Effect of Ovarian Hormones upon Milk Yield in the Rat (34425)

F. S. KNOX AND D. R. GRIFFITH
(Introduced by O. E. Tauber)
Zoology and Entomology Department, Iowa State University, Ames, Iowa 50010

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

Griffith and Turner (4) reported exogenous estradiol benzoate (EB) and progesterone (P), when administered to normal lactating dams in quantities sufficient to stimulate normal mammary gland growth (1 μg of EB + 3 mg of P), decreased the amount of milk obtained by day 14 suckling pups. In ovariectomized lactating animals, the hormones had only slight or no depressing action. It was suggested EB or EB and P could stimulate endogenous secretion of relaxin (R) and R alone or acting synergistically with EB and P depressed milk yield. The present study investigated the effect of R, EB, and P on the lactational performance of the intact and ovariectomized rat.

Methods and Materials. Primiparous Sprague–Dawley–Rolfsmeyer rats were employed in this investigation. Ovariectomy was performed on day 1 of lactation and litters were reduced to six pups on day 4. Lactating groups of normal (N) and ovariectomized (Nx) animals received sc injections of (a) 1 μg of EB and 3 mg of P, and (b) 90, 180, 360 guinea pig units (GPU) of R[1] alone or in combination with 1 μg of EB or 1 μg of EB and 3 mg of P from day 2 through day 13 of lactation. On day 14, milk yield was measured by method of Grosvenor and Turner (5). Immediately prior to nursing, the dam received 1 USP unit of oxytocin.

Results and Discussion. N and Nx groups: Litter weight (LW) and milk yield (M) values were 150 and 8.3 g, respectively, for N group while Nx group values were 142 g and 6.9 g, respectively (Table 1). Removal of ovaries had no significant effect upon subsequent lactational performance as judged by LW or M.

N and Nx + 1 μg of EB + 3 mg of P: EB and P administration to N group again demonstrates an inhibitory influence upon LW (105 g) and M (2.6 g) when compared to N. In contrast to a previous report (4), EB and P had a detrimental influence upon LW (111 g) and M (4.2 g) in the Nx group. It is possible in the present study EB and P stimulated release of R from the intact uterus, as has been reported for the rabbit (6). Both groups were significantly less than N ($p < .001$) with respect to LW and M, however, significance was not observed between these two experimental groups.

N and Nx + R, 180 GPU: R at 180 GPU did not adversely affect LW or M in either N or Nx animals. LW and M valves were 137 and 7.8 g and 139 and 8.8 g, respectively. The addition of EB alone or P alone with R (180 GPU) demonstrated no adverse effect upon LW or M. EB + R and P + R values for LW and M were 148, 8.9 g and 137, 8.9 g, respectively.

Nx + EB, P, R 90, 180, 360 GPU: The combination of all three ovarian hormones severely depressed LW and M. LW and M values for all Nx EB, P, and R groups were significantly less than the N group ($p < .001$). In addition, Nx EB, P, R 360 GPU group (LW 58 g, M 1.7 g) was markedly lower ($p < .001$) than Nx EB, P, R 90 GPU

[1] Received from Warner Lambert Research Institute.

TABLE I. Effect of Estradiol, Progesterone, and Relaxin upon Milk Yield.

