

THE RELATIONSHIPS AND HISTOGENESIS OF THYMUS-LIKE STRUCTURES IN AMMOCOETES¹

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THREE TEXT FIGURES AND FOUR PLATES

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INTRODUCTION

The question of the source and mode of development of the component structures of the thymus is one of the most difficult problems in anatomy. Although a vast literature has accumulated during the last fifty years some of the most fundamental phases of the problem still remain unsolved.

The thymus has been described in every group of vertebrate animals from the elasmobranchs up to and including man. The involvement of gill pouches in the formation of the gland has been established in almost all of the investigated forms. There

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is still disagreement on the purely epithelial origin of the reticulum and Hassal's corpuscles, and the cardinal problem, the source and nature of the development of the small thymus cells, has not been definitely settled up to the present time. Three fundamentally different views, each with its coterie of supporters, are held regarding the source of these cells. A large number of investigators believe that the small thymus cells are true lymphocytes which are formed from the epithelium by a process of transformation. An equally large number believe that these cells represent true lymphocytes which have wandered into the epithelial anlage from the mesoderm. A remaining smaller group of investigators believe that the small thymus cells have an epithelial origin and are different from true lymphocytes.

Even in the most primitive animals in which the thymus has been established, the elasmobranchs, the formation of the small thymus cells does not occur until mesodermal tissue is present in the epithelial anlage. The source of a new type of cell which forms in a mixed tissue would not be difficult to determine if the two tissues entering into the formation had different morphological characters and retained them. The methods employed up to the present time have not shown sufficient morphological differences in the mesodermal and endodermal cells present in the thymus anlage to establish the source of the small thymus cells.

The sudden appearance of the thymus as a well defined structure in the elasmobranchs, together with the probability of finding a solution to the question of the source of the small thymus cells, has stimulated a number of investigators to search for a homologous structure in the more primitive types of chordate animals. While the search has been a fruitless one in the ascidians and amphioxus, various structures have been described for a thymus in the cyclostomes. The evidence offered in these descriptions has not been sufficient to establish the organ in this group of animals. The failure to find the thymus or its homologue in the cyclostomes especially in the Petromyzontes, may be attributed largely to the peculiar nature of the branchial region in this primitive group of animals.

The bearing on the interpretation of thymus histogenesis in higher animals suggested by the development of the organ in a primitive type, led the author to undertake a systematic study of the branchial region of the petromyzon larva. The time and work which have been given to this study have, I believe, been amply repaid in the results obtained. Thymus-like placodes have not only been located in the position which makes them homologous with the thymus placodes of the elasmobranchs, but the placodes have also been found in a more primitive condition than they have been shown to exist in any other animal.

LITERATURE

The search for a thymus in the most primitive chordate animals has been undertaken by a number of investigators. Up to the present time the organ has not been established in any of these lower forms. Willey ('94) suggests that the tongue-bars occurring in the gill-slits of amphioxus represents the thymus gland. The position of these structures is apparently the only basis for this suggestion. Their gelatinous structure, however, would offset any argument that they were homologous with the thymus placodes of fishes. Stannius ('84) credits the discovery of the thymus in the myxinoids to Johannes Müller. Later investigators, however, have been unable to verify this discovery. Stockard ('07) in his study of the thyreoid in *Bdellostoma Stouti* was unable to find a thymus in this form. M. Schultze ('56) described a tortuous sac in the ventral wall of the branchial cavity of *Petromyzon planeri* which he thought represented a thymus. Schneider ('79) showed that a part of this structure disappears in the development of the animal while the remaining part changes into a group of follicles which represent the thyreoid.

Schaffer ('94) described structures in the lateral branchial wall of a 51 mm. larva of *Petromyzon planeri*, which he thought represented thymus anlagen. He found in all twenty-eight anlagen, seven pairs on each side which consisted of ventral and dorsal portions. These anlagen were connected with the epithelium of the branchial vestibules.

The minute structure of these buds is summed up in the following:

Was den feineren Bau dieser Knospen anlangt, so gestattet mir die mangelhafte histologische Conservierung (Alkohol) vorläufig nur folgende Bemerkungen zu machen: Von der kapsel dringen feine Bindegewebsbälkchen in das Innere ein, welche ein spärliches, reticulartiges Stützgerüst für die zelligen Elemente bilden. Diese selbst sind kleine Rundzellen von lymphoiden Aussehen, kaum wahrnehmbarem Protoplasma, stark färbbarem Kern und Kerngerüst. Zwischen denselben finden sich ausserdem grössere, blasse Kerne mit deutlicher Kernmembran, und Kernkörperchen, welche dem Stützgewebe anzugehören scheinen und rothe Blutkörperchen, von denen ich nicht sagen kann, ob sie frei zwischen den lymphoiden Zellen liegen oder eingeschlossen in Capillaren.

Die lymphoiden Zellen sind wahrscheinlich Abkömmlinge des Kiemenepithels, wie ihr director Übergang in das letztere vermuten lässt.

In a subsequent paper Schaffer ('06) withdrew his previous interpretation and said that he did not believe these structures represent thymus anlagen.

Giacomini ('00, 1 and 2) believed that "the lymphoid organ in the basalar region of the gill lamellae (in ammocoetes) might fulfill an analogous function to the thymus in the fishes."

Castellaneta ('13) describes the structures which Schaffer found, but insists that these structures correspond to lymphoid organs in general and suggests the name 'lymphoid formations' for them.

He further calls attention to the fact that on the one side these lymphoid formations are in contact with the peribranchial vessels and on the other with the epithelium of the branchial sac. He does not consider these structures as thymus anlagen insofar that there is not a reciprocal penetration of epithelial and lymphatic elements which should occur in a thymus. Castellaneta calls attention to the abundance of lymphoid cells in the general branchial region. He suggests that these special lymphoid accumulations of Schaffer may represent a primitive condition of the thymus in which the epithelium participates only to the extent of attracting the lymphoid cells.

These lymphocyte accumulations do not occur in the part of the branchial cavity which would make them homologous with

the thymus placodes in the fishes. While lymphocytes are present in these situations the evidence brought out in connection with them is not sufficient to establish their origin or the cause of their presence in these places.

A contribution on the ganoid thymus (*Lepisosteus*, *Amia*) has been submitted by Ankarsvärd and Hammar ('13). They found the organ a purely endodermal, unsegmented structure in a medial position in the dorso-caudal part of the epibranchial region. "It nevertheless has a segmented origin and the epibranchial unsegmented thymus structure represents a secondary alteration from the branchial segments." In the older developmental stages there is a rich infiltration of lymphocytes into the sub-thymic and perivascular connective tissue which stands out in striking contrast to the conditions in an earlier stage. The authors discuss the question whether this condition represents an immigration into the thymus or an emigration from it. They believe that the cells have migrated from the placode and represent the beginning of an accidental involution. A lobulization of the organ does not occur. In the adult *Lepisosteus* the thymus is strongly involuted.

The nature of the thymus in the ganoids as described by Ankarsvärd and Hammar is so suggestive of the conditions I have found in the ammocoetes that it appears to me to represent but a very small advance beyond a primitive form in the phylogenetic development of the organ. The origin of the lymphocytes which were supposed to originally migrate into the epithelial anlage in the *Lepisosteus* does not appear to have been especially determined by the authors.

MATERIAL AND METHODS

The material which is the basis for this work was generously supplied to me by Prof. Simon H. Gage. It consists of a series of specimens ranging from the segmentation sphere up to and including a transforming larva and the adult. These specimens undoubtedly represent two species, *Petromyzon marinus unicolor* and *Lampetra wilderi* (the lake and brook lamprey of central New York).

A number of the 5 mm. specimens in my collection were kindly given to me by Prof. C. R. Stockard. They were collected in Naples.

I wish to take this opportunity to express my thanks and appreciation to Professor Gage and Professor Stockard for this valuable material.

The specimens were fixed in various fixing fluids: Zenker, formol; Gilson's, Bouin's, picro-acetic, corrosive-absolute alcohol. After being imbedded in paraffin, transverse and frontal sections were cut from 4 micra to 15 micra in thickness. The sections were stained by various staining methods; methylene blue and eosin, Weigert's haematoxylin and eosin. Giemsa's eosin-azur, and haematoxylin (Delafield's) and eosin. The best differentiation was obtained by the use of the ordinary haematoxylin and eosin method.

A paraffined blotting paper model² of a part of the branchial region of a petromyzon larva prepared by Mr. Warburton was used in this study. A clay model of a part of the branchial region was also prepared to facilitate the study of the arrangement of the ciliated bands in the pharynx.

