Effects on Lactation of Progesterone Injections Administered During Late Pregnancy in the Rat[1] (35827)

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Cowie and Folley (1) have hypothesized that the onset of lactation is inhibited by high titers of circulating progesterone, such as those that characterize gestation. At or near parturition, when blood levels of progesterone decline, the block to lactation is removed, and milk secretion ensues. There have been no reports to date of a direct test of this view. The present experiment was designed to study the effects on lactation of progesterone injections administered during late pregnancy in the rat.

Materials and Methods. Virgin Sprague-Dawley rats, 60–70 days old and weighing 175–225 g, were bred with normal males and injected subcutaneously with 0.1-, 0.5-, or 2-mg doses of progesterone in 0.1 ml of sesame oil twice daily from days 18 through 23 of gestation. Control rats received only the oil. From day 18 on, the animals were housed in individual opaque Fiberglas cages 11 × 12 × 6-in. with San-i-cel bedding and food and water provided *ad libitum*. They were observed daily for the onset of birth, and on that day, the litters were reduced or brought up to 6 pups/litter and maintained daily at 6 pups for 5 days thereafter. Over this time, the mortality of the litters was scored daily as were the weight gains of the surviving young. In addition, the mammary glands were scored as positive for the presence of milk if: (a) from the day of birth on, milk was observed in the stomachs of the young; or (b) in cases of prolonged gestation, milk appeared evident in biopsy mammary samples from the inguinal region every other day for 4 days from day 23 of gestation on. For the latter, mammary samples were prepared and rated for secretory activity and degree of development by a modification of the methods of Benson *et al.* (2).

Over the first 5 days postpartum, the animals were also observed in standard nursing tests for 15-min each day in which the latency to begin crouching over 3 foster pups the same age as the litter was scored as was the duration. By removing the litter and replacing it during the nursing test with young that had been removed overnight from their mothers, a strong suckling stimulus was ensured. The nursing tests therefore served both as an index of motivation to nurse as well as for milk ejection. Milk ejection was scored as positive when active suckling by foster pups led to milk release as determined at the end of each test by their stomachs being distended with milk.

Results. Table I summarizes the lactational performance of rats injected with either 0.1-, 0.5-, or 2-mg doses of progesterone from days 18 through 23 of gestation. Deficits in performance were especially marked in the 0.5- and 2-mg progesterone groups, as evidenced in the significantly high mortality and weight loss among the young when contrasted with the sesame oil (control) group, particularly on postpartum day 5. On postpartum day 1, mortality of pups was significantly high in the 0.5- and 2-mg progesterone groups, but only the pups of the 2-mg progesterone group weighed significantly less than control young. Both the 0.1- and 2-mg progesterone groups showed significant delays in the onset of birth. By day 5, the pups of

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TABLE I. Lactational Performance of Rats Injected with Progesterone from Days 18 through 23 of Gestation.

		Postpartum day 1			Postpartum day 5			Nursing tests			
									Crouching behavior		
Dosage (mg)	Rats bred (no.)	Day of birth (mdn)	Ratio of dead to live pups (no.)	Wt change in litter between days 1 and 2 (g)	Ratio of dead to live pups (no.)	Wt change in litter between days 4 and 5 (g)	Mammary gland condition (% of rats with secretion)	(% of rats)	Latency (sec/10)	Duration (sec/10)	Milk ejection (% tests)
0.1 P[a]	4	26[c]	0/38	+8.7 ± 3.7[b]	1/23	+5.3 ± 1.2[g]	100	100	65.4 ± 14.1	23.9 ± 13.9	80
0.5 P	3	24	9/23[d]	−0.5 ± 9.8	9/9[d]	−2.2 ± 5.1[g]	67	100	58.8 ± 14.9	29.1 ± 14.2	13[d]
2 P	8	27[c]	30/82[d]	+0.1 ± 1.9[g]	12/36[d]	−3.7 ± 2.1[g]	88	100	61.8 ± 11.0	21.9 ± 9.9	2[d]
Sesame oil	14	23	0/174	+9.1 ± 0.6	0/84	+9.4 ± 0.6 $F = 8.6$[f]	100	100	61.4 ± 8.2 $F = .2$[h]	22.8 ± 8.0 $F = .5$[h]	97

