Separation and Partial Purification of the Protein Hormones from Human Pituitary Glands

By ANNE STOCKELL HARTREE

Department of Biochemistry, University of Cambridge

(Received 15 March 1966)

A method has been developed for separation and partial purification of four protein hormones from acetone-dried human pituitary glands. The major portion of growth hormone, follicle-stimulating hormone, luteinizing hormone and thyroid-stimulating hormone activities are each obtained in a separate fraction. Although the yields of the individual hormones are similar to those obtained by other investigators, the present procedure is simpler and a cleaner separation of the hormones has been achieved. Methods for further purification of each fraction are indicated including preparation of luteinizing hormone of very high potency (equivalent to 5.3 mg. of standard NIH-LH-S3/mg.).

The aim of the work described here has been to develop a simple and efficient procedure for separating the major portion of the growth hormone, FSH*, LH and TSH of relatively scarce human pituitaries into different fractions that could each be further purified if necessary. Because of species differences, human pituitary hormones rather than those obtained from animals are often required for clinical use, for production of specific antisera and for use as standards in immunological and biological assay. In addition, physicochemical studies of structure of purified human hormones are of value in attempting to explain their mechanism of action and in elucidating the differences between corresponding human and animal hormones.

Methods for the separation of growth hormone, FSH and LH from human pituitary glands have been published by Steelman, Segaloff & Mays (1958), Steelman, Segaloff & Andersen (1959), Li (1960), Wilhelmi (1961) and Reichert & Parlow (1964a). Hartree, Butt & Kirkham (1964) and Parlow, Condliffe, Reichert & Wilhelmi (1965a) obtained partial separation of human LH from TSH by chromatography on DEAE-cellulose at pH 9.5, a method previously developed by Bates & Condliffe (1960) for separation of the corresponding bovine hormones. In the present studies the use of an immunoassay for human LH based on its immunological cross-reaction with human chorionic gonadotrophin has greatly facilitated the development of simpler and more efficient fractionation procedures, which are, for the most part, modifications of the

methods described by Steelman *et al.* (1958, 1959) and Hartree *et al.* (1964).

MATERIALS AND METHODS

Source of glands and preparation of pituitary powder. Human pituitary glands were collected at autopsy in hospitals throughout the United Kingdom under a programme sponsored by the Medical Research Council. The glands were stored in acetone and periodically dispatched to Cambridge, where they were resuspended in fresh acetone and stored at 4°. Approximately once each year the accumulated glands were minced in an electric meat grinder (model AC22; Crypto Ltd., London, N.W. 10). The mince was thoroughly mixed, suspended in fresh cold acetone and stored at 4°. Acetone-dried powder was prepared as needed from a portion of the mince representing about 2500 glands. This was filtered with suction, washed with 5l. of acetone and spread out in a large tray to air-dry. It was periodically turned with a spatula and all lumps were broken up to ensure thorough drying. After 24–48 hr. the powder was transferred to an air-tight glass jar and stored at 4°. The yield of powder is approx. 100g./1000 glands.

Column chromatography. The ion-exchangers used were CM-cellulose powder (Whatman CM-70) and DEAE-cellulose powder (Whatman DEAE-50). Before use they were each soaked in M-ammonium acetate for at least 24 hr. to remove material absorbing at 280 m μ , after which they were washed repeatedly with water, fines being decanted each time. Before each chromatographic run, the ion-exchanger was washed with 2–3l. of distilled water in a Buchner funnel, mixed well with the starting buffer, and the pH of the mixture was adjusted to the original pH of the starting buffer by adding acetic acid or 2N-NaOH. The mixture was left for 15 min., after which the supernatant fluid was decanted, the ion-exchanger resuspended in the starting buffer and the procedure repeated two or three times. A slurry of the ion-exchanger in buffer was then

* Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

poured into a chromatographic column with a sintered-glass disk, and starting buffer was passed through until the effluent pH was the same as that of the buffer.

