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Growth of Mammary Glands of Hypophysectomized Rats Following Estrogen and Lactogen Administration.*

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It is well established that estrogens will stimulate duct growth of mammary glands in either normal or castrated animals (Turner[1]). Estrogens, however, are unable to cause growth of the mammary duct system in rats following ablation of the hypophysis and therefore a pituitary factor appears to be involved. In addition there is evidence that the pituitary factor is of a protein nature and that it may be the lactogenic hormone. The literature on this subject has been reviewed by Mixner and Turner.[2]

We have been interested in determining the effectiveness of estrogen in combination with the lactogenic hormone in producing mammary duct growth in the hypopyhysectomized rat. Previous experiments in the rat[3] were negative, but positive results were obtained in the mouse[4] and in the guinea pig.[5]

Forty-eight castrated and hypophysectomized rats were used in the study here reported. In general both operations were performed at the same time but in some instances a short interval occurred between operations. Body weight at the time of hypophysectomy ranged between 72 and 167 g.

In all cases, 5 days was allowed to elapse after hypophysectomy. The hormone or hormones were injected subcutaneously once daily for 10 days (in most cases), and the animals were autopsied 24 hours after the last injection. All pituitary capsules were serially sectioned, stained with Mallory's connective tissue stain, and examined microscopically. Only data from completely hypophysectomized rats are presented. At autopsy the right abdominal mammary gland was removed from each rat. These glands were stained and mounted *in toto*. The mammary glands were examined under a dissecting microscope and rated on the basis of response. Glands with either thin and bare ducts or thin ducts and a few regressing lateral buds were given a negative rating (—); a one plus (+) rating was assigned glands with lateral and end buds and glands with a thickened duct system either with or without lateral buds; and a two plus (++) was given to glands showing an extension of the duct system with enlarged end buds.

Estradiol dipropionate (Progynon—DP†) was the estrogen of choice; however, 3 different lactogenic hormone preparations were used. These preparations are designated as follows: lactogen A—a purified hormone (Prolactin—Schering†) containing 35 I.U. per mg; lactogen B—a purified hormone (Schering†) containing 14 I.U. per mg; and lactogen C—a purified hormone (Difco Prolactin‡) containing 25 I.U. per mg.

The experiments are considered by groups.

Group I. Three rats received no injections. Their mammary glands consisted of a thin and bare duct system (—).

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1 Turner, C. W., *Sex and Internal Secretions*, 1939, Chapter 11 (E. Allen, ed.), Williams and Wilkins Co., Baltimore.

2 Mixner, J. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1943, 378.

3 Nelson, W. O., and Tobin, C. E., *Anat. Rec.*, 1936, **67** (Suppl. 1), 111.

4 Gardner, W. U., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 590.

5 Gomez, E. T., *Endocrinology*, 1942, **31**, 613.

† The estradiol dipropionate (Progynon-DP) and two lactogenic hormone preparations (Prolactin-Schering and Schering) were generously supplied by Dr. E. Schwenk of the Schering Corp., Bloomfield, N.J.

‡ One lactogenic hormone preparation (Difco Prolactin) was generously supplied by Dr. Robert W. Bates of Difco Laboratories, Inc., Detroit, Mich.

TABLE I.
Mammary Gland Growth in Hypophysectomized Rats Following Estrogen and Lactogen Administration.

Group	Treatment: Estrogen, μg	Treatment: Lactogen, I.U.	Test period (days)	No. of rats	Mammary† response: —	+	++
I	Control		10	3	3		
II	1*	0	10	9	9		
III	0	35-A‡	9-10	4	2	2	
IV	1*	35-A	8-10	8	3	4	1
V	1		7-10	7	7		
VI	0	70-B§	10	2		1	1
VII	0	70-C¶	10	2		2	
VIII	1	70-A	7-10	3		2	1
IX	1	70-B	10	2			2
X	1	70-C	10	8			8

* Injected every other day.

† — = glands with either thin and bare ducts or thin ducts and a few regressing lateral buds; + = glands with lateral and end buds and glands with a thickened duct system either with or without lateral buds; and ++ = glands showing an extension of the duct system with enlarged end buds.

‡ A—A purified hormone (Prolactin-Schering) containing 35 I.U. per mg.

§ B—A purified hormone (Schering) containing 14 I.U. per mg.

¶ C—A purified hormone (Difco Prolactin) containing 25 I.U. per mg.

Group II. Nine rats were injected with 1 μg of estrogen every other day for 10 days. None of the mammary glands showed signs of growth (—), all glands showing involutionary changes. Six of the 9 glands were similar to those of the controls, whereas in the remaining 3 glands a few lateral buds were still present.

