

Experimental Mammary Gland Growth in Rabbits by Estrogen and Progesterone.*† (22408)

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The role of estrogenic hormones in stimulating growth of the duct system of experimental animals has been studied extensively in this laboratory. The mouse(4,13,14); the rat(4,16); the rabbit(11,12); the cat(10) and dog(15) all showed extended duct growth following estrogen administration. In the guinea-pig(5-14), lobulealveolar development could be stimulated with estrogens. When crude or purified preparations of progesterone became available, Turner and Frank(12) showed that combination of estrogen and progesterone would carry growth of the duct system to complete lobule-alveolar development. These observations were extended to mouse(14) and rabbit(12). The first quantitative study of amounts and proportions of these 2 ovarian hormones for optimal growth of lobule-alveolar system in the rabbit was reported by Lyons and McGinty(6) and Scharf and Lyons(7) who observed optimal synergism of the 2 hormones when 24 to 96 μ g of estrone were administered with 1 mg of progesterone daily for about 25 days. This is a ratio of 1:11 to 42. In subsequent studies of amounts and proportions of these 2 hormones on growth of glands in other experimental animals, the ratio was 1 of estrogen to 1,000 or more of progesterone in mice(1,8,17), rats(1,3) and dogs(9). The great discrepancy between the ratio of estrogen to progesterone for optimum lobule-alveolar growth in the rabbit and that required by other species is of considerable importance since in larger animals such as goats and cattle, use of narrow ratios has not been successful in growing the mammary gland. With development of a more objective measurement of mammary gland growth based upon nucleic acid values

(2,3) it seemed important to repeat the study of the rabbit to determine whether previous observations based upon visual observations of glands were accurate and thus to determine whether the rabbit was an exception in the narrow ratio of ovarian hormones required for mammary gland lobule-alveolar growth.

Material and methods. Normal New Zealand White male rabbits weighing 4½ to 7 lb were used. Hormones employed were estradiol benzoate and progesterone dissolved in sesame oil. The hormones were injected subcutaneously once per day for indicated period. Animals were sacrificed a day following the last administration of the hormones, skinned, and the fatty pads containing mammary glands were dissected free of lymph nodes and other tissues. All glands except the most anterior pair located in the loose skin of the neck were removed for nucleic acid determinations. Determinations of extent of mammary gland growth were primarily based on increase of mammary gland nucleic acid values, and especially upon DNA, believed to be related to cell multiplication (2,3). Mammary glands were also studied in whole mounts for visual verification of the extent of growth.

Experimental. To ascertain optimum levels of estradiol benzoate necessary to stimulate maximum extension of the duct system 6, 9, 12 and 15 μ g were administered daily for twenty days to groups of 5 rabbits each. All four levels of hormone showed good duct growth with almost negligible alveolar formation. The 9 μ g level was used as pretreatment dosage in the subsequent study due to the slightly higher nucleic acid values (Table I).

Groups of male rabbits were injected with 9 μ g of estradiol benzoate daily for 20 days to induce duct development. Since 1 mg of progesterone daily seemed to be adequate as

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TABLE I. Nucleic Acid Values of Male Rabbit Mammary Glands following Stimulation with the Ovarian Hormones. 5 animals/series.

Treatment μg EB	Days	DNA, mg/500 mg DFF glands	PNA, mg/500 mg DFF glands
0	—	$3.15 \pm .29$	$.50 \pm .18$
Estrogen only			
6	20	$3.47 \pm .62$	$1.39 \pm .83$
9	"	$3.72 \pm .38$	$1.82 \pm .52$
12	"	$3.56 \pm .49$	$1.64 \pm .36$
15	"	$3.61 \pm .87$	$1.76 \pm .97$
20 days pretreatment with estrogen			
5	30	9.25 ± 1.60	4.00 ± 1.94
10	"	$9.61 \pm .97$	4.20 ± 1.47
15	"	13.40 ± 1.33	7.99 ± 1.79
20	+ 1 mg P	10.36 ± 1.38	5.31 ± 1.49
30	"	11.00 ± 1.14	5.43 ± 1.54
60	"	12.58 ± 2.93	7.70 ± 2.72
90	"	11.50 ± 1.23	6.13 ± 1.37

DFF denotes dried fat-free glands.
EB denotes estradiol benzoate; P denotes progesterone.

shown in previous studies(6), the problem to be resolved was the proper level of estrogen which would synergize with progesterone. In order, levels of 5, 10, 15, 20, 30, 60 and 90 μg of estradiol benzoate plus 1 mg of progesterone were administered daily for 30 days to groups of rabbits.

