

Observations on Mammary Growth in Parabiotic Rats.*

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Although the use of parabiotically joined animals for investigations of the hormonal control of mammary growth has been limited,^{1,2} this method seemed to offer an approach to the problem of the nature of the anterior pituitary mammotropic substance and of the mechanism of interaction of hypophyseal and sex hormones on the mammary glands. The preliminary results of such a study on the mammary gland of the rat are here presented.

Methods. Littermate rats of the same or different sexes were joined parabiotically when 23 to 46 days old. In most cases one or both members of a pair were castrated a few days previously. A modification of the method of parabiosis described by Bunster and Meyer³ was employed. Coelomic cavities remained separate. After one to 3 weeks a castrated member of each pair was hypophysectomized. In most cases 10 γ of estradiol dipropionate⁴ were injected daily for 10 days into one member of the pair, usually the hypophysectomized one. Single hypophysectomized rats of comparable age served as controls. At autopsy the inguinal mammary glands were prepared as whole mounts, and thyroids and adrenals were weighed and fixed. Completeness of hypophysectomy was verified by histological examination of the sellae turcicae.

Results. Mammary glands of single hypophysectomized control rats consisted only of slender, naked ducts. In the mammary glands of 21 of 24 hypophysectomized rats living in parabiosis with rats with hypophysis intact new growth occurred or regression was pre-

TABLE I.
Mammary Responses in Estrogen-Treated Hypophysectomized Rats in Parabiosis with Rats with Intact Hypophyses.

Combination of parabionts			Mammary responses in hypophysectomized rat ¹					
Group	Hypophysectomized		Partner	++ + + + + +				
	rat			++	+	+	0	
I	♂ castrate		♀	5	4	2	0	
II	♂ "		♀ castrate	0	0	0	1	
III	♂ "	♂ "		0	0	5	0	
IV		♀ ²	♀	0	0	2	1	
V	♀ castrate		♀	1	0	0	0	
VI	♀ "		♀ castrate	1	0	1	1	

¹ Mammary responses have been evaluated as follows: +++, greatest growth; ++, moderate growth; +, slight growth or prevention of regressive changes; and 0, no prevention of regression.

² No estrogen injected.

vented (Table I). This response was evidenced by growth of ducts and end buds, thickening of the ducts, and the presence of numerous lateral buds. The cases of greatest growth were all confined to pairs wherein the animal with pituitary intact was a female or female castrate. Weights of adrenals and to a lesser extent of the thyroids of hypophysectomized parabionts exceeded those of single hypophysectomized rats of similar size although they did not equal those of normal animals.

Discussion and conclusions. Since estrogen does not stimulate mammary growth in hypophysectomized rats of the age and size of those used in these experiments,^{4,5,6} it may be concluded that some pituitary gland substance must have crossed from the partner with hypophysis intact to the hypophysectomized partner and there made possible the mammary growth observed. In this respect the hormone which possesses mammogenic potency is shown

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¹ Matsuyama, R., *Frankf. Z. Path.*, 1921, **25**, 436.

² Hill, R. T., *J. Exp. Zool.*, 1932, **63**, 203.

³ Bunster, E., and Meyer, R. K., *Anat. Rec.*, 1933, **57**, 339.

⁴ The hormone was supplied by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc., Summit, N. J.

⁴ Selye, H., Collip, J. B., and Thomson, D. L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1377.

⁵ Reece, R. P., Turner, C. W., and Hill, R. T., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 204.

⁶ Leonard, S. L., *Endocrinology*, 1943, **32**, 229.

to resemble other known pituitary hormones of protein nature. Such passage between parabiotic animals of gonadotropic, adrenotropic and growth hormones has been previously described^{7,8,9} and was also observed in the present experiments. The steroid hormone estrogen, on the contrary, failed to be transferred to the partner.⁸ If the pituitary mammotropic substance has a lipoid character

as is reported by Lewis and Turner¹⁰ it is perhaps surprising that its behavior in parabiosis seems to link it more with the protein hormones than with the chemically more similar estrogen.

Since the pituitary gland of one rat was serving both members of a pair the amount of mammotropic factor which crossed to the hypophysectomized partner must have been small; yet this quantity was sufficient to alter the mammary response to estrogen from an unqualified negative to a definite positive. Furthermore the fact that the greatest mammary growth occurred where a female was furnishing the mammotropic hormone to the hypophysectomized parabiont suggests a sex difference in production and release of this hormone.

⁷ Møller-Christensen, E., *Acta Path. et Microbiol. Scand.*, 1933, **10**, 296.

⁸ Van Dyke, H. B., *The Physiology and Pharmacology of the Pituitary Body*, University of Chicago Press, 1936, 1939.

⁹ Greep, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 214.

¹⁰ Lewis, A. A., and Turner, C. W., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 435.

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Cell Count, Rate of Flow, and Protein Content of Cervical Lymph in the Rat.*

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Previous studies^{1,2} have shown that it is possible to collect thoracic duct lymph from the anaesthetized rat in amounts sufficient to carry out cell counts and chemical analyses. It was noted in these studies that there was an evident flow of lymph from the cervical lymphatic trunk in the preparations employed. Since spontaneous flow of lymph from the periphery (head and neck or extremities) of quiescent animals is unusual,³ it is thought noteworthy that there is, in the anaesthetized

rat, a steady flow of lymph from the main lymphatic trunks draining the head and neck. The present report describes a method of cannulation of the cervical lymphatic trunk, and presents data on the rate of flow, white cell count, and protein content of the lymph obtained. This data is compared with further measurements of the same type for thoracic duct lymph.

Technic of collection of cervical lymph. Adult female rats of the Long-Evans strain, unfasted, weighing between 250-300 g, were anaesthetized by intraperitoneal injection of a 1% solution of sodium pentobarbital. A midline incision was carried through skin and subcutaneous tissue from the symphysis menti to the sternal angle. The incision was carried sufficiently deep to expose the sternohyoid muscles. All superficial neck tissues were retracted laterally. The manubrium sterni was split lengthwise in the midline, using bone

* Assisted by grants from the Research Board of the University of California and the Rockefeller Foundation of New York City.

¹ Reinhardt, W. O., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 123.

² Reinhardt, W. O., and Li, C. H., *Science*, 1945, **101**, 360.

³ Drinker, C. K., and Yoffey, J. M., *Lymphatics, Lymph and Lymphoid Tissue*. Harvard University Press, Cambridge, Mass., 1941.