COMPARATIVE ANATOMY OF THE AMMOCOETE PHARYNX

The phylogenetic position of the lamprey is still a matter of speculation. Various hypothesis have been advanced in regard to its position. Some place this animal between the amphioxus and the elasmobranchs, others claim that it represents a degen-

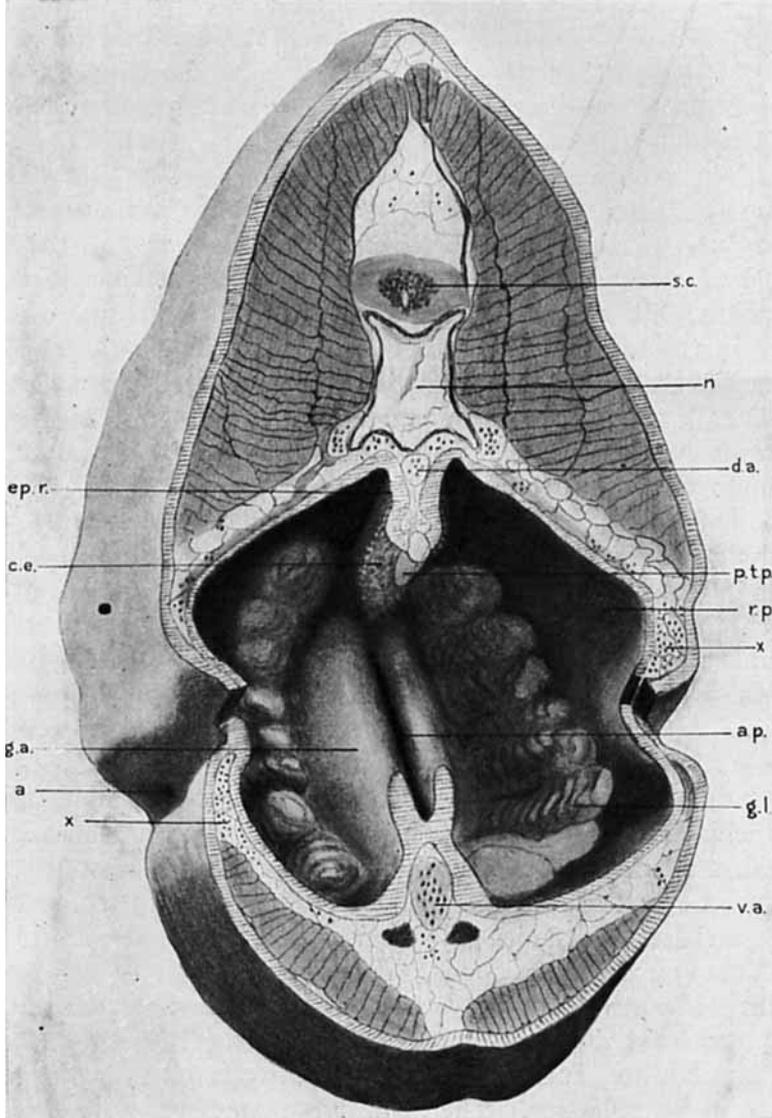
² A description of the method of reconstruction referred to above may be of interest to workers. This method is a modification of the late Mrs. Gage's blotting paper method. Sheets of blotting paper are dipped in melted paraffin and dried. The drawings are transferred to the paraffined paper by the usual methods when wax plates are used. The cutting is also done in the same way, the knife used, however, must have a thin but strong blade. In stacking the sections bank pins were used to hold the sections together. Small screws were also used occasionally to give firmness. When the stacking has been completed the sections may be smoothed down by means of any rounded instrument. A hot iron may also be used to cement the sections together. To give the best stability the complete model may be immersed in hot paraffin a few minutes. Models made in this way have a great firmness and are admirably efficient for class room use where a great deal of handling is necessary.

erate type. However, a comparative study of the branchial region of the lamprey larva with the same region in ascidians and amphioxus on the one hand, and with the elasmobranchs on the other, suggests that the branchial region of the lamprey larva represents a transitional stage between the amphioxus and the elasmobranch types.

A few comparisons between the pharyngeal region of the ammocoete and lower and higher forms may be found in the literature. Dohrn ('84, '85) discussed the homology of the thyroid of ammocoetes with the endostyle of ascidians and the hypobranchial ridge in amphioxus and the circumoral ciliated ring in the ammocoetes with that of the ascidians. Cunningham ('87) verifies the homologies Dohrn pointed out. Shipley ('87) calls attention to the homology of the dorsal ciliated ridge in ammocoetes and the dorsal lamellae of ascidians and the epipharyngeal groove of amphioxus.

The following considerations are based on my studies and include besides the homologies just quoted a comparison of the gills in these primitive animals:

The large branchial cavity with its medial gill arches of the lamprey larva (text fig. 1) is very suggestive of the conditions in the ascidians and amphioxus. In the ascidians there is a central pharynx surrounded by a peribranchial cavity. The two cavities communicate by means of numerous small pores, the stigmata. It is an unsettled problem whether the peribranchial cavity is derived from ectoderm or endoderm. In amphioxus there is a central pharynx which is partially surrounded by an atrium (peribranchial cavity). In this form the two cavities communicate by means of definite gill slits. The atrium of amphioxus is developed from ectoderm. In both forms there is a ventral endostyle and a structure homologous with the epipharyngeal ridge. The branchial cavity of the ammocoetes corresponds to a fusion of the two separate cavities in the ascidians and amphioxus. The primitive characters of these separate cavities, however, are still present. The central portion, that is the part bounded laterally by the gill arches (fig. 1, *a.p.*), corresponds to the pharynx of the ascidians and amphioxus.



It differs from the conditions in amphioxus in that the gill clefts are very much wider. It is probable that the ammocoete gill cleft represents the fusion of two or more gill clefts of amphioxus. There is an indication of such a fusion in the formation of the tongue-bar or secondary gill bar of amphioxus. The larger respiratory part of the ammocoete pharynx (text fig. 1, *r.p.*) corresponds to the peribranchial cavity of ascidians and amphioxus. The entire branchial cavity of the petromyzon larva is entodermal in origin. The ammocoete thus represents a phylogenetic stage in which the respiratory cavity, originally of ectodermal origin, is derived from the endoderm as it is in most higher animals.

The elasmobranch pharynx, it seems to me, represents an advanced stage of a modification which is already indicated in the ammocoete. This modification consists of a lateral migration of the dorsal and ventral attachments of the gill arches, resulting in a lateral enlargement of the central portion of the pharyngeal cavity and a consequent reduction of the respiratory part. This lateral migration is indicated by the dorsal attachments of the second pair of gill arches in the lamprey larva.

Attention may also be called to the fact that the primitive elongated character of the pharynx in the ammocoete tends to obscure its relation to the elasmobranch pharynx, in which the length has been reduced with a consequent reduction of the number and size of the gill slits.

It is necessary to determine the character of the gills in ammocoetes in so far that it has been established that the thymus in all higher forms has a more or less definite relationship to the gill pouches and gill arches.

Text fig. 1 Model of a segment of the branchial region of a 15 mm. lamprey larva. Cephalic aspect. The model shows the relationship of the primitive thymus placodes to the epipharyngeal ridge and the ciliated epithelium, as well as the relationship of the epipharyngeal ridge to the gill arches and general branchial cavity. *a.*, atropore; *a.p.*, alimentary pharynx; *c.e.*, ciliated epithelium; *d.a.*, dorsal aorta; *ep.r.*, epipharyngeal ridge; *g.a.*, gill arch; *g.l.*, gill lamellae; *n.*, notochord; *p.t.p.*, primitive thymus placode; *r.p.*, respiratory pharynx; *s.c.*, spinal cord; *v.a.*, ventral aorta; *x.*, position of accumulations of lymphocytes in lateral branchial walls.

Dohrn ('84) made the statement:

the great difference between the Selachian, Teleost and Ganoid branchial apparatus and that of the petromyzon consists therein that the gill septa and lamellae (Kiemen-blätter und -blättchen) of the former are directed outward while in the latter they are directed inward.

He further states that this arrangement in the petromyzon exists from the beginning. This interpretation of the gills of petromyzon has been accepted in some of the textbooks on Comparative Anatomy of Vertebrates. The basis for this interpretation is undoubtedly found in the position of the cartilaginous gill bars, which form a complicated branchial basket in the pharyngeal wall. The branchial artery, however, is situated in the medial gill arch. From this medial gill arch the gill septum extends caudo-laterally to its attachment in the lateral wall. The gill lamellae are situated on the anterior and posterior walls of the septum. The picture of a frontal section of the gills in the ammocoetes is so much like the picture of a similar section of the elasmobranch gills that it is difficult to consider them as directed in opposite directions. The question resolves itself into a choice between the cartilaginous branchial bars and the branchial aortic arches as a basis of interpretation. It is evident that the branchial basket of petromyzon is a special modification meeting the requirements of a specialized mode of breathing due to the life habits of the adult. The position of the cartilaginous gill bars must then be considered the result of a migration from a more medial position. Moreover, the presence of the ciliated bands in the medial gill arches point to a direct phylogenetic relationship to the gill arches of amphioxus. If we consider the gills of the ammocoete as directed inward it would be necessary to consider as the gill arch, the part of the respiratory portion of the lateral branchial wall to which the gill lamella is attached. This would be contrary to the arrangement of the gills in all other chordate animals.

ORGANOGENESIS OF THE PHARYNX IN THE AMMOCOETES

A complete detailed description of the development of the branchial region in the lamprey larva is apparently not to be found in the literature. Separate structures and the condition in a single or in a limited number of developmental stages, however, have been described by various investigators. These descriptions have been accurate with the exception of minor details, but having been limited to a single stage in most cases they do not include the changes which occur with the growth of the larva. There are consequently contradictory statements in the literature on the pharynx of the ammocoetes and especially in the part dealing with the ciliated grooves and bands. Further, the formation of structures which I interpret as primitive thymus placodes is closely linked with the changes which occur in the ciliated bands in the branchial lining.

