tion of results at 48 hours was complicated by the joint presence of persistently injured centrilobular liver cells and peripherally located liver cells which contained an increased number of ribosomes.

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Strength of Suckling Stimulus and Maintenance of the Mammary Gland.* (30220)

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Selye(1) first demonstrated the importance of the nursing stimulus in the maintenance of mammary parenchyma during lactation. He showed that involution which normally occurs upon removal of young from the mother could be delayed despite the fact that milk removal from the gland was prevented by ligation of galactophores. More recently, Tucker and Reece(2) have determined deoxyribonucleic acid (DNA) content of teat-ligated and non-ligated rat mammary glands and found that DNA content of ligated glands was significantly less than that of corresponding glands of intact lactating rats at comparable stages of lactation. However, they neglected to indicate whether or not the degree of involution of teat-ligated glands was comparable to that which normally occurs upon removal of the litter. Mizuno(3) found that DNA content of ligated glands of mice was comparable to corresponding glands of intact lactating control mice while the DNA content of the non-ligated glands was significantly greater than that of corresponding control glands.

Since there appears to be a discrepancy between the results obtained in rats and mice and since the number of young per litter employed in previous experiments was either not reported or was the same for all groups,

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TABLE I. Effect of Litter Size Upon Mammary Gland DNA of Lactating Rats with Unilateral Galactophore Ligation.

Galactophores ligated	No. of young/litter	No. of lactating rats	Body wt (g)	DFFT* (mg)		DNA/mg DFFT (μg)		Total DNA (mg/100 g)	
				Right	Left	Right	Left	Right	Left
Non-ligated	0	12	279.1	181.5	185.1	20.10 ± .64†	19.82 ± .63	1.30 ± .05[1]	1.31 ± .04
Idem	6	7	271.0	625.7	645.1	16.12 ± .50	16.44 ± .36	3.71 ± .16[2]	3.90 ± .12[7]
Right, ligated Left, non-ligated	0	12	268.5	167.0	182.4	20.40 ± .55	19.83 ± .77	1.26 ± .04[3]	1.34 ± .06
Idem	3	12	284.6	375.6	808.2	18.47 ± .49	16.52 ± .79	2.41 ± .15[4]	4.60 ± .24[8]
Idem	6	12	281.8	373.8	977.8	19.60 ± .59	14.94 ± .62	2.60 ± .10	5.13 ± .25
Idem	9	12	278.7	393.7	962.0	18.22 ± 1.04	16.98 ± 1.17	2.67 ± .14[5]	5.64 ± .36
Idem	12	12	291.3	516.1	1054.0	18.32 ± 1.21	15.71 ± .68	3.27 ± .18[6]	5.57 ± .25[9]

* Dry, fat-free tissue.
† Mean ± S.E.

Student's t test, level of significance

1-2	.001	2-5	.001
1-3	not significant	2-6, 7	not significant
		3-4	.001
		7-8	.05
		7-9	.001
		8-9	.025

it seemed of interest to determine whether these differences might be due to a variance in the nursing stimulus.

Methods. Holtzman rats undergoing their first lactation were kept in a room artificially illuminated 10 hours each day and maintained at a temperature of 76-80°F. Food and water were available at all times.

On day 3 postpartum, galactophores of the 6 mammary glands on the right side were ligated. The 6 glands on the left side were left intact. At this time, litters were adjusted to contain either 3, 6, 9 or 12 young. Controls consisted of intact lactating rats nursing 6 young; intact lactating rats in which the litter was removed on day 3 postpartum; and lactating rats in which both the litter was removed and the galactophores of the right glands ligated on day 3 postpartum.

Observations of the teats of lactating rats which appeared to have been suckled were made daily from days 4-11 postpartum. Teats which were elongated or teats in which the hair at the base appeared wet or matted were designated as contacted. Teats were numbered in an anterior-posterior direction with the odd-number teats on the right side and the even-numbered teats on the left. The observations were expressed as per cent number of days teats were contacted. Per cent number of days teats were contacted was determined by dividing the number of days each teat was contacted within each group of rats by the number of possible contact days for each teat times 100. Observations relating to the period of time young remained in contact with each teat or the number of times each teat was contacted per day were not made.

