THE ISOLATION AND CHARACTERIZATION OF GROWTH HORMONE FROM ANTERIOR LOBES OF WHALE PITUITARIES*

BY HAROLD PAPKOFF AND CHOH HAO LI

(From the Hormone Research Laboratory and the Department of Biochemistry, University of California, Berkeley, California)

(Received for publication, October 27, 1957)

Previous reports of the isolation of growth hormones (somatotropins) from beef, human, and monkey pituitary glands have been made (1-4). This communication describes the isolation and properties of growth hormone from another mammalian species, the whale.

Isolation Procedure

Starting Material—The anterior lobes used in these studies were obtained from mature humpback whales. The average interval between the death of the animal and removal of the pituitary was about 20 hours. Once removed, the gland was frozen as rapidly as possible and stored at -15° until used. The weights of the individual anterior lobes ranged from 6 to 40 gm.; those from females tended to be heavier than those from males.

Calcium Hydroxide Extraction and (NH₄)₂SO₄ Fractionation—The preliminary steps employed in the purification of whale somatotropin were essentially the same as those previously described by one of us (5) for the bovine hormone. In a typical experiment, 178 gm. of frozen anterior lobes of whale pituitaries are ground and stirred for 3 hours at a temperature of 0.5°, with 700 ml. of Ca(OH)₂ solution of pH 10.0. The suspension is centrifuged, the supernatant fluid is saved, and the residue stirred for 1 hour with 550 ml. of Ca(OH)₂ solution. This suspension is again centrifuged, the residue discarded, and the supernatant fluid combined with that obtained in the previous step. An equal volume of saturated ammonium sulfate solution is now added and the pH of the mixture adjusted to 7.0. The suspension is allowed to stand in the ice box for several hours.

* Taken from a dissertation submitted by H. Papkoff in partial satisfaction of requirements for a degree of Doctor of Philosophy, University of California, Berkeley, 1957.

1 We are indebted to Professor H. S. Olcott, through whose efforts the whale pituitaries were put at our disposal. In addition, we wish to acknowledge the cooperation of Mr. R. H. Gilmore, United States Fish and Wildlife Service, who personally excised the pituitaries at the Caito Brothers, the Western California Fish Company, San Francisco, California, and the Del Monte Fisheries, Point San Pablo, California.
to insure maximal precipitation and is then centrifuged. The resulting precipitate is dissolved in 800 ml. of distilled water and precipitated again with saturated ammonium sulfate solution as described above. The reprecipitation has been found to eliminate many of the pigments observed in the initial precipitate. The precipitate obtained in this manner is then suspended in 200 to 300 ml. of water and the suspension dialyzed against running tap water at 6–8°C for 24 hours. The dialyzed solution is brought to 0.2 saturation with respect to ammonium sulfate by the addition of an appropriate volume of saturated ammonium sulfate solution, and the pH adjusted to 6.8. The heavy precipitate obtained at this point contains some growth hormone activity and is saved for future fractionation. The supernatant fluid is next brought to 0.4 saturation by the addition of more saturated ammonium sulfate solution, and the precipitate formed is collected by centrifugation, dissolved in water, dialyzed against distilled water, and lyophilized. In this manner, 1.55 gm. of material are obtained from 178 gm. (wet weight) of whale anterior pituitaries.

**Chromatography on Cation Exchange Resin Amberlite IRC-50**—Further purification of the fraction obtained according to the procedure described above is effected by means of the chromatographic technique previously described (3). The material (1.55 gm.) is extracted twice, first with 150 ml. and then with 100 ml. of buffer of pH 5.1, and the clear supernatant fluid thus obtained is applied to a column 3 cm. in diameter, containing 220 ml. of Amberlite IRC-50 resin (XE-97) equilibrated with the same buffer. After the protein solution is applied, the column is washed with the buffer until no more material appears in the eluate, as indicated by the optical density read at 275 nm in a Beckman model DU spectrophotometer. The growth hormone activity was eluted with the buffer of pH 6.0. A typical chromatographic pattern may be seen in Fig. 1; the material from the active region (tubes 135 to 175) is precipitated by the addition of an equal volume of saturated ammonium sulfate solution. The resulting precipitate is dissolved in water, dialyzed against distilled water, and lyophilized. A yield of 0.5 gm. was obtained.

---

2 The clear supernatant fluid was brought to a concentration of 0.75 with respect to ammonium sulfate by the addition of solid (NH₄)₂SO₄; the resulting precipitate was saved for future investigations.