The following descriptions are based entirely upon my own material:

The transformations in the early larvae are very rapid so that in 6 and 7 mm. larvae gill lamellae have formed on the branchial septa, the pouches open to the outside, and the epithelium is represented by more than one layer. A system of ciliated epithelial grooves and bands are present in this stage of development of the pharynx. They form a connected system which may be looked upon as beginning in two rather deep diverticula in the caudal walls of the first pair of gill pouches. From each diverticulum two ciliated grooves originate, one passing ventro-caudally, the other dorso-caudally. These will be designated the ventral and dorsal grooves respectively.

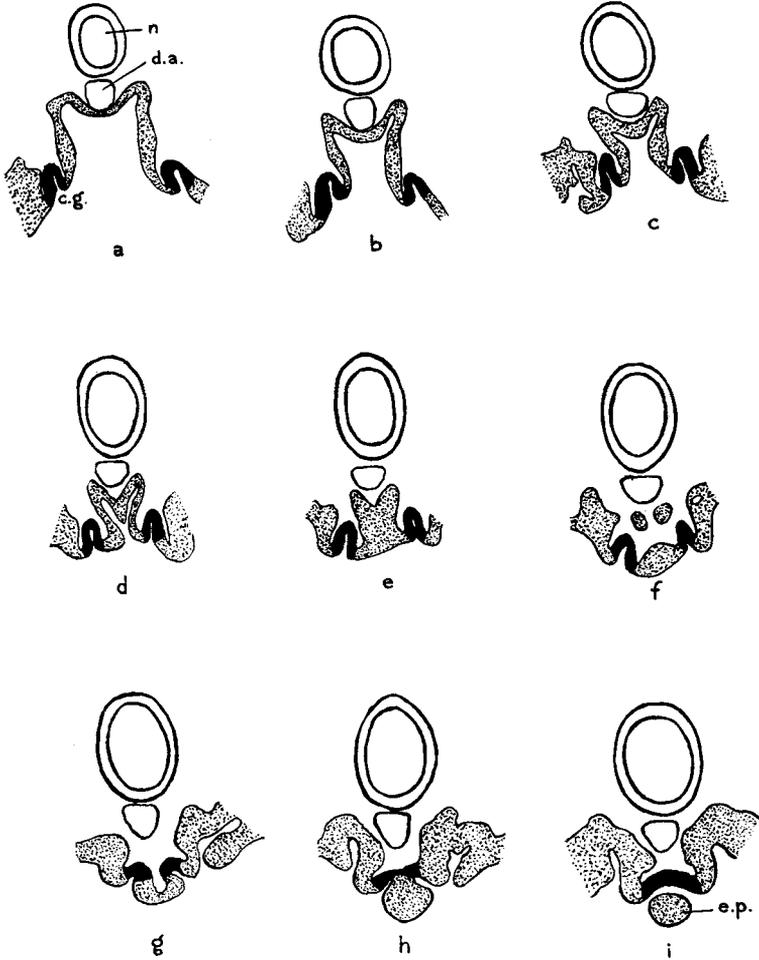
The two ventral grooves converge in a caudal direction as far as the third pair of gill pouches where they come to lie close together and parallel to each other near the median line. Between the two grooves is a median ridge of non-ciliated epithelium which disappears in the fourth pouch where the two ciliated grooves fuse to form a single one. A tubular diverticulum passes from the ventral groove into the thyroid a short distance caudad of the point where the grooves fuse. A second diverti-

culum connects the thyreoid with the fifth pouch. In the seventh pouch the ciliated groove ends. It is directly continued by non-ciliated epithelium which, in a few sections caudad, becomes a ridge. The ciliated groove is a continuous groove from the fourth to the seventh pouches. The ridge which begins in the seventh pouch gradually becomes high and stalked in the eighth pouch. The median columnar epithelium becomes invaginated and is directly continuous into the floor of the oesophagus. Surrounding the junction it seems to me there is evidence of a vestigial eighth gill arch in which an aortic arch is not present. The respiratory part of the branchial cavity extends a short distance caudad of the point of junction between the pharynx and oesophagus.

The dorsal ciliated grooves arising in the diverticula follow the course of the first aortic arch to the median dorsal line of the pharynx. They fuse at this point to form a single ciliated band which extends caudally the whole length of the branchial cavity and which is directly continuous into the roof of the oesophagus. A short distance caudad of the point of origin, this band forms a rounded ridge which extends to the seventh sac where it is converted into a groove. The aorta is lodged in the concavity of the rounded ridge. At the point where the two dorsal grooves of the first pouch fuse a tongue-like piece of non-ciliated epithelium is pinched off (text fig. 2). Schaffer apparently mistook this for ciliated epithelium and considered it the end of the fused ciliated bands.

The first pair of gill arches come together dorsally in the median line. Their ventral extremities, however, are far apart and end in the ventro-lateral part of the respiratory pharynx. Gill lamellae are present only on the caudal surface of the first gill septum. The second pair of arches are farther apart and thus they differ from the remaining caudal arches. Their dorsal attachments are in the angle between the epipharyngeal ridge and the dorso-medial part of the respiratory pharynx. Ventrally, the second gill arches are attached about midway between the mid-ventral line and the ventro-lateral angle of the respiratory pharynx. The dorsal attachments of the third and remain-

ing pairs of gill arches is the ventro-lateral angle of the epipharyngeal ridge. Ventrally, they are attached near the ventral median line, a little to the side of the endostyle. The second pair of arches are peculiar in that they contain no cili-



Text fig. 2 Camera lucida outline drawings to illustrate the course of the dorsal ciliated grooves (the ventral grooves are not shown) from the diverticula in the first pouch to the point where the grooves meet in the median dorsal line to form a single band of ciliated epithelium. *c.g.*, dorsal ciliated groove; *d.a.*, dorsal aorta; *e.p.*, epipharyngeal ridge; *n.*, notochord.

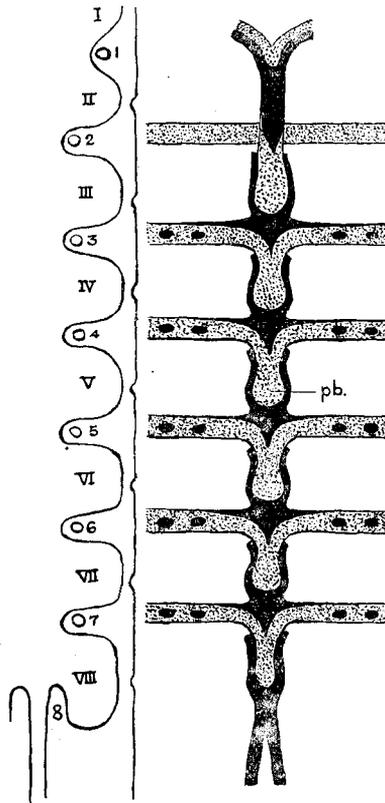
ated bands. The third and remaining arches have a broad ciliated band covering the medial and cephalic aspect. These bands are directly continuous with the ciliated band on the epipharyngeal ridge. They have no connection with the endostyle in this stage of development and I have been unable to determine whether such a connection exists or not in younger larvae.

The moving apart of the second pair of gill arches is very suggestive of an approach to the condition in fishes where the arches are attached in the lateral part of the roof of the pharynx. Accompanying this lateral migration there is a loss of the ciliated band on the arch.

The arrangement of the ciliated bands as described above does not persist in older larvae. This undoubtedly accounts for the contradictory descriptions given by Anton Schneider ('79) and Schaffer ('95, 1 and 2) and others. In a larva 9.5 mm. in length growth and differentiation of the epithelium of the gill arches has resulted in a new arrangement of the ciliated bands. This new arrangement has gained its permanent larval condition in a 15 mm. larva.

In the older larvae the median dorsal ciliated band which represents the fused continuation of the dorsal ciliated grooves of the first arch ends in the median dorsal line between the second pair of gill arches. Immediately caudad of the dorsal attachment of the second pair of gill arches two ciliated bands appear on the ventro-lateral part of the epipharyngeal ridge. Tracing these bands in a caudal direction, they are seen to come together and fuse in the median ventral part of the ridge at the caudal end of the dorsal equivalent of the third gill pouches. From this single band a branch is given to each of the third pair of gill arches. In the median line the ciliated band ends as a pointed process in the angle between the dorsal attachments of the third pair of gill arches. This arrangement of the ciliated bands is repeated in the remaining arches and dorsal equivalents of the gill pouches (text fig. 3, and *c.e.* in text fig. 1). In the eighth pouch, however, the median ventral fused part does not give off any lateral branches corresponding to the ones given off to each gill arch in the third to the seventh arches. This is

due to the nature of the dorsal attachment of what I have assumed must be the eighth vestigial gill arch. This arch, like the second, is not attached to the epipharyngeal ridge but to the angle between the ridge and the dorsal wall of the respiratory pharynx. Cilia, however, are present on the lateral side of the eighth gill arch. They undoubtedly represent the vestigial remains of a condition in which this arch had a dorsal attachment to the ridge or its equivalent. The median ciliated band



Text fig. 3 Diagram illustrating the arrangement of the ciliated bands on the epipharyngeal ridge and the gill arches in a 31 mm. larva. Ventral view. The gill arches which extend ventrally are here represented as extending laterally. The ciliated epithelium is represented in heavy black, the non-ciliated by the stippled part. Roman numbers indicate the gill pouches and Arabic numbers the gill arches. *pl.*, primitive thymus placodes.

of the eighth pouch is directly continuous into the oesophagus. It is interesting to find that the band divides into two portions within the oesophagus. These perhaps represent the branches which were given off to the eighth pair of gill arches in an ancestral form.

