Distribution and Partial Purification of Pituitary Gonadotropins of Human Plasma.* (22771)

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The presence of a substance or substances possessing gonadotropic activity, presumably of pituitary origin, in the serum of ovariectomized women was first demonstrated by Fluhmann(1). Subsequently, Cohn and his collaborators(2) reported the detection of two gonadotropic hormones in fractions prepared from certain pools of human plasma. Since these findings could not be fully confirmed in later studies,† we have reexamined the distribution of pituitary gonadotropic activity among various plasma fractions. The bulk of the gonadotropic activity has been located in Fraction II + III, and by subfractionation has been partially purified. Preliminary reports of these studies have appeared elsewhere (3,4).

Methods and materials. Postmenopausal plasma was employed exclusively in this study, preliminary bioassays having shown that gonadotropic activity is readily demonstrable in the whole plasma of postmenopausal women, but not in that of men or of adult women of menstrual age. The donors were healthy women 65 years of age or less who were not undergoing treatment with estrogenic preparations of any kind.‡ The blood was collected either with citrate or through cationic exchange resins in the ADL-Cohn blood fractionating machine. The data herein reported were obtained by fractionating a 10-liter plasma pool which comprised the donations of 40 women, and are identical with the results previously obtained from the fractionation of 20 individual or pooled plasma samples from 3-12 donors. The cold ethanol method VI described by Cohn *et al.* (6) was employed for the initial plasma fractionation. The following fractions were prepared: Fraction I containing the fibrinogen; II + III consisting of β-lipoproteins and gamma globulin; IV-1 containing the α-globulins and cholesterol; IV-4 consisting of α- and β-globulins and the metal-combining globulin; V, which contains the bulk of the albumin; and VI, the supernatant remaining after fractionation. Partial purification of the gonadotropically-active material in Fraction II + III was accomplished by precipitating the bulk of the biologically-inactive proteins with zinc. Fraction II + III was brought to the original plasma volume with 0.15 M sodium chloride and zinc glycinate added until a 5 mM zinc concentration was obtained at a pH of 6.8 and a temperature of 0°C. The supernatant was freed of zinc by dialysis against ethylene diamine tetra-acetic acid (EDTA) and sodium citrate. The EDTA and citrate were subsequently removed by dialysis against 0.15 M sodium chloride. The addition of 20% ethanol to the purified supernatant at a pH of 6.5 and a temperature of —5°C resulted in the precipitation of additional material which was biologically inactive, leaving the gonadotropins in a supernatant containing 2-4% of the proteins originally present in the fraction. In preparation for bioassay the fractions were redissolved in small volumes of 0.15 M NaCl and dialyzed for 24 hours against 0.15 M NaCl in order to remove residual alcohol and excess salt. All samples were stored at 2°C and their pH adjusted to 7.0 ± 0.4 immedi-

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‡ Previous experience has shown that unwitting ingestion of estrogen occasionally results in significant contamination of plasma pools. Therefore, the whole plasma and those fractions found to produce an increase in uterine weight of intact rats were assayed for estrogen in spayed rats by modification of method of Bulbring and Burn(5). No indication of estrogenic activity was found.

TABLE I. Relative Gonadotropic Activity of Whole Postmenopausal Plasma and of Its Individual Fractions.

Preparation	% plasma proteins	Plasma equivalence (ml)	No. of animals	Mean wt, mg* Uterus	Ovary
Whole plasma	100.0	15	3	24.6	17.2
		20	3	*91.5*	14.0
		40	6	*110.1*	*24.6*
I	4.1	400	3	15.2	11.3
II + III	25.0	15	2	23.3	14.8
		20	3	*39.7*	16.7
		40	3	*97.3*	16.1
		80	3	*131.4*	*21.8*
IV-1	7.5	400	3	23.9	15.9
		500	3	*58.6*	12.1
		666	3	*146.5*	15.7
IV-4	11.6	550	3	26.3	14.7
		700	3	*125.5*	18.8
V	50.0	600	3	24.9	18.9
		750	2	*73.0*	18.5
VI	1.0	700	3	33.2	16.9

