Studies on Maintaining the Lactational Diestrum after Early Litter Weaning[1] (34431)

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During lactation in the rat, vaginal diestrus is maintained provided an adequate number of pups are nursing (1, 2). This condition is known as the lactational diestrum and is believed to be brought about by the continued production of prolactin and the suppression of FSH and LH (2). Various stimuli given to the rat will induce either an acute or chronic pattern of prolactin production (3). Stimulation of the cervix either mechanically or electrically will result in continued prolactin production for a period of 12–14 days (4–6). On the other hand, prolactin production during lactation is maintained only if continued suckling occurred (2, 7). In the former case, prolactin production is chronic whereas in the latter it is acute. It appears that the brain of the rat possesses a biological clock which is engaged by cervical stimulation and which allows the continued secretion of prolactin for a period of approximately 2 weeks. The stimulus of suckling, however, does not initiate the biological clock and prolactin secretion is of an acute nature. Whether these patterns of prolactin production are due to the different receptor organs involved (cervix and teat), to the endocrine milieu bathing the nervous system at the time of stimuli, or to different CNS structures receiving the stimuli is not known. In the present study we have examined the effect of various forms of cervical stimulation and of hormone administration on extending the lactational diestrum after weaning.

Materials and Methods. Sprague-Dawley female rats, 180–200 g in body weight, were cohabited with males of proven fertility until palpated pregnant. They were then placed into individual solid bottom cages for the rest of the experiment. The day of parturition was called day 1 of lactation and litter was adjusted to 8 pups. Any mother not having at least 6 pups/ litter at the time of weaning was discarded from the experiment. Mothers whose litters were weaned on day 1 of lactation were placed into cages with a large mesh screening on the floor in late pregnancy. At parturition the pups fell through the mesh and did not suckle as evidenced by a lack of milk in their stomach. Vaginal smearing was initiated on the day of parturition and continued daily for 21 days. In some groups the initiation of vaginal smearing was delayed until the litter was weaned which was on day three of lactation. Those animals which did not exhibit a vaginal estrus until 10 days or more from weaning were allocated to the pseudopregnancy group.

Mechanical stimulation of the cervix was performed using a fire-polished glass tube. Electrical stimulation was accomplished using a bipolar copper electrode with a 1-mm separation at the tip; the electrode was inserted into the glass tube used for mechanical stimulation so that the noninsulated electrode tip touched only the cervix. Electrical stimulation was continued for 30 sec using either 10 or 30 V with 20 cps and a 20-msec delay. Both mechanical and electrical cervical stimulation as described above each induced pseudopregnancy in 5 out of 5 cyclic rats when performed at time of vaginal estrus (Gala, unpublished). Male rats with proven mating behavior were vasectomized and rested for 30 days. They were placed with a pregnant female when she was moved to an individual cage and left with her until the third day of lactation. Statistical analyses were performed using the analysis of variance and Duncan's multiple range test (8).

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TABLE I. Influence of the Time of Litter Removal upon Incidence of Extended Diestrus.

Day of lactation litter removed[a]	No. of pseudo-pregnant/no. of animals	Pseudopregnant animals		Nonpseudopregnant animals	
		Days p.p. to 1st estrus	Days weaning to 1st estrus	Days p.p. to 1st estrus	Days weaning to 1st estrus
1[b]	3/10	11.3 ± 0.7[d]	11.3 ± 0.7	5.7 ± 0.5	5.7 ± 0.5
3	6/20	13.5 ± 0.4	10.5 ± 0.4	7.0 ± 0.4	4.0 ± 0.3
6	0/9	—	—	11.0 ± 0.9	5.0 ± 0.9
9	0/10	—	—	16.7 ± 0.5	7.7 ± 0.5[c]
12	0/10	—	—	18.1 ± 0.6	6.1 ± 0.6

[a] All animals vaginally smeared from day 1 of lactation.

[b] Mothers did not nurse litter.

[c] Significantly different ($p < 0.05$) from the 3- and 6-day values; all other values are not significantly different ($p > 0.05$) from each other.

[d] ± standard error of the mean.

Results. Thirty percent of those animals whose litters were either not allowed to suckle, or suckled for 48 hr, showed an average extended diestrum of 11 days after the litter was weaned; vaginal smearing was initiated on day of parturition. None of the animals whose pups were removed on day 6, 9, or 12 of lactation showed an extension of diestrus. The time to the first vaginal estrus of mothers weaned on the ninth day of lactation was significantly longer ($p < 0.05$) than mothers weaned on day 3 or 6 of lactation (Table I).

