Ovarian Weight Changes in Immature Female Rats as a Function of Dose and Exposure Time to PMS[1] (37252)

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The assay of hypophyseal gonadotropins (FSH and LH) during the estrous cycle of the female rat have revealed only one period of elevated serum concentrations (1): Release of both hormones appears to occur on the afternoon of the day of proestrus. The question arises: Is this single burst, which increases hormone concentrations about five-fold, of significance to the animal? We have known for some time that ovulation of mature follicles occurs 10 to 12 hr after a rather brief (1–2 hr) exposure to gonadotropins (2). This has been offered as a good example of a hormonal action occurring long after the hormone has disappeared. In contrast to this kind of action, several studies have shown that the ability of follicles to ovulate requires an almost constant presence of gonadotropin [discussed in (3)], but this also refers to ovulation as an endpoint. We were particularly interested in two aspects of gonadotropin response; latency as related to dose and effects manifest after removal of the hormone. Earlier studies (4) had indicated a definite lag period for ovarian weight responses to FSH and HCG, regardless of the dose, and suggested the possibility of a strength–duration effect analagous to that for nerve or muscle.

Material and Methods. Immature (23 day) Holtzman-strain rats were used. They were kept in plastic cages with wire tops and given free access to Purina laboratory chow and tap water. The lights were on 14 hr/day between 6 AM and 8 PM.

Antihormone was prepared in rabbits by injecting pregnant mare's serum gonadotropin (PMS-Ayerst) suspended in complete Freund's adjuvent three times a week for 3 weeks followed by a booster 6 weeks after the last injection. The rabbits were exsanguinated one week after the booster dose. The serum was stored frozen until used. The potency of the antibody was assayed by injecting groups of immature females with 100 IU of PMS (subcutaneous) and various dilutions of antiserum (intraperitoneal); ovarian and uterine weights were obtained 48 hr later. The biological activity of the PMS was completely abolished by the equivalent of 25 μl of the antiserum (*i.e.*, a potency $>$ 4000 IU/ml). In all experiments, 25 μl/100 IU was used; the volume injected was 0.25 ml, obtained by isotonic saline dilutions of the serum.

Groups of 23-day-old females were given PMS intravenously at 9 AM on Day 1 of the experiment while controls received only saline. The animals were killed by chloroform on Day 3 (48 hr later) and the ovaries weighed. The biological action of the PMS was halted after various intervals by the intraperitoneal injection of anti-PMS serum. Special treatment regimens are explained with their results.

Results and Discussion. PMS was chosen for this study because: (a) it is a "complete" gonadotropin with both FSH and LH activity; (b) it has a long half-life (26 hr) in the serum (5); and, (c) it is a good antigen for the production of antibodies. The ovarian weight response might be considered insensitive but it has the advantages of easy measurement and relevance to gonadotropic function. The 48 hr allowed for the

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response was chosen so as to minimize the effect of endogenous gonadotropins; ovulation occurs about 60 hr after administration of PMS due to release of endogenous gonadotropin (6). The weight increases found in this study were the result of follicular and interstitial tissue growth; corpora lutea were not found.

Preliminary experiments compared the ovarian weights obtained after the administration of the antiserum with those obtained with autopsy at the time of the latter injection. This would indicate any delayed effect of the PMS which might occur after it was neutralized by the antiserum. For example, ovarian weight 24 hr after giving 50 IU of PMS was 27.8 ± 1.0 mg and after 36 hr it was 39.2 ± 2.2 mg. When the time for the response was held constant at 48 hr, but the exposure to the PMS varied by antihormone the weights were: 24 hr = 25.8 ± 0.3 mg; 36 hr = 42.5 ± 3.6 mg. Obviously growth, as reflected in ovarian weight, essentially stopped at the time of antibody administration. It is equally important that the weight did not decrease following the antibody injection.

Ovarion weights from groups of animals given various amounts of PMS were plotted against the time of anti-PMS administration and are shown in Fig. 1. Only one group (5 IU) was killed at 60 hr, because of the small weight increase found at 48 hr. In some groups (200, 300 IU) early growth changes were examined by giving antiserum at intermediate times (16, 18, 20 hr) not shown on the graph. As expected, a family of curves was obtained for the various doses of PMS. A line was drawn through these curves at a point which is double the value for terminal saline control ovarian weights. From this line a perpendicular line was dropped to the time scale so that the time of exposure to a particular dose of PMS necessary to produce a doubling of ovarian weight was obtained. This exposure time was then plotted against the dose of PMS and shown in Fig. 2: a strength–duration curve. This curve indicates that regardless of the amount of PMS given, about 22 hr of exposure are required for doubling of the ovarian weight. Actually a similar curve with only a small

