TERMINATION OF PREGNANCY AFTER ACCELERATED LACTATION IN THE RAT

II. RELATIONSHIP TO NUMBER OF YOUNG, DAY OF PREGNANCY AND LENGTH OF NURSING

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Summary. Pregnancy is terminated in rats that have mated at the post-partum oestrus if they are allowed to suckle a large litter after implantation. For consistent termination, the number of sucking young must be at least nine, the accelerated lactation must be begun before Day 10 of pregnancy, and must be continued for at least 2 days.

INTRODUCTION

Embryos of a rat that has mated at the post-partum oestrus and is nursing young from the previous pregnancy do not implant on Day 5 of pregnancy as usual, but enter a 'dormant' state and remain free in the uterus for a length of time that varies roughly with the number of sucking young (Weichert, 1940). We have previously reported that embryos of rats nursing two young from Day 0 to 9 of pregnancy did implant but were later resorbed when the size of the nursing litter was increased to between ten and fourteen on Day 9 of pregnancy (Veomett & Daniel, 1971). In this paper we report the results of further studies aimed at determining (1) the number of nursing young, (2) the time in pregnancy, and (3) the length of accelerated lactation necessary to cause termination of pregnancy.

MATERIALS AND METHODS

Animals

Adult female albino rats of the Holtzman strain were maintained at about 23°C with a 12-hr light/12-hr dark cycle. The length of gestation for non-lactating rats was 21 to 22 days. Males were introduced into the cages of pregnant females before parturition, and on the morning after parturition the animals were checked for post-partum mating by means of a vaginal smear. The day on which spermatozoa were found in the vaginal smear was designated Day 0 of pregnancy. At this time, the male was removed from the cage and young were removed to reduce the litter size to two, a number that usually permits implantation and maintains milk secretion (King, 1913; Weichert,

1940). The extra offspring were fostered to other nursing females kept for this purpose.

Lactation was accelerated by increasing the number of nursing young from two to the desired number. In experiments in which lactation was accelerated for only 1 or 2 days, the number of young was again reduced to two on the appropriate day. Offspring were weighed every 1 to 2 days and deaths were recorded. Occasionally, a rat would not nurse normally after accelerated lactation (the young lost weight and died) and such animals were removed from the experiment.

Examination of implantation sites

Implantation sites were examined at laparotomy performed at the start of the accelerated lactation. Laparotomies were performed through a mid-ventral incision under ether anaesthesia in clean, but not sterile, conditions. The number of implantation sites in each horn was counted and the size of one or two representative implantations was measured along one axis with a micrometer. Any animals without implantation sites or with sites that appeared abnormal or of inappropriate size at the initial laparotomy were discarded.

At autopsy the number of implantation and/or resorption sites was recorded and the size of implantation sites again measured to establish that they were of normal size for that day of pregnancy. If all implantation sites were obviously resorbed, they were not cut open. When implantation sites were present, at least two in each uterus were opened and examined for the presence of embryos, and for the presence of beating hearts after Day 10. The site was scored as resorbing if it contained only placental material and no embryo.

Some animals were maintained until Day 22, the expected time of parturition. If parturition had not occurred by Day 24, the animal was killed and the uterus examined for the presence and condition of the fetuses.

RESULTS

As shown in Table 1, increasing the number of nursing young from two to nine on Day 9 of pregnancy resulted in embryo resorption in all seven animals tested. When the number of nursing young was increased to eight, pregnancy was terminated in 3/6 animals. Pregnancy was terminated in 5/8 animals that nursed seven young. Although the results of the last two experiments are significant ($P < 0.05$ and $P < 0.01$, respectively), it is apparent that a litter size of seven or eight does not consistently result in termination of pregnancy. There were no apparent detrimental effects of nursing seven or eight young on those animals that were able to maintain pregnancy. The percentage of implantation sites which they maintained until autopsy was similar to that of controls and the sites were of normal size.

The number of implantation sites present on Day 9 of pregnancy did not influence the maintenance or termination of pregnancy. Animals with as few as two implantation sites failed to continue the pregnancy and animals with as many as sixteen implantation sites maintained pregnancy after accelerated lactation.

The effect of accelerating lactation on Days 8 or 10 is shown in Table 2. None of the eight animals whose lactation was accelerated on Day 8 maintained pregnancy. Pregnancy was subsequently terminated in only 4/8 animals that underwent laparotomy and accelerated lactation on Day 10 of pregnancy. Eight additional animals were subjected to accelerated lactation on Day 11, 12 or 13 of pregnancy, but pregnancy was terminated in only one.

Table 1. Effect of the number of nursing young on the termination of pregnancy in rats

Group	No. of nursing young after laparotomy	No. of rats	No. of I.S. at laparotomy	Condition at autopsy				Significance (χ^2 analysis)
				Day of autopsy	No. of rats pregnant	No. of I.S.	Maintenance (%)*	
I	7	8	93	13	3	31	88.6	I:IV, $P < 0.01$
II	8	5	53	13	3	28	85.3	II:IV, $P < 0.005$
	8	1	14	24	0	0	—	
III	9	7	60	13	0	0	—	III:IV, $P < 0.001$
IV	2	13	90†	13 to 17	12	86†	95.6†	

I.S., implantation sites.

