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Effect of Pituitary Growth Hormone on the Thymectomized Rat.*

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The problem of the relationship of the thymus gland to growth is one which has received considerable attention in the last decade. On the one hand, experimental work in the laboratories of Asher¹ and of Rowntree² employing thymus gland extracts has given presumptive evidence of a growth-promoting agent in the thymus gland. On the other hand, it has been observed in this laboratory³ that growth hormone preparations usually cause enlargement of the thymus gland in both hypophysectomized and normal animals. Further, adrenocorticotrophic hormone has been demonstrated to cause thymic atrophy while, at the same time, under certain experimental conditions, causing inhibition of somatic growth.⁴ The question has arisen, then, as to whether or not the growth hormone is "thymotropic" and whether its effect in stimulating growth is mediated by the thymus gland. The experimental evidence presented in this paper attempts to answer the latter part of this question.

Effect of growth hormone on hypophysectomized-thymectomized female rats. Female rats of the Long-Evans strain thymectomized when 8 days of age and weaned at 21 days of age, were allowed to grow until 84 days of age at which time they were hypophysectomized. The animals were allowed to plateau after hypophysectomy until the age of 104 days when injections of growth hormone were begun and continued for 20 days (excluding Sundays) at which time the animals were autopsied. The animals were weighed twice weekly to the time of autopsy. Hypophysectomized littermates which had been subjected to a sham thymectomy at the same time as the other experimental animals were also injected with the same growth hormone preparation under the same experimental condi-

* Aided by grants from the Board of Research of the University of California and the National Research Council Committee for Research in Endocrinology.

¹ Asher, L., *Endokrinologie*, 1930, **7**, 321.

² Rowntree, L. G., Clark, J. H., Hanson, A. M., and Steinberg, A., *Arch. Int. Med.*, 1935, **56**, 1.

³ Fraenkel-Conrat, H. L., Meamber, D. L., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1940, **27**, 605.

⁴ Crede, R. H., and Moon, H. D., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 44.

tions. Hypophysectomized-thymectomized littermates were used as uninjected controls. The animals were fed this laboratory's Diet XIV† throughout their life supplemented by wet Diet I‡ during the period of injection. At autopsy, the sites of the pituitary and thymus were carefully searched for evidence of remaining fragments. Any suspicious fragments were subjected to microscopic examination to check completeness of operation. None of the animals reported showed incomplete operations.

The growth hormone employed was a "globulin" fraction,⁵ prepared from the anterior lobes of beef pituitaries desiccated with acetone in the cold, extracted in alkaline solution, and precipitated with ammonium sulfate. The preparation contained adrenocorticotrophic, lactogenic and smaller amounts of interstitial cell stimulating and thyrotropic hormones. The injected animals each received intraperitoneal doses of an amount of hormone represented by 200 μ g of dry weight on the first day, 350 μ g on the second and third days, and 500 μ g daily from the fourth to the 20th day in 1.0 cc fluid. (The growth potency of the hormone preparation was 30 units⁶ per milligram when assayed in other hypophysectomized rats tested concurrently with the experimental groups.)

The results in the experimental groups of animals are presented in Table I. The growth curves of all the animals were similar to the time of injection. It is significant that there was no difference in the growth of the thymectomized animals as compared to the sham operated littermate controls.

The evidence presented indicates that in hypophysectomized animals growth hormone is equally effective either in the absence or presence of the thymus gland.

Effect of growth hormone on adult thymectomized female rats with intact pituitaries. In the second part of this experiment it was proposed to determine whether or not thymectomy would influence the response of plateaued female rats (with intact pituitaries) to a

† Diet I: 67.5% whole wheat (ground); 15% casein; 10% whole milk powder; 1% sodium chloride; 1.5% calcium carbonate; 5% milk fat (melted butter).

Diet XIV: 67% whole wheat (ground); 5% fish oil; 5% casein; 10% alfalfa leaf meal; 10% fish meal; 3% sodium chloride supplemented with fresh lettuce twice weekly.

⁵ Marx, W., The method, to be published in detail, is a modification of the procedure published in 3.

⁶ Evans, H. M., Uyei, N., Bartz, Q. R., and Simpson, M. E., *Endocrinology*, 1938, **22**, 483. A unit is defined as the amount of a growth hormone preparation which causes an average body weight gain of one g per day (10-day test, hypophysectomized rat).

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TABLE I.