Patches of ciliated epithelium are also present on the medial aspect of each gill arch from the third to the seventh inclusive. These ciliated patches undoubtedly were a part of the single ciliated band on the gill arches in the younger larva. With the growth of the non-ciliated epithelium of the gill arches, these patches were cut off from the ciliated band. In the older larva the ciliated band of the gill arch does not occupy the same relative position that it did in the young larva. The ventral end is situated on the lateral side of the gill arch. When traced in a dorsal direction it is found to take a slightly spiral course so that the dorsal extremity which is continuous into the ciliated band of the epipharyngeal ridge comes to occupy a cephalo-medial position. This change in the course of the ciliated band may also be looked upon as the result of the growth of the non-ciliated epithelium. The ciliated patches on the medial side of the gill arch may acquire a sensory function as Schaffer suggested.

The arrangement of the ciliated epithelium on the epipharyngeal ridge in the older larvae is also the result of the growth of the non-ciliated epithelium. In a larva between 8 and 9 mm. in length the non-ciliated epithelium of the dorso-medial part of the gill arches begins to invade the ciliated epithelium of the epipharyngeal ridge. As a result of this invasion the continuity of the ciliated band on the epipharyngeal ridge is lost. The two cords of invading epithelium from the opposing gill arches fuse in the median ventral line of the ridge. The invasion is continued in a caudal direction dividing the ciliated band into two portions which are pushed laterally. This fused portion (*pl.*, text fig. 3) becomes thicker and broader in a caudal direction and ends a short distance cephalad to the attachment of the next pair of gill arches. At the caudal end of this invading epithelium the subsequent growth does not divide the ciliated epithelium fur-

ther, but produced a tongue-like process which projects into the pharyngeal cavity (similar to the tongue-like non-ciliated epithelial process represented in text figure 2).

The non-ciliated epithelium which has invaded the ciliated epithelium of the epipharyngeal ridge begins to show histogenetic activities in a larva between 20 and 30 mm. in length. The nuclei of the cells of these areas, or placodes, wander out into the underlying connective tissues and are transformed into lymphocyte-like cells. A study of the histogenetic processes in these areas in various developmental stages leads to the conclusion that these areas represent specialized regions of the branchial epithelium which are suggestive of primitive thymus structures.

From the foregoing description it is evident that there are in all seven placodes. The seventh and the first are smaller than the remaining ones but they take part in the histogenetic processes and are therefore to be considered true functional placodes. The placodes increase in size with the growth of the larva. In the mature larva, however, they show a depletion of cells.

When the larva undergoes metamorphosis the whole structural arrangement of the branchial-region is altered. In the single specimen of a transforming larva of my collection, the adult arrangement has been attained, so I am unable to describe the nature of this process. In this transforming specimen I have also been unable to find any remains of the epithelial placodes of the larva. Serial sections of the branchial region of an adult lamprey have also been examined but with negative results. It is evident that an involution of the placodal organ has taken place as one would expect of a thymus. This involution began in the maturing larva and was completed in the early stages of metamorphosis.

HISTOGENESIS

*Early development of the pharyngeal wall in *Petromyzon marinus unicolor*, *Lampetra wilderi**

The search for a thymus in the lamprey larva has revealed an unusual accumulation of lymphocytes in the lateral walls of the branchial cavity. These accumulations were first observed by

Schaffer ('94) and later described more in detail by Giacomini ('00, 1 and 2) and Castellaneta ('13). The origin of these lymphocytes as well as the cause for their accumulation in these places has not been determined and consequently constitutes a problem to be solved in the consideration of a possible thymus structure in this animal. The descriptions in the literature have been limited to single stages of development and are consequently incomplete. The following descriptions are based on my own material and includes the essential developmental stages:

The branchial cavity of a 5 mm. larva represents a very simple condition. The gill pouches are present as wide evaginations extending to the ectoderm in the mid-lateral plane leaving the gills as simple projections of the lateral endodermal walls. Loose mesenchyma cells fill up the space within the gill. The aortic arches are forming near the free medial border of the gills, but the vascularization of the body of the gill has not begun as yet. Although the larva contains a great quantity of yolk in this stage the branchial region is quite free from it.

The epithelium lining the branchial cavity including the gills consists of a single layer of columnar cells. Dorsal and ventral to the mid-lateral plane of each gill pouch and corresponding to the position of the future lymphoid accumulations of Schaffer the endoderm shows a slight thickening (fig. 2). In these places which may be called placodes the cells have lost their columnar shape and their outlines have more or less disappeared. The area appears to be taking on a syncytial character in which the nuclei do not have any definite grouping.

Marked changes have occurred in these placodes in a 9.5 mm. larva. In general, the placodes have become enlarged both in thickness and area (fig. 3). Cell outlines are practically all obliterated. The cytoplasm is streaky in appearance suggesting a degeneration. The nuclei exhibit variations in character. Some are very dark with a large chromatin content while others are pale and contain a small amount of chromatin. Still others show amoeboid characters. I have not been able to determine whether all these nuclei are indigenous to the placode. The

general epithelium of the branchial cavity has also acquired a new character in this stage. Beneath the placodes and in direct contact with them the peribranchial blood channels are forming. It is difficult to distinguish an endothelial lining of these channels in all cases.

The degeneration of the placode has progressed farther in a 15 mm. larva (fig. 4). In places the cytoplasm has taken the stain very faintly. Scattered about in the placode are streaks of cytoplasm which are very deeply stained. Vacuoles are also present. The nuclei appear to be fewer in number than in the earlier stages. They appear more constant in their general appearance and chromatin content. The amoeboid character of the nuclei has also become more prominent. Some nuclei have taken up positions at the surface of the placode and the cytoplasm appears to be cutting off a layer of flattened cell (fig. 4, *s.l.*). The formation of a layer of flat cells at the surface of the general epithelium was begun in a much earlier stage of development.

The changes which occur in these placodes in older larvae approximate the character represented in the 63 mm. larva (fig. 1). It is a significant fact that cells in mitosis have not been seen in any stage of the development of these placodes. Furthermore, patches of epithelium giving the appearance of a degeneration are present in various other parts of the branchial lining, especially at the lateral attachments of the gill septa. Lymphocytes are present in the placodes in the older larvae, but they are also present in the general branchial epithelium. They do, however, occur in greater numbers at these placodes.

From these brief descriptions it is apparent that these placodes do not represent active anlagen of a future structure. Their development and structure do not suggest anything which might indicate their significance.

The lymphocyte accumulations in relation to the above described placodes are contained within vascular channels. These vascular channels contain red blood cells, and as has been shown by Mozejko ('10) and others they are in communication with

similar channels within the body of the gill as well as with the definitive blood vessels of the pharynx.

Lymphocytes begin to make their first appearance in the blood in larvae of about 9 mm. length. They increase in number with the growth of the larvae, but chiefly remain outside of the main blood vessels. They are especially abundant in the perivascular spaces of the gill arches.

The accumulations of lymphocytes in the lateral branchial walls are foreign to these situations so far as their origin is concerned. Furthermore, the epithelial placodes in these situations together with the lymphoid accumulations do not exhibit the characters which are essential in either a well-established or rudimentary thymus. I can offer no suggestion in regard to any special significance of these accumulations. They appear to be merely a part of a rich accumulation of lymphocytes in the connective tissue spaces of the branchial region. It is probable that the apparently degenerating placodes play a rôle of attraction for lymphocytes.

Epipharyngeal placodes

The placodes in the epipharyngeal ridge are present in an undifferentiated condition in a 15 mm. larva. They form distinct masses of cells in the mid-ventral part of the epipharyngeal ridge between the ciliated bands. They are very nearly circular in outline in a transverse section, producing a bulging into the interior of the ridge (figs. 5 and 6). A loose mesenchymatous tissue caps the dorsal surface of the placodes. Red blood cells are occasionally present in the spaces of the mesenchymal tissue. These spaces are apparently in communication with the dorsal aorta by means of minute apertures and are directly continuous with similar spaces in the connective tissue of the gill arches. Through the spaces of the gill arches a communication is also made with the peribranchial sinuses and the perivascular spaces in the gills.

Within the placode the cells are in an active state of proliferation. The nuclei of the resting cells are rather clear

structures with the chromatin generally collected into small lumps situated next to the nuclear membrane. The nuclei are smaller than those of the ciliated epithelial cells. Cell outlines are more or less distinct in the placodes. A peculiar type of vacuolization is in progress in some of the cells in which the complete cell becomes vacuolated leaving the protoplasmic remains as free bodies within the hollow cell. The protoplasmic bodies in these cases are small lumps of nucleated protoplasm in which the nuclear material generally stains an intense black and the cytoplasm a light red. These protoplasmic bodies are not limited to the epithelial placodes, but may be found everywhere in the branchial epithelium, especially in the 15 mm. larva.

A layer of flat cells clothes the surface of the placode. This layer is not distinct in every section and may easily be overlooked. At the connective tissue border of the placode a basement membrane sharply marks off the epithelium from the mesoderm (*b.m.*, fig. 5). Lymphocytes have not been found in the placodes in a 15 mm. larva although they are present in the blood. They may be seen, however, in the general branchial epithelium and also in the ciliated epithelium (*lm.*, fig. 6).