To maintain uniform conditions of chromatography the same batch of cellulose ion-exchanger was used repeatedly. After two chromatographic separations it was cleaned by stirring in Na_2PO_4 solution at pH 10.5 for about 2 hr. at room temperature, after which it was filtered on a Buchner funnel, washed thoroughly with water and equilibrated with the starting buffer.

Column chromatography was carried out at 4° at a flow rate of 50 ml./hr. per 100 ml. bed volume. Protein concentration was estimated from the $E_{280\text{ m}\mu}$ of the effluent. The protein was recovered from pooled fractions by precipitation with 5 vol. of 96% (v/v) ethanol at 4°. For complete precipitation of fractions eluted at low ionic strength it was necessary to add ammonium acetate to a concentration of approx. 50 mM. Fractions eluted at high pH were adjusted to pH 5.5 with acetic acid. The ethanol was added slowly with stirring and the mixture left at 4° for 2 days to complete precipitation. After centrifugation at 4°, the protein precipitate was washed once with cold 96% (v/v) ethanol, twice with diethyl ether (AnalaR grade) and dried *in vacuo*.

Extraction of glycoproteins. The procedure was essentially that of Steelman *et al.* (1958) for gonadotrophin extraction as modified by Butt, Crooke & Cunningham (1961b), and all steps were carried out at 4°. Acetone-dried human pituitary powder (100 g.) was extracted with a mixture of 600 ml. of 10% (w/v) ammonium acetate, adjusted to pH 5.1, and 400 ml. of 96% (v/v) ethanol. The mixture was stirred overnight and centrifuged. The pituitary residue was re-extracted once more and the protein was precipitated from the combined supernatant solutions by adding 2 vol. of cold 96% (v/v) ethanol slowly with stirring. Stirring was continued for another 30 min. and the mixture was left at 4° for 2 days to complete precipitation. The glycoprotein precipitate was recovered by centrifugation and was washed once with cold 96% (v/v) ethanol, twice with cold ether and dried *in vacuo*. The pituitary residue was mixed with cold acetone, washed on a Buchner funnel with about 5 l. of acetone and spread out in a tray to dry. It was then stored at 4° for subsequent extraction of growth hormone.

Preparation of growth hormone. Growth hormone was prepared from the acetone-dried pituitary residue (200 g.) by the procedure of Raben (1959).

Biological assays. Assays for FSH and LH were performed by Dr W. R. Butt of Birmingham by the augmentation method (Brown, 1955; Butt, Crooke, Cunningham & Evans, 1961a) and the ovarian ascorbic acid depletion method (Parlow, 1958, 1961; Hartree *et al.* 1964) respectively. Assays for TSH were carried out by Dr K. E. Kirkham of Edinburgh (Kirkham, 1962), who used a method *in vitro* that depends on the turnover of ^{131}I by thyroid tissue from guinea pigs pretreated with methylthiouracil. Growth hormone was assayed by Miss M. B. Thomas of Cambridge by its effectiveness in increasing the weight of hypophysectomized rats (Hartree, Kovačić & Thomas, 1965). The 95% fiducial limits for all bioassay results were no less than 50% and no greater than 200% of the potency value.

Immunological assays. The haemagglutination method of Read & Bryan (1960) as modified by Ehrlich & Randle (1961) was used. Antiserum to human growth hormone was prepared exactly as described by Ehrlich & Randle (1961) and was heated at 56° for 30 min. to inactivate complement.

