Isolation and Properties of the Electrophoretic Components of Human Growth Hormone by Sephadex-Gel Filtration and Preparative Polyacrylamide-Gel Electrophoresis

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1. Human growth hormone was prepared from acetone-dried residues after extraction of gonadotrophins from pituitary glands. 2. Crude growth hormone was purified by gel filtration on Sephadex, resulting in a product that is soluble in water or 0.5% sodium chloride. It is painless on injection and shows a twofold increase in biological potency. Aggregation of growth hormone on Sephadex columns can be avoided by the addition of urea (6M) and EDTA (1mM) to the buffer. 3. Growth hormone appeared as a single component from Sephadex and ion-exchange columns and sedimented as a single boundary in the ultracentrifuge. In the circular disk electrophoresis, however, the growth hormone showed one faster and two slower-moving anionic components. 4. These components were isolated by preparative electrophoresis on polyacrylamide columns. The purified growth hormone and its three components sedimented as single boundaries with coefficients 2.62, 2.66, 2.66 and 2.83s respectively. 5. Amino acid analyses of the purified growth hormone and its components were closely related. End-group analysis of purified growth hormone and its components showed only phenylalanine at both *N*- and *C*-terminals. 6. The purified growth hormone and its components were essentially free of other pituitary hormones, but contained significant prolactin activity. The biochemical similarities among the electrophoretic components of human growth hormone and the presence of the same three components in the growth hormone prepared from a single human pituitary gland suggest polymorphism of a biologically active protein molecule.

Preparations of human growth hormone have shown three or four components in agar-gel (Laron & Assa, 1962a), starch-gel (Ferguson & Wallace, 1961; Barrett, Friesen & Astwood, 1962), polyacrylamide-gel (Reisfeld, Lewis, Brink & Steelman, 1962; Reisfeld, Hallows, Williams, Brink & Steelman, 1963) and starch-grain (W. M. Hunter, personal communication) electrophoreses. Reports in the literature have also indicated biological (Laron, Assa & Menache, 1963), immunological (Laron & Assa, 1962b) and chemical (W. M. Hunter, personal communication) similarities between some of these components. The present paper describes the isolation and properties of the three electrophoretic components of a preparation of human growth hormone.

MATERIALS AND METHODS

Preparation of growth hormone. Human pituitary glands (supplied by the National Pituitary Agency, Baltimore,

Md., U.S.A.) were ground finely in a Waring Blendor and the homogenate was acetone-dried. The acetone-dried powder was extracted at 4° for 12 hr. in 35% (v/v) ethanol containing ammonium acetate (10%, w/v) adjusted to pH 6.1 with acetic acid. The supernatant was recovered by centrifugation in a high-speed refrigerated centrifuge. The gonadotrophins in the supernatant were recovered by precipitation with 2 vol. of acetone. The residue was acetone-dried and used in 200g. batches for the preparation of growth hormone by the method of Raben (1959).

A single human pituitary gland was also extracted for growth hormone by the method of Raben (1959). The growth hormone obtained was filtered through Sephadex G-200 and the growth-hormone fraction was analysed by polyacrylamide-gel electrophoresis.

Exclusion chromatography on cross-linked dextran (Sephadex). Sephadex G-200, G-100 and G-50 (Pharmacia, Uppsala, Sweden) were allowed to swell overnight in 0.9% NaCl, washed three times with water and equilibrated with 0.05M-borate-0.05M-KCl buffer, pH 8.6, by repeated washings. Eluates from the column were either scanned through a Uvicord instrument (LKB Instrument Co., Stockholm, Sweden) or examined in a spectrophotometer (Beckman Instruments, Fullerton, Calif., U.S.A.) for E_{278} .

Crude growth hormone (200mg.) was suspended in

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4–5 ml. of water, solubilized by the addition of 6–8 drops of 0.1N-NaOH and applied to a column (2.5 cm. $\times$ 36 cm.) of Sephadex G-200 equilibrated with 0.05M-borate–0.05M-KCl buffer, pH 8.6. The column was eluted at a flow rate of 12 ml./hr. Three fractions (I, II and III) were obtained (Fig. 1a) and assayed for growth-hormone activity. Fraction II, containing the growth hormone, was recovered by dialysis for 24 hr. against water followed by freeze-drying.

