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A Comprehensive Profile of Peripheral Progesterone Levels in Cyclic Hamsters Sequentially Bled at 2-hr Intervals (40646)

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The first assessments of the peripheral progesterone (P) profiles during the 4-day estrous cycle of the hamster were done nearly a decade ago (1, 2). The experimental design used in those and virtually all subsequent studies was either to make a single determination of P level in the blood at each of the first 3 days of the cycle and multiple determinations on the day of proestrous, Day 4 (1–4), or to examine in detail a restricted period (24 hr or less) of the cycle (5–7). The resultant limited profiles from these studies were relatively similar, even though (a) the assay methodology varied from gas–liquid chromatography (1) and competitive protein binding (2, 5) to radioimmunoassay (4, 6–8), and (b) the source of the analyzed peripheral blood varied from abdominal aorta (2, 5), vena cava (1), and mixed trunk blood (4, 6, 7) to ovarian venous blood (3). In contrast to the experimental design of those studies, a design used by Ridley and Greenwald (8) incorporated repetitive cardiac puncture of hamsters in groups and yielded a detailed profile of progesterone levels in the blood at 2-hr intervals throughout the estrous cycle. Their resultant profile of P indicated that a series of episodic fluctuations in P levels existed during the second half of Day 4 (proestrus) and continued through Day 1 of the next cycle. The significant fluctuations in P on Day 4 had not been found in the previous (1, 2, 5) or subsequent (4, 7) studies. In view of these collective reports, the present study was done to obtain a comprehensive profile of P levels in the blood by using a more direct experimental design. The rationale for the study was that the episodic fluctuations in P found in Ridley and Greenwald's study could have been a consequence of "animal variation" (8), which by chance was manifested through their particular experimental design.

Materials and methods. Female golden hamsters (Con Olson, Madison, Wis.), weighing 90–110 g, were maintained in an environmentally controlled room (22°, lighting from 0500 to 1900 hr daily, CST). The estrous cycle was monitored by the appearance of a characteristic vaginal discharge every fourth day, which was designated Day 1 of the cycle (the day of ovulation). After five consecutive 4-day cycles, eight hamsters (two at each day of the cycle) were concurrently bled (0.08 ml, 27-gauge needles) via cardiac puncture without anesthesia at 2-hr intervals for a 24-hr period (starting at 0800 hr). Bleedings were done in the animal room except during the period of darkness, when animals were rapidly but quietly removed to a very dimly lighted anteroom for at most a 10-min period. After the 24-hr period for blood collection, hamsters were monitored again for subsequent vaginal discharge. The experiment was replicated three times. Animals which failed to show two subsequent and normally timed discharges were excluded from the study. The collected blood was allowed to clot at 4° for 12–16 hr, centrifuged at 1200*g*, 4° for 0.5 hr, and then 10 μl sera was diluted with 0.5 ml distilled water and stored at −15°.

For determination of P, prediluted sera, nonradioactive P for reference standards, and control reference sera (with and without unlabeled P, or with labeled P for analysis of procedural loss through the extraction process) were extracted once with 3 ml anhydrous diethyl ether, and phase separation was facilitated by centrifugation at 500*g*, 22° for 40 sec. The aqueous phase was frozen (dry ice, ethanol bath); the ether phase was decanted into a 12 × 75-mm culture tube and then evaporated under air in a 45° water bath. The etheric extract was sequentially treated as follows: twice concentrated and dried via ether washes (0.5 and 0.25 ml); resuspended in 0.2-ml phosphate-buffered gelatine (PBG; 0.1 *M* phosphate, pH 7.0, containing a 0.14 *M* sodium chloride, 0.1% Knox gelatin, and

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0.1% sodium azide); gently agitated, and incubated at 45° for 20 min and then 22° for 10 min. To each culture tube was added 0.1 ml anti-P serum (1:62,000 dilution in PBG, antiserum AB12 kindly supplied and characterized by Dr. A. H. Surve and associates (9)), and then 0.1 ml [1, 2, 6, 7-^{3}H]labeled-P (equivalent of 19 pg P added; original SA = 105 Ci/mmole) in PBG. Tubes were agitated and then incubated at 22° for 16–20 hr and at 4° for 1 hr. Free and bound P were separated at 4° by the following sequence: addition of 0.5 ml dextran-coated charcoal suspension (0.4% Norit-A and 0.04% dextran in PBG) to each tube; contents agitated; incubation (15 min); (1500*g*, 15 min); decantation of supernatant into 6-ml scintillation vials and subsequent addition of 5 ml scintillation solution [15 g Omnifluor (New England Nuclear, Boston)/gallon of 10% dioxane in toluene]. After a 20- to 24-hr incubation, vials were counted for 10 min or 10^4 accumulated counts in a Packard Tri-Carb liquid scintillation counter, Model 3003. P content was calculated from the transformed standard curves (log–dose vs logit–response) after linear regression analysis and included correction for procedural loss. Cumulative characteristics for 19 assays were as follows: (a) lower limit of sensitivity = 5 pg; (b) mean (±SE) slope and the intercept at 50% binding were −2.465 ± 0.038 and 72.5 ± 1.8 pg, respectively; (c) percentage recovery of labeled internal standard after ether extraction was 83.8 ± 0.5% (n = 18); and (d) net recovery of nonradioactive P when 100 pg P was added to reference sera was 101 ± 3 pg. Levels of P in sera were analyzed by two-factor analysis of variance for repeated measure on one factor followed by Duncan's new multiple-range test (10), with a level of probability at less than 0.05 considered statistically significant.

