Fulton *et al*(11) reported a value of 0.269. The values for man do not appear to be too dissimilar to those observed in swine.

The LV + S/T ratio increased simultaneously with the decline in RV/T ratio (Fig. 1). Increase of this ratio was due principally to an increase in the left ventricular weight. The LV/RV ratio increased concomitantly, with an increase in the LV + S/T ratio. At birth the mean LV/RV ratio for the piglet is 0.860. This ratio increased to 1.812 at 7 days of age indicating that the left ventricle weighs nearly twice that of the right. Although the right ventricular pressure was not measured, the decline in the RV/T ratio in the piglet probably reflects a reduction in this pressure similar to that reported in the dog by Averill and co-workers(7). The data from the present study suggest that, in addition to weight ratios, the pulmonary arterial and systemic arterial pressures should be obtained to possibly relate pressure and ventricular weight changes.

Summary. Postnatal changes in the cardiac ventricles of 75 piglets are reported. The RV/T ratio reclines from a mean of 0.382 to 0.253 by 7 days of age. Concomitant with this decline the LV + S/T and the LV/RV ratios increased. Although such changes occur after birth more rapidly in the pig than in man, the magnitude of change in the swine cardiac ventricles appears to be quite similar to that reported for man.

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Evidence for the Existence of Human Growth Hormone-Ribonucleic Acid Complex in the Pituitary. (31088)

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Several widely different procedures for extracting human growth hormone (HGH) have been reported. Three of these procedures are quite drastic; for example, glacial acetic acid, $Ca(OH)_2$ and 0.1 N NaOH have been used (1,2,3). In another procedure, Lewis and Brink(4) extracted pituitaries with 0.3 M KCl at pH 5.5. The latter demonstrated that a further yield of HGH could be obtained from the insoluble residue by extraction at pH 11 in 0.3 M KCl. Elrick *et al*(3), in a more detailed study showed that a 2-hour extraction of acetone-dried pituitaries with 0.3 M KCl and 0.1% thioethanol at pH 5.5 resulted in a yield of about 6% of the HGH activity. Extraction for 20 hours increased the yield to about 25%. Treatment of the residue with 0.1 N NaOH solubilized the remaining 75% of the HGH. Because 25% of the HGH was extracted under mild condition and treatment of the residue with alkali yielded a HGH preparation which is soluble at neutral pH, it could be inferred that most of the HGH is present in the pituitary in an insoluble form which is hydrolyzed or cleaved by the more drastic procedures. If such were the case, HGH acitvity obtained by drastic procedures would be associated with different molecular species depending upon the degree of hydrolysis or cleavage. That the biologi-

cal activity in a given preparation of HGH is distributed among different components has been demonstrated by Ferguson and Wallace (5,6) and by Barrett, Friesen and Astwood (7). Furthermore, Kaplan and Grumbach(8) have presented evidence that the active moiety differs according to the method of extraction. They attributed these differences to chemical and physical changes brought about during the extraction. Even with a mild extraction, Roos, Fevold and Gemzell(9) present evidence that GH activity resides in more than one molecular species.

If the activity in the crude preparations resides in different molecules, the purification procedures employed with such a mixture could result in the activity being distributed among various fractions. Moreover, it would be difficult to reproduce many of the studies because the degree of fragmentation could vary with differences in time and temperature too small to be controlled in a routine, conventional manner.

It, therefore, seemed reasonable to study the chemical property of HGH activity obtained by mild hydrolysis with the object of selectively cleaving the molecule or aggregates by mild enzymatic digestion.

We have obtained evidence that several different biologically active moieties are present in an alkaline extract of human pituitaries. Moreover, we have demonstrated that this extract can be enzymatically hydrolyzed to give a preparation which is more homogeneous with respect to growth hormone activity than the crude extract. Evidence is presented that some of the HGH in the pituitary is bound to ribonucleic acid.