3 Growth hormone activity was assayed by means of the tibia test in female Long-Evans rats hypophysectomized at 28 days of age and used 14 days postoperatively (6, 7).

4 The composition of buffers used in the chromatography was as follows: (a) buffer, pH 5.1: 0.052 M NaH₂PO₄, 0.0025 M Na₂HPO₄, and 0.45 M (NH₄)₂SO₄; (b) buffer, pH 6.0: 0.18 M Na₂HPO₄, 0.085 M Na₃HPO₄, and 0.45 M (NH₄)₂SO₄.

5 The resin was prewashed by the procedure described by Hirs et al. (8) before use.
Isoelectric and Ethanol Fractionation—The fraction (0.5 gm.) obtained from the IRC-50 column is dissolved in 100 ml. of cold (0-3°C) distilled water, and the pH is adjusted to 3.0; a clear solution is obtained. The pH is then adjusted to 4.3 by the addition of 0.1 M NaOH. The precipitate that forms is removed by centrifugation, and the pH of the supernatant fluid is adjusted to 4.8. Once again, the precipitate that forms is removed by centrifugation. The supernatant fluid is now adjusted to an ethanol concentration of 5 per cent (v/v) by the slow, dropwise addition of cold 40 per cent (v/v) ethanol to the cold supernatant fluid. The precipitate is removed by centrifugation, dissolved in distilled water, and lyophilized. More ethanol is added to the supernatant fluid to make a concentration of 20 per cent (v/v); the precipitate obtained is dissolved in distilled water and the solution lyophilized. Both ethanol precipitates are highly purified proteins possessing comparable growth hormone activities. In the experiments to be described, the two fractions manifested identical behavior. The yield of these two precipitates amounted to 187 mg.

Fig. 1. Upper. Chromatography on IRC-50 resin of 0.2 to 0.4 saturated (NH₄)₂SO₄ precipitate. 3.0 cm. diameter column containing 220 ml. of resin; 1.05 gm. of protein in 250 ml. of buffer of pH 5.1 applied to the column; 15.5 ml. per tube collected. Lower. Chromatography on IRC-50 resin of purified whale somatotropin; 0.9 cm. diameter column containing 20 ml. of resin; 15 mg. of protein in 10 ml. of buffer of pH 5.1 applied to the column; the arrows indicate the same sequence of buffers as in the upper diagram; 3.0 ml. per tube collected.
Table I summarizes the yield of fractions obtained from each step of the isolation procedure, and the data obtained when they were submitted to bioassay. It may be seen that 0.1 gm. of whale growth hormone was obtained from 100 gm. of anterior lobes of whale pituitary glands.

**EXPERIMENTAL**

**Chromatography**—The purified protein hormone was submitted to chromatography on Amberlite IRC-50 resin under the same conditions as those employed in the chromatographic step of the isolation procedure. As shown in the lower chromatogram of Fig. 1, the hormone behaves as a homogeneous substance. When the peak was divided into three fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Amount</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material (anterior lobes)</td>
<td>178 gm.</td>
<td>mg.</td>
</tr>
<tr>
<td>0.2-0.4 saturated (NH₄)₂SO₄ ppt.</td>
<td>1.55</td>
<td>0.2</td>
</tr>
<tr>
<td>IRC-50 column chromatography, Tubes</td>
<td>135-175</td>
<td>0.5</td>
</tr>
<tr>
<td>5% ethanol ppt.</td>
<td>0.92</td>
<td>0.04</td>
</tr>
<tr>
<td>5-20% ethanol ppt.</td>
<td>0.95</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Total dose injected over a period of 4 days.
† Mean ± standard error.

and biological assay for growth-promoting activity was carried out according to the tibia test (6, 7), each fraction manifested identical biological potency.

**Electrophoresis**—Electrophoresis was performed in a Spinco model H electrophoresis diffusion apparatus at 1°, with buffers of ionic strength 0.1 and of pH ranging from 4.1 to 8.3. In every instance, the protein hormone behaved as a single substance; Fig. 2 presents a typical electrophoretic pattern, which was obtained with a solution containing 4 mg. of protein per ml. in an acetate buffer of pH 4.0 and ionic strength 0.03. Electrophoresis was performed with a potential gradient of 5.6 volts per cm. for 10,680 seconds.

Table II presents electrophoretic mobilities of the whale somatotropin in various buffers of ionic strength 0.1; from a plot of pH *versus* mobility, the isoelectric point can be located at pH 6.2, a value which was expected
from the observation that the point of minimal solubility of a salt-free solution of the protein hormone was in the vicinity of pH 6.2 to 6.4.

