A Simplified Augmented Ovarian Weight Assay for Follicle-Stimulating Hormone[1] (34513)

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The augmented rat ovarian weight method for assaying follicle-stimulating hormone (FSH) has been widely used since its introduction by Steelman and Pohley (1). While it is not a very sensitive bioassay for this hormone, it has the advantages of simplicity as well as a relatively long dose-response curve. The specificity of the assay has been well established, and luteinizing hormone contamination of extracts does not interfere with the response as it does in some assays for FSH. The original procedure specified that the total dose of hormone was to be administered by three daily subcutaneous injections (0.5 ml each) for 3 days with autopsy on Day 4; 72 hr after the beginning of the assay. Augmentation was accomplished by adding 20 IU of human chorionic gonadotropin (HCG) to the total dose of hormone given each animal. Maximal augmentation is not achieved with this dose, and now most workers use 50 IU of HCG. Parlow and Reichert (2) investigated the injection schedule and found that two daily injections for 3 days were just as good as three but that a single daily injection was inadequate.

In recent studies, using HCG augmentation of endogenous FHS, in immature female rats (3, 4) it became necessary to investigate the timing of the ovarian responses to mixtures of FSH and HCG. On the basis of those studies, we concluded that the hormone contained in the sixth and very probably the fifth injection in the Steelman-Pohley assay contributed little, if anything, to the final ovarian weight in the assay. This suggested that the assay could be shortened without a loss in sensitivity. The present report outlines a procedure for simplifying the assay by reducing the number of injections to two (given on the same day) and autopsying the animals after 48 or 54 hr.

Material and Methods. Immature female rats (24–26 days old) of the Holtzman strain were used. They were kept in air-conditioned and light-controlled (14 hr light between 6 AM and 8 PM) quarters and had free access to Purina Laboratory Chow and tapwater. The standards used were ovine FSH (S4 and S5) supplied by the Endocrinology Study Section of the NIH. The hormone to be tested was prepared so that 1 ml of saline contained the desired dose; one group in each assay received the saline without added hormone. To the 1 ml was added 0.5 ml of HCG (Pregnyl-Organon, Inc.; 100 IU/ml). The rats (5–6 per group) were injected subcutaneously twice: 0.8 ml at 9 AM and 0.7 ml at 4:30 PM at 25 days of age. At various times after the first injection the animals were killed with chloroform. The ovaries were dissected out, cleaned of adhering fat and oviducts, blotted on paper toweling, and weighed on a torsion balance. Statistical evaluation of the data was done using standard procedures (5).

Results and Discussion. The ovarian weight responses, in relation to the length of time from the first injection to autopsy, for varying amounts of FSH are shown in Fig. 1. With the largest dose of FSH (160 μg) ovarian weight doubled, over HCG controls, within 24 hr, but with smaller doses there was very little growth. During the next 24 hr, however, considerable increase in ovarian

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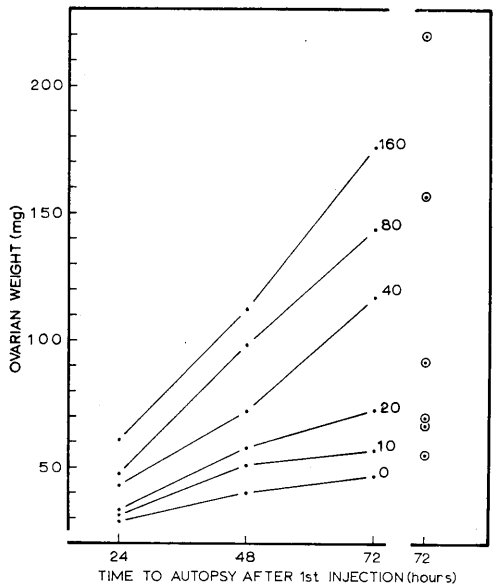