* Average % of implantation sites maintained in animals still pregnant at autopsy.

† The values given are those from 9/13 animals.

Table 2. Effect of accelerating lactation (to 10 young) on different days of pregnancy in rats

Group	Day of accelerated lactation	No. of rats	No. of I.S. at laparotomy	Condition at autopsy				Significance (χ^2 analysis)
				Day of autopsy	No. of rats pregnant	No. of I.S.	Maintenance (%)*	
I	8	8	73	13	0	0	—	I: IV, $P > 0.995$
II	10	2	25	14	2	21	85.0	II: IV, $P < 0.02$
	10	4	59	15	2	29	90.4	
	10	2	15	24	0	0	—	
III†	11	5	57	15	4	39	78.0	
	12	2	27	‡	2	(27)	100.0	
	13	1	12	‡	1	(11)	91.7	
IV	9	10	—	13 to 24	0	0	—	

I.S., implantation sites.

* Average % of implantation sites maintained in animals still pregnant at autopsy.

† Two animals in this group underwent laparotomy on Day 11 at the time of accelerated lactation. Laparotomy was performed on Day 9 of pregnancy for the remaining six animals.

‡ Allowed to give birth, producing 27 and 11 live young on Day 22.

The results of accelerating lactation for only 1 or 2 days are shown in Table 3. Six of seven animals that nursed ten young for only 1 day had normal fetuses on Day 13 of pregnancy; parturition was normal in another animal. Two of ten animals that nursed ten young for 2 days had normal fetuses on Day 13 of pregnancy. Of two additional animals allowed to go to the time of parturition, one animal gave birth normally on Day 22 of pregnancy; another, killed on

Day 24, contained no evidence of fetuses. In rats in which 1 or 2 days of accelerated lactation did not cause termination of pregnancy, there was no decrease in the average number of implantation sites maintained compared with those in animals nursing only two young.

Table 3. The length of accelerated lactation (to 10 young) necessary to cause termination of pregnancy in rats

Group	Length of accelerated lactation (days)	No. of rats	No. of I.S. at laparotomy	Condition at autopsy				Significance (χ^2 analysis)
				Day of autopsy	No. of rats pregnant	No. of I.S.	Maintenance (%) [*]	
I	1	7	81	13	6	60	87.0	I: III, $P > 0.70$
	1	1	16	†	1	(14)	87.5	
II	2	10	146	13	2	26	92.8	II: III, $P < 0.001$
	2	2	26	†	1	(10)	90.9	
III	0 (nursing 2 until autopsy)	13	90‡	13 to 17	12	86‡	95.6	

I.S., implantation sites.

* Average % of implantation sites maintained in animals still pregnant at autopsy.

† Allowed to give birth, producing twelve live+ two dead, and ten live young on Day 22.

‡ The values given are those from 9/13 animals.

DISCUSSION

The experiments described in this paper lead to a number of conclusions regarding the termination of pregnancy that occurs after accelerated lactation in the rat. Although the size of the nursing litter must be nine or ten in order for pregnancy to be consistently terminated, pregnancy is nonetheless terminated by fewer young (i.e. seven or eight) with greater frequency than occurs in control animals nursing two young throughout the experiment. A possible explanation for this finding is that a certain threshold of neural stimulation is required to cause the hormonal changes responsible for pregnancy termination. The size of the nursing litter needed to produce such changes could vary because of differences in the threshold necessary to cause pregnancy termination in individual rats, and to variability among litters in the number of young nursing concurrently and the strength of their suckling. It is considered unlikely that the threshold is related to the amount of milk removed by the young for two reasons. First, the weight of the litter did not influence the occurrence of pregnancy termination as long as the individual young were vigorous at the time they were introduced into the cage. Secondly, litters consistently lost weight (about 5% of the initial litter weight) during the first few days following accelerated lactation, indicating that milk production had not met the increased demand until after pregnancy termination had already begun.

The experiments performed to test the time at which accelerated lactation is able to cause pregnancy termination indicated a time about Day 10 of pregnancy, beyond which pregnancy was no longer adversely affected by any changes brought about by accelerated lactation. The fact that some animals

were still sensitive to accelerated lactation starting on Day 10 of pregnancy could be due to individual differences in the timing of hormonal changes normally occurring in pregnancy. That accelerated lactation is not detrimental to pregnancy from Day 10 onward may be related to the fact that the production of placental luteotrophin begins at about this time, and the presence of the pituitary is no longer required to sustain pregnancy (Sin *et al.*, 1971).

Although accelerated lactation begun on Day 10 of pregnancy did not consistently terminate pregnancy, animals that were subjected to accelerated lactation on Day 9 of pregnancy had to continue to nurse the large litter until Day 11 for termination of pregnancy to occur. The length of accelerated lactation needed to cause pregnancy termination may be shorter than 2 days, since many animals did not begin nursing the large litter immediately after their introduction to the cage. Most had begun nursing them, however, within 8 hr of introduction. Further experiments (Veomett & Daniel, 1975a, b) suggest that hormonal changes associated with the termination of pregnancy have begun within 1 day of accelerated lactation, but that these changes do not become irreversible until later.

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