THE METAMORPHOSIS OF THE ENDOSTYLE (THYROID GLAND) OF AMMOCOETES BRANCHIALIS (LARVAL LAND-LOCKED PETROMYZON MARINUS (JORDAN) OR PETROMYZON DORSATUS (WILDER)).*[1]

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Plates 66 to 70.

INTRODUCTION.

Wilhelm Müller in 1873 homologized the endostyle, or hypobranchial groove, of the Tunicate, Amphioxus, and the larval Petromyzon with the thyroid gland of all higher chordates. Subsequent investigations have served to strengthen this remarkable homology, which would have been impossible of demonstration but for the survival of a single class of vertebrates, the Cyclostomes, of which the Petromyzontidæ, or Lampreys, are the best known order. The lamprey embraces in its own life history both the fullest development of the endostyle mechanism[2] and the characteristic ductless

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[2] In addition to the ventral midline subpharyngeal glandular structure, or endostyle proper, there are accessory structures directly continuous with the endostyle epithelium developed in the pharyngeal mucosa that are believed to function as the path of conduction for the slime cord issuing from the orifice of the endostyle. This function has not, however, been observed in the Ammocoetes and is based on reasoning by analogy from Giard's (Giard, A., *Arch. de zoöl. expér. et gén.*, 1872, i, 525; 1873, ii, 481) and Fol's (Fol, H., *Morphol. Jahrb.*, 1874, i, 222) experiments with the endostyle structure of Tunicates, where they observed the cord of mucus issuing from the endostyle to pass forward, arch up around the sides of the pharynx to the dorsal midline, then pass back along this line to the esophagus. In the Ammocoetes the anterior part of this structure begins at the anterior margin of the large elliptical orifice of the

thyroid common to all Gnathostomes. The endostyle persists throughout the larval period of the animal's existence in full activity,[3] then at the metamorphosis, which was first made known to the zoological world by August Müller,[4, 5] the endostyle undergoes involution, all but a remnant disappearing, and other profound organic changes occur. This remnant assumes the form of ductless thyroid follicles and persists throughout the adult life of the lake lamprey (*Petromyzon dorsatus*) as a true ductless thyroid. It has been stated by some observers that the thyroid follicles may disappear entirely in old sea lampreys (*Petromyzon marinus*).

It is the purpose of this paper to record in some detail the series of changes that take place in the endostyle at metamorphosis and to

endostyle with which it is continuous and just posterior to the third gill arch pillar (figures 1a, 1b, and 1c). At the beginning it is a deep slit-like fold of the pharyngeal floor and roughly of the shape of an inverted T. Just anterior to the third pillar the groove widens and about the middle of the second gill pouch divides into right and left arms which continue forward with slight divergence to a point just anterior to the second gill arch pillar where the divergence becomes more marked. These grooves become shallower as they pass forward, outward, and upward around the branchial sac at its most anterior part to join the prominent dorsal midline fold where they fuse with the ciliated epithelium on the sides of this pendant-like fold and continue back along the midline to the esophagus. The ventral surface of this dorsal ridge or fold is covered with stratified squamous epithelium out of which the permanent esophagus will be formed. The posterior portion of the structure begins at the posterior lip of the endostyle orifice in the midline and extends backward along the midline of the pharyngeal floor as a shallow and narrow groove lined with ciliated epithelium continuous with that of the endostyle. This groove gradually gets smaller and narrower to end at the entrance of the larval esophagus.

The structure, then, that makes up this accessory endostylar mechanism is (1) the large, ventral midline duct-like ciliated groove issuing from the anterior lip of the gland orifice which divides into (2) two peripharyngeal ciliated grooves which terminate in the dorsal midline in the (3) ciliated dorsal ridge and (4) the shallow, narrow ventral midline groove from the posterior lip of the gland orifice to the esophagus.

[3] This persistence lasts at least for three, and possibly for four years, according to Gage (Gage, S. H., The Lake and Brook Lampreys of New York, Wilder Quarter-Century Book, Ithaca, 1893, 421).

[4] Müller, A., *Arch. f. Anat. u. Physiol.*, 1856, 323; *Ann. and Mag. Natural Hist.*, 1856, ser. ii, xviii, 298.

[5] According to von Siebold (von Siebold, K. T. E., Die Süsswasserfische von Mitteleuropa, Leipzig, 1863) this metamorphosis was known to Leonhard Baldner, a fisherman of Strassburg, in 1666.

ascertain what type or types of the endostylar epithelium persist as the lining epithelium of the ductless thyroid follicles, with the hope that it may serve as a step toward some clue to the thyroid function in this, which is seemingly the simplest form of the ductless thyroid.

THE NORMAL ANATOMY OF THE ENDOSTYLE.

The endostyle in the course of its development in the Ammocoetes acquires a complicated morphology which in section is not easily made out. On this account I have reconstructed the organ from a brook lamprey (*Lampetra wilderi*) in the collection of Professor S. H. Gage, of Cornell University[6] (Figures 1a, 1b, 1c, and 2).

This very prominent gland in the Ammocoetes lies just beneath the pharyngeal mucosa in the ventral midline and is contained within the cartilaginous framework of the branchial sac. It extends from the beginning of the first branchial pouch to the posterior border of the fifth branchial pouch and opens into the branchial sac by a large slit-like orifice just posterior to the third gill arch pillar. This orifice is situated in the ventral midline approximately at the middle of the long axis of the gland and is continuous with the posterior end of the deep pharyngeal groove. The gland is composed of right and left symmetrically opposed halves (figure 4). Each half consists of a single straight chamber anterior to the pharyngeal orifice (figure 3a). At the anterior border of the orifice each of the anterior chambers divides into an external and internal chamber (figure 3b). Each posterior arm contains approximately one half of the epithelial investment of the corresponding anterior chamber. The two external chambers extend directly posterior, and behind the posterior border of the coil of the internal chambers curve into the midline to extend a short way back side by side and end blindly just posterior to the bifurcation of the ventral aorta (figure 3c). The internal chamber of each half of the gland extends posteriorly side by side to a point approximately two thirds of the distance between the orifice and the posterior extremity of the two lateral chambers, then curves upward and forward, and reaches the vicinity of the gland orifice where it curves downward and backward and again upward

