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Hormones of the Vertebrates and Their Influence on the Growth and Development of Invertebrates

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Part I

Introduction

From earliest times the marked influence exerted by internal secretions has been recognized; however, the modern development of endocrinology may be said to have begun with the work of Bayliss and Starling in the isolation of secretin and their proposal to call this substance, and all other such substances, hormones -- meaning chemical messengers.

Since that time in the early part of the 20th Century much has been done especially in connection with the thyroid gland and the effect of its hormone thyroxine upon the growth and development of various vertebrates. Little, however, has been done upon the influence of vertebrate hormones upon the large class of invertebrates, and the purpose of this paper is to investigate the effect of thyroxine in this field. For the study the phylum Protozoa, a group of unicellular animals with neither organs nor tissues has been chosen, and the particular animal under observation is to be a ciliate, Paramecium multimicronucleatum.

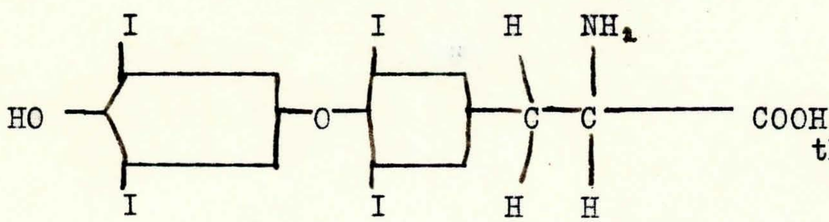
Before such a study can begin, it is necessary to investigate the origin and nature of the hormone thyroxine, which will conclude Part I. Part II will deal with the structure and physiological behavior of the Paramecium under normal conditions. Part III will include previous experimental work done on the effect of vertebrate hormones with various invertebrates and the experimental work itself upon which this paper is based. It will be concluded with a summary and interpretation of final results.

The thyroid gland from which thyroxine is obtained is found only in vertebrates; although earlier it was believed that the endostyle of Amphioxus and various tunicates was definitely homologous to the true thyroid gland found in higher animals. Further investigation has proven this to be highly improbable, and it is now regarded as more correct to attribute its development from cell elements of the pharyngeal floor of primitive vertebrates -- the elements being originally of unknown function. The evagination of cells takes place at the level of the hyoid arch between the primary tongue and the tuberculum impar. It early in its course of development separates from the floor becoming ductless and proliferates into a considerable mass of follicles, usually bilateral in structure. It is in the adult lamprey that the typical thyroid structure is first seen.

The physiologically active substance of the thyroid hormone is an iodine containing compound. The amount of iodine in normal thyroids varies from .1 to .33% of dry weight of the gland and tends to change with the species and the iodine content of the diet, as the thyroid has the power to select from the blood and store the very small quantities found in various foods.

Baumann, in 1896, prepared a product of the partial hydrolysis of thyroid protiens and named it thyroidin. It contained 9.3% iodine and through various tests has been proven to contain the active hormone of the thyroid gland. It has since been found that iodine is held in the thyroid in a protien band called thyreo-globulin. This protien, as does the entire thyroid substance, yields the free hormone when hydrolyzed in strong alkaline solutions.

In 1914, at the Mayo Clinic in Rochester, Minnesota, the thyroid hormone itself was isolated and identified by Kendall who named it thyroxine. His work was further extended by Harington who showed it to be an iodine derivative of a subclass related to tyrosine. Its formular is:



thyroxine

It contains 65% iodine in agreement with its structual formular.

Part II

The genus Paramecium of the phylum Protozoa is characterized by the oral groove appearing as a deep depression beginning at the anterior end and running obliquely back so that a cross section of the anterior half of the animal appears as crescentic in shape. The species used for this experiment is multimicronucleatum which ranges in length from .15 to .3 mm. It is aurelia in shape, having a resemblance to the outline of a shoe, being broadest behind the middle with its anterior portion elongated and the end rounded. The body is covered with a pellicle that appears to be sculptured into hexagonal depressions bounded by numerous ridges on which the trichocysts open. The greater part of the alveolar layer of the ectoplasm is filled with these objects which appear to be carrot-shaped bodies evenly distributed throughout the animal with their slender stems attached to ridges of the hexagonal structure of the pellicle. Upon certain kinds of stimulation they may be seen to be ejected into the exterior as long slender rods. The function and method of discharge of the trichocysts is unknown.

From each depression of the pellicle a cilium springs whose base pierces the covering and rests in the basal body situated in the alveolar layer of the ectoplasm. Except for a few long cilia located at the posterior end of the Paramecium, the ciliation may be said to be uniform.

