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Bioassay of Mouse Pituitary Gonadotropin by Induced Ovulation in Pregnant Mice. Evaluation and Biologic Application.* (20718)

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(Introduced by D. W. Fawcett.)

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The induced ovulatory reaction in pregnant mice has been suggested by Burdick and Whitney(1) as a bioassay for gonadotropins. We have recently shown this reaction to be a sensitive assay for gonadotropin content of the mouse pituitary(2), and have used it to show changes in synthesis and release of gonadotropin by the anterior pituitary during gestation(3). This report presents data on the responsiveness of the ovaries of pregnant mice to injections of a commercially standardized preparation of human chorionic gonadotropin. In addition, the differences in the responsiveness of the ovaries to injected homogenates of mouse pituitaries obtained at parturition and at the first postpartum ovulation are shown.

Materials and methods. Swiss female mice, 3 to 9 months of age and approximately 14 to 19 days pregnant, were used for assay animals. The care of animals and the procedure of bioassay of mouse pituitary for gonadotropins has been described earlier(2), so that they need only brief mention here. Commercially standardized human chorionic gonadotropin (HCG)§ was employed for the determination of the responsiveness of the pregnant animals' ovaries. The gonadotropin was made up in the desired concentrations in physiological saline solution and injected intraperitoneally in a uniform volume. Pituitary glands to be assayed were collected from animals which had been used in a previous study to determine the relationship between the time of parturition and ovulation(4). These glands were dehydrated in acetone, desiccated in vacuum and stored. Preparatory to assay they were homogenized, suspended in saline and serially diluted. The homogenates were injected intraperitoneally. The frequency with which ovulation was observed in groups of pregnant assay animals was adjusted by the statistical procedure recommended by Reed and Muench(5). This technic has the effect of augmenting the number of responses at the dosages on either side of the 50% end point. Since this design partially offsets chance variation, the method defines the end point more precisely than would otherwise be possible.

Results. The ovulatory responses of 144 pregnant female mice which received HCG in varying amounts are summarized in Table I. The dose of HCG that would have been required to produce a response in 50% of the animals has been interpolated from the adjusted values. This calculated 50% end

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TABLE I.
Ovulatory Responses of Pregnant Mice to Injections of Human Chorionic Gonadotropin.

Dosage, I.U.	No. of pregnant mice	No. induced to ovulate	% ovulation observed	% ovulation adjusted*	Total No. of ova	Avg No. ova/assay animal
.125	31	2	6.5	2.2	3	.10
.25	33	3	9.1	7.6	18	.55
.50	26	11	42.3	38.6	70	2.69
1.0	27	21	77.8	80.1	140	5.19
2.0	27	24	88.9	95.2	215	7.96

* Method of Reed and Muench(5).

TABLE II. Ovulatory Responses of Pregnant Mice to Mouse Pituitary Glands Obtained at Time of Parturition and at First Postpartum Ovulation.

Time of pituitary removal	Dosage in pituitary equivalents	No. of pregnant mice	No. induced to ovulate	% ovulation observed	% ovulation adjusted*
Parturition	1/8	12	1	8.3	2.9
	1/4	17	3	17.6	13.1
	1/2	19	9	47.4	41.7
	1	13	8	61.5	65.1
	2	10	7	70.0	86.5
Postpartum ovulation	1/2	13	1	7.7	2.9
	1	12	1	8.3	8.4
	2	21	9	42.9	42.0
	4	10	8	80.0	87.3

* Method of Reed and Muench(5).

point is 0.60 I.U. and agrees reasonably well with the 0.75 to 1.0 I.U. obtained by Burdick *et al.*(6) in diestrous mice.

Data on the responses of 127 assay animals which received varying equivalents of pituitary glands are presented in Table II. Homogenates of pituitary glands collected at the time of parturition indicated the 50% end point lying between ½ and one pituitary. The end point was calculated to be at the level of 0.58 pituitary equivalent. Pituitaries obtained at the time of the first postpartum ovulation showed the 50% response to be located between 2 and 4 pituitaries. The end point was calculated to be at the level of 2.25 pituitary equivalents. The difference between these end points indicates that almost a 4-fold decrease in the gonadotropic potency of the pituitary gland was detected during the 15-hour period between parturition and the first postpartum ovulation.

