH serum precipitated only 3 and 13%, respectively, of the ^{3}H-proteins in the medium. Following gel filtration of the medium on Sephadex, a single radioactive protein peak emerged with an elution volume which was not quite coincident with the elution volume of HGH and the protein peak. FSH, LH and TSH were present in earlier fractions and ACTH in later fractions. The total amount of all 4 hormones in the media was a thousandfold less than the amount of HGH in the media. Anti-HGH serum precipitated less than 5% of the ^{3}H-proteins in the tubes which contained HGH, yet the latter accounted for more than 80% of the proteins in these fractions. Hence, the specific activity of the remaining proteins present in these fractions was very much greater than that of HGH. In the tissue, anti-HGH serum precipitated ^{3}H-proteins of large molecular weight (mol wt greater than 100,000) and also ^{3}H-proteins which have the same elution volume as the HGH standard. Upon acrylamide gel electrophoresis the Rf of the major radioactive protein was 0.55 compared to an Rf of 0.6 for HGH and FSH. When rat anterior pituitary glands are incubated in a similar manner, the principal radioactive protein released into the medium is rat prolactin. We suggest that the principal radioactive protein secreted into the medium *in vitro* by human pituitary glands is also human prolactin, but the chemical amount present is extremely small relative to HGH. (*J Clin Endocr* **31:** 611, 1970)

WHEN rat anterior pituitary glands are incubated with radioactive amino acids, the principal radioactive protein synthesized and released into the medium during a short incubation period is prolactin (1, 2). Similar studies using primate pituitary glands have not been reported although the amount of immunochemical growth hormone and biologically active prolactin which is released into the medium by human fetal (3, 4) and monkey pituitary glands (5) has been measured. Brauman *et al.* (4) suggested that there is a preferential release of prolactin into the culture medium when human fetal pituitaries are incubated *in vitro* for a period of days, but Solomon *et al.* (5) were unable to confirm these observations. Nicoll *et al.* (6) reported that pituitaries obtained from female monkeys secreted much more prolactin into the media than males, whereas the amount of growth hormone secreted by both is the same. We have incubated human and rat anterior pituitary glands *in vitro* and compared the incorporation of ^{3}H-leucine into proteins and their release of radioactive proteins into the medium, and observed that, as in the rat pituitary, one principal radioactive protein is released into the medium by primate pituitaries. This protein is very similar to HGH in molecular weight and electrophoretic mobility, but fails to cross-react with antiserum to HGH. We suggest that this radioactive protein may be primate prolactin, but concur with Nicoll *et al.* (6) and Apostalakis (7) that ultimate proof that prolactin is a discrete entity will require its isolation and chemical and biological characterization. The availability of a ^{3}H-labeled protein, which may be prolactin, should facilitate the isolation of adequate quantities of this substance from pituitary powder to establish unequivocally whether or not it is prolactin.

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Materials and Methods

Antigens. HGH Raben (8) or human placental lactogen (HPL) Friesen (9) were used as antigens.

Antisera. Antiserum to HPL (anti-HPL) or antiserum to HGH (anti-HGH) for precipita-

tion studies was obtained following the immunization of albino rabbits. A crude gamma globulin fraction was obtained by precipitation with 40% ammonium sulfate. The precipitate was washed, redissolved in 0.1M ammonium bicarbonate, the proteins were separated upon Sephadex G-100, and the fractions with antibody binding activity were pooled.

Incubation of pituitary tissue. Sprague-Dawley rats, weighing approximately 180–200 g, were sacrificed by decapitation. The pituitary gland was removed, and the posterior pituitary body was dissected from the anterior pituitary gland, which was divided into small fragments and incubated in Krebs-Ringer bicarbonate buffer (KRB).

Fresh human anterior pituitary glands were obtained through the courtesy of the Department of Neurosurgery of the Notre Dame Hospital. The pituitary gland was removed intact through a transsphenoidal approach. Any capsular tissue which was attached to the pituitary body was rapidly dissected away from the gland, which was immediately divided into small fragments and placed into 5 ml of Krebs-Ringer bicarbonate buffer containing 10 mg of glucose, 200 μg of streptomycin sulfate, 200 units of sodium penicillin G and 250 μCi of ^{3}H-1,4,5-leucine (specific activity 5.5 Ci/mmole, New England Nuclear Corporation). The incubation was carried out in a Dubnoff incubator shaking at 80 cycles/min at 37 C in an atmosphere of 95% oxygen, 5% CO_2. The incubation medium was replaced with fresh medium at specified intervals.

Following the termination of each incubation period, 0.2 ml of a saturated solution of *l*-leucine was added to the medium, which was centrifuged immediately for 10 min at 3000 rpm at 4 C, and the supernatant was stored at −20 C. The tissue fragments were washed twice with cold KRB buffer and weighed and homogenized in 1–2 ml of 0.1M ammonium bicarbonate with 0.2 ml of cold *l*-leucine in a glass homogenizer.

Protein concentration. Protein concentration of aliquots was determined by the method of Lowry (10) or in an Aminco-Bowman Spectrofluorometer with excitation at 280 mμ and emission at 350 mμ using HPL or HGH as a standard (11).

Radioimmunoassay of pituitary hormones. ^{131}I-HGH was prepared according to the method of Hunter and Greenwood (12). HGH was measured by the double antibody method of Beck, Parker and Daughaday (13) using guinea pig antiserum to HGH and rabbit antiserum to guinea pig gamma globulin. HGH used as the standard was obtained from the Endocrinology Study Section, NIH, lot no. HS1216C with a potency of 1.45 U/mg. Assays of FSH, LH(14), TSH (15) and ACTH were performed through the courtesy of Drs. C. Faiman, S. Salisbury-Murphy and E. McGarry, respectively.

Incorporation of ^{3}H-leucine into proteins. Aliquots of medium and tissue were precipitated with an equal volume of 10% TCA and washed 3 times with 1 ml of 10% TCA. The precipitate was dissolved in 0.1 ml of hydroxide of hyamine (1 molar in methanol, Packard Instrument Company). The radioactivity in the solution was counted in 15 ml of Bray's solution (16) in a Tri-Carb Liquid Scintillation Spectrophotometer.

Radioactive proteins precipitated by antiserum to HGH. Optimal conditions for precipitating HGH with antisera were established by methods described earlier for the precipitation of HPL (17). The same precautions were observed to minimize nonspecific adsorption of radioactive proteins, and appropriate corrections for this were made. The amount of antisera added to each aliquot was sufficient to precipitate 80–90% of HGH-^{131}I in the presence of 0.5–5.0 μg of HGH added as carrier. When aliquots of medium or tissue were precipitated with antiserum the concentration of HGH was brought into this range. Antiserum to HGH and HPL were used interchangeably after it had been established that the amount of radioactive proteins precipitated by each was identical.

Gel filtration on Sephadex G-100. The tissue extract and the incubation medium were fractionated separately on columns of Sephadex G-100 (3×60 cm) at room temperature, with 0.1M ammonium bicarbonate as buffer. The fractions which were collected were stored at 4 C for subsequent analysis. The buffer contained 1% *n*-butanol as a preservative. Aliquots of the fractions were analyzed for proteins, HGH, ^{3}H-proteins and ^{3}H-HGH.

