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## THE MORPHOLOGY AND PHYSIOLOGY OF THE SALAMANDER THYROID GLAND

### II. THE ANTERIOR LOBE OF THE HYPOPHYSIS AS A CONTROL MECHANISM OF THE FUNCTION OF THE THYROID GLAND

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IN the study of the physiology of the thyroid gland it is becoming more and more evident that the function of this organ is composed of a number of component activities. Only some of them are controlled by intrinsic mechanisms, while the most important of them, the release of the active secretion product of the gland from the follicles into the blood circulation is effected by stimulation through an extrinsic "releasing mechanism." In warm-blooded animals the study of this releasing mechanism has received considerable attention, and although the question cannot by any means be considered a closed chapter of thyroid physiology, the work of O. A. Andersson<sup>(1)</sup>, Leo Asher<sup>(2)</sup>, Cannon and Fitz<sup>(3)</sup>, L. B. Wilson<sup>(4)</sup> and others, is generally credited with having demonstrated that the autonomous nervous system is the path over which the stimuli of the releasing mechanism reach the thyroid in warm-blooded animals.

The senior author of this paper has been interested, for some time, in the physiology of the thyroid of cold-blooded animals, and especially in that aspect of it which concerns the nature of the releasing mechanism. This work has now entered an advanced stage and certain definite conclusions are possible. Although the results, together with the records, will be published in a series of more extensive articles, it seems desirable that the chief results be made accessible at once.

Morphological as well as experimental work on salamanders, published by the senior author in previous papers<sup>(5)</sup> indicates that in the urodelan amphibians the thyroid function consists of two main phases, first of the elaboration of the active secretion product of the thyroid, the colloid, in the thyroid cells, of the excretion of the colloid from the cells into the lumen of the follicles and of the storage of the colloid within the follicles, and secondly of the excretion of the colloid from the follicles into the blood-circulation. The presence of two distinct steps of the secretion process is one of the most important characters distinguishing the thyroid from other glands and is the expression of the peculiar structure of this organ. After the secretion product has been excreted from the cells, it lies within the

completely closed lumen of the follicles, and in order to effect its excretion into the blood-circulation a special releasing mechanism is required. During the larval period the activities of the thyroid are confined entirely to those of the first phase (developmental or storage phase); the second or functional phase develops suddenly at the end of the larval period and is the immediate cause of metamorphosis. The activities of the developmental phase are elementary properties of the thyroid organ and are controlled by factors similar in nature to those controlling the activities of other glands; the display of the functional phase can be effected only by the action of an extrinsic releasing mechanism.

Starting from the neuro-secretory hypothesis of the thyroid function in warm-blooded animals and upon the basis of the technique employed in the study of the thyroid of mammals, the senior author, while still at the Rockefeller Institute, experimented on salamanders with drugs which are known to stimulate or inhibit the autonomous nervous system. Larvae of *Amblystoma tigrinum* were kept in solutions of pilocarpine, adrenaline and atropine. It was expected that, if these substances had any effect upon the autonomous nervous system, release of the colloid from the follicles of the thyroid and metamorphosis known to be the direct result of the action of the thyroid hormone should be enforced precociously or inhibited according to whether the particular drug had a stimulating or inhibiting influence upon the excretor-fibres which effect the colloid release in the thyroid gland. These experiments were, however, very disappointing and entirely negative; all larvae underwent metamorphosis at a normal time.

It might be suspected that the mere keeping of the larvae in the solutions of the drugs did not permit the drug to come in contact with the nervous system. But various other effects similar to those produced by the same drugs in higher animals were extremely pronounced. Pilocarpine f.i. produced an enormous excretion of slime from the glands of the skin, vomiting, excessive defaecation and visible contractions of the intestinal tract. Adrenaline caused contraction of the melanophores.

