Purification of Follicle-Stimulating Hormone from Human Pituitary Glands.* (24202)

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Highly purified follicle-stimulating (FSH) and interstitial-cell stimulating (ICSH) hormones have been prepared from sheep and hog pituitaries(1-3). Although it is known that human pituitary glands contain both FSH and ICSH activities(4), no purification of these two hormones from extracts of human pituitaries has been reported. In this paper is presented a procedure for the preparation of an FSH concentrate from human pituitaries.

Material and methods. Human pituitaries† were obtained at autopsy, one to 3 days after death; the whole pituitary was immediately frozen and lyophilized. The dried pituitary was cut into small pieces and ground before extraction. The assay method of Steelman and Pohley(5), based on augmentation of ovarian weight elicited by FSH and human chorionic gonadotropin (HCG) in intact rats, was used for estimating follicle-stimulating potency of the preparations. Immature female rats of the Long-Evans strain, 21 and 22 days of age, were injected, once daily for 3 days, intraperitoneally with HCG and subcutaneously with the FSH preparation to be tested. The dose of HCG was adjusted to give control ovaries weighing 30-35 mg. Autopsy was performed 24 hours after last injection. Biological activity of a preparation was given as a slope unit defined by the equation:

$$\text{Slope unit} = \frac{\text{Test ovaries (mg)} - \text{Control ovaries (mg)}}{\text{Total dose (mg)}}$$

The HCG preparation was a commercial product‡ and had an activity of 1000 I.U./mg. The preparations of both HCG and FSH were dissolved in distilled water for injection.

Purification procedure. All steps were performed at 0-2°C with the exception of the step involving ion exchange resin, conducted at room temperature. *$Ca(OH)_2$ Extraction and $(NH_4)_2SO_4$ Fractionation.* 10 g of human pituitary powder are stirred vigorously with 400 ml of $Ca(OH)_2$ solution at pH 10.3 for about 3 hours. The suspension is set aside for 16 hours and centrifuged; the residue is washed with 100 ml $Ca(OH)_2$ solution of pH 10.3. The supernatant fluid and washing are combined and brought to 1.9 M with respect to ammonium sulfate by addition of solid $(NH_4)_2SO_4$. The precipitate formed is centrifuged off and saved for isolation of growth hormone(6,7). The 1.9 M $(NH_4)_2SO_4$ supernatant fluid is brought to 3 M by addition of more solid $(NH_4)_2SO_4$ at pH 7, and the precipitate which forms is dissolved in water, dialyzed and lyophilized. The lyophilized product (*Fraction SI*) weighs 0.5 g and has an activity of 144 slope units. *Refractionation with $(NH_4)_2SO_4$.* 0.5 g of Fraction SI is dissolved in 50 ml distilled water; after removal of some insoluble material by centrifugation, the supernatant fluid is made to 1.9 M $(NH_4)_2SO_4$ by addition of saturated $(NH_4)_2SO_4$ solution at pH 7. If precipitation occurs, the solution is centrifuged and the precipitate discarded. The $(NH_4)_2SO_4$ concentration in supernatant fluid is increased to 2.7 M by further addition of saturated $(NH_4)_2SO_4$ solution. The precipitate which forms is dissolved, dialyzed, and lyophilized. The lyophilized production (*Fraction SII*) weighs 0.25 g and has an activity of 190 slope units. *Purification on Cation Exchange Resin.* Further purification of Fraction SII is effected on the carboxyl cation exchange resin Amberlite IRC-50 (XE-

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TABLE I. Protocol for Purification of Human FSH

Fraction	Wt, g	Procedure	Bioassay: Total dose, mg	Bioassay: No. of rats	Bioassay: Ovarian wt: HCG alone, mg	Bioassay: Ovarian wt: Test fraction + HCG, mg	Activity, slope units
A	10	Lyophilized whole pituitaries					
AS	4.9	Extracted with pH 10.5 CaO solution	1.0	8	33.2	63.5	30
SI	.5	1.9-3.0 M $(NH_4)_2SO_4$ precipitate	.2	13	37.6	66.5	144
SII	.25	Refractionation with $(NH_4)_2SO_4$	.2	13	32.4	70.4	190
CU	.14	Unadsorbed fraction from IRC-50 column	.1	21	32.9	67.5	346
F	.10	$(NH_4)_2SO_4$ fractionation as SII	.05	15	37.4	60.6	464

97). 250 mg of Fraction SII are dissolved in 50 ml of pH 5.1 buffer§ and applied to the resin column‖ (0.9 cm diameter column containing 50 ml resin), and the column is washed with 120 ml of the same buffer. The protein, which is adsorbed by the resin, is devoid of follicle-stimulating activity. The effluent obtained with the pH 5.1 buffer is brought to 3 M $(NH_4)_2SO_4$ by addition of solid $(NH_4)_2SO_4$. The precipitate formed is dissolved, dialyzed and lyophilized. The lyophilized product (*Fraction CU*) weighs 0.14 g and has an activity of 346 slope units mg. *$(NH_4)_2SO_4$ Fractionation.* 0.14 g of Fraction CU is dissolved in 15 ml of distilled water and fractionated with $(NH_4)_2SO_4$ according to the method described above for preparation of Fraction SII. The yield of the active fraction is 0.1 g; it possesses an activity of 464 slope units.