Acrylamide gel electrophoresis. Proteins in the supernatant and medium were separated upon acrylamide gel according to the method of Davis (18). The gels were stained with Amido Schwartz or divided into segments. Each unstained segment was eluted in 0.5–1.0 ml ammonium bicarbonate buffer for 24–48 hr. Aliquots were removed for the determination

of 1) proteins, 2) HGH, 3) total radioactive ^{3}H-proteins and 4) radioactive proteins precipitated by anti-HGH. The distribution of radioactive proteins was also determined after starch gel electrophoresis of the proteins according to the method of Suwa and Friesen (11).

Results

Table 1 shows the distribution of ^{3}H-proteins in the medium and tissue after incubating pituitaries from male and female rats. The total incorporation of ^{3}H-leucine by pituitaries from female and male rats was comparable, but at both 4 and 24 hours approximately 70% more radioactive proteins were present in the media of the females. Fig. 1 shows the distribution of radioactive proteins after gel filtration on Sephadex G-100 of the medium after incubating pituitaries from female rats for four hours. A single large radioactive protein peak was noted with a Ve/Vo of 1.75 and tubes 33–37 contained 74.5% of the radioactive proteins in the medium.

TABLE 1. Incorporation of ^{3}H-leucine into proteins by rat pituitary glands (TCA precipitate cpm/mg*)

Sex	Media		Tissue 24 hr	Total
	4 hr	24 hr		
Male	23,879	111,099	279,664	414,642
Female	40,309	195,693	186,244	422,246

* cpm/mg (wet weight of tissue).

Aliquots of tubes 33–36 were lyophilized, redissolved in the gel buffer, and the proteins were separated upon acrylamide gel electrophoresis and the distribution of radioactive proteins was compared with the distribution of proteins in a homogenate of female pituitary glands. Fig. 2 shows that, apart from the radioactive proteins localized in the first segment, a single large peak of radioactive protein was found in segment

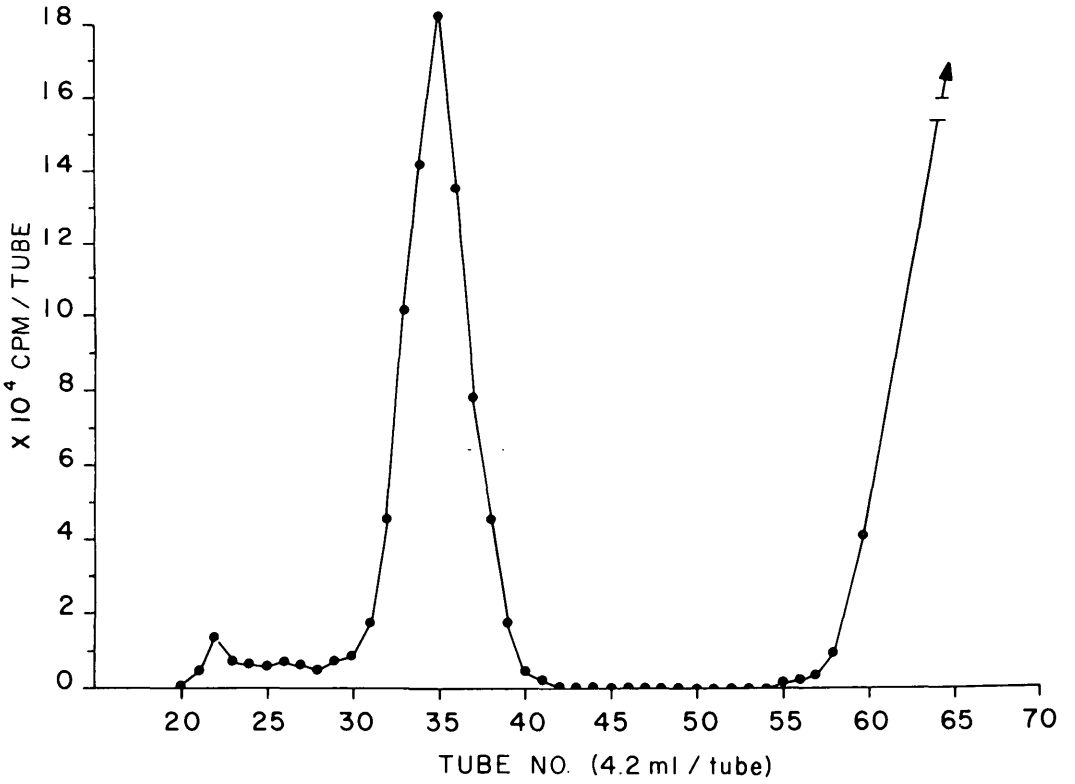


FIG. 1. Distribution of radioactivity after gel filtration of incubation medium (4 hr) from female rat pituitaries on Sephadex G-100. Tubes 33–37 contain over 75% of the radioactive proteins applied to the column. Free ^{3}H-1,4,5-leucine appears after tube 60.

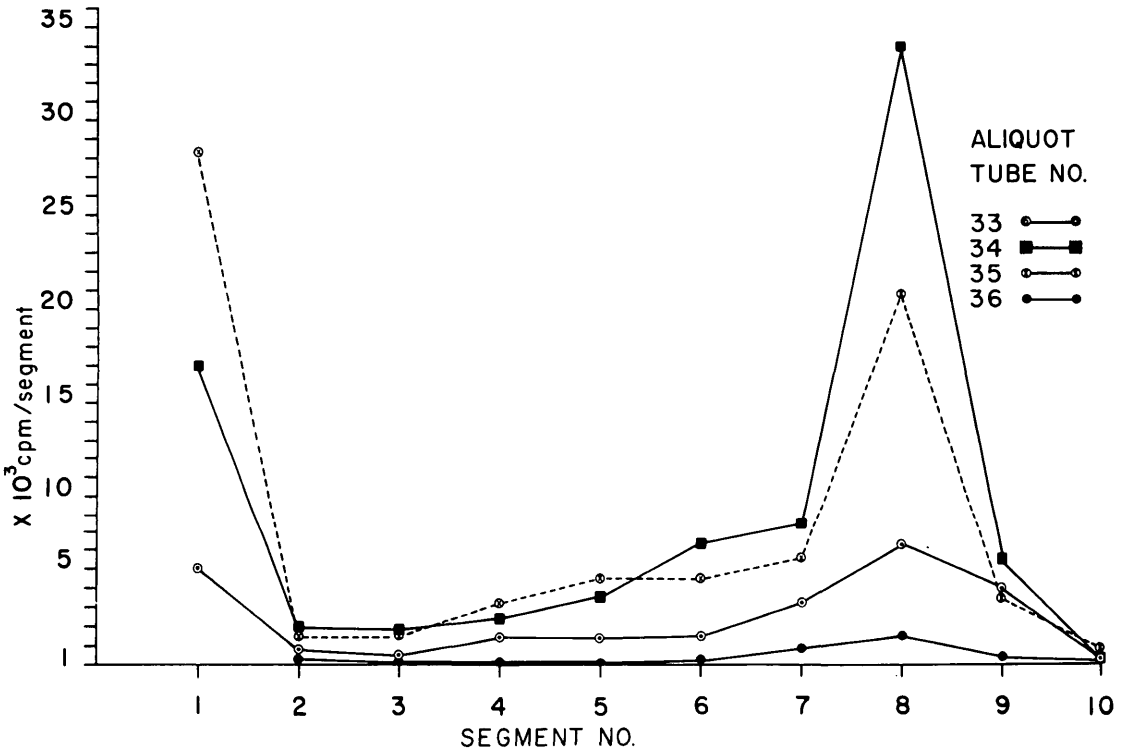


FIG. 2. Distribution of radioactive proteins after acrylamide gel electrophoresis of aliquots of tubes 33–36 from Fig. 1. Segment 8 contains the band identified by Jones *et al.* (16) as prolactin. Segment 3 contains growth hormone. Segment 10 is the most anodal segment.

