CHROMATOGRAPHY OF LUTEINIZING HORMONE FROM SHEEP PITUITARY GLANDS

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SUMMARY

Luteinizing hormone has been chromatographed on carboxymethyl cellulose and hydroxylapatite ion exchange agents. The carboxymethyl cellulose resolves two fractions with luteinizing hormone activity designated LH_1 and LH_2 in order of their emergence from the column. Re-chromatography demonstrated that LH_2 behaved as a single component, but LH_1 during processing was partially dissociated into LH_2 plus a fast-moving component not retained by the ion exchange column as determined by chromatography and bioassay.

The molecular weight of LH_2 was estimated at 28,000 from ultracentrifuge and viscosity data. Glucosamine was present in LH_1 (7.2 %) and LH_2 (8.0 %). Bioassays indicated that both LH_1 and LH_2 were not significantly different in potency from the Armour LH 227-80 with respect to behaviour in the hypophysectomized rat; LH_1 was less potent in the intact rat.

INTRODUCTION

Purification of luteinizing hormone (LH, ICSH) from sheep pituitary glands was reported by LI and co-workers in 1940[1]. Some physical and chemical properties of this material were described[1,2]. Recently LI AND SQUIRE[3] have reported that two fractions obtained by chromatography on IRC-50 resin possess ICSH activity. One of these fractions was further purified by zone electrophoresis and designated β-ICSH. The present study, which was presented in preliminary form[4], confirms the finding of two chromatographically distinct fractions using carboxymethyl cellulose as the ion exchange adsorbent. With a single chromatography, the conditions presented here allow a more complete resolution of the two active fractions than described by SQUIRE AND LI (*loc. cit.*).

METHODS

Preliminary fractionation

Frozen sheep pituitary glands obtained from the Armour Company, Chicago, Ill., were pulverized in dry ice. After sublimation of the CO_2, fractionation by the ethanol–acetate buffer procedure of KOENIG AND KING[5] was carried out. From 1 kg

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of whole, frozen sheep pituitary glands 1.4 g of gonadotropic fraction was obtained. This material was used for the chromatographic studies on carboxymethyl cellulose.

Chromatography

Carboxymethyl cellulose was prepared according to PETERSON AND SOBER[6]. This preparation had a capacity of 0.65 mequiv./g. For 100- to 150-mg samples, columns 1 × 25 cm or 1 × 60 cm were used containing 2.5 or 5 g of the carboxymethyl cellulose, respectively. For preparative scale chromatograms (1 g of the protein), columns 2.5 × 35 cm containing 18 g of the exchange agent were used. Gradient elution[7] was employed using a 50-ml mixing chamber with columns 1 cm in diameter or a 250-ml chamber with the 2.5 cm columns. All chromatography was carried out at room temperature (24°). Each run was completed within a period of 30 h or less.

The carboxymethyl cellulose was suspended in 0.005 *M* phosphate buffer at pH 6.0 (A), and the pH re-adjusted to 6 if necessary. The columns were then poured and equilibrated with at least 10 column volumes of buffer. The sample was dissolved and applied to the column in this same buffer (A). After development with 1 to 2 hold-up volumes, elution with 0.01 *M* phosphate buffer at pH 7.0 (B) and a gradient to 0.04 *M* borate buffer at pH 8.2 (C) was begun. After 3 ½ to 4 hold-up volumes the first fraction with luteinizing hormone activity was carried off the column. Application of a gradient to 0.04 *M* borate buffer, pH 8.2, containing 0.2 *M* sodium chloride (D) then eluted the second active fraction after 3 hold-up volumes.

Aliquots of the column effluent were analyzed by the method of LOWRY *et al.*[8] or by ultraviolet absorption at 275 mμ. Fractions under the peak areas were pooled, dialyzed in the cold room against distilled water (48 to 72 h), and finally lyophilized. The dried samples were stored at 5° in desiccators over P_2O_5.

