

## Effect of Estrogen and Progesterone Upon Milk Secretion in Normal and Ovariectomized Rats and on Mammary Gland DNA.\* (27673)

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Previous studies have demonstrated that estrogens, or estrogens and progesterone (P), when administered to normal or ovariectomized lactating rats, have adverse effects upon growth of litters. Various reasons have been suggested for depression of litter growth, including failure of milk secretion or "let-down," poor maternal behavior, effects of hormones in milk, and reversion of lactating mammary glands to pregnant state(1,2,3).

Recently, optimal levels of estradiol benzoate (EB) and P for mammary gland development have been reported as 1  $\mu$ g EB and 3 mg P(4,5). It was thought desirable to investigate influence of these levels of ovarian hormones upon lactation in the normal and ovariectomized rat and to study also their effect upon mammary gland desoxyribonucleic acid (DNA).

**Materials and methods.** One group of primiparous Sprague-Dawley-Rolfsmeyer rats were ovariectomized on day 2 of lactation. On day 4, each litter was reduced to 6 young. Groups of normal and ovariectomized animals received subcutaneous injections of (a) 1  $\mu$ g EB; (b) 1  $\mu$ g EB and 3 mg P; or (c) 3 mg P/day from days 2 to 13 of lactation.

Milk yield was measured on day 14 by method of Grosvenor and Turner(6). Six abdominal-inguinal mammary glands were then removed from the dams after nursing and DNA of these glands was determined as described previously(7).

**Results and discussion.** Present data confirm previous observations(1,2,3) that ovariectomy of lactating rats (day 2) does not interfere with intensity of milk secretion as shown by litter weight (156 g) and actual milk yield (8.2 g). Postparturient (lactational) growth of mammary gland also occurs

as shown by DNA value of 9.99 mg/100 g body weight (BW). It is clear that corpus luteum of lactation is unnecessary for early "lactational" mammary growth or milk secretion.

Milk yield and litter weights of normal uninjected controls were 8.7 g and 151 g, respectively. DNA/100 g BW was 10.88 mg (Table 1). This value was equal to that previously reported(7).

In normal lactating rat, injection of 1  $\mu$ g EB/day depressed lactation significantly ( $P < .01$ ) to 4.2 g, a decline of 51.7%. Litter weights decreased to 111 g, a decline of 26.1%. DNA (9.09 mg) was also markedly lower than controls ( $P < .02$ ).

When 1  $\mu$ g EB and 3 mg P were administered, lactation was depressed to 3.8 g ( $P < .01$ ), a reduction of 56.3%, and litter weight was reduced to 94 g, a decline of 37.7%. DNA (9.88 mg) was not significantly altered. EB and P at levels which stimulate lobule-alveolar growth in ovariectomized rats comparable to end normal pregnancy(7) seriously depressed lactation in normal animals without influencing amount of glandular tissue.

P alone depressed lactation slightly (7.8 g), but not significantly. Litter weight (152 g) and DNA (10.96 mg) were unaffected.

In ovariectomized rats 1  $\mu$ g EB depressed milk yield to 6.6 g, a decrease of 24.1%. However, litter weight (150 g) and DNA/100 g BW of 11.77 mg were not significantly altered. EB + P alone had no significant effect on milk yield, litter weight, or DNA. Respective values for EB + P were 7.7 g, 159 g, and 11.18 mg, and for P, 8.0 g, 152 g and 12.58 mg.

Data clearly indicate EB and EB + P injected into lactating animals with intact ovaries and corpora lutea of lactation has a depressing effect on lactation without influencing lobule-alveolar system. In ovariectomized

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TABLE I. Effect of Estrogen and Progesterone upon Milk Secretion and Mammary Gland DNA in Rats on Day 14.

| Treatment             | No. of animals | Wt of dam (g) | Wt of young (g) | Wt of* milk (g) | DFFT (mg) mean | DNA ( $\mu$ g/mg DFFT) | Total DNA (mg/100 g BW) |
|-----------------------|----------------|---------------|-----------------|-----------------|----------------|------------------------|-------------------------|
| Normal                | 21             | 294           | 151             | 8.7 $\pm$ .41   | 1269           | 24.6 $\pm$ .87         | 10.88 $\pm$ .59         |
| EB, 1 $\mu$ g         | 22             | 291           | 111             | 4.2 $\pm$ .40†  | 992            | 27.4 $\pm$ .98         | 9.09 $\pm$ .40‡         |
| " + P, 3 mg           | 15             | 305           | 94              | 3.8 $\pm$ .36†  | 1026           | 29.4 $\pm$ .97         | 9.88 $\pm$ .5           |
| P, 3 mg               | 19             | 303           | 152             | 7.8 $\pm$ .69   | 1210           | 27.3 $\pm$ .96         | 10.96 $\pm$ .71         |
| <i>Ovariectomized</i> |                |               |                 |                 |                |                        |                         |
| Normal                | 14             | 284           | 156             | 8.2 $\pm$ .45   | 1140           | 25.4 $\pm$ 1.06        | 9.99 $\pm$ .47          |
| EB, 1 $\mu$ g         | 11             | 276           | 150             | 6.6 $\pm$ .63†  | 1201           | 25.6 $\pm$ 1.4         | 11.77 $\pm$ 1.4         |
| " + P, 3 mg           | 9              | 295           | 159             | 7.7 $\pm$ .95   | 1122           | 28.5 $\pm$ .83         | 11.18 $\pm$ .35         |
| P, 3 mg               | 13             | 285           | 152             | 8.0 $\pm$ .26   | 1231           | 29.4 $\pm$ 1.4         | 12.58 $\pm$ 1.18        |

\* Determined by stomach contents of litters after nursing.

† Significant to normal lactating at .01 level.

‡ Significant to normal lactating at .02 level.

EB = estradiol benzoate.

P = progesterone.

animals, these hormones had only slight or no depressing action. It is clear these hormones influence lactation by some action upon ovaries and secretion of some substance detrimental to lactation rather than hormones acting directly upon secretion process. Since EB or EB + P may stimulate endogenous secretion of relaxin by ovaries(8), it appears possible that it may be involved.

Inhibitory substance appears to interfere with normal action of oxytocin on contraction of myoepithelial cells and milk removal. In normal rats receiving EB or EB + P mammary glands were still engorged with milk at end of nursing period. Since exogenous oxytocin was administered at beginning of nursing period, inhibitory substance could not have influenced secretion of oxytocin. Increased amounts of oxytocin up to 10 USP units were administered in some cases without beneficial effects. This lack of contraction was not a vascular problem, because topical administration of oxytocin did not affect myoepithelial cells.

**Summary.** The present study investigated influence of 1  $\mu$ g EB and 3 mg P alone or in combination upon lactation in normal and ovariectomized rat. EB and EB + P inhibited

milk let-down and litter weight significantly when given to normal lactating rats. Decrease in milk yield was 51.7 and 56.3%, respectively. EB and EB + P did not have such adverse effects in ovariectomized animals. P alone was without effect in both types of animals. This effect was apparently mediated through ovaries and apparently interfered with normal contraction of myoepithelial cells. Exogenous oxytocin intravenously or topically did not induce "let-down" in pre-treated animals.

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