[6] The total length of specimen 15 T, fourteen months old, was 39 mm. The total length of the endostyle was 3.08 mm.

and slightly forward to end blindly (figures 1a, 1b, 1c, and 2). Thus it describes about one and one half elongated coils. These two internal or coiled chambers if extended would be longer than the two lateral chambers. I have spoken of these coiled internal chambers as if they were separate, but they are not strictly separate chambers since the septum is not complete. They are separate, however, in that they arise from opposite halves of the organ. The median septum is complete anterior to the orifice, while at the orifice it becomes partial and remains so throughout the extent of the internal or coiled chambers. The anterior chambers then communicate with each other only at the orifice. The two lateral chambers posterior to the orifice also communicate only at the orifice, and the two internal chambers communicate throughout their length on account of the incomplete median septum. All chambers communicate with each other at the orifice.

The gland is contained within a well formed fibrous capsule which anterior to the orifice is composed of an outer layer surrounding both halves and an inner layer which passes into the septum. Posterior to the orifice the outer fibrous investment of the whole organ is well developed, but the septa dividing the four chambers are very atrophic.

The ventral aorta is continued forward in the midline from the conus arteriosus as a single tube on the dorsal surface of the posterior extension of the lateral chambers until it reaches the posterior edge of the coiled inner chambers. Here it divides into two trunks which are continued forward on either side of the coiled chambers and on the dorsal surface of the lateral chambers to the anterior end of the endostyle where they terminate in the first pair of gill arteries.

The Lining Epithelia.—Anterior to the orifice the most striking feature of the interior of each chamber is the large visceral invagination, or infolding (flask-shaped in transverse section (figure 3a)), from the ventrolateral wall carrying blood vessels and stroma, but composed for the most part of four large bundles (fan-shaped in cross section) of large cuneiform cells, two on each side of the midline of the invagination. These fan-shaped columns extend the entire length of the organ and are composed of long wedge-like cells with large nuclei lying at the base of the cells; *i. e.,* at the periphery of the columns. These long cells taper to a central core which opens on to the free surface by a small pyramidal projection. The finer cytology of these cells is not easily made out owing to the difficulty of obtaining good fixation. The nuclei are large, round, and vesicular. The cytoplasm also stains with basic dyes and one gets the impression that faintly staining granules and longitudinal striations are present, but, as Schneider remarks, this may be due to cytolysis. Each bundle is surrounded by a separate fibrous investment. These four bundles of gland cells make up the bulk of the interior of the invagination.

Covering the surface of the visceral invagination and thence reflected on to the septum and lateral wall of the chamber, one can distinguish four other types of epithelial cells. First on either side of the four small pyramidal openings of the large bundles there is a zone of narrow high columnar epithelium with long densely packed cilia. The nuclei of these cells stain densely, are much elongated, closely packed, and situated near the middle of the cell bodies. The cytoplasm stains with acid dyes. Extending up to the necklike constriction of the visceral invagination is the second type of epithelium,—high columnar, with coarser and shorter cilia. These cells are of the same length as those just described, but are broader. The nuclei are round, pale, and vesicular, and lie in the basal third. The cytoplasm stains a pale red with eosin and in addition contains large quantities of a yellow flocculent and granular pigment. The knob-like apex of the fold anterior to the orifice is covered with a third type directly continuous with the second type and composed of low columnar cells with short coarse cilia. The cells have darkly staining elongated nuclei situated about the middle of the cell bodies, and the cytoplasm, while taking acid dyes, lacks the yellow pigment of the second type. This type is continuous at the orifice with that lining the pharyngeal grooves. A small zone of this same type of epithelium also occurs at the base of the visceral invagination on each side and merges into the fourth type of epithelium which covers the parietal walls, septum, and lateral wall. This area is covered with low cuboidal or flattened endothelial-like cells arranged in a single layer, as are also all the other epithelia.

At the orifice, as stated above, each anterior chamber divides so that half of the flask-shaped invagination of each anterior chamber continues in each of the four posterior chambers, the outer half of each invagination continuing as the lateral chambers, and the inner halves going into the internal, or coiled, chambers (figure 2). There is no change in the types of epithelia, each posterior chamber containing all the types found in the anterior chambers, but the number of cells is reduced approximately by half.

The glandular portion of the endostyle then contains five distinct types of epithelium: first, the four large bundles of cuneiform cells of each half of the gland; second, the narrow, columnar, densely nucleated and densely ciliated cells on each side of the pyramidal openings of the cuneiform bundles; and third, the broad columnar cells with round pale nuclei containing abundant yellow pigment granules in the cytoplasm. All these forms are confined wholly to the visceral invagination. The fourth form occurs on the knob-like apex of the visceral folds in the anterior chambers which at the orifice fuses with the parietal epithelium and at the angles of the base of the fold merges into the parietal epithelium. These cells are also columnar, coarsely ciliated, with dense elongated nuclei, and contain none of the pigment of the third type. The fifth type forms the parietal lining of the chambers, and is composed of a single layer of large endothelial-like cells.

These five types of epithelia are quite distinct and offer no difficulty of recognition by ordinary methods. I have given a detailed description of these five types because of their importance in the changes associated with the metamorphosis. In order to avoid repetition in the descriptions of the fate of each type of epithelium from the inception to the completion of metamorphosis, the five epithelia will be designated as types as follows (figure 5):

Type I.—The large fan-shaped bundles of cuneiform cells.

Type II.—The narrow densely nucleated and ciliated high columnar cells extending for a short distance on each side of the narrow openings of the cells of type I.

Type III.—The broad columnar ciliated epithelium with yellow granular pigment.

