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Adrenal Rhythmicity in the Immature Pseudopregnant Rat (38626)

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Adrenal function is altered in the pseudopregnant rat. Adrenal weights are depressed after a period of pseudopregnancy (1, 2) and the stress response to ether is considerably blunted (3). Despite these signs of adrenal insufficiency, the production of adrenal progesterone is enhanced, and in fact probably accounts for the bulk of progesterone output in the last few days of pseudopregnancy (2). In an earlier study concerned with the development of the serum corticosterone rhythm in immature rats given pregnant mare's serum gonadotrophin (PMS), we obtained evidence suggesting that the daily corticosterone rhythm had been abolished by the induction of persistent corpora lutea (4). That study was done using only two sample intervals in each succeeding 24 hour period, a method which cannot distinguish between the loss of a daily rhythm and a time shift in the occurrence of the peak and trough. The following study was done to see whether PMS-treated pseudopregnant rats do have an adrenal rhythm.

Materials and Methods. Twenty-one day old female Sprague–Dawley derived rats were obtained from Sascho Laboratories on the day of weaning and were placed in groups of three in stainless steel hanging cages. The light cycle was maintained at 14 hr on: 10 hr off (on at 3 AM). At 26 days of age, groups of six rats each were injected at 9 AM or 4 PM with 25 IU PMS subcutaneously in 0.1 cc saline, a dosage that induces the formation of persistent corpora lutea (4). Controls either were handled without injection or were given 0.1 cc saline. The presence or absence of a daily corticosterone rhythm was determined by collecting blood samples at intervals of 4 hr throughout a 24-hr period 5 days after the PMS when both groups of PMS-injected rats had previously been found to lack a daily corticosterone rhythm (4). Blood was collected from the three rats in each cage by decapitation within 1.5 ± 0.4 min after the cage was removed from the animal room. Analysis of variance failed to reveal a correlation between the order of sampling and the corticosterone value obtained at any given time period. Six rats were sampled from each group at each time interval. The blood was allowed to clot at room temperature and the serum saved for analysis of corticosterone by a fluorometric procedure (5) and progesterone by a competitive binding assay (6). The presence of a rhythm was tested by means of analysis of variance.

Results and Discussion. The injection of saline at 26 days of age did not alter the corticosterone rhythm seen at 31 days of age. For this reason, the results from the two control groups (handled only and saline-injected) have been pooled in Fig. 1. The control rats in this experiment were very close to puberty. Other rats from the same strain showed vaginal opening at 35.4 ± 0.6 days during the time of this study (May–June). It can be seen in Fig. 1 that all three groups of rats had detectable peaks and troughs of corticosterone during the 24-hr period sampled. The rhythm in rats given PMS at 9 AM was sharper than that of the other two groups due to a failure of corticosterone to be elevated at noon in this group.

Figure 2 shows the levels of progesterone obtained in the same samples. There is a daily variation in progesterone in the control 31-day old rats with high levels during the light portion of the photoperiod and a fall during the dark. A similar rhythm, although reduced in amplitude, was found in 28-day old rats in a previous study (6). This rhythm was also found in ovariectomized rats and may therefore be of primarily adrenal origin. This does not correspond to the data of Mann and Barraclough (7) who reported high progesterone values shortly after the onset of light in adult females during the estrous cycle. In both PMS-treated groups, progesterone values were very high but there

