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Histological Study of Thyroid Gland of Hypophysectomized Rats Exposed to Cold.*

OPAL WOLF AND ROY GREEP. (Introduced by F. L. Hisaw.)

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Smith[1] reported atrophy of the thyroid glands of frog tadpoles and rats[2] following the removal of the anterior lobe of the pituitary. Early death of the hypophysectomized animals was attributed chiefly to their inability to maintain normal body temperature because death did not occur when they were kept in specially heated cages. Smith concluded, "Thyroids of hypophysectomized tadpoles are undoubtedly entirely non-functional because they do not metamorphose. It seems probable that in hypophysectomized rats these glands are likewise not functional."

Various investigators have reported, however, that differences of temperature affect the activity of the thyroid gland. In recent extensive experiments Kuschinsky[3] found that the histological picture of the thyroid of rats at 38°-40°C. was that of an inactive gland and exposure to 4°C. resulted in great activity. Moreover, the thyrotropic factor in the pituitary glands of these rats decreased in the warm room and increased up to 25 to 30 days' exposure to the cold.

The changes brought about in the thyroid glands of animals subjected to cold might be explained by a nervous stimulation of the anterior lobe of the pituitary resulting in an increased output of the thyrotropic hormone and in addition the activity might be increased by a nervous stimulation of the thyroid.

Evidence for a nervous stimulation of the thyroid has been contradictory. Recently Bachromejew and Ter-Ossipowa[4] stimulated the peripheral endings of the superior laryngeal nerve of the cat and found activity in the thyroid gland as evidenced by cytological changes. In carefully controlled metabolism studies Haney[5] found that stimulation of the cervical sympathetic of rabbits resulted in

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1 Smith, P. E., *Anat. Rec.*, 1916, **11**, 57.

2 Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

3 Kuschinsky, G., *Arch. f. Exp. Path. u. Pharmakol.*, 1935, **179**, 726.

4 Bachromejew, I. R., and Ter-Ossipowa, N. A., *Endokrinologie*, 1935, **15**, 404.

5 Haney, H. F., *Am. J. Physiol.*, 1932, **102**, 249.

an energy metabolism 22% above the normal at 5 days and concluded that the stimulation produced a secretory effect upon the thyroid gland. Nonidez,[6] however, found no secretory nerve endings in the thyroid of the dog but many terminations in walls of the blood vessels. He suggested that the blood flow through the gland is under the control of the nervous system and thus indirectly may influence the secretory phenomena and removal of the hormone produced by the gland.

The present investigation was undertaken to study the histological picture of the thyroid glands of young albino male rats which were hypophysectomized and subsequently placed in a cold room at 2°-4.5°C.

Seven young male rats 22 days of age were placed in a lighted cold room at a temperature of 2° to 4.5°C. for a week, hypophysectomized, kept in a warm room for 2 days and returned again to the cold room. Thirty-one males of the same age which had been kept in the warm animal room were hypophysectomized and on the third day 13 were placed in the cold room and 18 were left in the warm room. Each cage contained one or 2 normal animals. These experiments were carried on during the summer months so that the temperature varied in the animal room, reaching 29°C. during the middle of the day. Some of the controls were kept at room temperature in a small room which was lighted continuously. However, the animals in these lighted rooms, both cold and warm, were given ample cotton waste so that they could avoid the light. Immediately after hypophysectomy the animals were given access to glucose and water for several hours and then given the regular food consisting of ground grain mixed with a high percentage of brewer's yeast. In addition, a dish of glucose was kept in the cages. A large amount of brewer's yeast was given to stimulate the appetite of the animals and glucose was placed in the cage so that there would be a carbohydrate available which could be quickly assimilated.

At the conclusion of the experiment the thyroid and adrenal glands were fixed in Bouin's, dehydrated by the dioxan method,[7] stained in Delafield's hematoxylin and eosin and cut in serial sections at 7 micra. The sella of each animal was examined under a binocular for evidence of incomplete hypophysectomy and serial sections were made of those which survived the longest.

Two of the hypophysectomized rats (checked by serial sections

[6] Nonidez, Jose F., *Am. J. Anat.*, 1935, **57**, 135.

[7] Guyer, M. F., *Animal Micrology*, Univ. of Chicago Press, Chicago, Ill., fourth edition.

through the sellae) were killed after surviving 34 days in the cold room. With the exception of 3 animals killed on the 10th day, 10 animals died after 2-10 days' exposure. Five survived 12 to 16 days and 2 were killed on the 34th day of exposure. The animals which survived longest in the cold had been subjected to the cold one week before hypophysectomy.

Those animals which survived the longest were able to keep the body temperature at levels slightly below normal rats in the cold for 16 days, followed, however, by a gradual fall until just before death, when a drop of 1.6°-2.8° occurred.

The rectal temperatures of the animals were as follows:

Normal animals at room temperature	37.75°-38.3 °C.
'' '' in cold (at 2° to 4.5°C.)	36.4 °-37.2 °C.
Hypophysectomized animals 16 days in cold	35 °-36.15°C.
'' '' at room temperature	35.3 °-36.15°C.
'' '' 20 days in cold	34.65°-35.35°C.
'' '' 29 '' '' ''	34 °-35.45°C.
'' '' 34 '' '' ''	32.65°-33.35°C.
'' '' 33 '' at room temperature	36.55°

The 2 animals which survived 34 days in the cold (age 64 days and weighing 64 and 59 gm.) felt cold to the touch, showed asthenia and had not eaten for some time.

