Isolation of Gonadotropin from Human Term Placenta (34382)

S. S. KOIDE

The Population Council, The Rockefeller University, New York, New York 10021

Human chorionic gonadotropin (HCG) obtained from the urine of pregnant women is generally used in studying the physicochemical properties and the metabolic effects of gonadotropin of human placenta (1–6). The HCG is capable of inducing ovulation associated with uterine stimulation, increase in ovarian weight and follicular hemorrhages (1–6). The production of follicular hemorrhages has been attributed either to pituitary follicle-stimulating hormone (FSH) occurring as a contaminant (2–4) or, to an intrinsic follicle-stimulating property (5). It has been suggested that HCG occurs as a complex since several fractions having different degrees of activities are obtained during the purification procedure (7–9).

Only a few reports have been published on the characteristics of gonadotropin isolated from human placenta (10). The decline of urinary gonadotropin with the duration of pregnancy has deterred attempts to isolate gonadotropin directly from human term placenta (6, 11). In the present paper, a procedure for the isolation of gonadotropin from human term placenta is presented. The gonadotropin activity and potency were assayed and compared with HCG.

Materials and Methods. Preparation of crude gonadotropin. Human term placenta was obtained immediately from the delivery room and washed with 0.85% saline. All subsequent steps were performed in the cold (4–5°). The vessels were cut open, clots were removed, and vessels were perfused and washed with 0.85% saline. The placenta was cut into pieces, passed through a meat grinder, and homogenized in 0.85% saline (g/2 ml) in a Waring Blendor at full speed for 5 min. The homogenate was filtered through coarse and fine stainless steel sieves and the filtrate centrifuged at 10,000*g* for 30 min. The supernatant fraction was adjusted to pH 5.5 with glacial acetic acid, stirred in the cold for 2 hr and centrifuged at 10,000*g* for 30 min.

To the supernatant solution, solid ammonium sulfate was added slowly to a concentration of 3.0 molality under constant stirring. After standing overnight, the supernatant solution was removed by suction and centrifuged at 10,000*g* for 10 min. The sediment was dissolved in distilled water (10% of original volume). The pH of the mixture was adjusted to 5.5 with glacial acetic acid and centrifuged at 10,000*g* for 15 min. Ammonium sulfate was added to a concentration of 3.0 molality and the mixture was centrifuged at 10,000*g* for 10 min. The supernatant solution was discarded. The sediment was dissolved in distilled water (5% of original volume) and stirred for 1 hr and centrifuged at 10,000*g* for 15 min. To the supernatant solution 2 vol of cold acetone were added, the mixture was stirred for 30 min, and centrifuged at 10,000*g* for 15 min. The sediment was resuspended in cold acetone, filtered, and dried on a Buchner funnel. The acetone powder was dissolved in water (g/10 ml) stirred overnight and the solution centrifuged at 10,000*g* for 15 min. Two vol of acetone were added to the supernatant fraction, stirred for 30 min, and centrifuged at 10,000*g* for 15 min. The sediment was resuspended in cold acetone, filtered, and dried on a Buchner funnel. The acetone powder was dissolved in water (g/10 ml), stirred overnight, and centrifuged at 10,000*g* for 15 min. The supernatant solution was lyophilized. The dried product was designated as crude human placental gonadotropin (HPG), placed in a desiccator, and stored in the cold. The mean yield was about 1.5 g/placenta.

Gel filtration. Weighed samples of the lyophilized HPG preparation was dissolved in 3

M NaCl (100 mg/ml), stirred overnight, and the solution was centrifuged at 15,000*g* for 10 min. The supernatant fraction was placed on a Sephadex G-100 column (3.0 × 140 cm) which was previously equilibrated and washed with 3 *M* NaCl. The column was eluted with 3 *M* NaCl. Fractions were collected and the absorbance was read at 278 mμ. Three distinct peaks were observed (Fig. 1). The tubes under each peak were pooled and dialyzed against distilled water until the diffusate gave a negative test with 1% silver nitrate solution. The retentate was lyophilized and assayed for gonadotropic activity. The two fractions with activities were dissolved in 3 *M* NaCl (100 mg/ml) and gel filtration repeated as described above. Fractions were collected, measured for absorbance, pooled, dialyzed, lyophilized, and assayed.

