pantothenic acid. This indicates that the slight activity was destroyed along with the pantothenic acid by the alkali treatment.

Two preparations of factor W were tested at levels equivalent to 250 mg of liver extract per day. The first preparation was made by extracting liver extract thoroughly with dry butanol and then precipitating out considerable material by adding an equal volume of hexane.[11] The second, a much more purified preparation, was an "acetone eluate" preparation, kindly prepared for us by Mr. Simon Black.[11] Both of these preparations were inactive. The factor which prevents achromotrichia was undoubtedly lost in the early stages of the above preparations.

In confirmation of the results of Lunde and Kringstad[2] we have observed the "rusting" of the fur of albino rats fed the above and other purified diets and also have not been able to correlate the appearance of achromotrichia with the growth of the animals.

Thus it appears that the dietary factor which prevents nutritional achromotrichia is distinct from all factors of the vitamin B complex which have thus far been identified and associated with specific function in the nutrition of the rat. Further the factor does not seem to be involved in the growth of the rat.

10878 P

Hypersecretion of Gonadotropic Hormone of Pituitary Gland of Rats Resulting from Treatment with Antigonadotropic Serum.*

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Thompson and Cushing[1] have reported that the anterior pituitary gland of a dog chronically injected over a period of months with pituitary extract had an increase in the number of basophiles associated with an atrophy of the gonads. Collip, Selye and Williamson[2] also found similar changes in rats injected with pituitary gonadotropic extracts, and Severinghaus and Thompson[3] noted a correspond-

[11] Black, S., and Elvehjem, C. A., unpublished data.

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[1] Thompson, K. W., and Cushing, H., *Proc. Roy. Soc. B*, 1934, **115**, 88.

[2] Collip, J. B., Selye, H., and Williamson, J. E., *Endocrinology*, 1938, **23**, 279.

[3] Severinghaus, A. E., and Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 627.

ing atrophy of the gonads and basophilism of the anterior pituitary of dogs injected with an antigonadotropic serum. The increase in the basophile cells of the pituitary glands of these animals suggests there might be an increase in the gonadotropic hormone secretion in contrast to the atrophic condition of the gonads. Nevertheless no attempt has been made to determine whether or not the pituitary glands of such animals secrete an increased quantity of gonadotropic hormone.

The work to be reported here is concerned with the effect upon endogenous secretion of the pituitary gonadotropic hormone in single and parabiotic rats following treatment with antigonadotropic serum.

The serum was obtained from rabbits which had been injected daily for a period of 2 months or longer with an aqueous extract of whole dried pituitary gland of sheep. Tests made with immature female rats showed that this serum can inhibit the action of gonadotropic hormone contained in the pituitary glands of human beings, sheep, horses, and rats. In a previous publication we have shown that such rabbit serum will also prevent the action of endogenous gonadotropic hormone secreted by the pituitary gland of a castrated rat in parabiosis with a normal female.[4]

Experimental Procedure and Results. 1. Twenty-four female rats were injected subcutaneously from the 10th to the 20th day of life with 0.5 cc per day or a total dose of 5 cc of antigonadotropic rabbit serum. After discontinuing the injections the day of vaginal opening of the treated rats was noted and all animals were killed 5 days after the vagina had opened. Littermate control rats were autopsied at the same age as the experimental animals. The average age of the treated rats at the time of vaginal opening was 28.5 days and the average ovarian weight 5 days later was 42 mg. The littermate control rats autopsied at the same age had an average ovarian weight of 16.1 mg. The ovaries of the experimental rats showed large cystic follicles, corpora lutea and blood points, whereas the ovaries of the control animals were juvenile in appearance.

2. Ten rats were injected from the 1st to the 11th day of life with 0.1 cc daily or 1 cc total dose of antigonadotropic rabbit serum. The vaginas of these rats opened at an average age of 21.9 days and the uteri were enlarged. The average weight of the ovaries of these animals was 16.7 mg. The ovaries of the untreated female littermates, which were killed at the same age as the experimental rats, averaged 14.2 mg. Thus, as a result of the treatment with the antigonadotropic rabbit serum the opening of the vagina was produced

[4] Kupperman, H. S., Meyer, R. K., and Hertz, R., *Endocrinology*, 1939, **24**, 115.