Antiserum to human chorionic gonadotrophin was provided by Dr W. R. Butt (Butt *et al.* 1961b). Sheep erythrocytes were washed three times with saline solution buffered at pH 7.2 (77 mM-NaCl-18 mM- KH_2PO_4 -57 mM- Na_2HPO_4) and incubated for 10 min. at 37° as a 4% (v/v) suspension in 10 ml. of the same buffer containing 0.35 mg. of tannic acid. The cells were washed again with buffer and incubated for 15 min. at 37° as a 2% (v/v) suspension containing 10 μg . of antigen/ml. The cells were washed three times with diluent (normal rabbit serum previously heated at 56° for 30 min. and diluted 1:200 with saline-phosphate buffer) and made up to a 1% (v/v) suspension with diluent. The coated cells could be stored at 4° for up to 72 hr. and were washed and resuspended in diluent just before use. For assay of growth hormone the antigen attached to the cells was human growth hormone (preparation MY-61 of Hartree *et al.* 1965). For assay of LH the cells were sensitized with human chorionic gonadotrophin of potency 2790 i.u./mg. provided by Dr W. J. Tindall of Organon Laboratories or with a preparation of human pituitary LH (DEAE-I-IRC-2 described below). Test tubes for assay were 5 cm. x 1 cm., hand-blown, supplied by R. B. Turner and Co. Ltd.

For each preparation of coated cells the antiserum titre was determined from doubling dilutions of 1:100 antiserum in diluent (0.2 ml.) to which was added 0.1 ml. of cell suspension. When complete agglutination of the cells occurred they appeared as a pink mat spread evenly over the bottom of the tube. If no haemagglutination occurred the cells formed a small red circle. The degree of agglutination was graded from 0 for none to 4+ for complete agglutination. The antiserum concentration used in the assays was 1.5 times that of the most dilute solution showing 4+ haemagglutination. For the assay two solutions of standard and two of each unknown, in diluent, were set up with concentrations in the ratio 1:0.66. A series of nine doubling dilutions (0.1 ml.) was made from each solution, and to these were added 0.1 ml. of dilute antiserum and 0.1 ml. of prepared sheep cells, with thorough mixing after each addition. The end points could be read within 2-3 hr. or on the following day, and end points of 2+, 3+ and 4+ agglutination were determined for each row. The concentration of hormone in each unknown was determined by assuming that the dilutions of unknown and standard with corresponding end points were of equal hormone concentration. The standards used were human growth hormone preparation MY-61 (0.89 i.u./mg.) and for LH assays human chorionic gonadotrophin (2790 i.u./mg.). The reproducibility of the method was estimated from repeat assays performed on different days on some of the preparations. When each of 31 LH assays on 14 preparations was expressed as a percentage of the geometric mean of duplicate or triplicate assays, the 95% fiducial limits of the assay values were from 66% to 152% of the mean value for each preparation. Similar calculations for ten growth hormone assays on four preparations indicated that the 95% fiducial limits for this assay were from 70% to 143% of the mean value. Results with cells coated with LH were 1.6 times those obtained with chorionic-gonadotrophin-coated cells, and so the results with LH-coated cells were corrected by this factor.

RESULTS

Extraction of glycoproteins and preparation of growth hormone. A large number of 100 g. batches

Table 1. *Immunoassays and weight yields of glycoprotein extracts and growth hormone*Where appropriate, results are given as means $\pm$ S.D. with the numbers of observations in parentheses.

Fraction	Growth hormone immunoassay (% of standard)	LH immunoassay (% of standard)	Wt. yield of material (g./100 g. of pituitary powder)	Recovery of growth hormone (%)	Recovery of LH (%)
Acetone-dried powder	15	0.51	100	100	100
Glycoprotein ppt. [ethanol (40%)-ammonium acetate (6%)]	1.48	8.91 $\pm$ 2.08 (6)	2.15 $\pm$ 0.28 (54)	0.21	37.6
Supernatant from glycoprotein ppt.	—	—	—	—	4.55
Acetone-dried pituitary residue	—	0.03	98	—	5.75
Glycoprotein ppt. [ethanol (30%)-ammonium acetate (7%)]	4.31	4.52	3.0	0.86	26.6
Glycoprotein ppt. from freeze-dried glands	—	3.7	3.7	—	26.8
Growth hormone extracted at 70°	84.9 $\pm$ 13.0 (6)	—	4.34 $\pm$ 0.43 (20)	24.5	—
Growth hormone extracted at 65°	61.8 $\pm$ 11.6 (2)	—	5.27 $\pm$ 0.50 (5)	21.7	—
Growth hormone extracted at 65° without prior extraction of glycoprotein	—	—	5.73	—	—