The two active fractions were also studied with respect to their chromatographic behaviour on calcium phosphate columns. The calcium phosphate was prepared according to TISELIUS *et al.*[9]. Columns 1 × 11 cm were used for this chromatography. Starting buffer was 0.005 *M* phosphate at pH 6.8; development was by gradients to increasing molarity of this same buffer—0.1 *M*, 0.15 *M*, 0.2 *M*, etc.—through a 50-ml mixing chamber.

Bioassay

In much of the early work, sperm release by the male frog (*Rana pipiens*) within 4 h after injection was used as a qualitative means of locating luteinizing hormone activity during chromatography[10]. The frogs* were kept for periods up to two months under conditions suggested by the work of KONTAXIS *et al.*[11]. Frogs were screened for hormone sensitivity as suggested by the latter authors.

Quantitative assays of luteinizing activity were made using the weight increase of the ventral lobe of the prostate in both normal and hypophysectomized male Sprague-Dawley rats of approximately 50 g body weight[12]. Hypophysectomized rats were obtained from Hormone Assay Laboratories, Chicago, Ill. Although the assay animals are much more sensitive to the hormone if injections are started within 48 h after hypophysectomy, difficulties in transportation arrangements from Chicago usually have necessitated beginning the assays 72 h or more after operation. Under these conditions 2 to 4 times more hormone was required to produce a satisfactory

* Frogs were obtained from the J. R. Schettle Frog Farm, Route 1, Stillwater, Minnesota.

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weight increase for the ventral prostate. In order to furnish a basis for comparison of assays with this uncontrolled time-of-dose variable, a "standard" group of animals has been included each time using Armour's LH 227-80 as a reference preparation. The hormone was injected twice a day, subcutaneously, for 4 days and the animals sacrificed on the fifth day. Doses were expressed as total quantity of hormone injected over the four-day period.

RESULTS

Chromatography

Fig. 1 shows the chromatogram obtained when 120 mg of the gonadotropic fraction from the KOENIG AND KING procedure were chromatographed on a 1 × 25 cm column of carboxymethyl cellulose. The first peak emerging after one hold-up volume contained virtually all the follicle-stimulating hormone (FSH). The material emerging between 50 and 90 ml effluent volume represents a mixture of minor impurities. By delaying the application of the gradient from buffer B to C the apparent peak in this area disappears. The peak emerging from 100 to 115 ml stimulated release of sperm by the frog when aliquots were injected into the dorsal air sac. The material in this area was pooled and designated LH_1. The peak emerging from the column from 130 ml to 160 ml effluent also stimulated release of sperm by the frog. Material from this peak was pooled and designated LH_2. Based on the Folin-Lowry color, equivalent amounts of LH_2 gave a more intense response than LH_1, grading response as per BURGOS AND LADMAN[10]. Differences in potency judged in this manner may be rather subjective, however. In another chromatogram the application of the final elution gradient (C to D) has been delayed for an additional 50 ml of effluent. In this case the LH_1 emerged in the same position but the LH_2 was displaced 50 ml later; thus the two fractions are clearly different in their chromatographic behavior.

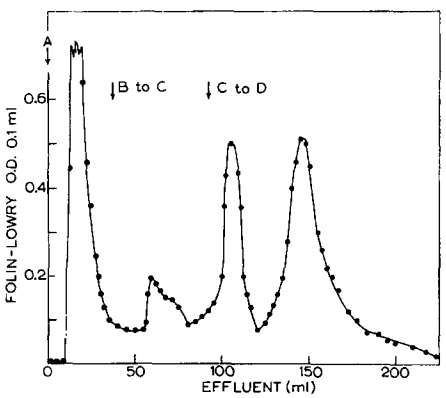

Fig. 1. Chromatogram, 120 mg KOENIG AND KING[5] gonadotropic fraction on carboxymethyl cellulose (2.5 g, 0.65 mequiv./g). Column size 1 × 25 cm. Hold-up volume, 18 ml; flow rate, 10 to 16 ml/h. Buffers: A. 0.005 *M* phosphate, pH 6.0; B. 0.01 *M* phosphate, pH 7.0; C. 0.04 *M* borate, pH 8.0; D. 0.04 *M* borate, pH 8.0, plus 0.2 *M* sodium chloride. Arrows mark the point of application of the buffer designated. "B to C" and "C to D" designate a gradient to the second buffer through a 50-ml mixing chamber. O.D. for 0.1 ml aliquots of effluent with Folin-Lowry reaction[8].

