Normal Growth of Rat Mammary Glands During Pregnancy and Lactation.* (25337)

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Previous studies of normal and experimental development of mammary gland have used morphological qualitative indices to measure extent of duct and lobule-alveolar growth. Kirkham and Turner(1) first suggested the use of desoxyribonucleic acid (DNA) as a quantitative index of mammary gland cellular proliferation in rats. More recently studies have been conducted on rats(2,3), rabbits(4) and mice(5,6,7). It has been shown that DNA/nucleus is constant during pregnancy but drops 42.8% during early lactation(8). With a quantitative estimate of mammary gland growth in individual animals available, it is possible to determine the mean and normal variation in total mammary proliferation in individual rats at end of pregnancy. These data will serve as a standard of comparison in study of extent of mammary gland development in experimental animals stimulated with estrogen and progesterone(2). It will be possible, also, to determine quantitatively the synergistic influence of growth hormone, thyroxine, glucocorticoids, etc., on normal mammary gland growth during pregnancy and lactation.

Methods. Data were obtained from primiparous Sprague-Dawley-Rolfsmeyer rats during late pregnancy (18-20 days) and on days 5, 10, 14 and 21 of lactation. Rats ovariectomized on day 2 of lactation and sacrificed on day 14 were included. Six abdominal-inguinal mammary glands were removed from each rat and placed in deep freeze for 4 days, extracted in 95% ethanol for 3-5 hours and in ether for an additional 3-5 hours. Dry, fat-free tissue (DFFT) was weighed and ground to a fine powder in a Wiley mill. DNA of a 25 mg aliquot of tissue was determined by the method of Webb and Levy(9). Product of quantity DNA/mg DFFT and DFFT/100 g body weight was estimated as "total DNA." Five animals were anesthetized with nembutal and all nipples were covered except the 3 right abdominal-inguinal glands. The animals were then injected with oxytocin and 6 young were allowed to nurse for a sufficient time to insure complete milk removal from untaped glands. This procedure allowed one to compare DNA of lactating glands with and without milk.

Results. In initial experiments, separate DNA determinations were made on 4 pectoral glands and 6 abdominal-inguinal glands. Sheets of muscular tissue located between pectoral glands were impossible to remove; therefore, only abdominal-inguinal glands, which are free of muscle, were used in estimating DNA content. There was no significant difference between mean DNA of pectoral glands (9.01 mg/100 g) and abdominal-inguinal glands (8.46 mg/100 g) of pregnant rats. Highly significant differences (P .001) exist between total DNA of late pregnancy and various stages of lactation (Table I). Total DNA on day 10 of lactation and day 14 in ovariectomized rats approached significance (P .05) as compared to day 5 of lactation. Statistically significant difference (P .01) was observed between glands containing milk and those without milk. Average total DNA in nursed glands was 5.81 mg/100 g (5.36-6.39); those with milk averaged 7.31 mg/100 g (6.52-8.54 mg/100 g). Dry, fat-free milk did not interfere with color reaction in determination of DNA.

Discussion. It has been considered that mammary gland growth occurs primarily during the first ⅔ of pregnancy, followed by gradual initiation of lactation during final trimester. It has not been possible to determine by morphological methods additional growth of alveoli during the latter part of pregnancy or lactation since secretion of colostrum and milk obliterates the individual alveoli. Re-

* Contribution from Mo. Agr. Exp. Sta., Journal Series No. 2037. Approved by the Director.

† This investigation supported in part by grant from American Cancer Soc.

TABLE I. Total Mammary Gland DNA during Late Pregnancy and Lactation.

No. of animals	Stage of gestation or lactation	Body wt (g), mean	DFFT (mg/100 g body wt), mean	DNA (μg/mg DFFT), mean ± S.E.	Total DNA (mg/100 g), mean ± S.E.
19	18-20 days of pregnancy	324.3	230.9	33.2 ± .84	7.63 ± .40
10	5th day of lactation	301.5	319.3	34.1 ± .73	10.8 ± .52*
10	10th *Idem*	302.2	390.8	31.8 ±1.2	12.26 ± .25*
25	14th "	298.1	417.5	26.3 ± .5	10.9 ± .39*
9	21st "	304.7	396.6	28.3 ± .45	10.31 ± .02*
18	Castrate, 2nd day; lactation, 14th day	290.4	572.7	22.3 ± .4	12.75 ± .64*

* Significant at .1% level when compared to pregnant level. DFFT = Dry, fat-free tissue.