Treatment[c]	No. of rats	Body wt (g)	Litter wt (g)	Milk yield (g)
N	14	254	150.0 ± 20.0	8.3 ± 1.4
Nx	5	257	142.0 ± 20.5	6.9 ± 2.8
N, EB-P	5	269	105.0 ± 1.5[a]	2.6 ± 1.3[a]
Nx, EB-P	12	265	111.0 ± 40.0[a]	4.2 ± 2.0[a]
N, R_{180}	4	290	137.0 ± 46.0	7.8 ± 4.3
Nx, R_{180}	11	232	139.0 ± 18.0	8.8 ± 0.9
Nx, R_{180}EB	8	287	148.0 ± 19.0	8.9 ± 2.0
Nx, R_{180}P	9	274	137.0 ± 22.0	8.9 ± 1.5
Nx, R_{90}EB-P	6	273	117.0 ± 39.0[a]	4.6 ± 2.0[a]
Nx, R_{180}EB-P	11	256	92.0 ± 24.0[a]	3.6 ± 2.0[a]
Nx, R_{360}EB-P	6	270	58.0 ± 14.0[a]	1.7 ± 1.6[a]
Nx, pp	4	245	132.0 ± 16.0	7.5 ± 3.0
Nx, ppEB-P	7	262	114.0 ± 14.0[b]	4.5 ± 1.6[a]

[a] Significant to N group: $p < .001$; [b] $p < 0.01$.

[c] N = normal (intact); Nx = ovariectomized; R = Relaxin (guinea pig units/day); EB = estradiol benzoate (1 μg/day); P = progesterone; pp = low potency polypeptide fraction (0.5 GPU/mg).

values (LW 117 g, M 4.6 g). Significance was not observed between the Nx EB, P and EB, P R 90 GPU values, indicating R at the 90 GPU level does not enhance the inhibitory influence of EB and P. R at the 360 GPU level in combination with EB and P reduced LW and M to their lowest values. This suggests R acts synergistically with EB and P to give maximal inhibition of milk yield.

Because relaxin is not a chemically pure substance, Steinetz (6) suggested that all investigational results should be compared against the action of a low potency protein fraction (pp). Nx animals which received pp fraction (LW 132 g, M 7.5 g) did not differ from N or Nx animals. Significance was not observed between Nx pp EB P and Nx EB P groups, and the observed inhibition in these groups must have been entirely to EB and P.

Several factors alone or in combination may be responsible for this hormonal inhibition of milk yield. Cowie *et al.* (7) reported relaxin may reduce lobule–alveolar tissue of the goat during experimental lactation. Griffith and Turner (4) reported that EB and P did not reduce mammary gland deoxyribonucleic acid of the rat during lactation. This indicates EB and P do not bring about mammary gland involution during lactation in the rat.

The ovarian hormones may interfere with milk synthesis and/or milk let-down. This assumption results from the fact that copious amounts of milk were observed in mammary glands after the nursing period and from the fact that exogenous oxytocin had no influence upon milk removal.

It is also suggested that R in combination with EB and P may be responsible for inhibiting milk let-down until after parturition. It may also explain the reduction in milk yield during the latter stages of a concurrent pregnancy when the relaxin level reaches it maximal level.

Summary. EB, P, and R significantly reduced milk yield in the day 14 lactating rat. Relaxin acts synergistically with the steroid hormones to reduce milk yield below that observed with EB and P alone. It is suggested the ovarian hormones act by inhibiting the normal action of oxytocin on the mammary gland during milk-ejection reflex.

1. Young, W. C., "Sex and Internal Secretions," Vol. 1, p. 590. Williams and Wilkins, Baltimore (1961).

2. Kon, S. K. and Cowie, A. T., eds., "Milk: The Mammary Gland and its Secretion," Vol. 1, p. 182. Academic Press, New York (1961).

3. Cole, H. H. and Cupps, P. T., "Reproduction in Domestic Animals," Vol. 1, p. 539. Academic Press, New York. (1959).

4. Griffith, D. R. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **110**, 862 (1962).

5. Grosvenor, C. E. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. **100**, 162 (1959).

6. Hisaw, F. L., Zarrow, M. X., Money, W. L., Talmage, R. V. N., and Abramowitz, A. A., Endocrinology **34**, 122 (1944).

7. Cowie, A. T., Cox, C. P., Folley, S. J., Hosking, Z. D., and Tindal, J. S., J. Endocrinol. **31**, 165 (1965).

Received July 2, 1969. P.S.E.B.M., 1970, Vol. 133.