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## Effect of Exogenous Androgen on the Steelman-Pohley Assay for Follicle Stimulating Hormone\* (33730)

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The most widely used assay for follicle stimulating hormone (FSH) is the ovarian weight method of Steelman and Pohley (1). In this assay the effect of endogenously released luteinizing hormone (LH), or LH contained in the test material, is overridden by an excess of human chorionic gonadotropin (HCG), used to augment the action of FSH on the ovary. However, other pituitary hormones, particularly FSH, of the assay animal obviously play a role in the response since hypophysectomized animals are much less sensitive than intact animals. The major inadequacy of the assay is its lack of sensitivity. The lower portion of the dose-response curve has a shallow slope which differs from the steep slope of the most usable portion of the curve (2). If a single slope could be obtained, materials with very low FSH potency, such as plasma, might be assayed more reliably.

The objective of the present study was to partially inhibit the output of endogenous pituitary FSH in order to decrease the ovarian weight obtained with low doses of exogenous FSH. It was hoped that a linear dose-response curve, with a significant slope could thus be obtained. Since estrogen affects the response to HCG in intact animals (3)

by direct and indirect actions, an androgen, testosterone propionate (TP) was chosen for the study. The results demonstrate that the slope was changed by the androgen, but the direction was opposite to that expected.

*Materials and Methods.* Holtzman strain female rats, housed in air-conditioned and light controlled (14 hr light/day) quarters and given free access to water and Purina laboratory chow were used. In each assay, animals were assigned randomly to treatment groups (usually 5-6/group) when 25 days old. The standard FSH (NIH-FSH-S4 was a gift of the Endocrinology Study Section of the NIH) was dissolved in normal saline and given twice daily for 3 days. The total volume given each animal (1.5 ml) also contained 50 IU of human chorionic gonadotropin (HCG). When steroids were used they were dissolved in sesame oil and administered subcutaneously. In some assays, the control animals were given oil 24 or 48 hr prior to, or during, the assay period, but since the results of these trials were in no way different from saline injected animals the data were combined.

In two assays animals were hypophysectomized prior to the beginning of the assay. At the time of hypophysectomy 0.25 mg of cortisone acetate (aqueous suspension) was given each animal, and during the assay a 5% solution of glucose was substituted for drink-

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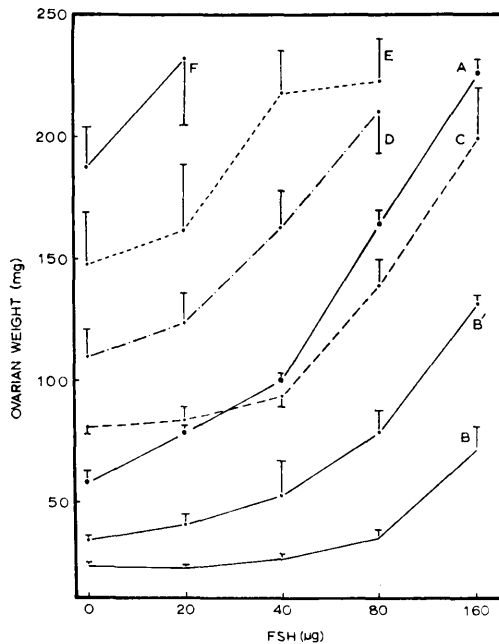


FIG. 1. Effect of various pretreatments in the Steelman-Pohley assay for FSH: A = composite of 8 control assays using NIH-FSH-S4 plus 50 IU of HCG; each point represents the average of about 40 animals; standard errors are indicated by vertical lines; Hypophysectomy 24 (B) or 0 (B') hr prior to the assay; TP 24 hr prior to, and daily during, the assay, C = 1 mg; D = 100  $\mu$ g; E = 1 mg of TP 48 hr before assay, and F = 1  $\mu$ g of EB 24 hr before assay; at least 5 animals /group.

ing water. The completeness of pituitary removal was determined visually. At autopsy, the ovaries were removed, blotted on paper toweling, and weighed on a torsion balance.