The placodes have increased considerably in size in a 31 mm. larva. The mesenchymatous tissue which was present above the placode in the 15 mm. stage has changed to connective tissue (fig. 7). Large spaces containing various kinds of blood cells are present in this connective tissue. Larger and smaller nuclei may be seen in the walls of these spaces. The smaller undoubtedly represent the nuclei of endothelial cells. The larger, however, are apparently derived from the placode and are in a stage of migration into the vascular spaces. It is doubtful whether these spaces should be considered true blood channels. While red blood cells are quite abundant in the spaces in this stage of development they are practically absent in them in the full grown larva. It is probable that they represent a primitive type of lymph vessels, as has been suggested by various authors. The connective tissue is of the fibrous variety in which the individual fibers are quite slender. The fibers interlace to

form a loose mesh work. Figure 7 represents a transverse section through the cephalic part of a placode with its neighboring connective tissue and the ciliated epithelium of one side. In such a region the nuclear elements are very scarce in the connective tissue when compared to the region above the central part of a placode. The basement membrane which is present only on the right-hand side in the section illustrated in figure 7 (*b.m.*) bridges across the entire placode a few sections cephalad of the one illustrated. In the central part there is no line of demarcation between the placode and the connective tissue. The cytoplasm of the placode in this place is directly continuous with the connective tissue.

The cells within the placode have greatly increased in numbers in this stage of development. Near the free surface of the placode they are loosened from each other, displaying their rounded outlines distinctly. Toward the deeper part of the placode the cells become oblong in shape. Near the connective tissue border the cell outlines are lost which gives the appearance of a syncytium. The nuclei of the cells in the placode are not unlike the nuclei of the ciliated and the general branchial epithelium in their general morphological characters, except in size. They are smaller than the nuclei of the ciliated and general epithelium. The chromatin of the nuclei, for the most part, is collected into a single lump which stains a reddish-purple with the haematoxylin-eosin stain. The nuclei also change from a circular to an oblong outline from the free surface of the placode to the connective tissue border. At the place where the cytoplasm of the placode is continuous with the connective tissue, the nuclei become quite elongated, having the appearance suggestive of a migration into the connective tissue. This migratory appearance is more prominent at the central part of the placode. Figure 10 represents a part of a transverse section from the central region of the placode. The two lower nuclei marked *a* in the figure lie in the cytoplasm of the placode. All the nuclei and cells above this level are in the connective tissue and spaces above the placode. The nuclei in the connective tissue show degrees of gradual variations in morphological

characters from the typical epithelial nucleus of the placode to the mature lymphocyte-like cell. Nuclei showing these degrees of variation may all be found in a single section. Figures 10 and 11 show the most obvious stages in this gradual variation. The nuclei marked *a* are typical placode epithelium nuclei apparently in a state of emigration. The nucleus marked *b* is in the connective tissue. The chromatin in this nucleus is apparently breaking up into a number of granules, a process which has proceeded farther in nucleus *c*. In nucleus *d* the chromatin granules are apparently arranging themselves on the nuclear membrane, an arrangement which has been completely attained in nucleus *e*. Nucleus *e* further shows a tendency towards acquiring a circular outline which becomes more manifest in nucleus *f*. Nucleus *f* also shows a reduction in size. Nucleus *g* (fig. 11) has a circular outline and further shows a change in the character of the protoplasm. The nucleus *h* shows a still further reduction in size, the protoplasm stains darker as does also the chromatin. The chromatin, further, forms a continuous layer at the periphery. In *i* (figs. 10 and 11) the nuclei have acquired a thin covering of cytoplasm which is not visible at all points of the nuclear surface. The cytoplasm stains a gray-blue. The nucleoplasm and the chromatin of these cells take the stain more intensely than the nucleus *h*. These cells have also become free from the connective tissue mesh work. In the cells *j* the nucleoplasm stains a deep purple. The chromatin appears to have left the nuclear membrane and is now present as granules scattered about in the nucleus. In some nuclei the chromatin granules are connected together by slender processes, in others this is apparently not the case. Still other cells show nuclei in which the chromatin is represented by a single large lump. These cells (*j*, figs. 10 and 11) represent the typical lymphocyte-like cell in this region of the 31 mm. larva. Some of these cells may be found in which the nucleoplasm stains a gray-blue (*k*, fig. 10). They are similar to the lymphocytes in older larvae and may either represent a final stage in the development of the cells, or they may represent cells foreign to this locality.

From the above described transitional conditions and from a study of the stained sections, I can draw no other conclusion than that, the nuclei of the epithelial placode transform into lymphocyte-like cells. It is a significant circumstance that the nuclei alone migrate from the placode, i.e., no cytoplasm is visible. Complete cells bearing epithelial characters may be found in the connective tissue spaces. However, I have never found them migrating from the placode while I have found migrations of the complete cell from the epithelium of other regions.

Cells in mitosis may be seen occasionally in the placodes of the 31 mm. larva. Cells in a state of amitotic division, however, are quite abundant in a 44 mm. larva, suggesting that cell-proliferation takes place chiefly by simple fission. Figure 15 shows the nucleus of a placode cell apparently in a process of simple fission. Mitotic cells are especially scarce in the connective tissue above the placode. A single instance has been found and is represented in figure 18. It is quite evident from the lack of mitotic or amitotic cells or nuclei in the connective tissue that cells or nuclei are not being formed in any significant quantities in this situation.

Transformation stages have not been found within the placode in the 31 mm. larva. Lymphocyte-like cells, however, are present in the placodes. Their presence may be accounted for by means of an immigration from the connective tissue.

The further development of the placodes is a repetition of the above-described processes except that the transformation is more rapid and begins within the placode. Figure 8 represents a part of a transverse section of the placode and the connective tissue above it in a 44 mm. larva. The illustration was drawn to the same magnification as figure 7. The nuclei in the placode are elongated and show amoeboid characters. They also appear to be in an active state of emigration. The transformation process appears to have begun in the placode in this stage. The nuclei near the connective tissue border have taken on characters which approach the characters of some of the nuclei in the connective tissue. This change is shown in the staining reaction, the condition of the chromatin, and the shape of the

nuclei. The nuclei at the border show a tendency to stain blue, the chromatin takes a darker stain, and in some cases is broken up into granules, and the nuclei approach the globular shape. The transformation of the nuclei in the connective tissue appears to be of the same character as in the 31 mm. stage, but apparently more rapid. Nuclei may occasionally be found in the placode which show phagocytic properties. Figure 14 represents a placode nucleus in the act of engulfing protoplasmic bodies.

The spaces in the connective tissue in the 44 mm. larva appear to be smaller than in the 31 mm. stage. Some of them have a distinct wall while others appear like transient spaces in the connective tissue. Red blood cells are only occasionally seen in the connective tissue spaces of this stage.

The placodes in a 63 mm. larva are larger in area but thinner than in the preceding stages. The nuclei of the epithelial cells of the placode have lost their original character. The chromatin is no longer represented by a single large lump, but is present in the form of granules corresponding to the chromatin in the nuclei which had migrated into the connective tissue in the 31 mm. stage. The number of lymphocyte-like cells has increased considerably within the placode. All the stages of transformation from epithelial nucleus to the mature lymphocyte-like cell may be found within the placode in this stage of development. A basement membrane is re-forming at the connective tissue border of the placode. The 'vascular spaces' of the connective tissue are now chiefly limited to the peripheral part of the whole connective tissue within the ventral half of the epipharyngeal ridge. The mature lymphocyte-like cells are chiefly located in these channels, leaving the central connective tissue core quite free from cells. The central core consequently has a much lighter appearance. Some nuclei are present in the central core, the morphological characters of which are similar to the characters of connective tissue nuclei in other parts of the body. Other nuclei may occasionally be seen in which the characters agree with the various transformation stages of the lymphocyte-like formation shown in younger larvae.

The activities within the placodes of the full grown larva (120 mm.) have diminished and are apparently approaching a condition of cessation. The number of epithelial nuclei has been reduced considerably. Transitional stages may be found, but are quite scarce. Mature lymphocyte-like cells are also present, but not in great numbers. A definite basement membrane is now present at the connective tissue border of the placode. Figure 9 represents a portion of a transverse section of the placode and the tissue above it in a 120 mm. larva. The section is taken near the cephalic end of the placode. In such a region a peculiar formation has occurred in the connective tissue, the significance of which I am quite unable to explain. This formation consists of what appears to be red blood cells held in the meshes of the connective tissue (*x*, fig. 9). The cells have the morphological characters of the red blood cells. The cytoplasm has a decided yellow tint, while the pale nuclei have a green tint. In some cases what appears to be the nuclei have morphological characters similar to the lymphocyte-like cells. These formations are present in the periphery of the whole connective tissue. A section through the central part of the placode would show the same character that was indicated in the 63 mm. larva, that is, a central core of connective tissue in which there are no 'vascular channels' surrounded by a 'vascular area.' The tissue between the 'vascular channels' in the 120 mm. larva consists entirely of the peculiar tissue just described.

Development of lymphocytes

A brief description of the general development of lymphocytes in the petromyzon larva is here given since the nature of this formation in the advanced larvae has a direct bearing on the interpretation of the histogenetic processes in the above described placodes. My observations do not include the first appearance and development of the blood in the embryo, but begin with the development in the 5 mm. larva. The nature of the blood formation in this stage of larval development need not be described here for the reason that it occurs at a time when the placodes have not begun to form. However, in larvae

ranging from 9.5 mm. in length up to the mature individual, blood cells develop from the epithelial cells of the gills, gill arches, and probably the branchial wall by a process of transformation. It is the blood formation occurring in the gills and gill arches which is of especial interest in connection with the histogenesis in the placodes. The description of this formation will be limited to the formation of lymphocytes only, in the 31 mm. larva.