Table I summarizes the purification procedure together with the yield and potency of each fraction. A 15-fold increase in biological activity can be achieved by the procedure with a yield of 100 mg of purified FSH from 10 g of lyophilized pituitaries; the follicle-stimulating activity of the product (*Fraction F*) has been assayed in graded doses by the augmentation test(5) and results are given in Table II. It is evident that the preparation contains a potency of approximately 600 slope units/ml, an activity comparable to that of ovine FSH preparation obtained by zone electrophoresis on starch(9).

When Fraction F was assayed in hypophysectomized male rats(10), the preparation elicited an increase of ventral prostate weight from 7 mg to 15 mg at a total dose of 0.1 mg, indicating that the fraction is contaminated with ICSH.¶ The preparation is practically free from thyrotropic, lactogenic, adrenocorticotropic and growth-promoting activities, according to tests by conventional assay procedures. Physicochemical investigations indicate that Fraction F is not homogeneous; further purification of this fraction is in progress.

TABLE II. Follicle-Stimulating Activity of Purified Human FSH (Fraction F) According to Augmentation Test.

Total dose of fraction F, μg*	No. of rats	Body wt, g		Ovarian wt, mg	
0	11	52.4	1.0†	30.5	2.3
25	9	49.1	.8	48.9	3.0
50	13	54.2	2.6	61.0	4.1
100	11	51.8	1.3	79.5	3.5

* A constant daily dose (20 μg) of HCG was also administered to all experimental animals.

† Mean ± stand. error.

§ Composition of the buffer is as follows: 0.052 M NaH_2PO_4, 0.002 5 M Na_2HPO_4 and 0.45 M $(NH_4)_2SO_4$.

‖ For conditions used and for details of the operation of the resin colum, see Papkoff and Li(8).

¶ Purification of ICSH from human pituitary glands will be reported elsewhere; a total dose of 0.005 mg of purified human ICSH will elicit an increase of 100% in weight of ventral prostates of hypophysectomized rats over the controls.

Summary. A procedure for preparation of follicle-stimulating hormone from human pituitaries has been described. Approximately a 15-fold purification could be achieved by a procedure involving fractionation with $(NH_4)_2SO_4$ and a cation exchange resin.

Addendum: FSH fraction SII has been tested for gonad-stimulating activities in human subjects by Dr D. M. Bergenstal of National Cancer Institute, Bethesda, Md. A female subject (age 32), one day after cessation of menstruation, was injected subcutaneously with 2.5 mg FSH concentrate every 6 hours for 6 days and followed by castration. Ovaries were highly stimulated with the appearance of multiple follicular cysts. Details of these clinical studies will be reported by Dr. Bergenstal elsewhere.

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Association of Pyridoxine Deficiency and Convulsions in Alcoholics.*† (24203)

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Alcoholic withdrawal syndrome includes convulsions. It is generally believed that these seizures relate to idiopathic or post-traumatic epilepsy or alcohol withdrawal(1,2). As infants fed a formula deficient in Vit. B_6 (3) or adults with chemically induced Vit. B_6 lack (*i.e.*, administration of isoniazide(4) or desoxy-pyridoxine(5,6)) have occasional *grand mal* seizures, we wondered if seizures in alcoholics might not be associated with Vit. B_6 deficiency, particularly as their diets are grossly inadequate. The term "rum fit" has been used to describe these seizures occurring in the immediate 36- to 72-hour period after cessation of an alcoholic spree. Henceforth this nomenclature will be used to describe these patients. This study purports to determine if rum fitters may be Vit. B_6 deficient.

Materials and methods. Five rum fitters, 2 patients with alcoholism and associated epilepsy§, 6 patients in various phases of alcoholism but without fits, and 1 normal non-alcoholic individual were studied. The group consisted of 13 males and 1 female. All were seen immediately on admission to the hospital. They were given a Vit. B_6-free diet consisting of "Dexin"‖ in water mixture and daily vitamin supplement excluding Vit. B_6.¶ An initial urine was collected. Following this, the patients were fed 10 g dl-tryptophane mixed with Dexin in water, either orally or by gas-

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§ These patients also had convulsions when not drinking and when their diets were good.

‖ A partial starch hydrolysate, Burroughs-Wellcome Co., Tuckahoe, N. Y.

¶ The daily vitamin supplement consisted of: thiamine 50 mg (i.m.) and 10 mg riboflavin, 100 mg niacin, and 100 mg ascorbic acid (p.o.).