During the experiment, young which appeared to be in a weakened condition were replaced by pups which were comparable in size and weight to healthier members of the liter.

On day 14 postpartum, the left and right abdominal-inguinal glands were removed for determination of DNA and the posterior pair of pectoral glands was used for histological study. Details of the procedure for extraction and determination of DNA have been reported previously(4).

Results. Total DNA of the left and right abdominal-inguinal glands of rats without galactophore ligation and nursing 6 young was comparable (Table I). The right and left glands of lactating rats whose litters were removed at day 3 postpartum also exhibited comparable mammary DNA values irrespective of galactophore ligation. In galactophore ligated animals nursing 3-12 pups, the DNA content of ligated glands was significantly less than that of contralateral non-ligated glands.

DNA content of ligated glands of rats

TABLE II. Percent Number of Days in Which Ligated and Non-Ligated Mammary Glands Were Contacted by Suckling Young.

No. of lactating rats	No. of young/ litter	% of days teats contacted							
		Ligated gland No.				Non-ligated gland No.			
		5	7	9	11	6	8	10	12
12	3	4	8	10	16	98	94	92	61
12	6	23	32	48	34	100	100	96	94
12	9	17	38	60	27	98	100	92	91
12	12	45	52	60	53	100	100	99	92

nursing either 3, 6 or 9 young was 28-35% less than that of corresponding glands of control rats nursing 6 young but without the right galactophores ligated, whereas total DNA of ligated glands of rats nursing 12 young was comparable to that of glands of controls. Furthermore, DNA content of the left, non-ligated glands of these animals was 18-43% greater than that of corresponding glands of non-ligated control animals. Thus, the decrease in DNA content of the ligated glands was compensated for by a corresponding increase in DNA of the contralateral non-ligated glands so that the combined DNA value for both the ligated and non-ligated glands was either comparable to or greater than that of the right and left glands of control animals. In all cases, total DNA of either the ligated or non-ligated glands of nursing animals was greater than that of corresponding glands of rats whose litters were removed at day 3 postpartum.

Histologically, the glands of animals whose litters were removed on day 3 postpartum exhibited varying degrees of involution. For the most part, the parenchyma of these glands consisted of essentially ducts with isolated areas of alveolar tissue (Fig. 1). Ligated glands of animals nursing 3-9 young revealed the characteristic structure of a lactating gland in the early phases of involution. In these glands leucocytes were present within the lumina of the alveoli and many areas exhibited disrupted and collapsed alveoli. In rats nursing 12 young, ligated glands (Fig. 2) were similar to those of rats without galactophore ligation and nursing 6 young (Fig. 3) except for the presence of leucocytes within the alveoli of the ligated glands. However, the non-ligated glands (Fig. 4) of animals nursing 3-12 young appeared more highly proliferated than those of control animals and areas of considerable hyperplasia were common.

The per cent number of days teats were contacted is shown in Table II. It is apparent that the ligated glands (#5, 7, 9, 11) of rats nursing only 3 young were contacted less frequently than those of animals nursing either 6, 9, or 12 young, whereas contact with the non-ligated glands (#6, 8, 10, 12) appeared comparable, irrespective of litter size. A notable exception, however, was gland #12 of animals nursing 3 young. Gland #12 was apparently contacted less frequently than the other non-ligated glands of rats within this group.

