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Preparation of Highly Potent Human Pituitary Gonadotrophins.* (28892)

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There have been numerous reports on the preparation of human pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH, ICSH) having relatively high degrees of purity(1,2,3,4). Recently, Reichert and Parlow(5) described a simple, efficient method for a high degree of separation and purification of human pituitary FSH and LH, involving differential adsorption on DEAE-Cellulose followed by stepwise elution with NaCl. The resulting preparations of lyophilized human FSH (29 times as potent as NIH-FSH-S1) and LH (0.9 times as potent as NIH-LH-S1) were more active than any previously isolated(5). Since then, it has been possible to obtain human FSH and LH fractions which are even more active than those of our previous report.

Methods and results. LH activity was determined by the method of Parlow (ovarian ascorbic acid depletion in pseudopregnant rats)(6). FSH activity was determined by the HCG-augmentation method of Steelman and Pohley(7). Descriptions of the methods, design, and statistical analysis of these bioassays have been reported(8,9). *Gel filtration.* Five mg of a partially purified human LH fraction, F-1-2, (0.9 times as potent as NIH-LH-S1), prepared by chromatography on DEAE-Cellulose(5), was applied to a 0.9 × 120 cm column of the hydrated dextran gel Sephadex G-100, previously equilibrated with 0.005 M phosphate buffer, pH 6.0. This buffer was also the solvent for the protein fraction. The column was developed at a flow rate of 6 ml/hour, with collection of 1.5 ml samples per tube. This and all other experiments were conducted at 3-5°C. The ultraviolet elution profile, measured at 275 mμ, is shown in Fig. 1. Protein content of fractions in solution was determined by the ultraviolet absorption method of Waddell (10) and Murphy and Kies(11). A detailed outline of this procedure as we use it has appeared elsewhere(12). The results of bioassays of various fractions are shown in Fig. 1 and Table I. The most potent fraction, #23, was 2.9 times more active than NIH-LH-SI, representing a 3-fold increase in activity over its precursor.

Three mg of a partially purified human FSH fraction F-1-4 (initial potency: 29 times NIH-FSH-S1), prepared by chromatography on DEAE-Cellulose(5), was treated under conditions identical to those described above for gel filtration of the human LH fraction. The ultraviolet elution pattern at

TABLE I. Gel Filtration of Human Pituitary LH on Sephadex G-100.

Fraction	No. of assays	Mean potency (units/mg)*	95% limits
Precursor F-1-2	5	.9	.8–1.1
Tube 17	1	<.2	
18	1	<.2	
19	1	.5	.3– .8
20	2	.9	.6–1.5
23	3	2.9	2.1–4.0
26	2	1.4	.8–2.2
29	1	.3	.2– .5
38	1	<.1	

* One unit = activity in one mg NIH-LH-S1.

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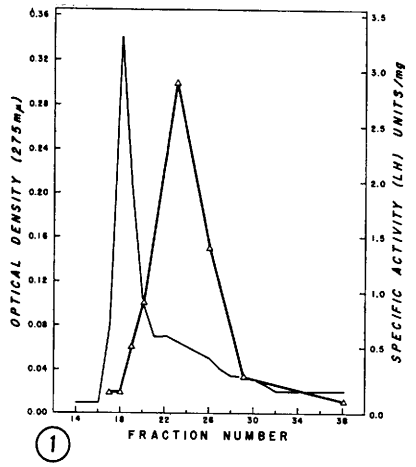

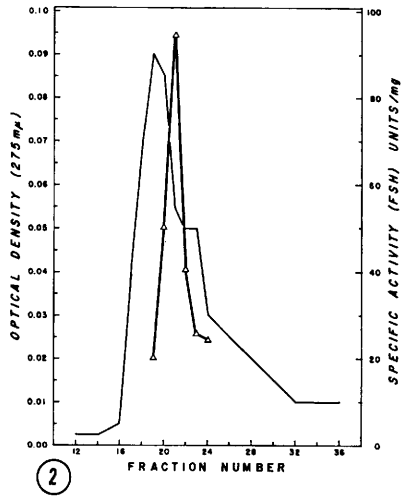

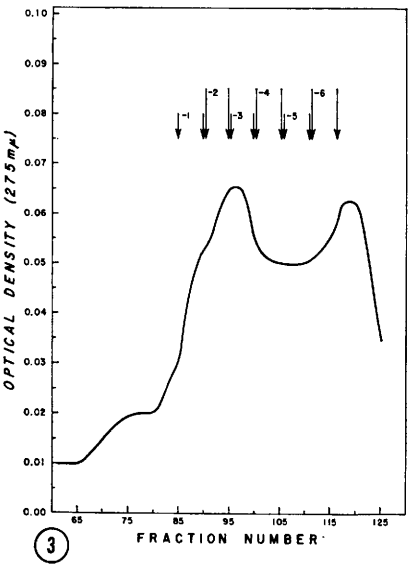

TABLE II. Gel Filtration of Human Pituitary FSH on Sephadex G-100.

