Effect of Interrupted Nursing on Lactation in Rats[1] (36385)

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Milk secretion rates in cows decrease curvilinearly as the interval between milking increases, especially after 12–16 hr (1, 2). Our studies (3, 4) indicate that omission of a few milkings on the days immediately following parturition may adversely affect milk secretion to a greater extent than similar treatments imposed at other periods in lactation. For example, the omission of the first 4 milkings immediately postpartum reduced subsequent milk yields 9.9%, whereas the comparable reduction following the omission of 4 milkings in midlactation was 1.0%. These findings assume added importance when it is recognized that the efficiency of milk removal during the first few days postpartum is frequently erratic especially in cattle lactating for the first time.

Although imposition of a single between-nursing interval of up to 16 hr does not alter markedly milk secretion rates in rats (5), chronic application of long intervals markedly reduces litter weight gains (6), mammary cell numbers [deoxyribonucleic acid (DNA)] and metabolic activity as measured by ribonucleic acid (RNA) content (7).

It was not apparent from the work in cattle whether the reduction in subsequent milk yields was associated with reduced mammary cell numbers or reduced metabolic activity. And in rats it was not known if the deleterious effects of long between-nursing intervals on lactational performance could be reversed by resumption of *ad libitum* nursing. The purpose of the present experiments was to study the effects of interrupted nursing on litter weight gains and mammary nucleic acid content of rats immediately following parturition and at midlactation.

Materials and Methods. A total of 129 primiparous Spartan-strain rats (Spartan Research Animals, Haslett, Michigan) were used. They were housed in individual cages under conditions of controlled temperature (25°) and light (for 14 hr daily), and they had *ad libitum* access to water and a normal laboratory diet (Wayne Lab-Blox, Allied Mills Inc., Chicago, IL). The teats of the thoracic mammary glands were ligated approximately 7 days prepartum.

Experiment I. Litters were separated immediately from dams at parturition by allowing them to fall through large mesh wire bottom cages. Litters were replaced 3, 6, 9, 12 or 24 hr later and permitted to nurse *ad libitum* throughout lactation. Litters of control rats were not separated from the dams.

Experiment II. Dams were permitted to nurse their litters *ad libitum* until 9:00 a.m. on day 10 of lactation when they were separated for 0 (control), 6, 12 or 24 hr. Litters and dams were reunited after these intervals and allowed *ad libitum* nursing.

Experiment III. Ad libitum nursing of the six functional abdominal-inguinal mammary glands was allowed until 9:00 a.m. of day 10 of lactation. At this time three abdominal-inguinal teats (either left or right sides) were covered with adhesive tape for 12 hr to prevent suckling of these glands. The litter size was reduced to three pups during this interval, and nursing of the nontaped glands was granted. After 12 hr, the adhesive tape was removed and litter size was restored to 6 pups.

Pups in all experiments were placed with nurse dams during periods of interrupted suckling. Litters were adjusted to six pups immediately following parturition in Expt. I

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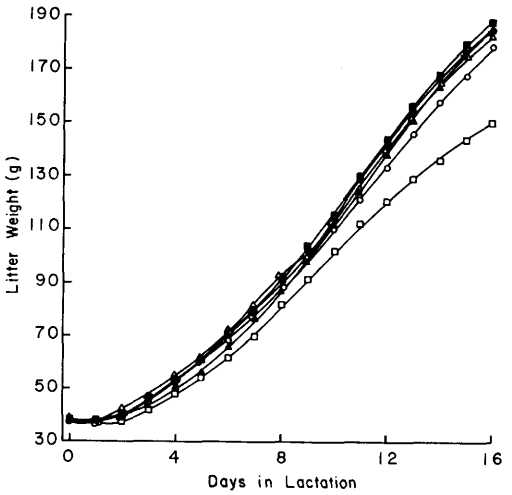

FIG. 1. Litter weights of rats permitted to nurse their litters *ad libitum* after separation from dams for 0 (●), 3 (○), 6 (▲), 9 (△), 12 (■) or 24 (□) hr immediately following parturition.

and on day 3 postpartum in Expts. II and III. Pups dying after litter size adjustment, were replaced by pups of comparable weight and age.