Fraction II from Sephadex G-200 filtration in batches of 100 mg. was chromatographed on columns (2.5 cm. $\times$ 44 cm.) of Sephadex G-100 in 0.05M-borate–0.05M-KCl buffer, pH 8.6. Fractions IIA, IIB and IIC (Fig. 1b) were obtained and assayed for growth-hormone activity. The growth-hormone activity was recovered in fraction IIB.

Fraction IIB (30 mg.) was refiltered through Sephadex G-100 in 0.05M-borate–0.05M-KCl buffer, pH 8.6, without (Fig. 1c) and with (Fig. 1d) urea (6M) and EDTA (1 mM).

A 30 mg. portion of fraction IIB was chromatographed on a column (2 cm. $\times$ 34 cm.) of Sephadex G-50 in 0.05M-borate–0.05M-KCl buffer, pH 8.6, prepared both without (Fig. 1e) and with (Fig. 1f) urea (6M) and EDTA (1 mM). Growth-hormone fractions appeared unretarded from Sephadex G-50 columns.

Ion-exchange chromatography. Fraction IIB (30 mg.) was studied further by ion-exchange chromatography on DEAE-cellulose with a discontinuous pH gradient between 0.05M-sodium phosphate buffer, pH 7.7, and 0.05M-sodium acetate–acetic acid buffer, pH 3.5, in a Varigrad (Buchler Instrument Co., Fort Lee, N.J., U.S.A.). Growth-

Fig. 1. Gel filtration of: (a) human growth hormone on a column (2.5 cm. $\times$ 36 cm.) of Sephadex G-200 in 0.05M-borate–0.05M-KCl buffer, pH 8.6 (growth-hormone activity was associated with fraction II); (b) fraction II on a column (2.5 cm. $\times$ 44 cm.) of Sephadex G-100 in 0.05M-borate–0.05M-KCl buffer, pH 8.6; (c) fraction IIB on a column (2.5 cm. $\times$ 44 cm.) of Sephadex G-100 in 0.05M-borate–0.05M-KCl buffer, pH 8.6; (d) fraction IIB on a column (2.5 cm. $\times$ 44 cm.) of Sephadex G-100 in 0.05M-borate–0.05M-KCl buffer, pH 8.6, containing urea (6M) and EDTA (1 mM); (e) fraction IIB on a column (2 cm. $\times$ 34 cm.) of Sephadex G-50 in 0.05M-borate–0.05M-KCl buffer, pH 8.6; (f) fraction IIB on a column (2 cm. $\times$ 34 cm.) of Sephadex G-50 in 0.05M-borate–0.05M-KCl buffer, pH 8.6, containing urea (6M) and EDTA (1 mM). Sephadex G-200, G-100 and G-50 columns were eluted at the rate of 12 ml./hr.

hormone fraction IIB (30mg.) was chromatographed on columns of DEAE-Sephadex as described by Free & Sonenberg (1965) for bovine growth hormone and on Amberlite IRC-50 resin according to the procedures of Papkoff & Li (1958) and Li & Papkoff (1956) for bovine and human growth hormones respectively. The growth hormone was eluted from these columns in single fractions.

Ultracentrifugal analysis. Since growth-hormone fraction IIB appeared from Sephadex and ion-exchange columns as a single fraction, a 1% (w/v) solution of fraction IIB in 0.02M-phosphate buffer, pH 7.0, was examined in a Beckman Spinco model E ultracentrifuge. Sedimentation coefficients were calculated from the results obtained at five different concentrations of growth hormone and extrapolated to zero concentration. Temperature was maintained at $20 \pm 0.02^\circ$ with the RTIC (rotor temperature indicator control).