8, which is the segment that contains the prolactin band (19), whereas rat growth hormone is found in segment 3.

Similar studies were carried out following the incubation of human pituitary tissue. Table 2 summarizes the incorporation of ^{3}H-leucine into proteins and HGH in medium and tissue in five experiments. Four hours after incubation only 3.2% of the radioactive proteins present in the medium were precipitated by antiserum to HGH. A gradual increase in the proportion of the

TABLE 2. Incorporation of ^{3}H-leucine into proteins and HGH by human pituitary glands

Exp no.	Pit. wt, mg	Media						Tissue	
		4 Hours		24 Hours		48 Hours			
		^{3}H-proteins cpm/mg*	^{3}H-HGH cpm/mg	^{3}H-proteins cpm/mg	^{3}H-HGH cpm/mg	^{3}H-proteins cpm/mg	^{3}H-HGH cpm/mg	^{3}H-proteins cpm/mg	^{3}H-HGH cpm/mg
I	540.2	8,366	402	34,364	3450			25,893	14,594
II	207.8	10,767	284	39,699	4029			260,577	40,521
III	218.8	6,786		47,469	5717	39,446	9019	314,243	45,329
IV	209.2	3,678	48	17,191	3164	31,605	4064	258,881	144,714
V	239.4	9,139	372	55,890	8186	33,707	5384	432,361	55,469
Mean		7,747	277	38,923	4910	34,920	6156	258,400	60,125
±SD		4,171	139	13,195	1862	3,493	2095	132,250	43,241
% ^{3}H-HGH/ ^{3}H-proteins		Mean ±SD	3.2±1.2	13.0±3.2		17.2±4.1		31±20.6	

* cpm/mg (wet weight of tissue).

TABLE 3. Concentration and specific activity of proteins and HGH in the incubation medium

Media										
	4 Hours					24 Hours				
Exp no.	Prot* μg/mg†	HGH μg/mg	% HGH / Prot	SA‡ Prot cpm/μg	SA HGH cpm/μg	Prot μg/mg	HGH μg/mg	% HGH / Prot	SA Prot cpm/μg	SA HGH cpm/μg
I	24	10	42	349	40		8.7			396
II	59	11	18.6	183	26	19.0	6.9	36.3	2089	584
IV	11	9	82	334	5	9	5.7	60.6	1829	555
V	21	15	71	435	25	11	8.0	73.4	5128	1023
Mean	29	11	53	325	24	13	7.3	57	3016	640
±SD	18.1	2.3	25	92	13	4	1.1	15	1497	230

* μg/mg (wet weight of tissue).
† Prot = protein.
‡ SA = specific activity.

radioactive proteins precipitated with anti-HGH serum was noted with time. At 24 and 48 hours a mean of 13 and 17.0% of the radioactive proteins in the medium was precipitated by anti-HGH, while in the tissue 31% was precipitated. There was considerably more variation in the proportion of ^{3}H-proteins precipitated by antiserum in the tissue compared with the medium.

In Table 3 the protein and HGH contents of medium at different periods of incubation are shown along with the calculation of the specific activity of proteins and HGH. The results of experiment III are not outlined because the medium was added to acetone-dried pituitary powder for subsequent extraction of HGH according to the method of Raben (8). HGH comprised approximately 50% of all the protein present in the media at 4 and 24 hours (Table 3). It is apparent that the average specific activity of total proteins in the medium is much greater than that of HGH at both 4 and 24 hours.

The proteins in the incubation media and tissue extracts of human pituitaries were separated by gel filtration on Sephadex G-100 using 0.1M ammonium bicarbonate buffer. Fig. 3 shows the distribution of proteins, HGH and radioactive proteins present in the medium after incubating human pituitary fragments for four hours. A single large peak of radioactive proteins emerged (tubes 35–45) which accounted for 70% of all the radioactive proteins present in the medium at four hours. The ^{3}H-leucine which was not incorporated emerged subsequently in tubes 55–70. Only a very small proportion (2%) of the radioactive proteins in tubes 35–45 was precipitated with anti-HGH serum. Anti-HPL precipitated the same amount of radioactive protein and failed to precipitate any additional radioactive protein in aliquots of the same tubes. Moreover, no additional protein was precipitated when more anti-HGH serum was added to the supernatant. The peak concentration of HGH by immunoassay (tube 42) was not coincident with the maximum concentration of radioactive proteins which was found in tube 41.

Fig. 4 indicates the distribution of FSH, LH, TSH, HGH, ACTH and radioactive proteins in the same column shown in Fig. 3. The three glycoprotein hormones were eluted before (Ve/Vo = 1.5–1.6), and ACTH appeared after tube 47. The elution of HGH (Ve/Vo = 2.0) paralleled the elution of one principal radioactive protein peak very closely, but the peaks of each differed by one tube. The amount of HGH present was approximately 1000 times the weight of any other pituitary hormone. HGH accounted for at least 75% of the proteins present in tubes 38–45.

Fig. 5 shows the distribution of the radioactive proteins ^{3}H-HGH, HGH, and pro-