Table 2. *Chromatography of glycoprotein fraction on CM-cellulose*

Buffer	Column dimensions	Wt. of starting material (g.)	LH unadsorbed (%)	LH adsorbed (%)
10 mM-Ammonium acetate, pH 6.0	5 cm. $\times$ 20 cm.	2.5	56.5	43.5
4 mM-Ammonium acetate, pH 6.3	1.1 cm. $\times$ 30 cm.	0.28	52.2	47.8
4 mM-Ammonium acetate, pH 5.5	1.1 cm. $\times$ 30 cm.	0.28	21.9	78.1

of human pituitary powder have now been extracted for glycoproteins before preparation of growth hormone. Data on immunoassay for growth hormone and LH and recovery of these hormones in various fractions are given in Table 1. Treatment of pituitary powder by this method [ethanol (40%, v/v)-ammonium acetate (6%, w/v)] results in extraction of about 40% of the LH but only 0.2% of the growth hormone. Approx. 5% of the LH originally present remained in the pituitary residue after extraction and another 5% could be accounted for in the supernatant solution after precipitation with ethanol. One extraction with ethanol (30%, v/v)-ammonium acetate (7%, w/v) resulted in a 50% higher weight yield of glycoprotein precipitate; its LH content was not increased, however, and there was a fourfold increase in the amount of growth hormone extracted. One batch of freeze-dried pituitary glands, provided by Dr W. M. Hunter of Edinburgh, yielded nearly twice as much glycoprotein, but again the LH content was not increased and assay for FSH by Dr W. R. Butt indicated that there was no increase in the total amount of this hormone.

When growth hormone was prepared from the

pituitary residue by the Raben (1959) method the yield of hormone was approx. 4.3% of the weight of the original pituitary powder. Since the rather drastic conditions of extraction (acetic acid at 70°) might be expected to decrease the hormone activity, more recent preparations have been extracted at a lower temperature (65°), which results in a 20% increase in yield but no increase in recovery of growth hormone as measured immunologically. One preparation was made without prior extraction of glycoproteins, and the yield was not significantly altered.

Separation of FSH and LH. The method of Steelman *et al.* (1959) was used initially to separate these hormones. The glycoprotein fraction after chromatography on CM-cellulose under their conditions (pH 6.0, 10 mM-ammonium acetate) yielded an LH fraction (adsorbed) low in FSH activity (Butt *et al.* 1961b), but the FSH fraction (unadsorbed) contained about half of the total LH activity (Table 2). Therefore small-scale experiments with altered pH and ionic strength were performed in an attempt to improve the separation of hormone activities. After equilibration of ion-exchanger with the starting buffer the glycoprotein

was applied as a 5% (w/v) solution, a small amount of insoluble material having been previously removed by centrifugation. The column was washed with more buffer until the $E_{280\text{ m}\mu}$ of the effluent decreased to the base line; the adsorbed fraction was then eluted with m-ammonium acetate. The LH content of each fraction was assayed immunologically and the results are given in Table 2. It is apparent that more LH is adsorbed by the ion-exchanger at lower pH. As a result of these experiments the conditions for chromatography with this particular batch of CM-cellulose were modified and ion-exchanger was equilibrated with 4mm-ammonium acetate, pH 5.5. Bioassay for hormone activity in the unadsorbed (CM-1) and adsorbed (CM-2) fractions indicated that 96% of the FSH activity recovered from the column was in fraction CM-1. Similarly fraction CM-2 contained 89% of the LH and 99% of the TSH activity recovered from the column.

For large-scale separation of FSH from LH, 4.0g. of glycoprotein precipitate is extracted overnight at 4° with about 60ml. of buffer (4mm-ammonium acetate, pH 5.5). After centrifugation the insoluble material is washed twice with the same buffer and the supernatant solutions are combined and applied to a CM-cellulose column (5cm. x 30cm.). When the column is washed with the same buffer approx. 2g. of protein passes through unretarded in a volume of 1.5l. and is freeze-dried (fraction CM-1). The remaining protein (fraction CM-2) is eluted with m-ammonium acetate in a volume of 1l. The effluent is adjusted to pH 5.5 and protein recovered by precipitation with ethanol.