[a] Progesterone.
[b] Mean ± standard error.
[c] $p < .01$ by Fisher's test.
[d] $p < .01$ by x^2.
[e] $p < .05$ by t.
[f] $p < .05$ by the F test.
[g] $p < .05$ by the Duncan's multiple range test applied to a significant F.
[h] $p > .05$ by the F test.

the 0.1-mg progesterone group gained in weight, but they were still significantly lower than control young. Despite these differences, milk was evident in the mammary glands of a majority of rats in all the progesterone groups. Only one rat in the 0.1- and 0.5-mg progesterone groups, respectively, delivered young on day 23 and successfully proceeded to nurse them. The remaining progesterone rats, 13 in all, gave birth late, between days 24 and 27. In 11 of these animals, secretion lay throughout their mammary samples distending alveoli as well as ducts.

With respect to nursing behavior, there were no significant differences between any of the progesterone treatments and the control group, either in the percentage of rats crouching over young or the latency to begin and the duration of the behavior. However, in both the 0.5- and 2-mg progesterone groups, the percentage of tests in which active suckling by foster young led to the release of milk was significantly reduced.

Discussion. These data indicate that progesterone injections during late pregnancy effectively inhibit lactation in the rat. They indicate further that 0.5- and 2-mg doses of progesterone twice daily from days 18 through 23 of gestation produce more of an inhibitory effect than 0.1 mg of progesterone similarly administered, particularly with regard to mortality and weight changes among the young, and the release of milk in nursing tests.

Progesterone can inhibit lactation by one of two (or both) routes; by affecting milk secretion and/or milk release. With regard to these two processes of lactation, Cowie and Folley have particularly stressed the former: "lactogenic doses of estrogen may be deprived of their lactogenic action by suitable doses of progesterone . . . At parturition the relative fall in the progesterone to estrogen ratio removes the inhibition (1, p. 607)" and milk secretion ensues. We maintained high progesterone levels in rats by injecting them during late pregnancy when endogenous progesterone levels are known to decline (3, 4). That milk secretion was inhibited appears unlikely on the basis that our biopsy samples revealed the presence of milk. Furthermore, progesterone is not known to inhibit either the lactogenic content of the adenohypophysis or the development of the mammary glands. Reece and Bivins (5) reported that, in the ovariectomized rat, subcutaneous injections of progesterone plus estrogen augmented anterior pituitary lactogenic hormone content as much as injections of estrogen alone. In the hypophysectomized–adrenalectomized–ovariectomized rat, Lyons *et al.* (6) showed that progesterone in synergism with estrogen was a more potent stimulator of mammogenesis than estrogen alone.

That milk ejection was inhibited appears the more likely alternative. Our nursing tests revealed a marked effect on milk release, suggesting that progesterone may act to block lactation by inhibiting an oxytocin effect. This alternative could account also for the delay in the onset of labor, or conversely, the prolongation of gestation, which we (Table I) as well as others have observed (7, 8). In early work on the rabbit, Zarrow (7) found that daily injections of progesterone into pregnant animals markedly delayed parturition. Later, Csapo (8) showed that pre-priming the 31-day pregnant rabbit with progesterone inhibited the induction of labor that typically followed a single injection of oxytocin. On this basis, he concluded that progesterone injections delayed parturition by inhibiting an oxytocin effect.

Progesterone may also inhibit lactation by reducing the motivation to nurse. Moltz *et al.* (9) performed cesarean sections on primiparous rats on day 21 of gestation and injected them twice daily with 2-mg doses of progesterone from days 19 through 23. They found that 50% of the animals accepted and reared to weaning the foster young with which they were subsequently housed. However, under the conditions of our experiment, in which cesarean delivery was not performed, we found that nursing behavior remained intact.

Summary. Progesterone injections administered during late pregnancy effectively inhibit lactation in the rat. Subcutaneous doses of 0.5 and 2 mg of progesterone in sesame oil twice daily between days 18 and 23 of gestation produced a greater inhibitory effect than

0.1 mg of progesterone similarly administered, particularly with regard to mortality and weight changes of the young, and the release of milk in nursing tests. The primary inhibition appears to rest on processes of milk ejection rather than milk secretion or the motivation to nurse.

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