Type IV.—The low columnar ciliated cells occupying three sites: (1) the angle between the visceral fold and the parietal wall; (2*a*) the similar angle at the apex of the visceral fold posterior to the orifice; and (*2b*) the knob-like free apex of the visceral fold anterior to the orifice.

Type V.—The flat epithelium lining the parietal walls and septum.

THE METAMORPHOSIS.

I have found no publications dealing specifically with the metamorphosis of the endostyle, although several observers have referred to it in a general way. Rathke[7] was the first to describe the endostyle of Ammocoetes as a gland, and in 1856 August Müller[8] observed that it disappeared at the metamorphosis. He thought that the structure became the muscular portion (tongue) of the sucking apparatus. Schneider[9] gives the first account of the metamorphosis of the endostyle as ascertained from two metamorphosing specimens of *Ammocoetes branchialis* given him by von Siebold. He noted that the large bundles of cuneiform cells disappeared during the change, while the connective tissue framework and lining ciliated epithelium remained. He stated that the fibrous tissue increases and forms a dense wall about the epithelium and gradually compresses it, although the epithelium holds to its orderly arrangement in the tube until at last the fibrous tissue growth cuts the tubules into segments and thereby obliterates all its original endostylar structure and creates the ductless thyroid follicles. W. Müller in his earlier paper,[10] while recognizing the thyroid gland in the adult *Myxine glutinosa,* seems to have mistaken the salivary gland for the thyroid in *Petromyzon fluvialis.* In a later paper[11] he states that only at metamorphosis the large glandular endostyle atrophies and only a small remnant of its epithelial lining remains as the ductless thyroid follicles of the sexually mature lamprey.

The extensive papers of Schneider's pupil, Kaensche,[12] and of Bujor,[13] give no account of the changes taking place in the endostyle at metamorphosis.

My observations are based on the study of twenty-four metamorphosing specimens taken from the mud and sand of Cayuga Lake Inlet at Ithaca, New York. Specimens for microscopic study were taken from the stream twice each week from June 17 to August 11, 1912.

Metamorphosing specimens in excess of those needed at the time of taking were stored in laboratory aquaria which were half filled with mud and through which running water was continually passing. After August 11 one of these laboratory specimens was removed

[7] Rathke, M. H., Bemerkungen über den inneren Bau des Bricke, Danzig, 1826.

[8] Müller, A., *loc. cit.*

[9] Schneider, A., *Ann. and Mag. Natural Hist.,* 1873, ser. iv, xi, 236.

[10] Müller, W., *Jenaische Ztschr.,* 1871, vi, 428.

[11] Müller, W., *Jenaische Ztschr.,* 1873, vii, 327.

[12] Kaensche, C. C., *Zoöl. Beitr. von A. Schneider,* 1890, ii, 219.

[13] Bujor, P., *Rev. biol. du nord de la France,* 1890–1, iii, 301; 1891–2, iv, 41.

every fifth day until September 20. I was able therefore to study an unbroken series from June 17 to September 20, under natural conditions until August 11, and modified by laboratory residence from August 11 to September 20.

The first specimen showing external signs of metamorphosis was taken on July 19. In a specimen taken July 16 and showing no external signs of metamorphosis, microscopic examination revealed in the endostyle changes that in the light of subsequent studies of the series as a whole must be considered as the earliest evidences of metamorphosis; namely, a thickening of the fibrous capsule and a slight reduction in the volume of the cells of type I. The specimen of July 19 shows, on careful examination, external changes. The dentaphores are visible as a stipple-like roughening on the inner surface of the upper leaflet of the hood and there is partial obliteration of the normal sharp angle at the juncture of the upper and lower leaflet of the buckle hood. The eyes could not be seen in the fresh specimen, but after a day's fixation in formalin, round whitish spots in the location of the eyes were visible through the skin. The prominent endostyle changes are the same as, but slightly more marked than those recorded for the specimen of July 16.

The specimen of July 23 shows obvious external changes. The body length is 14.7 cm., the eyes are clearly visible, the color is slate gray on the dorsal surface, and brownish yellow on the ventral surface. Dentaphores are visible, and there are slight elongation of the head and partial obliteration of the angles of the buccal hood.

The endostyle (figure 6) has undergone a striking reduction in size, measuring 1.006 mm. in diameter at the level of the orifice, as compared with 1.518 mm. for the normal endostyle of an ammocoetes of the same size. The fibrous capsule is sharply outlined as a thick band closely investing the gland and involving the septum as well. There is also a new formation of fibrous tissue in the periglandular zone. The interval between the gland and the pharyngeal mucosa is increased by this new tissue formation which is in the location of the future tongue. Of the epithelia, the cells of type I are reduced one third of their original volume, although they retain their wedge shape and arrangement. The nuclei of these cells have migrated from the base of the cells, *i. e.*, the periphery of the fan-shaped formation, and occupy the middle third of the cells. All four columns are affected to about the same extent. The cells of types II, III, and IV are somewhat compressed, but their general appearance is but little altered. The cells of type V have become more cuboidal owing to reduction in the size of the chambers.

The specimen of July 26 is 14.4 cm. in length. The eyes are distinct, the nasal pore large and patent, and the pineal eye white. The head and branchial region appear reddish and semitranslucent from the increased vascularity. The color on the dorsal surface is a dark slate gray and on the ventral surface it is tinged with yellow. The endostyle measures 0.627 mm. in diameter at the level of the orifice and exhibits reductions in the volume of all its component parts (figure 7). The sharp line of demarcation between the thickened capsule and the newly formed tissue external to it, which was a prominent feature in the specimen of July 23, is absent and the two tissues merge. The whole area is much more vascular and contains large blood spaces. The tongue anlage is visible as a rounded bundle of large closely set elongated cells. The cells of type I are still detectable in the fan-shaped areas, although connective tissue has begun to invade them. The cell boundaries are for the most part lost. There is clumping of the cell masses. The nuclei are scattered irregularly through the area and present many stages of dissolution. The surface connection of these cell bundles is lost although the site of this connection is recognizable as slight notches in the cells of type II between which these bundles normally opened. The cells of type II are more compressed though still ciliated and high columnar. The nuclei of these cells while normally dense and closely set are through compression so closely packed that their limiting membranes are barely visible. The cells of type III have undergone a great reduction in size and probably also in number. The cilia are still present. The characteristic yellow pigment granules are more in evidence, probably as a result of a more rapid destruction of the cells than of the pigment. The cells of type IV are the least affected of all the types. There is possibly some slight reduction of their volume and a loss of cilia. The cells of type V, as a result of the further shrinkage of the capsule, are more cuboidal. Their positions are slightly altered and the first evidence of piling up and overlapping is seen.