Data on yields and immunological assay for LH are given in Table 3. Approx. 35% of the glycoprotein precipitate is insoluble in the starting buffer, but this material contains only about 9% of the total LH. A similar amount is in the CM-1 fraction, but most of the LH is found in the CM-2 fraction, where its specific activity is increased sixfold over that of the starting material.

Separation of LH and TSH. Fraction CM-2 can be further fractionated by chromatography on DEAE-cellulose at pH 9.5 as described by Hartree *et al.* (1964). To avoid the use of gradient elution the ionic strength of the starting buffer has been increased so that most of the LH is unretarded. Ion-exchanger equilibrated with 50mm-glycine buffer, pH 9.5, adsorbed 96% of the protein and most of the LH activity of fraction CM-2. When the glycine concentration of the starting buffer was increased to 0.1M about one-third of the protein was unretarded (fraction DEAE-I) and this fraction contained 70% of the recovered LH. Bioassay for FSH and TSH indicated negligible contamination of fraction DEAE-I by these hormones, but the adsorbed fraction (DEAE-II) was potent in TSH activity. These latter conditions have been used for large-scale separation of LH from TSH. Fraction CM-2 (1.2g.) is dissolved in 30ml. of glycine buffer (0.1M adjusted to pH 9.5 with n-sodium hydroxide) and left overnight at 4°. A small amount of insoluble material is removed by centrifugation and the solution applied to a DEAE-cellulose column (5cm. x 30cm.) previously equilibrated with the same buffer. The column is washed with more buffer and approx. 300mg. passes through

Table 3. *Yield and LH content of fractions after CM-cellulose chromatography of 4.0g. of glycoprotein precipitate*

Where appropriate, results are given as means $\pm$ s.d. with the numbers of observations in parentheses.

Fraction	Wt. yield (g.)	Immunoassay for LH (% of human chorionic gonadotrophin standard)	Recovery of LH (%)
Insoluble residue from glycoprotein fraction	1.37 $\pm$ 0.14 (15)	2.3 (1)	8.8
CM-1	2.03 $\pm$ 0.23 (15)	1.65 $\pm$ 0.84 (5)	9.4
CM-2	0.51 $\pm$ 0.11 (15)	52.6 $\pm$ 29.4 (5)	75.2

Table 4. *Yield and LH content of fractions after DEAE-cellulose chromatography of 1.2g. of fraction CM-2*

Where appropriate, results are given as means $\pm$ s.d. with the numbers of observations in parentheses.

Fraction	Wt. yield (mg.)	Immunoassay for LH (% of human chorionic gonadotrophin standard)	Recovery of LH (%)
DEAE-I	303 $\pm$ 56 (7)	114.7 $\pm$ 15.0 (7)	55.1
DEAE-II	535 $\pm$ 43 (7)	24.0 $\pm$ 6.9 (6)	20.3

unretarded in a volume of 1 l. The remaining protein (fraction DEAE-II) is eluted with *m*-ammonium acetate in a volume of 800 ml. Protein is recovered from each fraction by adjusting to pH 5.5 with acetic acid and precipitating with ethanol.

Data on yields and immunoassay for LH are given in Table 4. The specific activity of the LH recovered in fraction DEAE-I is increased twofold over that of the starting material. The TSH fraction (DEAE-II) still possesses appreciable LH activity.

The overall procedure for fractionation of human pituitary glands is outlined in Scheme 1 together with average yields of products and results of bioassay of one batch of each. Another batch of LH (fraction DEAE-I) was assayed by Dr A. F. Parlow (ovarian ascorbic acid depletion method), who obtained a potency equivalent to 2.5 mg. of standard NIH-LH-S1/mg. The same preparation was

assayed for TSH (promotion of ^{32}P uptake by chick thyroid) by Dr L. E. Reichert, who obtained a potency of 0.030 i.u./mg. Information on weight yields and potency estimates of the starting material and intermediate fractions is given in Table 5.