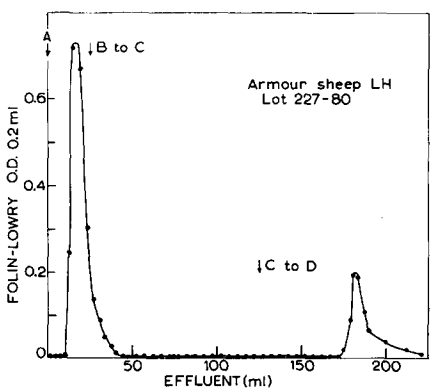

Fig. 2. Chromatogram, 23 mg Armour Sheep LH 227–80 on carboxymethyl cellulose. Column dimensions and buffers were the same as Fig. 1 except the last buffer gradient was started 35 ml later.

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The Armour "standard" LH preparation 227-80 was next submitted to this same type of chromatography. Fig. 2 shows the chromatogram obtained with 23 mg of this material. The peak emerging after one hold-up volume gave a very high Folin-Lowry reaction compared to the luteinizing hormone. However, after dialysis this peak represented only 25 % (5.8 mg) of the starting weight while the smaller peak contained 65 % (15 mg). From this chromatogram it is apparent that the Armour Sheep LH preparation does not contain material similar to the LH_1 fraction obtained from chromatography of the KOENIG AND KING gonadotropic fraction; instead, the major component appears identical with the LH_2 material.

The KOENIG AND KING gonadotropic fraction was next submitted to chromatography on a preparative scale. Fig. 3 shows a chromatogram in which 1 g of material was chromatographed on a 2.5 × 35 cm column. The LH_1 peak was at 660 to 690 ml effluent and the LH_2 peak at 895 to 950 ml effluent. The ultraviolet absorption at 275 mμ was also followed in this chromatogram and showed a pattern identical to that obtained with the Folin-Lowry reaction. Based on 5 chromatograms carried out in this manner, on a weight basis after dialysis, recovery of material applied to the column ranges from 65 to 90 % with an average of 75 %. Material in the peak emerging after 1 hold-up volume accounts for 26 to 28 %; the LH_1 peak, 11 to 20 %; and the LH_2 peak 21 to 27 % of the starting material. An additional 8 to 15 % may be accounted for in the intermediate areas between peaks. During dialysis of the LH_1 peak obtained from the preparative scale chromatograms, a globulin fraction precipitates. Thus, from 215 mg total material in the LH_1 area 30 mg of the insoluble

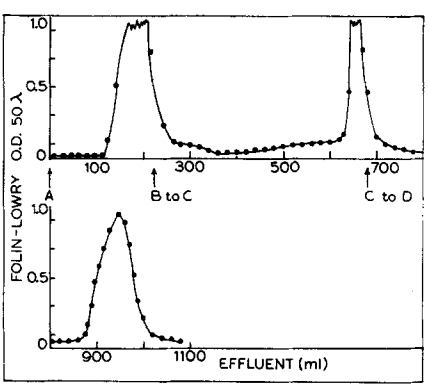

Fig. 3. Preparative scale chromatogram, 1 g KOENIG AND KING[5] gonadotropic fraction on carboxymethyl cellulose (18 g, 0.65 mequiv./g). Column size 2.5 × 35 cm. Hold-up volume, 105 ml; flow rate, 50 ml/h. Buffers as per Fig. 1 but gradient was applied through a 250-ml mixing chamber.