Nevertheless we repeated these experiments last summer in Woods Hole; this time we injected intraperitoneally pilocarpine and adrenaline (in addition larvae of *Amblystoma maculatum* were kept in solutions of ergotamine). Again the larvae of *Amblystoma tigrinum* were employed in many of the experiments with pilocarpine and adrenaline, again they showed a typical and, if the dose was high enough, even violent response to pilocarpine and adrenaline; in case of adrenaline the melanophores remained contracted for 20 hours and more. Yet metamorphosis remained completely unaffected.

Before deciding, whether the thyroid, in these experiments, had actually failed to respond by a release of the colloid or whether the colloid had been released, but, in the larval thyroid, does not yet contain the active principle, it was necessary to examine the structure of the thyroid gland.

In a previous histological study a very characteristic structural appearance was found to be correlated with the release of the colloid at the time of metamorphosis, indicating an extraordinarily high degree of functional activity of the thyroid cells,

Finally a method was worked out which permits the study of some of these structures (high columnar cells, a conspicuous gathering of the secretion granules at the apical pole of the cells and especially the presence of large, distinctly visible secretion vacuoles—"Anderson Vacuoles"—within the cells) in the fresh thyroid gland immediately after its removal from the animal. With this method the functional state of the gland can be determined very rapidly and without great loss of time. Ultimately another method was developed which permits the exact counting of the individual follicles composing each thyroid as well as the study of the size and shape of each individual follicle. The thyroids of larvae kept in or injected with solutions of pilocarpine, adrenaline and ergotamine were examined in this fashion, and it was found that they did not show any noticeable difference as compared to the controls. From these experiments it is concluded that in the larval thyroid of salamanders the release of the colloid and the active principle can be neither enforced nor inhibited by drugs known to stimulate or inhibit the sympathetic and parasympathetic fibres in warm-blooded animals.

We suspected, and in fact hoped, that these results might mean that in the salamander, during the larval period, the nervous releasing mechanism is not yet established and that it does not become established until immediately before metamorphosis. Such an arrangement would explain the very sudden development of the functional phase at the time of metamorphosis. If this conclusion were correct, drugs stimulating the autonomous nervous system should cause an increased colloid release in the metamorphosed, adult salamander. Adult animals especially of the species *Amblystoma tigrinum* and *Notophthalmus torosus* were injected with pilocarpine and adrenaline. But although in both cases a general response to the drugs was very pronounced, just as in the larvae, the thyroids of the injected animals remained entirely unaffected. Evidently then, the thyroid gland cannot be stimulated either in the larval or adult salamander by the drugs employed.

It seems to us that this is a very interesting result as it indicates an essential difference between the warm-blooded animals and the cold-blooded salamanders. Apparently in the salamander the thyroid is either not connected up with the autonomous nervous system, or else the latter does not respond to these drugs in the same way as it does in the warm-blooded organism.

If now the effect of these drugs is compared with the effect of extracts of the substance of the anterior lobe of the hypophysis, a striking difference is noticed.

In 1922 P. E. Smith and I. P. Smith<sup>(5)</sup> reported experiments showing that fresh anterior hypophyseal extracts retard metamorphosis of the Colorado axolotl. In 1923 Hogben<sup>(4)</sup> published a paper in which just the opposite effect of anterior lobe substance was claimed; in the Mexican axolotl injected with "Armour's anterior lobe tablets" metamorphosis was enforced. In a recently communicated article Smith<sup>(6)</sup> maintains that the effect of Armour's anterior lobe tablets, as observed by Hogben, is probably caused by admixtures of thyroid substance; Smith supports his view by experiments which showed that metamorphosis is hastened by feeding the Colorado axolotl on Armour's anterior lobe tablets. Recently Spaul<sup>(7)</sup>

repeated Hogben's experiments in the Mexican axolotl, but used anterior lobe extracts made up freshly in the laboratory. His extracts differed from Smith's extracts in that they were made in acid saline solutions and were kept as free as possible from admixtures of posterior lobe. These extracts had the same effect as Armour's anterior lobe tablets, producing metamorphosis in the Mexican axolotl.