**Results and Discussion.** Curve A (Fig. 1) shows a composite log-dose response curve for eight assays of FSH. Obviously there are two different slopes: one from 0 to 40  $\mu$ g and one from 40 to 160  $\mu$ g of FSH. Plotting the data using an arithmetic dose scale gave a curve which was linear from 0 to 40  $\mu$ g of FSH, but not above this dose. The lower part of the curve appears to fit the requirements of a slope-ratio assay, while the upper portion is better as a parallel line assay.

Hypophysectomy 24 hr before the assay resulted in a significant reduction in response to FSH (curve B, fig. 1) (1). With removal of the pituitary delayed until a few minutes

before the first injection an increased response was obtained (curve B') but this was still significantly below that of intact animals. Quite obviously complete lack of endogenous FSH is undesirable for this assay. However, if exogenous steroid could reduce some of the endogenous gonadotropin it might be possible to lower the responses seen with HCG alone and yet keep the responsiveness to exogenous FSH.

Testosterone propionate (TP) has no direct effect upon the ovary (4) and does not augment the action of exogenous FSH (5). This hormone does however, affect pituitary gonadotropins; in general it increases pituitary FSH content but does not favor FSH release (6).

With a dose of 1 mg of TP given 24 hr prior to, as well as on each day of the assay (total dose = 4 mg) the response to FSH was not greatly changed from control (curve C, Fig. 1). The most important result of this treatment was the elevation of ovarian weight in HCG treated animals. No further increases in ovarian weight were obtained until a dose of 40  $\mu$ g of FSH was reached but above this dose the curve was essentially the same as control. The increased response obtained with HCG alone was further augmented when the dose of TP was reduced from 1000 to 100  $\mu$ g (curve D, Fig. 1) given on the same schedule (total dose = 400  $\mu$ g). The dose-response curve was linear from 20 to 80  $\mu$ g of FSH, and the slope was not significantly different from control.

When administered as a single dose 48 hr prior to the assay, 1 mg of TP elevated the curve further than the daily treatment (curve E, Fig. 1). With this priming, maximal ovarian weight was obtained with only 40  $\mu$ g of FSH. The responses with the various doses of FSH were quite variable and the resulting curve was totally useless as an assay.

One  $\mu$ g of estradiol benzoate (EB) given 24 hr before the assay, produced ovaries of nearly maximal size with HCG alone (curve F, Fig. 1). Only two points were plotted in this curve, since all the weights obtained with higher dose of FSH did not differ from these seen with 20  $\mu$ g of FSH. Smith and Bradbury

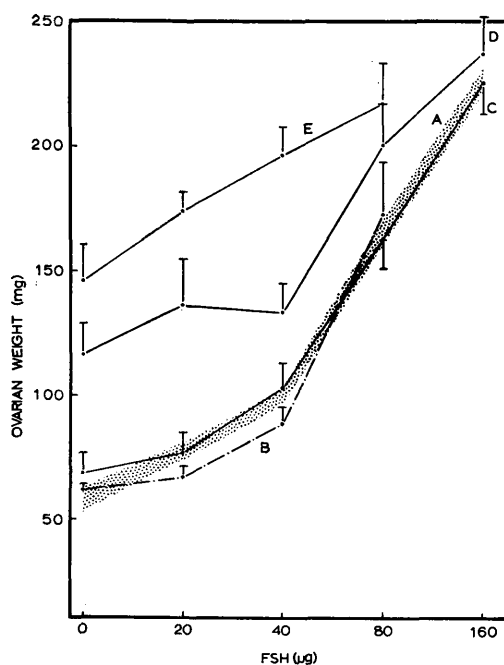


FIG. 2. Effect of 25  $\mu\text{g}$  of TP: control is indicated by shaded area; B = a single injection of TP at start of assay; C = TP daily during assay (total = 75  $\mu\text{g}$ ); D = 24 hr prior to assay; E = 48 hr before assay; standard error obtained with 5 animals/group indicated by vertical lines.

(3) reported that priming of intact animals for 2 days with large doses of diethylstilbestrol increased the response obtained with HCG.

