

Limited Effects of Certain Steroid Hormones on Mammary Glands of Hypophysectomized Rats.*

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The controversial nature of the reports on mammary growth of hypophysectomized mammals treated with steroid hormones has apparently been caused in part by species differences and by different physiological conditions of the animals subjected to treatment. Certain steroid hormones have induced mammary growth in hypophysectomized mice¹ and rabbits,² and under particular circumstances, similar results were reported in hypophysectomized rats.³ While the mammary glands of hypophysectomized rats gave specific growth effects with testosterone regardless of age or when treatment was begun following ablation of the pituitary, they did not respond to estrogen unless the rats were immature and under 70 grams body weight, and the injections were begun immediately following the operation.

In view of the reported favorable mammary growth in hypophysectomized mice and rabbits with progesterone and the negative effects of this hormone in hypophysectomized rats,^{4,5} an investigation of the influences of this hormone alone or in combination with estrogen was undertaken. Studies were also made on the effects of desoxycorticosterone acetate under conditions which permit estrogen to stimulate mammary growth in hypophysectomized rats.

Methods. Male and female rats of the Long-Evans strain, ranging from 26-38 days

of age and weighing from 50 to 98 g at the time of operation were used. As far as possible, comparisons were made between glands of animals within the same litter. An adequate diet supplemented with a 20% glucose solution was fed *ad libitum*.

Experiments were designed to include both immediate and postponed treatment following hypophysectomy. The total amounts of the hormones injected for a 10-day period were progesterone (Lutocylin), 10-30 mg; α -estradiol dipropionate (Di-Ovocylin), 50 γ ; desoxycorticosterone acetate (Percorten), 7.50-10.0 mg.[†] At autopsy, the right abdominal mammary gland was removed and stained *in toto* with Mayer's hemalum. Observations were made on adrenal, testis, and body weights, and all operations were verified as complete by examining the sellæ turcicæ histologically. Sixty-one completely operated animals were used in these experiments.

Results. Progesterone was observed to stimulate growth of the end buds and to thicken slightly the ducts of the mammary glands in 3 of 5 hypophysectomized rats when dosages of 30 mg in 10 days were injected, beginning immediately after the operation. In 2 of 3 rats receiving 15 mg dosages of progesterone under the same conditions, only a slight enlargement of the ducts could be observed and only when compared with their littermate controls. If treatment was postponed for 4-8 days after hypophysectomy, the mammary glands of 2 animals receiving 30 mg of hormone could not be differentiated from the controls.

When progesterone was given in combination with a constant dose of estrogen, the amount of new mammary growth obtained in

* Aided by a grant from the Sackett Fund of the Trustee-Faculty Committee on Research.

¹ Gardner, W. U., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 835.

² Asdell, S. A., and Seidenstein, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 931.

³ Leonard, S. L., *Endocrinology*, 1943, **30**, 229.

⁴ Gomez, E. T., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1937, 259.

⁵ Selye, H., *Anat. Rec.*, 1940, **78**, 253.

[†] Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., Summit, N.J., kindly furnished these hormones.

rats treated immediately after hypophysectomy varied with the dosage of progesterone. A combination of 1 mg of progesterone plus 5 γ of estrogen per day for 10 days resulted in mammary growth superior to that in rats receiving estrogen alone in only 1 of 3 rats. When 2 or 3 mg of progesterone were given with the same amount of estrogen, in all of 7 cases, the mammary growth was superior to that obtained with estrogen alone. This was characterized by more numerous stimulated end buds, increased lateral buds and branches, and decidedly thickened ducts, in contrast to fewer end buds and practically no duct enlargement when estrogen alone was given. The stimulated glands showed a summation of the effects of the individual hormones.

If treatment with the combined hormones was not begun immediately after hypophysectomy, no growth was obtained. Six rats hypophysectomized for 4-8 days and receiving 3 mg progesterone plus 5 γ of estrogen daily for 10 days, failed to show any evidence of growth. In no way could they be distinguished from control glands receiving only estrogen or from those of uninjected controls.

In the next experiment, desoxycorticosterone acetate was injected in daily doses of .75-1.0 mg for 10 days into 5 immature hypophysectomized rats starting immediately after the operation. These rats were of such age and weight as to be sensitive to injections of estrogen or progesterone under the conditions of the experiment. This hormone was without effect, for the mammary glands of the injected rats could not be distinguished from those of the uninjected controls.

The androgenic action of progesterone as reported by Nelson⁶ was also observed in the rats receiving immediate treatment in that the testes of the hypophysectomized injected animals were 250-300% heavier than those of the untreated hypophysectomized controls. No effect was noted on the ovarian or adrenal weights.