Discussion. The DNA content of galactophore ligated mammary glands in rats nursing 3-9 young was 28-35% less than corresponding glands of animals nursing 6 young but without galactophore ligation (controls). On the other hand, the ligated glands exhibited sinificantly greater quantities of DNA than glands of rats in which the litter was removed at day 3 postpartum. Furthermore, the ligated glands of rats nursing 12 young were comparable to that of controls. The DNA content as well as the histological structure of the ligated glands indicates that the mammary parenchyma may be at least partially maintained as long as the suckling stimulus is continued even though milk is not removed from the gland. The data of the present study are in agreement with those of Selye(1) for the rat and of Mizuno(3) for the mouse. Tucker and Reece(2) reported that the suckling stimulus without milk removal did not prevent loss of mammary cells in rat. Although the ligated glands of animals nursing 3-9 young of the pesent study exhibit cellular loss (decreased DNA), the

degree of involution is less pronounced than that of glands of rats in which litters are removed on day 3 postpartum. If, however, the suckling stumulus is sufficiently intense, as is the case for rats nursing 12 young, mammary DNA and histological structure is maintained at a level comparable to that of rats nursing 6 young but without the right galactophores ligated.

Undoubtedly, infiltration of leucocytes into the ligated glands contributes to the total DNA content of these glands. However, the histological structure of the ligated glands indicates that the contribution of leucocytes to total DNA is not critical and that the greater quantity of DNA of ligated glands when compared to non-suckled involuted glands is due in fact to at least partial main-