Materials and methods. The alkaline extract of human pituitaries was obtained from the Human Protein Hormone Bank, Veterans Administration Hospital, Denver, Colorado. The glands were processed as follows: Pituitary glands which had been collected at autopsy and preserved in acetone containing 0.1% thioethanol and 1% acetic acid were minced in fresh acetone-0.1% thioethanol. The mince was centrifuged, washed with ether and dried under vacuum. The dry powder was ground further in a Wiley Mill and extracted with 0.3 M KCl containing 0.1% thioethanol at pH 5.5 for 4 hours at 2-5°C. The residue was reextracted for 2 hours. The residue from the second extraction was then extracted with 30 ml of 0.1 Na NaOH per gram of residue for 5 minutes at room temperature, pH 11.6-12.0. The residue was reextracted with 15 ml of 0.1 N NaOH for 5 minutes. The supernatants were combined and the pH lowered to 7.0 with 1 N HCl. The precipitate that formed was removed by centrifuging at $29{,}000 \times g$ for 20 minutes. The final supernatant was dialyzed and lyophilized. The dried alkaline extract (extract #2) was used in the experiments reported below.

The dried alkaline extract was put into solution with dilute ammonia (pH 10-11) or 1 M K_2HPO_4 and neutralized with dilute acids (1 M) to the required pH, depending upon the experimental procedure reported. Frequently a precipitate occurred upon neutralization which was removed by centrifuging. The precipitate contained little or no growth hormone activity.

The supernatant of extract #2 was applied to Sephadex columns equilibrated with 0.1 M ammonium formate pH 6.3. The column eluate was continuously monitored at 278 mμ. Upon lyophilization most of the ammonium formate was removed. The final traces of ammonium formate were removed by dialysis against distilled water in a rocking arm dialyzer and the material was again lyophilized. Analyses for RNA and DNA of the resultant fractions were performed using the Dische method(10) and by the diphenylamine procedure(10). Protein was determined by the method of Lowry *et al*(11).

The GH activity was estimated by rat tibia assay(12) using a standard bovine GH (U.S. P.).

The DEAE-cellulose columns were prepared in the usual manner and equilibrated with 0.052 M $Na_2B_4O_7$ pH 9.14. The sample was dissolved in 0.052 M $Na_2B_4O_7$ pH 9.14 and applied to the column. Gradient elution was performed starting with 200 ml of 0.052 M $Na_2B_4O_7$ and mixing with 0.52 M $Na_2B_4O_7$ in 0.5 M KCl.

Enzymatic digestion of the alkaline extract was performed as follows: For 100 mg of extract, 0.35 ml of 1 M K_2HPO_4 was added

TABLE I. Chromatogram of Extract #2 on Sephadex G100.

Fraction	Tibia assay, U/mg	% Recovered of total Wt	Activity	Protein, %	RNA, %	260/280 OD ratio
		80.8 mg of undigested extract				
1	.23	23	9	85	3.0	1.11
2	.68	44	42	90	.96	.96
3	.67	28	32	95	.92	.92
Recovery		95	83			
		67.9 mg of digested* extract				
1	.0	20	0	—	4.3	1.18
2	.74	21	18	—	.0	1.01
3	1.2	48	67	—	.0	.95
Recovery		89	85			

Column 20 × 450 mm eluted with 0.1 M KCl in .05 M phosphate buffer pH 6.5.
* Extract #2 digested 1.5 hr, pH 7.6—37°C with 5% ribonuclease.

with thorough mixing. This was followed by 1.66 ml of 0.3 M KCl and mixed; 1.64 ml of water was then added and the pH adjusted to 7.6 with 1 M NaH_2PO_4 (approximately 0.2 ml). Five mg of ribonuclease* as a dry powder was added. The material was incubated for one hour at 37°C. When a further digestion by spleen phosphodiesterase* was performed, solid EDTA was added to give a 0.01 M solution and the pH lowered to 6.6 with 1 M NaH_2PO_4. Two units of enzyme in 1 ml water were added. The mixture was digested for 1 hour at 37°C.

In those experiments in which the presence of salts was undesirable, the digested extract was dialyzed and lyophilized.