The similarity of the cytoplasm of the gill epithelium to the cytoplasm of some of the blood cells was early noticed. This similarity was found to be due to an actual relationship between the two kinds of cells and thus not a mere coincidence. This relationship was demonstrated when epithelial cells were found migrating through the walls into the lumen of the blood channels in the gill. Figure 13 represents a part of the gill epithelium and a blood vessel and shows an epithelial cell beginning its migration into the vessel. Figure 12*a* shows another epithelial cell in the state of migration, almost half of the cell in this case is inside of the vessel. The cells to the left in figure 12 represent blood cells (in the vessel) in various stages of transformation. In this figure, the chief stages in the transformation of the epithelial cells to lymphocytes are represented. The lettering *a* to *h* in the figure shows the line of transition from an epithelial to the mature lymphocyte.

In this formation of lymphocytes, it is noteworthy that the entire epithelial cell migrates from the 'epithelium' and takes part in the transformation. The transformation consists of a reduction in the size of the nucleus and also in the amount of cytoplasm. The cytoplasm retains its staining qualities through these changes so that even in the mature lymphocyte a cytoplasmic ring which stains red may be seen in many instances. It is very seldom that a lymphocyte containing a cytoplasm which stains a blue or gray-blue is seen in these situations. All the transforming cells have a cytoplasm which stains red with the haematoxylin-eosin stain.

Although some of the epithelial cells in this transformation migrate directly into the blood vessels, the great majority wan-

der into the perivascular spaces and undergo their transformation in these places. The sluggish character of the blood flow in these spaces must account for the retention of the large number of transforming and mature lymphocytes which are present in these situations. The entrance of these cells into the main blood vessels is of a slow nature.

The tall epithelial cells in the dorsal part of the epipharyngeal ridge also enter into the blood formation. Figure 16 shows a cell taken from the space in the connective tissue of the dorsal part of the epipharyngeal ridge. The nucleus has the morphological characters of the epithelial nuclei. It appears to be in a state of simple fission. The cell in figure 17 was taken from the same locality. Two nuclei are present in this cell which still show the epithelial character.

Histogenetic comparisons between cells arising from placodes and lymphocytes

In the study of the histogenetic processes in the placodes it was shown by means of various transitional stages that the epithelial cells of the placode transform into lymphocyte-like cells. Lymphocytes were shown to develop from the 'epithelial' cells of the gills and gill arches. The lymphocyte-like cells formed from the placodes do not have the same mode of development nor do the transitional forms have the same morphological characters as the lymphocytes and transitional forms developed from the gill and gill arch 'epithelium.' In the placode the nuclei alone migrate away from the original epithelial bed and the transformation occurs in the connective tissue meshwork. The complete cell migrates away from the epithelial bed in the gills and gill arches, the transformation occurs in the perivascular spaces and the blood vessels. A small amount of cytoplasm becomes visible in the placode 'lymphocyte' just before it attains its maturity. This cytoplasm stains a gray-blue. The cytoplasm of the gill and gill arch lymphocytes represent the original cytoplasm of the 'epithelial' cells and stains red. These important differences in the lymphocytes and lymphocyte-like cells

occur in the same section and thus cannot be attributed to difference of technique. A lymphocyte with red cytoplasm may occasionally be found in the epipharyngeal ridge just as a 'lymphocyte' with gray-blue cytoplasm may occasionally be found in the gill region. The great majority of 'lymphocytes' in the placode region, however, contain cytoplasm which stains gray-blue. It was also pointed out above that the lymphocytes in the gill region are chiefly the type which have red cytoplasm. The presence of the lymphocytes with red cytoplasm in the placode region and the type with gray-blue cytoplasm in the gill region may be accounted for by migration from their seats of origin. They may also be brought to these situations by the flow of the blood.

On account of the morphological difference of the developing lymphocytes in the gill region and lymphocyte-like cells of the placodes, the conclusion seems justifiable that the placodes are segregated portions of the 'epithelium' representing individual organs which produce cells of a lymphocyte appearance, but differing from the lymphocytes formed in the 'epithelium' of the gills.

DISCUSSION

The data submitted in the consideration of the lymphocyte accumulations in the lateral branchial wall of the lamprey larva does not supply any evidence that these formations represent primitive thymus anlagen. Although placode-like formations are present in the lateral branchial wall, similar formations are also present in other parts of the pharyngeal epithelium.

An important component of the thymus of higher animals is a reticulum. In my study of the thymus-like placodes in the lamprey larva, I have been unable to find any undisputable evidence of a reticulum in the placode. At the connective tissue border of the placode the epithelial cytoplasm apparently has a fibrous character (fig. 10). I have been unable to determine whether this represents connective tissue or transformed epithelial cytoplasm. Judging by its appearance and position it probably represents connective tissue which has been invaded by cyto-

plasm from the placode. The connective tissue outside the placode plays the role of a reticulum insofar that the transformation of the epithelial nuclei occur within its meshes.

Hassal's corpuscles, or any structures comparable to them have not been found in the placodes or in the connective tissue outside of the placode.

The history of the placodes in the successive developmental stages indicates a gradual involution of the placodes. The maximum size of the placodes occurs in a larva of 50 to 60 mm. in length. From this stage of development the placodes diminish in size so that in the mature larva very few lymphocyte-like cells remain. In the transformation of the larva, Nestler ('10) maintains that the oesophagus of the adult is formed by a transformation of "the under edge of the dorsal fold in the branchial chamber" (the epipharyngeal ridge). If such a process occurs, it is only after the histogenetic activities in the placodes have ceased and consequently does not affect the status of an earlier thymic function in these placodes.

An examination of the descriptions given in the preceding pages give the impression that the primitive thymus placodes and lymphocytes are formed from an endodermal epithelium. While I am not ready at this time to supply the evidence, the changes which occur in the general branchial epithelium in the early stages of development seem to point to a general fusion of the original endoderm with the underlying mesenchyma. The character of the epithelium in the more advanced larvae has such an important bearing on the interpretation of the histogenesis of the primitive thymus cells and lymphocytes that a separate and detailed study of this process seems warranted.

In a recent article on the Development of the Human Pharynx, Kingsbury ('15) discusses the intrinsic and extrinsic factors in thymus formation and challenges the view that the thymus is a branchiomic organ definitely located in the branchial epithelium. The basis for this interpretation

is found in the recognition that it is a structure whose appearance is determined by extrinsic factors of relation and position and not intrinsic factors located in any particular group of cells. In support of

such an interpretation and giving us, I believe, a better comprehension of its morphologic significance, we have the fundamental plan of its histogenesis.

The true nature of the endodermal-mesenchymal relationship in the ammocoete pharynx has not been definitely determined. Whatever these extrinsic factors may be, they are apparently of the same nature in the thymus-like placodes and the lymphocyte-forming 'epithelium' of the branchial arches. The products of these two regions, however, are not similar and it seems to me that this dissimilarity can only be explained on the basis of an intrinsic value or specificity of the 'epithelium' of the placode.

The nature of the formation of lymphocytes and the primitive thymus placodes in the lamprey larva point to an ontogenetic relationship in the histogenesis of thymus cells and lymphocytes. The branchial region of the lamprey larva may be looked upon as possessing general lymphocyte-forming properties in which the primitive thymus placodes represent specialized regions of the general lymphocyte-forming 'epithelium.'

SUMMARY

From the evidence obtained in this investigation of the ammocoetes the following conclusions seem justified:

The placodes in the lateral branchial wall are apparently patches of degenerating epithelium and have nothing to do with a thymus structure. The collection of lymphocytes at these places are foreign to this situation so far as their origin is concerned.

The gills in the ammocoetes are homologous with and extend in the same direction as the gills in elasmobranchs.

The branchial 'epithelium' does not represent a pure endodermal epithelium. This 'epithelium' develops haemopoetic properties in the advanced larva.

'Epithelium' from the gill arches invades the ciliated epithelium of the epipharyngeal ridge and produces placodes. These placodes have a relationship to the gill arches and gill pouches which makes them homologous with the thymus placodes of

elasmobranchs and are to be considered primitive thymus structures.

The lymphocyte-like cells which originate in the primitive thymus placodes have different morphological characters and have a different mode of formation than the lymphocytes which are formed in the gill arches and lamellae.

This investigation has been pursued in the laboratories of anatomy at Cornell University Medical School and Marquette University Medical School. While I hope to have established a primitive thymus structure in the ammocoetes, many of the important problems of the histogenesis of the lymphocytes and primitive thymus cells must be left undecided until more exhaustive investigations can be completed.