Mechanical stimulation of the cervix on day 1 of lactation to animals whose litters were subsequently removed on day 3 did not alter the incidence of extended diestrus from that of controls (25 vs. 30%). Electrical stimulation performed under similar conditions resulted in a greater incidence of extended diestrus (50 vs. 30%). It was also observed that in those animals which did not show an extended diestrum the time to the first estrus was somewhat delayed but was not statistically different from the control group (Table II). Mothers mechanically stimulated on the day of parturition and weaned on day 6 of lactation did not differ in their incidence of extended diestrus when compared to nonstimulated controls. The time to first estrus, however, appeared shorter but the difference was not significant (Table II).

In those animals in which the initiation of vaginal smearing was delayed until weaning (day 3 of lactation) no incidence of extended diestrus was observed (Table III). Vasecto-

TABLE II. Influence of Mechanical or Electrical Stimulation of the Cervix on Day 1 of Lactation on Incidence of Extended Diestrus after Litter Removal.

Day pups removed[a]	Type of cervical stimulation	No. of pseudo-pregnant/no. of animals	Pseudopregnant animals		Nonpseudopregnant animals	
			Days p.p. to 1st estrus	Days weaning to 1st estrus	Days p.p. to 1st estrus	Days weaning to 1st estrus
3	None[b]	6/20	13.5 ± 0.4[c]	10.5 ± 0.4	7.0 ± 0.4	4.0 ± 0.3
	Mechanical	5/20	13.0 ± 0.0	10.0 ± 0.0	7.8 ± 0.7	4.8 ± 0.7
	Elect, 10 V	6/10	14.3 ± 0.3	11.2 ± 0.4	8.8 ± 0.8	5.8 ± 0.8
	Elect, 30 V	4/10	13.8 ± 0.5	10.8 ± 0.5	8.7 ± 0.9	5.7 ± 0.9
6	None[b]	0/9	—	—	11.0 ± 0.9	5.0 ± 0.9
	Mechanical	1/10	18	12	9.3 ± 0.8	3.2 ± 0.8

[a] All animals vaginally smeared from day 1 of lactation.

[b] Data from Table I for comparative purposes.

[c] ± standard error of the mean.

TABLE III. Influence of the Time of the Initiation of Vaginal Smearing on the Incidence of Extended Diestrus (litter removal on day 3).

Day of lactation smearing initiated	No. of pseudo-pregnant/no. of animals	Pseudopregnant animals		Nonpseudopregnant animals	
		Days p.p. to 1st estrus	Days weaning to 1st estrus	Days p.p. to 1st estrus	Days weaning to 1st estrus
1[a]	6/20	13.5 ± 0.4[b]	10.5 ± 0.4	7.0 ± 0.4	4.0 ± 0.3
3	0/11	—	—	7.7 ± 0.4	4.7 ± 0.4

[a] Data from Table I for comparative purposes.
[b] ± standard error of the mean.

mized male rats cohabited with females during late pregnancy and early lactation did not alter the occurrence of extended diestrus from that observed of control animals. The administration of a single dose of 50 μg of estradiol on the first day of lactation resulted in a pseudopregnancy-like smear in 60% of the animals; these animals were weaned and vaginal smearing was initiated on day 3 of lactation. The combination of 50 μg of estradiol and mechanical stimulation on day 1 of lactation produced a slight decrease in the incidence of extended diestrus when compared alone (40 vs. 60%). The time to the first estrus in the doubly treated animals, however, was significantly lengthened (Table IV).

Discussion. Zeilmaker (9) reported that the litter must suckle 2–3 days before extended diestrus and induced deciduomata can be observed after weaning. Another investigator noted that when pups were removed after 1–2 days of suckling, 50% of the animals were found to be pseudopregnant (2). In both of these studies animals were smeared from the day of delivery. Our results indicate that even though suckling does not occur a small percentage of animals (30%) will exhibit an extended diestrum (Table I, day 1 of lactation). The application of a strong suckling stimulus for 2 days does not increase the percentage of animals with pseudopregnancy-like smears and if the duration of suckling is extended further no animals give such a response. Alloiteau (10) pointed out that vaginal smearing alone early in lactation was capable of inducing pseudopregnancy after the litter was weaned. Our data substantiate his observation and lead us to believe that the small percentage of extended diestrus observed of animals weaned on day 1 and 3 of lactation was probably due to the increased sensitivity of the animal to vaginal smearing. Continuation of suckling to day 6 of lactation

TABLE IV. Influence of Estradiol (E) and/or Mechanical Cervical Stimulation on Day 1 of Lactation on the Incidence of Extended Diestrus after Litter Removal and Vaginal Smearing was Performed on Day 3 of Lactation.