**Sedimentation**—Sedimentation measurements were made in a Spinco model E ultracentrifuge at a speed of 59,780 r.p.m. and at temperatures of 22° ± 2°. The solvent used was the borate buffer of pH 9.93 and ionic strength 0.2 employed by Li and Pedersen (9) for ultracentrifugal studies of bovine somatotropin. In each instance, only a single, symmetrical sedimenting boundary was observed, indicating a high degree of homogeneity. A typical series of patterns may be seen in Fig. 3. The sedimentation rate (s20) determined at various concentrations is shown in Table III. Analysis of the data by the method of least squares yielded the following equation: $s_{20} = 2.84 + 0.084 C$, where $C$ is the concentration in gm. per 100 ml. of solvent. Thus, at zero concentration, the sedimentation constant is 2.84 S.

**Diffusion**—Diffusion experiments were carried out at 1° in a Spinco model H electrophoresis diffusion apparatus with an 11 ml. cell; the boundary

<table>
<thead>
<tr>
<th>pH</th>
<th>Buffer</th>
<th>Mobility ($\times 10^5$ cm.$^2$ per sec. per volt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Acetic acid-NaOH</td>
<td>+4.16</td>
</tr>
<tr>
<td>5.0</td>
<td>&quot;</td>
<td>+2.26</td>
</tr>
<tr>
<td>5.7</td>
<td>&quot;</td>
<td>+0.80</td>
</tr>
<tr>
<td>7.1</td>
<td>Cacodylic acid-NaOH</td>
<td>-0.99</td>
</tr>
<tr>
<td>7.3</td>
<td>Tris(hydroxymethyl)aminomethane-HCl</td>
<td>-1.75</td>
</tr>
<tr>
<td>8.3</td>
<td>Boric acid-NaOH</td>
<td>-2.91</td>
</tr>
</tbody>
</table>
was sharpened by the technique of Kahn and Polson (10). The protein was dialyzed against the buffer before establishment of the diffusion boundary; the buffer was the same as that used for the sedimentation experiments; namely, borate buffer of pH 9.93 and ionic strength 0.2. Diffusion coefficients \( (D_{20,w}) \) were calculated by the maximal ordinate-area method (11). The hormone preparation was used in two different concentrations, 0.3 and 0.2 per cent. For 0.3 per cent, a diffusion coefficient of \( 6.81 \times 10^{-7} \) cm.\(^2\) per sec. was obtained, and for 0.2 per cent, \( 6.31 \times 10^{-7} \) cm.\(^2\) per sec., giving an average value of \( 6.56 \times 10^{-7} \) cm.\(^2\) per sec.

From the sedimentation and diffusion coefficients \( (s_{20} = 2.84, \text{ and } D_{20,w} = 6.56 \times 10^{-7}) \), together with an assumed specific volume of 0.73 ml. per gm., the molecular weight of whale somatotropin may be computed according to the Svedberg equation (12). A value of 39,000 was obtained.

**Tyrosine and Tryptophan Content**—The ultraviolet absorption spectrum of whale somatotropin was examined in both acid and alkaline solutions in a Beckman model DU spectrophotometer. A solution containing 0.36 mg. of protein per ml. was used for these determinations. In an acetate buffer of pH 4.0, a minimum was observed at 253 m\(\mu\) and a maximum at 278 m\(\mu\). In 0.1 m NaOH, the expected shift of spectra was observed, with

---

**Fig. 3.** Schlieren patterns of whale somatotropin obtained in the ultracentrifuge at 59,780 r.p.m. and 20\(^\circ\); 15.7 mg. of protein per ml. of solvent, borate buffer of pH 9.93; sedimentation from right to left; the pictures were taken 45, 61, 93, 109, and 124 minutes after attainment of full speed; bar angle 65\(^\circ\).

---

<table>
<thead>
<tr>
<th>Concentration, gm. per 100 ml.</th>
<th>( S_{20} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>2.95</td>
</tr>
<tr>
<td>1.57</td>
<td>2.98</td>
</tr>
<tr>
<td>1.00</td>
<td>3.03</td>
</tr>
<tr>
<td>0.80</td>
<td>2.98</td>
</tr>
<tr>
<td>0.60</td>
<td>2.94</td>
</tr>
<tr>
<td>0.40</td>
<td>3.07</td>
</tr>
<tr>
<td>0.30</td>
<td>2.64</td>
</tr>
<tr>
<td>0.23</td>
<td>2.77</td>
</tr>
</tbody>
</table>

* All runs were performed at 59,780 r.p.m. and at a temperature of 20–23\(^\circ\).
the minimum appearing at 272 m\(\mu\) and a maximum at 289 m\(\mu\). It may be seen in Fig. 4 that an isosbestic point is located at 277 m\(\mu\), as would be expected for proteins containing tyrosine and tryptophan as the only ultraviolet-absorbing moieties in this region (13).