Since large doses of testosterone could be converted into estrogens within the assay animal and possibly produce the results seen, the effect of a smaller amount of TP (25  $\mu\text{g}$ ) was examined (Fig. 2). When given as a single injection at the beginning of the assay (curve B) or daily (curve C) the response was unaltered when compared to control. However, pretreatment 24 hr before the assay had a pronounced effect and the ovarian weight obtained with HCG alone was double the control value (curve D). With doses above 40  $\mu\text{g}$  of FSH, the curve was essentially parallel to that of the standard. A linear log-dose response curve, having a shallow slope, was obtained when the 25  $\mu\text{g}$  of TP was given 48 hr before the assay (curve E).

The results of these experiments suggested

that the effects of exogenous FSH were being added to the effects of increased endogenous FSH. Both estrogen and androgen appeared capable of increasing the endogenous FSH, but only when given prior to the assay. In a third series (Fig. 3) animals were pretreated with pregnant mare serum gonadotropin (PMS), a hormone which would cause production of endogenous estrogen and yet, because of its long half-life (7), have persisting FSH activity. A single priming dose of 2.5 IU given 24 hr prior to the assay increased the responses seen with the zero and lower doses of FSH, but not with 80  $\mu\text{g}$  of FSH (curve B, Fig. 3). The response was quite similar to that obtained with 100  $\mu\text{g}$  of TP given 24 hr early and every day of the assay (curve C, Fig. 3). When the treatments were combined, the resulting curve (D, Fig. 3) indicated that the effects of PMS and TP were additive, giving further support to the conclusion that circulating endogenous FSH had been increased by the androgen priming.

The results of these studies clearly indicate that the dose-response curve in the Steel-

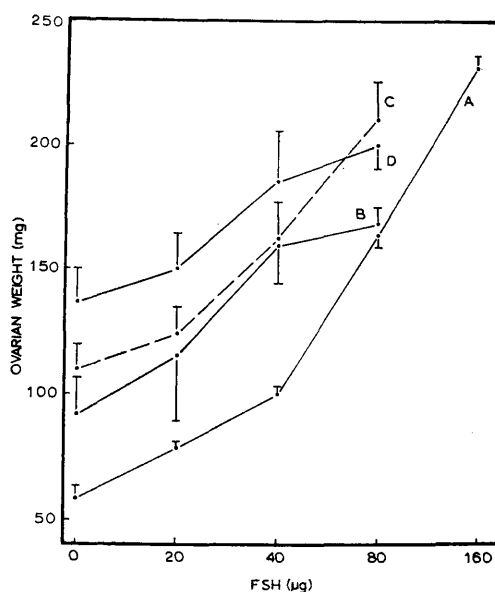


FIG. 3. Effect of PMS and TP: A = control; B = 2.5 IU of PMS 24 hr early; C = 100  $\mu\text{g}$  of TP 24 hr before, and daily during, assay; D = combined PMS and TP; 5 animals/group with vertical lines indicating standard error.

man-Pohley assay can be changed by the use of exogenous androgen. However, the mechanisms involved appear complex, and with only the limited information available from these experiments a detailed discussion is unwarranted. A great many questions are raised by the results. The important effect of the steroid pretreatment was the increase in output of pituitary FSH. Presumably this effect was mediated via the hypothalamic-hypophyseal axis. When exogenous FSH was added either as purified ovine hormone, or as PMS, to the endogenous FSH, an increased response was obtained. Since the maximal ovarian weight attainable in this assay is limited to about 250 mg, the slope of the dose-response curve necessarily must become more shallow as the intercept with zero dose is elevated. Further experiments will be necessary to elucidate the effects of acute androgen treatment on the hypothalamic-hypophyseal control of pituitary FSH.

*Summary.* A single injection of 25, 100, or 1000  $\mu$ g of testosterone propionate given 24

or 48 hr prior to the administration of chorionic gonadotropin (HCG) plus various doses of FSH altered the slope of the log-dose-response curve. Since the ovarian weight obtained with HCG alone was greatly increased in pretreated animals the slope of the curve was reduced. The results suggest that androgen priming caused an increased output of endogenous FSH in the assay animal.

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