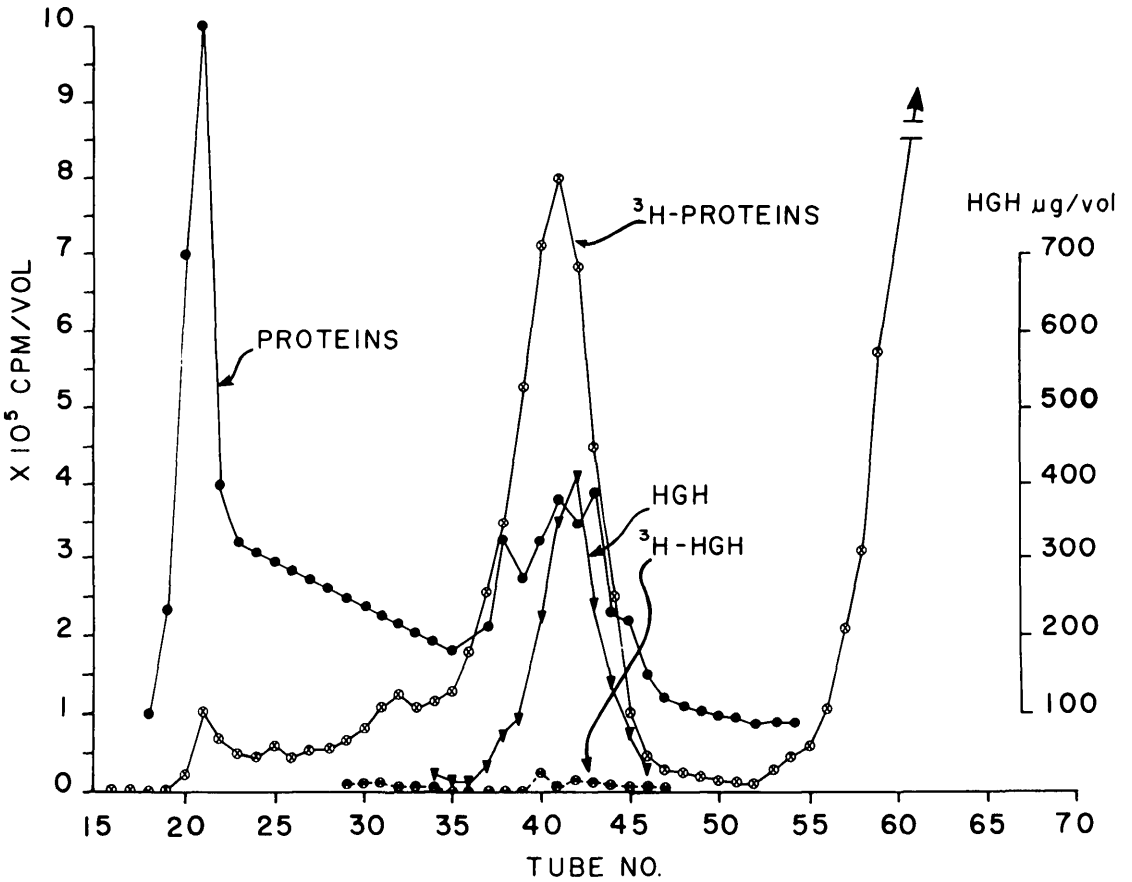


FIG. 3. Gel filtration pattern of Sephadex G-100 of the medium obtained after 4-hr incubation of a human pituitary gland. The distribution of proteins, HGH, ^{3}H-proteins, ^{3}H-HGH is shown. A single large radioactive protein peak (tubes 35–45) encompasses the tubes containing HGH. The maximal concentration of ^{3}H-protein is found in tube 41, while the maximal concentration of HGH is in tube 42.

teins in the medium after 24 hours of incubation. One principal radioactive protein peak is apparent with an elution volume similar to HGH. Less than 15% of the radioactive proteins in tubes 38–45 was precipitated, yet this pool contains 70% of the total TCA precipitable proteins in the medium. HGH again accounts for 80% of the proteins present in tubes 37–45.

A suitable amount of gamma globulin obtained from rabbit anti-HGH serum was added to duplicate aliquots from tube 42 (Fig. 5), the fraction which contained the maximum radioactivity and HGH after gel filtration of the medium after 24 hours of incubation. A trace amount of ^{131}I-HGH was added to one of the two tubes as well. The contents of the tubes were allowed to incubate overnight and the antibody bound ^{3}H-protein and/or ^{131}I-HGH was separated from the unbound fraction by passing the incubation mixture through a column of Sephadex G-100 (Fig. 6). Less than one third of the ^{3}H-proteins were associated with large molecular weight proteins, presumably antibody bound at least in part, but over 90% of the ^{131}I-HGH added as a tracer was antibody bound. Immunoassay of growth hormone of the appropriate fractions showed that less than 10 ng HGH remained in these fractions although 30,000 ng had been applied to the column. The elution volume of the remaining protein was the same in the presence or absence of

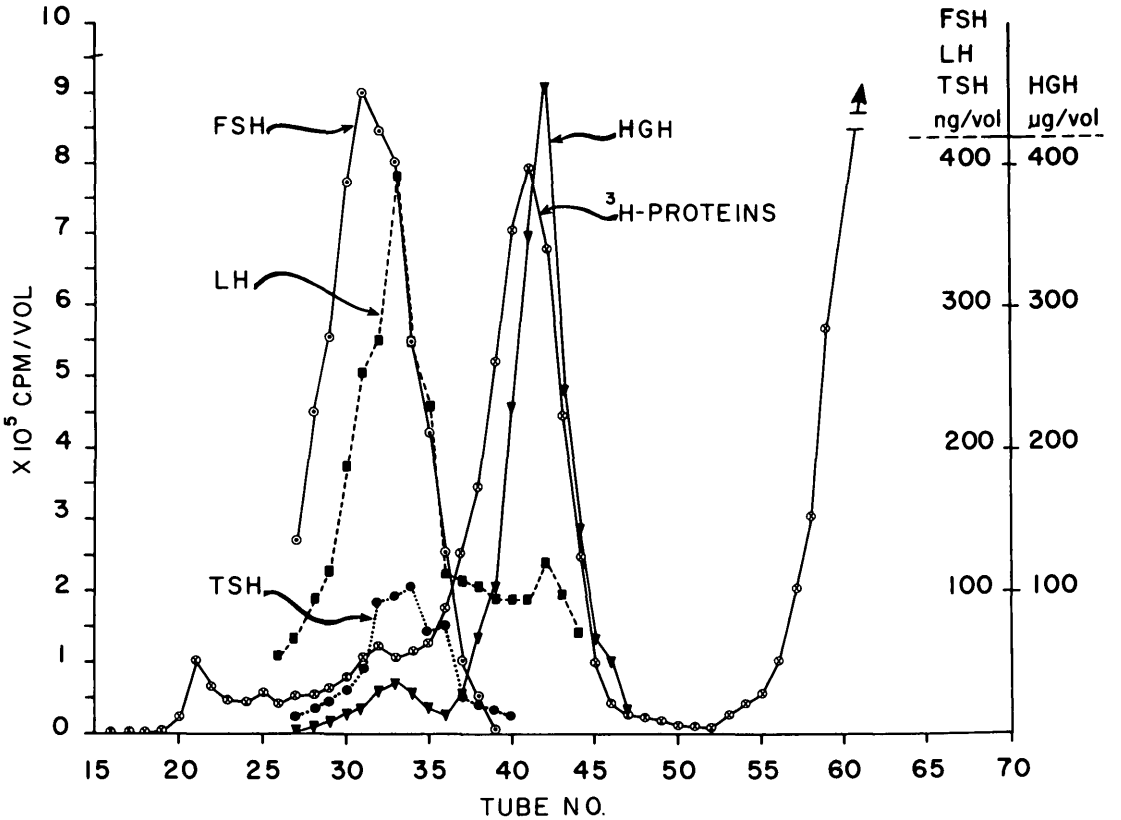


FIG. 4. Gel filtration of the 4-hr medium shown in Fig. 3. The distribution of FSH, LH, TSH, HGH and ^{3}H-proteins is shown. For the conversion of hormone concentrations to ng the following potencies were used: 1) HGH, 1.45 U/mg, 2) NIH, FSH-S_1 150 U/mg, 3) LH, NIH, LH, S_1 5 U/mg, 4) TSH, 20 U/mg, 5) ACTH, 120 U/mg. The maximum concentration of ACTH was found beyond tube 46.

HGH, suggesting very strongly that the highly labeled radioactive protein was not simply adsorbed to HGH. Moreover, the electrophoretic mobility of the residual protein remained the same after all the HGH had been removed.