The sedimentation coefficients of the three electrophoretic components IIB₁, IIB₂ and IIB₃ obtained from the polyacrylamide-gel column (see below) were also determined in the same way.

Disk electrophoresis in polyacrylamide gel. Growth-hormone fractions IIB from Sephadex G-100, DEAE-cellulose, DEAE-Sephadex and Amberlite IRC-50 columns were subjected to analytical disk electrophoresis (Ornstein & Davis, 1961) in polyacrylamide gel (Canalco, Bethesda, Md., U.S.A.) in 0.05 M-tris-glycine buffer, pH 8.6, with a current of 5 ma for 30 min. at 125 v/tube (7 cm. $\times$ 0.5 cm.). The gels were stained with Amido Black 10B and excess of stain was removed by electrophoresis in 15% (v/v) acetic acid. Fraction IIB from the Sephadex G-100 and ion-exchange columns showed one faster and two slower-moving anionic components (1, 2 and 3 in Fig. 2a). These components were isolated by preparative polyacrylamide column electrophoresis and re-examined by analytical disk electrophoresis (Figs. 2b, 2c and 2d).

Preparative polyacrylamide-gel electrophoresis. The three electrophoretic components of human growth-hormone

fraction IIB were separated on a preparative polyacrylamide-gel column (catalogue no. 3-1700; Buchler Instruments Inc.) with the tris-urea discontinuous buffer system of Jovin, Chrambach & Naughton (1964). In a typical experiment 60 ml. of lower gel and 20 ml. of upper gel were used. A total of 30 mg. of fraction IIB was equilibrated with tris-HCl buffer, pH 8.1 (upper buffer), by overnight dialysis. The sample volume (20 ml.) containing 3% (w/v) of polyacrylamide and 5 drops of tracking dye was applied to the upper gel by the aid of micro-pump. Electrophoresis was performed at 820 v and 40 ma. The column was cooled to -1° . The column was eluted at a flow rate of 1 ml./min. and an automatic fraction collector was used. The eluate was scanned through a LKB Uvicord for E_{255} . The faster-moving component IIB₁ and the slower-moving components IIB₂ and IIB₃ (Fig. 3) obtained from the polyacrylamide column were dialysed against water for 48 hr. and freeze-dried. The remaining fractions contained no protein or growth-hormone activity. Disk-electrophoretic patterns of the components IIB₁, IIB₂ and IIB₃ are presented in Figs. 2(b), 2(c) and 2(d).

Amino acid analysis. Amino acid analyses of growth-hormone fraction IIB and the electrophoretic components IIB₁, IIB₂ and IIB₃ were performed according to the method of Spackman, Stein & Moore (1958) on an automatic amino acid analyser (model K-5000A; Phoenix Precision Co., Philadelphia, Pa., U.S.A.). A weighed sample of freeze-dried material was hydrolysed under vacuum in Pyrex glass tubes with 6N-HCl at 110° for 20 hr. The hydrolysate was concentrated to a small volume *in vacuo* in the presence of NaOH and freeze-dried. The freeze-dried material was dissolved again in a small amount of water and freeze-dried again to remove residual HCl. This sample was dissolved in 5 ml. of 0.2 N-sodium citrate buffer, pH 2.2, and filtered through glass wool into a 10 ml. volumetric flask. The glass wool was washed twice with 2 ml. of buffer and the final volume made up to 10 ml. Samples (2 ml.) were applied to columns (1 cm. $\times$ 140 cm. and 1 cm. $\times$ 50 cm.) of

Fig. 2. Polyacrylamide-gel disk electrophoresis of human growth-hormone fractions: (a) IIB; (b) IIB₁; (c) IIB₂; (d) IIB₃. Experimental details are given in the text.

Dowex 50 (X2) for amino acid analysis. The amount of protein applied to the column was calculated from nitrogen analyses of the sample of the hydrolysate. Tryptophan was determined spectrophotometrically as described by Dreze & Reith (1956).

