Separation and Properties of Multiple Components of Bovine Growth Hormone

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SUMMARY

Starch gel electrophoresis at alkaline pH of bovine growth hormone preparations from a number of sources has revealed a common pattern characterized by a major anionic component and two minor faster moving components. Gel filtration studies of the hormone indicate that all three components are of similar molecular weight. A method has been developed for separation of the components by chromatography on diethylaminoethyl Sephadex. All three components were biologically active and displayed similar amino acid compositions.

In this paper, electrophoretic patterns of a number of different bovine growth hormone preparations are compared. Gel filtration studies of the hormone indicate that all three components are of similar molecular weight. A method has been developed for separation of the components by chromatography on diethylaminoethyl Sephadex. All three components were biologically active and displayed similar amino acid compositions.

The advent of gel electrophoresis techniques has allowed the detection of heterogeneity (1, 2) in preparations of bovine growth hormone, a protein previously considered homogeneous by various available criteria of purity (3). Presence of growth hormone activity in the various components of such preparations was demonstrated by Ferguson and Wallace (2) following a partial separation of the components by chromatography. More recently, Reusser (4) has submitted evidence of similar immunological activity in each of a series of bovine growth hormone components separated by electrophoresis on a basic polyacrylamide gel. The heterogeneity raises a question concerning the relationship among the multiple forms of the hormone, a problem requiring separation of the components into electrophoretically pure fractions suitable for comparative studies.

In this paper, electrophoretic patterns of a number of different bovine growth hormone preparations are compared. A chromatographic method for separation of the electrophoretic components has been developed, and evidence will be presented indicating the similarity of the components with respect to molecular size, biological activity, and amino acid composition.

EXPERIMENTAL PROCEDURE

Growth Hormone Preparations—BGH1 was prepared by the method of Dellacha and Sonenberg (5), except that Sephadex G-100 rather than G-75 was employed for the final gel filtration step. Other preparations were obtained by an alternate procedure, with the use of a bovine pituitary extract rich in growth hormone ("crude growth hormone powder," purchased from Parke, Davis) as starting material. At temperatures of 0-4°C, 10 g of "crude growth hormone powder" were suspended in 200 ml of 0.036 M sodium acetate buffer, pH 4.0. After centrifugation for 15 min at 35,000 ¥ g to remove the insoluble fraction, the supernatant was dialyzed against distilled water and lyophilized to yield 3 to 4 g of product. Appropriate quantities of the product were redissolved in 0.036 M sodium acetate buffer, pH 4.0, and subjected to gel filtration on columns of Sephadex G-100 eluted with the same buffer (Fig. 1). Protein in the eluting fractions was determined by measurement of absorbance at 278 nm, appropriate dilutions of the fractions being made when absorbance was too high to allow measurement. Growth hormone was obtained by dialysis and lyophilization of the second peak to emerge from the columns. The yield of BGH was approximately 15% in terms of the original quantity of "crude growth hormone powder" employed. The nature of the material in the first peak has not been extensively studied. It contains only low levels of growth promoting activity, however, and yields a diffuse pattern upon starch gel electrophoresis at pH 9.5.1 It may be noted that the gel filtration step represents an identical stage of purification in each of the isolation procedures described above. Additional BGH preparations were donated by the Endocrinology Study Section of the National Institutes of Health.

Starch Gel Electrophoresis—Electrophoresis experiments were performed in a cold room at 4°C, in horizontal gels 25 cm long and 7.5 ¥ 0.7 cm in cross section. Techniques for gel preparation, sample introduction, and staining were those described by Smithies (6). Glycine buffer, 0.1 M, pH 9.50, was prepared by adjustment of the pH at 25°C with 5 M NaOH. Glycine buffer,

1 The abbreviations used are: BGH, bovine growth hormone; NIH-GH-B1, etc., bovine growth hormone preparations supplied by the Endocrinology Study Section of the National Institutes of Health.