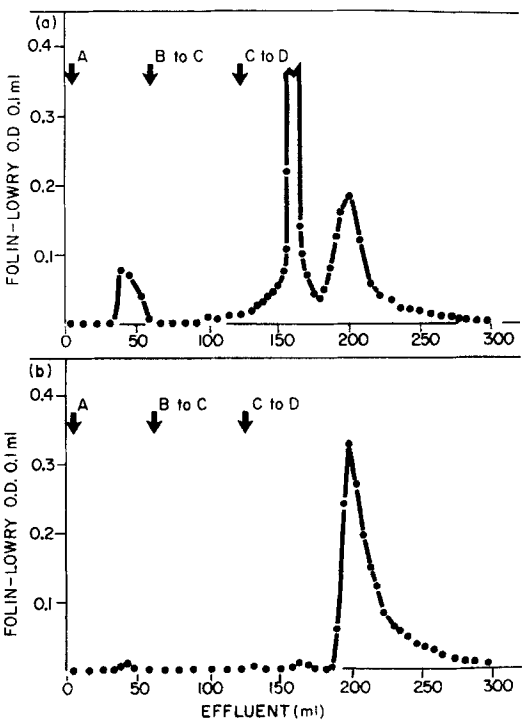

Fig. 4. Comparison of LH_1 and LH_2 (see text) after re-chromatography on carboxymethyl cellulose. Column dimensions 1 × 60 cm containing 5 g of exchange agent. Hold-up volume, 40 ml; flow rate, 12 ml/h. Buffers and mixing chamber are the same as in Fig. 1. Fig. 4(a) represents the chromatogram obtained with 50 mg of LH_1, 4(b) with 52 mg of LH_2.

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globulin were obtained. This fraction has been routinely removed so studies on the LH_1 fraction below refer to the soluble material. This insoluble fraction was not detected during dialysis of the LH_1 fraction from the small chromatograms.

Re-chromatography of the LH_1 and LH_2 fractions from the preparative scale chromatograms was studied on carboxymethyl cellulose columns 1 × 60 cm. In Fig. 4 two such chromatograms are presented. Fig. 4-a represents the chromatogram obtained when 50 mg of LH_1 was subjected to a second chromatography and Fig. 4-b an identical chromatogram with 52 mg of LH_2. Since both LH_1 and LH_2 were obtained from the peak area of a chromatogram as shown in Fig. 3 it is apparent that LH_1 (Fig. 4-a) does not behave as a single component, but suggests that LH_1 (150–170 ml effluent) may dissociate into LH_2 (185–225 ml effluent) plus a component not adsorbed by the ion exchange agent (35 to 55 ml effluent). Since the LH_1 material still remaining forms such a sharp band, it seems likely that any dissociation must occur during processing prior to chromatography. Bioassay of the fractions from the chromatogram shown in Fig. 4-a are consistent with this interpretation (see below). The recovery of material in the 3 fractions obtained from Fig. 4-a after dialysis was as follows: first peak (35–55 ml effluent), 9.5 mg; LH_1 peak (150–170 ml effluent), 20 mg; and LH_2 peak (185–225 ml effluent), 19 mg. Thus, approximately 60 % of the LH_1 appears to have been transformed into the LH_2 plus the unidentified protein. Similar results have been obtained with other LH_1 preparations.

This dissociation is being studied further. So far, it has been observed with columns loaded with 20 to 100 mg of LH_1; thus, it does not appear to be a simple concentration effect. Also, trichloroacetic acid precipitation and re-solution does not greatly increase the amount of dissociation.