Further purification of hormone preparations. The specific activity of human growth hormone prepared by Raben's (1959) method can be increased twofold by methods described by Hartree *et al.* (1965).

Further purification of FSH (fraction CM-1) has been accomplished by Butt and co-workers (Butt, Cunningham & Hartree, 1964; Butt, Crooke & Wolf, 1965), who increased the potency tenfold by chromatography on DEAE-cellulose and calcium phosphate followed by partition between urea, ammonium sulphate and ethylCellosolve.

Chromatography of LH (fraction DEAE-I) on Amberlite IRC-50 (pH 7.5 phosphate buffer, 5 mm

Scheme 1. Flow sheet for fractionation of human pituitary glands. The fractionation of human pituitary glands is described in the text and the average yields from 100 g. of pituitary powder were calculated from Tables 1, 3 and 4. The bioassay data on growth hormone are quoted from Hartree *et al.* (1965). The other bioassays were performed by Dr W. R. Butt and Dr K. E. Kirkham as described in the Materials and Methods section.

Table 5. Potency estimates of glycoprotein hormones in starting material and intermediate fractions in the purification of human pituitary hormones

Fraction	Average wt. yield (g./100 g. of starting material)	FSH (mg. of NIH-FSH-S1/mg.)	LH (mg. of NIH-LH-S1/mg.)	TSH (i.u./mg.)
Acetone-dried pituitary powder	100	0.08*	0.006†	0.002‡
Glycoprotein fraction	2.2	1.1	§	0.08‡
CM-cellulose-adsorbed fraction (CM-2)	0.28	0.085	0.35	0.34

* Data of Butt *et al.* (1961b) assuming that 1 mg. of NIH-FSH-S1 is equal in potency to 180 mg. of International Reference Preparation of Human Menopausal Gonadotrophin.

† Data of Parlow *et al.* (1965b).

‡ Data of Kirkham (1962).

§ Data not available.

Fig. 1. Gel filtration of LH purified on Amberlite IRC-50 (preparation DEAE-I-IRC-2) on Sephadex G-100. The LH preparation (25.7 mg.) was dissolved in 3 ml. of 0.2M ammonium acetate (adjusted to pH 8.0 with dilute ammonia) and applied to a Sephadex G-100 column (4.25 cm. x 80 cm.) previously equilibrated with the same buffer. The elution volume, V_e , of the major peak was 613.0 ml.

in Na^+) as described by Hartree *et al.* (1964) results in a further increase in specific activity. Under these conditions LH is adsorbed by the ion-exchanger and can be eluted at high ionic strength (1M). Immunoassay of three preparations purified in this way gave an average potency of 174% of the chorionic gonadotrophin standard (range 161–181%). One preparation (DEAE-I-IRC-2) assayed by Dr E. T. Bell (ovarian ascorbic acid depletion method) had a potency equivalent to 5.3 mg. of standard NIH-LH-S3/mg. Another batch prepared similarly and assayed by Dr Parlow had a potency equivalent to 4.8 mg. of standard NIH-LH-S1/mg. Gel filtration on Sephadex G-100 of the hormone purified on Amberlite IRC-50 indicated

that most of the protein migrated as a single peak (Fig. 1). Immunoassay and bioassay of the protein recovered from the hatched area by ethanol precipitation showed no increase in potency over the starting material.

The TSH activity of fraction DEAE-II can be further increased by chromatography on Amberlite IRC-50 (Hartree *et al.* 1964) and by gel filtration on Sephadex G-100 (Condliffe, 1963; Parlow *et al.* 1965a). However, these procedures do not increase the TSH/LH ratio.