2 Unpublished experiments.
0.033 M, pH 9.5, was obtained by dilution of the 0.1 M buffer. Starch gels were prepared from suspensions of 14 g of starch (“hydrolyzed”) (Connaught Medical Research Laboratories) per 100 ml of 0.033 M glycine buffer, and were covered with Saran Wrap 20 min after pouring. Protein samples were prepared by precipitation with 0.1 M glycine buffer. Electrode vessels contained 0.1 M glycine buffer, and were connected to the gel through sponge bridges. The duration of electrophoresis runs was 3 hours or longer at 30 mA, after which the gels were bisected horizontally and stained with Amido black 10B.

**Gel Filtration Experiments**—For experiments involving comparison or estimation of the molecular weights of BGH components, a Sephadex G-100 column, 1.9 X 92 cm, was employed. All experiments were conducted in a cold room at 4°. The buffer employed was 0.1 M Tris, pH 8.80, prepared at 25° by addition of concentrated HCl to the proper pH. For the estimation of molecular weights, the Tris buffer was 0.1 M with respect to KCl to diminish any possible ion exchange effects which might occur between the proteins and Sephadex. Effluent fractions of 5 or 10 ml were collected, and protein content was measured by absorbance at 278 mµ. In the experiments designed to estimate molecular weights, peak elution volumes were measured to the nearest milliliter by use of a recording ultraviolet absorption meter and continuous flow cell connected to the bottom of the column. Measurement of void volume was made with blue dextran 2000 (Pharmacia), a dextran derivative of molecular weight 2 X 10⁶ which absorbs strongly at 278 mµ. A number of standard purified proteins were used in the estimation of molecular weights. These included crystalline preparations of bovine pancreatic ribonuclease, soybean trypsin inhibitor, and ovalbumin. Other preparations and their sources were: bovine serum albumin (Fraction V), Armour Pharmaceuticals; human γ-globulin (Fraction II), Hyland; ovine luteotropin (prolactin), Lot 71713, Squibb; and equine heart cytochrome c, Mann. Molecular weights of these proteins were taken from recent literature (7). For prolactin, a molecular weight of 23,400 was used (8).

**Chromatography on DEAE-Sephadex**—Chromatography was performed with the use of 0.1 M Tris buffer, pH 8.80, prepared at 25° by addition of concentrated HCl. Buffers for stepwise elution were prepared by addition to the 0.1 M Tris buffer of NaCl to concentrations of 0.03, 0.10, or 1.0 M. DEAE-Sephadex A-50 (Medium) (Pharmacia) was washed with 0.5 M HCl and 0.5 M NaOH, and equilibrated with 0.1 M Tris, pH 8.80, before being poured into columns. Beginning with column pouring, all operations were conducted at 4°. Prior to chromatography, the DEAE-Sephadex columns were washed with at least 5 column volumes of 0.1 M Tris buffer to assure complete equilibration.

Samples of BGH were suspended in quantities of 0.1 M Tris, pH 8.80, sufficient to allow nearly complete dissolution (the solubility of most preparations was approximately 5 mg per ml at 4°). When significant quantities of material remained undissolved following extraction and centrifugation, one or more additional extractions were performed to increase the yield. Following adsorption of the dissolved BGH on the columns, stepwise elutions were performed with the 0.1 M Tris-NaCl solutions. Flow rates of 7 to 14 ml per cm² per hour were maintained. Protein in effluent fractions of 10 ml was measured by ultraviolet absorption at 278 mµ. To recover protein, the pooled fractions were exhaustively dialyzed against distilled water and then lyophilized.

**Bioassay of Growth Hormone**—Growth hormone activity was determined in hypophysectomized rats (7 to 10 per group) by the method of Marx, Simpson, and Evans (9), which uses weight gain during a 10-day treatment interval. Daily injections at two dose levels from 15 to 80 µg per day were used for all preparations. Growth hormone potency was expressed in terms of the U.S.P. reference standard, which was similarly administered at two dose levels in each assay. All assays were performed by Biometrics, Inc., Chicago.

**Amino Acid Analyses**—The determinations of amino acid composition were performed at the Institute for Organic Chemistry, University of Basel. Protein samples were hydrolyzed in 6 N HCl at 110° under reduced pressure for 24 and 72 hours. Aliquots of hydrolysates were analyzed on an amino acid analyzer, according to the techniques of Spackman, Stein, and Moore (10).

