## Ovarian Hemorrhages Induced in Immature Mice with Human Placental Gonadotropin

The induction of follicular hemorrhages by human chorionic gonadotropin (HCG) obtained from the urine of pregnant women has been attributed to the presence of pituitary follicle-stimulating hormone (FSH) as a contaminant or to an intrinsic FSH-like property[1]. In a previous study crude gonadotropin isolated from human term placenta (HPG) was administered to immature mice. Uterine weights were increased without inciting ovarian hemorrhages[2]. In the present study, further purification of HPG was carried out by chromatography on CM-cellulose and DEAE-cellulose and gel filtration of Sephadex G-100. Fractions of HPG were obtained which induced ovarian hemorrhages when administered to immature mice.

HCG (A.P.L., 100 IU per ml) was purchased from Ayerst Labs, Inc. One unit was equivalent to 1.279 μg of standard preparation of National Institute for Medical Research, England. Materials to be assayed were dissolved in 0.85% NaCl solution. Immature female mice (body wt. 8–10 g) were injected s.c. with 0.1 ml of samples at intervals of 24 h for a total of 3 injections and killed 24 h after the 3rd injection. The mean body and uterine wt. of 36 control untreated mice was 11.9 ± 0.6 g and 8.0 ± 2.9 mg, respectively.

Acetone powder of human term placenta was prepared. The placental powder was weighed and suspended in cold deionized water (g/20 ml). Subsequent steps were performed in the cold. The mixture was stirred for 2 h, filtered through a sieve and centrifuged at 10,000 *g* for 10 min. The supernatnat solution was placed in a Visking casing and equilibrated for 24 h against a saturated solution of ammonium sulfate containing solid ammonium sulfate to maintain saturation. The retentate was centrifuged and the sediment dissolved in a minimum vol of deionized water. The mixture was dialyzed against deionized water until the dialysate gave a conductivity reading of less than 10 μmhos. The retentate was centrifuged at 10,000 *g* for 10 min and the supernatant solution lyophilized. The dried product was dissolved in deionized water (50 mg/ml), stirred for 1 h and centrifuged at 10,000 *g* for 15 min. The supernatant solution was lyophilized. The product was designated as crude HPG.

The lyophilized material was dissolved in cold 5 m*M* potassium phosphate buffer (pH 6.0) (10 mg/ml). The solution was passed through CM-cellulose column (2.0 × 25 cm), equilibrated in 5 *mM* potassium buffer (pH 6.0). Absorbance of each fraction was measured at 278 nm. Fractions showing greater than 0.05 absorbance unit were pooled. The pooled solution was chromatographed on a DEAE-cellulose column (2.0 × 25 cm). The column eluted stepwise with 5, 20, 50 and 100 m*M* potassium phosphate buffer (pH 6.0). Absorbance of each fraction was measured. Fractions showing greater than 0.05 absorbance unit were pooled and dialyzed against deionized water. The retentate was centrifuged at 10,000 *g* for 10 min and the supernatant lyophilized. The biologically active fractions from DEAE-cellulose chromatography were dissolved in 50 m*M* phosphate buffer (pH

Assay of human placental gonadotropin

| Samples | Total dose administered (μg) | Average uterine wt. (mg) | Ovarian hemorrhages |
|---|---|---|---|
| HCG | 1.5 | 12.3 | 0 |
| | 1.9 | 15.3 | 0 |
| | 3.8 | 19.7 | 0 |
| | 7.7 | 22.5 | 4 (6) |
| | 9.6 | 25.3 | 4 (4) |
| HPG-II-B | 3 | 8.1 | 0 |
| | 10 | 12.7 | 0 |
| | 50 | 30.5 | 0 |
| | 78 | 17.1 | 1 |
| | 252 | 16.7 | 1 |
| | 390 | 20.4 | 1 |
| | 1722 | 25.1 | 2 |
| HPG-III-A | 4 | 7.6 | 0 |
| | 12 | 7.5 | 0 |
| | 19 | 10.1 | 0 |
| | 35 | 10.9 | 0 |
| | 94 | 21.6 | 0 |
| | 106 | 19.9 | 0 |
| | 531 | 45.0 | 0 |
| | 2559 | 37.3 | 0 |
| HPG-III-B | 5 | 7.4 | 0 |
| | 25 | 11.8 | 0 |
| | 58 | 23.6 | 0 |
| | 125 | 36.1 | 0 |
| | 291 | 34.9 | 0 |
| | 624 | 16.5 | 1 |
| | 1452 | 19.9 | 3 |