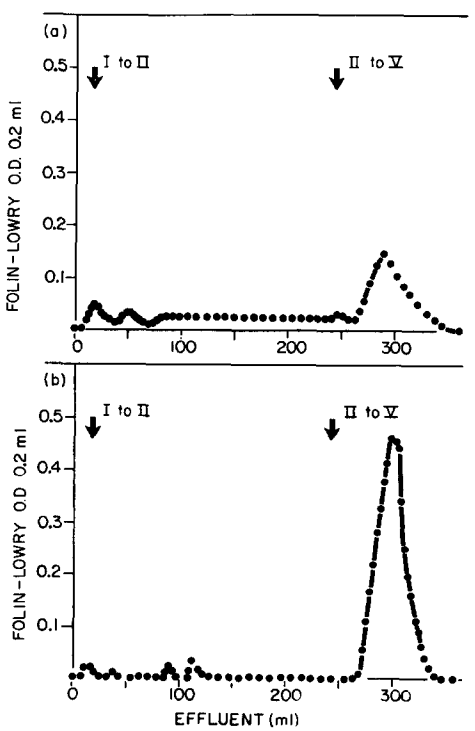

Fig. 5. Comparison of LH_1 and LH_2 (see text) after chromatography on hydroxylapatite columns. Column dimensions 1 × 11 cm. Hold-up volume, 5 ml; flow rate, 8 to 10 ml/h. Development was by gradient through a 50-ml mixing chamber to the buffer indicated, starting at the point marked by the arrows. Buffers: I. 0.001 *M*-phosphate, pH 6.8. II. 0.05 *M*-phosphate, pH 6.8. V. 0.2 *M* phosphate, pH 6.8. (Direct elution with 0.5 *M* phosphate after chromatography elutes an additional trace of protein—less than 5 % of the starting material—with each hydroxylapatite chromatogram run with either LH_1 or LH_2.)

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Besides the apparent difference in chromatographic behaviour, the LH_2 was approximately twice as soluble in distilled water as LH_1.

Preliminary studies[4] using diethylaminoethylcellulose[6] had shown that the luteinizing hormone activity was not significantly retained at a pH near neutrality. Therefore, hydroxyapatite was tried next as an exchange agent for further chromatography[9]. Fig. 5 shows comparable chromatograms for LH_1 and LH_2 on this type of column. LH_2 (Fig. 5-b) exhibits a rather clean, sharp peak during gradient elution at pH 6.8. In the chromatogram shown the gradient was to 0.2 *M* phosphate buffer, although a gradient to 0.15 *M* will also elute the material as a single, broader peak. The LH_1 peak (Fig. 5-a) was considerably less than the LH_2 peak as judged by Folin-Lowry reaction, although both emerged with approximately the same effluent volume.

The LH_1 material (Fig. 5-a) also showed a considerable quantity of material giving a Folin-Lowry reaction throughout the development of the column prior to the emergence of the peak. Since this ion exchange agent does not seem useful for the study of the two luteinizing hormone activities, the fractions from this chromatography have not been studied further with respect to chemical and physiological properties.

Bioassay

Table I summarizes representative assays of the two luteinizing hormone preparations, LH_1 and LH_2, together with similar data on the Armour LH 227-80. Each series designated in this table was carried out on a single group of rats received at the same time and dosed over the same period. All rats were of the Sprague-Dawley strain.

In series 1, values obtained with 10, 20, and 40 μg doses in intact rats are shown. Body weight showed an average increase of approximately 20 g in all groups during the 5 day test period. LH_2 and the Armour LH 227-80 appear equipotent in this test animal with respect to ventral prostate, seminal vesicle, and testes growth stimulation. However, the intact rat did not show any stimulation for growth of these same organs when dosed with LH_1 at similar levels. (This difference in potency was also suggested in a qualitative manner by the sperm release response of the intact frog as noted above). However, it was apparent that the intact rat is not a good test animal and within-group variance is high.

In series 2 the results were in marked contrast. The hypophysectomized rat showed an equal response to LH_1, LH_2 and Armour 227-80, with respect to ventral prostate and testes growth stimulation. The seminal vesicle showed only slight increases over control animals and it was apparent that in this strain of rat and under the conditions employed, the seminal vesicle was very insensitive to luteinizing hormone stimulation. In none of the assays carried out to date based on ventral prostate growth in the hypophysectomized rat has LH_1, LH_2 or Armour LH 227-80 shown any significant difference in potency. In some instances ventral prostate growth equivalent to that shown for the 24 μg dose level have been obtained with as little as 6 μg of these preparations (the smallest dose tried to date). However, with the high variability in sensitivity of test animals under the conditions of procurement available, such a result was not readily reproducible.