We repeated these experiments in the larvae of *Amblystoma tigrinum*, using Armour's anterior lobe powder and obtained the same result reported by Hogben and by Spaul; metamorphosis of the larvae is distinctly accelerated. Next we examined carefully the thyroids of the control and experimental animals and found that during metamorphosis of the larvae injected with anterior lobe substance the thyroid goes through the whole cycle of functional changes characteristic for the thyroids of the normal metamorphosing larvae. At first the cells increase considerably in height, taking on a high columnar shape, and a massing of the secretion granules at the apical cell end takes place. Finally secretion vacuoles develop within the cells. We repeated this experiment in the Mexican axolotl; unfortunately we had only two larvae at our disposal and no control could be run. Yet as compared with other normal axolotls the behaviour of these two larvae was extremely striking. The first animal died before any metamorphic changes had become visible externally. The second animal was killed at a time at which metamorphosis was in an advanced stage. No other animal of the same stock has ever metamorphosed spontaneously up to the present time. The thyroids of both animals were examined. In the thyroids of animals of the same stock, which the senior author examined previously, all cells are characterised by an extreme flatness; the secretion granules are scattered throughout the whole cell. In the thyroids of both of the two experimental animals the cells were on the contrary conspicuously high columnar and the secretion granules were massed at the apical pole. No secretion vacuoles were found, but the animals were killed at a stage at which the presence of secretion vacuoles could not yet be expected.

It is certain that the anterior lobe substance in these animals did not enforce metamorphosis by direct action, but indirectly by the intermediation of the thyroid gland, stimulating it and enforcing its functional activity.

Further experiments were carried out to decide whether the action of Armour's anterior lobe powder is the result of the specific principle of the anterior lobe or whether it is caused, as Philip Smith claims, by traces of thyroid substance contained in Armour's preparation. In order to test this point we metamorphosed the larvae of *Amblystoma tigrinum* by keeping them in solutions of Bayer's iodothyrene and examined their thyroids at various periods after the beginning of the experiment. The thyroids of these animals showed at no time any signs of functional activity; in general it may be said that after the beginning of the iodothyrene administration these thyroids remained at the stage at which they were at the beginning of the experiment. Hence it is evident that thyroid substance administered to the larvae of salamanders enforces metamorphosis directly by its action upon the tissues of the animals, without the intermediation of the animal's own thyroid gland, and therefore behaves entirely differently from the anterior lobe substance.

It might still be suspected that the action of Armour's preparation was due to

inorganic iodine contained in it. The senior author showed in previous experiments that, in salamanders, inorganic iodine has no accelerating influence upon metamorphosis. Nevertheless we repeated these experiments, feeding various quantities of inorganic iodine to the larvae of *Amblystoma tigrinum*. Again the animals failed to show an acceleration of metamorphosis. In addition we examined this time the thyroids of the larvae fed iodine and compared them with control thyroids. We found that the action of inorganic iodine is entirely different from the action of both anterior lobe substance and thyroid substance, and is indeed very specific. In the main it causes an enormous swelling of some follicles, a complete disintegration of other follicles and a conspicuous diminution of the number of follicles.

We believe that these experiments permit a further analysis of the physiology of the thyroid gland of salamanders. No nervous control, but a distinct control of the thyroid function by the anterior lobe substance could be demonstrated. The action of the anterior lobe powder (Armour's preparation) is not due to the presence of iodine or thyroid substance in this preparation, but to a specific principle of the anterior lobe of the hypophysis. The anterior lobe of the animal's own hypophysis is an integral component of the peculiar releasing mechanism of the salamander thyroid; it elaborates a specific substance which, in some unknown way, stimulates the thyroid, thereby effecting the release of the thyroid hormone. On the basis of previous findings we propose, as a guide in further experimentation, the hypothesis that this active principle of the anterior lobe is not elaborated during the larval period, and that for this reason release of the colloid and metamorphosis do not take place at an earlier period of life.

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