Fig. 7 shows the distribution of radioactive proteins, anti-HGH precipitable protein and HGH after gel filtration of the tissue extract, which was obtained after incubating the pituitary gland for 24 hours. Two major radioactive protein peaks emerged, the first immediately after the void volume, and the second, much smaller peak, which was coincident with HGH, and corresponded to the elution volume of the major radioactive protein peak in the medium. Anti-HGH serum precipitated ^{3}H-proteins in both radioactive peaks, which were coincident with the two protein peaks in tubes 18–23 and tubes 37–46. The total amount of HGH present in the fractions in tubes 39–47 accounted for virtually all the proteins present. Indeed, in some assays, the estimates of HGH concentrations were equal to or greater than the protein concentration, as in tubes 41–43 (Fig. 7), suggesting that the standards were not entirely appropriate for measuring HGH in the tissue extract. The nature of the large molecular weight proteins precipitated by anti-HGH is not known, but the immunoassay also detected small amounts of HGH in these fractions (tubes 18–22).

Fig. 8 shows the electrophoretic pattern of the proteins found in the fractions with the maximum radioactivity after gel filtration of the medium at 4 and 24 hours and

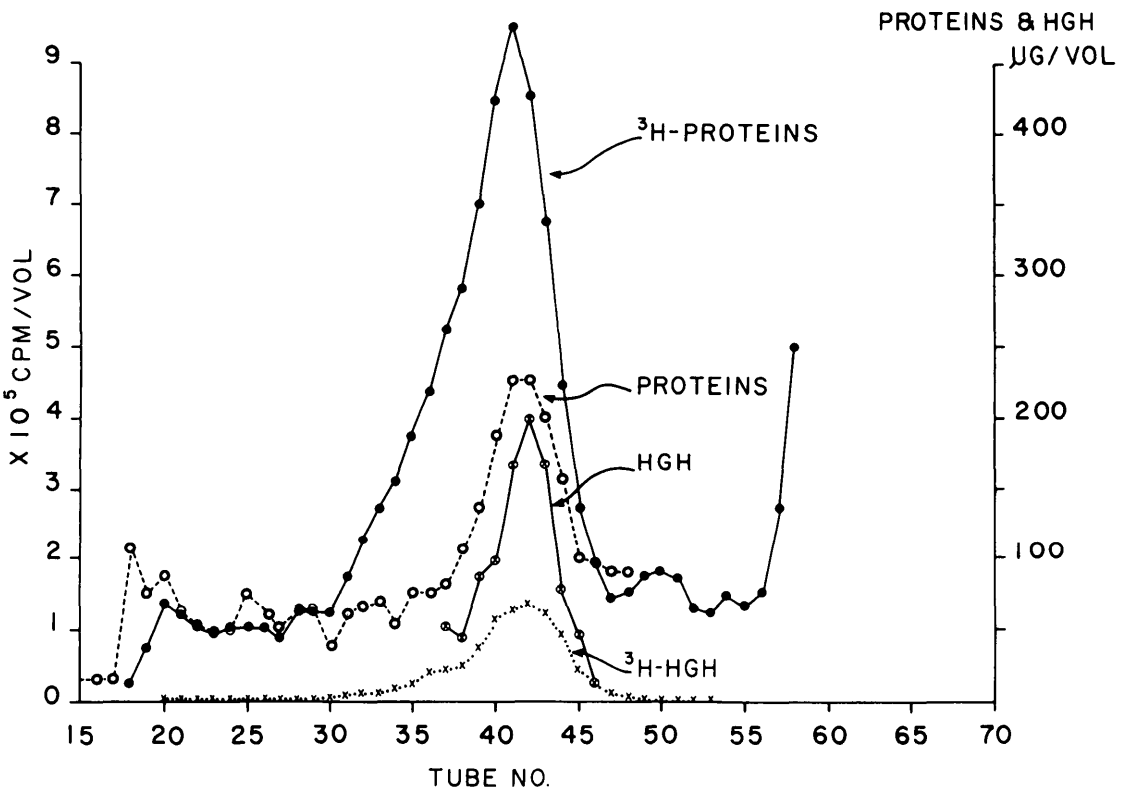


FIG. 5. Gel filtration of the medium obtained after a 24-hr incubation period showing the distribution of proteins, HGH, ^{3}H-proteins and ^{3}H-HGH.

tissue extract. The major stained band has the same electrophoretic mobility as the principal band of HGH, namely, an Rf of 0.60, which was run as a control and appears indistinguishable from the pattern of the tissue and medium.

Fig. 9 shows the distribution of radioactivity in eluants of gel segments after electrophoresis of aliquots from tubes 42 and 43 from the 24 hour medium shown in Fig. 6. The maximum HGH content is in a more anodal segment (Rf = 0.6) than the segment containing the maximal radioactivity (Rf = 0.55). Again, less than 15% of the radioactive proteins in the eluants from these acrylamide gel segments were precipitated with anti-HGH serum. The electrophoretic mobility of FSH was coincident with that of HGH, that is, the segment corresponding to an Rf of 0.60. Unfortunately, protein determinations could not be carried out because the concentration that was present was too low to be detected.

The radioactive proteins from experiment III corresponding in size to HGH were lyophilized and added to 1 g of acetone-dried human pituitary powder. The pituitary powder was extracted for HGH according to Raben's method, after Hartree's pre-extraction of gonadotropins (20) had been applied. Table 4 shows the distribution of the radioactive proteins remaining at each stage in the extraction procedure. The lyophilized pituitary media contained approximately 8,200,000 cpm. Of this radioactivity, 15.8%, that is, 1,300,000 cpm, was precipitable by anti-HGH serum. A small proportion, 8.5% of the total count, was found in the gonadotropin and thyrotropin fraction obtained when the ethanol concentration was increased to 80% to obtain the B ppt. In the growth hormone fraction, A_4 ppt 1,700,000 cpm were found but only 500,000 cpm

