bility) values for test *vs.* control mice were: (A) for mice injected at zero hour, 0.01 or less at 6, 24, 48 and 120 hours thereafter, and (B) for mice injected 15 minutes after zero hour, 0.01 or less ONLY at 6 hours thereafter.

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Normal and Experimental Growth of Rat Mammary Gland.* (25096)

R. C. MOON,† D. R. GRIFFITH AND C. W. TURNER
(With technical assistance of Mary E. Powell)
Dept. of Dairy Husbandry, University of Missouri, Columbia

It is generally accepted that estrogenic hormones stimulate growth of mammary gland duct system while estrogen and progesterone stimulate lobule-alveolar growth in intact and ovariectomized rats. Many investigators have attempted to determine levels of these hormones which produce optimal mammary gland growth(1). Differences in extent of lobule-alveolar growth have been evaluated by visual examination of whole mounts of glands. Kirkham and Turner(2) first suggested the use of desoxyribonucleic acid (DNA) content as an objective index of mammary gland cellular growth. Griffith and Turner(3) have shown that mean DNA content/nucleus of rat mammary gland remained constant during pregnancy. Determination of total mammary gland DNA permits quantitative estimation of gland growth during normal pregnancy and following growth under experimental conditions. In the present study, frequency distribution of total mammary gland DNA of rats pregnant 18-20 days has been compared with that of adult ovariectomized rats treated with 1 μg estradiol benzoate in synergism with graded levels of progesterone for 19 days.

Materials and methods. Groups of pregnant and ovariectomized rats of Sprague Dawley-Rolfsmeyer strain with initial weight of 220-270 g were used. Animals killed 18-20 days of pregnancy and ovariectomized rats killed 34 days postcastration served as controls. Remaining ovariectomized rats received daily subc. injections of 1 μg estradiol benzoate (EB) alone and with 1-10 mg progesterone (P) for 19 days commencing 14 days after ovariectomy. They were killed one day after last injection, skinned rapidly and abdominal-inguinal glands removed and frozen for 4 days. Tissues then were thawed and fat extracted in boiling 95% ethanol for 6 hours followed by ether extraction for another 6 hours. DNA was extracted from 25 mg aliquots of finely ground, dry, fat-free tissue (DFFT) by the method of Schneider(4) with exclusion of fat extraction and cold trichloroacetic acid extraction steps. DNA was determined according to method of Webb and Levy(5). The product of the quantity DNA/mg DFFT and DFFT, expressed as unit body weight, was estimated as total DNA/posterior 6 glands. Visual observations were made for presence of lobule-alveolar development but

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† Postdoctoral Fellow Nat. Cancer Inst.

TABLE I. Mammary Gland DNA of Adult Rat Ovariectomized 14 Days Followed by 1 μg Estradiol Benzoate (EB) and 1-10 mg Progesterone (P) for 19 Days.

Treatment (amt/day)	No. of rats	Body wt (g)	DFFT* (mg/100 g)	DNA/mg DFFT (μg)	Total DNA posterior 6 glands (mg/100 g)
Ovariectomized controls	15	273.7	132.4	23.20 ± .54‡	3.05 ± .14[1]
EB alone	5	246.8	197.0	22.86 ± 1.27	4.48 ± .15[2]
EB + 1 mg P	5	254.8	210.1	27.30 ± 1.10	5.71 ± .23[3]
+ 2 "	20	291.4	188.4	39.33 ± .67	7.41 ± .31[4]
+ 3 "	19	269.0	219.0	35.65 ± 1.09	7.72 ± .25
+ 4 "	21	274.6	219.0	35.70 ± 1.01	7.72 ± .23
+ 5 "	21	281.2	235.3	33.17 ± 1.22	7.63 ± .28
+ 6 "	10	278.4	225.9	35.50 ± 1.53	7.95 ± .34
+ 7 "	5	279.6	264.6	33.73 ± .74	8.87 ± .55[5]
+ 8 "	5	272.4	225.7	36.18 ± .63	8.16 ± .60
+ 9 "	5	267.4	226.1	37.29 ± 2.41	8.43 ± .56
+ 10 "	5	280.8	219.0	41.01 ± 1.20	8.96 ± .36[6]
Pregnant controls	19	324.3†	230.9	33.24 ± .84	7.63 ± .40
Aggregate, EB + 2-10 mg P	111	278.3	219.9	36.09 ± .45	7.83 ± .12

* Dry, fat-free tissue.
† Corrected for wt of fetuses.
‡ Stand. error of mean.

Student's "t"	Probability
1-2	.001
2-3	.005
3-4	.025
4-5,6	.05

no attempt was made quantitatively to determine the extent of growth.