End-group analysis. The C-terminal residues of fraction IIB and the electrophoretic components IIB₁, IIB₂ and IIB₃ were determined by digestion with di-isopropyl phosphorofluoridate-treated carboxypeptidase A (Fraenkel-Conrat, Harris & Levy, 1958) obtained from Worthington Chemical Corp., Freehold, N.J., U.S.A. The amino acids liberated were dinitrophenylated and identification of the DNP-amino acids was achieved by two-dimensional paper chromatography (Levy, 1954). Samples obtained every 30 min. for a period of 3 hr. from fraction IIB and preparations of components IIB₁, IIB₂ and IIB₃ showed the release of only phenylalanine as C-terminal residue. Phenylalanine appeared as the main product of carboxypeptidase activity from all preparations. The N-terminal residues of fraction IIB and components IIB₁, IIB₂ and IIB₃ were determined by the fluorodinitrobenzene method of Sanger (1945) and also found to be phenylalanine for all preparations.

Biological activity in rats. Crude growth hormone, fraction II and fraction IIB were immunoassayed by Dr Mary L. Parker, Washington University School of Medicine, St Louis, Mo., U.S.A., for growth-hormone content by the haemagglutination of cells coated with growth hormone (Read & Bryan, 1960). Crude human growth hormone, fraction IIB and components IIB₁, IIB₂ and IIB₃ from the polyacrylamide column were also assayed by the Endocrine Laboratories, Madison, Wis., U.S.A., for growth-hormone

activity by the tibia test (Greenspan, Li, Simpson & Evans, 1949), for prolactin by crop-sac stimulation in pigeons (Lyons & Page, 1935), for adrenocorticotrophic hormone by the adrenal ascorbic acid depletion method of Sayers, Sayers & Woodbury (1948) and for thyrotrophic hormone by the increase in the thyroid weights of 1-day-old cockerels. The follicle-stimulating-hormone and luteinizing-hormone activities were determined by the methods of Steelman & Pohley (1953) and Parlow (1961), with NIH-FSH-S2 and NIH-LH-S4 as standards respectively.

Biological activity in man. Crude growth hormone and fractions I, II and IIB were administered intramuscularly in 5 or 10 mg. daily doses to two patients with hypopituitarism and to one normal subject during constant diets and constant living conditions. Urines were analysed for nitrogen, phosphorus, calcium, sodium and potassium as described previously (Reifenstein, Albright & Wells, 1945; Henneman, Forbes, Moldawer, Dempsey & Carroll, 1960).

RESULTS AND DISCUSSION

There is increasing interest in the recovery of all hormones during extraction of human pituitaries. In the present study, gonadotrophins were extracted before growth hormone. The growth-hormone yields of 3.56 g. and 3.84 g./100 g. of acetone-dried powder for two separate batches (Table 1) were not significantly different from the yields of 3.1–4.6 g. reported by Raben (1959). The yield of the crude gonadotrophin fraction was 3.35 g./100 g. of acetone-dried powder and contained $243 \pm 53.1\%$ of the standard NIH-FSH-S2 activity as determined by the augmentation assay of Steelman & Pohley (1953).

The results from 13 Sephadex G-200 columns indicated that inactive fraction I and growth-hormone fraction II (Fig. 1a) contained 22 and 78% respectively of the material recovered from the column (Table 1). Fraction III did not show any yield as protein and was biologically inactive. This distribution of protein in fractions I and II was constant for loads of crude growth hormone ranging from 60 to 200 mg. applied to Sephadex G-200 columns. When fraction II was chromatographed on Sephadex G-100 columns (Fig. 1b) the average of seven experiments showed that 60% of the material appeared in fraction IIB, containing

Fig. 3. Electrophoretogram of a 30 mg. sample of fraction IIB on a preparative polyacrylamide-gel column. Experimental details are given in the text. Growth-hormone fractions IIB₁, IIB₂ and IIB₃ were obtained. Dye and the preceding fraction showed no protein yield or growth-hormone activity.

Table 1. Yield and activity of human growth hormone

Experimental details are given in the text. Bovine growth hormone (Wilhelmi no. 503) was used as standard for the activity assays. Results are given as means $\pm$ s.e.m. where appropriate, with the numbers of experiments in parentheses.