Discussion. The present experiments provide further demonstration that the induced ovulatory response of pregnant mice is an immediate and sensitive indicator of the potency of both human chorionic and mouse pituitary gonadotropins. This response has been separated into (a) a qualitative estimate based upon the proportion of animals ovulating at a given dosage level and (b) a quantitative evaluation based upon the number of ova extruded from the ovaries at any given dosage level (Table I). Application of the quantitative response has been reported previously(3). Our present report applies the qualitative response in order to determine changes in the gonadotropic potency of the mouse pituitary gland between parturition and the postpartum ovulation (Table II).

Advantages of establishing dosages at which half the animals will respond have been summarized by Gaddum(7). This end point is less affected by chance variations than is any other qualitative end point. The frequencies of ovulation are given in Tables I and II and are graphed in Fig. 1. The steepness of these curves both for the standard preparation and for the 2 types of pituitary glands indicates the sensitivity of the assay animals. In addition, the slopes of the 3 curves are comparable thereby attesting to the uniformity in response of the assay animals. An assay procedure

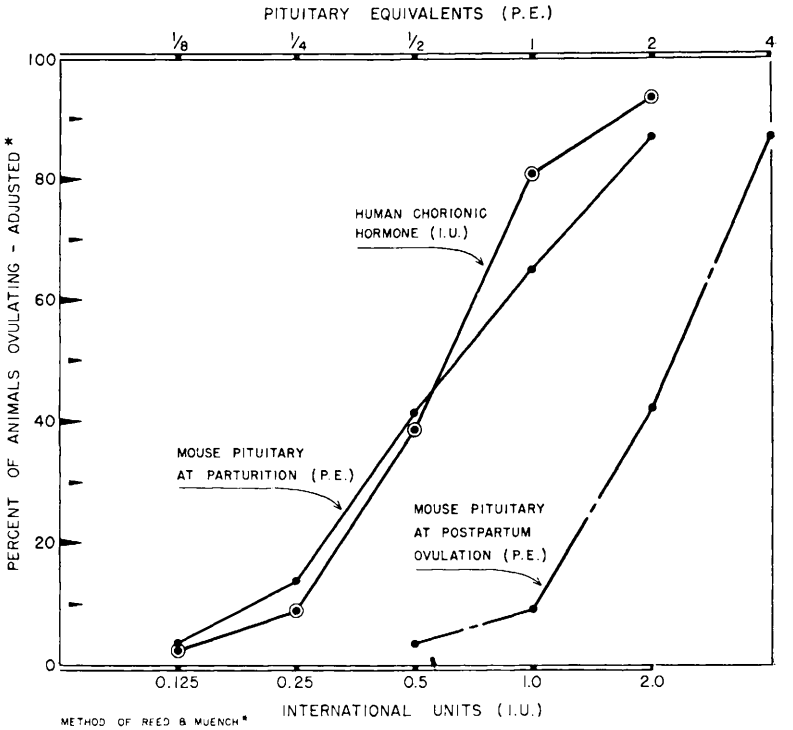

FIG. 1.
Ovulatory responses of pregnant mice to human chorionic and mouse pituitary gonadotropin.

having a steep slope and a reproducible dose-response relationship engenders confidence and lends precision to the method.

Assays of pituitary gonadotropin at parturition and at ovulation, permit the following deduction. The pituitary gland shows a 4-fold decrease within 15 hours after parturition. Burdick and Whitney(1) had reported and we have confirmed (unpublished) that the shortest interval between administration of gonadotropin and ovulation is 12 hours. Therefore, it follows that the gonadotropin that elicits the first postpartum ovulation must be released within 3 hours after parturition.

Summary. The ovulatory responses of pregnant mice were evaluated with human chorionic gonadotropin. From these observations the end point response for this material was calculated to be 0.60 I.U. Assays of mouse pituitaries demonstrated a 4-fold decrease in gonadotropin content between parturition and the first postpartum ovulation. This decrease is interpreted as indicating that amounts of pituitary gonadotropin capable of inducing ovulation are released within 3 hours after parturition.

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