The specimen of August 1 is 12.5 cm. in length. The eyes are large and clear and produce slight disc-like prominences on the sides of the head as in the adult. The color on the dorsal surface is a dark slate gray, and that on the ventral surface is tinged with yellow. The hood is much elongated. The sucking disc is now complete and all outlines of the larval hood are absent. The pharyngeal grooves can still be made out and the gland duct, much elongated, is still patent notwithstanding the extreme atrophic changes in the endostyle. The pericapsular tissues are vascular. The dense fibrous tissue of the thickened capsule, so evident in specimens of July 16, 19, and 23, has disappeared and the rather loose, palely staining fibrous tissue extends to the gland epithelium lining the chambers. The septum between the two halves of the endostyle is now a wide band of proliferating fibrous tissue separating the two halves as well as rotating their dorsal portions outward. At the level of the orifice the gland diameter is 0.462 mm. The individual chambers anterior to the orifice are about 0.2 mm. in diameter, or slightly smaller than the diameter of each ventral aorta at the same level.

The fan-shaped bundles of type I are no longer distinguishable as such. Connective tissue ingrowths from the base of the visceral fold and from the fibrous tissue within the fold itself have broken up these areas so that one sees only shrunken and distorted cell masses with or without visible nuclei (figure 8). These areas are, in general, lighter in color. The cells of type II are still ciliated, and are considerably reduced in length and extent. All evidence of the openings of the cells of type I is gone. The nuclei are basal, very dark, and closely packed. The cells of type III are much reduced in size and number. The yellow pigment granules are relatively more abundant and are contained for the most part in the basal portion of the cell bodies, but are also present above the nucleus. The scattered cilia are still to be made out on these cells. The cells of type IV are well preserved although the cilia are absent. Their extent is slightly reduced by compression. Their nuclei are large, darkly staining, and closely packed. The cells of type V, owing to the great contraction of the chambers, are irregularly distributed, piled up, in places two or more in thickness, and their nuclei show many stages of degeneration.

The specimen of August 5 is 14.9 cm. in length. The anterior portion of the endostyle is separated from the pharynx by the now large muscular anlage of the tongue which lies between the aortæ and the endostyle. The duct is elongated, and while its outline is visible, its pharyngeal connection has been closed at the level of the tongue anlage. All the pharyngeal grooves are gone and, in cross section, the branchial sac is a smooth walled oval chamber with the permanent esophagus anlage embedded above it in the dorsal midline. The endostyle chambers are still discernible in both the anterior portions as clefts, about 20 μ in width. A capsule as such is no longer visible. The fibrous tissue has become uniform so that no line of demarcation exists between the visceral fold and the parietal wall. The diameter of the endostyle at the level of the duct is 0.146 mm. All the epithelium of type I has been absorbed. The cells of type II have either atrophied beyond recognition or fused with the lower area of type IV. It appears as if this epithelium had gone, but I lack sufficient gradations to exclude a fusion. The cells of type III are now represented by a few atrophic cells containing a great abundance of yellow pigment granules (figures 9 and 10). The cells of type IV remain prominent. They are more cuboidal and their nuclei are probably paler than in previous stages. The cells of type V appear as an extruded mass of large, distorted, and disintegrating cell masses lying to the outer side of the cleft-like remnant of the chambers.

The specimen of August 9 is 12.5 cm. in length. All evidences of the true endostyle are absent in this specimen. The duct is obliterated. The small follicles and nests of epithelial cells lie in a rather uniform area of connective tissue. These follicles and cell nests are grouped into right and left bundles corresponding to the right and left halves of the old endostyle, and by the obliteration of the septum and ingrowth of fibrous tissue they are relatively widely separated. Anterior to the old orifice there are three to four follicles or nests of cells in each group corresponding to each half of the endostyle; while posterior to the old orifice and corresponding to the coiled portion of the inner chambers the follicular and cell nest remnants of the two halves of the old endostyle are closely approximated, and one can make out as many as twelve to fifteen follicles or cell nest areas. Of the epithelia, type I is completely absent, and type II is either completely absorbed or, becoming atrophic, has fused with the lower area of cells of type IV. Type III is still recognizable as a few nuclei surrounded by clumps of yellow pigment, in some levels extruded toward the lumina of the future follicles and in others pushed backward in the connective tissue stroma. Type IV is now the only prominent epithelium. The upper or dorsal area has in places assumed definite follicular shapes, utilizing the remnant of the original chamber as their lumina. In places the epithelial layer is slightly curved while in others it has completed the formation of the follicle. The lower mass of epithelium of type IV and possibly the fused cells from type II appear as a more or less curved layer of well preserved cuboidal cells surrounding the remnant of the original chambers. The cells of type V may still be made out as a small mass of more brightly staining cell remnants in the connective tissue opposite the concavity of the cell layers of type IV (figures 11, 12, and 13).

From this stage on, the further involution is largely a matter of the absorption of the remnants of the disappearing types of epithelia, the completion of the follicle formations out of the persisting type of epithelium (type IV), the development of the tongue and of the branchial sac or bronchus, the formation of the great blood spaces about the tongue through the absorption of the connective tissue, and the rotations and possible migrations, distortions, and secondary constrictions of the original follicular tubes by changes in the interfollicular stroma.

EXPERIMENTAL OBSERVATIONS.

