

rats poisoned with carbon monoxide. 3. Methylene blue injections appeared to be detrimental rather than beneficial to white rats suffering from carbon monoxide poisoning.

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A Thyreotropic Hormone from Pituitary Extracts.*

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The anterior pituitary substance responsible for thyroid hypertrophy and hyperplasia has been attributed to various anterior lobe hormones or to a separate thyreotropic hormone.¹ By heating a pituitary extract (Phyone), the thyreotropic substance has been separated from the contained gonad and growth stimulating substances.†

To determine the effect of heat on the activity of the growth hormone, young mice, 18 to 23 days of age, were used. 172 animals were divided into 5 groups: Group I injected subcutaneously with phyone; group II injected with phyone heated 60°C. 15 minutes; group III injected with phyone heated 80°C. 15 minutes; group IV injected with phyone heated to boiling 15 minutes. Group V was used as controls. All animals were injected with 0.1 cc. extract daily for 5 days. The average increase in body weight in 6 days was as follows: group I, 56%; group II, 55%; group III, 37%; group IV, 34%; controls, 35%. These data indicate that the growth hormone contained is not thermostable and is inactivated at some temperature between 60° and 80°C.

To determine the effect of heat upon the activity of the gonad stimulating hormone, female rabbits, 11 to 12 weeks of age, were used. These were divided into 3 groups of 7 each: group I, injected with 0.5 cc. of phyone, twice daily for 5 days; group II, with

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¹ Riddle, O., Bates, R. W., Dykshorn, S. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 794. Anderson, E. M., and Collip, J. B., *Ibid.*, 1933, **30**, 680. Crew, F. A. E., and Wiesner, B. P., *Brit. J. Med.*, 1930, **1**, 777.

† Dr. David Klein, Wilson Laboratories, kindly supplied us with the Phyone used in this work.

1 cc. of phyone heated to boiling 15 minutes, twice daily for 5 days; group III, served as controls.

In group I all animals had hypertrophied uteri, and 6 showed from 2 to 4 corpora hemorrhagica in each ovary. No corpora hemorrhagica were present in group II, and in 6 animals the uteri were small and characteristically infantile, while the uterus of one animal, though small, showed a condition of hyperaemia. It is evident from these experiments that the gonad-stimulating hormone is inactivated by subjecting the extract to boiling temperatures for 15 minutes.

That the thyreotropic principle in phyone is still active even though the extract be heated to boiling, was determined by injection of this material into tadpoles. Injections of 0.1 cc. daily for 5 days into *R. catesbiana* larvae produces hypertrophy and hyperplasia of the thyroid gland. These glands show an emptying of the follicles of their contained colloid and a change in the follicular epithelium from the flattened to the columnar condition, indicating active secretion.

Heat treatment decreases slightly the effectiveness of the thyreotropic substance. Phyone, and phyone heated to boiling for 15 minutes, were made up to dilutions of 1:3, 1:6, 1:12, 1:24, 1:48, and 1:96. Each sample was injected into 6 tadpoles in doses of 0.1 cc. daily for 5 days. Hypertrophy and hyperplasia of the thyroid occurred in all animals receiving phyone up to and including dilutions of 1:48. Thyroids of animals receiving heated phyone showed similar hypertrophy and hyperplasia in dilutions up to and including 1:24, indicating a slight reduction in the activity of the thyreotropic substance.

Since the thyreotropic factor has been shown to be thermostable, it can be separated from the growth and gonad-stimulating substances by heat. These findings support the view that pituitary control of the thyroid is due to neither of these 2 hormones, but more probably to a separate and distinct thyreotropic hormone.