Ribonuclease was assayed by the method of Kalnitsky *et al*(13) and spleen phosphodiesterase by the method of Hilmoe(14).

Electropartition was performed by the method of Leaver(15). The chambers are numbered from the anode as number 1 toward the cathode. They were separated from one another by Bac-T-Flex† membranes of known pore size and from the anode and cathode by Visking cellulose dialyzing membranes (5 mμ pore size). When glycine was used as a buffer, the cathode solution was constantly mixed with the anode solution. The running pH is the pH in the sample chamber after equilibrium has been established (15 minutes).

Results. Fig. 1, curve A, gives the chromatogram pattern when the alkaline extract was passed through a Sephadex G100 column. When the 3 fractions were rechromatogrammed individually, the activity appeared in the same volume of eluant as in the original chromatogram (curves 1, 2 and 3, Fig. 1). In curve 1 it can be seen that the activity of fraction 1 appeared in the same volume of eluant as in curve A. The same is true of curve 2, the rechromatogram of fraction 2, and curve 3 of fraction 3. This indicates that the GH activity in the extract is found in at least three different molecules which differ markedly in molecular properties.

Table I gives the analyses of fractions 1, 2 and 3 obtained from another chromatogram on Sephadex G100 of the extract. It can be seen that all fractions contained GH activity. It is also noted that all fractions contain RNA. Table I also gives the analyses of fractions 1, 2 and 3 when an aliquot of the alkaline extract was digested with ribonuclease and chromatogrammed. No loss of hormonal activity following ribonuclease digestion could be detected by the tibia assay. This is also evident from the activity recovery. It can be seen that the GH activity of ribonuclease-digested material was eluted in greater volume than the undigested material. Compare 42% of the total activity in fraction 2 of the undigested extract with 18% for the digested; and for fraction 3, 32% and 67%.

* Ribonuclease, spleen diphosphoesterase was obtained from Worthington Biochemical Corp., Freehold, N. J.

† Bac-T-Flex membranes were obtained from Carl Schleicher and Schuell Co., Keene, N. H.

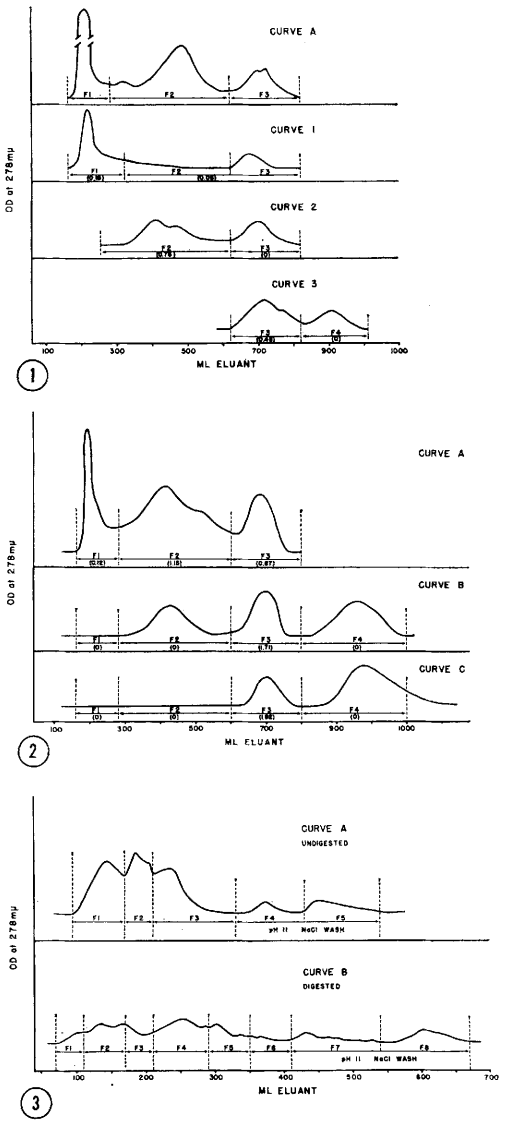

FIG. 1. A Sephadex G100 column 40 x 430 mm bed volume was used for curves A, 1, 2 and 3. The elution was 0.1 M ammonium formate pH 6.3. Curve 1 is the rechromatogram of fraction 1, curve A. Curve 2 is the rechromatogram of fraction 2, curve A, and curve 3 the rechromatogram of fraction 3, curve A. Figures in parenthesis and U/mg of GH activity of the fraction are indicated by arrows. The ml of eluant is comparable for all columns. For curve A, 147.9 mg of extract #2 was chromatogrammed, for curve 1, 68.1 mg, curve 2, 31.8 mg, and for curve 3, 36.7 mg.