Recovery of other hormones from side fractions. Assays for melanocyte-stimulating hormone by Dr H. B. F. Dixon and adrenocorticotrophic hormone by Dr M. P. Stack-Dunne have shown that a portion of each hormone is extracted with the supernatant solution after precipitation of glycoprotein. The remainder is adsorbed by oxycellulose during preparation of growth hormone (Raben, 1959). At present these hormones are not isolated in the routine procedure.

DISCUSSION

The 'gonadotrophin precipitate' of Steelman *et al.* (1958) has been designated 'glycoprotein fraction' in the present paper. This term seems more appropriate since assays performed by Kirkham (1962) have demonstrated that virtually all of the TSH of acetone-dried human pituitary powder is found in this fraction.

The use of immunoassay to measure hormone concentrations is open to criticism because it is not necessarily a measure of biological activity. However, since a good correlation between biological and immunological LH assays has been reported (Hartree *et al.* 1964) it appeared to be useful for the present study. Bioassays of LH on preparations described in the present paper are also in good agreement with the immunoassay results. Correlation between immunological and biological assay of

Table 6. *Comparative yields of hormones obtained from 100g. of acetone-dried pituitary powder prepared by two different methods*

Hormone	This paper		Parlow <i>et al.</i> (1965b)	
	Fraction	Total yield of activity	Fraction	Total yield of activity
FSH	CM-1	3700 mg. of NIH-FSH-S1	R-470-4	6000 mg. of NIH-FSH-S1
LH	DEAE-I	250 mg. of NIH-LH-S1	R-470-3	180 mg. of NIH-LH-S1*
Growth hormone	Raben HGH	4300 i.u.	Bb3N	4400 i.u.

* Based on 44% recovery of LH from fractions A and Bb4N.

human growth hormone is less good (Hartree *et al.* 1965). However, immunoassay of various batches for both hormones and data on weight yields of fractions have been useful as a measure of the reproducibility of the fractionation procedures.

The procedures that have been evolved are efficient and relatively simple to carry out. Four human pituitary hormones, growth hormone, FSH, LH and TSH, are each obtained in a separate fraction, and, in addition, adrenocorticotrophic hormone and melanocyte-stimulating hormone can be recovered from side fractions. Methods for separation of these same hormones have also been developed by Wilhelmi and co-workers (Wilhelmi, 1961; Parlow *et al.* 1965a; Parlow, Wilhelmi & Reichert, 1965b). Their extraction procedures, employing ammonium sulphate fractionation, result in excellent recovery of hormone activity in the initial stages but appear to be more complicated and time-consuming than the methods described in the present paper. After further purification their overall recoveries of hormone activities are comparable with mine when preparations of similar potencies are compared (Table 6). Preparation R-470-4 is more potent in FSH activity and the total recovery of hormone is higher than in fraction CM-1. However, the latter preparation contains much less LH and TSH contamination. The LH fraction obtained by the present method is of higher specific activity than that described by Parlow *et al.* (1965b). Moreover, the total recovery of activity in fraction DEAE-I is somewhat higher and there is less contamination with FSH and TSH than in preparation R-470-3. Growth hormones prepared by both methods are comparable in terms of yield and potency. The TSH activity is recovered in a single fraction in the present method, but is found in small amounts in several fractions in the procedure of Parlow *et al.* (1965b). Preparation of TSH of very high potency from fraction A has been described by Parlow *et al.* (1965a) but the percentage recovery of activity was not stated. Although the reported potency of their TSH is much higher than that of fraction DEAE-II, a recent comparison of biological assays of human TSH by a number of

Laboratories using different methods indicates that assays on a given sample may vary as much as 100-fold, depending on the method used (Bakke, 1965). Thus a direct comparison of the TSH preparation of Parlow *et al.* (1965a) and fraction DEAE-II cannot be made.

Although freeze-dried human pituitary glands might be expected to be a more potent source of hormone activity than acetone-preserved glands, my results suggest that at least for LH and FSH this is not the case. The glycoprotein fractions from freeze-dried and acetone-dried glands have comparable FSH and LH activities. Similar findings were reported by Parlow *et al.* (1965b), who observed that the total FSH and LH activities extracted from fresh frozen human glands were no greater than that obtained from acetone-dried material.