Running parallel to the body surface in the alveolar stratum of the ectoplasm is located the neuromotor system. It is of primitive nature and consists of interciliary fibrils, each of which is composed of a longitudinal strand connecting the basal bodies of the cilia. Pellicular markings and fibrils of the neuromotor system continue into the cytopharynx where the latter are seen to form a system of radiating and circular fibrils passing through the basal bodies in the form of a network. To the left dorsal wall of the cytopharynx is attached the neuromotorium consisting of two masses from which numerous fibrals may be traced into the entoplasm. Near the inner portion of the cytopharynx in the dorsal wall, lies a chain of about five granules from which a bundle of postesophageal fibers enters posteriorly into the entoplasm.

In addition to the neuromotor fibrilla system, there have been described in Paramecium two systems of ectoplasm networks, an inner and outer, both believed to be of supporting nature. The outer is located in the pellicular ridges and the other is situated at the level of the basal bodies.

The vascuolar system, located in the innermost layer of the ectoplasm, consists usually of two vascuolar apparatuses -- one in each body half -- but in Paramecium multimicronucleatum there are frequently one to five extra. Each consists of a pore and of radiating canals and

and are often of considerable length tapering into fine canals that receive fluid from many minute vacuoles. In general, the canals may be said to run parallel to the surface. A swollen portion, the ampulla, is located at the proximal end of each canal from which a fine injector canal proceeds towards the pore. Drops of fluid emitted from the ends of the canals unite to form the contractile vacuole which is a structure of temporary nature formed after each discharge.

The cytopharynx at the posterior end of the oral groove leads into the entoplasm and possesses a complex ciliation. The outer portion of the cytopharynx has, in general, the same structure as the ectoplasm except that at times two cilia are found in each depression and the posterior edge is devoid of cilia. The next main portion of the pharynx is a curved tube devoid of cilia on the right side. On the left is seen the penniculus composed of a curved band with eight rows of cilia in parallel arrangement of two groups of four rows wach. In the dorsal wall is located the quadrulus which is four rows of long cilia paralleling the penniculus and serving to direct the finer particles of food into the esophagus, while the larger objects are handled by the penniculus. The innermost portion is the esphagus and is very short. Its entrance is considered by some to be the true mouth that has sunken inward. The cytopyge is a definite pore situated on the right side near the inner portion of the cytopharynx.

The macronucleus is a large oval body located in the entoplasm near the middle of the animal. There are two to four micronuclei -- each with a central endosome.

The entoplasm of Paramecium has no features of special interest but consists of fat and glycogen. The food of Paramecium consists of bacteria but at times larger objects may be included in their diet.

As to the behavior of Paramecia, they swim in spiril paths, rotating upon the longitudinal axis and makiing one spiril turn to each rotation of the animal, which appears to be to the right in this species. This behavior is due to the fact that in ordinary forward progression the ciliary beat passed backward in a diagonally directed wave taking a spiril course, and as a result the animal would swim only in circles were it not for the fact that it rotated its body in the opposite direction as a counter action. The coordination in the action of the cilia and the changes in the beats under certain conditions are due, in all probability, to the neuromotor apparatus.

Paramecia are sensitive to heat, chemicals, and contact but, in general, not to light. The cilia serve as receptors while the non-motile cilia at the posterior tip are steering aids. As to its action towards chemicals it appears to have an avoiding reaction.

The ability of Paramecium to distinguish food from other particles has been given much study and as will be later seen, is of some importance in the experiment to be presented. At first appearance, it seems that they will ingest foreign particles and swallow them, but upon further investigation it is shown that they are capable of discrimination even as to different species of bacteria. The rejection of particles is accompanied by the reversal of the action of cilia used for food getting. It is believed that the discrimination depends in part on the tactual properties such as size, shape and surface texture of the available food, and in some cases on the chemical properties.

As to the reproduction of Paramecium, it is almost exclusively asexual by means of binary fission, and transverse in nature. The process is initiated by the micronuclei each of which draws apart into two halves by mitosis. The macronucleus divides by a simple amitotic division. As the nuclei separate, a cytosomal constriction appears, and as it deepens, cuts the animal in half. As to the organelles, each half retains those which fall within its borders and regenerates the others at an early stage. At times it is possible to notice a new mouth, cytopharynx, etcetera, commonly appearing before fission is entirely completed by means of budding of the original structure.