BIBLIOGRAPHY

- ANKARSVÄRD, G., UND HAMMAR, J. 1913 Zur Kenntnis der Ganoidenthymus. Zool. Jahrb. Abt. f. Anat. u. Ontog. der Tiere, Bd. 36, p. 3.
- CASTELLANETA, V. 1913 Sulla questione del timo in 'Ammocoetes.' *Monitore zool. Italiano*, Anno 24, pp. 161-174.
- CUNNINGHAM, J. Y. 1887 Dr. Dohrn's inquiries into the evolution of organs in the chordata. *Quart. Journ. of Micr. Science*, vol. 27, pp. 265-266.
- DOHRN, A. 1884 Studien zur Urgeschichte des Wirbeltierkörpers. IV. Die Entwicklung und Differenzierung der Kiemenbogen der Selachier. *Mitteil. a. d. Zool. Station zu Neapel*, Bd. 5.
- 1885 Studien zur Urgeschichte des Wirbeltierkörpers; VII. Entstehung und Bedeutung der Glandula Thyreoids; VIII. Die Thyreoidea bei Petromyzon, Amphioxus und den Tunikaten, *Mitteil. d. Zool. Station zu Neapel*, Bd. 6, pp. 44-92.
- GIACOMINI, E. 1900 a Sulla Strutturra dells branchiedei Petromyzonti. *Monit. Zool. Ital.*, Anno 11, Suppl. 9-10.
- 1900 b *Ibid.* (cited from Oppel '05).
- GOETTE, A. 1875 Die Entwicklungsgeschichte der Unke. Leipzig (cited from Hammar, '10).
- 1890 Entwicklungsgeschichte des Flussneumauges. Hamburg and Leipzig.
- MOLLIER, S. 1906 Die Entwicklung von Blut und Gefassen. In Hertwig's *Handbuch der vegl. u. exp. Entwicklungsgeschichte der Wirbeltiere*, Jena.
- MOZEJKO 1910 Über die Injektion des Vascularsystems von Petromyzon fluviatilis. *Zeitschr. f. wiss. Mikrosk.*, Bd. 27.
- 1911 Über den Bau und den morphologischen Wert des Vascularsystem der Petromyzon. *Anat. Anz.*, Bd. 40.

- RABL, C. 1886 Zur Bildungsgeschichte des Halses. Prager Med. Woch., Bd. 11, p. 52 (cited from Hammar, '10).
- RATHKE, H. 1827 Bemerkung über den inneren Bau des Querder (*Ammocoetes branchialis*) und des Kleinen Neunauges (*Petromyzon Planeri*) (cited from Opper, '05).
- SCHAFFER, J. 1894 Über die Thymusanlage bei *Petromyzon Planeri* Sitzungsber. d. K. Akad. d. Wiss. Wien., Bd. 103, p. 3.
1895 a Zur Kenntnis des Histologischen und Anatomischen Baues von *Ammocoetes*. Anat. Anz., Bd. 10, pp. 697-708.
1895 b Über das Epithel des Kiemensarmes von *Ammocoetes* nebst Bemerkungen über intraepitheliale Drüsen. Arch. f. Mikr. Anat., Bd. 45, pp. 294-338.
1906 Berichtigung, die Schilddrüse von *Myxine* betreffend. Anat. Anz., Bd. 28.
- SCHNEIDER, A. 1879 Beitr. zur Vergl. Anatomie und Entwicklungsgeschichte der Wirbeltiere, Berlin.
- SCHULTZE, M. 1856 Die Entwicklungsgeschichte von *Petromyzon Planeri*. Naturkundige Verhandl. van d. hollandsche Maatschappij. d. Wetenschappen te Haarlem, II Versam, d. 12, p. 28 (cited from Hammar, '10).
- SHIPLEY, A. E. 1887 On some points in the development of *Petromyzon fluviatilia*. Quar. Journ. of Nat. Sc., vol. 27, pp. 325-371.
- STANNIUS, H. 1854 Handbuch der Anatomie der Wirbelthiere (cited from Schaffer, '94).
- STOCKARD, C. R. 1906 The development of the thyroid gland in *Bdellostoma Stouti*. Anat. Anz., Bd. 29.
- WHEELER 1899 The development of the urogenital organs of the lamprey. Zool. Jahrb., Bd. 13.
- WILLY, A. 1894 Amphioxus and the ancestry of the vertebrates. New York.

PLATE 1

EXPLANATION OF FIGURES

All figures were drawn with the aid of the camera lucida. Higgin's carmine and true blue inks were used to reproduce the colors of the stained sections represented in the colored plate.

1 Lymphoid accumulation in the lateral branchial wall of a 63 mm. larva. *Ep.*, Endodermal epithelium; *End.*, endothelium of blood sinus; *pb.b.s.*, peri-branchial blood sinus; *trab.*, connective tissue trabecula in blood sinus. ($\frac{1}{12}$ oil immersion obj., ocular No. 3.)

2 Portion of a frontal section of a 5 mm. larva showing the epithelial placode in the lateral branchial wall. *Ect.*, ectoderm; *End.*, endoderm; *Mes.*, mesenchyma. ($\frac{1}{12}$ oil immer. obj., ocular No. 3.)

3 Epithelial placode in lateral branchial wall of a 9.5 mm. larva. Frontal section. *Am.n.*, amoeboid nuclei; *b.c.*, red blood cells; *lm.*, longitudinal muscle fibers; *tm.*, transverse muscle fibers. ($\frac{1}{12}$ oil immer. obj., ocular No. 3.)

4 Epithelial placode in lateral branchial wall of a 15 mm. larva. Frontal section. *pb.b.s.*, peri-branchial blood sinus; *s.l.*, layer of flat cells forming at surface of placode. ($\frac{1}{12}$ oil immer. obj., ocular No. 3.)

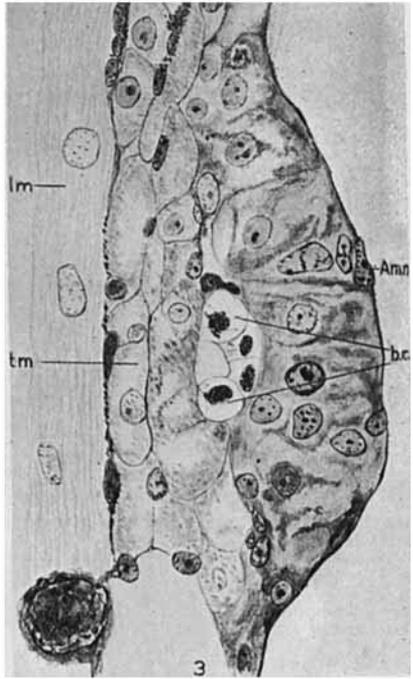
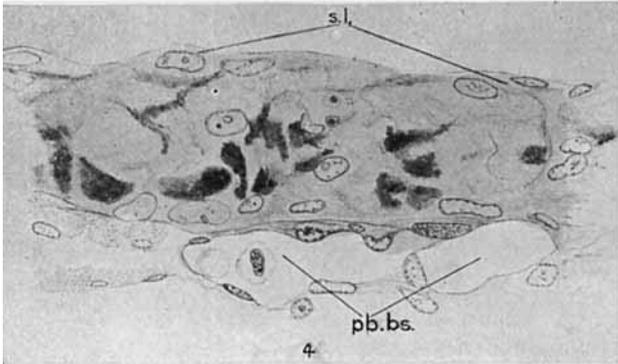
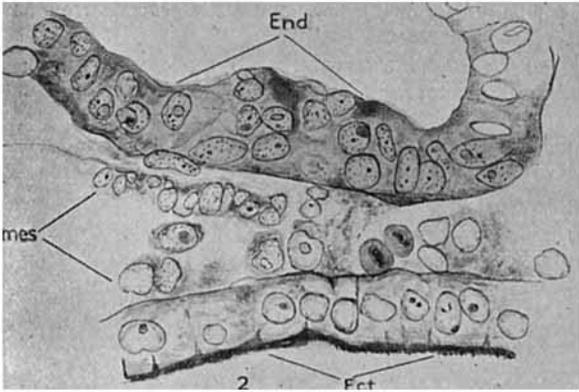
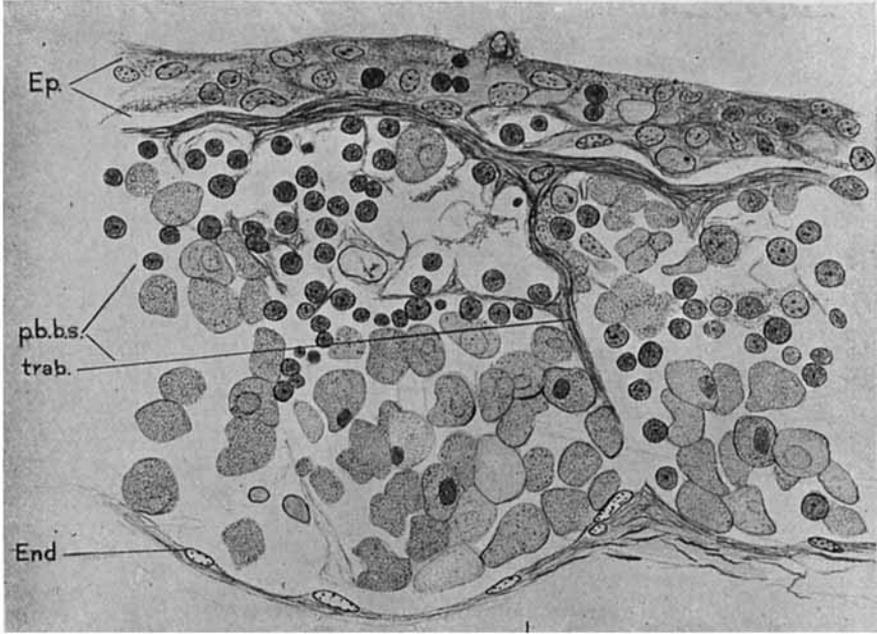


PLATE 2

EXPLANATION OF FIGURES

5 Epipharyngeal ridge containing primitive thymus placode from a 15 mm. larva. *d.a.*, dorsal aorta; *mes.*, mesenchyma containing vascular spaces; *pl.*, primitive thymus placode; *b.m.*, basement membrane separating placode from mesenchyma. ($\frac{1}{1\frac{1}{2}}$ oil immer. obj., ocular No. 3.)