FIG. 2. A Sephadex G100 column 40 x 430 mm bed. Volume eluant was 0.1 M ammonium formate pH 6.3. Curve B is the chromatogram of fraction 3, curve A after digestion by ribonuclease. Figures in parenthesis are U/mg of GH of the fraction indicated. For curve A, 204 mg of extract #2 was used. For curve B, 67.5 mg and for Curve C, a 10 mg aliquot of fraction 3, curve A.

FIG. 3. 90 mg of sample dissolved in 0.0052 M sodium borate pH 9.14 was placed on a DEAE column 20 x 400 mm bed volume. The DEAE was equilibrated with 0.0052 M $Na_2B_4O_7$ pH 9.14. The columns were gradient eluted by mixing 400 ml of 0.5 M NaCl in 0.0052 M borate with 200 ml of 0.0052 M borate pH 9.14. The columns were finally washed where indicated with 0.0052 M borate pH 11 in 3 M NaCl.

There was also some increase in specific activity by tibia assay of fraction 3 of the digested extract as compared to fraction 3 of the undigested (0.67 as compared with 1.2). It is to be noted further that no RNA could be detected in fractions 2 and 3 of the digested material but the 260/280 OD ratio was still quite high indicating the possible contamination of DNA. This was borne out in a subsequent experiment in which DNA was detected by chemical analysis. Three experiments with different preparations of the crude extract indicated marked differences in the amount of material and activity found in fractions 1, 2 and 3. Digestion by ribonuclease always resulted in a decrease of GH activity in fraction 2 and an increase in fraction 3.

Fig. 2 also shows a marked decrease in molecular size of the material containing GH activity when the fractions of undigested extract #2 from a Sephadex column were digested with ribonuclease and rechromatogrammed. It can be seen that all the activity of fraction 2 in curve A appeared as fraction 3 in curve B, with an increase of biological activity from 1.15 to 1.71 U/mg. However, digesting fraction 3 of curve A and rechromatogramming (curve C) resulted in all the activity being located in essentially the same position as the undigested, but with increase in specific activity (1.92 from 0.87 U/mg). It is evident from this experiment that ribonuclease digestion brings about a marked cleavage of the larger aggregates of HGH. It is also evident from the increase in specific activity when fraction 3 is digested that considerable impurities are removed. Hence ribonuclease digestion of the crude GH results in the activity being located in molecules more homogenous with respect to size.

Additional evidence of this change by ribo-

TABLE II. Analysis of Fractions from DEAE Column Given in Fig. 3.

Fraction	Tibia assay, U/mg	% Recovery Wt	% Recovery Activity	260/280 OD ratio
		Undigested extract		
1	1.4	38.6	80.6	.77
2	1.6	20.1	47.8	1.09
3	—	8.1	—	1.55
4, 5	—	11.6	—	1.04
Total		78.4	128.4	
		Ribonuclease digested extract		
1	.0	12.8	.0	
2	.0	22.6	.0	
3	.25	2.2	8.2	1.06
4	1.1	29.3	48.1	.94
5	1.5	3.3	7.3	1.20
6, 7, 8	—	32.3		
Total		102.5	63.6	
Initial	.67			1.24

Fractions 4 and 5 of the undigested and fractions 6, 7 and 8 of the digested extract were pooled.