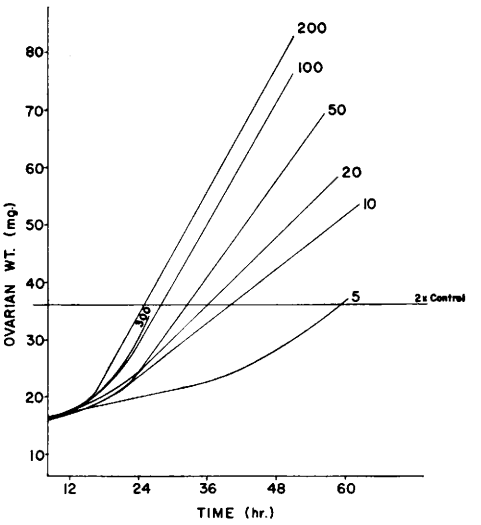

FIG. 1. Ovarian weight obtained with various doses of PMS which were neutralized by anti-PMS antiserum after varying periods (hr). IU of PMS indicated at the end of each curve. The 5 IU group was killed at 60 hr, but all others at 48 hr after the injection of PMS. A line is drawn through the curves at twice the ovarian weight of saline control females.

reduction in minimal time of exposure would be obtained by choosing a smaller increase in ovarian weight as an endpoint.

Recently, Goldenberg, Vaitukaitus, and Ross (7) have shown that large doses of diethylstilbestrol enhance the activity of FSH, presumably by increasing the number of receptor sites for the gonadotropin. The delay in response to PMS found in the present study could mean that estrogen must be produced by the ovary before the PMS could increase ovarian weight appreciably. This hypothesis was tested by giving PMS to animals that had been pretreated with estrogen. In the first trial 10 μg of estradiol benzoate, dissolved in oil, was injected subcutaneously 24 hr before a 100 IU dose of PMS. The antiserum was given at 6, 12, 18, 24, 30, or 36 hr after the PMS. The resulting curve had the same shape as that obtained without the estrogen pre-treatment and failed to show any effect of the estrogen on the latency period. The amount of estrogen used however, may not have been sufficient to have a direct ovarian effect. Therefore, the experiment was done twice using diethylstil-

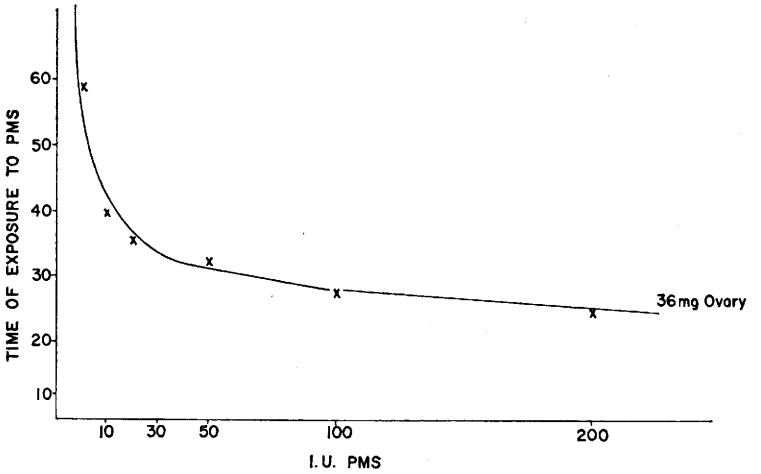

FIG. 2. The time of exposure to PMS necessary to produce a doubling of ovarian weight vs the dose of hormone; a strength–duration curve.

bestrol. In the first trial 1 mg of the hormone suspended in saline was used and in the second 2 mg dissolved in sesame oil. The hormone was given exactly 48 hr prior to a dose of 50 IU of PMS. The results are shown in Figs. 3a and b. Higher levels of endogenous FSH would be expected (4) within 24 hr of the estrogen injection, which would account for the elevated ovarian weights of the starting controls. The response to PMS was enhanced to some extent by the estrogen pretreatment and larger ovaries were found at 48 hr. The important point however is that the time course of the response was unchanged; the curves are parallel for the first 24 hr. The effect of stilbestrol on tritiated thymidine (DNA synthesis) by the ovary is maximal at 60 hr (7) which is 12 hr after the PMS injection in the present experiments. Therefore, if about 24 hr on the receptor site is actually required for the activity of PMS we would expect to find the largest deviation from the controls at 36–48 hr, and indeed we do. One cannot, however, ignore the possible role of endogenous gonadotropins in producing the greater responses in the estrogen-treated females. Increased output of FSH from the pituitary would be maximal between 48 and 60 hr (4, 8). Applying this to the present experiment, an increased amount of FSH would be expected about 12 hr after the injection of PMS (stilbestrol given 48 hr earlier) and if the activity of this endogenous hormone had the same latency as PMS, then its effect would be observed at about 36 hr. Separate receptor sites for PMS and FSH have been previously proposed to interpret results obtained with the use of antihormone (9). Experiments involving the neutralization of endogenous FSH would be required to clarify which of these mechanisms was more important. For the present discussion however, we need only be concerned with the failure of estrogen to hasten the ovarian response to PMS. We interpret the results as indicating that, regardless of the amount of hormone present, a distinct minimum amount of time is required before a weight change is manifested. Furthermore, removal of the stimulating hormone stops the effect. A note of caution is necessary however, when dealing with antihormones. The problem of possible removal of the hormone from the target by stronger binding to antibody than to receptor must be considered. If in fact this does occur, interpretation of results obtained by neutralizing gonadotropins with antibodies becomes complicated; half-life at the receptor site may be much more important than in any of the body fluids.