In series 3, Table I, the assay data on the fractions obtained after re-chromatog-

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TABLE I

EFFECT OF LUTEINIZING HORMONE PREPARATIONS DURING 4-DAY TREATMENT PERIOD ON INTACT AND HYPOPHYSECTOMIZED MALE RATS

*Series**	*Material injected***	*Total dose (μg)*	*Number of rats*	*Average weight gain during treatment*	*Ventral prostate weight (mg)****	*Seminal vesicle weight (mg)****	*Testes weight (mg)****
1	Saline (control)	—	6	20.4	41.6 (± 9.2)	13.4 (± 2.4)	441 (± 80)
	Armour LH						
	No. 227-80	10	5	22.8	49.3 (± 5.3)	15.8 (± 1.9)	537 (± 36)
	No. 227-80	20	5	20.0	55.8 (± 10.6)	16.4 (± 2.5)	564 (± 65)
	No. 227-80	40	5	20.6	61.8 (± 10.5)	19.9 (± 3.9)	484 (± 82)
	LH_1	10	5	21.8	43.4 (± 12.9)	13.3 (± 1.8)	506 (± 115)
		20	5	19.6	46.7 (± 2.8)	13.5 (± 0.8)	473 (± 61)
		40	5	20.0	41.8 (± 5.1)	15.4 (± 3.0)	448 (± 90)
	LH_2	10	5	24.0	47.7 (± 8.8)	15.5 (± 2.4)	557 (± 59)
		20	5	24.2	49.7 (± 6.5)	17.2 (± 2.1)	563 (± 78)
		40	5	23.8	64.2 (± 6.9)	19.8 (± 1.9)	578 (± 66)
2	Saline (control)	—	5	§	6.9 (± 0.6)	6.3 (± 0.4)	167 (± 27)
	Armour LH						
	No. 227-80	6	5		8.2 (± 1.5)	7.2 (± 0.8)	186 (± 17)
	No. 227-80	12	5		10.3 (± 1.3)	8.3 (± 0.5)	214 (± 28)
	No. 227-80	24	6		16.5 (± 1.6)	6.9 (± 0.9)	232 (± 26)
	LH_1	6	5		7.6 (± 0.9)	6.4 (± 0.4)	175 (± 14)
		12	5		11.1 (± 2.1)	7.6 (± 0.5)	206 (± 43)
		24	6		16.3 (± 3.0)	7.4 (± 1.2)	239 (± 36)
	LH_2	6	5		8.6 (± 1.3)	5.6 (± 0.9)	175 (± 25)
		12	5		10.8 (± 2.3)	7.7 (± 1.1)	184 (± 31)
		24	6		18.0 (± 4.3)	8.6 (± 1.0)	250 (± 37)
3	Saline (control)	—	6	—1.2	8.5 (± 0.9)	7.3 (± 0.9)	188 (± 21)
	Armour LH						
	No. 227-80	12	5	2.0	14.8 (± 2.9)	7.7 (± 0.9)	216 (± 31)
	Peak 1 (Fig. 4a)	12	5	1.8	7.5 (± 0.9)	6.8 (± 0.3)	165 (± 11)
		24	5	2.4	7.7 (± 1.5)	7.1 (± 1.1)	182 (± 20)
	Peak 2 (Fig. 4a)	6	5	0.4	10.7 (± 1.5)	6.5 (± 0.1)	186 (± 7)
	(original LH_1)	12	4	5.0	11.6 (± 3.8)	6.1 (± 1.3)	198 (± 18)
	Peak 3 (Fig. 4a)	6	5	—1.8	11.0 (± 2.7)	7.4 (± 1.3)	194 (± 22)
	(converted LH_2)	12	5	1.0	15.4 (± 1.9)	6.7 (± 0.5)	228 (± 65)

* Series 1: Intact male rats, 21 day old, Sprague-Dawley, 40–50 g at start.
Series 2: Hypophysectomized male, Sprague-Dawley, 21 day old rats obtained from Hormone Assay Laboratories. Injections started 72 h after operation.
Series 3: Same as Series 2, but injections were started approximately 8 h sooner after operation.