Results and discussion. Three hamsters were excluded from the study (two at Day 2, one at Day 3) because they failed to exhibit a vaginal discharge at the expected, *second* postbleeding Day 1. The remaining 21 females appeared healthy throughout the study and exhibited two normally timed vaginal discharges after the bleedings.

The results are presented in Fig. 1. Overall P levels were similar in hamsters bled over a 24-hr period initiated on Days 1 or 2 but were significantly greater in Day-1 or -2 hamsters than the P levels in Day-3 animals. Also, the levels of P in animals in which serial bleeding was initiated at 0800 hr on Day 4 were significantly elevated compared to 24-hr levels in the groups of hamsters bled at the other days of the cycle. A noteworthy point is that both the levels of P and the pattern of peripheral P levels found between 1000 hr, Day 2

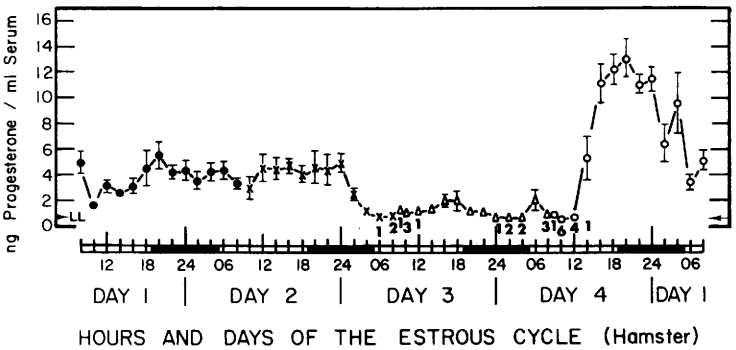

FIG. 1. Peripheral levels of progesterone in the cyclic hamster. Animals in each of four groups were bled (0.08 ml/bleeding) by cardiac puncture without anesthesia at 2-hr intervals over a 24-hr period beginning at 0800 hr on Day 1 ((●); n = 6), Day 2 ((×); n = 4), Day 3 ((△); n = 5), or Day 4 ((○); n = 6), respectively. Each symbol represents the mean (±SE) progesterone concentration; at some hours the symbol for the mean overlapped the limit of the SE. LL on the ordinate indicates the lower limit of detection of progesterone, in 10 μl of serum, on a concentration basis. The numbers beneath the symbols at Days 3 and 4 indicate the number of hamsters that had nondetectable levels of progesterone at the particular hours (these animals were not included in the calculation of the mean progesterone level depicted by the symbol at the corresponding hour). The dark bars along the abscissa represent the darkness periods of the lighting cycle.

and 1000 hr, Day 3 in the present study using the cardiac puncture technique are virtually identical to the levels and pattern recently determined in sera from decapitated hamsters for this particular time frame (6).

In contrast to patterns of P levels reported by Ridley and Greenwald (8), no significant episodic fluctuations over successive 4-hr periods were found on either Days 4 or 1 in the present study, when hamsters in a *single group* were serially bled at 2-hr intervals during either 24-hr period. In the former study (8), the multiple peak levels of P on Days 4 and 1 were virtually from two groups of animals serially bled at 4-hr intervals over consecutive 8-hr periods (2000, 2400, 0400 hr, and 0600, 1000, 1400 hr, respectively) while the reciprocal *low* levels of P on these days were from two other groups of animals serially bled in like design (1800, 2200, 0200 hr, and 0800, 1200, 1600 hr, respectively). Comparisons of the designs and results of these two studies and results of other studies, which assessed P levels primarily on Day 4 (1, 3, 5, 7) and with which the present results agree, suggest that the series of episodic fluctuations found by Ridley and Greenwald were most probably due to chance variation in tonic peripheral P levels between aforementioned groups of hamsters and were manifested by their experimental design. The same explanation appears equally plausible for the significant elevations found on Day 3 (8), which contrast with levels in the present study and in sera from decapitated hamsters (Bast, unpublished). Although only the composite profiles are given in Fig. 1, the 24-hr profile of individual animals at various stages of the cycle contained an occasional sporadic elevation, which, however, did not consistently occur in all hamsters at particular hours during the day. Thus, the results of the present study indicate that the peripheral P levels are relatively stable within each day of the cycle, at least when determined at 2-hr intervals in serially bled animals, except during delineated transitional periods of altered ovarian function (e.g., the preovulatory rise in P after 1200 of Day 4; during luteinization on the first half of Day 1; and during a period of rapid luteolysis early on Day 3).

Summary. A composite profile was made of peripheral progesterone (P) levels at 2-hr intervals over the 4-day estrous cycle of the hamster. Specifically, each of four groups of animals was bled via cardiac puncture (0.08 ml blood/bleeding), without anesthesia, at 2-hr intervals for a 24-hr period starting at 0800 hr on different days of the cycle. P was measured in etheric extracts of serum by RIA. After a fairly steady threefold increase in P from 1000 to 1800 hr on Day 1 (day of ovulation and vaginal discharge), P levels remained relatively stable at 4–6 ng/ml until 2400 hr on Day 2. During the first 6 hr on Day 3, P levels declined to near nondetectable values (<1 ng/ml) and thereafter fluctuated little until the dramatic preovulatory rise began between 1200 and 1400 hr on Day 4 (to 11–13 ng/ml by 1600 hr). After 2400 hr on Day 4, levels of P declined to 4–6 ng/ml by 0600 hr on Day 1. Series of close-interval, episodic fluctuations in P levels were not found.

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