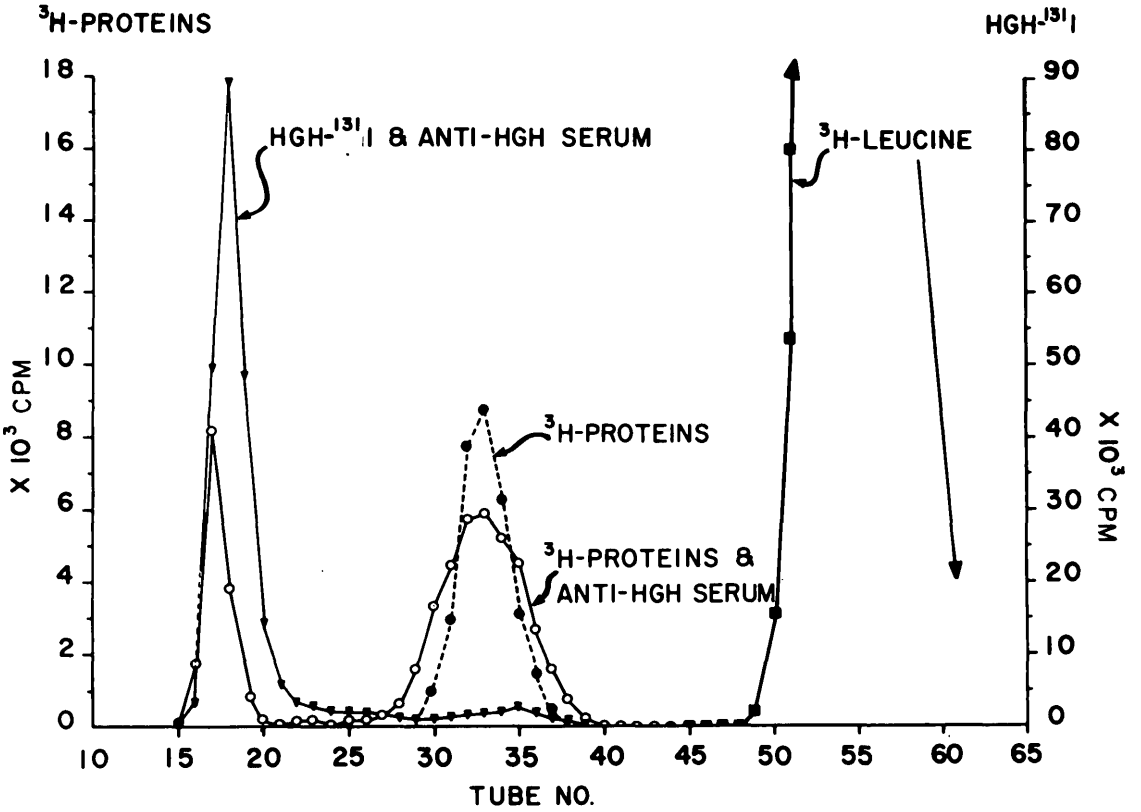


FIG. 6. Gel filtration of an aliquot of tube 41 (Fig. 5) on a small column of Sephadex G-100 (0.5×60 cm). The ^{3}H-proteins (solid circles) emerged with the same Ve/Vo of 2.0 as found originally in Fig. 5. Maximum radioactivity at tube 33. Antibody bound ^{131}I-HGH or ^{3}H-proteins are present in tubes 15–20. Only 30% of the ^{3}H-proteins emerged with the antibody fraction, while over 90% of the ^{131}I-HGH was bound to antibody.

could be precipitated with anti-HGH serum. Therefore, the over-all recovery of ^{3}H-HGH was approximately 38%. If the recovery of the unknown radioactive protein paralleled that of ^{3}H-HGH, and if most of it upon extraction and fractionation were contained in the A_4 ppt, then, if no losses had occurred, approximately 3,000,000 cpm would have been expected in this fraction. This represents approximately 50% of the ^{3}H-proteins in the medium. The major point of the fractionation scheme is to emphasize that the bulk of the radioactive protein is found in the hot glacial acetic acid extract, and even after oxycellulose adsorption approximately 56.5% of the original radioactive protein in the hot glacial acetic acid extract is present, but only a small part of this cross-reacts with antiserum to HGH.

Discussion

Our experiments show conclusively that the human pituitary synthesizes and releases proteins into Krebs-Ringer bicarbonate buffer for a 48–72 hour incubation period. The principal pituitary hormone present in the medium is HGH. This point is well illustrated in Fig. 4, where the amounts of FSH, LH and TSH are shown in the same figure with the amount of HGH. The scale for HGH is in μg/vol, whereas, for the other four pituitary hormones, it is in ng/vol. Indeed, Table 3 shows that HGH accounts for 50 to 70% of

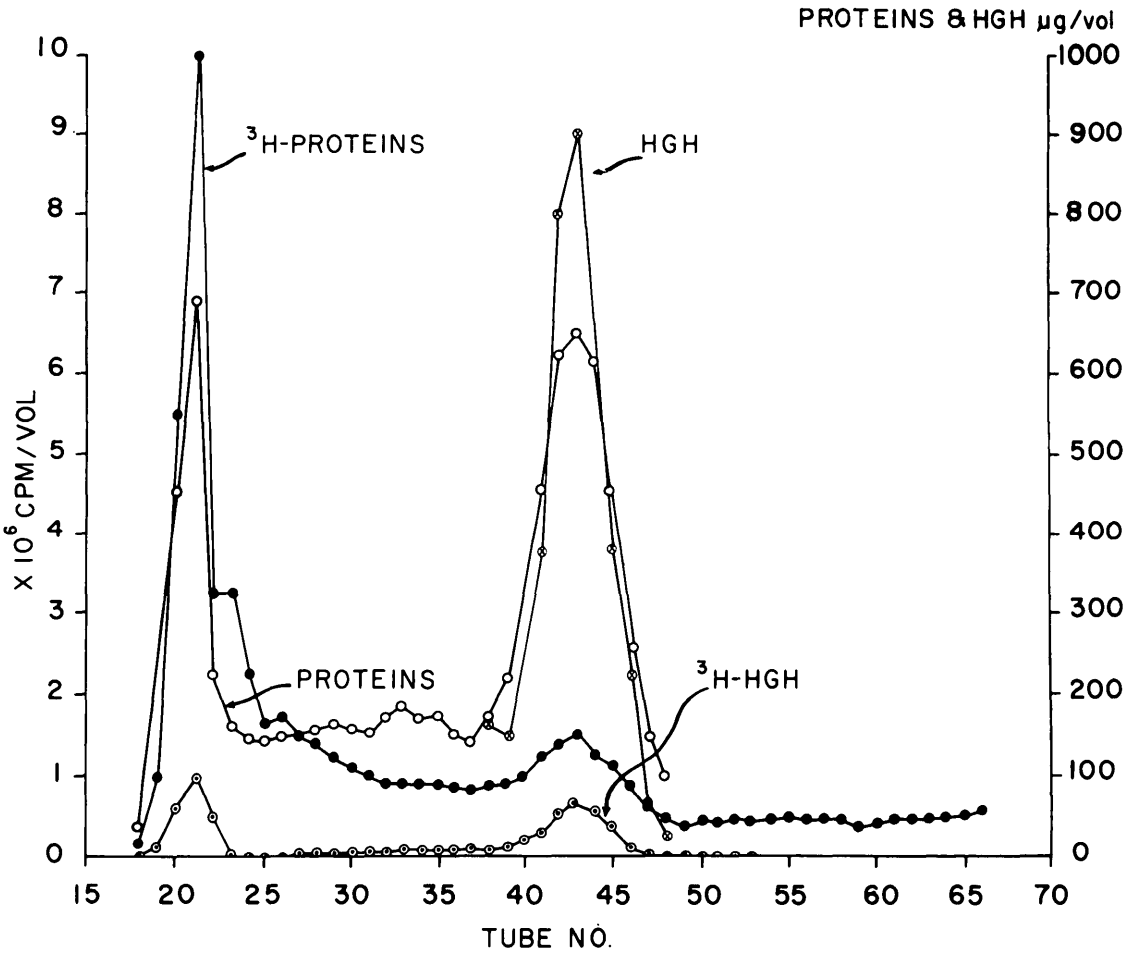


FIG. 7. Distribution of proteins, HGH, ^{3}H-proteins and ^{3}H-HGH, after gel filtration of human pituitary tissue extract; anti-HGH serum precipitated radioactive proteins in tubes 19–23 as well as in tubes 39–46.

all the protein present in the medium. It is clear that the specific activity of HGH in the medium is considerably lower than that of the residual protein. The latter is defined as the protein accounting for the difference between the total protein and HGH concentrations. The specific activity of HGH in tube 41, Fig. 5, is 7270 cpm/µg, whereas the specific activity of the residual protein is 50,125 cpm/µg, approximately seven times the specific activity of HGH. From Table 3 it is apparent that the mean ratio of specific activity of protein to HGH is greater than 12. Presumably, the very high specific activity of the residual protein which is similar in size to HGH is due to its rapid rate of synthesis and equally rapid release into the medium. In contrast, newly synthesized growth hormone may first enter a large tissue pool of HGH before it is released; hence, its specific activity is especially low.

