

Effect of Simultaneous Administration of Growth Complex and Estradiol on Mammary Gland of Hypophysectomized Rat.

IRA T. NATHANSON, DARREL T. SHAW AND CLIFFORD C. FRANSEEN.
(Introduced by Joseph C. Aub.)

From the Laboratories of the Collis P. Huntington Memorial Hospital, Harvard University.

In a study of the effect of sex hormones on the growth and development of the mammary gland of the rat, it was found by Reece, *et al.*, that estrogens were not capable of stimulating the gland in the absence of the hypophysis.¹ It was postulated by these investigators that the hormones acted upon the mammary gland by way of the pituitary gland. On the other hand, Astwood, *et al.*,² suggested that this failure to react to a known stimulus might be due in part to the nutritional deficiency which resulted as a consequence of hypophysectomy. The present experiment was undertaken therefore to ascertain whether the mammary gland could be stimulated in the hypophysectomized rat by the simultaneous administration of growth complex and estradiol benzoate. | A possible effect from injection of estrogens on the rate of secretion of other pituitary hormones in intact animals is eliminated by the injection of a constant amount of growth complex in hypophysectomized animals.

A total of 70 stock rats was used in this experiment. Thirty-six of these were females, of which 22 were immature. Of the 34 males, 20 were immature. These were divided further into the subgroups which are outlined below. The immature animals weighed between 50 and 70 g. The mature weighed from 150 to 175 g.

Hypophysectomy on the animals which were subjected to the procedure was performed by the parapharyngeal route, using a technic previously described.³ At the completion of the experiment the pituitary fossa of each of the animals was examined with a magnifying loupe to verify the fact that hypophysectomy had been

¹ Reece, R. P., Turner, C. W., and Hill, R. T., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 204.

² Astwood, E. B., Geschickter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, **61**, 379.

³ Franseen, C. C., Brues, A. M., and Richards, R. L., *Endocrinology*, 1938, **23**, 292.

complete. Daily weights were recorded on all animals during the course of the experiment. The gonads in both sexes showed complete atrophy at the termination of the experiment.

Estradiol benzoate was injected subcutaneously in daily doses of 1000 I.U. in 0.1 cc of sesame oil. The growth complex, after dilution with normal salt solution, was administered intraperitoneally daily in 2 divided doses equivalent to 1/50 cc of the extract (2 rat units).*

The hormones were given to the various animals in periods varying from 3 to 5 weeks. They were then sacrificed at intervals during the latter period when one could be reasonably certain that the changes resulting from hypophysectomy *per se* were complete. Whole mounts of the breasts were then made and examined.

Results: Group I—Control. Mature males, 2. Immature males, 2. Mature females, 3. Immature females, 3. The breast in this group showed typical development dependent upon the sex and stage of maturity.

Group II—Hypophysectomy Only. Mature males, 2. Immature males, 2. Mature females, 2. Immature females, 2. In this series the breast showed almost complete atrophy when the animals were sacrificed 3 to 4 weeks after hypophysectomy.

Group III—Hypophysectomy plus Growth Hormone. Mature males, 2. Immature males, 3. Mature females, 2. Immature females, 2. Injections of growth complex as outlined above were started 6 hours after hypophysectomy and continued for 3 to 4 weeks. The mature rats maintained their pre-treatment weight levels. The immature animals gained about 2 g per day until about the third week. Both groups after this period began to lose weight. Each animal was sacrificed as soon as weight loss became evident. The breasts of these animals showed almost complete atrophy.

Group IV—Estradiol Benzoate only. Mature males, 2. Immature males, 2. Mature females, 2. Immature females, 2. Injections

* We are indebted to Mr. Joseph Hutchinson of Ayerst, McKenna & Harrison for a generous supply of growth complex, and to Drs. Gregory Stagnell and Max Gilbert of the Schering Corporation for liberal quantities of estradiol benzoate. The growth complex, according to information received from the manufacturers, contained 100 Collip units of growth factor, 10 Collip units of adrenotropic factor, and 10 bird units of prolactin per cc. One growth unit is defined as that quantity which, when administered in two divided doses over a period of 10 to 15 days by intraperitoneal injection into recently hypophysectomized but otherwise untreated rats weighing between 80 to 120 g, causes an increase in body weight of one g per day. Our experiments check their findings in regard to growth hormone.

of estradiol benzoate were given daily as outlined. All animals were sacrificed between 3 and 5 weeks after the onset of the experiment. Examination of the breasts revealed marked ductal and acinar proliferation and in some instances cyst formation.