Group III. Four rats received daily for 9-10 days 35 I.U. of lactogen A. Two of the mammary glands were similar to those of the controls (—), whereas the remaining 2 glands had lateral buds and a slightly thickened duct system (+).

Group IV. Eight rats received 35 I.U. of lactogen A daily for 8-10 days and 1 μg of estrogen every other day. The results are inconsistent. Three glands had not been stimulated (—), 3 showed a thickened duct system with many lateral buds (+), one showed many lateral and end buds (+), and one gland exhibited growth similar to that seen in the intact rat following estrogen treatment (++).

Group V. Seven rats were injected with 1 μg of estrogen daily for 7-10 days. The mammary glands of 5 rats consisted of a bare duct system (—); and, in the remaining 2 rats the glands were involuting, though lateral buds were still present (—).

Group VI. Two rats received 70 I.U. of lactogen B daily for 10 days. The glands from both rats showed a slightly thickened duct system, one gland having many lateral buds (+) and the other gland numerous lateral buds as well as proliferating end buds (++).

Group VII. The daily injection of 70 I.U. of lactogen C for 10 days into 2 rats caused a slight thickening of the ducts which were without buds (+).

Group VIII. Three rats received 70 I.U. of lactogen A and 1 μg of estrogen daily for 7-10 days. Lateral buds were present in 2 of the glands (+), and the third gland showed numerous lateral buds and enlarged end buds (++).

Group IX. The mammary glands of 2 rats injected daily for 10 days with 70 I.U. of lactogen B and 1 μg of estrogen showed stimulation similar to that seen in intact rats following estrogen injections (++).

Group X. The daily injection of 70 I.U. of lactogen C and 1 μg of estrogen into 8 rats for 10 days produced duct growth of the mammary glands in each instance. Five of the mammary glands exhibited enlarged end buds and numerous lateral buds (++). Enlarged end buds were observed in the remaining 3 glands (++), otherwise the

ducts were bare. Two of these 3 glands were from rats that had lost weight during the experiment. This latter condition of the mammary glands is similar to that observed by Reece and Leonard[6] following either estrogen injection into partially hypophysectomized (minute fragment) rats or estrogen and growth hormone injections into hypophysectomized rats. The results are summarized in Table I.

These results indicate again that estrogens will not stimulate duct growth of the mammary glands of hypophysectomized rats. The main action of lactogen, when injected alone, appears to be that of causing a thickening of the duct system along with a tendency to prevent the regression of the lateral buds. In a single case, lactogen injection caused end buds to proliferate. Our observations suggest that the lactogenic hormone is the pituitary factor which permits mammary duct growth in the rat. These observations agree with other known facts, since it has been shown that the lactogen content of the pituitary gland increases considerably at sexual maturity,[7] a time when the mammary duct system is rapidly proliferating. Moreover, estrogen injections into ovariectomized rats augment the lactogen content of the pituitary gland,[8] making possible the development of the duct system.

Summary. In castrated-hypophysectomized rats estrogen did not stimulate mammary duct growth. The injection of lactogenic hormone caused a thickening of the duct system and in combination with estrogen it induced mammary duct growth similar to that seen in intact rats following estrogen injections.

[6] Reece, R. P., and Leonard, S. L., *Endocrinology*, 1941, **29**, 297.

[7] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1937, 266.

[8] Reece, R. P., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 283.

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Serologic Agglutination of Antigen-Coated Dye Particles.

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Previous reports[1,2] have indicated the possible utility of a serologic reaction wherein the antigen as *e.g.*, horse serum or neurotropic virus, has been adsorbed to heat killed bacterial cells (*Serratia marcescens*) and in this form, employed as an agglutinable suspension. The technic of the test (B.A. or bacterial agglutination method) in its present state of development[3] is one, which for satisfactory operation, particularly with viral antigens, requires that scrupulous attention be given to various details in its performance. Some difficulty may be experienced also in observing a reaction, even under optimal conditions, since the masses of agglutinated bacteria are very small and may not be easily recognized. It was considered that certain of the critical limitations or deficiencies of the B.A. method could be obviated by substituting for *S. marcescens* cells, other types of particulate matter possessing similar adsorptive capacities but of units larger in dimension. Also it was expected that a particulate substance might be found which would serve the purpose without involving some of the disadvantages encountered in the use of collodion pellets[1] as for instance, the necessity for careful removal of electrolyte from antigenic material, by dialysis, as a preliminary to its adsorption. Preliminary experiments indicated that par-

[1] Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 11.

[2] Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 52.

[3] Roberts, E. C., *J. Immunol.*, 1945, **50**, 55.