Experiment 1.—September 4, 1911. Sixty-five specimens were collected and provisionally classified as follows:

9 of the hatch of 1911, measuring 1.9 to 2 cm. in length.
34 of the hatch of 1910, measuring 6 to 6.5 cm. in length.
20 of the hatch of 1909, measuring 11 to 12 cm. in length.
2 of the hatch of 1908, measuring 14 to 15 cm. in length (metamorphosis complete).

The specimens were taken to Cleveland and placed in jars half filled with mud and sand in which some hay was placed, and running lake water was supplied continuously to each jar. On September 10 the twenty specimens of the hatch of 1909 were divided into four lots of five each and placed in separate jars. To two of the lots one drop of Lugol's solution was added daily, while the remaining two lots were kept as controls. On the 14th of each month, beginning September 14, 1911, and ending June 14, 1912, a specimen from the lots exposed to iodin and one of the controls were taken for histological examination. The object of this experiment was to see whether iodin might influence the endostyle structure and possibly afford a chemical connecting link with the ductless thyroid gland which is so markedly influenced by iodin. The results were entirely negative as regards any morphological change. This negative evidence, however, in so far as it is of value, supports the hypothesis of Müller, Dohrn, and others, based on morphological changes, that the physiology of the endostyle is quite different from that of the ductless thyroid.

Experiment 2.—Attempts were made to destroy the endostyle organ in ammocoetes varying in length from 10 to 16 cm., (1) by means of injecting paraffin into the gland lumen, and (2) by cauterizing with a fine electric needle. Ether vapor was found to produce a safe and quick anesthesia from which the animals rallied in a few minutes. Chloroform vapor and several solutions of chloretone and magnesium sulphate were tried, but proved to be too toxic.

The operation was performed by placing the anesthetized ammocoetes with its ventral surface up in a grooved pad of water-soaked absorbent cotton. For the paraffin injections, a fine hypodermic needle with screw adjustment for graduating the pressure was used. A mixture of paraffin oil and 52° paraffin was tried. The method was abandoned on account of the hardening of the paraffin in the needle as soon as it came in contact with the cold surface of the ammocoetes.

With the fine electric needle and the animal in the dorsal position the following procedure was carried out. The skin and musculature were burned through in the ventral midline between the second and fourth gill openings and the tip of the cautery was allowed to extend into the branchial sac. The entrance of the cautery into the sac is easily detected. The cautery was then extended slightly forward and backward from this point. This produced some destruction of the endostyle between the right and left branches of the ventral aorta in the region of the gland orifice. Ten ammocoetes were thus operated upon on July 13. All recovered and were kept until August 11, when the experiment was interrupted. The ammocoetes were all vigorous and active at the time they were killed. The cautery wounds had healed in all and the sites were marked by white, non-pigmented scars.

Histological examination of these ten specimens shows that the endostyle was only partially destroyed in each. The large bundles of cuneiform cells (type I) were destroyed at the cauterized site and exhibited no evidence of regeneration. There was an inflammatory reaction in the visceral invagination extending for a considerable distance beyond the cauterized area and containing eosinophiles. In none of the specimens were the chambers destroyed, as I hoped they might be, and it appears that an actual regeneration of at least two types of epithelium takes place: type IV, or that lining the duct and continuous with the pharyngeal grooves, and also type V, or the endothelial-like lining of the parietal walls. There is no evidence that the yellow pigment-bearing cells of type III regenerate. The duct was not destroyed in any of the specimens, although in two specimens evidence of severe injury was present, yet the epithelial lining had regenerated.

These observations, although meager, show that gross injury may be induced experimentally without death of the animal, and that some of the endostylar epithelia have little or no regenerative capacity, while others exhibit considerable regeneration within the period of a month. Further experiments extending over a longer time so as to include the effects of metamorphosis on animals which have been operated on in this way seem highly desirable.

SUMMARY.

The first specimen of *Ammocoetes branchialis* that showed histologically any atrophic changes in the endostyle was taken on July 16. These changes proceeded relatively rapidly for about a month, after which the endostyle as such was no longer recognized. All specimens examined after August 15 showed in cross section the characteristic ductless follicles more or less completely formed. More gradual and minor changes in the way of further absorption of cell remnants and completion of the follicles continued at least until September 1. Two specimens taken from the creek on September 4, 1911, showed complete follicle formation with some stainable colloid (figures 14 and 15). There was still yellow granular pigment in the fibrous tissue between the follicles. In two specimens[14] taken on October 14, 1909, the pigment was absent and the follicles were more closely set, larger, and contained homogenous colloid. In the twenty-four specimens of ammocoetes studied, there were variations in the time of the onset of metamorphosis. There may also be variations in the rate of progress of the changes in different specimens. There is no evidence that removal of the animals from their native environment to the laboratory either increases or decreases the rate of metamorphosis. Schneider states that he was unable to get specimens kept in the laboratory to undergo metamorphosis. Gage, however, has repeatedly observed the metamorphosis under laboratory conditions, and the six of our specimens kept in the laboratory—some for forty days—remained in excellent condition and the metamorphosis proceeded as well as in those living in the creek. I know of no observations bearing on the question as to whether the metamorphosis may be hastened or delayed as it can be in tadpoles and other amphibia. It is probable, however, that physical conditions greatly influence the transformation.

These observations as to the length of time from the inception to the completion of metamorphosis indicate that a month and probably longer is necessary for the lake and brook lampreys of Central New York. This is in agreement with the observations of Gage[15] and of Müller[16] on metamorphosis in general, but is at variance with the views of Bujor,[17] who states that the process takes place within three to four days.

The first endostylar changes are a gradual shrinkage in the whole organ with thickening of the capsule and septum and proliferation of the connective tissue in the periendostylar zone. The tongue anlage is developed in this thickening just dorsal to the endostyle and anterior to the gland orifice. The size of the chambers progressively decreases and with the thickening of the septum the halves of the endostyle are both absolutely and relatively more separated.

[14] These two specimens were presented to me by Professor Kingsbury for serial sections.