Fraction	No. of assays	Mean potency (units/mg)*	95% limits
Precursor F-1-4	2	28.9	21.9– 38.1
Tube 19	2	19.7	14.7– 26.4
20	2	51.9	39.6– 68.0
21	2	94.2	57.4–154.5
22	2	41.2	31.0– 54.8
23	1	27.0	17.9– 40.8
24	1	24.0	16.0– 36.0

* One unit = activity in one mg NIH-FSH-S1.

275 mμ is shown in Fig. 2. Table II summarizes the results of bioassays of various fractions. The most active fraction, #21, was 94 times more potent than NIH-FSH-S1, or 172 times more potent than the Armour Standard FSH (264-151-X).

Fraction F-1-4 was also purified by gradient elution from a column of DEAE-Cellulose with sodium chloride. Three mg of F-1-4 were dissolved in 2 ml of 0.007 M phosphate-0.003 M borate buffer pH 8.0, and applied to a 0.9 × 24 cm column of DEAE-Cellulose which had been previously exhaustively equilibrated with the solvent buffer. The flow rate throughout the run was 6.0 ml per hour and samples of 1.5 ml were collected per tube. After washing with solvent buffer to remove traces of unadsorbed protein, the column was developed with the sodium chloride gradient (beginning at fraction #41). The gradient was prepared by allowing 50 ml of 0.2 N NaCl in solvent buffer, contained in a 125 ml aspirator bottle, to flow into 50 ml of the solvent buffer in an identical container, in a manner producing a linear salt gradient. The gradient was completed by fraction #101, at which time a final stepwise elution with 0.2 M NaCl-solvent buffer was carried out. The ultraviolet elution pattern (275 mμ) is shown in Fig. 3, while the bioassay data are

FIG. 1. Gel filtration of fraction F-1-2 on Sephadex G-100. △——△ Refers to specific activity (LH). See Table I for detailed bioassay data.

FIG. 2. Gel filtration of fraction F-1-4 on Sephadex G-100. △——△ Refers to specific activity (FSH). See Table II for detailed bioassay data.

FIG. 3. Chromatography of fraction F-1-4 on DEAE-cellulose. Tubes between arrows were pooled. See Table III for detailed bioassay data.

TABLE III. Purification of Human Pituitary FSH by Gradient Elution from DEAE-Cellulose.

Fraction	No. of assays	Mean potency (units/mg)*	95% limits
Precursor F-1-4	2	28.9	21.9– 38.1
Tubes 85– 90	1	85.6	56.7–129.3
91– 95	1	23.1	15.2– 35.1
96–100	1	14.2	9.5– 21.2
101–106	1	15.9	10.7– 23.7
107–112	1	4.9	3.3– 7.4
119–124	1	5.4	3.6– 8.0

* One unit = activity in one mg NIH-FSH-S1.

summarized in Table III. The fraction representing pooled tubes 85-90 exhibited FSH activity 85 times greater than NIH-FSH-S1, similar to that of fraction #23 obtained by gel filtration of the same precursor (Table II, Fig. 2).

Discussion. Human FSH and LH fractions with high biological activity prepared by the method of Reichert and Parlow(5) were not regarded by them(5) as pure or homogeneous, since even at that time biological evaluation revealed that each preparation contained significant contamination with the other. That ultimate purity had indeed not been achieved with the preparation of those fractions is supported by the description here of human FSH and LH fractions considerably more active than our previous preparations.

Expression of the potency of the principal FSH and LH fractions in terms of reference preparations other than NIH-FSH-S1 and NIH-LH-S1 can be achieved by use of conversion factors listed elsewhere(5). Thus, FSH fraction #21, obtained by gel filtration, is 314 times more potent than Pergonal-23 and 18,840 times more potent than HMG-24. Similarly, LH fraction #23 exhibited activity 1381 times greater than NIH-HPG-UE. Both of these FSH and LH fractions are at least 3 times more active than any previously described(5). No attempt has been made as yet to isolate these fractions from solutions.

Summary. Human FSH and LH fractions, equal in potency to any previously reported, have been further purified by gel filtration and ion-exchange chromatography. The most potent of these new fractions was 94 times more active than NIH-FSH-S1 and 2.9 times more active than NIH-LH-S1, respectively.

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