Group V—Hypophysectomy plus Estradiol Benzoate. Mature males, 2. Immature males, 2. Mature females, 3. Immature females, 3. Estradiol benzoate was injected daily commencing one day after operation. All animals were sacrificed between the third and fifth week. The breasts of all animals showed the usual atrophy, thus corroborating the work of the other investigators.

Group VI—Hypophysectomy plus simultaneous administration of growth complex and estradiol benzoate. Mature males, 4. Immature males, 7. Mature females, 4. Immature females, 9. Injections of growth complex were commenced 6 hours after operation, and continued daily thereafter along with estradiol benzoate in the usual dose. The injections were continued for 3 to 5 weeks. The animals were sacrificed at intervals during the last 2 weeks of the experiment. This group can be subdivided on the basis of weight change.

Subgroup A. Eight animals in which there was a definite gain in weight following operation. These were sacrificed at the first discernible sign of weight loss. Examination of the breasts in the group revealed a degree of development and activity which approached those of the untreated controls.

Subgroup B. Six animals,—no change from preoperative weight. The breasts in this group were intermediate between the normal controls and the atrophic changes seen after hypophysectomy alone.

Subgroup C. Ten animals—in which weight loss varied from 8 to 26 g from the preoperative level; the mature animals having the greatest weight loss. Examination of the breasts in this group revealed changes which varied in degree between those seen in subgroup B and the group with hypophysectomy only.

It would seem from the results given above that the nutrition of the animal is an important factor in determining the effect of estrogens upon the growth and development of the mammary gland in the rat. This is obvious since breast development and maintenance of the fully developed gland after simultaneous injection of growth complex and estradiol benzoate in the hypophysectomized group closely paralleled weight gains and losses. We have no evidence that it is the sole factor, however, since in no instance did the breasts of these animals approach the degree of proliferation noted in intact animals treated with estrogen alone. It seems, therefore, that the

hypothesis formulated by the other workers that the sex hormones act through the pituitary to produce typical changes in the breast is a likely possibility for at least a part of the changes.

We have no explanation for the wide variations in weight in the hypophysectomized animals which were treated at the same time with identical doses of growth complex and estradiol benzoate. Although no microscopic examination was made, the pituitary fossa was examined carefully at autopsy in each instance for evidence of fragments of the anterior pituitary gland. As has been stated above, many of the animals gained or maintained their initial weight after the administration of growth complex. After about 3 weeks, however, they commenced to lose weight rather rapidly. It is reasonable to assume, therefore, that the hypophysis was effectively removed. Several possibilities suggest themselves: (a) The large doses of estrogen may be toxic to the animal; (b) estrogens may inhibit the action of the growth hormone; (c) the animal may become refractory to growth complex. In another experiment we have evidence that the second view is the more tenable. This is also fortified by the work of Zondek, who was able to stunt the growth of immature mice by injecting rather large doses of estrogenic hormone, which probably reduced the anterior pituitary secretion. Experiments to clarify these points are being carried out at present by using much smaller doses of estrogen in combination with the same dose of growth complex.

Conclusion. Nutrition of the animal is an important factor in the effects noted on the breasts of hypophysectomized rats after injection of estrogenic hormone. It is probable, however, that this hormone exerts a part of its effect on the breast by way of the hypophysis.

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Natural Vitamin K and Synthetic Vitamin K₁.

S. ANSBACHER, ERHARD FERNHOLZ AND H. B. MACPHILLAMY.

From The Squibb Institute for Medical Research, New Brunswick, N. J.

Several investigators¹⁻⁴ described in preliminary form the synthesis of 2-methyl-3-phytyl-1,4-naphthoquinone and its identification with vitamin K₁. In regard to their respective biological ac-

¹ Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 2557.

² Binkley, S. B., Cheney, L. C., Holcomb, W. F., McKee, R. W., Thayer, S. A., MacCorquodale, D. W., and Doisy, E. A., *J. Am. Chem. Soc.*, 1939, **61**, 2558.

³ Fieser, L. F., *J. Am. Chem. Soc.*, 1939, **61**, 2559.

⁴ Fieser, L. F., *J. Am. Chem. Soc.*, 1939, **61**, 2561.