[15] Gage, S. H., *loc. cit.*

[16] Müller, A., *loc. cit.*

[17] Bujor, P., *loc. cit.*

All the five types of epithelia are affected, the first to show the change being type I, the four fan-shaped bundles of cuneiform cells of each half of the endostyle. These disappear totally quite early. The next type to show marked changes is type III, or the cells with yellow pigment granules. Here the change is progressive and these cell groups in different stages of atrophy may be traced through to the fully developed follicles. The epithelium of type V, or the endothelial-like lining of the parietal walls of the chambers, is piled up and extruded laterally as the chambers contract or shrink. These cells in different stages of atrophy may be followed until the metamorphosis is nearing completion. It is certain that the cells of types I, III, and V play no part in the formation of the ductless follicles. With types II and IV the question is not so easily settled as it is from one or the other or from both of these types that the permanent follicles arise. One can say definitely that type IV plays the major rôle, but whether the cells of type II after fusion with the basal group of type IV do not also share in the formation of the ventral follicle of the given chamber, I cannot decide, but from the evidence obtainable this seems probable. It is significant that the cells of type IV are continuous with, and indistinguishable from, the cells lining the orifice and are continued anteriorly in the deep pharyngeal groove and peripharyngeal grooves as well as posteriorly from the orifice in the small pharyngeal groove. As to the fate of this extraglandular epithelium of type IV I have no data save that with the closing of the orifice and the formation of the permanent branchial sac these grooves with their ciliated epithelium disappear and the whole sac comes to be lined with plain stratified epithelium. The fact that the cells of the pharyngeal grooves and the lining cells of the gland orifice are continuous with the cells of the endostyle from which the permanent thyroid follicles are formed is not without significance in relation to the development of the thyroid of the higher chordates. One or more very large follicles are formed from the lower portion of this orificial epithelium of type IV.

Four ductless follicles are the maximum number that may be formed primarily in each half of the endostyle from the four areas of epithelium of type IV. From the specimens studied this maximum is frequently not obtained. Posterior to the orifice where four chambers exist, each corresponding to one half of an anterior chamber, but two follicles may be formed from each chamber, but in the coil these are proportionately increased, in cross section. Most of the detailed studies here recorded have been made on the part of the endostyle posterior to the coil where the simplest conditions exist. Here two follicles are ordinarily formed from each chamber.

In cross sections the follicles are at first only long tubules whose cavities are the remnants of the original endostyle chambers, but when the metamorphosis is completed each of these primary tubules is cut up into several elongated closed sacs corresponding to the true ductless follicles of all higher chordates.[18] New follicles also arise by budding from these primary ones, and this process is probably of normal occurrence at the metamorphosis.

[18] Dr. H. D. Reed, of the Department of Zoölogy of Cornell University, gave me twelve specimens of adult lake lampreys taken at spawning time during the spring of 1912 from the Cayuga Lake Inlet. In examining histologically the thyroid areas of these sexually mature lampreys certain features worthy of mention were noted. (1) The thyroid area still shows the division into the right and left halves corresponding to the endostyle halves. This separation is most marked in the anterior half of the thyroid area. (2) The increase in number of follicles due to the coil of the inner chambers of each half of the endostyle can be made out. (3) In two specimens the follicles were much larger and the number of follicles was increased above that which obtains just after the metamorphosis, suggesting that both hypertrophy and hyperplasia of the epithelium lining the ductless follicles have occurred. This epithelium was originally that of type IV of the endostyle. This is of interest in suggesting that these cells, after involuting to a given level as part of the destruction of the endostyle, assume a resting stage from which they may again undergo hypertrophy. (4) In one of the twelve specimens (a large adult female) the thyroid follicles were very large, some measuring 0.214 mm. in diameter, although there was no increase in the number over that obtaining just after the metamorphosis. The lining epithelium was very high columnar and for the most part, if not completely, ciliated. The nuclei of these very large high columnar cells were large, rounded, and palely stained. In each follicle there was a homogeneous red staining colloid-like mass. The significance of this observation is probably greater than one can estimate from the few facts obtainable from this accidental specimen. The fact that the endostylar epithelium from which these follicles arise is ciliated, and that these cilia usually disappear rather early in the metamorphosis, suggests that some physiological factor is involved either by way of preventing normal involution through sustained physiological activity of the larval cells, or by inducing a regeneration of the involuted cells to the larval type.

EXPLANATION OF PLATES.

PLATE 66.

FIG. 1a. Photograph of a wax model with the pharyngeal mucosa attached, showing (*a*) the pharyngeal opening of the endostyle, (*b*) deep anterior pharyngeal groove, (*c*) branching of the anterior groove into the two peripharyngeal grooves, (*d*) shallow posterior pharyngeal grooves, (*e*) left terminal branch of the aorta and (*f*) opening into branchial pouch.

FIG. 1b. Photograph of a wax model with the pharyngeal mucosa removed (dorsal view), showing (*a*) endostyle orifice, (*b*) coiled internal chambers, (*c*) left external chamber, (*d*) median raphe and septum, (*e*) conus arteriosus, (*f*) bifurcation of the aorta, and (*g*) gill arch artery.

FIG. 1c. Photograph of a wax model with the pharyngeal mucosa removed (lateral view).

FIG. 2. Photograph of transverse sections of the model, (*a*) anterior to the orifice, showing visceral invagination and the arrangement of the large bundles of cuneiform cells, (*b*) just posterior to the orifice, (*c*) through the coiled portion, and (*d*) through the posterior edge of the coil.

PLATE 67.

FIG. 3a. Photomicrograph of a transverse section of the endostyle anterior to the orifice.

FIG. 3b. Photomicrograph of a transverse section of the endostyle through the orifice.

FIG. 3c. Photomicrograph of a transverse section of the endostyle posterior to the coiled chambers.

PLATE 68.

FIG. 4a. Schematic diagram of the left half of the endostyle. The single anterior chamber branches at the anterior edge of the orifice into an inner coiled chamber and an outer straight chamber. In the diagram the inner chamber is distorted inward and rotated to the left from its normal upright position.