**RESULTS**

**Heterogeneity of BGH Preparations**—The gel electrophoresis patterns at pH 9.5 of BGH samples prepared by the method of Dellacha and Sonenberg (5), isolated from “crude growth hormone powder,” or obtained from the Endocrinology Study Section, National Institutes of Health, are shown in Fig. 2. The three patterns are similar, each containing a major anionic component and two minor components of greater mobility. In Fig. 3, a similar comparison is made of five BGH preparations from the Endocrinology Study Section. Preparations NIH-GH-B6, -B7, and -B9 display identical patterns, again characterized by three electrophoretic components in the same proportion as shown in Fig. 2. The patterns of NIH-GH-B1 and NIH-GH-B2, however, are characterized by a greater proportion of more
FIG. 2. Starch gel electrophoresis of BGH preparations at pH 9.5. From the top, the samples are BGH prepared by the method of Dellacha and Sonenberg (6), BGH isolated from “crude growth hormone powder” (Parke, Davis), and NIH-GH-B6. The duration of the run was 190 min.

FIG. 3. Starch gel electrophoresis of NIH-GH preparations at pH 9.5. The duration of the run was 3 hours.

FIG. 4. Gel filtration at pH 8.80 of BGH on a column, 1.9 X 92 cm, of Sephadex G-100. The soluble fraction of 102 mg of BGH in 20 ml of 0.1 M Tris buffer, pH 8.80, was applied to the column; elution was carried out with the same buffer. Fractions of 5 ml were collected at a rate of 13 ml per hour.

FIG. 5. Starch gel electrophoresis at pH 9.5 of fractions of BGH obtained from gel filtration on Sephadex G-100 at pH 8.80. The duration of the run was 210 min.

rapidly migrating bands and poorer resolution due to smearing. The three principal components common to the BGH preparations shown in Fig. 2, as well as to most of the NIH-GH preparations, will be designated Components 1, 2, and 3, in order of increasing electrophoretic mobility.

Gel Filtration of BGH—Inasmuch as BGH Components 1, 2, and 3 emerge as one peak during the final gel filtration step of purification, their similarity with respect to molecular size is indicated. Nevertheless, a more sensitive experiment was designed to exploit any slight differences in size or shape which might distinguish the components. A solution of BGH in 0.1 M Tris buffer, pH 8.80, was subjected to gel filtration on Sephadex G-100 equilibrated with the same buffer. As shown in Fig. 4, a nearly symmetrical peak was obtained, with a very small shoulder emerging on the leading edge. Four pooled fractions were obtained, from which protein was recovered by dialysis and lyophilization. Starch gel electrophoresis of the four fractions is illustrated in Fig. 5. Although Fraction I, the material from the shoulder, gave a diffuse pattern, the similarity of the three fractions from the main peak indicates that no separation of components occurred during the gel filtration. Thus no difference in molecular weights of the components was apparent at either acidic or basic pH.

Additional experiments were performed to estimate the molecular weight of the BGH components by a comparison of their gel filtration behavior with that of other well characterized
reference proteins. Such applications have been described recently by Whitaker (11) and Andrews (7). The results are shown in Fig. 6, plotted in terms of peak volume/void volume $\left(\frac{V_e}{V_0}\right)$ against log molecular weight (11). Interpolation with the $\frac{V_e}{V_0}$ obtained for the largest BGH sample (19 mg) yielded a value of 41,000 for the molecular weight of the hormone. Evidence for a concentration dependence was found, however, possibly indicative of dissociation of the molecule, inasmuch as samples of 11 and 2 mg yielded molecular weight values of 38,000 and 35,000, respectively.

Chromatography on DEAE-Sephadex—Fig. 7 illustrates the partial fractionation by chromatography of 180 mg of BGH on a column of DEAE-Sephadex. Elution of the peaks was performed by stepwise increase in the NaCl concentration in the eluting buffer, each change being applied following complete elution of the preceding peak. Of the protein applied to the column, approximately 94% was recovered in the collected fractions as measured by absorbance at 278 nm. This included 77% and 13% of the material in the two peaks eluted by 0.03 M and 0.10 M NaCl, respectively. Fractions were pooled as shown, dialyzed, and lyophilized.