Results. Results of daily administration of EB alone and with graded doses of P for 19 days are presented in Table I. Total DNA of glands of ovariectomized rats receiving EB alone was significantly greater than that of ovariectomized controls. Administration of EB and 1 mg P increased total DNA above that of EB alone and upon increasing the P level to 2 mg, a further increase in total DNA was obtained. Treatment of rats with EB and 3-10 mg P did not significantly increase total DNA above that resulting from administration of EB and 2 mg P. Mean total DNA of glands of rats receiving EB and 2-10 mg P did not differ significantly from that obtained with rats pregnant 18-20 days. DNA values of experimentally developed glands (2-10 mg P levels) were grouped to determine the frequency distribution. Coefficient of skewness (g_1) of DNA values obtained with pregnant rats and those of rats receiving EB and 2-10 mg P were .02 and .14, respectively. Since g_1 values did not differ significantly from a g_1 of zero, DNA values of both pregnant and experimentally treated animals fell within a normal frequency distribution (Fig. 1). Analysis of data also indicated a highly significant correlation (r = .74) between body weight and DFFT weight.

Visual examination of mammary glands of rats ovariectomized 2 weeks, indicated an atrophic duct system with occasional end buds but no alveoli. EB treatment resulted in increased duct growth and end-bud formation. Rats receiving EB and 1-10 mg P exhibited lobule-alveolar growth. The extent of growth, however, was not rated.

Discussion. Numerous investigators have attempted to determine the synergistic level

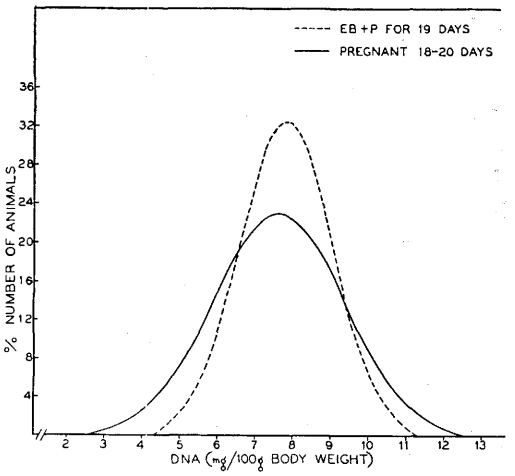

FIG. 1. Frequency distribution of DNA of rat mammary gland. EB + P – 1 μg estradiol benzoate + 2-10 mg progesterone.

of estrogen and progesterone which would produce optimal mammary gland growth in the rat(6-11). Results, however, have been conflicting due in part to age of animals used, interval and duration of treatment and difficulties encountered in visual estimation of extent of growth.

Data of present study indicate daily administration of 1 μg EB and 2 mg P for 19 days resulted in mammary gland growth comparable to that of rats pregnant 18-20 days. Increasing the P level to 10 mg daily had little, if any, further beneficial effect upon growth. The highly significant correlation between body weight and weight of DFFT appears to implicate body weight as a contributing factor in estimation of total DNA of the rat mammary gland. Harkness and Harkness(12) have shown that collagen content and presumably amount of connective tissue of the rat mammary gland do not change during pregnancy and that collagen content is relatively the same between animals when expressed as unit body weight. Since connective tissue possesses relatively fewer cells than glandular tissue/unit mass and, therefore, less DNA, it appears that differences in body weight of animals under similar conditions reflect greater differences in weight of DFFT rather than in quantity of DNA. Total DNA calculated from quantity DNA/mg DFFT and total DFFT, expressed as unit body weight (DFFT/100 g), appears, therefore, to be a more reliable index of total cellular proliferation of mammary tissue.

The lower standard error (Table I) and decreased range (Fig. 1) of DNA values of experimentally developed glands indicate decreased variability in gland growth as compared with that of pregnant rats. The decreased variation may result from administration of EB and P to animals which do not normally secrete these hormones in quantities necessary for optimal gland growth. Variation in total DNA/100 g of animals under the influence of optimal amounts of EB and P may be due, in part, to inherent variation in responsiveness of mammary epithelial cells to ovarian hormones. On the other hand, much of the variability observed may result from variation in endogenous secretion rate of thyroxine(13), growth hormone(1), glucocorticoids(1), etc., which synergize with ovarian hormones in stimulating mammary gland growth. Determination of mammary gland DNA affords a method whereby the synergistic action of these hormones alone and in combination, upon variation in total gland growth may be studied.

Summary. 1) Daily administration of 1 μg estradiol benzoate (EB) and 2 mg progesterone (P) to mature ovariectomized rats for 19 days resulted in mammary gland growth (mean total DNA/100 g) comparable to that of rats pregnant 18-20 days. Increasing the progesterone level to 10 mg daily has little further beneficial effect on mammary gland growth. 2) Variability in growth of experimentally developed gland was less than that of pregnant controls. 3) Total DNA appears to be a more reliable index of mammary gland growth when expressed as unit body weight.

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