nuclease is illustrated in Fig. 3, which gives the comparison of the 2 different elutions of GH from a DEAE column. The divisions of the fractions collected are indicated on the curves. Table II gives a summary of the analyses of the fractions. It can be seen that the majority of the activity appeared immediately after the hold-up volume in curve A (undigested) indicating little or no retardation by the DEAE. With the digested material, however, the initial fractions had no activity. The activity did not appear until quite late in the elution, indicating a marked molecular change following digestion. The retarding of digested GH activity by DEAE might be expected from the results obtained with Sephadex chromatography(16). If the GH activity of crude extract is bound to RNA it might be considered to be a neutral compound and, therefore, it would be eluted earlier in the chromatogram than the free GH which is reported to have an isoelectric point of 4.9. However, one cannot draw a strict parallel in behavior of pure proteins on an ion exchanger to that of conjugated proteins, for factors other than the isoelectric point may dominate the elution pattern. Regardless of the interpretation, the DEAE elution patterns indicate a marked change in the properties of the GH due to ribonuclease digestion.

TABLE III. Migration of Tibia Activity in an Electrical Field.

Chamber	Partition 1 undigested % Wt	Partition 1 undigested U/mg	Partition 2 digested % Wt	Partition 2 digested U/mg	Partition 3 RNAse digest of chamber 1, partition 1 % Wt	Partition 3 RNAse digest of chamber 1, partition 1 U/mg
1	77.8	.88	17.4	—	55.9	.76
2	14.1	<.20	22.6	—		
3	4.7	<.20	33.0	.49	33.0	.90
Recovery	96.6		73.0		88.9	

For partition 1, 150.0 mg of the sample was placed in chamber 2; for partition 2, 100.8 mg; for partition 3, 63.0 mg. Initial activity of the sample of partitions 1 and 2 was 1.1; of partition 3, 0.88. Chamber #1 is nearest the anode; chamber #2, center; chamber #3, nearest the cathode. A 5 μ membrane separated chambers #1, #2 and #3. Chambers #1 and #3 are separated from electrodes with a cellulose dialyzing membrane (5 mμ pore size). Chamber #2 of the undigested extract, partition 1, ran at pH 7.6. The digested extract, partition 2, ran at pH 7.6. Chamber #1 ran at pH 6.8; chamber #3 at pH 8.4. Partition 3 was at pH 7.6. The voltage gradient ranged from 10 V/cm to 60 V/cm during the 6 hr run. Partitions 2 and 3 were digested by 5% ribonuclease at pH 7.6 and 37° for 1 hr. Partitions 1 and 2 were run in 0.005 M PO_4 buffer. Partition 3 was run in 0.02 M glycine buffer.

To obtain direct evidence for changes in isoelectric point brought about by ribonuclease digestion, the migration of the GH in an electrical field was studied. Table III gives the results of a typical experiment.

Using the apparatus previously described by Leaver(15), a preparation of crude extract was placed in the center chamber (chamber No. 2). This chamber was separated from chamber No. 1 (nearest the anode) and from chamber No. 3 (on the cathode side) by a 5 μ Bac-T-Flex membrane. For partition 2, an aliquot of the extract was digested with ribonuclease and run under identical conditions to partition 1. Of the undigested material, 77.8% (Table III) moved toward the anode and was recovered from chamber No. 1, whereas only 17.4% of the digested extract (partition 2) moved toward the anode. It is also to be noted that 4.7% of the undigested extract was recovered from chamber No. 3 but 33% of the digested material moved to chamber No. 3. Almost 30 times more GH migrated to the cathode when the extract was digested as compared to the undigested ex-

