Effects on Lactation of Progesterone Injections Administered After Parturition in the Rat (36673)

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When administered to normal lactating rats, estrogens, or estrogens and progesterone, are known to have adverse effects upon the growth of the young. Among the reasons advanced to explain the depression of litter growth have been a disturbance of secretory events in the mammary glands (*e.g.*, biochemical changes in the milk, or no milk secretion at all due to involution of the mammary glands or some other event), the failure of milk release, and poor maternal behavior (1–5). Generally speaking, progesterone alone, without the estrogens, had no marked effect on litter growth (4–6).

Cowie and Folley (1), among others (7), have hypothesized that the onset of lactation is inhibited by high titers of progesterone in circulation, 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. In a recent test of this view, we injected late pregnant rats with progesterone and found a marked suppression of litter growth after birth (8). In that report, the depression of litter growth appeared due to a failure of milk release. The question arises as to whether progesterone injections of the sort that suppress lactation when administered during late pregnancy might not also suppress lactation when administered after parturition.

Materials and methods. Virgin Sprague–Dawley rats, 60–70 days old and weighing 175–225 g, were mated and injected subcutaneously with 2-mg doses of progesterone in 0.1 ml of sesame oil twice daily either for the first or the second week postpartum. Control rats received only the oil. From Day 18 of gestation on, animals were housed and fed as before (8) and on the day of birth, typically Day 23 in this strain, the litters were reduced to 6 pups/litter to keep litter size constant.

Over the respective treatment weeks, lactational performance was scored in terms of daily weight gains in the litters. Nursing behavior was also scored daily with standard observational techniques (8). In 15-min behavior tests, the latency to assume a crouching position over the pups was scored as was the duration. The test began when three foster pups the same age as the litter were introduced into the cage each morning, 30 min after the female's own litter had been removed. For the next 15 min, nursing behavior was scored continuously and then analyzed in 10-sec blocks of time. Latency of nursing behavior was defined as the time that elapsed between the presentation of the foster pups and crouching contact with at least one pup. Duration was the amount of time the behavior occurred. After the behavior test, the female's own young were reintroduced.

Results. Table I summarizes the lactational performance and nursing behavior of rats injected with either progesterone or oil during the first or the second week of lactation. It can be seen that progesterone treatments during the first week of lactation only moderately affected lactational performance as well as nursing behavior whereas during the second week, they affected only the nursing behavior.

For postpartum Days 1–7, litters with progesterone-injected females consistently gained less weight from day to day than did litters with oil-injected rats. However, the weight gains were significantly less on one day only, on postpartal Day 3.

TABLE I. Lactational Performance and Nursing Behavior of Rats Injected with Progesterone during the First or Second Week Postpartum.

Postpartum period	Dosage (mg)	Rats (no.)		Lactation: Weight change in litters from day to day (g), Days: 2nd	3rd	4th	5th	6th	7th	Total, 1–7	Nursing behavior: Latency (sec/10), Total, days 1–7	First 3 days	Second 3 days	Duration (sec/10), Total, days 1–7	First 3 days	Second 3 days
Days 1–7	2 P[a]	5	$\bar{X}$	3.66	5.04	7.28	8.28	9.80	8.62	7.11	71.06	74.13	71.26	12.25	10.80	10.26
			SE	1.18	.33	.88	.78	.63	1.06	1.36	4.84	4.56	5.54	8.00	8.42	7.33
	Sesame oil	5	$\bar{X}$	4.98	8.22	9.04	9.06	10.80	10.50	8.77	57.22	54.60	61.52	22.14	24.00	19.93
			SE	.44	.67	.69	.92	.96	1.22	1.28	7.85	7.45	7.12	11.51	12.12	10.47
			t	1.17	4.74**	1.75	.72	.98	1.30	.99	1.68	2.50*	1.20	.79	1.00	.85
Days 6–12	2 P	5	$\bar{X}$	9.65	10.13	12.40	9.65	10.20	11.42	10.58	53.96	63.00	42.33	27.75	21.75	35.83
			SE	1.44	.73	.83	.45	.13	1.78	1.17	6.56	4.88	5.61	10.11	8.81	10.24
	Sesame oil	5	$\bar{X}$	9.60	9.08	10.40	10.08	10.88	9.54	9.93	69.97	68.13	74.13	17.25	16.20	15.06
			SE	.50	1.27	1.02	.90	1.23	1.86	1.14	7.30	9.12	3.81	11.31	11.99	9.42
			t	.04	.80	1.70	.48	.62	.82	.44	1.82	.55	5.24**	.77	.42	1.67

[a] Progesterone. * $p < 0.025$. ** $p < 0.005$.

