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Appearance of Vacuolated Cells in Hypophysis of *Triturus torosus* Following Bilateral Thyroidectomy.*

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This paper is a report of studies made upon the morphological response of the anterior lobe of the hypophysis following bilateral thyroidectomy in the salamander, *Triturus torosus*. The work was undertaken as a prelude to physiological studies upon amphibia designed to throw light upon the possibility of a reciprocal action between the thyroid and hypophysis. It seemed necessary to determine first whether or not the anterior lobe of a typical amphibian shows morphological changes after thyroidectomy such as have been reported by numerous investigators in the mammalia. (See Severinghaus¹ for bibliography.)

The technic of thyroidectomy was essentially that recommended by Taylor.² Darkening of the integument^{2, 3} occurred usually some 15 to 25 days after operation. Animals were killed by decapitation after periods ranging from 5 to 114 days.

All glands were fixed in Heidenhain's "Susa" (Sublimate-Trichloroacetic Formalin) and imbedded by the dioxan method. Sections were cut at 5 μ and stained in hematoxylin, acid fuchsin, and anilin blue. In several cases sections of the hypophyses from control and thyroidectomized animals were mounted on the same slide to ensure uniform treatment.

Glands from 8 controls and 33 operated animals were studied. It appears that the most conspicuous change in the anterior lobe of *Triturus torosus* following thyroidectomy is the development of vacuoles in the cytoplasm of certain basophilic cells. The exact classification of these cells has not yet been worked out but it is supposed that they are degranulated basophiles.

The vacuoles apparently first make their appearance in small clusters in one or 2 localized areas of the cytoplasm. They may be in close association with the nucleus or considerably removed from it, all on one side of the nucleus or scattered around it. From their

* I wish to express my sincere appreciation of the generous advice and encouragement given by Dr. Bennet M. Allen during the course of this study.

¹ Severinghaus, A. E., *Physiol. Rev.*, 1937, **17**, 556.

² Taylor, A., *J. Exp. Zool.*, 1936, **73**, 153.

³ Adams, A. E., Kuder, A., and Richards, L., *J. Exp. Zool.*, 1932, **63**, 1.

distribution it is hard to see how the Golgi material could be concerned in their formation. Cells in this early stage of vacuolation tend to be more numerous after short periods of thyroidectomy but are regularly present also after longer periods.

The small vacuoles seem to coalesce fairly rapidly in later stages into larger ones of usually perfectly circular outline. These vary in size and number. In extreme cases they may occupy very nearly the entire cytoplasm and press in upon the nucleus. As many as 3 of these large vacuoles have been observed in a single cell.

The time of the beginning of the vacuolation process has been roughly estimated. The thyroidectomized animals may be segregated into 2 groups, 10 animals kept 20 days or less after operation, and 23 animals kept 26 days or more after operation. In the group of shorter duration only 2 animals developed vacuoles. Of these 2 one had been kept 15 days and the other 20 days after operation. In both cases the vacuolation was slight. Careful study of the glands of 8 other animals kept 13 to 20 days after thyroidectomy, as well as of 8 normal controls, revealed no significant vacuolation.

In the group of longer duration of thyroidectomy (26 to 114 days) only 4 out of 23 animals failed to show marked vacuolation. These 4 were also the only animals in the group which did not undergo the darkening of the integument characteristic of the thyroidectomized salamander.⁴ On this basis, and because thyroid remnants were found in one of these animals, it is believed that they were incompletely thyroidectomized.

Thus it may be said that 19 animals kept longer than 26 days after operation and rendered sufficiently hypothyroid to induce darkening of the integument showed vacuolation, while 8 animals kept less than 21 days failed to show vacuolation and 2 only bare beginnings. It is concluded that the vacuolation process in the anterior lobe first becomes evident about 3 weeks after thyroidectomy.

A second change has been noted in these same basophilic cells in 8 of the 19 glands which showed cytoplasmic vacuolation. The nuclei of certain of these cells, never more than 4 or 5 in a section and usually less, develop small clear areas in the midst of the chromatin material. In later stages this area enlarges and becomes basophilic, frequently with a mass of heavily basophilic material at the center. The phenomenon has all the appearances of a disintegrative process.

⁴ Adams, A. E., and Grierson, M. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **30**, 341.

This nuclear change has been observed in cells both with and without cytoplasmic vacuoles. It has never been observed in an acidophile nor in the basophiles of a non-vacuolated gland, whether control or following a short period of thyroidectomy. It seems highly probable that there is some correlation between the nuclear change and the cytoplasmic vacuolation and that the former, like the latter, is a characteristic response to thyroidectomy in *Triturus*.

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**Choline and Pancreas Extract on Fatty Livers and Ketosis
Due to Ant. Pituitary Extract.**

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These experiments were carried out when there was reason to believe that the pancreas extract described by Dragstedt, *et al.*,¹ under the name of lipocaic was a new fat metabolizing hormone. Recent developments^{2, 3} suggest that this substance owes its activity to the choline and protein content and somewhat diminish the original intent of this work. However, the data reported here are still of interest in connection with the further differentiation of factors influencing fat transport to and from the liver.

The albino rats used were removed from the stock diet, fasted for 24 hours, weighed and urine collections commenced. The administration of 2 cc of 5% NaCl per day to each rat insured good urine volumes. The rats comprising a given group through the 6 experiments were all treated simultaneously. The ketogenic anterior pituitary extract was prepared according to the method of Best and Campbell.⁴ The rats in experiments 2, 3, and 5 received 4 cc per rat per day subcutaneously. The rats receiving choline hydrochloride were given 2 cc of 10% solution by stomach tube at the beginning of each 24-hour period of urine collection. The pancreas extract was prepared according to the method of Dragstedt, *et al.*,¹ and was ad-

¹ Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 175.

² Best, C. H., and Ridout, J. H., *Am. J. Physiol.*, 1938, **122**, 67.

³ MacKay, E. M., and Barnes, R. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 410.

⁴ Best, C. H., and Campbell, J., *J. Physiol.*, 1936, **86**, 190.