

Lactogenic Hormone Requirement for Pseudopregnancy in Normal and Hysterectomized Rats* (32784)

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Inhibition of estrous cycles by daily injections of lactogenic hormone has been demonstrated in mice (1,2) and rats (3). Lahr and Riddle (3) found that rats receiving 15 IU of lactogenic hormone per day had diestrous intervals of 13–16 days followed by estrous and then a second diestrous interval of equal length. In rats treated beyond the second extended diestrus, the estrous cycles were normal. This means of inducing pseudopregnancy in the rat seems unique based on the pattern of interrupted pseudopregnancies succeeding each other. Since hysterectomy has been found to result in extension of the life span of corpora lutea in pseudopregnant rats (4,5), it was felt that the response of hysterectomized rats to lactogenic hormone injections might provide some insight into the phenomenon of uterine luteolysis.

The data reported here provide evidence suggesting that lactogenic hormone may be secreted for only 5–7 days to produce pseudopregnancy. They also suggest that ovine lactogenic hormone is rendered ineffective by the rat after constant injections of 3 weeks and that the refractory period to lactogenic hormone is lost after 2 weeks.

Materials and Methods. Sprague-Dawley (Holtzman) female rats were maintained in an animal room illuminated artificially for 14 hours per day. Water and commercial pelleted feed were provided *ad libitum*. Hysterectomy was performed by a ventral abdominal approach several estrous cycles prior to initiation of experimental vaginal smearing. The smears were made daily by water lavage. Two normal estrous cycles were followed prior to the initiation of lactogenic hormone injections. Lactogenic hormone or lactogen (ovine, Armour) was dissolved in physiological saline solution and injected daily by subcutaneous route in a volume of 0.2 ml per 17 IU of

lactogenic hormone starting on the day of estrous.

The relationship between injected lactogen and duration of pseudopregnancy was studied in 14 normal and 14 hysterectomized rats given 17 IU of lactogen daily.

The minimum duration of lactogenic hormone injection to induce pseudopregnancy was examined as follows: (a) 6 normal animals injected with 85 IU of lactogenic hormone for 1 day, (b) 6 normal animals injected with 17 IU of lactogenic hormone/day for 1, 3, 5, or 7 days, (c) 6 hysterectomized rats injected with 17 IU of lactogenic hormone/day for 1, 3, 5, 7, 9, or 11 days.

The duration of pseudopregnancy was determined by counting the first day of an estrous smear as day 0 and the first day of the succeeding proestrous smear as the last day of pseudopregnancy.

Results. The injection of lactogenic hormone daily to 14 normal rats resulted in consecutive pseudopregnancies, the first of 14.8 days duration and the second of 12.6 days (Table I). Continuation of injections beyond the end of the second pseudopregnancy resulted in only 2 animals showing a third pseudopregnancy, both of which were of 12-days duration. All cycled normally following pseudopregnancy.

The 14 hysterectomized rats given 17 IU of lactogenic hormone/day had pseudopregnancies of 20.7 days. This was significantly longer than those of normal rats. When the lactogen was continued, only 3 of the 14 rats exhibited second pseudopregnancies averaging 20 days. The other 11 rats cycled normally.

When lactogenic hormone injections were discontinued for three estrous cycles and then reinitiated, pseudopregnancies were again produced in all normal and hysterectomized rats. These averaged 13.4 days in the normal rats and 21.3 days in the hysterectomized rats.

Injection of 85 IU of lactogenic hormone

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TABLE I. Effect on Estrous Cycles of Daily Subcutaneous Injection of 17 IU of Lactogenic Hormone to Normal and Hysterectomized Rats.

Group	Treatment	No. of rats	No. of pseudo-pregnancies	Duration of pseudopregnancies (days) ^b	Significance 1%
A	Normal rats, 17 IU lactogenic hormone/day 1st pseudopregnancy	14	14	14.8 ± 0.6	D
B	2nd pseudopregnancy	14	13 ^a	12.6 ± 0.7	
C	3rd pseudopregnancy	14	2	12.0	
D	Hysterectomized rats, 17 IU lactogenic hormone/day 1st pseudopregnancy	14	14	20.7 ± 0.8	A, B
E	2nd pseudopregnancy	14	3	20.0 ± 1.0	

^a Rats which did not become pseudopregnant had normal 5-day estrous cycles.^b Mean ± SE.

TABLE II. Effect of Duration of Daily Lactogenic Hormone Injections on Estrous Cycles and Pseudopregnancies in Normal and Hysterectomized Rats.