Preparation	Yield (g./100 g. of acetone-dried powder)	Activity	
		(units/mg.)	(total units)
Crude growth hormone	3.7 (2)	0.78	2886
Sephadex G-200 fraction II	2.9 ± 0.07 (13)	1.5	4350
Sephadex G-100 fraction IIB	1.7 ± 0.1 (7)	0.87	1479

the growth hormone, and that 40% of the material was in the unretarded inactive fraction IIA.

When fraction IIB from the Sephadex G-100 column was rechromatographed on a Sephadex G-100 column (Fig. 1c) 20% of the material again appeared unretarded. However, when 30mg. of fraction IIB was equilibrated with 0.05M-borate-0.05M-potassium chloride buffer, pH 8.6, containing urea (6M) and EDTA (1mM) by overnight dialysis and chromatographed on a column (2cm. $\times$ 40cm.) of Sephadex G-100 (Fig. 1d) the growth-hormone protein appeared as a single retarded fraction with no indication of unretarded material. From the Sephadex G-50 column (Fig. 1e) fraction IIB appeared unretarded with a slight faster-moving shoulder, indicating the formation of high-molecular-weight material. The effect of 6M-urea in preventing the formation of high-molecular-weight material was confirmed by the appearance of a single symmetrical retarded component when 30mg. of fraction IIB from the Sephadex G-100 column was rechromatographed on a column (2cm. $\times$ 40cm.) of Sephadex G-50 (Fig. 1f) in 0.05M-borate-0.05M-potassium chloride buffer, pH 8.6, containing urea (6M) and EDTA (1mM). These results suggest aggregation of fraction II into a high-molecular-weight material during

dialysis, freeze-drying and refractionation through Sephadex G-100, and further suggest that this aggregation of growth hormone can be prevented by the addition of urea (6M) and EDTA (1mM) to the 0.05M-borate-0.05M-potassium chloride buffer, pH 8.6. W. M. Hunter (personal communication) has also obtained evidence for the transformation of fraction II-like material into high-molecular-weight fraction I by reheating to 70° the acetic acid-acetone extract of whole acetone-dried pituitaries.

Sedimentation of fraction IIB as a single boundary in the analytical centrifuge with a coefficient of 2.62s indicated that filtration through Sephadex columns has been successful in selecting protein of similar molecular weight. The $S_{20,w}$ value 2.62s is in good agreement with the coefficient 2.5s for human growth hormone described by Squire & Pederson (1961).

Fraction IIB and growth-hormone fractions from Sephadex, DEAE-cellulose, DEAE-Sephadex and Amberlite IRC ion-exchange columns showed three anionic components, IIB₁, IIB₂ and IIB₃ (Fig. 2a), in polyacrylamide-gel disk electrophoresis. When the components from the preparative polyacrylamide-gel columns were re-examined in analytical gels, component IIB₁ appeared as a

Table 2. *Amino acid composition of human growth hormone*

Experimental details are given in the text. The results are expressed as residues/mol. of mol.wt. 27100 (Wilhelmi, 1959).

Growth-hormone preparation . . .	Amino acid composition (residues/mol.)					Wilhelmi (1959)
	IIB	IIB ₁	IIB ₂	IIB ₃	Dixon & Li (1962)	
Lys	12	10	12	12	12	12
His	5	5	5	5	4	5
Arg	14	11	12	13	13	13
Asp	25	24	27	25	25	31
Thr	14	11	13	13	13	14
Ser*	21	22	20	21	21	20
Glu	29	25	31	27	32	36
Pro	8	5	8	7	12	12
Gly	10	14	13	13	12	15
Ala	10	9	9	9	11	11
CyS	5	4	4	6	5	4
Val	10	9	10	10	11	9
Met	3	4	4	4	4	6
Ile + Leu	39	36	40	42	38	38
Tyr	9	5	8	9	9	9
Phe	18	14	16	16	14	14
Trp†	1	1	1	1	1	1
NH ₃	37	38	44	46	30	
Total‡	233	209	234	232	237	250

* Values corrected for 10% loss during hydrolysis.