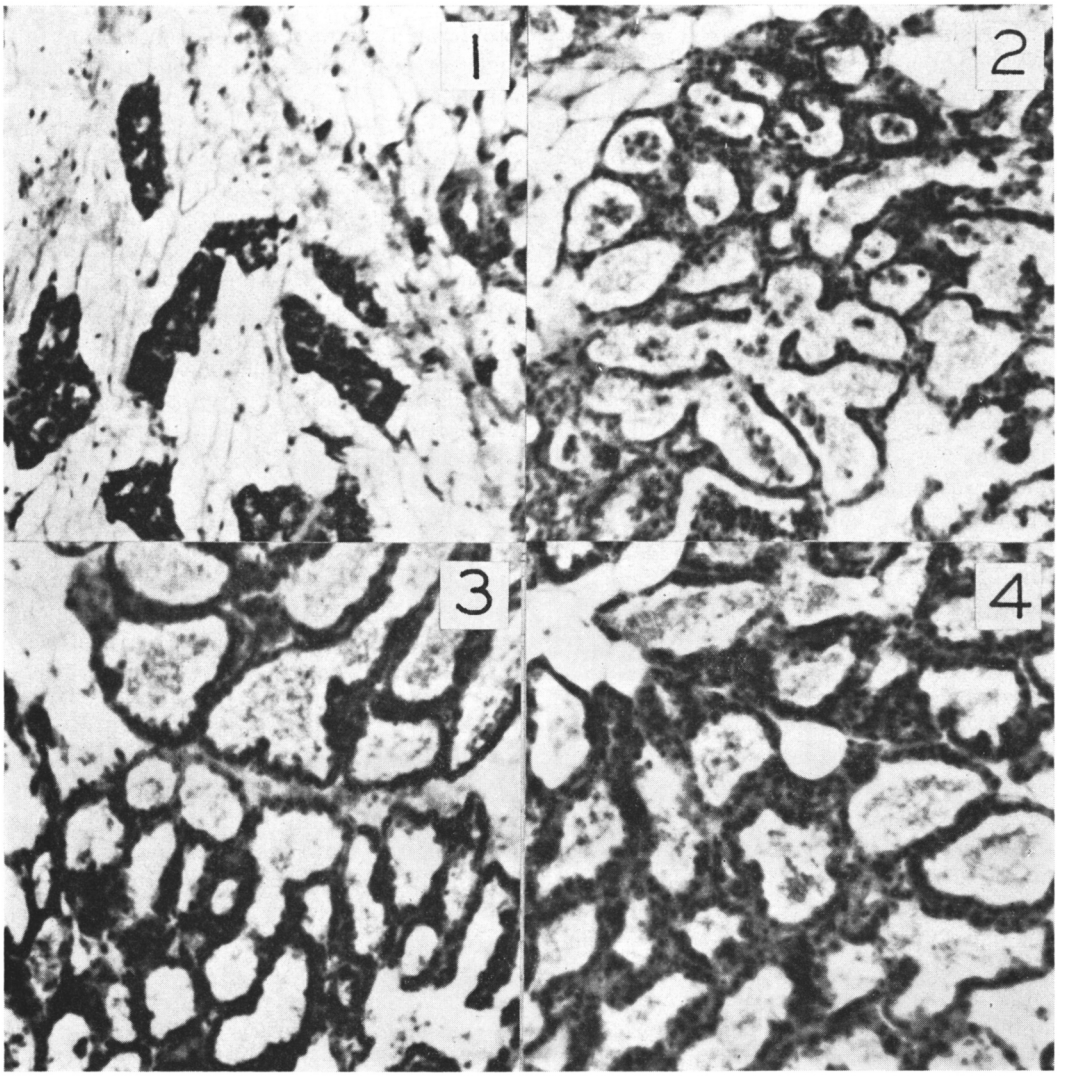

FIG. 1. Galactophore ligated mammary gland of rat whose litter was removed on day 3 postpartum; sacrificed day 14 postpartum. 172 ×.

FIG. 2. Galactophore ligated mammary gland of rat nursing 12 young; sacrificed day 14 postpartum. 172 ×.

FIG. 3. Mammary gland of rat without galactophore ligation and nursing 6 young; sacrificed day 14 postpartum. 172 ×.

FIG. 4. Non-ligated mammary gland of rat with galactophore ligation and nursing 12 young; sacrificed day 14 postpartum. 172 ×.

tenance of the mammary parenchyma.

It is interesting to note that the DNA content of non-ligated glands is significantly greater than either the contralateral ligated glands or the corresponding glands of rats without galactophore ligation irrespective of the number of suckling young. Furthermore, the non-ligated glands of rats nursing 9 or 12 young contain significantly more DNA than those of rats nursing only 3 young. These observations appear to suggest that either the more frequent removal of milk or a stronger or more sustained suckling stimulus results in a compensatory cellular proliferation of the non-ligated glands. Although the observations on per cent number of days that teats are contacted by the suckling young are rather limited, it is apparent that the ligated glands (#7, 9, 11) of rats nursing 3 young are contacted less frequently than those of rats nursing 12 young. If this is the case, then it appears that mammary cellular proliferation during lactation is more closely related to the strength of the suckling stimulus than to the frequency of milk removal for DNA content of both the ligated and non-ligated glands of animals nursing 12 young is significantly greater than that of rats nursing 3 young.

It is generally accepted that suckling results in the reflex release of pituitary prolactin(5-7). Furthermore, evidence has been presented indicating that suckling also results in the discharge of somatotropin from the adenohypophysis(8). Since either prolactin (3) or STH(9) stimulate mammary cellular proliferation in the lactating animal, a more frequent or a more prolonged release of these adenohypophyseal hormones could account for at least partial maintenance of the mammary parenchyma in rats subjected to the stronger suckling stimulus.

Summary. Galactophores of the right 6 mammary glands of rats were ligated on day 3 postpartum and litters were adjusted to contain either 3, 6, 9 or 12 pups. Mammary glands on left side were not ligated. Rats nursing 6 young but without galactophore ligation served as controls. Animals were sacrificed on day 14 postpartum and DNA content of both left and right abdominal-inguinal glands was determined. The posterior pair of pectoral glands was used for histological study. In galactophore-ligated rats, ligated glands were significantly smaller than contralateral non-ligated glands. DNA content of ligated glands of rats nursing 12 young was similar to that of corresponding control glands whereas DNA content of glands of rats nursing 3-9 young was less than control glands. In animals with galactophore ligation, non-ligated glands were significantly larger than corresponding glands of rats without galactophore ligation. It appears that the strength of the suckling stimulus is directly related to maintenance of the mammary parenchyma during lactation.

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