tract. The lower weight recovery of partition 2 (73%) as compared to partition 1 (96.6%) is due to loss in dialysis of small fragments produced by ribonuclease digestion. The crude extract used in the above experiments was obtained by a very short contact with alkali at approximately 10°C. With other extracts prepared as given in the methods section, as much as 50% of the material by weight migrated to the cathode before ribonuclease digestion. This indicates that the alkali treatment itself produces a marked change in the GH aggregates. However, digestion always resulted in more migration of the GH to the cathode. To secure further evidence that the isoelectric point of GH changes after ribonuclease digestion, the material found in chamber No. 1 of partition 1 was digested with ribonuclease and repartitioned. Since this material had previously migrated to the anode indicating an isoelectric point below 7.6 only two chambers were employed. The sample was placed in the chamber nearest the anode. It was found that 33% of this material now moved to the cathode when digested with ribonuclease. The specific activity was 0.90 U/mg. Sixty per cent of the material remained in the sample chamber and had a specific activity of 0.75 U/mg. This partition was run for 1½ hours at 10 V/cm. This indicates that 33% of the growth hormone now has an isoelectric point greater than 7.6 demonstrating that ribonuclease digestion not only brings about a change in molecular weight, but also a change in isoelectric point. Since 2 chambers of the ribonuclease digested extract contained appreciable hormonal activity, it is evident that even though this digestion results in GH activity being more homogeneous in respect to molecular size it is still heterogeneous in respect to isoelectric point. The isoelectric point for a "purified" HGH has been estimated to be 4.9(17). The high isoelectric point of HGH found in our study suggested that a base was still attached to the molecule. Analysis of the material in chamber 2 by Fiske-Subbarow method(18) indicated 19 μg P/mg of material. It seemed possible that the base was attached to the GH molecule by a phosphoester bond. Therefore, a further digestion by spleen phosphodiesterase was performed. Table IV gives the results when an aliquot of the crude alkaline extract was digested by ribonuclease and spleen phosphodiesterase. The digestion by the diesterase did not result in any loss of GH activity. It can be seen that less material migrated toward the cathode at pH 7.6 of the diesterase digested GH. Conversely, more material (by weight) migrated at pH 3.5 to the cathode with the diesterase-ribonuclease digested extract (16.8%) than with ribonuclease digested extract (9.6%). Hence, this final enzymatic digestion results in the largest yield of low molecular weight material with an isoelectric point below 7.6. The additional digestion of the alkaline extract by phosphodiesterase, therefore, results in a preparation more homogeneous in respect to isoelectric point.

TABLE IV. Effect of Phosphodiesterase on GH Migration in an Electrical Field.

Chamber	Ribonuclease digest		Ribonuclease and diesterase digest	
	mg	% of total	mg	% of total
1	38.6	35.5	93.2	44.1
2 (pH 7.6)	7.6	7.0	6.7	3.2
2 (pH 3.5)	10.4	9.6	35.5	16.8
Total recovered	56.6	52.0	135.4	64.0

290 mg of extract #2 was digested with 5% ribonuclease (RNAse) at pH 7.6 for 1 hr at 37°. A fraction of this digest (180 mg) was further digested with spleen phosphodiesterase at 37°, pH 6.6 for 1 hr in 0.01 M EDTA. Both preparations were dialyzed and lyophilized. Figures given are on the basis of the initial weight of extract #2. The sample was placed in chamber #1 (nearest anode). A 5 μ membrane separated chambers #1 and #2. The electropartition was run in a 0.02 M glycine buffer whose pH was adjusted with KOH or HOAc. The cathode chamber's contents were continuously mixed with anode chambers. The sample ran 2 hr at 20 V/cm pH 7.0, and 2 hr at 20 V/cm pH 3.5. The temperature was maintained below 10°C. The run totaled 80 V hr/cm.

The low total recovery of material in these experiments is due to dialysis of small fragments through the cellulose dialyzing membranes. This was demonstrated by dialyzing the ribonuclease digested extract against various salts. Table V gives the results. It can be seen that upon dialysis against water, no material was lost; however, electrodialysis resulted in 24 to 35% loss. When dialyses

TABLE V. Dialysis of Small Fragments Produced by Ribonuclease Digestion of GH Extract.