Litter weights were recorded daily at approximately 10:00 a.m. Dams were killed by decapitation on completion of 16 days lactation and the carcass, anterior pituitary, adrenal and 6 abdominal-inguinal glands were weighed. The right and left abdominal-inguinal mammary glands of animals in Expt. III were removed separately. Mammary glands were stored at —20° until analyzed for DNA and RNA as previously described (8).

Within experiments, rats were assigned to treatments on the basis of parturition date and analyses of individual treatment values were calculated only if analysis of variance on all treatments indicated a significant difference.

Results. Litter weights. Mean litter weights of rats separated for 24 hr immediately postpartum were significantly lower ($p < .05$) throughout lactation than values for each of the other treatments in Expt. I (Fig. 1); litter weight of rats on the other treatments did not differ significantly ($p > .05$). In Expt. II, litter weights of rats on all treatments were similar until day 10 of lactation when pups were removed for 0 to 24 hr (Fig. 2). After day 10 of lactation, values for control rats and rats separated for 6 hr did not differ significantly ($p > .05$), whereas litter weights of rats separated for 12 or 24 hr were reduced so that by day 16 of lactation the litter weights were 144 and 129 g, respectively. These values were significantly different ($p < .05$) from one another and from the mean weight (160 g) of control litters.

Anterior pituitary, adrenal and mammary gland weight. Average values for weights of anterior pituitary (mg), adrenal (mg) and mammary glands (g) were, respectively, 11.0, 77.2 and 17.2 in Expt. I; 10.1, 78.2 and 15.9 in Expt. II; and 10.5, 77.7 and 15.1 in Expt. III. Values for individual treatments, within experiments, did not differ significantly ($p > .05$).

Body weight, mammary RNA and DNA content of lactating rats. Individual treatments did not significantly affect ($p > .05$) body weight or DNA content of the 6 abdominal-inguinal mammary glands on day 16 of lactation in Expt. I (Table I) or Expt. II (Table II).

Interrupted suckling for 24 hr immediately postpartum (Table I) or in midlactation (Table II) significantly reduced ($p < .05$) RNA content of the 6 abdominal-inguinal mammary glands at day 16 of lactation but withdrawal and replacement of pups for 12 hr or less had no significant effect ($p > .05$).

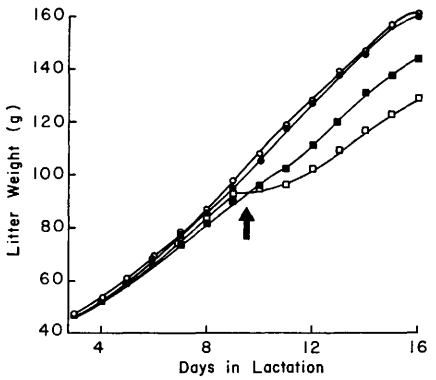

FIG. 2. Litter weights of rats permitted to nurse their litters *ad libitum* except for a 0 (●), 6 (○), 12 (■) or 24 (□) hr separation on day 10 of lactation (↑).

TABLE I. Body Weight and Nucleic Acid Content of 6 Abdominal-inguinal Mammary Glands of Lactating Rats Permitted to Nurse Their Litters *ad libitum* After an Initial Separation for 0 (control), 3, 6, 9, 12 or 24 hr at Parturition.[a]

Non-nursing interval (hr)	No. of rats	Body wt[b] (g)	RNA (mg)	DNA[b] (mg)
0	10	301 ± 12	193 ± 12	41 ± 2
3	12	290 ± 4	206 ± 13	38 ± 2
6	9	295 ± 5	213 ± 14	36 ± 2
9	10	298 ± 5	196 ± 8	39 ± 2
12	9	298 ± 7	213 ± 7	44 ± 2
24	10	308 ± 8	161 ± 5[c]	43 ± 2

[a] Mean ± standard error of mean.
[b] Means not significantly different ($p > .05$).
[c] Significantly less ($p < .05$) than other values in column.

