Nucleic Acid Levels During Functional Development of Mouse Mammary Gland[1] (36489)

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It is well established that DNA and RNA synthesis occur during secretory development of the mammary gland (1, 2). DNA changes provide a useful index of tissue cellularity, and the ratio RNA/DNA, an index of the functional status of the cells. Although there is general agreement that nucleic acid synthesis plays an important role in mammary differentiation, the patterns and magnitudes of change reported for nucleic acids are not in complete accord (3–11). These discrepancies could be due to species differences, differences in procedures to estimate DNA and RNA, differences in expressing results (*i.e.*, total content, concentration, labeling indices), or other factors as discussed below.

Because the milk contained in the lactating mammary gland contributes to gland weight, the milk correction factor (12) has often been used in the calculation of nucleic acid content to correct for "retained milk". However, this correction factor, which is based on lactose content, assumes a correlation between lactose and milk protein and between lactose and visible milk that does not seem justifiable (10).

The number of suckling young can also influence nucleic acid metabolism in the mammary gland (13, 14). But in many experiments with laboratory rodents the litter number is reduced to a constant number, which is less than the number of mammary glands (3, 5, 13). Certain glands may therefore be suckled more frequently than others, resulting in variable states of metabolic activity among the different glands.

To circumvent some of these problems, the lactating mice used in this study were provided with a constant stimulus of 10 suckling young to ensure suckling of all glands. For tissue weight estimations, the technique described by Kopelovich *et al.* (15) was used. With these modifications in procedure, the changes in DNA and RNA concentration and content of mouse mammary glands were investigated from midpregnancy through lactation to determine the patterns of tissue cellularity and function during the secretory cycle. The results of this study emphasize the importance of tissue preparation in the assessment of changing levels of nucleic acids, a finding of significance not only for mammary gland studies but for other secretory tissues as well.

Materials and Methods. Animals were 2- to 3-month-old Swiss albino mice (Spartan Research, Haslett, MI) in their first pregnancies. Date of pregnancy was determined by selecting females at estrus, leaving them individually overnight with one male and if conception occurred, the following day was considered day 1 of pregnancy. To provide a constant maximum suckling stimulus for the lactating mice, each female was allowed to suckle 10 young, this species having 5 pairs of mammary glands. In cases where females gave birth to less than 10 young, they were further provided with young of the same age from other females.

Total glands were removed randomly from either the left or right sides of each animal, since no significant differences in the results were found between right and left glands. For tissue weight determinations, the mammary glands were dissected free of lymph

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TABLE I. Nucleic Acid Changes During Functional Development of Mouse Mammary Gland.

Stage[a]	No.of mice	Wet wt (mg)	% Dry wt (DW)	RNA (mg/g DW)	DNA (mg/g DW)	RNA/DNA	Total RNA (mg)	Total DNA (mg)
P 10	6	175 ± 13	4.0 ± 0.6	24.4 ± 0.8	21.4 ± 1.4	1.14 ± 0.09	0.17	0.15
P 15	4	305 ± 56	3.4 ± 0.8	30.6 ± 0.5	23.3 ± 0.6	1.31 ± 0.01	0.32	0.24
P 18	4	668 ± 55	6.6 ± 0.2	38.0 ± 1.4	23.1 ± 1.7	1.65 ± 0.07	1.68	1.02
L 1	9	695 ± 60	7.4 ± 0.5	73.7 ± 4.8	38.5 ± 2.1	2.05 ± 0.07	3.79	1.98
L 3	4	685 ± 72	7.8 ± 0.9	83.0 ± 6.9	34.8 ± 2.7	2.41 ± 0.07	4.43	1.86
L 5	6	656 ± 46	12.0 ± 1.0	107.0 ± 5.1	30.5 ± 2.8	3.67 ± 0.39	8.42	2.40
L 7	7	797 ± 51	14.5 ± 1.1	113.6 ± 6.6	31.2 ± 1.1	3.65 ± 0.14	13.13	3.61
L 10	5	822 ± 55	12.1 ± 0.8	114.0 ± 2.7	25.9 ± 0.6	4.41 ± 0.13	11.34	2.57
L 15	5	983 ± 76	14.6 ± 0.2	88.4 ± 3.8	23.0 ± 0.8	3.88 ± 0.15	12.69	3.30
L 21	4	903 ± 54	10.9 ± 0.7	95.1 ± 5.0	26.7 ± 1.9	3.18 ± 0.12	9.36	2.63