Starch gel electrophoretic patterns of the preparations, shown in Fig. 8, indicate that Component 1 emerges in the initial peak and that Component 2 constitutes a large proportion of the material in the trailing edge of the peak. The second peak, eluted with 0.10 M NaCl, contained predominantly Component 3. A small amount of additional material eluted with 1.0 M NaCl was insoluble in the gel electrophoresis system.

Additional similarity in the heterogeneity of the BGH prepared in this laboratory and that of a National Institutes of Health preparation, NIH-GH-B6, was observed when the three principal components of the latter preparation showed chromatographic and electrophoretic behavior similar to that of the BGH preparation shown in Figs. 7 and 8. The NIH-GH-B6 differed, however, in that the peak eluted with 1.0 M NaCl contained at least three electrophoretic components not found in the BGH samples which had undergone a gel filtration step. When chromatographic fractionation was attempted with NIH-GH-B1, the components were eluted less sharply from the column and no separation was obtained. Thus it appears that the components in this preparation not only are present in a different proportion, but may also differ qualitatively from those in later NIH-GH preparations.

Further purification and evidence of stability of the partially separated BGH components are shown in Fig. 9. By rechromatography under conditions identical with those employed during the initial chromatography, the respective components were eluted at salt concentrations identical with those employed during the initial chromatography. Purified Component 1 revealed negligible contamination with other peaks. Rechromatography

Fig. 6. Plots of elution volume/void volume ($\frac{V_e}{V_0}$) against log molecular weight for a series of proteins on Sephadex G-100. The column, 1.9 X 92 cm, was eluted at a rate of 10 ml per hour with 0.1 M Tris, pH 8.80, containing 0.1 M KCl. The BGH sample was 19 mg; other samples varied from 6 to 12 mg. Each sample was applied in a 5-ml volume of buffer.

FIG. 7. Chromatography of BGH on DEAE-Sephadex. A 40-ml sample containing 180 mg of protein was applied to the column, 1.85 X 19 cm, and eluted by stepwise increases of NaCl concentration in 0.1 M Tris buffer, pH 8.80, as shown by arrows. Fractions were pooled as indicated by Numerals, I to VI.

FIG. 8. Starch gel electrophoresis at pH 9.5 of BGH Fractions I to VI obtained by chromatography on DEAE-Sephadex (see Fig. 7). The duration of the run was 210 min.
Multiple Components of Bovine Growth Hormone

Vol. 241, No. 21

0.8

0.6

0.4

0.2

0.0

200 400 600 800

M effluent

Fig. 9. Rechromatography of BGH fractions on DEAE-Sepha-
dex. In A, a 30-ml sample containing 72 mg of Component 1 was
subjected to rechromatography. The sample volume in B was 50
ml, containing 82 mg of a mixture of Components 1 and 2. C
illustrates rechromatography of a sample of 25 ml containing 60
mg of Component 3. The columns, 1.85 × 15 to 18 cm,
were eluted
by stepwise increase of NaCl concentration in 0.1
M
Tris, pH 8.80,
as shown by the arrows.
Purified components were isolated from
fractions indicated by shading.

of a mixture of Components 1 and 2 resulted in a peak with a
pronounced shoulder, elution of which was accelerated by application
of 0.10 M NaCl. Rechromatography of a sample rich in
Component 3 revealed complete absence of the first peak. The
shaded areas in the diagram indicate the fractions which were
pooled, dialyzed, and lyophilized prior to electrophoresis and
amino acid analysis.

Electrophoretic patterns of the rechromatographed components
appear in Fig. 10. Whereas Components 1 and 3 appear
as nearly homogeneous electrophoretic zones, Component 2
reveals some contamination.

Biological Activity—Growth-promoting potency assayed by
weight gain in hypophysectomized rats is shown in Table I for
a number of preparations of the separated BGH components. It
is evident that the minor components possess growth-promoting
levels at least as great as that of Component 1, the main constituent of most BGH preparations.