The highest nucleic acid values were obtained in glands from rabbits receiving 15 μg of estradiol benzoate plus 1 mg of progesterone. As substantiated by whole mounts of the glands, these amounts and proportions of the 2 hormones brought out full extension and development of the duct system and lobule-alveolar proliferation, which most closely approximated lobule proliferation attained during the first two-thirds of pregnancy or that developed by normal pregnancy.

The levels of 30, 60 and 90 μg of estradiol benzoate plus 1 mg of progesterone daily produced mammary glands with high nucleic acid values and generally with good lobule formation. The two lower amounts (5 and 10 μg of estradiol benzoate plus 1 mg of progesterone) produced only fair lobule development. Alveoli seemed to be in process of multiplication and the lobules were irregular and isolated. At 20 μg level daily extension of the duct system associated with lobule-alveolar system appeared greater.

Discussion. The present study using DNA content of mammary gland in conjunction with visual examination as indications of extent of growth stimulation confirms previous work indicating that the rudimentary duct system of the male rabbit can be caused to grow extensively with minimum lobule-alveolar growth following the injection of 6 to 15 μg daily of estradiol benzoate for 20 days. Our data indicated that 9 μg of estradiol benzoate daily for 20 days was optimal in the development of the duct system. Since estradiol benzoate is a more potent estrogen than estrone, these results and those of Lyons and McGinty(6) using 12 μg of estrone per day are considered in good agreement.

The most favorable level of estradiol benzoate to synergize with 1 mg of progesterone daily to obtain optimal lobule-alveolar growth was 15 μg based upon the DNA content of the glands. Smaller amounts of estradiol produced glands lower in DNA and visually inferior in lobule development. Estradiol in amounts varying from 20 to 90 μg with 1 mg of progesterone produced glands with good lobule-alveolar development. In comparison, Scharf & Lyons(7) reported that estrone in amounts from 24 to 96 μg daily synergized best with 1 mg of progesterone. Considering the difference in the biological activity of the estrogens used, the results, again, are in good agreement.

From these data, it is concluded that the rabbit differs greatly in the ratio of synergism of the 2 ovarian hormones (estrogen; progesterone) in stimulating lobule-alveolar mammary gland growth in comparison with the mouse, rat and dog. In relation to body weight of these species, the amount of estrogen required by the rabbit is relatively high in comparison to the dog (dog, 10 μg ; rabbit, 15 μg) whereas progesterone requirement of the rabbit is low, 1 mg as contrasted to 0.5 mg for the mouse, 2-5 mg for the rat, and 10 mg for the dog.

The daily injection of 15 μg of estradiol benzoate and 1 mg of progesterone (ratio of 1:67) for 30 days, found to be effective for optimal lobule development in the male rabbit pretreated with 9 μg of estradiol benzoate

for 20 days has been shown to be even more effective in ovariectomized female rabbits in our laboratory as evidenced by the more pronounced overlapping of the adjacent mammary glands at their peripheries. These glands were comparable to the mammary glands of pseudo-pregnant rabbits routinely prepared preliminary to lactation studies in our laboratory.

Summary. 1. Daily injection of 9 μ g of estradiol benzoate for 20 days induced extensive development of the mammary duct system of male rabbits with negligible lobule formation. 2. With male rabbits pretreated with estrogen to develop the duct system, the daily injection of 15 μ g of estradiol benzoate combined with 1 mg of progesterone (synergistic ratio 1:67) induced the extensive development of the lobule-alveolar system as indicated by the DNA content and by visual examination of whole mounts of the glands. 3. These observations indicate that the optimum synergistic ratio of the ovarian hormone in lobule-alveolar development in the rabbit differs widely from the ratio of 1:1000 or more observed in the mouse, rat and dog.

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Convulsant Effects of Semicarbazide in Epileptic Monkeys.* (22409)

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Semicarbazide (amino-urea) has been described as a potent convulsant and its action in normal monkeys has been reported(1). The present investigation was undertaken to study the comparative effects of its administration to chronically epileptic as well as normal monkeys.

Method. Semicarbazide was injected by the rapid intravenous route at intervals of one or more weeks to a group of 8 *Macaca mulatta* (3.5-6.0 kg). Five monkeys of this

group had been made chronically epileptic by application of alumina cream to the brain (2), and 3 were unoperated normal controls. Each monkey was observed for periods up to 6 hours following injection. Clinical observations and electroencephalographic studies were made.

Results. Threshold convulsant dosages which provoked clinical motor seizures in the 3 normal monkeys were 80, 80, and 60 mg/kg respectively. Time of onset of clinical seizures was approximately 3 hours after intravenous injection in 2 monkeys and 4 hours in the third (60 mg/kg).

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