† Determined spectrophotometrically (Dreze & Reith, 1956).

‡ Ammonia not included.

single band (Fig. 2b); components IIB₂ and IIB₃ showed, however, one major and a light diffuse band (Figs. 2c and 2d).

Fractions IIB and IIB₁, IIB₂ and IIB₃ sedimented as single boundaries in the analytical ultracentrifuge with sedimentation coefficients 2.62, 2.66, 2.66 and 2.83s respectively. This observation implies that fractions IIB₁, IIB₂ and IIB₃ may exist as separable entities in precursor fraction IIB. The disk-electrophoretic pattern of human growth hormone prepared from a single pituitary and filtered through Sephadex G-200 also showed three anionic components similar to those of the growth hormone prepared from a large batch of glands. One may conclude from this that each pituitary contains these three peptides with growth-hormone activity. The possibility remains that chemical procedures may cause the appearance of three subfractions of a single protein molecule.

The amino acid compositions of fractions IIB₁, IIB₂ and IIB₃ were compared with that of fraction IIB and with the published data of Wilhelmi (1959) and Dixon & Li (1962) on growth-hormone preparations (Table 2). The total amide nitrogen residues in components IIB₁, IIB₂ and IIB₃ were 38, 44 and 46 respectively. The amide nitrogen residues imply six and eight more carboxyl groups in IIB₁ than in IIB₂ and IIB₃ respectively, and two more carboxyl groups in IIB₂ than in IIB₃. These differences may be related to the electrophoretic mobilities of the components in the polyacrylamide gel. Since the amino acid analyses of fractions IIB, IIB₁, IIB₂ and IIB₃ are not widely divergent, the differences in total nitrogen residues might be due to loss of one or more side-chain amide groups. The similarity of the amino acid compositions of fraction IIB to those of the growth-hormone preparations of Wilhelmi (1959) and Dixon & Li (1962) is noted. W. M. Hunter (personal communication) and Meisinger, Cirillo, Davis & Reisfeld (1964) have also shown the similarity in the amino acid compositions of the growth hormone and electrophoretic components obtained by starch-grain and polyacrylamide-gel electrophoresis.

Preliminary end-group analyses of fractions IIB, IIB₁, IIB₂ and IIB₃ indicated the presence of phenylalanine at both the N- and C-terminals. During C-terminal analysis phenylalanine appeared as the main product of carboxypeptidase digestion.

Biological activity in rats. Bioassay revealed 0.78 unit of growth hormone/mg. in crude growth hormone, 1.5 units/mg. in fraction II and 0.87 unit/mg. in fraction IIB. These results indicate that the filtration of crude growth hormone on Sephadex G-200 resulted in the elimination of 22% of biologically inactive high-molecular-weight material with approximately twofold increase in biological activity (Table 1). Reisfeld *et al.* (1963) and W. M.

Hunter (personal communication) have also claimed a twofold increase in the specific activity of the growth hormone by purification on Sephadex G-200 columns. Sephadex G-100 filtration apparently resulted in decreased activity that may be

Table 3. *Bioassay of preparations of human growth hormone*

Results are given as means $\pm$ S.E.M. where appropriate, with the numbers of animals in parentheses.