FIG. 4b. Composite diagram of the right and left halves with the two coiled chambers rotated outward from their normal upright positions.

FIG. 4c. Schematic diagram of the right half of the endostyle.

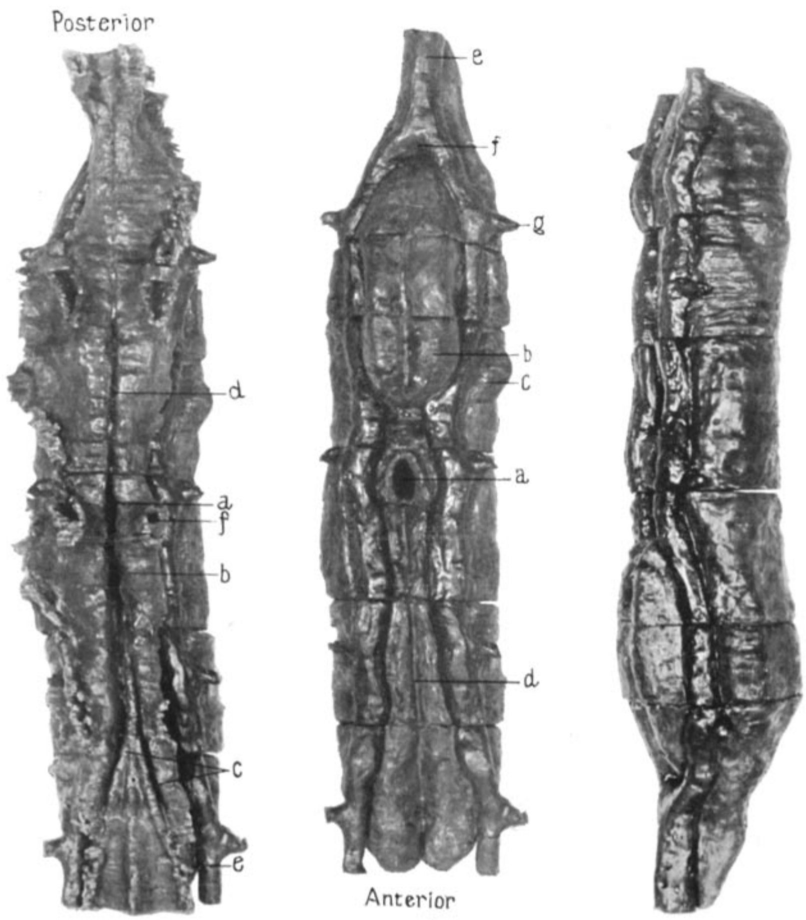
FIG. 5. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing the arrangement and distribution of the five types of epithelium in the normal fully developed endostyle. × 107½.

FIG. 6. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing the earliest changes in the epithelia at metamorphosis. × 367½.

PLATE 69.

FIG. 7. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing a slightly later stage of metamorphosis. × 367½.

FIG. 8. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing a slightly later stage of metamorphosis. × 367½.



a *b* *c*

FIG. 1.

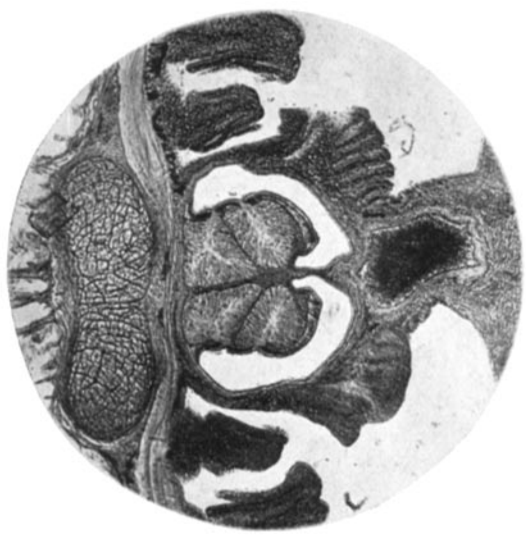
d *c* *b* *a*

FIG. 2.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

a

b



c

FIG. 3.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

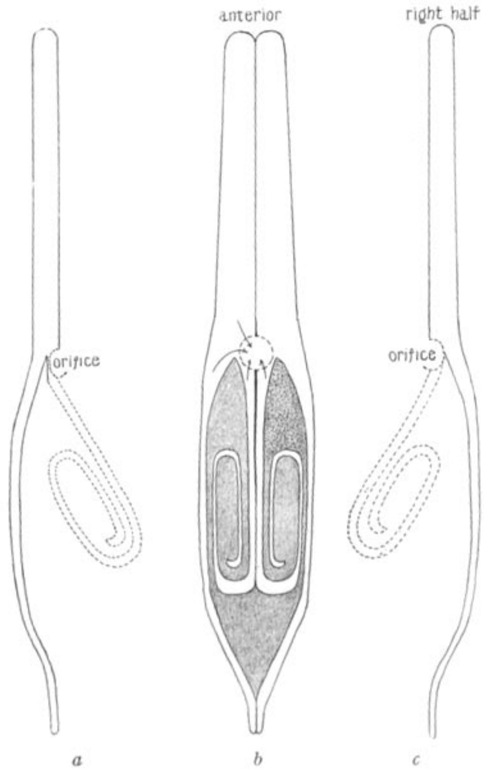


FIG. 4.

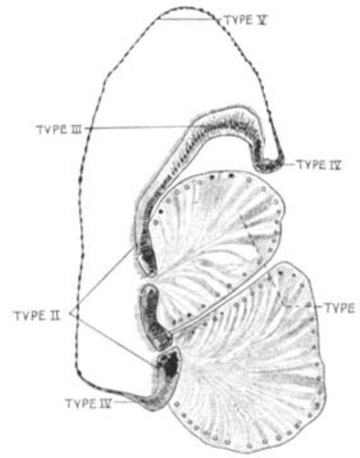


FIG. 5.