Tyrosine and tryptophan were determined quantitatively by the spectrophotometric procedure of Goodwin and Morton (14); the results are summarized in Table IV. It can be seen that average values of 5.60 and 1.32 per cent, respectively, were obtained when five different preparations of the whale hormone were analyzed for tyrosine and tryptophan.

**N-Terminal Residue**—The \(N\)-terminal residuc of the hormone protein was identified by paper chromatography by the fluorodinitrobenzene method (15, 16) whereby a quantitative determination of the yield of the dinitrophenyl (DNP) derivatives of the amino acids in the acid hydrolysates of DNP-somatotropin is obtained. Determinations were performed on two different preparations of whale growth hormone, and in each instance phenylalanine was found to be the sole \(N\)-terminal residue. As shown in Table V, DNP-phenylalanine occurs in an amount of nearly 1 mole per mole of the whale hormone, on the basis of the assumption that

![Fig. 4. Ultraviolet absorption spectrum of whale somatotropin. Curve A, 0.36 mg. of protein per ml. of acetate buffer of pH 4.0; Curve B, 0.36 mg. of protein per ml. of 0.1 M NaOH.](http://www.jbc.org/)

1.32 per cent, respectively, were obtained when five different preparations of the whale hormone were analyzed for tyrosine and tryptophan.

---

**Footnotes:***

1.32 per cent, respectively, were obtained when five different preparations of the whale hormone were analyzed for tyrosine and tryptophan.

\(N\)-Terminal Residue—The \(N\)-terminal residuc of the hormone protein was identified by paper chromatography by the fluorodinitrobenzene method (15, 16) whereby a quantitative determination of the yield of the dinitrophenyl (DNP) derivatives of the amino acids in the acid hydrolysates of DNP-somatotropin is obtained. Determinations were performed on two different preparations of whale growth hormone, and in each instance phenylalanine was found to be the sole \(N\)-terminal residue. As shown in Table V, DNP-phenylalanine occurs in an amount of nearly 1 mole per mole of the whale hormone, on the basis of the assumption that
the whale hormone has a molecular weight of 40,000. It will be seen that this assumption is consistent with the molecular weight computed from molecular kinetic data.

Table IV

Content of Tyrosine and Tryptophan in Whale Somatotropin As Determined Spectrophotometrically*

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>Tyrosine</th>
<th>Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>1</td>
<td>5.82</td>
<td>1.40</td>
</tr>
<tr>
<td>2</td>
<td>5.59</td>
<td>1.19</td>
</tr>
<tr>
<td>3</td>
<td>5.63</td>
<td>1.42</td>
</tr>
<tr>
<td>4</td>
<td>5.29</td>
<td>1.22</td>
</tr>
<tr>
<td>5</td>
<td>5.66</td>
<td>1.36</td>
</tr>
</tbody>
</table>

5.60 ± 0.00† 1.32 ± 0.05†

* According to the procedure of Goodwin and Morton (14).
† Mean ± standard error.

Table V

N-Terminal Residues in Whale Somatotropin

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>N-Terminal residue</th>
<th>N-Terminal residue per mole of hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mole</td>
</tr>
<tr>
<td>1</td>
<td>Phenylalanine</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>0.70</td>
</tr>
</tbody>
</table>

* Uncorrected for hydrolytic destruction and chromatographic losses.

Table VI

Bioassay of Whale Somatotropin

<table>
<thead>
<tr>
<th>Total dose*</th>
<th>No. of rats</th>
<th>Tibia width</th>
</tr>
</thead>
<tbody>
<tr>
<td>µ</td>
<td></td>
<td>microns</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>220 ± 4†</td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>260 ± 2</td>
</tr>
<tr>
<td>120</td>
<td>5</td>
<td>260 ± 4</td>
</tr>
</tbody>
</table>

* Total dose injected over a period of 4 days.
† Mean ± standard error.

Biological Purity—The growth-promoting activity of whale somatotropin has been assayed by the tibia test (6, 7). The results, summarized in Table VI, indicate a potency comparable to that of the bovine hormone
For example, a total dose of 0.02 mg administered over a period of 4 days elicited an increment of the uncalcified cartilage of the tibia in hypophysectomized rats (operated upon at 28 days of age and injected 14 days postoperatively), amounting to 63 μ over the control.

