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Desoxyribosenucleic Acid as Index of Mammary Gland Growth of Mice.*† (23253)

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Several methods have been proposed to estimate quantitatively mammary gland growth (1.2). Since the suggestion(3) that desoxyribosenucleic acid (DNA) may be constant in the somatic cells of various tissues of any species, DNA has been employed as a measurement of somatic growth and as an index of mammary gland growth in rats(4) and rabbits(5). Recently Griffith and Turner (6) have shown that DNA is constant per nucleus in mammary gland and in other somatic tissue cells of rats and mice during pregnancy. Further, it has been shown that changes in collagen content of the mammary gland are small in comparison to changes in DNA, indicating that most of DNA change during growth is due to mammary parenchymal increments(7). With the establishment of DNA as an accurate measure of mammary gland growth and a sensitive technic of determination available(8), the use of male mice was explored as assay animals for the determination of mammary gland stimulating hormones.

Methods. Male albino mice weighing 16-18 g at start of the experimental periods were used. In first experiment, groups of mice were fed a ration containing 1.23 mg diethylstilbestrol/kg ground mouse food for periods of 0, 1, 2, 3 and 4 weeks, and sacrificed after each period. In second experiment, mice were maintained on a similar diet for 4 weeks in order to insure good development of the mammary duct system(9), then divided into groups receiving graded amounts of estradiol benzoate plus progesterone subcutaneously for 10 days, using a 1:1000 ratio of the 2 substances. This particular ratio has been reported to be optimal in the production of mammary lobule-alveolar development in mice(10,11). Estradiol benzoate (0.75 μg/day) alone was injected into one group for the same length of time. Mice were sacrificed approximately 24 hours following the last injection, skinned, and ¾ of subcutaneous fascia, including mammary glands, were removed for DNA determination. The tissues were quickly frozen by placing the tissue container in an alcohol dry ice bath. The frozen tissues were then lyophilized, fat extracted in hot alcohol and then ether for 12 hours, and ground to a fine powder in a Wiley mill. DNA was extracted from 30 mg dry, fat-free tissue samples by procedure of Schneider(12) with exclusion of the fat extraction step. The tissue was extracted twice with 5 ml of cold 10% trichloroacetic acid (TCA), in a cold room held at 4°C, centrifuged and supernatant discarded. The residue was extracted with 10 ml of hot 5% TCA, and then 5 ml, for periods of 20 minutes. The supernatants were pooled, brought up to a volume of 15 ml, and DNA determined on 2 ml aliquots by the p-nitrophenylhydrazine method of Webb and Levy(8) in preference to the older diphenylamine method of Dische(13). The same purified standard DNA preparation was used throughout all experiments; however, the phosphorous content and the nitrogen content of the preparation were not checked. The remaining quarter of tissue was fixed in Bouin's fluid, washed in water, stained in Delafeld's hematoxylin, differentiated in acid-

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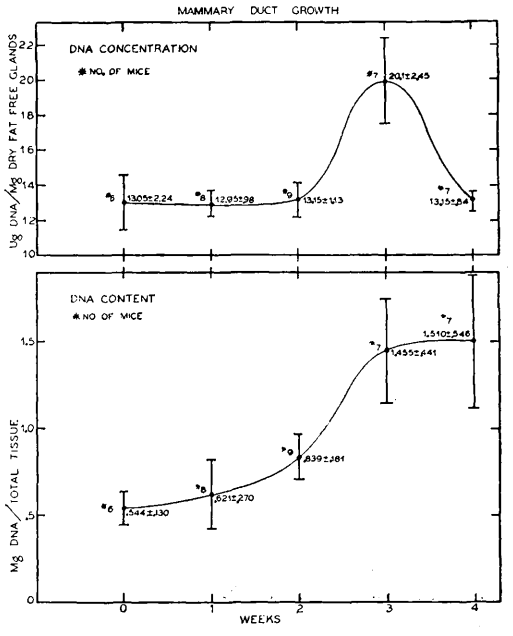

FIG. 1. Relative amounts of DNA in mouse mammary gland during various stages of duct growth. Mice were fed a ration containing 1.23 g diethylstilbestrol/kg. Range represents one stand. error. Note that concentration curve (above) approximates a lagging differential of content curve.

alcohol, and kept in alcohol for visual examination prior to mounting(1).

Results. The DNA of the mammary glands of male mice receiving oral estrogen for varying lengths of time was determined to see if growth could be detected in the developing duct system by this method. Total DNA increased somewhat from the first to the second week, but not until the third week did the DNA rise significantly ($p<.02$) (Fig. 1). There was no difference noted between the total DNA of mammary glands of mice receiving oral estrogen for 3 or 4 weeks. The DNA concentration, expressed as μg/mg dry, fat-free tissue, remained essentially at the same level until after mice were fed estrogen for 3 weeks. At this time a significant ($p<$ .001) increase was observed, after which the concentration decreased to the original level. Whole mount observation indicated progressive, though variable, development of the mammary duct system throughout the feeding period.