Conditions of dialysis	Initial extract		Nondialyzable residue		
	Wt, mg	U/mg	Wt, mg	U/mg	% Dialyzable
Distilled water	105.0	1.1	104.7	1.2	0
	51.6	1.0	51.4	.94	0
.5 M urea	61.3	.71	22.0	.58	64.1
.03 M ammonium acetate	18.9	.43	11.7	.39	38.1
Electrodialysis against water	30.5	1.2	23.2	.6	24.0
	24.1	1.1	15.8	1.6	34.5
Undigested controls					
.5 M urea	20.7	.71	18.7	.7	10.0
.03 M ammonium acetate	14.7	.43	13.9	.4	5.4

The dialyzable material was recovered in all experiments except when dialyzed against urea. Recoveries of 92% or better were obtained in all experiments. Ten ml of solution was placed in a cellulose dialyzing bag and dialyzed 18 hr at 4°C, with stirring against 400 ml of solution under conditions given in the table. No material was dialyzable with the crude undigested extract except in the presence of urea. In the presence of urea 10.0% of the crude extract was dialyzable.

were performed against ammonium ion or urea, the losses were much higher—38% for ammonium ion and 64.1% when dialyzed against urea. In fact, there was a 10% loss when undigested crude extract was dialyzed against urea. Hence, a large fraction of the ribonuclease digested extract has a molecular weight probably below 10,000. Previously it was observed that 70 to 80% of the undigested extract failed to pass through a 1 μ membrane in an electrical field indicating a molecular weight greater than 250,000(14). Also the growth hormone activity of the crude extract appears early in the elution from a Sephadex column but just before the internal front after digestion demonstrates a several-fold reduction in molecular size.

Discussion. Roos *et al*(9) have demonstrated that HGH could be found in several fractions obtained from a Sephadex column. We have found 3 discrete fractions containing GH activity when extract #2 was partitioned by a Sephadex column, confirming the suggestion by Roos *et al*(9) that the GH activity resides in different molecular species. The elution patterns of GH activity varied greatly from one preparation to another. This observation also confirms Kaplan and Grumbach's (8) suggestion that the different molecular species containing GH activity result from the physical and chemical changes brought about by the extraction procedures.

Analysis of our fractions from Sephadex chromatography revealed the presence of nucleic acid. This was to be expected as extract #2 is an alkaline extract of the pituitaries. The presence of nucleic acid as being vital for biological activity could be discounted since several workers have demonstrated the absence of nucleic acids in their preparations (19,20). That the nucleic acid was tightly bound to the growth hormone was evident from the observation that they could not be separated by rechromatography, electrophoresis, electropartition, or isoelectric precipitation. We, therefore, attempted to remove the nucleic acid as a contaminant by enzymatic hydrolysis with ribonuclease. Upon enzymatic hydrolysis of the crude material, complete biological activity remained, but there were surprising changes in molecular weight as demonstrated by Sephadex chromatography and electropartition.

That some RNA escapes digestion by ribonuclease is not unexpected for Markham and Smith(21) have demonstrated that this enzyme rapidly hydrolyzes RNA to yield nucleotides but an RNA "core" remains which resists further hydrolysis. The fact that GH of the digested extract can be readily separated from the "core" nucleic acid is further evidence that the binding between GH-RNA in the crude alkaline extract is not an artifact of salt formation or aggregation. The RNA comprised 3 to 5% of the total material yet the hydrolysis of this small fraction led to cleavages which resulted in a several-fold decrease in molecular weight. In fact, 30 to

40% of the material was now dialyzable in the presence of relatively low concentrations of amines or ammonia. Accompanying this fragmentation were marked changes in the isoelectric point. These observations provide the basis for suggestions that the GH as well as other small fragments are bound together by RNA to form macro molecules or aggregates. It is attractive to suggest these macro aggregates occur in the pituitary and the acetone treatment destroys naturally occurring enzymes which bring about de-aggregation in fresh glands. The isolation of GH even under mild conditions may then result in several different molecules depending upon the degree of de-aggregation. Various hydrolytic enzymes are known to be present in the pituitary; among these are 2 proteolytic enzymes first described by Smith(22). Under mild extraction conditions these enzymes or other unknown enzymes may bring about partial de-aggregation to produce GH activity in several different molecular species. If such enzymes are active, then extraction under mild conditions may result in a greater degradation than extraction by alkali. Under these conditions it would be unlikely that a given preparation of GH is the same molecule as that circulating in the body, or that it would be enzymatically active in a purified *in vitro* system. The question naturally arises whether the marked fragmentation of the macro aggregates can be solely attributed to the action of ribonuclease in our preparation. We were unable to detect the presence of any proteinases in the alkaline extract. Also ribonuclease was effective in bringing about further fragmentation of any fraction from the Sephadex columns or electropartition. It would seem likely that one of these procedures would have resulted in the separation of an unknown enzyme. If such an enzyme were present then it too must be bound to RNA and be distributed in various size molecules in a very similar fashion to GH. The unknown enzyme also does not complete the de-aggregation, for we found that phosphodiesterase digestion was necessary to cleave a base from the GH to give a preparation more homogeneous in respect to isoelectric point.

