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Effect of Estrogen on Mammary Gland Growth of Immature Female Rats.* (31015)

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Previous studies of mammary gland growth in the rat using deoxyribonucleic acid as an index have involved sexually mature females during pregnancy(1,2,3,5), and ovariectomized animals stimulated with estrogen and progesterone for 19 days(4). Earlier studies of mammary gland growth in immature rats were concerned with visual examination by means of whole mounts of the glands from birth to sexual maturity(6,7,8). During this period the rudimentary duct system develops extensively into the fatty pad. It has been suggested that duct growth is at first isometric, *i.e.*, the same rate as body growth; but after sexual maturity, duct growth is allometric, *i.e.*, more rapid than body growth(9-11).

The present study was designed to determine the rate of growth of the duct system of young normal female rats compared to a similar group administered the estrogenic hormone as measured by DNA.

Materials and methods. Twenty-day-old female rats were divided into 2 groups. One group, serving as controls, received 0.2 ml of sesame oil, whereas each animal of the second group received 1 μg of estradiol benzoate (EB) in 0.2 ml of sesame oil daily. Animals of each group were sacrificed 20, 40 and 60 days after the first injection. The initial mean body weights of the 2 groups were similar, about 50 g. They were maintained on Purina Lab Chow with tap water *ad libitum* in a constant environmental temperature of 78 $\pm$ 1°F. Six abdominal-inguinal mammary glands were collected on ice from each animal and the DNA was estimated by the method previously described(1,6).

Results. The mean DNA/100 g body weight (BW) of the control female mammary glands at 40 days was 2.60 $\pm$ 0.16 mg, at 60 days 3.42 $\pm$ 0.12 mg, and at 80 days 3.62 $\pm$ 0.26 mg (Table I).

The daily injection of 1 μg EB for 20 days resulted in no difference in the mean body weights of the treated and control groups. However, after 40 and 60 days of EB a decrease of 15 and 39 g in mean body weights respectively was observed, compared to the controls.

The mean DNA/100 g body weight of the treated group at 40 days was 2.81 $\pm$ 0.14 mg, significantly higher ($P < 0.001$) than its control; at 60 days 3.45 $\pm$ 0.15 mg; and at 80 days 3.0 $\pm$ 0.18 mg, about 20.6% less than the corresponding control ($0.02 > P < 0.01$).

Discussion. Based upon the visual observation of duct growth in immature female rats, it has been suggested that early duct growth is isometric in relation to body weight gain until puberty, then duct growth is stimulated at a greater rate by recurring estrous cycles(9,10). Since this strain of rats reaches puberty at between 30 and 40 days, the mean control value at 40 days would represent isometric growth. The increased growth of the duct system of the EB injected group indicates that the duct system is susceptible to allometric growth at this time. At 60 days of age, the control and experimental groups showed the same DNA/

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TABLE I. Comparison of Normal Growth of Mammary Gland Duct Syste m of Female Rats and by Estrogen as Measured by DNA from Weaning to Maturity.

Group No.	Treatment	No. of animals	Age (days) At beginning	Age (days) At time of sacrifice	Mean body wt (g) Initial	Mean body wt (g) Final	Dry fat free tissue mean ± S.E. (mg)	DNA/mg DFFT mean ± S.E. (μg)	Total DNA mean ± S.E. (mg)	DNA/100 g BW mean ± S.E. (mg)
1	Control	14	20	40	50.80	123.00	160.00 ± 12.70	20.38 ± .46	3.23 ± .23	2.60 ± .16[1]
	1 μg EB/day for 20 days	16	20	40	51.60	121.50	175 ± 20.97	24.22 ± .77	4.12 ± .43	2.81 ± .14[2]
2	Control	19	20	60	44.00	169.10	291.20 ± 14.74	20.32 ± .73	5.82 ± .02	3.42 ± .12
	1 μg EB/day for 40 days	19	20	60	44.50	154.50	208.47 ± 14.57	26.10 ± .58	5.41 ± .30	3.45 ± .15
3	Control	18	20	80	52.30	199.80	428 ± 12.85	18.04 ± 1.33	7.54 ± .64	3.62 ± .26[3]
	1 μg EB/day for 60 days	19	20	80	51.10	160.30	225.50 ± 11.02	21.20 ± .76	4.83 ± .60	3.00 ± .18[4]

EB = Estradiol benzoate
DFFT = Dry fat-free tissue
BW = Body weight
DNA = Desoxyribonucleic acid
S.E. = Standard error

Student's "t" probability
1 *vs* 2 $P < .001$
3 *vs* 4 $.02 > P < .01$

100 g BW, indicating marked duct growth in both groups. This indicates that the recurring estrous cycles stimulated duct growth comparable to that stimulated by 1 μg/day of EB. Since the ovaries of the experimental group were infantile, this level of estrogen depressed gonadotropin secretion and the growth of the ducts was dependent upon exogenous estrogen.

