would be able to present it in simpler form. The Drosophila work has been studied carefully and is presented in all its theoretical aspects well. On the whole, the book is a distinct and welcome contribution to the literature of genetics.

W. E. CASTLE

SPECIAL ARTICLES

THE SECRETION GRANULES AND THE VACUOLES IN THE LIVING THYROID GLAND

SEVERAL years ago the writer made an extensive study of the development of the various structures of the thyroid gland of the salamander *Ambystoma opacum*. The method employed in this study was the usual histological technique. The histological slides representing the records of this work were demonstrated at several meetings as well as to a number of individual investigators here and abroad and several short articles outlining the results were published.[1] The manuscript of the final paper, which has gone through the hands of editors for the last two years, is now in press. As the present study on the living thyroid gland corroborates the essential findings of the histological study, brief reference to the results of the latter must be made here.

Through the work on amphibian metamorphosis as conducted during the last fifteen years in American and European laboratories the fact has been well established that the metamorphosis of an aquatic salamander larva into a terrestrous animal is the direct result of the function of the thyroid gland. Therefore the structure of thyroid glands taken from metamorphosing salamander larvae should be expected to be representative of the structure of a functioning thyroid gland. Study of such thyroid glands should yield conclusive information as to which of the various known structures of the thyroid gland are the expression of the specific function of this organ. Upon comparing sections through the glands of metamorphosing animals with sections through the thyroids of earlier stages it was found that the most conspicuous characters of the thyroid at the time of metamorphosis are the presence of large numbers of unstainable vacuoles in the cells (these vacuoles I called Anderson vacuoles after Anderson, who was the first one to describe them),[2] the presence of similar vacuoles in the colloid, the scarcity of stainable colloid in the lumen of the thyroid follicles and the collapse of the follicles into frequently solid and star-shaped masses of epithelium. These characters do not develop gradually, but on the contrary come about in a very sudden manner, taking not more than approximately twenty-four hours for their development. The structural peculiarities enumerated above are among the characters typical for the thyroids of exophthalmic goiter patients. Similar resemblances between the thyroids of metamorphosing salamanders and those of exophthalmic goiter patients were found in nearly every other respect.

Although Anderson in 1894[3] described vacuoles in the cells of the thyroid and saw that the colloid vacuoles, under certain conditions, communicate with the vacuoles in the cells, anatomists and pathologists interpret the colloid vacuoles as artefacts due to shrinkage of the colloid in the fixation fluids. In the thyroid of metamorphosing salamanders the colloid vacuoles are found in a condition indicating clearly that they are an unstainable material excreted by the cells in especially large numbers during stages of highly increased functional activity and identical with the unstainable material contained in the Anderson vacuoles of the cells, with which, in the salamander gland, they are found to be in open communication. Careful fixation of human material showed the same relation to be true for the colloid vacuoles in the human thyroid gland. Through the kindness of Professor Martin Heidenhain I had at my disposal, for comparison with my own material, a human thyroid fixed and stained by Professor Heidenhain himself after the Heidenhain-Mallory method, a technique which I found particularly useful in the study of the thyroid. This thyroid shows exactly the same condition as my own material; in many places colloid vacuoles are found in direct communication with the Anderson vacuoles.

From these observations the conclusion was drawn that the colloid vacuoles are not an artefact, but represent an important secretion of the thyroid gland and are derived from the Anderson vacuoles of the thyroid cells.

The precursor of the stainable colloid was found in an acidophilic substance accumulated in the apical cell ends. The so-called secretion granules were found accumulated in the apical cell end and imbedded in the acidophilic substance. As these granules were never found to lie in the follicular lumen and as no other sign indicating that they are excreted could be noticed, their nature as true secretion granules was

[1] Uhlenhuth, E., Proc. Soc. Experim. Biol. and Med., 1923, XX, 494; *Anat. Rec.*, 1924, XXVII, 222; *J. Gen. Physiol.*, 1924, VI, 597; *Zeitsch. wiss. Zool.*, 1925, CXXV, 483.

[2] Anderson, O. A., *Arch. Anat. und Physiol.*, Anat. Abt., 1894, 177.

[3] *Ibid.*

doubted and, in accordance with other observations, it was assumed that these granules play some other rôle in the elaboration of the colloid.