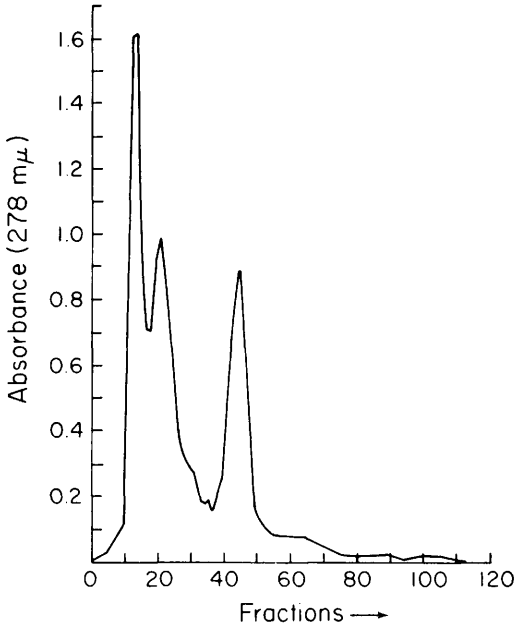

FIG. 1. Gel filtration of human placental gonadotropin on Sephadex G-100 (3.0×140 cm). Void volume was 335 ml. Each fraction contained 6.5 ml and was collected at a rate of about 30-40 ml/hr. A sample containing 210 mg of crude HPG dissolved in 3 ml of 3 *M* NaCl was applied to the column with a recovery of 71%. The protein content of the pooled samples of the three major peaks were 10, 35, and 76 mg for the initial, HPG-A and HPG-B, respectively.

Bioassay. The gonadotropic activity was assayed as described by Kikutani and Tokuyasu (12). Each immature mouse (21 days) was injected subcutaneously with 0.1 ml of samples dissolved in 0.85% NaCl solution at intervals of 12 hr for five doses. The mice were killed 72 hr after the first injection. The uteri were weighed and the ovaries were observed for hemorrhagic spots. Protein was determined by the spectrophotometric method (13, 14). Antuitrin "S" (500 units/ml) purchased from Parke, Davis and Co., Detroit, Michigan, was used as HCG in the assay.

Results. Figure 1 shows the pattern of gel filtration of crude HPG on Sephadex G-100 column. The pooled sample from the initial peak had none to minimal biological activity. The latter two peaks showed gonadotropic activities and were designated as HPG-A and HPG-B, respectively. The mean body and uterine weights of the immature mice were 14.9±1.7 g and 9.4±2.8 mg, respectively. The doses ranging from 13 to 400 μg of HPG-A and from 12 to 180 μg of HPG-B were assayed (Table I). The minimal dose of the crude HPG, HPG-A, and HPG-B which induced a significant increase in uterine weight (2 SD) was 65, 21, and 63 μg, respectively. Ovarian hemorrhage was not observed in any of the mice injected with HPG. In contrast, ovarian hemorrhages were consistently produced with the administration of 2.5 units of HCG/mouse.

Discussion. The result of the present study that HPG did not produce ovarian hemorrhages when administered to immature mice supports the contention that the usual preparation of HCG is probably contaminated with pituitary FSH rather than due to an intrinsic follicle stimulating property (1–6). During the purification of HCG these contaminants may not have been separated (7, 9, 15–17). An alternate explanation is that HPG may be a precursor and distinct from HCG. It is possible that at very high doses of HPG, or with more potent material, ovarian hemorrhages may be induced.