Preparation	Total dose/ animal	Results
Growth hormone: (a) immunoassay by the method of Read & Bryan (1960)		
		Activity (units/mg.)
Crude growth-hormone prep. 1		0.78
Fraction II		1.5
Fraction IIB		0.87
Standard (Wilhelmi no. 503)		1.0
Growth hormone: (b) tibia test in hypophysectomized rats		
		Epiphyseal cartilage width (μ)
Crude growth-hormone prep. 2	200 μ g.	232 $\pm$ 5.5* (5)
Fraction IIB	75 μ g.	232 $\pm$ 18.5* (2)
Component IIB ₁	100 μ g.	209 $\pm$ 7.8* (5)
Component IIB ₂	200 μ g.	256 $\pm$ 6.4* (5)
Component IIB ₃	200 μ g.	223 $\pm$ 5.6* (5)
Standard (NIH-GH-B7)	100 μ g.	208 $\pm$ 29.4* (3)
Control	0.9% NaCl	137 $\pm$ 4.7 (6)
Prolactin: stimulation of pigeon crop sac		
		Degree of stimulation
Crude growth-hormone prep. 2	100 μ g.	4.0+ (4)
Fraction IIB	50 μ g.	3.5+ (3)
Component IIB ₁	100 μ g.	3.8+ (4)
Component IIB ₂	100 μ g.	4.0+ (4)
Component IIB ₃	100 μ g.	3.6+ (4)
Standard (NIH-P-S3)	0.1 unit	3.3+ (9)
Thyrotrophic hormone: increase in the thyroid weight of 1-day-old cockerels		
		Wt. of thyroid (mg.)
Fraction IIB	300 μ g.	4.03 $\pm$ 0.3 (10)
Standard (USP-TSH)	0.6 unit	6.88 $\pm$ 0.35 (10)
Control	0.9% NaCl	4.05 $\pm$ 0.24 (10)
Adrenocorticotrophic hormone: depletion of rat-adrenal ascorbic acid		
		Ascorbic acid (mg./100g. of tissue)
Fraction IIB	200 μ g.	172.38 $\pm$ 14.7 (4)
Standard ACTH (Parke, Davis and Co.)	0.05 unit	75.86 $\pm$ 9.3 (5)
Control	0.9% NaCl	167.6 $\pm$ 8.1 (5)

* $P < 0.001$.

due to the polymerization of growth hormone into high-molecular-weight inactive protein. Crude growth hormone and the electrophoretic components were free of thyrotrophic-hormone and adrenocorticotrophic-hormone activities. Crude growth hormone, fraction IIB and components IIB₁, IIB₂ and IIB₃ contained significant amounts of prolactin activity, indicating no apparent separation of prolactin and growth-hormone activities in electrophoretic components (Table 3). Dose levels of 75 and 150 μ g. of fraction IIB did not show any follicle-stimulating-hormone activity in the Steelman & Pohley (1953) assay. Also, 9 and 45 μ g. of fraction IIB failed to deplete ovarian ascorbic acid (Parlow, 1961), indicating the absence of luteinizing hormone at these dose levels.

Biological activity in man. In a 42-day complete balance study in a 17-year-old white male with idiopathic hypopituitarism crude growth hormone and fractions I, II and IIA and IIB were compared.

Fraction II was more active than crude human growth hormone in increasing plasma free fatty acids and urinary calcium and in decreasing urinary nitrogen, phosphorus, sodium and potassium. Fraction IIA was inactive and permitted urinary values to return towards control levels. Fraction IIB was slightly more active than fraction II in increasing urinary calcium and decreasing urinary nitrogen. Thus, in spite of the recognized tendency for the metabolic responses to human growth hormone to decrease with time, it was apparent that fraction IIB was slightly more active than fraction II, which in turn was much more active than crude human growth hormone. Fractions IA and IIA were virtually inactive.

Crude human growth hormone and fractions I and II were studied in a 15-year-old boy with idiopathic hypopituitarism. Fraction II was more active than crude growth hormone in decreasing urinary nitrogen, phosphorus, potassium and hydroxyproline. Fraction I was virtually inactive.

Fractions I and II were compared in a 48-year-old obese normal woman. Fraction II was more active than fraction I in decreasing urinary nitrogen, phosphorus and potassium and in increasing urinary calcium. Fraction I was virtually without effect.

Solubility of purified fractions. Growth hormone prepared by Raben's (1959) procedure is usually solubilized by the addition of 0.1N-hydrochloric acid, which makes the preparation painful on injection. Filtration of crude growth hormone through Sephadex G-200 results in a product that is freely soluble in water or 0.5% sodium chloride and that is painless on injection.

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