The study of the living thyroid gland was undertaken with the hope to obtain further information about the nature of the Anderson vacuoles, of the colloid vacuoles and of the granules of the thyroid cells. The salamander *Ambystoma maculatum* was used in this work. Up to the present time only the thyroids of adult animals and of larvae before metamorphosis have been examined. Before examination the thyroids were stained with neutral red. In adult animals the best results were obtained by injecting the dye intraperitoneally. The larvae were stained in the manner described by Fischel[4] and by McClure.[5] After the tissues had become sufficiently imbibed with the dye, the thyroids were removed and examined on the slide under the microscope immediately after extirpation.

Vacuoles in the cells were found in both the adult and larval glands. If the thyroids of adult animals are removed cautiously, no vacuoles are usually present. If, however, rough handling, during removal or otherwise, precedes examination, formation of vacuoles takes place. In this respect the thyroid behaves very differently from other epithelial organs. In the epithelial cells of the skin of the same animals or of the larvae no vacuoles can be made to appear by mechanical stimulation. In the thyroid the vacuoles are located between the nuclei of the cells. If cell borders are not visible, it is impossible to say whether the vacuoles are extracellular or intracellular. In many thyroids the cell borders were visible and of extreme clearness; in these instances the vacuoles were intracellular. They are especially distinct, if the follicles are viewed from the surface, but may be seen also in optical sections of the cells. The vacuoles are located in largest numbers at the basal pole of the cells and are often found even between the nucleus and the peripheral wall of the cell, but smaller numbers of vacuoles are found also at the apical cell end. Immediately after removal of the gland they are of small size (approximately of the size of a nucleolus), but may increase in size later on and attain half or even fully the size of a nucleus. The formation of new vacuoles and the increase in the size of the old ones ceases after the organ has been exposed on the slide for thirty minutes to an hour.

In the thyroids of young larvae no vacuoles comparable to those seen in adult glands could be found, even if the glands were handled and pressed severely. Instead small vacuoles of yellowish green color are present, in moderate numbers, in the cell plasma. In contradistinction to the other kind of vacuoles they stain red with Sudan III and Scarlet Red and are fat vacuoles derived probably from the yolk. The first vacuoles similar in appearance to those of adult glands were observed in thyroids taken from larvae of 11.5 mm body length and forty-one days of age. One or several vacuoles may be found in such glands, which are located at the periphery of the follicle just between two neighboring cells and apparently extracellular. No other vacuoles are present at that stage. This condition persists nearly up to metamorphosis. In animals just about ready to shed their skin for the first time conditions are decidedly different from either the adult or the larval gland. In addition to the vacuoles characteristic for the adult gland, and independent of mechanical stimulation, small vacuoles are present in these glands, which are about twice as large as a nucleolus and are surrounded by very tiny dust-like granules. The granules are closely attached to the walls of the vacuoles and, in the Neutral Red specimen, stain a dark reddish brown. In high cuboid or cylindrical cells they are spread throughout the apical half of the cell; in low cuboid cells they are frequently arranged in one or two rows parallel with, and located just below, the apical cell wall and apical to the secretion granules of the cell. Especially these latter vacuoles resemble in a very striking manner the Anderson vacuoles as observed in sections through the thyroid gland.

The colloid vacuoles were studied so far only in the thyroids of larval animals. In early stages they are absent. The first colloid vacuoles were found in an animal of 11.9 mm body length and forty-seven days of age. Only in larvae which are immediately before the first skin shedding do the colloid vacuoles become of real significance; at that stage the number of these vacuoles is considerable. It was of especial importance to ascertain whether, as deduced from the histological sections, the colloid vacuoles are excreted from the cells. So far four vacuoles were observed during the process of excretion from the cell. In one instance a vacuole was pushed forth from the cell into the follicular lumen, while the cell was under observation. It attained rapidly about half the size of a nucleus and then stopped increasing. No break of the cell wall could be noticed in this instance. In another thyroid a small colloid vacuole was attached to the cell by a clear hollow stalk penetrating the cell wall and extending into the cell plasma. This vacuole increased slowly in size till it attained about the size of a nucleus. It resembled greatly the communicating colloid vacuoles observed in the sections.

[4] Fischel, A., *Anat. Hefte*, 1901, XVI, 415.

[5] McClure, C. F. W., *Am. Anat. Memoirs* (of the Wistar Inst.), No. 8, 1918.