Experimental conditions	No. of pseudo-pregnant/no. of animals	Pseudopregnant animals		Nonpseudopregnant animals	
		Days p.p. to 1st estrus	Days weaning to 1st estrus	Days p.p. to 1st estrus	Days weaning to 1st estrus
Control[a]	0/11	—	—	7.7 ± 0.4[c]	4.7 ± 0.4
Vasectox ♂	1/10	15	12	6.8 ± 0.6	3.8 ± 0.6
50 μg of E	6/10	14.7 ± 0.7	11.7 ± 0.7	6.5 ± 0.9	3.5 ± 0.9
50 μg of E + cervical stim.	4/10	14.8 ± 0.6	11.8 ± 0.6	9.5 ± 1.0	6.5 ± 1.0[b]

[a] Data taken from Table III for comparative purposes.
[b] Significantly different ($p < 0.05$) from the vasectomized male and 50 μg of E alone values but not from the control value.
[c] ± standard error of the mean.

and beyond in some way negates the effect of early vaginal smearing. The similar lengths of the extended diestrum of animals weaned on day 1 and 3 of lactation lends some support to this suggestion and does not foster the possibility that the smear-induced pseudopregnancy ran its normal course concurrent with the suckling diestrum. The extended diestrus smears in these instances (day 1 and 3 of lactation) were additive to the suckling diestrum (see Table I).

The single injection of high amounts of estrogen at vaginal estrus in cyclic animals has been reported by a number of workers to induce pseudopregnancy in the rat (3). We observed a similar effect when estradiol was administered to lactating rats and weaned 2 days later. The incidence of extended diestrus, however, was not as great as expected if an estrous cycle animal was used (10, 11). The inability of various stimuli both of a physical and hormonal nature to induce a pseudopregnancy-like diestrum in more than 60% of the animals leads one to wonder why the nursing rat does not retain the capacity to respond as well as estrous cycle animals. This lapse of "memory" on the part of lactating rats will be the point of origin for future studies.

Summary. The litters of lactating rats were adjusted to 8 pups on the day of parturition (day 1 of lactation) and various methods of cervical stimuli were performed. The litters were weaned at regular intervals and vaginal smears were examined for the return of estrous cycles. Thirty percent of mothers who either were not suckled or were suckled for 48 hr showed diestrus smears for 10 days or more. Mothers whose litters were removed on days 6, 9, and 12 of lactation did not exhibit an extended diestrum. Mechanical stimulation of the cervix of mothers weaned on day 3 of lactation did not increase the incidence of extended diestrus from the controls; electrical stimulation, however, almost doubled the number responding (50 vs. 30%). If the initiation of vaginal smearing was delayed from the first day of lactation until the third, none of the animals had pseudopregnancy-like vaginal smears. Estradiol administration on day 1 of lactation resulted in 60% of the animals exhibiting an extended diestrum. Combining both estradiol injection and mechanical cervical stimulation resulted in a slightly lower percentage (40%). The suckling stimulus is believed to induce an acute pattern of prolactin secretion while cervical stimulation induces a chronic pattern. The summation of these two secretory patterns may be observed early in lactation.

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1. Hain, A. M., Quart. J. Exptl. Physiol. **25**, 303 (1935).

2. Rothchild, I., Endocrinology **67**, 9 (1960).

3. Everett, J. W., Pituitary Gland **2**, 166 (1966).

4. Long, J. A. and Evans, H. M., Mem. Univ. Calif. **6**, 1 (1922).

5. Shelesnyak, M. C., Anat. Record **49**, 179 (1931).

6. Haterius, H. O., Am. J. Physiol. **103**, 97 (1933).

7. Selye, H. and McKeown, T., Proc. Soc. Exptl. Biol. Med. **31**, 683 (1934).

8. Duncan, D. B., Biometrics **11**, 1 (1955).

9. Zeilmaker, G. H., Acta Endocrinol. **49**, 558 (1965).

10. Alloiteau, J. J., Biol. Med. **51**, 250 (1962).

11. Dury, A. and Bradbury, J. T., Am. J. Physiol. **139**, 135 (1943).

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