Each group consisted of 3 mice or more (given in parenthesis). See text for designation of HPG-II-B, HPG-III-A, and HPG-III-B. The amount of HCG was calculated by multiplying the units used with the factor 1.279. Mean uterine wt. of control untreated mice was 8.0 ± 2.9 mg.

[1] A. Albert, Endocrinology *29*, 1504 (1969).
[2] S. S. Koide, Proc. Soc. exp. Biol. Med. *132*, 1137 (1969).

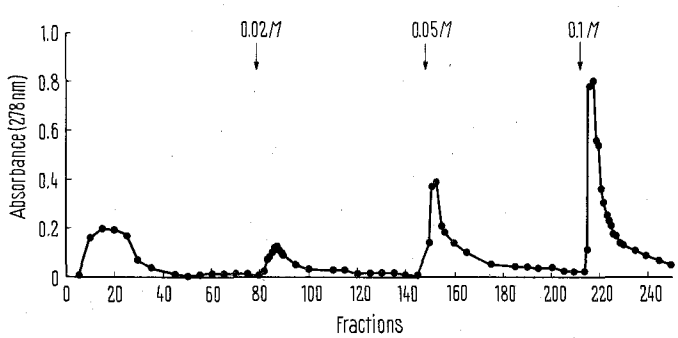


Fig. 1. Chromatography of crude HPG on DEAE-cellulose column. A 500 mg sample of crude HPG dissolved in 50 ml of 0.005 *M* potassium phosphate buffer, pH 6.0, was applied to a CM-cellulose column (2.5 × 12 cm) equilibrated with the same buffer. Fractions showing absorbance greater than 0.05 unit were pooled (92 ml). The pooled fraction was applied to a DEAE-cellulose column (2.5 × 12 cm) equilibrated in the same buffer. Elution was started with 0.005*M* phosphate buffer, pH 6.0, and changed to 0.02, 0.05, and 0.1 *M* phosphate buffer, pH 6.0.

7.5) (100 mg/ml) and centrifuged. The supernatant solution was placed on a Sephadex G-100 column (2.5 × 180 cm) and eluted with 50 m*M* potassium buffer (pH 7.5). Absorbance of each fraction was measured at 278 nm. Fractions composing a peak were pooled and dialyzed against deionized water. The retentate was centrifuged at 10,000 *g* for 10 min. The supernatant solution was lyophilized.

The average yield of crude HPG from human term placenta by the described procedure ranged from 60–240 mg of lyophilized powder per 100 *g* (wet wt.) of tissue. When crude HPG was fractionated on DEAE-cellulose, biologically active material was eluted with 20 and 50 m*M* phosphate buffer (pH 6.0) and designated as HPG-II and III, respectively (Figure 1 and Table). Occasional fractions eluted with 100 m*M* potassium phosphate buffer (pH 6.0) possessed biological activity. Materials eluted subsequently with 0.5 and 1.0 *M* NaCl did not possess any biological activity.

HPG-II and III were purified by gel filtration on Sephadex G-100 (Figure 2). Two peaks were obtained and designated as A and B. HPG-III-A and B and HPG-II-B administered to immature mice induced an increase in uterine weights; whereas HPG-II-A did not (Table). HPG-II-B and HPG-III-B at high doses induced ovarian hemorrhages (Table) and ovulation (Figure 3). HPG-III-A was recycled through Sephadex G-100. The administration of recycled HPG to immature mice (128 and 239 µg/mouse) induced increase in uterine weights. Ovarian hemorrhages were observed in 1 out of 3 mice.

Recycled HPG subjected to disc electrophoresis on polyacrylamide gel[3,4] separated into 1 major and 2 to 3 minor bands. Since the biological potency of HPG was considerably less than that of HCG (Table), one of the minor bands might be the biologically active material rather than the major band.