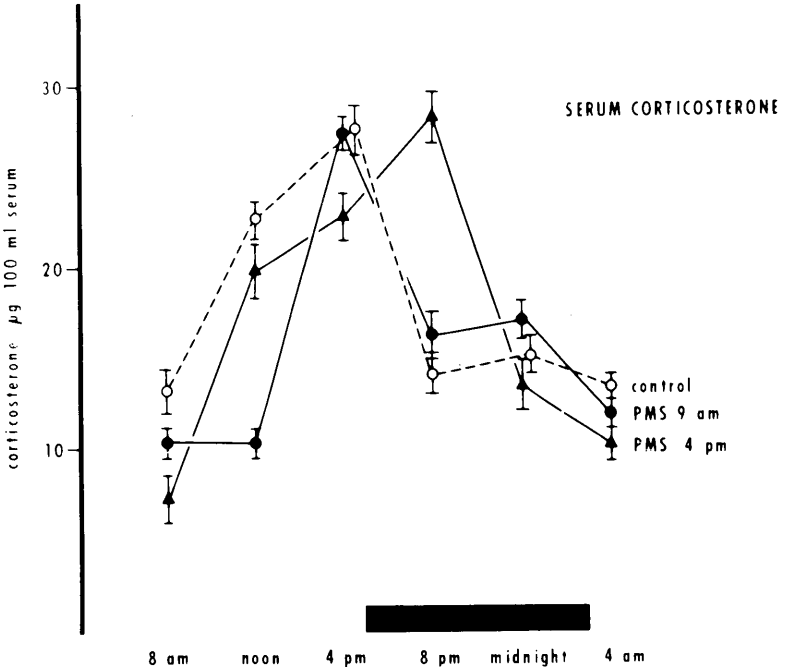

FIG. 1. Serum corticosterone levels after PMS treatment. 25 IU PMS was injected at 9 AM (●), or at 4 PM (▲) on day 26. Controls received saline or were handled only (○). All rats were 31 days on day of sampling. Data expressed as mean ± standard error, six rats per group (PMS 9 AM and 4 PM) or 12 rats per group (controls). Dark portion of the photoperiod indicated by a solid bar.

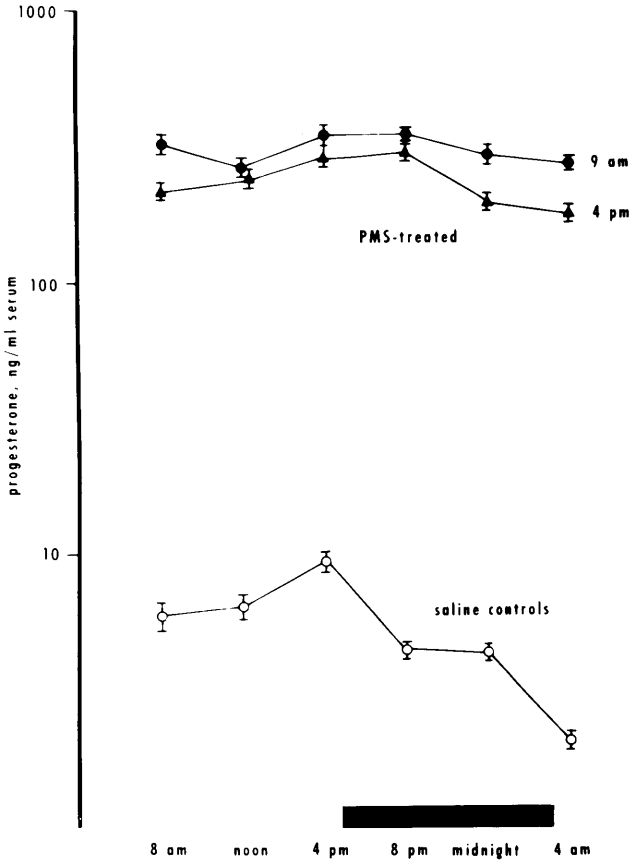

FIG. 2. Serum progesterone after PMS treatment. For explanation, see Fig. 1.

was no evidence of a daily rhythm. In adult rats made pseudopregnant by sterile mating or cervical stimulation, a circadian rhythm of progesterone secretion from the ovary was evident with a peak at the midpoint of the light phase (8) on day 7 of pseudopregnancy. Peripheral progesterone was not measured. It is possible that this ovarian progesterone rhythm is balanced by a later adrenal rhythm which maintains high progesterone during the entire light interval.

It can be concluded that there is a daily rhythm of corticosterone maintained during PMS-induced pseudopregnancy in the rat. This occurs despite a depression in stress–responsiveness during this period (3) and a reduction in adrenal weight (1, 2).

Summary. A daily rhythm of serum corticosterone persists in 31 day old immature female rats in which pseudopregnancy has been induced by means of 25 IU pregnant mare's serum gonadotrophin (PMS) given at 26 days of age. There is, in addition, a daily rhythm of progesterone in 31-day old controls with a rise during the day and a fall during the dark portion of the photoperiod. PMS-injected rats had a much higher level of progesterone but no rhythm.

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