The secretion granules observed in thyroid sections correspond in every respect to the granules staining with Neutral Red. The first neutral red granules were found in thyroids in which the differentiation of the solid cell column into primary follicles had just started. During the larval period the granules are spread throughout the cell, but are especially dense at the apical pole. In several larvae which were close to metamorphosis the granules showed a tendency to become massed at the apical pole; no granules at all were present at the basal cell end. Although colloid is excreted from the cells into the lumen of the follicles throughout the larval period, an excretion of the granules into the follicular lumen has never been noticed; no granules whatsoever could be detected in the colloid. Moreover, a careful comparison made, in the vitally stained larvae, between the granules of the thyroid cells and the true secretion granules (the mucine granules) of the Leydig cells of the skin showed that there is no resemblance between the reactions of these two kinds of granules towards the Neutral Red.

Although the study of the living thyroid gland, as compared to the histological study, so far has not disclosed any new facts, it has at least been instrumental in demonstrating that the Anderson vacuoles and the colloid vacuoles are not artefacts, but are structures characteristic of the living thyroid gland. Furthermore, it seems to be certain that the so-called secretion granules of the thyroid cells are not actually excreted from the cells.

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A POTATO NECROSIS RESULTING FROM CROSS-INOCULATION BETWEEN APPARENTLY HEALTHY POTATO PLANTS

WHILE conducting investigations on the manifestation of virus disease symptoms on different potato varieties and seedlings[1] in the Washington greenhouses during the winter of 1923–24, the writer found a necrosis on certain seedlings. The symptoms of these necrotic or streaked seedlings resembled those of so-called "streak" in potato. This malady is distinguished in the early stages by necrotic spots on the leaf parenchyma; later, necrosis appears along the veins, producing a streaked appearance. Likewise, linear necrotic lesions appear on the petioles and stems, usually resulting in the death of the shoot in a short time; shoots from four to ten centimeters above ground may die in a few days. Frequently only one side of the shoot or even of a compound leaf manifests these symptoms. Necrotic areas may develop on very young actively growing shoots and leaves, as well as on apparently full-grown plants. These facts suggest that the inciting agent is transmitted through the conducting elements.

Necrosis developed on certain seedlings as a result of tuber grafts on mild mosaic and spindle tuber Green Mountains. Other seedlings from different parentage and other varieties treated similarly at this time manifested only mild mosaic and spindle tuber symptoms. All the healthy controls from seed pieces from the same tubers as the grafted seed pieces remained healthy. Also the mosaic and spindle tuber Green Mountain plants in these grafts disclosed only mosaic and spindle tuber.

Similar necrosis again developed on the same seedlings and on an additional seedling when these investigations were continued in the fall of 1924 under the same conditions as before. At this time studies also were initiated on the reaction of some apparently healthy foreign potato varieties, *viz.*, Duke of York, Bravo, Paul Kruger, Koksiaan[2] and Arran Comrade,[3] to the mosaic, leaf roll and spindle tuber diseases in Green Mountains. On these foreign varieties necrosis, as on the seedlings, developed when grafted on mosaic, spindle tuber and leaf roll Green Mountains. Some of the shoots became necrotic when but a few centimeters above ground, without manifesting the symptoms of the particular disease represented in the Green Mountains. Other shoots plainly disclosed either mosaic, leaf roll or spindle tuber. The controls, plants from the seed pieces from the same tuber as the grafted seed pieces, remained healthy. Likewise, no necrosis appeared on the mild mosaic, spindle tuber or leaf roll Green Mountains. In a similar series of mild mosaic Green Mountain tuber grafts on healthy Green Mountains, Rural New Yorkers, Irish Cobblers, Spaulding Rose, Early Rose and Bliss Triumphs, no necrotic symptoms developed.

From these peculiar manifestations and in view of Professor James Johnson's[4] recent discovery of faint mottling or irregular necrotic areas produced on tobacco by cross-inoculations with juice from apparently healthy potatoes, it was surmised that this necrosis might be due to the reaction between certain

[1] Potato seedlings were furnished through the courtesy of Dr. C. F. Clark, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Obtained through the courtesy of Dr. H. M. Quanjer, Wageningen, Holland.

[3] Obtained through the courtesy of Dr. George H. Pethybridge, Harpenden, England.

[4] Johnson, James, "A virus from potato transmissible to tobacco," *Phytopathology*, V. 15, No. 1, p. 46–47. 1925.