In Expt. III, the average RNA and DNA content (mg) of the three abdominal-inguinal glands nursed *ad libitum* were, respectively, 71 ± 5 and 20 ± 1 while comparable values for glands in which suckling was interrupted for 12 hr were 62 ± 5 and 20 ± 1, respectively. The reduction in RNA content was not significant ($p \cong .10$).

Discussion. Interrupted suckling for 12 hr or less, either in early or midlactation, had no significant effects on milk secretion in the lactating rat. This agrees with our observation in cattle that the deleterious effects of milk accumulation must exceed 12 hr (3, 4).

However, the data suggest that interruption of nursing for periods greater than 12 hr at either parturition or midlactation depresses subsequent litter weight gains and mammary RNA content at day 16 of lactation. Whether the reduced metabolic activity in the mammary gland alters the milk secretion rate and/or milk composition to reduce the litter weight is not known. These inhibitory effects as a result of interrupted nursing at parturition and at midlactation are in contrast to our results in cows (3, 4) where failure to remove milk adversely affected subsequent milk synthesis only at parturition. The differences in response between the two species at the two stages of lactation may be associated with the fact that milk secretion rates in rats are relatively lower during early lactation.

The data show that milkings omitted for 12 to 24 hr reduce mammary gland metabolic activity as measured by RNA content, but have no significant influence on mammary cell numbers (DNA). These results are in agreement with our report that RNA begins to decline in advance of cell losses during postlactational involution (9). And it would appear that resumption of *ad libitum* nursing for up to 15 days does not restore this loss in RNA.

Our finding that the mammary RNA content was greater ($p \cong .10$) in the three nursed abdominal-inguinal glands in comparison with the contralateral nonnursed glands suggests that the effects of interrupted nursing probably occur locally in the mammary gland. This would be in agreement with the findings of Smith and Convey (10) that milk accumulation may directly reduce the ability of mammary tissue to synthesize protein. We speculate that a nonnursing interval greater than 12 hr would be needed to show a significant reduction in RNA between the contralateral glands because as listed in Table II the values at 12 hr are intermediate between the ineffective 0–6 hr separations and the significant reduction observed at 24 hr.

Summary. Interruption of suckling in rats for a single period of up to 12 hr in the immediate postpartum period or on day 10 of

TABLE II. Body Weight and Nucleic Acid Content of 6 Abdominal-inguinal Mammary Glands of Lactating Rats Permitted to Nurse Their Litters *ad libitum* Except for a 0, 6, 12 or 24 hr Separation on Day 10 of Lactation.[a]

Non-nursing interval (hr)	No. of rats	Body wt[b] (g)	RNA (mg)	DNA[b] (mg)
0	13	285 ± 9	184 ± 6	45 ± 2
6	14	283 ± 6	187 ± 10	39 ± 1
12	16	266 ± 7	162 ± 9	40 ± 2
24	14	272 ± 6	134 ± 11[c]	38 ± 3

[a] Mean ± standard error of mean.
[b] Means not significantly different ($p > .05$).
[c] Significantly less ($p < .05$) than values for rats subjected to nonnursing intervals of 0 and 6 hr.

lactation had no significant effect on subsequent litter weights or mammary nucleic acid content. Interruption for 24 hr in both early and midlactation reduced litter weights and mammary gland RNA content but had no significant effect on weight of anterior pituitary, adrenal, or mammary gland DNA content. The results suggest that the effect of interrupted nursing is to reduce mammary gland metabolic activity without altering cell number and furthermore, that the effect probably occurs locally within the mammary gland.

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