* Mean uterine weights in excess of 38.3 and mean ovarian weights in excess of 21.1 mg are regarded as differing significantly from control weights ($p < 0.01$) and are italicized.

ately prior to assay. "Gonadotropic activity," which presumably comprises the action of two substances, pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH), was assayed biologically. Immature female rats of the Sprague-Dawley strain, weighing 45-55 g, were injected subcutaneously with the material to be tested twice daily for 4 days and were killed on the fifth day. The wet weights of the ovaries and the fluid-free uterus were employed as indices of gonadotropic activity, the minimum quantity of whole plasma and of the individual fractions required to induce statistically significant increases in the weights of these organs being ascertained. In 23 control animals, the mean ovarian weight was 15.1 ± 3.7 and the mean uterine weight 28.2 ± 6.2 mg. Therefore, that volume of plasma which just sufficed to increase the mean ovarian weight of the three test animals above 21.1 or the mean uterine weight above 38.3 mg was regarded as the minimal effective dose ($p<0.01$).

Results. The relative gonadotropic activity of whole plasma and of its various fractions is shown in Table I. The gonadotropic potency of whole plasma ($M.E.D._{ut.\ wt.}$, 20 ml; $M.E.D._{ov.\ wt.}$, 40 ml) accords well with Fluhmann's observations(1). Employing as his test animal the immature female mouse, which is approximately 6 times as sensitive to human menopausal gonadotropin as the rat (7), Fluhmann demonstrated gonadotropic activity in 3-5 ml of the serum of ovariectomized women. The finding that appreciably greater gonadotropic stimulation is required for ovarian growth than for the induction of ovarian secretory activity is in agreement with many previous observations.

The bulk of the gonadotropic activity is seen to be concentrated in Fraction II + III, although traces of activity are demonstrable in Fractions IV-1, IV-4 and V. The potency of Fraction II + III would appear to be somewhat more than half that of whole

TABLE II. Relative Gonadotropic Activity of Fraction II + III, and of Its Subfractions.

Preparation	% proteins in Fraction II + III	Plasma equivalence (ml)	No. of animals	Mean uterine wt (mg)*	Mean ovarian wt (mg)*
II + III	100	20	3	*39.7*	16.7
		40	3	*97.3*	16.1
		80	3	*131.4*	*21.8*
5 mM Zn^{++} precipitate	94	300	3	35.7	14.1
" supernatant	6	20	3	26.3	18.2
		40	3	*126.1*	18.1
		80	3	*141.9*	*35.8*
20% ethanol precipitate from 5 mM Zn^{++} supernatant	3	100	3	25.8	14.7
20% ethanol supernatant from 5 mM Zn^{++} supernatant	3	40	2	*98.8*	15.4
		80	3	*115.7*	*34.9*

* Mean uterine weights in excess of 38.3 and mean ovarian weights in excess of 21.1 mg are regarded as differing significantly from control weights ($p < 0.01$) and are italicized.

plasma, whether gauged by the $M.E.D._{ut.\ wt.}$ or the $M.E.D._{ov.wt.}$ Fractions I and VI appear to be devoid of gonadotropic activity.

The relative gonadotropic potency of Fraction II + III and of its subfractions is shown in Table II. It will be noted that zinc precipitation effectively frees the gonadotropically-active material from the majority of the contaminating proteins and that re-precipitation with 20% ethanol removes additional contaminants.

Summary. The location and approximate concentration of gonadotropic activity in various fractions of human postmenopausal plasma has been determined. The plasma was fractionated by means of the cold ethanol method VI of Cohn and was assayed in immature female rats by estimating ovarian growth and secretory activity. Although traces of activity were found in Fractions IV-1, IV-4 and V, the bulk of the gonadotropically-active material was located in Fraction II + III. Subfractionation of Fraction II + III by successive precipitations with zinc and 20% ethanol freed the gonadotropins from the majority of the contaminating proteins.

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