FIG. 1. Ovarian weight responses to various doses FSH in relation to time. The total dose of hormone (given at the end of each curve) was administered subcutaneously in two injections, 7 hr apart on the same day. The time shown indicates the hours from the first injection to autopsy. The circled dots at the second 72-hr point indicate ovarian weights obtained with the same amount of hormone given in six injections in 3 days (typical Steelman-Pohley assay).

size occurred. This rapid rate of growth was maintained with 40, 80, and 160 μg of FSH for the succeeding 24 hr also, but with 20 μg the rate slowed, and with 10 μg there was no increase in weight between 48 and 72 hr. The weights obtained using the same amount of hormone given in six injections over 3 days are also included in Fig. 1. With the lower doses, multiple injections had no advantage over two injections in bringing about heavier ovaries: With 40 μg, the multiple-injection schedule was less efficient and produced distinctly smaller ovaries.

Because 48 hr appeared to be a suitable length of time for the response in the animals, this schedule was followed for several assays. The results of a typical assay, using male rat pituitary for an unknown, are shown in Fig. 2. The response curves are similar—parallel—and the assay has an acceptable precision. The curve for the standard is linear for 20-160 μg of FSH: In our experience (2), as well as that of others (6), the regular Steelman-Pohley assay is linear from about 40–160 μg.

In an attempt to produce a steeper dose-response curve, we allowed an additional 6 hr between the first injection and autopsy (total time of 54 hr). We found that with the five doses tested the additional time resulted in an average increase in ovarian weight of 48%. Apparently the rapid growth phase extends beyond 48 hr even with the lower doses of FSH. Using this 54-hr schedule, we obtained the results shown in Fig. 3. The responses obtained with rat pituitary (23-day female) as well as a human urinary gonadotropin extract (Humegon; Organanon, Inc.) are included. The slopes of the curves are slightly greater than when using 48 hr for the response. We have repeated this assay several times using pituitary extracts as well as the second international reference preparation for urinary gonadotropin (IRP-II), and in all cases the curves were parallel with the standard, and all had acceptable indices of precision (lambda values less than 0.200).

Hypophysectomized animals are unsuitable for the Steelman-Pohley assay because of a great loss in sensitivity. Even when the hypophysectomy was performed immediately before the first injection of hormone, meaningful increases in ovarian weight were not

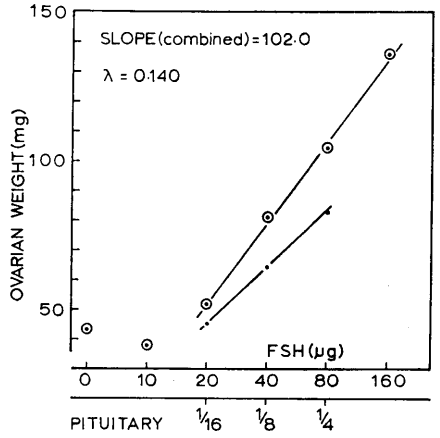

FIG. 2. Log dose-response curves for NIH–FSH–S4 and male rat pituitary extract. The material was given in two injections with autopsy 48 hr after the first injection.

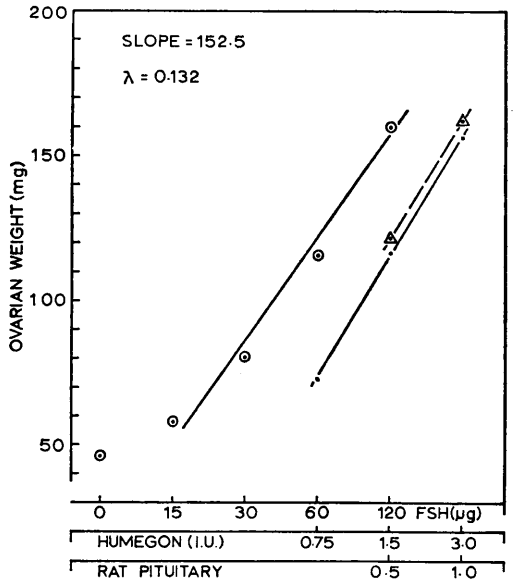

FIG. 3. Log dose-response curves for FSH (S4) (circled dots), human urinary gonadotropin (Humegon; Organon, Inc.) (dots-solid line) and immature female rat pituitary extract (dashed line). The material was given in two injections on Day 1, with autopsy 54 hr after the first injection.