Group	Lactogenic hormone injection		No. of rats	Duration of estrous cycle or pseudo-pregnancy (days) ^a	Significance 1%
	(days)	(IU/day)			
Normal rats					
A	1	85	6	4.7 ± 0.3	D,E,H,I,J,K
B	1	17	6	4.7 ± 0.3	D,E,H,I,J,K
C	3	17	6	6.7 ± 0.7	D,E,H,I,J,K
D	5	17	6	11.0 ± 0.8	A,B,C,F,G,I,J,K
E	7	17	6	12.7 ± 0.5	A,B,C,F,G,I,J,K
Hysterectomized rats					
F	1	17	6	5.1 ± 0.3	D,E,H,I,J,K
G	3	17	6	6.0 ± 0.4	D,E,H,I,J,K
H	5	17	6	13.2 ± 2.8	A,B,C,F,G,I,J,K
I	7	17	6	20.0 ± 0.9	A thru H
J	9	17	6	21.3 ± 0.7	A thru H
K	11	17	6	23.0 ± 1.1	A thru H

^a Mean ± SE.

in one subcutaneous injection on the day of estrous, or approximately 12 hours after ovulation which normally occurs at 2 a.m. on the day of estrous, was without effect on cycle length (Table II).

The injection of 17 IU of lactogenic hormone for 1 day did not lengthen normal estrous cycles, while the prolongation of daily injections to 3 days resulted in cycles of 6 or 7 days in normal and hysterectomized rats. When the injections were given daily for 5

days, one-half of the normal rats and one-half of the hysterectomized rats had greatly extended diestrous periods or pseudopregnancies. Mean periods to the next cycle were 11.0 ± 0.8 and 13.2 ± 2.8 days, respectively. Daily injections of lactogenic hormone for 7 days resulted in the exhibition of pseudopregnancies in all normal and hysterectomized rats. The durations were 12.7 ± 0.5 days in normal rats and 20.0 ± 0.9 days in hysterectomized rats. Lactogen for 9 days or 11 days in

hysterectomized rats produced pseudopregnancies of 21.3 ± 0.7 and 23.0 ± 1.1 days, respectively.

Discussion. Since the study of Lahr and Riddle (3), showing that continuous injections of lactogenic hormone were not effective in preventing lysis of corpora lutea for more than 15 days in the rat, speculation as to the mechanism of action of lactogenic hormone has arisen. The present data concerning daily injection of lactogenic hormone support the work of Lahr and Riddle (3) in showing that a pseudopregnancy of no more than 14 or 15 days is possible in normal rats with a second beginning immediately after the first.

The present study shows that while two successive pseudopregnancies occur in normal rats subjected to daily lactogenic hormone injection, only one extended pseudopregnancy can be produced in rats having no uterus. Rothchild (6) has suggested that progesterone secretion inhibits gonadotropin release from the pituitary and, therefore, prolongs the corpora lutea lifespan. He suggested that the eventual refractoriness of the hypothalamus to progesterone inhibition results in gonadotropin secretion which has a luteolytic effect on the existing corpora lutea. This hypothesis, however, does not explain the extension of pseudopregnancy after hysterectomy. The work of De Jongh and Wolthuis (7) concerning the metabolism of progesterone by the uterus would help to explain Rothchild's hypothesis. Not to be dismissed is the concept that lactogenic hormone may be both luteotropic and luteolytic, depending upon the age of the corpora lutea (8).

When rats are rendered pseudopregnant by mating with a vasectomized male, they have pseudopregnancies of 13–15 days if normal and 21–26 days if hysterectomized (4). They may also be stimulated with a glass rod to initiate pseudopregnancy and will then have succeeding pseudopregnancies whether hysterectomized or normal (personal observations). If succeeding pseudopregnancies are possible under the influence of endogenous

luteotropin, it would seem plausible that one pseudopregnancy in hysterectomized rats and two in normal rats following lactogenic hormone injections may be the result of inactivation of the exogenous ovine hormone by an immune reaction.

The fact that lactogenic hormone injection for 7 successive days enabled corpora lutea to survive an additional 7 days (intact uterus) or 14 days (hysterectomized) suggested that luteotropin stimulation was not necessary throughout the entire lifespan of the corpora lutea. This observation verifies the suggestion of Zeilmaker (9) that lactogenic hormone secretion lasts for about 8 days following stimulation of the cervix.

Summary. Female albino rats were injected subcutaneously with 17 IU of ovine lactogenic hormone/day continuously. While normal rats had 2 consecutive pseudopregnancies of 14 and 12 days, respectively, hysterectomized rats had only one of 21 days after which normal estrous cycling occurred in spite of the continued lactogen. Injection of lactogenic hormone for 5–7 days from the day of estrous was required to induce pseudopregnancies in normal or hysterectomized rats, the latter always having diestrous periods of about 7 days longer than normal rats. When lactogenic hormone was discontinued for 3 estrous cycles and reestablished, pseudopregnancies again resulted in both normal and hysterectomized rats.

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