The tissue content of HGH was difficult to assess accurately by radioimmunoassay because in some instances there was nonparallelism between the standard and serial dilutions of the tissue extracted. At very high dilutions HGH content was overestimated by a factor of at least 2-fold when our estimates of HGH content are compared with values of 20 and 60 µg/mg reported by others (21, 22).

It is of some interest that anti-HGH precipitates ^{3}H-proteins of large molecular

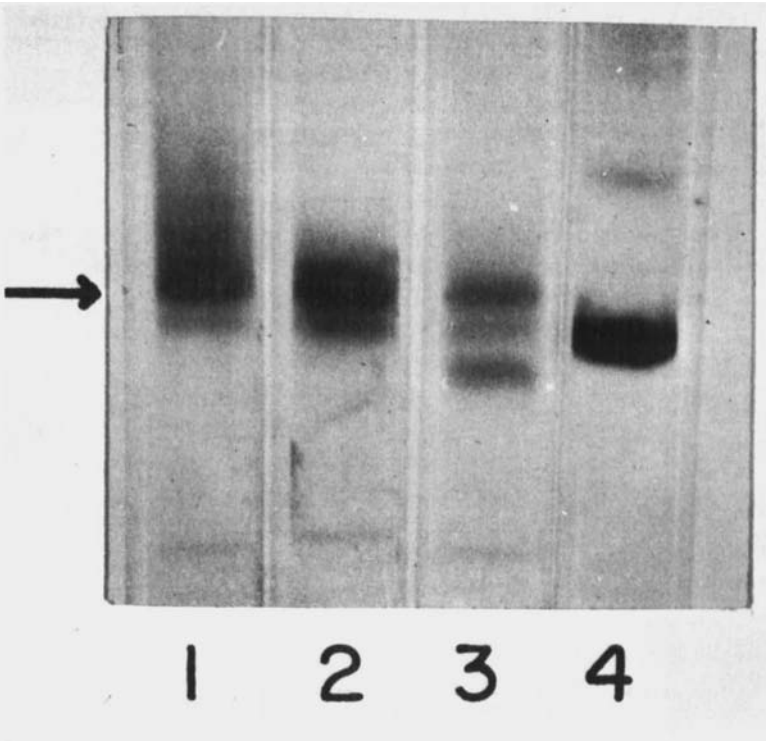


FIG. 8. Protein pattern after acrylamide gel electrophoresis of aliquots of 1) tissue extract, 2) incubation medium (24 hr), 3) 4-hr incubation medium and 4) serum albumin. The arrow points to the band which has the same mobility as the major band of HGH.

weight obtained after gel filtration of the tissue extracts shown in Fig. 7. The total amount precipitated in the tubes containing most of the HGH (tubes 37–45) is approximately equal to the amount precipitated in tubes 19–23; yet the HGH content of this fraction is almost negligible. Hence, it is difficult to calculate the specific activity of ^{3}H-HGH in the tissue. These findings are very similar to the results we observed in studies on HPL biosynthesis (23), where anti-HPL also precipitated two molecular species of ^{3}H-HPL. Indeed, in these studies with prolonged incubation (48–72 hr), the large molecular weight species of ^{3}H-HPL formed over 50% of the total radioactive proteins precipitated in the medium. Perhaps this large molecular weight species is a precursor in the biosynthesis of HGH.

What is the nature of the principal radioactive protein(s) released into the medium by human pituitary glands? Its total elution volume is similar to that of another homogeneous protein, namely, HGH. Upon acrylamide gel and starch gel electrophoresis only one major radioactive protein peak was found. It is unlikely to be any of the three glycoproteins because their elution volume and electrophoretic mobility are different, but more importantly very little of the radioactive protein is pre-extracted into the glycoprotein fraction by Hartree's method (8.5%). It is not ACTH because the oxycellulose-adsorbed fractions contain less than 5% of the radioactive protein and the elution volume of ACTH differs from the principal radioactive component. The elution volume and electrophoretic mobility differ slightly from HGH but one might argue that the substance is a precursor of HGH. Yet it would be strange if the small number of additional amino acid residues which might be present would so drastically alter the HGH molecule that it would not react at all with antiserum to HGH or HPL. Proinsulin, for example, reacts less well with diluted antiserum but reacts nevertheless (24). Moreover, if the radioactive protein were a prohormone, after 24 and 48 hours the predominant form should