Type of animal and treatment	No. of animals	Avg initial B.W.(g)	Gain in B.W. (g)*	Avg gain in % of initial B.W.
Hypophysectomized, treated with growth hormone	4	155	50 43 <i>49</i> 53 50	32
Hypophysectomized—thymectomized, treated with growth hormone	6	153	34 30 <i>42</i> 52 50 52 36	28
Hypophysectomized—thymectomized, untreated	5	149	—4 0 <i>0</i> 3 —3 4	0

*Figures in italics represent the average weight gain of the group.

potent growth hormone preparation. Female rats thymectomized at the age of 8 or 9 days and weaned at 21 days of age were allowed to grow until the usual plateauing of the growth curve had occurred. At the age of about 6 months, the animals were injected with the same preparation of growth hormone, receiving daily intraperitoneal injections of 1.0 cc solution containing 2.5 mg of the preparation for 20 days (excluding Sundays). The animals were weighed weekly until the time of injection and twice weekly during the time of injection. Thymectomized and sham operated littermates served as untreated controls and in addition one group of sham operated littermates was injected with the same amount of growth hormone to determine any difference in response which might occur depending on the presence or absence of the thymus gland. At autopsy, all suspicious tissue in the neck regions of the thymectomized animals was subjected to microscopic examination to check the completeness of operation. In no case in the groups reported was a fragment of thymus gland found in the normal position. A few of the animals suffered from superficial wound infections during the first postoperative week. The growth of these animals did not differ from that of their controls.

The growth curves of all the animals were similar, there being no significant difference up to the time of growth hormone injection between the thymectomized animals and their sham operated littermate controls. The response of these groups to the injection of growth hormone is summarized in Table II.

As indicated, there is no significant difference in growth either in

TABLE II.

Type of animal and treatment	No. of animals	Avg initial B.W. (g)	Gain in B.W. (g) *	Avg gain in % of initial B.W.	
Normal control, untreated	5	256	33	24	9
			26		
			26		
			24		
			11		
Thymectomized, untreated	7	238	28	26	11
			18		
			32		
			18		
			17		
			26		
			42		
Normal, treated with growth hormone	6	225	44	57	25
			63		
			54		
			68		
			68		
			47		
Thymectomized, treated with growth hormone	8	239	60	64	27
			50		
			45		
			49		
			71		
			81		
			61		
			98		

*Figures in italics represent the average weight gain of the group.

the treated or untreated groups when the thymectomized animals are compared with their controls. The increase in weight of the control groups after a time in which they were plateaued may, in all probability, be ascribed to the change in environment and diet. At the beginning of the experimental period of growth hormone injection the diet of all animals was changed from Diet XIV to a combination of Diet XIV and Diet I, and the animals were moved to a different location in the colony.

Discussion. The experimental evidence presented allows one to state that under the conditions of the experiment, the presence of the thymus gland is not essential to the normal growth in body weight of the female rat in the time limits employed (8 days to 6 months). Nor is the thymus gland necessary for the marked increase in body weight produced by a potent growth hormone preparation when injected into female rats either in the presence or absence of the animal's own pituitary gland. The evidence presented here does not bear on the claim that one may extract from the thymus gland a substance or substances possessing growth promoting properties.

Whatever these may be, it is evident that they have not played a detectable rôle in the experiments here reported.

Summary. 1. The growth in body weight of female rats thymectomized at the age of 8 days and followed to the age of 6 months is the same as that of sham operated littermate controls. 2. The response of hypophysectomized-thymectomized female rats to a preparation of the pituitary growth hormone is the same as that of similarly treated hypophysectomized littermate controls which had been subjected to a sham thymectomy. 3. The response of plateaued thymectomized female rats with intact pituitaries to a potent growth hormone preparation of the anterior pituitary is the same as that of sham operated littermate controls. 4. Under the conditions of this experiment, therefore, the thymus gland was not necessary either for the growth in weight of otherwise normal animals or for the marked increase in body weight produced by the administration of anterior pituitary growth hormone.

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Absorption of 2-Methyl-1,4-Naphthoquinone and Phthiocol by Bile Fistula Rats.*

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It is now well established that deoxycholic acid is necessary for the absorption of vitamin K. The fact that certain water soluble compounds possess vitamin K activity has raised the question as to whether or not these substances are absorbed from the intestinal tract in the absence of bile. Smith and Owen¹ have shown that oral administration of 4-amino-2-methyl-1-naphthol to patients suffering from chronic obstructive jaundice led to an increase in the level of prothrombin. Similar results were reported by Warner and Flynn² on administration of the potassium salt of the disulfuric acid ester of 2-methyl-1,4-naphtho-hydroquinone to vitamin K depleted bile-

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¹ Smith, H. P., and Owen, C. A., *J. Biol. Chem.*, 1940, **134**, 783.

² Warner, E. D., and Flynn, J. E., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 607.