Purification of Human Pituitary Follicle Stimulating (FSH) and Luteinizing (LH) Hormones.* (24976)

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A number of investigators reported partial purification of human gonadotropin and follicle stimulating hormone (FSH)(1,2,3). Steelman *et al.*(3) showed that a simple extraction of acetone dried pituitaries and precipitation with alcohol resulted in a 50-100 fold purification of gonadotropins. Since the initial publication it has been possible to separate FSH and luteinizing hormone (LH) activities and prepare highly active fractions.

Methods and results. FSH was assayed by the method of Steelman and Pohley(4) based upon ovarian weight increase in intact immature female rats treated for 3 days with human chorionic gonadotropin. In all assays samples were dissolved in 0.9% saline containing 0.5% gelatin at pH 6-7. LH was measured in immature hypophysectomized male rat using increase in ventral prostate weight as index of activity. Animals were injected (24 hours after operation) once daily for 4 days and sacrificed on fifth. Total gonadotropin activity was determined by increase in uterine and/or ovarian weights in immature female rats and mice. The assay schedule consisted of 2 subcutaneous injections a day for 3 days and autopsy on fourth day. Chromatography with *carboxymethyl cellulose* (CMC). Sixty mg of human gonadotropin concentrate(3) was dissolved in 60 ml of water and adjusted to pH 6.1 with dilute ammonium hydroxide. This was placed on a column of CMC (0.4-0.5 meq/g) (10 x 200 mm) previously equilibrated at pH 6.1 with 0.01 M ammonium acetate buffer (Fig. 1). The sample was followed with pH 6.1, 0.01 M ammonium acetate. Elution of retained fraction was accomplished with 1 M ammonium acetate and was designated fraction CMC-B and contained LH. The non-retained fraction (CMC-A) had about 80% of protein and all FSH activity. Fraction CMC-B was lyophilized and reworked on CMC under identical conditions, except a gradient (1 M NH_4Ac through 200 ml reservoir of 0.01 M NH_4Ac) elution was employed. This resulted in 2 well separated peaks—CMC-B-1 and CMC-B-2. Fraction CMC-B-1 which emerged first contained practically all the LH activity, while CMC-B-2 had very low activity. *DEAE-Cellulose.* Fraction CMC-A was adjusted to pH 7.0 with dilute ammonium hydroxide and placed on a DEAE-cellulose (0.5 meq/g) column (8 x 150 mm) equilibrated at pH 7.0 with 0.01 M ammonium acetate (Fig. 2).

All FSH activity and most of protein were adsorbed. After washing with 0.01 M buffer, a gradient of 0.75 M NH_4Ac through 200 ml of 0.01 M NH_4Ac was applied. The FSH fraction emerged first and was extremely active (50 x standard). Fraction B was less than 10 times standard. Although optical density at 280 mμ was used to estimate protein content of individual tubes, the protein content of fractions from pools of tubes was determined by a microbiuret method(5) using crystalline bovine albumin as a standard. All activities were expressed in terms of biuret protein. Folin-Lowry determinations(6) were made on selected samples and in general agreed with biuret values, although they tended to be slightly higher.

Attempts to rechromatograph DEAE fraction A resulted in considerable loss in biological activity and no increase in specific activity of any fraction. This confirms observations made with swine FSH(7).

Table I summarizes gonadotropic activities of the various fractions. It was of particular

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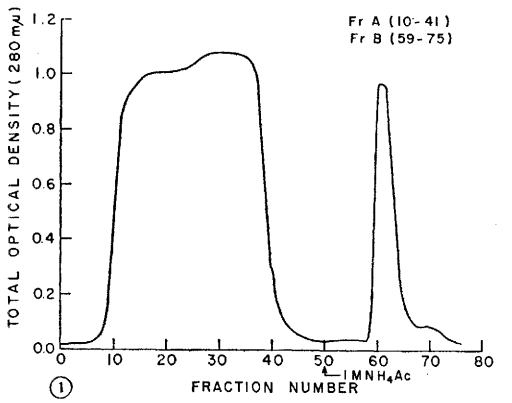

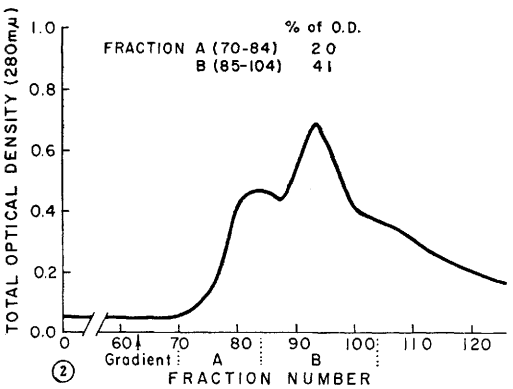

FIG. 1. Chromatography of human gonadotropin on CMC. Fraction volume was 2 ml and total optical density was observed optical density (280 mμ) multiplied by sample volume.