HPG-A and HPG-B were obtained following gel filtration and may be variants (isohormone) or subunits of placental go-

TABLE I. Effect of Human Placental Gonadotropin on Mouse Uterine Weight and Ovary.[a]

Materials	Total dose	Increase in uterine wt (2 SD)	Ovarian hemorrhages
HCG	0.25 IU	0	0
	2.5	+	+
Crude HPG	400 μg	+	0
	149	+	0
	80	+	0
	65	+	0
	27	0	0
	16	0	0
	13	0	0
HPG-A	103	+	0
	52	+	0
	47	+	0
	21	+	0
	9.4	0	0
	4.0	0	0
	1.9	0	0
HPG-B	179	+	0
	175	+	0
	80	+	0
	63	+	0
	47	0	0
	35	0	0
	12	0	0

[a] Each group was composed of 3–5 mice. See text and Fig. 1 for designation of crude HPG, HPG-A and HPG-B.

nadotropin. The lack of interconversion of HPG-A and HPG-B following subsequent purification which included dialysis, lyophilization, and gel filtration suggests that they are variants rather than subunits or dimers.

Summary. Gonadotropin was isolated from human term placenta by precipitation with acetone and ammonium sulfate and by gel filtration on Sephadex column. The product was designated as human placental gonadotropin (HPG). The HPG induced an increase in uterine weight without producing ovarian hemorrhages. The result suggests that HPG and human chorionic gonadotropin may be different forms of chorionic gonadotropin.

The author wishes to express his gratitude to Mr. Gilbert Martinez and Mrs. Maria T. Elvira for their technical assistance and to Dr. F. Fuchs for making available the placenta used in this study.

1. Loraine, J. A., *in* "Hormones in Blood" (C. H. Gray and A. L. Bacharach, eds.), 2nd ed., Vol. 1, p. 313. Academic Press, New York (1967).

2. Crook, A. C. and Butt, W. R., J. Obstet. Gynaecol. Brit. Empire **66**, 297 (1959).

3. Simpson, M. E., *in* "Human Pituitary Gonadotrophin" (A. Albert, ed.), p. 127. Thomas, Springfield, Illinois (1961).

4. Saxena, B. B. and Henneman, P. H., J. Clin. Endocrinol. **24**, 1271 (1964).

5. Cole, H. H. and Bigelow, M., Anat. Record **157**, 19 (1967).

6. Butt, W. R., *in* "The Chemistry of the Gonadotrophin," p. 46. Thomas, Springfield, Illinois (1967).

7. Bell, J. J., Canfield, R. E., and Sciarra, J., J. Endocrinology **84**, 298 (1969).

8. Hamashige, S., Astor, M. A., Arquilla, E. R., and Van Thiel, D. H., J. Clin. Endocrinol. **27**, 1690 (1967).

9. Bahl, O. P., J. Biol. Chem. **244**, 567 (1969).

10. Kikutani, M., J. Pharm. Soc. Japan **77**, 370 (1957).

11. van Hell, H., Matthijsen, R., and Homan, J. D. H., Acta Endocrinol. **59**, 89 (1968).

12. Kikutani, M. and Tokuyasu, C., J. Biochem. (Tokyo) **57**, 598 (1965).

13. Layne, E., Methods Enzymol. **3**, 451 (1957).

14. Murphy, J. B., and Kies, M. W., Biochim. Biophys. Acta **45**, 382 (1960).

15. Wilde, C. E. and Bagshawe, K. D., *in* "Gonadotropins: Physico-chemical and Immunological Properties" (G. E. W. Wolstenholme and J. Knight, eds.), p. 46. Little, Brown, Boston, Massachusetts (1965).

16. Taymor, M. L., Todd, R., and Blatt, W. F., J. Endocrinol. **36**, 417 (1966).

17. Bruner, J. A., J. Clin. Endocrinol. **11**, 360 (1951).

Received July 30, 1969. P.S.E.B.M., 1969, Vol. 132.