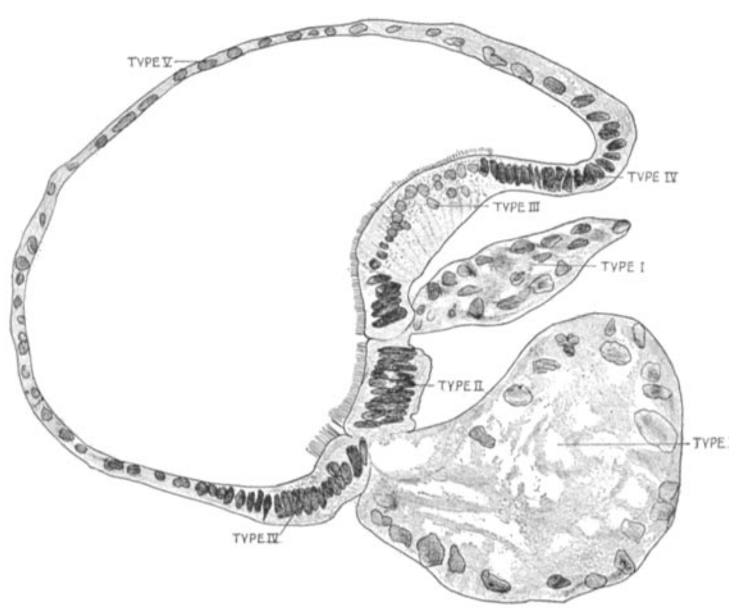


FIG. 6.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

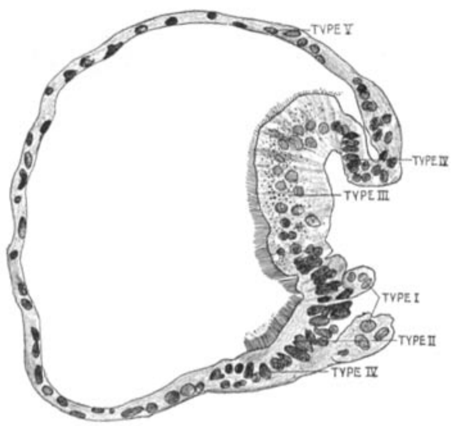


FIG. 7.

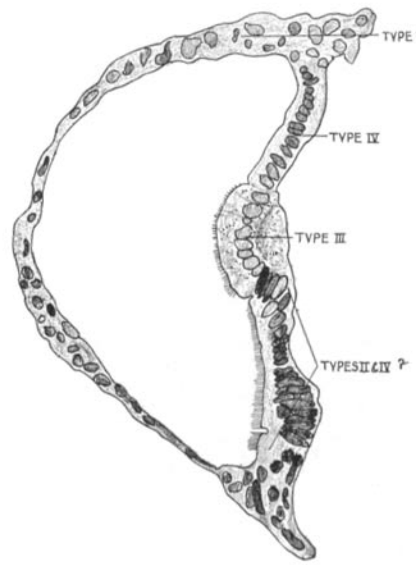


FIG. 8.

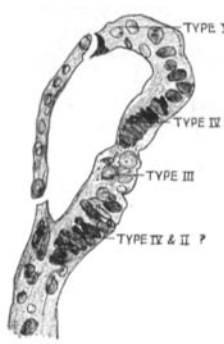


FIG. 9.

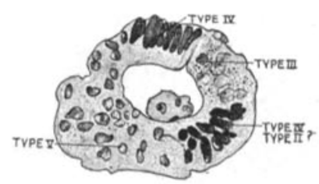


FIG. 10.

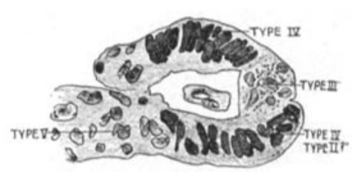


FIG. 11.

FIG. 12.

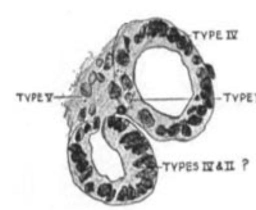


FIG. 13.

Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

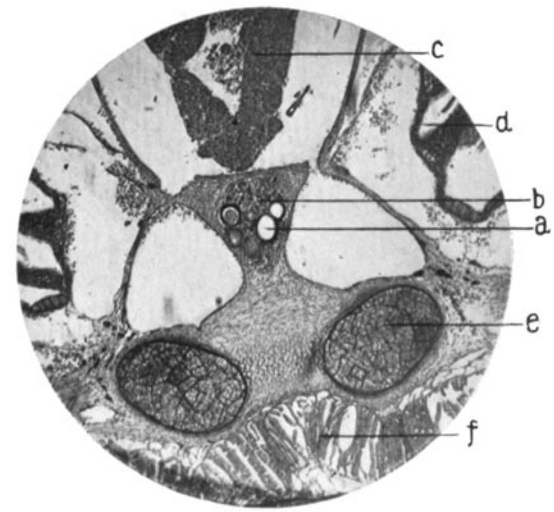


FIG. 14.

FIG. 15.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

FIG. 9. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing a still later stage of metamorphosis. $\times$ 367½.

FIG. 10. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating the last stages of the metamorphosis. $\times$ 367½.

FIG. 11. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating a slightly later stage of the metamorphosis than is shown in figure 10. $\times$ 367½.

FIG. 12. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating the earliest ductless thyroid formation. $\times$ 367½.

FIG. 13. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating two distinct follicle formations from the single chamber. $\times$ 367½.

PLATE 70.

FIG. 14. Photomicrograph of a transverse section of the thyroid area from a specimen removed from the creek on September 4, 1911, in which the metamorphosis was complete, showing (*a*) the thyroid area with complete follicle formations, (*b*) pigment and cellular debris, (*c*) tongue, (*d*) gills, (*e*) cartilaginous framework of the branchial sac, and (*f*) somatic musculature.

FIG. 15. Photomicrograph of the thyroid area shown in figure 14. Higher magnification.