found until the dose of FSH was increased to about 80 μg (3). In the shorter schedule (48 hr) we found that hypophysectomized animals could be used without a loss in sensitivity. The dose-response curve for an assay using hypophysectomized animals is shown in Fig. 4. In this assay the animals were hypophysectomized by the parapharyngeal approach using ether anesthesia. Immediately after the last operation the animals were divided into groups of six and given the first injection of FSH plus HCG. The second injection was given 7 hr later, and the animals were killed 48 hr after the first injection. Animals with pituitary remnants were discarded from the assay. The dose-response curve (arithmetic) is linear, but the slope is shallow, and consequently it offers no advantage over the use of intact animals.

Steelman and Pohley (1) calculated their results using the slope ratio method. They pointed out that with higher doses of FSH the results could be calculated by methods for parallel-line assays. Because of the relative simplicity of this method of calculation, many investigators prefer it over the slope ratio method. With a computer, however, the results can be easily calculated by either, or both, methods. A computer program (C. Hendley, unpublished) which analyzes portions as well as the entire dose- and log dose-response curves has shown that in several assays (54 hr) the highest correlation coefficients are obtained using the former. However, with responses greater than those obtained with 12.5 μg FSH (S4 or equivalent) and ignoring the response to HCG alone (O FSH), completely satisfactory results can be obtained with the log dose-response curve.

The procedure which has been adopted for the assay is as follows: The animals are received in the laboratory when 24 days old. The next morning (Day 1 at 9 AM) they received the first injection (0.8 ml) of standard or unknown. The second injection (0.7 ml) is given at about 4:30 PM of the same day. The animals are killed at 3 PM on Day 3, and the ovaries are weighed. Since the ovaries in the animals, especially those treated with large doses of FSH, are in a very active growth phase, it is necessary to com-

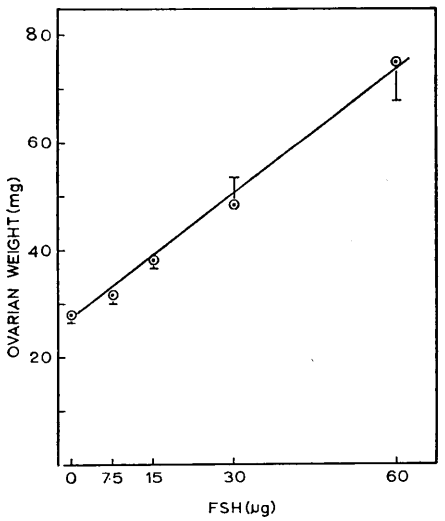

FIG. 4. Dose-response curve for FSH (S5) in hypophysectomized animals. The rats were given the hormone in two injections with autopsy 48 hr after the first injection. Each point represents the average of at least five animals with the standard error indicated by the vertical lines.

plete the autopsy quickly (within about 1 hr). Using this schedule, an assay can be started every day without progressively increasing the number of animals to be injected.

Summary. A simplified schedule for the augmented ovarian weight assay of FSH is presented. Standard amounts of FSH or unknowns are mixed with 50 IU of HCG in a total of 1.5 ml per assay rat. The rats are injected subcutaneously when 25 days old with 0.8 ml of material at 9 AM and with 0.7 ml at 4:30 PM. The animals are autopsied 48 or 54 hr after the first injection, and the ovarian weight is plotted against the dose of hormone. This method reduces the amount of handling of the animals, shortens the assay by 1 day, and increases its sensitivity.

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