Nucleic Acid Content of Mammary Glands of Lactating Rats Simultaneously Pregnant.* (29065)

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A post-partum estrus occurs in mice and rats and, if the animals are mated, pregnancy may exist concurrently with lactation. Mammary glands of rats lactating and pregnant showed very little mitotic activity whereas hyperplasia was extensive in glands of pregnant rats(1). Involution, based on histology, respiratory activity, and nucleic acid content, of mammary glands of lactating mice, following unilateral ligation of inguinal galactophores with continued suckling of contralateral glands, was markedly retarded by concurrent pregnancy(2,3).

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content was significantly greater in glands of lactating, pregnant mice than that in glands of lactating, non-pregnant mice; however, RNA/DNA ratio, litter gain, and rate of litter growth were not significantly different(4). Thus, stimulus for mammary growth can coexist with stimulus for lactation(4). Rats, however, that were mated after periods of 72 to 105 days of continuous lactation had pregnancies of normal length, but concurrent lactation always failed despite continued suckling(5). The purpose of this investigation was to determine the effect of concurrent lactation and pregnancy on rat mammary gland development (DNA content) and protein synthetic activity (RNA content and RNA/DNA ratio).

Methods. Rats in advanced gestation were cohabitated with a male rat. On day 3 of lactation litter size was adjusted to 8, and the male rat removed. At autopsy the 6 abdominal-inguinal mammary glands were removed from rats lactating either 18, 21, 24, or 28 days while simultaneously pregnant. No attempt was made to control time of implantation and stage of pregnancy was estimated by comparing fetuses with those of pregnant rats in which matings had been timed.

In a second experiment, implantation time was controlled by subcutaneously injecting lactating rats which had mated on the post-partum estrus with 0.2 μg estradiol-17$_B$ on the fifth day of lactation. All animals were sacrificed on day 21 of lactation and only rats simultaneously pregnant were used; fetal size was similar to that of rats pregnant, but not lactating, 18 to 20 days. At sacrifice both groups were separated into subgroups with 6 or fewer fetuses (6—) or 7 or more fetuses (7+). Control animals, except for the fact that they were not cohabitated with male rats, were treated similarly to experimental animals. Nucleic acid content of 6 abdominal-inguinal mammary glands of all groups was determined as previously reported(6). Analysis of variance was used to compare means(7).

Results and discussion. DNA content (mg/100 g body wt) of mammary glands was greater in lactating, pregnant rats than in lactating rats on day 18 but less on day 24; no differences were noted on days 21 and 28. Trends within the 24- and 28-day stages of lactation suggested that mammary development declined with advancing stages of concurrent pregnancy.

A highly significant increase in total RNA was observed in glands of pregnant rats lactating 18 days when compared with glands of non-pregnant rats lactating 18 days(8). No significant difference was found between glands of pregnant rats and non-pregnant rats lactating 21 days (Table I). Within days 24 and 28 of lactation total RNA decreased markedly with each advance in stage of concurrent pregnancy.

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TABLE I. Nucleic Acid Content of Mammary Glands of Lactating Rats Simultaneously Pregnant.

Days of lact	No. animals	Avg body wt in g*	DEEET† (mg, mean)	Total DNA/100 g body wt (mg)‡	Total DNA (mg)‡	Total RNA (mg)‡	RNA/ DNA‡
18	12	251	1268.4	11.88 ± .22§	29.81 ± .74¶	84.34 ± 2.16¶	2.83 ± .07
21	12	239	1146.7	11.55 ± .30	27.61 ± .91	75.57 ± 2.30	2.74 ± .06
24	8	227	898.4	9.81 ± .32‖	22.27 ± 1.11‖	53.92 ± 5.74	2.42 ± .21
28	7	221	743.3	9.51 ± .41	21.02 ± .46	39.16 ± 7.24	1.86 ± .33

* Body wt corrected for fetal weight.
† Dried, ethanol-ether extracted tissue.
‡ Mean and S.E.
§ Significantly greater, ‖ significantly less ($P < 0.05$) and ¶ highly significantly greater ($P < 0.01$) than non-pregnant rats lactating same number of days (Tucker & Reece, 8).

TABLE II. Nucleic Acid Content of Mammary Glands of Lactating Rats Simultaneously Pregnant 19 to 20 Days and Grouped on the Basis of Number of Fetuses in Utero.