At 80 days of age, the control group showed further slight duct growth as indicated by DNA. The experimental group showed a depressed body growth rate due to estrogen and a reduced level of duct growth in comparison with the controls. The reduced level of DNA at 80 days compared to 60 days is not interpreted as an indication of involution of the duct system, but rather due to the variability in the extent of duct growth in individual animals of the two groups. In a comparable study of normal and experimental duct growth in male rats, it is interesting to note that EB for 60 days stimulated duct growth to the same extent as in the females of the present experiment(12). This observation indicates that the duct systems of the mammary glands of male and female rats are capable of being stimulated equally by estrogen.

Summary. The normal growth of the mammary glands of immature female rats of the Sprague-Dawley-Rolfsmeyer strain was compared to that induced by 1 μg/day of estradiol benzoate (EB) from 40 to 80 days of age by the DNA method. The mean DNA/100 g BW of normal female rats at 40 days of age was 2.60 ± 0.16 mg, at 60 days 3.42 ± 0.12 mg, and at 80 days 3.62 ± 0.18 mg. The rats injected with EB for 20 days (age 40 days) showed mean DNA/100 g of 2.81 ± 0.14 mg, significantly higher ($P < 0.001$) than the control, with no difference in BW. After 40 days of EB (age 60 days), the mean DNA/100 g BW was 3.45 ± 0.15, the same as the control, but had a lower mean body weight of 15 g. After 60 days of EB (age 80 days), the mean DNA/100 g BW was 3.0 ± 0.18 mg, significantly different from the control ($0.02 > P < 0.01$), but had a lower mean body weight of 39 g. The treated rats plateaued in their body weight after 40 days of treatment, whereas the controls showed continuous growth.

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Effect of Growth and Lactogenic Hormone on Tumor Induction by Methylcholanthrene in 4-Month-Old Female Rats.*† (31016)

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An optimum yield of mammary tumors occurs when methylcholanthrene (Mc) is administered *per os* to female rats 30-60 days of age(1); successive daily doses of the carcinogen, including those given after the rats are 4 months of age or older, continue to contribute to the final tumor yield(2). If, however, administration of the initial dose is delayed until the rats are 4 months of age, the tumor yield is markedly reduced(3). These data indicate that the age of the animal when the carcinogen is first administered, governs its tumor-inducing, but not its growth-promoting, properties. Since the period of greatest susceptibility to tumor induction is coincident with the period of greatest growth rate, an attempt was made to increase tumor induction in 4-month-old female rats by addition of growth hormone to the daily regimen. Prolactin was administered to control rats since it is the only one of the minute quantities of contaminating hormones not normally found in virgin rats(4).

Method. Female Wistar rats were divided into 4 groups of 20 animals each and treated according to the plan in Table I. The rats were caged singly in wire mesh cages and were given Wayne Lab-Blox *ad libitum*.

TABLE I. Tumor Incidence in 4 Groups of Experimental Female Rats.

Group	Age (mo)	Treatment*	No. of animals	No. of animals with tumor	No. of tumors
A	4	GH & Mc	19	9	35
B	4	Prolactin & Mc	19	9	25
C	4	Mc	16	11	38
D	1	Mc	19	18	108

* Growth hormone ovine. NIH-GH-S6.
Prolactin ovine. NIH-P-S6.

Methylcholanthrene dissolved in olive oil was administered five days weekly through an esophageal catheter. All 4-month-old rats received doses of 5 mg daily whereas the 30-day rats received 2 mg daily for the initial 30 days of experiment, after which the dose was increased to 5 mg daily. Hormones were administered subcutaneously twice daily for the initial 4 months of the experiment. The total daily dose of growth hormone was 3.2 USP units and that of prolactin of 0.8 I.U., the latter an estimate of prolactin contamination in the growth hormone preparation. The experimental plan and results are shown in Table I.

Results and discussion. The administration of growth hormone resulted in a constant increase in weight of the recipient adult rats, whereas the animals receiving prolactin failed to gain at the same rate as those treated with methylcholanthrene alone (Fig.

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