Such multiple requirements for a contaminating enzyme seem to be unlikely. It appears more reasonable to postulate the direct action of ribonuclease on a ribonucleic acid-growth hormone complex which produces small fragments of growth hormone bound to a base and some free growth hormone and other small fragments. Phosphodiesterase digestion completes the cleavage of the base to give free growth hormone.

Summary. Evidence was presented which indicates that HGH activity obtained by an alkaline extract of pituitaries is distributed in at least 3 different molecules or aggregates which varied widely in respect to size. Ribonucleic acid was also shown to be present in these fractions. The nucleic acid could not be removed from the GH by chromatography, electropartition or isoelectric precipitation. It could be removed by digestion with ribonuclease. Upon digestion by ribonuclease the activity was located in molecules or aggregates more homogeneous with respect to size, but with different isoelectric points. A further digestion by phosphodiesterase resulted in the activity being located in a fraction more homogeneous with respect to isoelectric point. It was suggested that the majority of the HGH in the pituitary is bound to ribonucleic acid as a macromolecule.

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Separation of Human Growth Hormone into Two Components—GHA and GHB. (31089)

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Korner(1) and other investigators(2,3) have found that growth hormone (GH) stimulates nucleic acid synthesis when given *in vivo*. GH can also stimulate protein synthesis (4,5,6). It has also been reported that GH stimulates protein synthesis *in vitro* without stimulation of nucleic acid synthesis(7). A logical interpretation of these data would be that there are two factors comprising GH: one factor responsible for the direct stimulation of protein synthesis and the other for nucleic acid synthesis. In the absence of evidence relating to this possibility, a study was undertaken to evaluate this interpretation.

The present study provides evidence that the GH is comprised of two components; are necessary for maximum tibia response.

Materials and methods. The alkaline extract (extract #2) of human pituitaries was obtained from the Human Protein Hormone Bank, Veterans Administration Hospital, Denver, Colo. The dried alkaline extract was dissolved in dilute ammonium hydroxide (pH 10-11) or 1 M K_2HPO_4 and neutralized with dilute acid (1 M) to the required pH. If a precipitate formed during the neutralization, it was removed by centrifuging. The precipitate contained little or no GH activity.

Enzymatic digestion of the alkaline extract was performed as follows: For 100 mg of extract, 0.35 ml of 1 M K_2HPO_4 was added with thorough mixing. This was followed by 1.66 ml of 0.3 M KCl and mixed; 1.64 ml of water was added and the pH adjusted to 7.6 with 1 M NaH_2PO_4. Five mg of ribonuclease as a dry powder was added. The mixture was incubated for 1 hr at 37°C. If a further digestion by spleen phosphodiesterase was performed, solid EDTA was added to give 0.01 M solution and the pH lowered to 6.6 with 1 M NaH_2PO_4. Two units of enzyme in 1 ml of water were added. The mixture was digested for 1 hr at 37°C.

Electro-membrane partition was performed by the method previously reported(8), using glycine as a buffer. The solution in the cathode chamber was continuously mixed with the anode. Membranes of known pore size were inserted between the chambers so that a separation by molecular size was obtained.

Extract #2 was applied to Sephadex G100 chromatogram columns equilibrated with 0.1