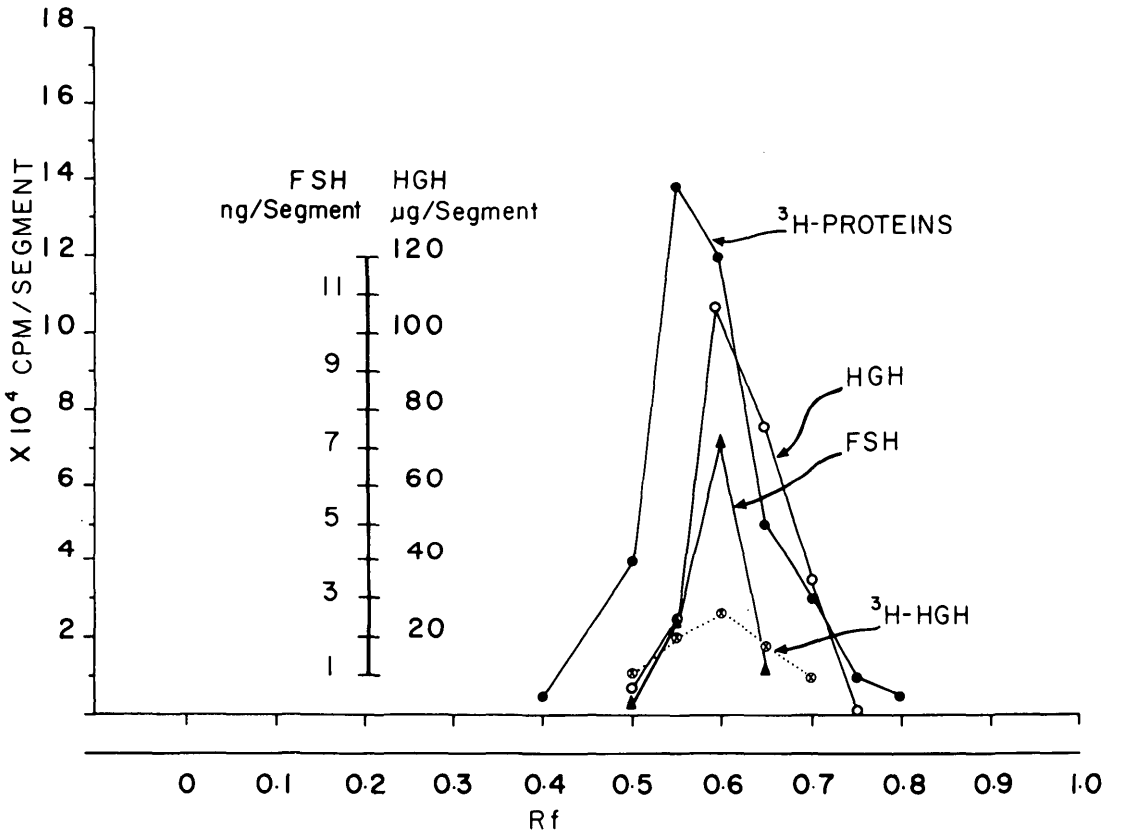
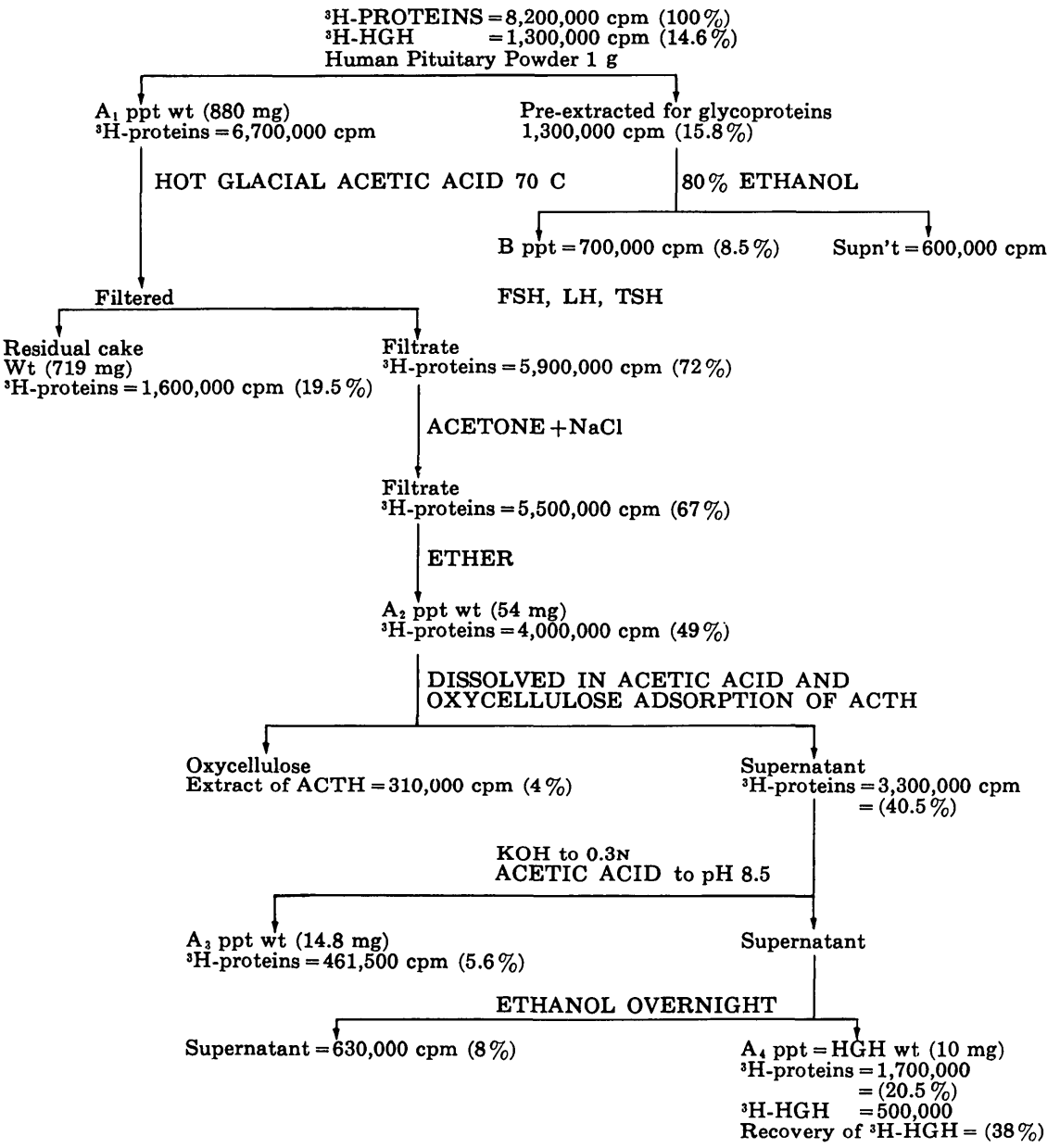


FIG. 9. Distribution of HGH, FSH, ^{3}H-proteins and ^{3}H-HGH after acrylamide gel electrophoresis. The maximum concentration of radioactive proteins is in segment corresponding to an Rf of 0.55, while the maximum HGH concentration is in segments corresponding to an Rf of 0.60.

be the authentic hormone that is secreted, and hence should react with antiserum. Another possibility accounting for the data might be that the radioactive proteins have been altered by the incubation procedure, but in this case, with prolonged incubation periods, less of the radioactive proteins should react, whereas we observe that at four hours 3% is precipitated and at 24 hours, 13%. Moreover, the amount of HGH which reacts with antisera in a radioimmunoassay precludes the possibility of major destruction, because HGH in the medium already accounts for 50 to 60% of the total protein and in the fractions obtained after gel filtration for 80 to 90% of the proteins. Therefore, the over-all destruction during a 24-hour period probably is not greater than 10 to 20%.

If the radioactive protein which is released rapidly into the medium is not radioactive HGH, what is it? We suggest that it may be prolactin for several reasons: First of all, in the rat, using an identical experimental procedure, one principal radioactive protein accounting for 70% of the labeled protein is released into the medium which upon electrophoresis migrates as prolactin. Moreover, Adiga *et al.* (25) reported that, with polysomes obtained from bovine anterior pituitary glands, approximately 33% of the ^{3}H-leucine was incorporated into prolactin compared to 8% into growth hormone. In the case of the human pituitaries, approximately 70% of the radioactive proteins are also found in a single peak almost coincident with HGH, and upon electrophoresis most of the radioactive proteins migrate as a single peak with HGH, yet it is different immunologically.

TABLE 4. Distribution of ^{3}H-proteins from the incubation medium during the extraction of HGH from acetone-dried pituitary powder



Kleinberg and Frantz reported that antiserum to HGH did not inhibit serum prolactin activity but inhibited the prolactin activity of HGH (26). Peake *et al.* (22) also reported that anti-HGH serum did not inhibit all the prolactin activity of pituitary extracts. Hence, it seems that anti-HGH serum is very useful in dissociating HGH from prolactin.

Our data would rationalize some of the difficulties that have been encountered in attempts to purify prolactin free of HGH. The two proteins appear to be very similar in size, isoelectric point and solubility, the three main points upon which purification steps are based. The problem is compounded by the fact that HGH is present in concentrations 500 to 1000 times that of

at least four other pituitary hormones. If the concentration of prolactin is similar to the other four hormones, then it is easy to see how difficult the problem of purification would be. We suggest that, after immunochemical adsorption of HGH in fractions containing most of the radioactive proteins, it should be possible to obtain sufficient amounts of this fraction, free of HGH, for immunoprecipitation studies using antiprolactin serum and for bioasay of prolactin to determine directly whether this fraction has prolactin activity. If the assay were positive, then it is likely that the radioactive proteins are human prolactin. If the fraction has no prolactin activity it is possible that the pituitary secretes an additional as yet unidentified hormone.

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