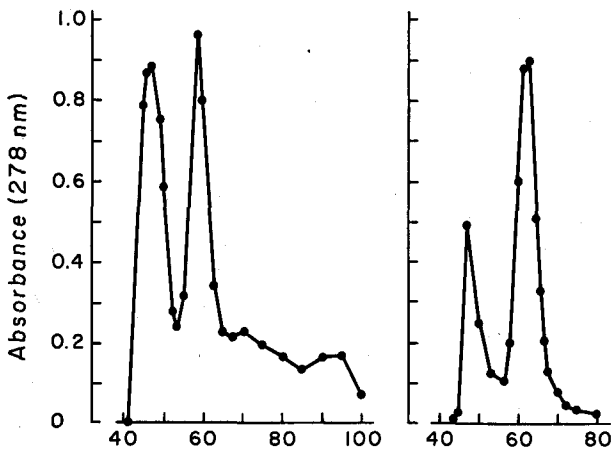


Fig. 2. Gel filtration of HPG on Sephadex G-100. Fractions II (171 mg, left) and III (161 mg, right) from DEAE-cellulose column were dissolved in 1 ml of 0.05*M* phosphate buffer, pH 6.0, and applied to a column of Sephadex G-100 (2.5 × 180 cm). The column was eluted with the same buffer. Void volume was 272 ml. Fractions of 6.5 ml per tube were collected.

The present finding that HPG administered to immature mice induced ovarian hemorrhages and ovulation supports the contention that HCG possessed intrinsic FSH-like property[1]. The isolation of 2 fractions with biological activity on gel filtration supports the report of ASHITAKA[5] that 2 types of gonadotropin exist in human placenta. One possessed LH-like and the other FSH-like activities[5]. Further purification of HPG is being carried out to resolve definitively whether or not variants of HPG exist and that one of them possessed principally LH or FSH-like activity.

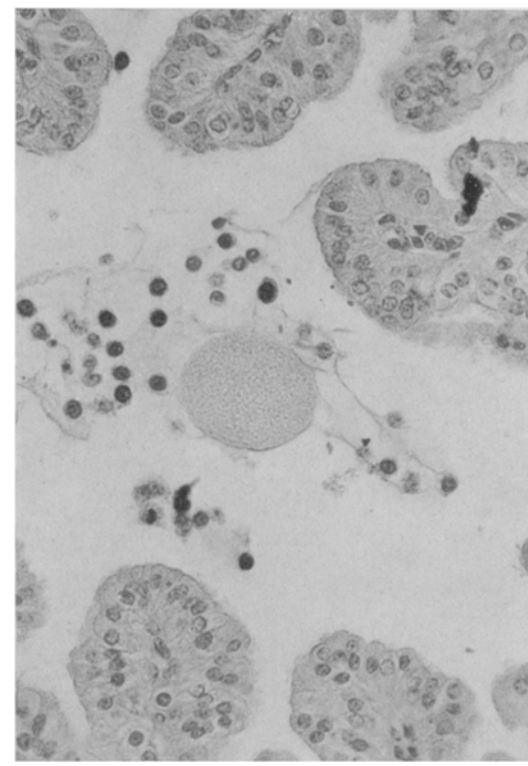

Fig. 3. Ovum in oviduct of immature mouse following the administration of 1722 µg of HPG. × 101.

*Zusammenfassung.* Gonadotropin, durch Fällung mit Aceton und Ammoniumsulfat aus menschlicher Placenta (HPG) isoliert und durch Chromatographie an CM- und DEA-Cellulose sowie durch Gelfiltration an Sephadex G-100 gereinigt, ergab Fraktionen, die in hoher Dosis bei jungen Mäusen Blutungen im Ovar hervorriefen, was zeigt, dass GPH eine FSH-ähnliche Wirkung bewirkt.

S. S. KOIDE[6]

*Bio-Medical Division,*
*The Population Council,*
*The Rockefeller University*
*New York (N.Y. 10021, USA), 28 June 1971.*

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