6 Primitive thymus placode in 15 mm. larva. *lm.*, lymphocyte; *mes.*, mesenchyma; *v.*, vacuoles containing protoplasmic bodies. ($\frac{1}{1\frac{1}{2}}$ oil immer. obj., comp. ocular No. 12.)

7 Primitive thymus placode in a 31 mm. larva (ventral surface to the left). *ep.*, nuclei derived from the placode; *c.n.*, connective tissue nuclei; *b.m.*, remains of basement membrane. ($\frac{1}{1\frac{1}{2}}$ oil immer. obj., ocular No. 3.)

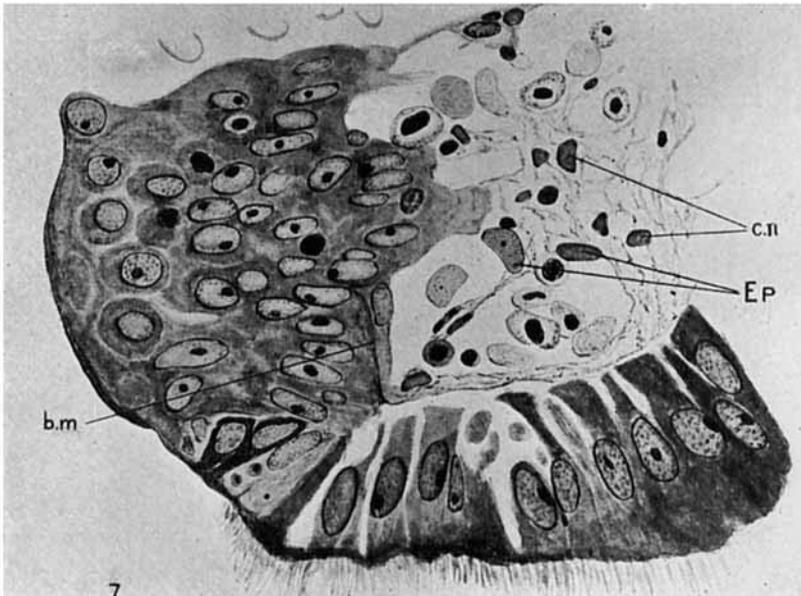
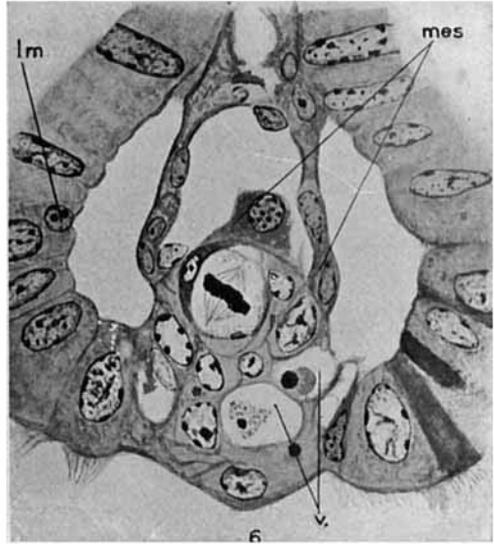
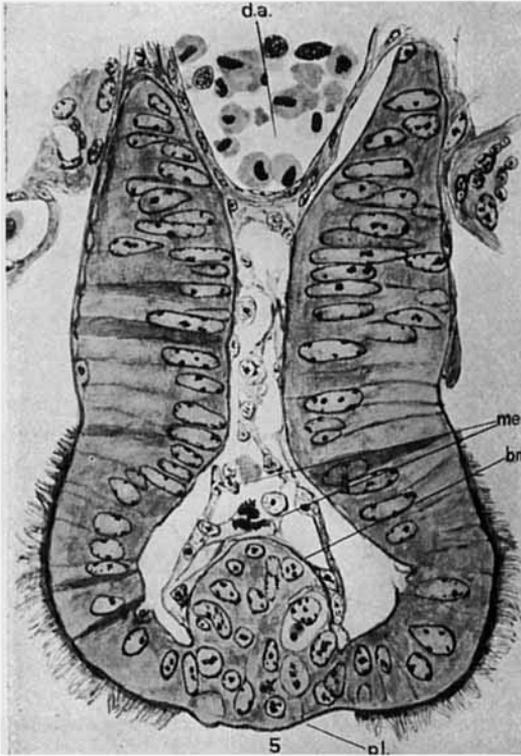


PLATE 3

EXPLANATION OF FIGURES

8 Primitive thymus placode in a 44 mm. larva. Ventral surface to the right. ($\frac{1}{2}$ oil immer. obj., ocular No. 3.)

9 Portion of primitive thymus placode in a 120 mm. larva. *b.m.*, basement membrane; *x.*, cells which are apparently red blood cells held in the connective tissue. ($\frac{1}{2}$ oil immer. obj., ocular No. 3.)

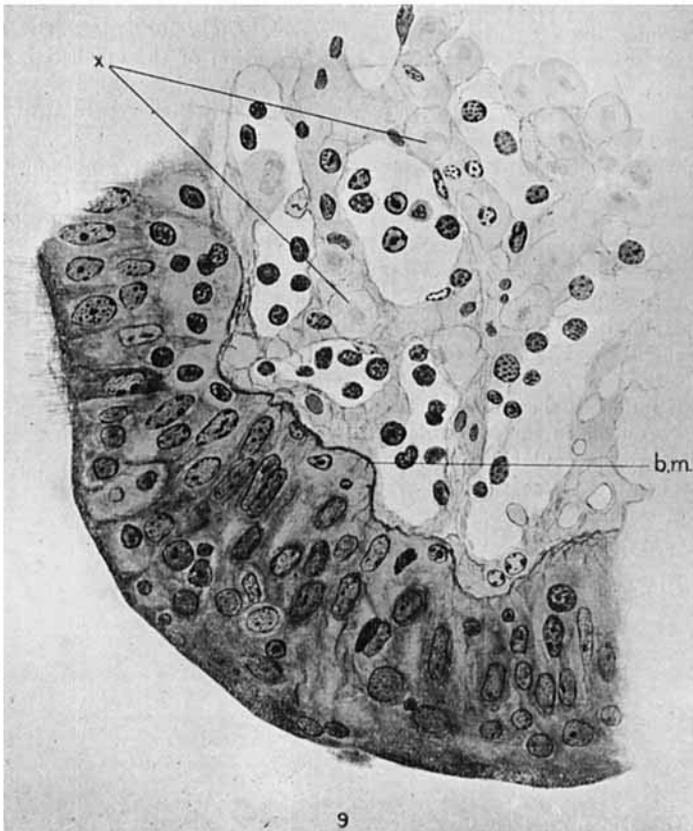
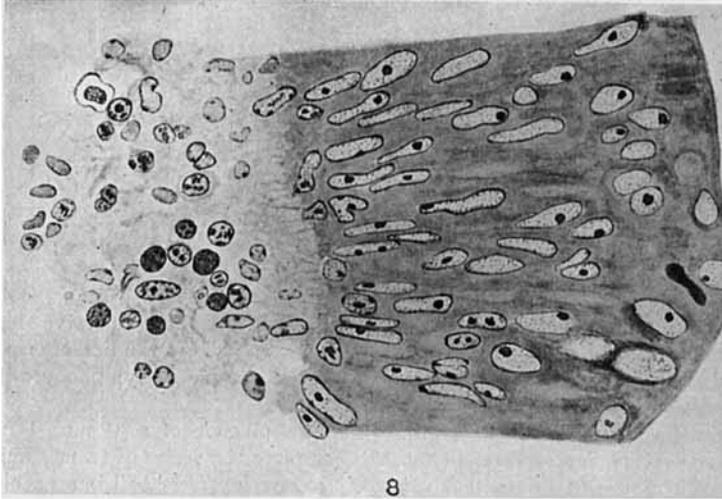


PLATE 4

EXPLANATION OF FIGURES

10 A small portion of a primitive thymus placode and the connective tissue in relation to it in a 31 mm. larva. The lettering *a* to *j* shows the line of transition from the typical epithelial nucleus (*a*) to the completed lymphocyte-like cell (*j*). ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

11 Transforming cells from primitive thymus placode of a 31 mm. larva. Cells *g* and *h* (not represented in fig. 10), complete the series of transforming cells shown in figure 10. *c.t.c.*, connective tissue nucleus. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 3.)

12 Portion of the epithelium of a gill in a 31 mm. larva showing the migration of an epithelial cell (*a*) into a blood vessel. Cells in the left part of the figure (*a* to *h*) show various stages of transformation of the epithelial cell to lymphocyte. *ery.*, red blood cells; *i*, blood cell in vessel in which the nucleus appears to be dividing; *j.*, blood cell in vessel in which there are two nuclei. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

13 Epithelial cell beginning migration into blood vessel. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

14 Nucleus in primitive thymus placode showing phagocytic properties. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

15 Nucleus in primitive thymus placode dividing by simple fission. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

16 Epithelial cell found in a connective tissue space in dorsal part of epipharyngeal ridge. Nucleus beginning to divide. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

17 Cell found in a connective tissue space in dorsal part of epipharyngeal ridge. Two nuclei in the cell which still retains epithelial characters. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

18 Connective tissue nucleus dividing by simple fission. ($\frac{1}{2}$ oil immer. obj., comp. ocular No. 12.)