The thyroid glands of the normal controls in the cold showed a high columnar epithelium, loss of colloid in the small vesicles and in the larger vesicles many absorption vacuoles were present in the periphery of the colloid. Normal control animals in the warm room did not have as high an epithelium or as many absorption vacuoles as the animals in the cold.

Hypophysectomized rats in the warm room had atrophic glands with very low cuboidal to squamous epithelium. However, in the peripheral vesicles a few showed a high cuboidal epithelium and absorption vacuoles. The hypophysectomized animals in the cold room (killed on the 34th day) had glands which were atrophic in the center with small vesicles showing a low epithelium and no absorption vacuoles but the peripheral vesicles had high cuboidal to columnar epithelium. A considerable loss of colloid was seen in some vesicles and in others many absorption vacuoles were present in the periphery of the colloid. The adrenals of these animals also showed some atrophy. The cytoplasm of the cells of the *zona fasciculata* in places had very large vacuoles and there was a wide area with pycnotic nuclei in the *zona reticularis*.

It is of interest to note that surviving hypophysectomized rats

were able to maintain body temperature for 16 days in the cold at approximately the same level as similar rats in a warm room. Normal rats are imperfectly homoiothermic. In the cold room rectal temperature dropped to the level of the rats hypophysectomized and placed either in the cold or heat for 16 days. That cold stimulates the metabolism of rats in the absence of the thyroid gland has been shown by Ring's[8] studies. However, Grollman and Firor[9] found that in a warm room the fall in body temperature of white rats with chronic adrenal insufficiency was due to the failure of the anterior lobe of the pituitary to secrete sufficient thyrotropic hormone. By injections of either a thyrotropic hormone, or the feeding of thyroxin they maintained body temperature at approximately normal levels. However, it has been shown by Hartman, *et al.*,[10] and Cannon, *et al.*,[11] that epinephrine is secreted when animals are subjected to cold and the lack of this hormone may be one of the contributing factors in causing the early death of recently adrenalectomized animals which were exposed to the cold.†

In the present experiment the ability of surviving hypophysectomized animals in the cold to maintain body temperature for 16 days at nearly the level of the normal rats at the same temperature indicates a secretion of hormone from the thyroid gland. However, the histological picture of the thyroid gland of the animals 34 days in the cold did not present a picture comparable to that of normal animals at the same temperature. The removal of the anterior lobe of the pituitary had resulted in an atrophy in the central region of the gland. That some hormone was still being released was probable because some peripheral follicles showed considerable loss of colloid, others showed the typical high epithelium with absorption pseudopods or absorption vacuoles in the periphery of the colloid as described by Sevringhaus.[12] This picture is not characteristic of the thyroid gland of hypophysectomized animals. It is evident, how-

[8] Ring, G. C., *Abst. Am. J. Physiol.*, 1936, **116**, 129.

[9] Grollman, A., and Firor, W. M., *Am. J. Physiol.*, 1935, **112**, 310.

[10] Hartman, F. A., McCordock, H. A., and Loder, M. M., *Am. J. Physiol.*, 1923, **64**, 1.

[11] Cannon, W. B., and Querido, A., *Proc. Nat. Acad. Sciences*, 1924, **10**, 245.

† During this experiment 2 adult male rats doubly adrenalectomized 1½ days (19 days having elapsed between the removal of the right and left gland, one animal having gained 20, the other 15 gm. by the day of the second (left) adrenalectomy) were placed in the cold room. At the end of 7 hours one animal was dead and the temperature of the other dropped from 36.5° to 26.25°C. This animal was returned to room temperature and at the end of 12 hours had a temperature of 25.5°C. and died 8 hours later. Unpublished data of Robert Kroc.

[12] Sevringhaus, A. E., *Z. f. Zellforsch. u. Mik. Anat.*, 1933, **19**, 653.

ever, from this experiment that cold in the absence of the anterior lobe of the pituitary is not an adequate stimulus to release sufficient hormone from the thyroid gland to maintain a temperature characteristic of a normal rat in the cold.

The fall in temperature, asthenia and failure to grow indicates undoubtedly a multi-glandular disfunction due to hypophyseal insufficency.

Summary. 1. Normal young albino rats had a temperature of 36.4°-37.2°C. in the cold, in a warm room 37.7°-38.3°C. 2. Surviving hypophysectomized rats maintained their body temperature for 16 days in a cold room (2°-4.5°C.) at 35°-36.15°C. From the 20th to 34th days the temperature fell approximately 1.3°-2.7°C. 3. The thyroid glands of normal animals in the cold showed activity as previously described by other investigators. Those in the warm room had a lower epithelium and fewer absorption vacuoles. 4. The glands of hypophysectomized rats 38 days in the heat showed atrophy. Similar animals placed in the cold room 34 days showed signs of atrophy in the central part of the gland but absorption of colloid was indicated in the peripheral vesicles by the high epithelium, loss of colloid in the smaller vesicles, and many absorption vacuoles in the peripheral colloid of the larger vesicles. 5. The drop in body temperature of the hypophysectomized rats after 16 days in the cold as well as the histological picture of the thyroid gland indicates that cold in the absence of the anterior lobe of the pituitary is not an adequate stimulus to maintain either body temperature or a histological picture of the thyroid gland comparable to that of a normal rat.

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Effect of Calcium on the Digitalized Heart.*

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The action of calcium upon the mammalian heart has certain similarities to that of digitalis. Slowing of the heart rate, A-V block of varying degree, and ectopic beats have been observed fol-

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