** See text for designation of LH_1 and LH_2.

*** Figures in parentheses are the standard deviation of the mean.

§ Not measured for Series 2.

raphy of the LH_1 materials is presented. For the assay the peaks were designated as 1, 2 and 3 in order of their emergence from the column (Fig. 4-a). The first peak to emerge showed no activity at the 24 μg level, while the peak coming off in the area of the original LH_1 was active at the 6 and 12 μg level, as was the "new" peak emerging in the area of LH_2, designated as "converted" LH_2 in the Table. Thus, the bioassay data was consistent with the appearance of the chromatogram (Fig. 4-a) suggesting a conversion of LH_1 to LH_2.

In view of the above noted conversion of LH_1 to LH_2, further studies have been directed largely toward the characterization of the LH_2 material.

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The potency of an LH_2 preparation has been assayed in the weaver finch[13] and showed a positive response at the 5 μg dose level and negative at 2.5 μg dose. Again, this was in agreement with a potency equal to that of the Armour LH 227-80[13].

Follicle-stimulating hormone (FSH) activity has been assayed using a 1 mg dose of LH_2 according to the method of STEELMAN AND POHLEY[14]. This quantity of LH_2 gave a response less than one-tenth of the Armour FSH standard. In terms of the highest potency of FSH obtained to date[15] this would represent a contamination of less than 0.3 % FSH on a weight basis.

Thyroid-stimulating hormone (TSH) activity has been assayed for an LH_2 preparation as 0.6 to 0.75 USP units per mg using the ^{32}P uptake method in 2 day old chicks[16]. Since the most potent TSH preparations so far obtained have a potency of 30 USP units per mg* this would represent a contamination of approximately 2 % TSH on a weight basis. Chromatographically we have been unable to demonstrate such a contaminant using diethyl amino ethyl cellulose as the ion exchange agent as per CONDLIFFE AND BATES[17]. Under these conditions luteinizing hormone passes through the column without retention, an observation in agreement with the findings of CONDLIFFE AND BATES*.

Physical and chemical properties

A study of the behaviour of LH_2 during centrifugation was carried out by Mr. JOHN COOPER, Department of Biochemistry, Baylor University Medical College, Houston, Texas. Fig. 6 shows the pattern obtained at two different concentrations. A single component was observed. Viscosity determinations were carried out on the same solutions used for centrifugation. The sedimentation data, corrected to zero protein concentration and 20°, gave the value $S_{20} = 2.32 \pm 0.07$. Assuming a partial

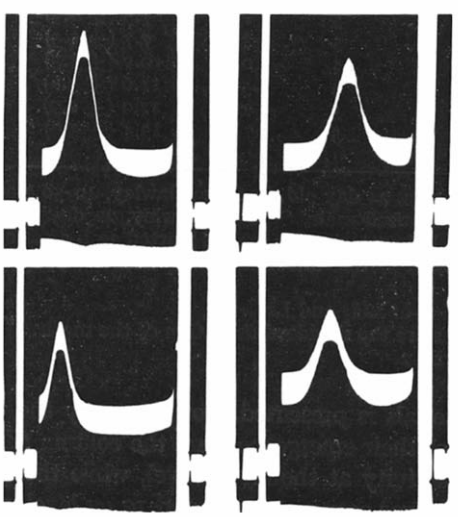

Fig. 6. Ultracentrifuge pattern of LH_2. The photographs on the left were taken after 84 min at 260,000 × g (Spinco Model E) and those on the right after 132 min. Upper photographs were made on a 3 % solution in glycine buffer, pH 6.0, $\mu = 0.1$. Lower photographs were made with a 1 % solution in this same buffer.

* Personal communication, Dr. PETER G. CONDLIFFE, National Institutes of Health, Bethesda, Md.

