Normal Growth of Mammary Gland in Pregnant and Lactating Mice.* (25384)

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Students of mammary gland growth in the past have been able to distinguish only lobule-alveolar development by morphological means (1,2). By measuring the amount of desoxyribonucleic acid (DNA)/gland(3,4,5), quantitative estimations of cell multiplication throughout pregnancy in the mouse(6) and further proliferation during lactation in the rat(7) have been observed. Our study presents a more detailed study of mammary gland development during pregnancy and lactation in albino mice. DNA values thus obtained can be used as a standard of reference in future studies concerning experimental growth of gland.

Materials and methods. Virgin, primipara, pregnant and lactating Webster-Swiss mice, fed a standard laboratory feed, were maintained at 78 ± 1°F. Ovariectomized lactating mice were operated on day 2 of lactation and sacrificed on day 10. Mice weighing 28-30 g were bred and pregnancy dated from morning vaginal plug. Animals mated but lacking fetuses were classified as pseudopregnant. Lactating mice were housed in individual cages with reduction of young to 5 shortly after parturition. Animals were sacrificed, skinned and 7 of the mammary glands of each mouse taken for DNA determination at time indicated (Table I). Remaining 3 glands were used for whole-mount observations. Glands were frozen immediately to inhibit enzyme (DNA-ase) activity(8). Tissues were thawed in 95% ethanol(4) and fat extracted from 5-8 hours, depending on adiposity of gland, placed in ethyl ether 4 hours, dried and ground to a fine powder in a Wiley mill. Standard sample (30 mg) of gland was extracted twice with 5 ml of hot trichloracetic acid to remove DNA which was then determined by the method of Webb and Levy(9).

Results. Highly significant differences (P .001) exist between the virgin total DNA level and that of 12 and 18 days pregnancy. Between 12- and 18-day pregnant mice a highly significant difference (P .001) was observed also.

There is a slight but non-significant increase in DNA of 6-day pregnant as compared to pseudopregnant mice. At 12 days pseudopregnancy, no further increase in DNA was observed, whereas DNA of 12-day pregnant mice increased 51.9%. Similar total DNA levels were noted between present results and earlier data obtained with virgin, pseudopregnant and pregnant mice(6). These comparisons indicate repeatability of mean DNA under standard laboratory conditions and the same strain. Total DNA increased gradually after parturition to maximum level of 10.211 ± .426 at 14 days lactation. DNA of days 5, 10, and 14 of lactation were statistically highly significant when compared to 18th day pregnancy. Decline in DNA on day 21 may result from partial glandular involution. In the lactating rat there was no significant decline in DNA from days 14 to 21 (7).

Lactating ovariectomized mice exhibited cellular proliferation comparable to normal lactators at 10 days.

Discussion. Past studies using morphological methods have been interpreted as indicating complete lobule-alveolar development by the first ½-⅔ of pregnancy. These data indicate, however, that lobule-alveolar growth continues throughout pregnancy and well into lactation. Of the total cellular increase, 30.6% occurred during the first 12 days of pregnancy, a further 47.5% was observed during second half of pregnancy and a final 21.9% increase noted by day 14 of lactation.

It had previously been assumed that growth during first ½ of pregnancy and pseudopregnancy was similar. These data show that growth is complete at 6 days in pseudo-

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TABLE I. Normal Growth of Mammary Gland in Pregnant and Lactating Mice.

Reproductive state	Stage of pregnancy or lactation	No. of mice	DFFT,† mean (mg)	DNA (μg) mg of DFFT, mean ± S.E.	Total DNA (mg), mean ± S.E.	Final body wt, mean (g)	Total DNA (mg)/10 g final body wt, mean ± S.E.
Virgin		16	40.6	44.1 ± 2.1	1.757 ± .022	29.6	.590 ± .002
" *		7	44.0	38.8 ± 3.5	1.704 ± .180	30.8	.544 ± .051
Nullipara	6 days after sterile coitus*	15	52.5	42.9 ± .5	2.269 ± .126	31.6§	.715‖ ± .038
	12 days after sterile coitus	13	51.9	41.8 ± 1.2	2.147 ± .116	32.5	.661 ± .047
	*Idem**	5	54.8	39.8 ± 1.4	2.202 ± .159	32.8§	.778‖ ± .082
Primipara	6 days of pregnancy*	10	63.2	46.3 ± 2.0	2.853 ± .153	31.4§	.904‖ ± .049
	12 " " "	14	95.3	46.9 ± 1.9	4.344 ± .261	36.9‡	1.171 ± .047
	12 " " " *	10	80.1	51.4 ± 2.8	4.118 ± .319	32.8§	1.253‖ ± .091
	18 " " "	15	125.6	57.0 ± 1.7	7.007 ± .281	38.5‡	1.832 ± .021
	18 " " " *	10	121.8	49.4 ± 1.6	5.965 ± .353	32.0§	1.858 ± .070
Primipara lactating	Immed. postpartum	20	202.2	43.2 ± 1.7	8.358 ± .308	35.5	2.355 ± .080
	5 days lactating	14	247.8	42.1 ± 3.0	9.758 ± .396	35.9	2.719 ± .104
	10 " "	15	317.0	32.1 ± 1.1	9.988 ± .494	39.1	2.559 ± .105
	10 " " ¶	10	269.7	34.5 ± 1.4	9.135 ± .627	36.7	2.489 ± .045
	14 " "	11	336.9	31.3 ± 1.8	10.211 ± .426	37.5	2.756 ± .114
	21 " "	16	245.0	33.4 ± 1.7	7.742 ± .516	37.9	2.032 ± .113

* Data obtained from Wada and Turner, *J. Dairy Sci.*, 1959, v42, 1198.
† DFFT = Dry, fat-free tissue. ‡ Body wt corrected for wt of fetus. § Initial body wt.
‖ Total DNA (mg)/10 g initial body wt. ¶ Ovariectomized, day 2 of lactation.

pregnant mice, whereas in pregnancy the development of mammary gland continues. It is believed, therefore, that something associated with pregnancy is contributing to the stimulation of growth of gland not present in pseudopregnancy.

It was thought that corpora lutea of lactation contributed to additional lactational growth. However, the ovariectomized lactating animals sacrificed at 10 days exhibited DNA levels comparable to normal lactators. Therefore, growth of mammary gland during lactation is not dependent on the corpora lutea of lactation.

Some variability may be eliminated due to variation in body weight by using total DNA/body weight. In present experiment final body weights were quite similar, whereas for purposes of comparison with mice of varying body weight it is suggested total DNA be corrected for body weight.

Summary. 1) Using DNA as index of mammary gland growth, a study of changes from virgin throughout pregnancy and lactation in Webster-Swiss mice was conducted. Previous morphological studies were interpreted as indicating complete lobule-alveolar development by the first ½-⅔ of pregnancy. Our data show, however, only 30.6% of total growth during first 12 days of pregnancy, 45.7% in latter half of pregnancy and 21.9% further proliferation from parturition to day 14 of lactation. 2) Growth of the gland continues throughout pregnancy, but in pseudopregnancy it is completed by day 6. 3) DNA level of ovariectomized lactating mice is similar to normal lactators, indicating corpora lutea of lactation does not contribute to additional growth of mammary gland.

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