23-28 days before the normal time for rats of our colony. In this experiment the ovaries of the treated rats were not significantly larger than the controls but the ovaries were stimulated as shown by the effect on the vagina and uterus.

3. Five male and 5 female rats were treated from the 17th to the 27th day of life with 0.5 cc antigonadotropic serum per day. At 31 days of age, 4 days after the injections were discontinued, the treated rats were placed in parabiosis, according to the technic of Meyer and Hertz,[5] with a littermate female in such a manner that the injected experimental rat was the right hand partner and the intact littermate female the left partner. It is to be emphasized that no other treatment was given these animals. The rats were autopsied on the 41st day of life after having been in parabiotic union for 10 days. In the female-female pairs the average ovarian weight for the left untreated female was 72 mg while the gonads of the right female which received the antigonadotropic serum before being united in parabiosis, averaged 41 mg. The ovarian weight of the left female partner in the 5 female-male pairs was 92 mg while the testes of the right partner averaged 982 mg. The average ovarian weight of 7 single littermate female rats autopsied on the 41st day of life was 17 mg. The average testicular weight of 8 single littermate male rats autopsied when 41 days old was 1344 mg.

The results obtained with the single and parabiotic rats indicate that following a short time treatment of rats with an antigonadotropic rabbit serum there is a hypersecretion of gonadotropic hormone from the pituitary gland of the treated rat. This effect is not the result of the presence of gonadotropic hormone in the serum of the rabbit since it causes atrophy of the gonads as long as the injections are given. The injection of 0.5 cc of normal female rabbit serum per day into 14 rats from the 10th to the 20th day of life did not accelerate or inhibit the development of the ovaries or hasten vaginal opening.

The explanation of the precocious development of the ovaries following the treatment with antigonadotropic serum is based in part on the fact that the serum prevents the action of the gonadotropic hormone produced by the pituitary gland of the rat. As a result the gonads receive little, if any, stimulation and consequently they produce very little, if any, gonadal hormone. The absence of the gonadal hormones results in a condition analogous to castration which causes the increase of gonadotropic hormone in the pituitary gland and the blood.[5] However, the increase in the gonadotropic hormone is rendered ineffective by the excess quantity of anti-

[5] Meyer, R. K., and Hertz, R., *Am. J. Physiol.*, 1937, **120**, 232.

gonadotropic serum injected and the gonads remain in an atrophied condition. When the antigonadotropic serum is discontinued the increased gonadotropic hormone being produced by the pituitary gland is not neutralized and as a result the gonads are stimulated to develop from an atrophied condition to one much greater than that found in normal animals of the same age. Further experiments are being made to determine whether the stimulation of the gonads resulting after the treatment with antigonadotropic serum is temporary or permanent.

Summary. Pretreatment of young female rats with antigonadotropic serum induced a hypersecretion of the gonadotropic hormone of the animal's pituitary. This effect was determined by the precocious development of the ovaries of the treated rats after discontinuing the injections, and by the ovarian hypertrophy occurring in female rats paired to pretreated male or female littermates. A theory is offered to explain the results obtained.

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On the Site of Formation of Citric Acid in the Dog.

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With the advent of methods of analysis suited to the determination of small amounts of citric acid,[1-3] interest in this compound and its salts as factors in intermediary metabolism has increased. The wide distribution of citric acid in the animal body has been indicated by Smith and Orten.[4] In addition, these authors[5] have shown that, in the dog following the rapid intravenous injection of the sodium salts

* Chas. Pfizer and Company Fellow, Yale University, 1936-37.

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1 Thunberg, T., *Biochem. Z.*, 1929, **206**, 109.

2 Kuyper, A. C., and Mattill, H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 863.

3 Pucher, G. W., Sherman, C. C., and Vickery, H. B., *J. Biol. Chem.*, 1936, **113**, 235.

4 Smith, A. H., and Orten, J. M., *J. Nutr.*, 1937, **13**, 601.

5 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1937, **117**, 555.