Discussion. Progesterone, like estrogen, was found capable of inducing some mammary

growth in immature hypophysectomized rats in a 10-day period, provided the dosage was adequate and was begun immediately after the operation. The type of growth, on the whole, resembled that obtained with estrogen in that end buds were stimulated, but in addition, the thickening of the duct system was characteristic of progesterone action. If the treatment was postponed following the operation, no growth was observed.

✓ The combined estrogen and progesterone injections induced greater growth than either hormone alone in the animals receiving immediate treatment, but it should be emphasized that under no circumstances did the gland development approach that found in a normal animal. The effects of these hormone injections became significant only when the glands of treated animals were compared with those of untreated controls where, in the latter group, nothing but naked ducts were found. When treatment was postponed, the combined hormones were unable to stimulate growth. ✓

Desoxycorticosterone acetate, which is capable of stimulating mammary growth in normal mice⁷ or rats,⁸ or in hypophysectomized mice,¹ was found inactive in hypophysectomized rats. The mammary glands of the rats selected for these experiments were capable of responding to estrogen, yet this cortical hormone was ineffective. Previously, it was reported that older hypophysectomized rats would not respond to desoxycorticosterone.⁸

Species differences between hypophysectomized rats and mice in their responses to estrogen³ also occur for progesterone. Gardner¹ reported that estrogen plus progesterone or progesterone alone, was capable of stimulating the hypophysectomized mouse mammary gland any time from 1-89 days after the operation. In the rat, the conditions seem to be limited in which these hormones will stimulate the glands.

In the normal or castrate rat with pituitary

⁷ Van Heuverswyn, J., Folley, S. J., and Gardner, W. U., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 389.

⁸ Leonard, S. L., and Reece, R. P., *Endocrinology*, 1942, **30**, 42.

⁶ Nelson, W. O., *Anat. Rec.*, 1936, **67**, 110 (Suppl.).

intact, it had previously been found^{9,10} that total doses of 16-48 mg of progesterone with or without estrogen over periods of 8-20 days were ineffective in stimulating mammary development above that of estrogen alone. Inasmuch as the growth obtained with similar doses in the present work with hypophysectomized rats was relatively minimal compared to normal growth, possibly any effect that might have been present in the normal rat would have been masked by the greater overall development of the normal gland. That this is likely is suggested by the fact that doses of 150 mg of progesterone over 10 days in spayed rats, or 225 mg over 15 days in rats spayed 9 days before injections, produced distinct mammary development with lobule formation.¹¹

Other workers have reported negative effects of progesterone on hypophysectomized rat mammary glands.^{4,5} In the latter work a total amount of 100 mg of progesterone over a 10-day period or 200 mg over a 20-day period was used without securing growth. As the weights of the rats employed were some-

what heavier than those used in the experiments reported here, it would seem that the conditions necessary for new growth with progesterone are similar to those required for estrogen stimulation.

Progesterone exhibited androgenic activity in that it prevented the normal rate of atrophy of the testes following hypophysectomy, an action similar to that of testosterone. Although testosterone³ and progesterone were found capable of increasing the thickness of the mammary ducts, testosterone was found to be more potent. A dosage of 6.25 mg of testosterone gave better results than 30 mg of progesterone in this respect.

These studies, while indicating that the hypophysectomized rat mammary gland may be stimulated by certain steroids, emphasize the necessity of adequate pituitary hormones to produce a fully developed mammary gland.

Summary. Hypophysectomized rats were treated immediately or several days after the operation with progesterone alone or in combination with estrogen and with desoxycorticosterone acetate. The mammary glands of these animals were stimulated with progesterone and estrogen alone or in combination only when treatment was begun immediately. The effects of the combined hormones revealed a summation of the individual responses. Desoxycorticosterone was ineffective.

⁹ Selye, H., Browne, J. S. L., and Collip, J. B., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 472.

¹⁰ Astwood, E. B., Geschichter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, **61**, 373.

¹¹ Selye, H., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 343.

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Local Maintenance of Spermatogenesis in Hypophysectomized Rats with Low Dosages of Testosterone from Intratesticular Pellets.*

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The maintenance of spermatogenesis in hypophysectomized rats by androgen administration, first reported by Walsh *et al.*,¹ has been repeatedly confirmed. Reinitiation of

spermatogenesis by this treatment has also been reported by Nelson.² In all of these experiments the androgen has been administered in regions of the body distant from the testes. It seemed not improbable that androgen of testicular origin (testosterone) might

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¹ Walsh, E. L., Cuyler, W. K., and McCullagh, D. R., *Am. J. Phys.*, 1934, **107**, 508.

² Nelson, W. O., *Anat. Rec.*, 1941, **79**, Suppl., 2, 48.