[a] P = pregnancy; L = lactation.

nodes, sliced into very thin sections (approx 0.5 mm), and washed several times according to the procedure of Kopelovich *et al.* (15), except that cold 0.15 *M* KCl was used as the washing medium instead of distilled water. Special care was taken with lactating glands to wash the thin sections until the washes no longer appeared milky. Preliminary studies revealed that the nucleic acid values of the "unwashed" sections of lactating glands were significantly higher than "washed" sections.

The glands were defatted and dehydrated as described before (16), and the dry weights were recorded as percentage of tissue wet weight. The procedures for DNA and RNA estimations have also been described (16, 17).

Results and Discussion. The results are summarized in Table I. The total wet weights of the 5 unilateral glands increased 280% from days 10 to 18 of pregnancy but only 40% from days 1 to 15 of lactation. This indicates increased water uptake by the mammary cells during pregnancy and is in accord with the previous finding (18) that the average wet weight of the mammary gland attains a maximum at late pregnancy and remains roughly constant throughout lactation. Dry weights rose from an average of 4% at midpregnancy to 14% at midlactation, the increase presumably due to higher intracellular protein content of the secretory cells. That this was not the result of milk retention by the tissue is indicated by the lack of a corresponding increase in tissue wet weight during lactation.

The peak concentration of RNA occurred on days 7 to 10 of lactation, which represents a 367% increase from day 10 of pregnancy. A large proportion (310%) of this increase occurred between day 18 of pregnancy and day 7 of lactation. In contrast, there was only a 56% increase in RNA from days 10 to 18 of pregnancy. Thus most of the RNA accumulates postpartum as in the rat (18) and as observed earlier (19), there is a correlation between the intensity of secretion and the amount of RNA in the mammary tissue. Total RNA of the 5 glands was highest on day 7 of lactation at the time of peak RNA concentration. Banerjee, Rogers and Banerjee (20) reported that the rate of incorporation of labeled uridine by mouse mammary glands was higher on day 5 than on day 10 of lactation. However, as suggested previously (16, 17), the rates of synthesis and accumulation of nucleic acids appear to be separate phenomena.

The highest concentration of DNA occurred on the first day of lactation although the total content of DNA was greatest on day 7 postpartum. This observation suggests that a "burst" of cellular proliferation occurs shortly after parturition as previously noted by some investigators (8, 9, 21) but not by others (3, 5, 6). Recent results (22) indicate that some of the DNA synthesized in early lactation may not actually represent pre-

mitotic DNA replication. However, the results in Table I indicate that cell proliferation continues, although at a slower rate, at least through day 7 postpartum. DNA concentration and content increased 67 and 94%, respectively, between day 18 of pregnancy and day 1 of lactation. On the other hand, the concentration of DNA *decreased* by 19% whereas the total content of DNA *increased* 82% from day 1 to day 7 of lactation. These latter results are not incompatible in view of the dry weight increases during lactation which lowers, in effect, the concentration of DNA. The ratio RNA/DNA, near unity at midpregnancy, reached a peak value of 4.41 at midlactation and supports the observation of augmented protein-synthesizing capacity of mammary gland ribosomes during the secretory phase (19).

The decline in DNA and RNA contents at 21 days postpartum parallels the decline in function of the mammary gland at this time. Thus, the decline in secretory activity may be attributed to decreased cell numbers as well as to decreased functional activity of the remaining cells.

Summary. The content and concentration of nucleic acids in the mouse mammary gland were examined at various stages of secretory development. The total content of DNA and RNA attained maximal values on day 7 of lactation. The highest concentration of RNA occurred on days 7 to 10, whereas that of DNA occurred on day 1 of lactation. The decline in secretory activity of the mammary gland at the end of lactation apparently results from a decrease both in the number of cells and in their functional capacity.

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