The second experiment was devoted to determining the levels of DNA in the mammary gland at various stages of development of the lobule-alveolar system. Total DNA in mammary glands of mice injected with a control amount of estrogen remained at a low level, while in mammary glands of mice receiving graded doses of estrogen plus progesterone, it increased generally as the log-dose of the hormones (Fig. 2). DNA concentration, however, rose rapidly from the level obtained in the estrogen treated group to the level in group receiving .06 μg estradiol benzoate (E.B.) plus 0.06 mg progesterone (P). From this point it rose to the peak amount seen in the group receiving 0.125 μg E.B. plus 0.125 mg P and gradually came down in each successively higher dose until it reached the low level seen in the group receiving 0.75 μg E.B. plus 0.75 mg P. Whole mount observation again indicated progressive, though variable, development of the mammary lobule-alveolar system with increasing doses of estrogen and

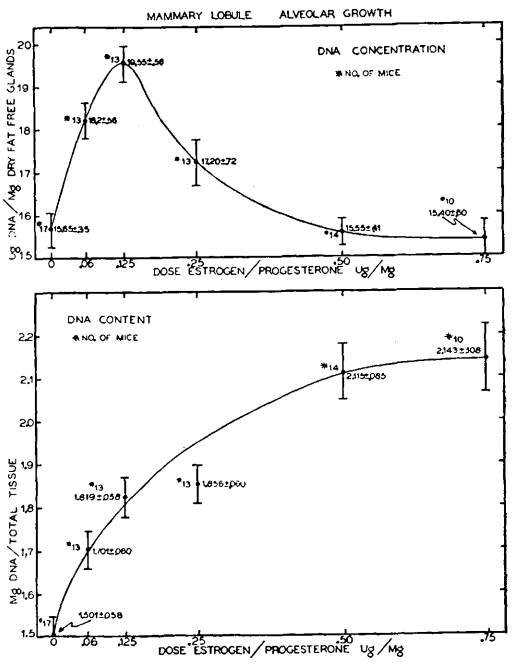

FIG. 2. Relative amounts of DNA in mouse mammary gland at various stages of lobule-alveolar development, stimulated by inj. various levels of estradiol benzoate and progesterone (a constant ratio of 1:1000 was kept) each day for 10 days. Range represents one stand. error. Again concentration curve approximates a differential of content curve.

progesterone.

Discussion. Kirkham and Turner(4) found that DNA content (total DNA) of the mammary glands of rats increased during the first two-thirds of pregnancy, after which time it increased only slightly. This period is generally regarded as the time of greatest mammary gland growth. The results, showing an increase of total DNA during periods of both duct and lobule-alveolar growth, agree well with these findings, in that amounts of total DNA per gland are related to the stage of growth. Further, total DNA accumulation seems to follow a logarithmic progression, in that an approximately straight line is obtained when mean DNA content values are plotted on semilogarithmic paper.

Leslie and others(14) demonstrated that DNA concentration (expressed in terms of DNA/mg protein nitrogen) may be roughly related to rate of growth of tissues, a fact which may be due to the lag in cytoplasmic protein accumulation in rapidly dividing cells. This may be based on the idea that as increasing numbers of cells divide, the DNA increase is much greater than increase in cytoplasmic protein, until the protein "catches up" with the DNA. This seems to agree with results which show that DNA concentration, expressed in terms of μg DNA/mg dry, fat-free tissue, approximates a differential of the DNA content curves.

Several factors may be operative in production of variance in DNA. Among these are the differing responses of the pituitary to progesterone, the differing responses of the mammary gland to pituitary mammogen, and varying secretion rates of other hormones influencing mammary gland growth. Mouse weight was not a factor, in that no significant correlation could be obtained between mouse weight and DNA content of mammary gland. The coefficients of variation compared favorably with previous work(1,5).

Summary. 1. Total DNA of the mouse mammary gland is an index of mammary gland development while concentration (μg DNA/mg dry, fat-free tissue) seems to be an index of growth rate. 2. Feeding 1.23 mg diethylstilbestrol/kg mouse food caused most rapid mouse mammary duct development between the second and third weeks. 3. Rate of mammary gland growth is not constant. At the end of a 10-day period, mice injected with lower doses of lobule-alveolar developing substances seemed to be developing at a faster rate, whereas those receiving a higher dose had a greater amount of absolute growth, although the rate of development had passed the peak.

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