There occurrs at times in Paramecium a form of sexual reproduction known as conjugation. The animals seem to become sticky and adhere in couples, fusion taking place at the mouth region. This lasts for a number of hours during which time definite nuclear changes occur. The macronucleus, of vegetative function, breaks up into fragments which disappear gradually during the course of change leaving the two to four macronuclei to play the important role. All divide twice, corresponding to the maturation division in higher animals. Next all the micronuclei except one disappear in each conjugant. The one remaining divides in two, one becoming the stationary and the other the wandering micronucleus. The latter in each animal crosses the protoplasmic bridge into the other where it fuses with the stationaly body to form a synkaryon. This structure divides with some parts becoming a new macronucleus and others forming the micronuclei. The conjugates separate after this exchange of nuclear matterial and are known as exconjugates.

Part III

Previous experimental work on vertebrate hormones and their effect upon invertebrates was done in 1927 by the Russian scientist, M. S. Reznichenko and again in 1930 under the leadership of Jan Zavrel at the University of Bren. Portions of both works have since been translated, and summaries of their conclusions follow.

In rearing the larvae of Chironomus Marmoratus in extracts of the thyroid gland (.025 - .1%) Zavrel found a considerable acceleration of their growth and development. It was not, however, believed to be necessarily due to the action of the hormone as similar results were obtained by an increase in the temperature. He came to the conclusion that the hormones of vertebrates have no specific effect on the growth and development of invertebrates and that inconsistencies in the behavior of different invertebrates to the hormone was due to diferent degrees of phyletic development.

Reznichenko administered thyreoidin to a form of fly, Drosophila Melanogaster, in the proportions of .5 - 1.%. From all results, he concluded that there was absolutely no change in the development, growth, or rate of reproduction and concluded that thyreoidin did not influence the development of these flies because the thyroid hormone is non-specific for invertebrates.

The Experiment

Thyroideum siccum, a light brown powder prepared by drying the thyroid gland of sheep, was fed to Paramecium multimicronucleatum. The chief constituent of this substance is a proteid known as thyreoglobulin the active principle of which contains 9.3% of iodine and .5% of a phosphorus and is known as thyroidin or thyroxin, the hormone of the thyroid gland.

Four test cultures were run, each with its own control. The four test cultures were as follows:

(1.) 1 gram of thyroideum siccum to 20,000 cc. pond water

(2.) 1 gram to 10,000 cc. pond water

(3.) 1 gram to 5,000 cc. pond water

(4.) 1 gram to 1,000 cc. pond water

The cultures were partly covered and placed in subdued light. Observations were taken every two to three days for a period from April 18 through May 6, at which time the experimental work reached its end point and was concluded.

Until April 22 no change of any sort occurred in any of the cultures, but at that date there appeared in cultures (3) and (4) to be a slight increase in the number of Paramecia over the third and fourth control cultures. There was, however, no change in the actual appearance or behavior of the Paramecia at this time. On April 26 there appeared to be a slight increase in the number of animals in cultures (1) and (2), but (3) and (4) had not increased in number beyond the stage they had reached on

April 22. On April 28 there appeared in all four test cultures to be a definite increase in the size of the contracting vacuoles over those of the controls and a decided decrease in the number of bacteria in the test cultures which was not evident in the controls. By April 30 the number of Paramecia in all the cultures was less, but the number in the test cultures was still greater than in the controls, and the bacteria in the test cultures had entirely disappeared whereas they were still numerous in the controls. At this time the size of the vacuoles had begun to decrease in size and were again approaching normal. By May 6 the Paramecia in all cultures had died out and the experiment was concluded. During the progression of the experiment no changes in the methods of reproduction were noticed in either the tests or controls and that which did take place was asexual in nature. Two physiological changes were noticed. The first, an increased rate of reproduction and secondly, the appearance of enlarged vacuoles indicating, perhaps, an increased food intake in the cultures fed on thyroid. This, however, did not result in a permanent increase in size of the Paramecium.

Summary and Conclusions

The results obtained in the above experiment show that the thyroid hormone, administered in the form of thyroideum siccum, to the invertebrate Paramecium multimicronucleatum tended to slightly increase the rate of asexual reproduction and the rate of food intake as was indicated by the temporary increase in size of the vacuoles and the decrease in the number of bacteria found in the test cultures. Except for these no other noticeable anatomical or physiological changes occurred. The fact that Paramecia are selective in their choice of food may have caused less apparent results than might have been obtained if they had freely engulfed the hormone which was evident that they did not do.

It may be concluded that thyroxine, taken as a typical example of a vertebrate hormone, has a limited effect on the invertebrate Paramecium multimicronucleatum. This is in contrast to the results obtained by other experimenters, for from their research they conclude that vertebrate hormones have no specific results on either the growth or development of invertebrates and that any changes occurring during the experimentation were due to the phyletic development of the animal.

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