Human pituitary LH prepared by chromatography of fraction DEAE-I on Amberlite IRC-50 appears to be of higher potency than any other preparation described thus far. Its specific activity, equivalent to 5.3 mg. of standard NIH-LH-S3/mg., is somewhat higher than the purified hormone prepared by Reichert & Parlow (1964b) and Parlow *et al.* (1965a), which had a potency equivalent to 3.2 mg. of standard NIH-LH-S1/mg.

This work would not have been possible without the co-operation of other workers, and particularly Dr W. R. Butt and Dr K. E. Kirkham, in performing biological assays. The competent technical assistance of Miss M. B. Thomas, Mrs S. Earl, Mrs W. Thorn and Mrs B. Brown is gratefully acknowledged. Human pituitary glands were collected through the co-operation of many pathologists throughout Great Britain. The Medical Research Council supplied the glands and a grant in support of this work. I am also indebted to Professor F. G. Young for his encouragement and helpful discussions and to Dr A. F. Parlow for providing a copy of his manuscript before publication.

REFERENCES

- Bakke, J. L. (1965). *J. clin. Endocrin. Metab.* **25**, 545.
 Bates, R. W. & Condliffe, P. G. (1960). *Recent Progr. Hormone Res.* **16**, 309.

- Brown, P. S. (1955). *J. Endocrin.* **13**, 59.
- Butt, W. R., Crooke, A. C. & Cunningham, F. J. (1961*b*). *Biochem. J.* **81**, 596.
- Butt, W. R., Crooke, A. C., Cunningham, F. J. & Evans, A. J. (1961*a*). *Biochem. J.* **79**, 64.
- Butt, W. R., Crooke, A. C. & Wolf, A. (1965). *Ciba Found. Study Group*, no. 22, p. 85.
- Butt, W. R., Cunningham, F. J. & Hartree, A. S. (1964). *Proc. Roy. Soc. Med.* **57**, 107.
- Condliffe, P. G. (1963). *Endocrinology*, **72**, 893.
- Ehrlich, R. M. & Randle, P. J. (1961). *Lancet*, ii, 230.
- Hartree, A. S., Butt, W. R. & Kirkham, K. E. (1964). *J. Endocrin.* **29**, 61.
- Hartree, A. S., Kovačić, N. & Thomas, M. (1965). *J. Endocrin.* **33**, 249.
- Kirkham, K. E. (1962). *J. Endocrin.* **25**, 259.
- Li, C. H. (1960). *Ciba Found. Colloq.: Endocrinology*, **13**, 46.
- Parlow, A. F. (1958). *Fed. Proc.* **17**, 402.
- Parlow, A. F. (1961). In *Human Pituitary Gonadotrophins*, p. 300. Ed. by Albert, A. Springfield, Ill.: Charles C. Thomas.
- Parlow, A. F., Condliffe, P. G., Reichert, L. E., jun. & Wilhelmi, A. E. (1965*a*). *Endocrinology*, **76**, 27.
- Parlow, A. F., Wilhelmi, A. E. & Reichert, L. E., jun. (1965*b*). *Endocrinology*, **77**, 1126.
- Raben, M. S. (1959). *Recent Progr. Hormone Res.* **15**, 71.
- Read, C. H. & Bryan, G. T. (1960). *Recent Progr. Hormone Res.* **16**, 187.
- Reichert, L. E., jun. & Parlow, A. F. (1964*a*). *Endocrinology*, **74**, 236.
- Reichert, L. E., jun. & Parlow, A. F. (1964*b*). *Endocrinology*, **75**, 815.
- Steelman, S. L., Segaloff, A. & Andersen, R. N. (1959). *Proc. Soc. exp. Biol., N.Y.*, **101**, 452.
- Steelman, S. L., Segaloff, A. & Mays, M. G. (1958). *Arch. Biochem. Biophys.* **78**, 262.
- Wilhelmi, A. E. (1961). *Canad. J. Biochem.* **39**, 1659.