FIG. 2. Chromatography of human FSH on DEAE-cellulose.

interest that human pituitary LH was approximately 10 times as active as pure sheep LH. A total dose of 500 mμg gives at least 100% increase in ventral prostate weight in hypophysectomized male rats. Sufficient human LH has not been available to conduct adequate physicochemical studies; however, the CMC-B-1 did not appear to be homogeneous. Steelman and Segaloff(8) reported that purified equine gonadotropin concentrates have LH activities of 5-7 times the Armour Standard (227-80).

Human pituitary FSH appeared to be at least as potent as swine and sheep FSH(8). On the basis of published data, the FSH preparation reported here is at least 10-20 times as active as those of Li(1) and Gemzell(2). Unfortunately, the same reference standard was not used by the 3 laboratories. Li(1) has proposed the use of a slope unit based on the assay of Steelman and Pohley(4). Unfortunately, this predicates the use of a biological end point rather than a standard reference preparation. The variation in responsiveness of the animals renders this of doubtful value as a quantitative measure of FSH activity. Twelve consecutive FSH assays conducted in the laboratory of one of the authors (SLS) were analyzed and the slope unitage determined for the reference preparation (264-151-X). A range of 200-750 units/mg was found. Fraction DEAE-A possessed 10,000-20,000 slope units per mg. It was estimated that a total dose of 2 μg of DEAE-A will, under normal assay conditions, produce approximately the same response as 50 μ of the Li Fraction F. Table II summarizes a typical FSH assay of 2 separate DEAE-A fractions of human FSH. The slopes of the dose response curves of the standard and unknown do not differ significantly.

It has been possible to prepare human LH which was relatively free of FSH. Sufficient LH has not been available to assay for FSH at very high dosage levels. So far it has not been possible to prepare FSH free of LH. Even recycling the FSH fraction from the CMC column on fresh CMC did not remove additional LH. Either the remaining LH has different properties from the one retained by

TABLE I. Gonadotropic Activities of Human Pituitary Fractions.

Fraction	FSH X Standard*	LH X Standard†	Gonadotropin R.U./mg‡	Gonadotropin M.U./mg§
Starting material	8–10	3–5	200– 400	
CMC–A	10–12	.5–1.0	100– 200	
–B-1	<.5	10	500–1000	300–500
DEAE–A	50	1–1.5	500–1000	300–500

* Armour Standard 264-151-X. † Armour Standard 227-80. ‡ One rat unit = 100% increase in uterine wt. § One mouse unit = 100% increase in uterine wt.

TABLE II. Activities of Highly Purified Human FSH, 5 Animals/Series.

Preparation	Total dose* (μg)	Ovarian wt (mg) †	Potency X standard
Control		39.0 ± 3.9	
Standard (264-151-X)	100	79.6 ± 8.8	
Idem	200	102.6 ± 5.5	
FMA	2	80.1 ± 6.7	54
"	4	110.6 ± 9.9	
FJC	2	75.5 ± 6.5	56
"	4	125.6 ±18.2	

* Each animal received total of 40 I.U. of human chorionic gonadotropin plus indicated amount of FSH.

† ± stand. error of mean.

CMC or, it is inherent in the molecule of FSH. It should be recalled that pregnant mare serum gonadotropin has both FSH and LH activity. Indeed, the preparations reported by Li(1) and Gemzell(2) both contained considerable LH. Ward *et al.*(9) and Li and Squires(10) have reported 2 types of LH from sheep pituitaries. In the case of Ward *et al.*(9) both were adsorbed on CMC under the conditions cited in this paper. In fact, it was proposed that one was derived from the other, and it is possible that they are identical with the α and β LH of Li and Squires(10).

Our important finding was that both human FSH and LH were highly active in causing increases in uterine weights of rats and mice. For many years, the standard method for determining gonadotropin content of human urine has been the increase in uterine weight in immature mice(11). This has erroneously been called FSH. As can be clearly seen in Table I, both FSH and LH were active. Recently(10) it has been shown that human urinary gonadotropins can be separated into FSH and LH fractions, and that both have uterine weight activity. The two hormones had chromatographic behavior similar to their precursors from the pituitary. Thus, it should be possible physically to separate and biologically assay FSH and LH in the urine of humans. This would materially aid the clinician in accurate diagnosis of many gonadal and pituitary dysfunctions.

Summary. FSH and LH from human pituitaries have been isolated with high specific activities. Both FSH and LH had uterine weight increasing activity in immature rats and mice. The FSH had approximately the same activity as porcine and ovine FSH, however, LH was 10 times as active as pure ovine LH.

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