Amino Acid Composition—Amino acid analyses of rechromato-

graphed preparations of Components 1, 2, and 3, isolated as
shown in Fig. 9, are presented in Table II. The number of
residues was calculated from single analyses of 24-hour hydrolysates, except for those amino acids for which the values decreased
or increased with time. For the amino acids that decomposed,
appropriate corrections were applied by extrapolation to zero
time from the values obtained from both 24- and 72-hour hydrolysates. The values for isoleucine and leucine were taken
from 72-hour hydrolysates. The molecular weight of 45,700
used in calculating the number of residues was derived from
values originally employed by Li (3) and Parcells (13) for the
same purpose. For comparison, analyses of BGH published by
two other laboratories are included. The results of Parcells (13)

Fig. 10. Starch gel electrophoresis at pH 9.5 of BGH and Com-
ponents 1, 2, and 3 isolated by rechromatography experiments
shown in Fig. 9. The duration of the run was 3 hours.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Potency ± S.E. (U.S.P. units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component 1</td>
<td></td>
</tr>
<tr>
<td>F-II-133</td>
<td>0.87 ± 0.21</td>
</tr>
<tr>
<td>F-II-174</td>
<td>1.20 ± 0.28</td>
</tr>
<tr>
<td>F-II-222-I</td>
<td>0.78 ± 0.18</td>
</tr>
<tr>
<td>F-II-224-I</td>
<td>1.34 ± 0.36</td>
</tr>
<tr>
<td>F-II-273-I</td>
<td>0.80 ± 0.16</td>
</tr>
<tr>
<td>F-II-275-I</td>
<td>0.66 ± 0.20</td>
</tr>
<tr>
<td>F-II-286-I</td>
<td>2.01 ± 0.42</td>
</tr>
<tr>
<td>Average</td>
<td>1.04</td>
</tr>
<tr>
<td>Component 2</td>
<td></td>
</tr>
<tr>
<td>F-II-177-II</td>
<td>2.39 ± 0.56</td>
</tr>
<tr>
<td>F-II-282-III</td>
<td>0.88 ± 0.18</td>
</tr>
<tr>
<td>Average</td>
<td>1.04</td>
</tr>
<tr>
<td>Component 3</td>
<td></td>
</tr>
<tr>
<td>F-II-72-IV</td>
<td>0.77 ± 0.21</td>
</tr>
<tr>
<td>F-II-173-II</td>
<td>1.42 ± 0.28</td>
</tr>
<tr>
<td>F-II-280-I</td>
<td>0.53 ± 0.18</td>
</tr>
<tr>
<td>Average</td>
<td>1.04</td>
</tr>
</tbody>
</table>
were obtained from three samples of BGH prepared by an early method of Li (14). The values obtained by Wolfenstein, Santomé, and Paladini (12) were from a BGH sample prepared by the procedure of Dellacha and Sonenberg (5). Comparison of the data for the three BGH components reveals a similarity in their compositions. Moreover, the composition of Component 1 agrees well with those of BGH preparations analyzed by the other laboratories.

Stability of Bovine Growth Hormone—During the handling of the preparations, no interconversions were noted among the components. Transformations of BGH components in alkaline solution have been observed by Lewis (15), however, and it was therefore of interest to examine a purified BGH preparation for evidence of such behavior. A 0.5% solution of BGH was incubated at 25° in a 0.025 M Na₂CO₃ buffer, pH 10, a medium used by Lewis (15) to promote interconversion. After 48 hours of treatment, the BGH revealed no alteration of electrophoretic pattern and no decrease in biological activity.

Because an early step in the isolation procedure for BGH involves extraction with Ca(OH)₂ at pH 11.5, the ability of this medium to cause transformation of purified BGH was examined. A 0.5% solution of BGH Component 1 was dialyzed against 0.005 M Ca(OH)₂, pH 11.5, for 24 hours at 4°. Following recovery of the treated material, no change was observed in its electrophoretic pattern. Treatment of 0.1% or 0.2% solutions of Component 1 with NaOH was subsequently performed to determine the limit of stability of the protein in alkaline. Incubation in 0.01 M NaOH at 4° for 24 hours was without effect on the electrophoretic pattern. A partial conversion of Component 1 to faster moving zones at 4° within 24 hours was noted only when the NaOH concentration was increased to 0.1 M. The latter transformation was accelerated at room temperature, as evidenced by complete conversion of Component 1 to faster moving components within 24 hours.