cently Wada and Turner(7), using DNA as an index of growth, have indicated that in mouse a continued proliferation of epithelial cells occurs from day 12 to 18 of pregnancy. The present study is believed to indicate cellular proliferation during lactation on the basis of a 41.5% increase in DNA of lactating glands in comparison with those of late pregnancy. Growth appears to continue until day 5 or 10 of lactation and cellular replacement may occur until weaning since no reduction in total DNA was observed by day 21. A complicating factor in the measurement of proliferation during lactation is the 42.8% reduction in DNA/nucleus during lactation as compared to pregnancy values. Decrease in DNA/nucleus coupled with the 41.5% increase in total DNA during lactation would indicate a tremendous growth during this period. The difference between milked and unmilked glands indicates the possibility that some DNA component is present in milk. It has been demonstrated that desoxyribonuclease (DNA'ase) activity increases 100% on day 1 of lactation, while the acid-soluble components of supernatant decrease at this time(10). This observation would indicate possibility that enzymatic products of DNA may be involved in milk synthesis and lower estimation of total DNA in milked glands. Milk within gland does not mask the mammary proliferation during lactation since total DNA of the nursed glands when doubled equals values normally obtained on day 5 of lactation.

Rats normally ovulate shortly after parturition. If conception does not occur, corpora lutea of lactation form and persist until weaning. It was considered that ovarian hormones secreted by corpora lutea of lactation might be responsible for additional gland growth. However, the failure of mammary glands of ovariectomized lactating rats to return to pregnancy values indicates that gland growth in early lactation is not dependent upon the ovarian hormones. It has been suggested that lactogen(11) and growth hormone (12) have capacity for stimulating mammary gland growth. Recently, in this laboratory a lactogen-free anterior pituitary fraction (mammogen) has been extracted which is effective in producing lobule-alveolar growth in mice(13). It is suggested that mammary gland growth observed during early lactation may be due to the release of mammogen as a result of the nursing stimuli comparable to release of lactogen(14,15). This growth may be synergized by increased secretion of thyroxine(16), growth hormone, and other hormones secreted in increased amounts during lactation(17).

Summary. With DNA as a measure of mammary gland growth in rat, it was shown that there was a 41.5% increase between 18-20th day of pregnancy and day 5 of lactation. This increase occurred in spite of the fact that DNA/nucleus of lactating cells is reduced 42.8% in comparison with cells of pregnant animals. Increased total DNA for 6 abdominal-inguinal glands/100 g body weight was maintained until day 21 of lactation. Animals ovariectomized on day 2 of lactation showed equal increases on day 14 of lactation. It is concluded that marked glandular proliferation occurs following parturition (5 to 10 days) which is not interfered with by ovariectomy. It is suggested that post-parturient mammary gland growth, instead of depending upon ovarian hormones, may be due to direct

release of pituitary mammogen associated with release of oxytocin and lactogen by periodic nursing stimuli.

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Received August 11, 1959. P.S.E.B.M., 1959, v102.

Glucagon Antibodies and Their Use for Immunoassay for Glucagon.* (25338)

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Clarification of the physiologic status of glucagon may well depend upon development of a practical and specific method for its detection in body fluids. Bioassay technics of "glucagon-like" activity have recently been developed(1,2,3) but are fraught with many serious objections(4). It seems that an immunoassay for glucagon, similar to those being developed for insulin(5,6,7), would provide greater specificity. The present report deals with efforts to develop an immunoassay for glucagon.

Methods. Female rabbits were given monthly subcutaneous injections of crystalline "insulin-free" beef-pork glucagon (Lilly†) suspended in Freund's adjuvant. A control group received either Freund's adjuvant alone, Freund's adjuvant plus insulin, or no injections at all. At intervals of several weeks specimens of serum were obtained and tested for development of non-precipitating antibodies to glucagon by means of Berson-Yalow radiochromatographic and electrophoretic technics used by these authors to detect insulin antibodies(8). The Berson-Yalow procedure(8) was adapted without major change, except that glucagon-I^{131} was substituted for insulin-I^{131}. In general 0.01 μg of glucagon-I^{131} was added to 1 cc of rabbit serum and incubated with shaking for 2 hours at 37° C. Ten to 50 μl were applied to cathodal end of Whatman No. 3 mm filter paper strip and chromatographed in barbital buffer at pH 8.6 for one hour. In some instances this was followed by 16 hours of electrophoresis. After heat drying for 1 hour at 120°C, staining with naphthalene blue black, and destaining in methyl alcohol and 10% acetic acid for 10 minutes, the strips were divided and segments counted in well-type scintillation

* This work supported by U. S. Public Health Service.

† Supplied through generosity of Dr. W. R. Kirtley, Eli Lilly Co. Indianapolis, Ind., and considered to be very pure(4) with at most no more than 1% insulin contamination.