No. fetuses*	No. of rats	Body wt (g, mean)†	DEEET‡ (mg, mean)	Total DNA/100 g body wt (mg)§	Total DNA (mg)§	Total RNA (mg)§	RNA/ DNA§
6 −	7	235	1071.0	11.26 ± .39	26.45 ± 1.09‖	76.41 ± 3.86	2.89 ± .09
7+	18	238	851.6	9.78 ± .14¶	23.27 ± .50	53.27 ± 2.79¶	2.29 ± .09¶
None (control)	23	225	1060.5	10.79 ± .14	24.27 ± .33	68.42 ± 1.75	2.82 ± .05

* 6 − = 6 or fewer fetuses; 7+ = 7 or more fetuses.
† Body wt corrected for fetal wt.
‡ Dried, ethanol-ether extracted tissue.
§ Mean and S.E.
‖ Significantly greater ($P < .05$) and ¶ highly significantly less ($P < .01$) than none (control).

RNA/DNA ratios in glands of pregnant rats lactating 18 and 21 days were not significantly different from those in glands of non-pregnant rats in corresponding stages of lactation(8). Protein synthetic activity, however, of glands of rats lactating either 24 or 28 days decreased greatly as stage of concurrent pregnancy advanced.

Results in the 18-day-lactating, pregnant rat were interpreted as indicating that pregnancy stimulated mammary glands to grow and to increase proportionately in protein synthetic activity. An equilibrium between the stimulus to grow and the stimulus to lactate was reached on day 21 of lactation since concurrent pregnancy did not influence mammary growth or protein synthesis. DNA content and protein synthetic activity decreased in mammary glands after 24 and 21 days of normal lactation, respectively(8), and in lactating, pregnant rats as pregnancy advanced within days 24 and 28 of lactation. These data suggest that fetuses have priority over lactating, mammary tissue for available nutrients. This observation is somewhat biased, however, because at later stages of lactation litters normally suckle less than at earlier stages.

When implantation time was controlled by injecting estrogen, DNA content (mg/100 g body wt) of the 6— group was similar to that of the control group whereas DNA content of the 7+ group was highly significantly less than that of the control group. Similar relationships were found for total RNA and RNA/DNA ratios. Hence rats carrying 7 or more fetuses had glands which were less well-developed and less able to synthesize protein than glands of non-pregnant, lactating rats (Table II).

The results of Reece and Warbritton(1) and those of the present study concur that the stimulation of mammary growth by concurrent pregnancy was much less than that in rats pregnant but not lactating(9). Mizuno(5) found a significant increase in DNA and a proportionate increase in RNA of mouse mammary glands during concurrent pregnancy. These results agree with observations on day 18 of lactating, pregnant rats,

and in lactating, pregnant rats carrying 6 or fewer fetuses. It would appear, however, that the mammary glands of the lactating, pregnant rat do not respond to the stimulus of pregnancy, and may begin to involute as nutrient requirements increase, due either to fetal size or numbers.

Summary. Concurrent pregnancy significantly enhanced mammary DNA and RNA in rats lactating 18 days when compared with non-pregnant rats lactating 18 days, but the RNA/DNA ratio was not changed. Concurrent pregnancy in rats lactating 21, 24, and 28 days produced, respectively, no change, significant decrease, and no change in nucleic acid content when compared with lactating, non-pregnant rats. Within days 24 and 28 of lactation, nucleic acid content decreased markedly with advancing pregnancy. Standardization of gestation length showed that glands of rats with 7 or more fetuses contained highly significantly less DNA and RNA than glands of lactating, non-pregnant rats.

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Nucleic Acid Content of Suckled and Non-Suckled Mammary Glands Within Lactating Rats.* (29066)

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Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content have been used to estimate mammary gland cell numbers and protein synthetic activity, respectively(1,2, 3). Kuramitsu and Loeb(4) and Hesselberg and Loeb(5) observed in the guinea pig that ligation of one teat, with continued suckling of the contralateral, intact teat, resulted in rapid involution of the ligated gland, but the suckled gland continued to secrete milk. Selye(6) and Selye and McKeown(7), however, were of the opinion that the cause of mammary involution following litter removal in rats and mice was the absence of the suckling stimulus rather than the accumulation of milk. Griffith and Turner(8) found significantly less DNA in suckled glands than in non-suckled glands of the same rat, and suggested that some component of DNA was present in milk.

The present experiments were designed to estimate, in lactating rats, the effects of an increasing non-suckling interval on the DNA and RNA content and RNA/DNA ratio of suckled and non-suckled mammary glands.

Methods. Litters were separated from mothers on 13th to 14th day of lactation for 0, 3, 6, 9, or 12 hours (non-suckling interval). At the end of this separation mothers were anesthetized with sodium pentobarbital (Nembutal-30 mg/kg body wt), and all teats, except 3 abdominal-inguinal teats, were taped to prevent suckling. One international unit oxytocin (Pitocin-Parke, Davis and Co.) was injected 3 times at intervals of 20 minutes, and the litter replaced each time. The first 2 oxytocin injections were administered subcutaneously and the last in-

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