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specific volume of 0.75 ml/g and the frictional ratio ($f/f_0 = 1.30 \pm 0.10$) obtained from the viscosity measurements and assuming a prolate ellipsoid of revolution, the molecular weight was estimated to be 28,000 ± 4,000. Equations for this calculation have been detailed by LUNDGREN AND WARD[18].

TABLE II

GLUCOSAMINE CONTENT OF LUTEINIZING HORMONE PREPARATIONS OBTAINED FROM CHROMATOGRAPHY ON CARBOXYMETHYL CELLULOSE

Sample	*Glucosamine content* %
LH_1	7.2
LH_2	8.0
Globulin precipitated during dialysis of LH_1	6.0

Glucosamine was reported in preparations of luteinizing hormone in the original work of LI *et al.*[1]. They reported a value of 5.85 % for their preparation of ICSH. Various fractions from the preparative scale chromatograms (Fig. 3) have been analyzed using the method of JOHNSTON *et al.*[19] modified to employ a 1 *N* HCl hydrolysis. Table II lists the per cent glucosamine observed for the various fractions obtained after chromatography on carboxymethyl cellulose (Fig. 3). The fractions showed slightly higher content of glucosamine than originally reported by LI *et al.*[1]. However, slight variations in the method tend to give lower values. The conditions of hydrolysis appear to be most critical. Thus, the noteworthy point was not so much slight differences in reported glucosamine content but rather the fact that the hormone has such a remarkably high glucosamine content.

Finally, an attempt has been made to characterize the N-terminal amino acids in the LH_2 preparation. The fluorodinitrobenzene method as detailed by FRAENKEL-CONRAT *et al.*[20] has been used except for the substitution of a new solvent for the first development of the paper chromatograms. Instead of the "toluene" solvent, a solvent consisting of 15 parts *tert.*-amyl alcohol, 5 parts chloroform, 7.5 parts ethanol, and 22.5 parts 0.8 *M* ammonium hydroxide has been employed. The upper phase was used to develop the chromatogram. This solvent gave essentially the same resolution of DNP amino acids as the "toluene" solvent, but was easier to prepare. The principal N-terminal groups obtained have been serine and threonine with lesser quantities of glycine, lysine, and glutamic and/or aspartic acid. However, the minimum molecular weight calculated from the yield of DNP-serine or threonine varies from 118,000 to 250,000, depending on the length of time used for the hydrolysis. (This has been investigated over the period ½-hour to 12-hours hydrolysis.) Thus, the maximum yield of N-terminal amino acid ranges from 11 to 24 % of theoretical based on the molecular weight estimated from the ultracentrifuge data. In view of this discrepancy, no conclusion concerning the N-terminal amino acids is yet possible.

DISCUSSION

Although SQUIRE AND LI[3] have described a β-ICSH and presumably an α-ICSH (which has not yet been reported in detail), it still remains for direct comparison

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to determine whether their preparations correspond to the LH_1 and LH_2 reported here. If we are to judge from the chromatographic behaviour of β-ICSH as reported for Amberlite IRC-50, then the LH_2 reported in the present paper appears to show a similar behaviour on carboxymethyl cellulose. SQUIRE AND LI report stimulation of ventral prostate growth at 1/3 to 1/12 the dose we have used. But it must be noted that they were using animals dosed sooner after hypophysectomy and thus more sensitive assay animals. Also, a different strain of rats was used, and considerable differences in response to a single gonadotropic preparation has been shown for different rat strains[21]. In order to decide whether LH_2 has a different potency than β-ICSH will require a simultaneous determination of potency in a single group of animals, or at least a comparison with a common standard such as the Armour LH *227*-80. With respect to molecular weight, the 30,000 figure of SQUIRE AND LI[3] is within experimental error for the 28,000 molecular weight estimated in the present paper.

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Technical assistance was rendered by Miss E. E. NEU during the early stages of this study, and Mr. JOSEPH D. PUTCH during recent studies.

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