Summary. A dose-exposure time relationship was studied using PMS effect upon ovarian weight in immature female rats. The biologic activity of the hormone was neutralized by use of a potent antiserum prepared

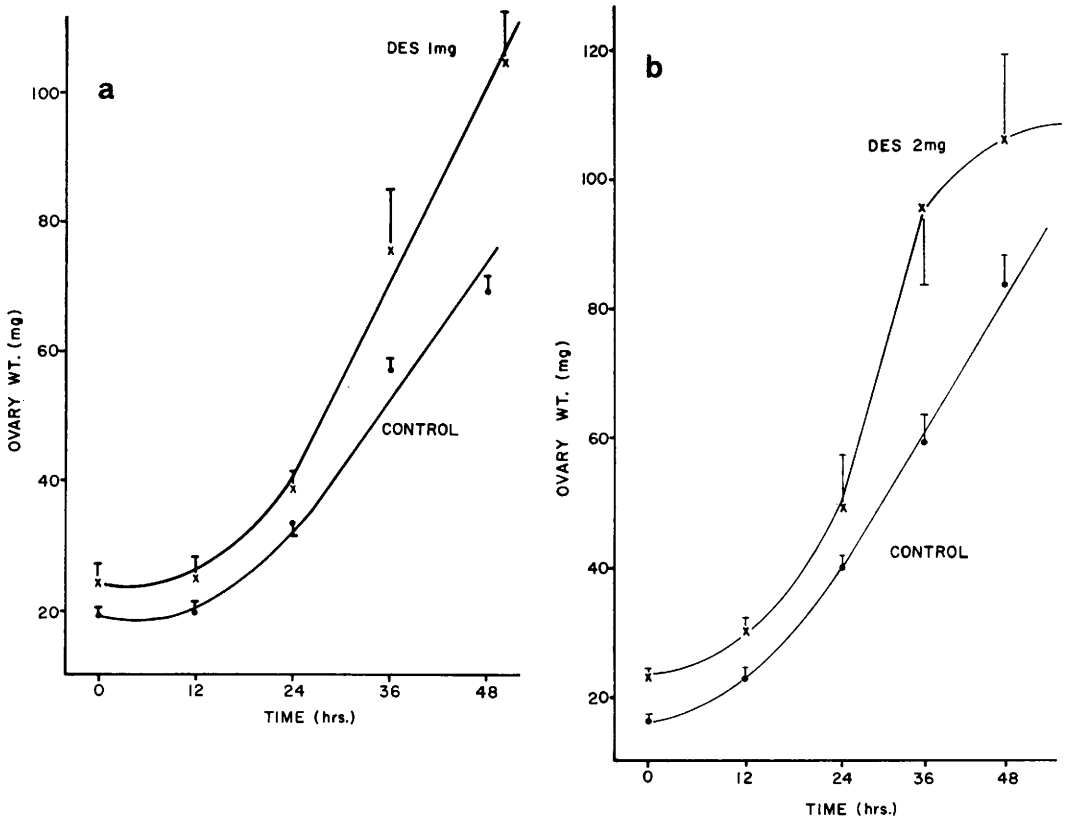

FIG. 3a. Ovarian weights obtained in animals treated with 1 mg of diethylstilbestrol (aqueous suspension) 48 hr before a 50 IU dose of PMS. Anti-PMS antiserum was given at the times indicated and all of the animals killed at 48 hr. Each group consists of 6 animals; standard error is indicated by vertical line at each point. (b) Effect of 2 mg of diethylstilbestrol (oil solution) given 48 hr before 50 IU of PMS. Other conditions the same as in 3a.

in rabbits. Regardless of the dose of PMS injected, about 22 hr were required for doubling of ovarian weight. Estrogen production by the ovary appeared to be unnecessary for the ovarian response to PMS since pretreatment with a large dose of stilbestrol did not alter the response time.

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