**TABLE II**

<table>
<thead>
<tr>
<th>Component</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Wolfenstein et al. (12)</th>
<th>Parcells (13)</th>
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<tbody>
<tr>
<td>Lysine</td>
<td>23.1</td>
<td>23.2</td>
<td>23.2</td>
<td>26.6</td>
<td>22-23</td>
</tr>
<tr>
<td>Histidine</td>
<td>6.5</td>
<td>6.4</td>
<td>6.8</td>
<td>6.7</td>
<td>7</td>
</tr>
<tr>
<td>Arginine</td>
<td>26.1</td>
<td>26.0</td>
<td>25.3</td>
<td>26.8</td>
<td>24-26</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>33.4</td>
<td>34.1</td>
<td>34.6</td>
<td>33.0</td>
<td>35-36</td>
</tr>
<tr>
<td>Threonine</td>
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<td>25.2</td>
<td>25.4</td>
<td>24.0</td>
<td>24-26</td>
</tr>
<tr>
<td>Serine</td>
<td>25.8</td>
<td>26.2</td>
<td>26.7</td>
<td>25.1</td>
<td>24-28</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>47.7</td>
<td>48.8</td>
<td>49.0</td>
<td>50.7</td>
<td>50-52</td>
</tr>
<tr>
<td>Proline</td>
<td>13.4</td>
<td>13.4</td>
<td>15.4</td>
<td>12.7</td>
<td>13-15</td>
</tr>
<tr>
<td>Alanine</td>
<td>21.0</td>
<td>21.0</td>
<td>23.3</td>
<td>21.8</td>
<td>22-23</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>29.8</td>
<td>29.5</td>
<td>29.4</td>
<td>30.3</td>
<td>29-30</td>
</tr>
<tr>
<td>Valine</td>
<td>10.1</td>
<td>8.2</td>
<td>10.2</td>
<td>9.7</td>
<td>8</td>
</tr>
<tr>
<td>Methionine</td>
<td>14.0</td>
<td>13.8</td>
<td>15.2</td>
<td>13.8</td>
<td>14</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>14.3</td>
<td>14.7</td>
<td>14.7</td>
<td>14.9</td>
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<tr>
<td>Leucine</td>
<td>53.5</td>
<td>55.2</td>
<td>51.3</td>
<td>52.5</td>
<td>49-54</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>13.2</td>
<td>11.7</td>
<td>10.3</td>
<td>13.5</td>
<td>12-13</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>25.0</td>
<td>25.5</td>
<td>23.7</td>
<td>25.4</td>
<td>25-26</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>25.0</td>
<td>25.5</td>
<td>23.7</td>
<td>25.4</td>
<td>25-26</td>
</tr>
</tbody>
</table>

* Extrapolated to zero time.
* Calculated for 72-hour hydrolysate.

A comparison of the starch gel electrophoresis patterns at pH 9.5 of a number of BGH preparations has revealed the prevalence of a pattern in which a major component and at least two minor components are present. Similar patterns of heterogeneity for the hormone have been described by other investigators (1, 4, 15). Two considerably different isolation methods were employed in the preparation of BGH in this laboratory. One method (5) was based on an ethanol fractionation procedure, and the other utilized a partially purified starting material obtained by an ammonium sulfate extraction procedure. Although a gel filtration step at pH 4.0 was employed in each of the methods, it may be eliminated as a source of the heterogeneity, in that the NIH-GH preparations were not subjected to such a procedure. A process common to all of the preparation methods, extraction of ground pituitary glands at pH 10 to 12, would not appear to be directly responsible for any transformation of the components, inasmuch as purified BGH was unaffected by exposure to similar conditions. The lack of appreciable variation in the ratio of components in many consecutive preparations, or with variations in isolation procedure, also detracts from the likelihood that the components may represent artifacts arising during isolation of the hormone from frozen glands. Alternatively, the possibilities exist that the components may result from physiological action of pituitary proteinases (16) or that they may represent genetic variants (2).

