Changes in Concentration of Serum LH and FSH Associated With Estrogen-Advanced Ovulation in 4-day Cyclic Rats (36227)

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It has been established that an ovulatory surge of gonadotropin occurs between 2:00 and 4:00 PM on the day of proestrus as determined by hypophysectomy, lesion of basal median eminence, administration of neuropharmacologic drugs (1–4), or antibodies against gonadotropin (5, 6), bioassays and radioimmunoassays of gonadotropins (7–10). Recently, advancement of ovulation with a single injection of estradiol benzoate (EB) in 4-day cyclic rats has been demonstrated in this (11) and other (12) laboratories. Furthermore, experiments with phenobarbital administered at 2:00 or 5:00 PM the day after EB injection revealed a "critical period" similar to that occurring between 2:00 and 4:00 PM on the day of proestrus as described by Everett and Sawyer (3, 4) for cyclic adult rats. The present experiments were designed to measure serum LH and FSH at different stages of the EB-shortened estrous cycle to determine if a surge of gonadotropin could be detected during the "critical period."

Materials and Methods. Mature female Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. The animals were fed Purina laboratory chow and water *ad lib.* The lights were automatically turned on at 5:00 AM and off at 7:00 PM, producing a 14:10-hr light:darkness period. The midpoint of the dark period is considered as "midnight, colony time" and all times are reported in relation to that. Vaginal smears were taken daily before 11:00 AM for at least three consecutive cycles before any treatment was administered. Only animals showing regular 4-day cycles were used in the experiments. The stages of the 4-day cycle are defined as estrus, 2 days of diestrus, and proestrus. Estradiol benzoate (50 μg) dissolved in 0.1 ml sesame oil was administered subcutaneously at 10:00 AM on the first day of diestrus. Three blood samples of 1 ml each were taken by cardiac puncture under light ether anesthesia, usually one in the morning, one in the afternoon of the second day of diestrus; and one in the morning of proestrus. Also, cardiac blood samples collected concurrently at the same times in the oil-treated group were included in this study. Serum samples were analyzed for LH and FSH using the radioimmunoassay kits available from the National Institutes of Health with minor modifications. The LH and FSH values are expressed as nanograms of NIAMD-Rat LH-I-1 and FSH-I-1 per ml of serum, respectively. Data were analyzed by analysis of variance and Duncan's new multiple range test.

Results. EB treatment casued a significant ($p < 0.01$) increase in serum LH (Table I) which coincides with the "critical period" determined by barbiturate experiment (11). Serum FSH increased slightly but significantly ($p < 0.05$) at 3:00 PM on the second day of diestrus. The oil-treated animals showed no significant fluctuation in either LH or FSH. Results obtained from a representative sample of 8 EB-treated animals (Fig. 1) demonstrated that the peak LH concentration occurred at 3:00 PM on the presumptive second day of diestrus. Thereafter, the con-

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TABLE I. Changes in Serum LH and FSH Levels After a Single Injection of EB on Day 1 of Diestrus in 4-Day Cyclic Rats.

Time of blood collection	LH (ng/ml)[a]		FSH (ng/ml)[a]	
	Oil-treated	EB-treated	Oil-treated	EB-treated
9:00 AM (Diestrus-2)	2.4 ± 0.19	5.2 ± 0.58[b]	0.4 ± 0.11	2.9 ± 0.62[b]
3:00 PM (Diestrus-2)	1.2 ± 0.54	15.9 ± 0.90[bc]	0.7 ± 0.58	6.3 ± 0.95[bc]
9:00 (Proestrus)	2.6 ± 0.24	2.1 ± 0.61[b]	0.6 ± 0.34	2.5 ± 0.64[b]

[a] $N = 10$; standard error of means. [b] Significant from the oil controls. [c] Significant from other measurements of EB-treated animals.

centration of serum LH declined rapidly. Examination of the oviducts in the morning of the expected proestrus showed that 8 of 10 EB-treated rats ovulated an average of 7.5 ova, while none of the oil-treated animals showed any incidence of ovulation.

Discussion. The results of this study support the concept that estrogen is capable of stimulating the ovulatory surge of gonadotropin, mainly LH. This positive feedback effect probably results from the action of the steroid on the hypothalamus (13–15), however, a direct action on the pituitary had not been excluded. Regardless of the mechanism of action, this effect is significant from the standpoint of explaining how estrogen, either endogenous or exogenous, induces ovulation in immature rats or advances ovulation in cyclic rats.

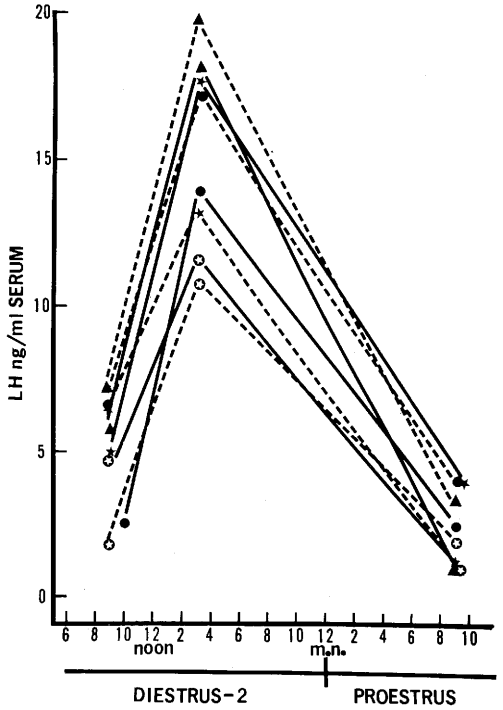

FIG. 1. Serum LH changes which occurred in a representative sample of 8 4-day cyclic individual rats after a single injection of EB on the first day of diestrus.

It is of interest to note that the LH levels at 9:00 AM were higher in EB-treated rats than in those of oil-treated controls ($p < 0.05$). By measuring the ovarian secretion of progesterone at 10:00 AM on the second day of diestrus, Krey and Everett (12) reported a marked increase only in the EB-treated rats with advanced ovulation. The reciprocal stimulatory relationship between a steroid such as estrogen or progesterone and gonadotropin(s) has been observed frequently (16, 17). It is possible that the slightly elevated LH led to the secretion of estrogen and progesterone in quantities sufficient to facilitate the surge of ovulating hormone and consequently the advancement of ovulation.

Summary. Serum LH and FSH were determined by radioimmunoassay in 4-day cyclic rats at approximately 24, 29 and 47 hr after a single injection of EB. Ovulation was determined by the presence of ova in the oviduct 48 hr after EB. Serum LH was elevated 24 and 29 hr and drastically reduced at 47 hr after EB. Serum FSH showed only a slight increase at 29 hr after EB. It is suggested that a surge of ovulatory gonadotropin secretion, primarily LH, occurs 24 hr ahead of schedule in EB-treated 4-day cyclic rats.

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