With regard to nursing behavior, the latency measures showed that for Days 1–7, progesterone-injected females were consistently slower to initiate nursing behavior than were oil-injected ones. However, a more detailed analysis of the latency data revealed that the progesterone females were only significantly slower for the first 3 days postpartum, and not the second 3 days. With regard to the duration of nursing behavior, progesterone females spent consistently less time over young than did control rats. However, the differences were not significant, either for the first full week or for either grouping of Days 1–3 or 4–6.

For postpartal Days 6–12, the weight gains among litters with progesterone-injected females were neither consistently nor significantly different from that among litters of control rats. With regard to nursing behavior, progesterone females were quicker than control ones to begin crouching over the young during the second week postpartum, but that difference was not significant. Progesterone females were only significantly quicker than control ones during the second half of that treatment week, from postpartal Days 9–11 ($p < 0.005$). Although progesterone females consistently spent more time crouching over young than did control rats, there were no significant differences in the duration of nursing behavior either for the full second week of lactation or for its first or second part.

Discussion. Two-mg doses of progesterone twice daily during the first or the second week postpartum did not markedly suppress lactation, as evidenced by the fact that there were no marked differences in weight between experimental and control young. Thus contrary to our previous report that 2-mg doses of progesterone twice daily for 6 days in late pregnant rats significantly reduced litter growth (8), like doses offered for equivalent days but during either the first or the second week postpartum were without marked effect.

At least three factors, singly or in combination, could explain the differences in results between the prepartal and postpartal administrations of progesterone. One is that unlike the effects of prepartal injections, which markedly disrupted birth (8), birth ensued normally in the present case before proges-

terone was introduced. Another factor is that the hormone state of the animals was considerably different. And still a third factor is that in the postpartal study, young were already present.

One event implying that differences in hormone state might have contributed to differences in pre- and postpartal results rests on the fact that progesterone did depress litter growth significantly on one postpartal day, on Day 3 postpartum only (Table I). It is interesting to note that at that time endogenous levels of progesterone are undergoing a marked shift in direction, just as they do before parturition. At or near the time of birth, however, progesterone levels are on the decline, reaching a low point around parturition. Thereafter, they rise and continue to elevate, stabilizing again at some high point about 72 hr after birth (9, 10).

Our results confirm past reports that progesterone injections after parturition do not markedly suppress lactation (4–6). Folley and Kon (4) injected lactating rats with high doses of progesterone ("1 mgm-crystalline semisynthetic progesterone in . . . 0.2 ml. arachnis oil," p. 1107) from Days 5–15 postpartum and found no difference in mean growth-rates for the suckling experimental and control young. Walker and Matthews (5) reported that neither 2-mg nor 5-mg doses of progesterone per day from Days 2–16 postpartum inhibited lactation; again the mean growth-rates of experimental and control litters were not significantly different. Finally, in a study in which among other treatments, progesterone was introduced alone, Griffith and Turner (6) reported that 3-mg progesterone per day from Days 2–13 of lactation affected neither the weight of the litters nor the DNA content of the mammary glands of the dams.

It should be mentioned that in the previous reports in which treatments began on Day 2 (5, 6), the effects on lactation were not scored daily but rather were presented after some block of treatment time had passed. This may be one reason why unlike the current report there is no mention in past reports (5, 6) of a depression of litter weight on Day 3 postpartum.

Prepartum injections of progesterone depressed litter growth and survival of young without disrupting nursing behavior (8). Thus poor maternal behavior could not be implicated as a cause of the depression of litter growth. In the current study, it is not possible to dissociate an effect on nursing behavior from an effect on lactation alone. During the first week postpartum, for example, progesterone-injected rats were significantly slower than control ones to assume a lactation posture over young. They also spent less time over the pups, although not significantly so (Table I). Thus the depression in litter growth scored during the first week could be due to the reduction in nursing behavior also observed at that time; or vice-versa; or to the fact that the two events are not causally related at all and that some other factor(s) were responsible.

It should be remembered that as time since parturition advances, not only do the hormone states of the dam undergo change but so too do the stimuli afforded by the pups, and the experience with them. These factors, or some combination of them, could also explain the differences in results between pre- and postpartal administrations of progesterone. Some precedent for this view has already been set by the work of Grosvenor (11) with rats which showed that exteroceptive cues associated with pups can directly release prolactin from the pituitary only at Day 14 postpartum and not before. His data (12) further suggest that the suckling stimulus is the primary means by which prolactin is released in the early lactating rat, but in the later lactating one, the suckling stimulus becomes conditioned to stimuli emanating from the pups so that these exteroceptive stimuli alone evoke prolactin release.

Summary. Progesterone injections administered during the first or second week after parturition did not markedly suppress lactation in the rat. Subcutaneous doses of 2-mg progesterone in 0.1 ml sesame oil twice daily on postpartum Days 1–7 only significantly reduce weight gains in the young on one day, on postpartum Day 3. Because nursing behavior was also suppressed, it was not possible to dissociate an effect of progesterone on

lactation alone. Like doses of progesterone from Days 6–12 had no effect on lactation.

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