Two exceptions to the common electrophoretic pattern described above were observed in preparations NIH-GH-B1 and NIH-GH-B2. These preparations differ from later NIH-GH preparations in that the former were lyophilized from alkaline rather than neutral solution. The patterns of the exceptional preparations might therefore be related to those obtained by transformation of BGH in 0.1 M NaOH.

The possibility that the electrophoretic components of BGH might result from association or dissociation phenomena was not supported by the results obtained with gel filtration studies on Sephadex G-100. In experiments conducted at both pH 4.0 and pH 8.80, the three principal components were eluted from the columns as a single peak. Estimation by gel filtration of the molecular weight at pH 8.80, with the use of a sample quantity closest to those used in preparative experiments, gave a value of 41,000, in fairly good agreement with previously established values for the protein (3). Experiments with smaller samples, however, revealed a concentration dependence, in which the lowest molecular weight of 35,000 was obtained with a 2-mg sample. The observations are in partial agreement with those of Andrews (17), who reported that gel filtration revealed a minimum molecular weight of 26,000 for BGH samples of approximately 1.0 mg at pH 7.5. Dellacha, Enero, and Faiernan (18), using gel filtration and ultracentrifugation, found a molecular weight for BGII of 20,000 at pH 3.6. The aforementioned behavior may indicate a dissociation of BGH to subunits at low pH.

5 The crude growth hormone powder was obtained with an ammonium sulfate extraction procedure by Wayne Donaldson, Parke, Davis and Company.

A. E. Wilhelmi, personal communication.
concentration or pH. During preparative gel filtration procedures, or those such as described in Fig. 4, however, a particle of molecular weight in excess of 40,000 appears to predominate.

Although attempts to fractionate the BGH components by exploitation of molecular weight differences were unsuccessful, a separation based on charge differences proved effective; the ease of elution of the components from columns of DEAE-Sephadex was inversely proportional to electrophoretic mobility at pH 9.5.}

Chromatography of the column fractions indicated that no interconversion of components occurred under the conditions that prevailed during chromatography.

Assay of growth-promoting activity in preparations of the components isolated by chromatography revealed that all were biologically active. Although a similar observation was made by Ferguson and Wallace (2), who demonstrated biological activity in a number of BGH fractions partially separated by anion exchange chromatography, the latter experiments were carried out with NIH-GH-B2, which differed electrophoretically from the BGH samples employed in our studies. Early experiments conducted by Ellis and Simpson (19) also showed that at least two active BGH peaks could be eluted from DEAE-cellulose. Although no electrophoretic data on the peaks were obtained, it appeared that the heterogeneity resulted from an interconversion of the peaks during chromatography (10).

The amino acid composition of Component 1, the major constituent of BGH preparations investigated in this laboratory, indicated no significant differences from the composition data obtained by Parells (13), with the exception of a higher apparent content of 10 residues of half-cystine. The latter value was also obtained in the analyses performed by Wolfenstein et al. (12). Comparison of the amino acid compositions of all three components revealed several minor differences in residues per molecule, the largest of these involving half-cystine, tryptophan, proline, glycine, and phenylalanine. Of these differences, those involving the latter three amino acids also appeared in analyses of a different group of similar preparations from our laboratory (12), and may represent valid differences in composition of the components.

The studies described in this paper indicate that a single electrophoretically and chromatographically homogeneous component accounts for the major portion of biologically active protein in BGH isolated by a variety of methods. In addition, at least two minor components were always present, each of which appeared similar to the major component except with respect to charge. Inasmuch as both alkaline-induced and enzymatic transformations of the hormone generally appear to produce electrophoretic components that migrate more rapidly (15, 20, 21) it may be postulated that Component 1 is the primary form of the hormone encountered in the pituitary gland. Prior to verification of this speculation, the chromatographically purified component should serve as a useful standard for biological and chemical studies of the hormone.

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