When 1 mg of whale somatotropin was injected intravenously into hypophysectomized rats for the estimation of corticotropin (ACTH) contamination by the procedure of Sayers et al. (17), no evidence of adrenal ascorbic acid-depleting activity was found. The whale hormone has also been tested for thyrotropic (TSH) contamination. By the method of Bates and Cornfield (18), one preparation of whale somatotropin was shown to contain 0.07 unit of TSH per mg. Other studies at a total dose of 0.5 mg. showed that the whale hormone is free from lactogenic, follicle-stimulating (FSH), and interstitial-stimulating (ICSH) activities.

**DISCUSSION**

The immensity of the whale is sufficient to stimulate searching questions regarding the animal’s basic biochemistry, physiology, endocrinology, and other facets of its life history. Surprisingly little is known about this mammal, perhaps in part owing to the difficulty in studying live specimens. With respect to pituitary research, the whale is an interesting animal in that it does not possess an intermediate lobe, and the anterior and posterior lobes are very distinctly separated. The anatomy of the whale pituitary has been described by Valsö (22) in 1934 and more extensively by Geiling (23) in 1935. In addition, observations on the hormonal content of whale pituitaries were presented by these investigators, who reported extensively on the occurrence of oxytocic and vasopressor activities in the posterior lobe of the whale pituitary (22-24). In the anterior lobe, Geiling (23) finds evidence for gonadotropic, lactogenic, adrenocorticotropic, and melanocyte-stimulating activities. This was confirmed by Valsö, who, in addition, reported that the content of growth-promoting activity in the anterior lobe of the blue whale pituitary approached that found in cattle pituitaries (24).

In the present investigation, we employed the anterior lobes of the humpback whale pituitary glands and were able to isolate 0.1 gm. of growth hormone from 100 gm. of fresh anterior pituitaries by a procedure involving

---

6 We wish to thank Dr. S. Hier of The Wilson Laboratories for the ACTH assay.
7 We wish to thank Dr. R. W. Bates of the National Institutes of Health for the TSH assay.
8 The following procedures were used for the detection of these activities: for FSH that of Simpson et al. (19), for ICSH that of Greep et al. (20), and for prolactin that of Lyons (21). We wish to express our thanks to Dr. A. J. Lostroh for performing these assays.
chromatography on Amberlite IRC-50 resin and isoelectric and ethanol fractionations. Investigations of the purified product by means of chromatography, ultracentrifugation, electrophoresis, and N-terminal residue analysis reveal that it possesses a high degree of homogeneity. The molecular weight of whale growth hormone as computed from molecular kinetic data is lower than that of the bovine hormone (25) but higher than those of human and monkey somatotropins (3, 4). It may also be noted that the isoelectric point of whale somatotropin lies between the value for bovine growth hormone (25) and the values for the primate hormones (3, 4). However, the bovine hormone possesses two N-terminal residues (25), whereas the primate somatotropins (3, 4) have only one N-terminal group, as does the whale hormone herein reported. At the C-terminus, preliminary investigations of whale somatotropin by means of digestion with carboxypeptidase and by hydrazinolysis have yielded phenylalanine as the sole terminal residue. The somatotropins thus far isolated from all the various species likewise have been found to possess only one C-terminal residue (3, 4, 25), which has been identified as phenylalanine in all instances.

SUMMARY

A procedure has been described for the isolation of growth hormone in highly purified form from anterior pituitaries of humpback whales. The biological activity of whale growth hormone as measured by the tibia test was found to be comparable to that of bovine growth hormone. The isoelectric point of whale somatotropin was determined to be 6.2 and the molecular weight calculated from molecular kinetic data was found to be 39,000. The N-terminal residue determined by the fluorodinitrobenzene reaction was phenylalanine in an amount of nearly 1 mole per mole of the hormone protein. The hormone was further characterized by quantitative determination of tyrosine and tryptophan and by its ultraviolet absorption spectrum.

The authors wish to express appreciation to the American Cancer Society, and to the Albert and Mary Lasker Foundation, for grants in partial support of this work.

BIBLIOGRAPHY

24. Valsø, J., Hvelvaadets Skrifter (Oslo), 16, 6 (1938).
THE ISOLATION AND CHARACTERIZATION OF GROWTH HORMONE FROM ANTERIOR LOBES OF WHALE PITUITARIES
Harold Papkoff and Choh Hao Li


Access the most updated version of this article at http://www.jbc.org/content/231/1/367.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/231/1/367.citation.full.html#ref-list-1