

The development of the heart in shad (*Alosa sapadissima*, Wilson) : with a note on the classification of teleostean embryos from a morphological standpoint / by H.D. Senior.

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**The Development of the Heart in
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Wilson).**

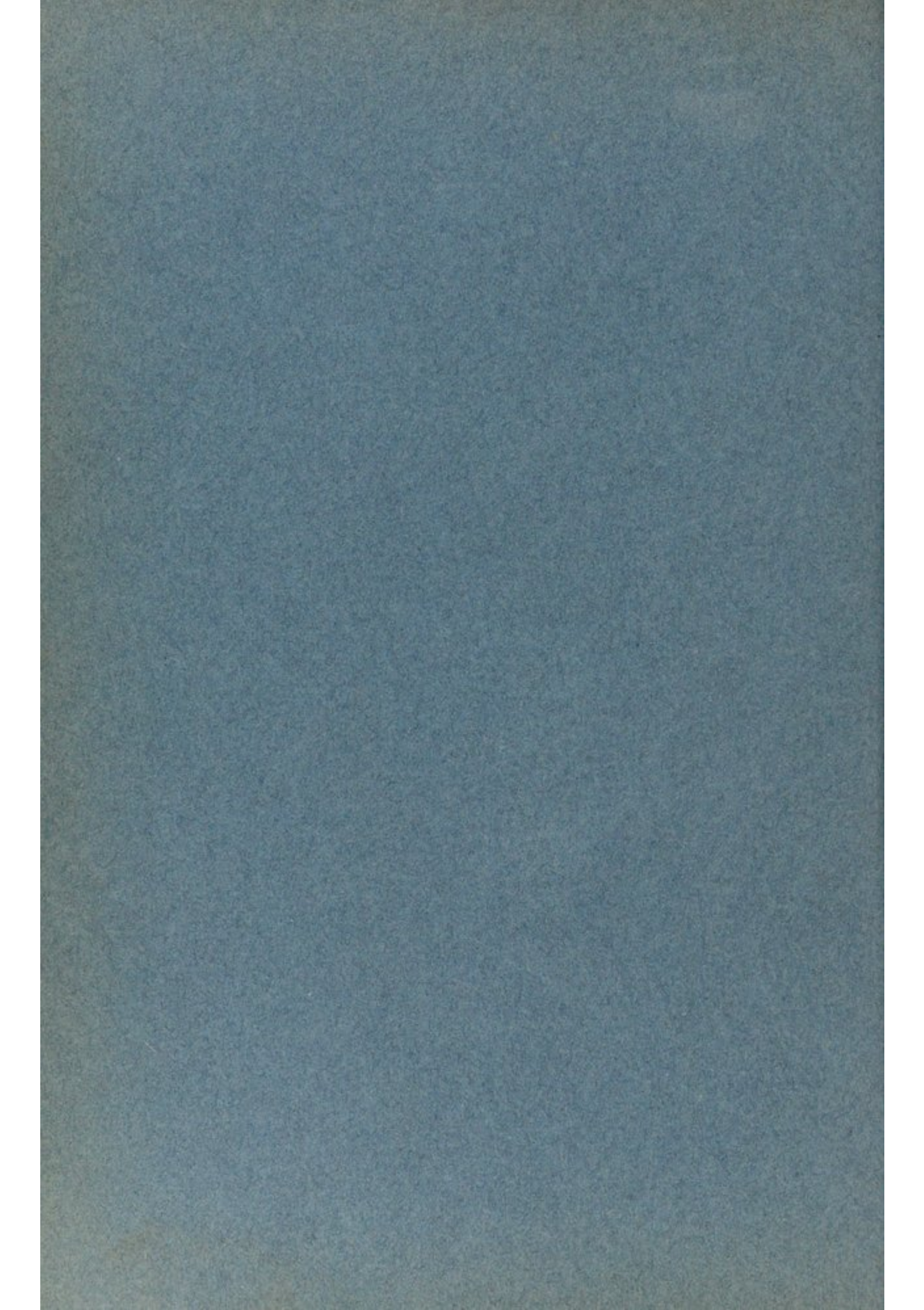
With a Note on the Classification of Teleostean
Embryos from a Morphological Standpoint.

By H. D. SENIOR

From The Wistar Institute of Anatomy and Biology, Phil-
adelphia, and the Department of Anatomy in the
College of Medicine, Syracuse University.

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WITH 27 FIGURES.

CONTENTS.

	PAGE.
Introduction—A brief description of the heart and great venous channels of a recently hatched shad.....	212
Contrast drawn between embryos of the shad type and those in which a vitelline vessel-network occurs. Suggested classification of teleostean embryos into morphological types depending on the relation of the ventral vessel system to the yolk.....	215
Statement of the object in investigating the development of the heart in shad, and of the scope of this investigation.....	219
Material and Methods	219
Development of the Heart.....	222
Period 1. Formation of the heart anlage.....	222
Review of the evidence bearing on the relation of the endocardium to the vascular endothelium of the head in general	232
Period 2. Lasting until rhythmical contraction begins in the partially formed heart-tube	237
Period 3. In which the heart-tube is completed, to form conus, ventricle, and atrium; and assumes the adult position.	
Stage of 6.2 mm.....	249
Correlation of the stages estimated by the number of somites with those designated by the length of the embryo.....	244
Mechanism of the circulation in different stages of development	244
Stage of 6.2 mm.....	246
Stage of 7.3 mm.....	249
Stage of 8.75 mm., and a comparison of the heart with that of the stage described in the introduction.....	250
Period 4. Formation of the sinus venosus and hepatic vein.....	252

INTRODUCTION.

*A Brief Description of the Heart and Great Venous Channels
of a Recently Hatched Shad.*

In examining a recently hatched specimen of shad, it will be found that the heart (Fig. 1) is apparently widely open at the venous end. The atrium is separated from the ventricle in the usual way, but, on tracing the atrial wall back to where the sinus venosus should be, no sinus venosus, as such, is found to exist. The heart wall immediately succeeding the somewhat constricted venous end of the atrium represents the anterior wall of the future sinus venosus. This flares out abruptly, and its circumference, having reached the body-wall, is reflected forward upon the latter as the parietal pericardium. These relations are shown in Fig. 1; the general arrangement of the circulation, at this period, is diagrammatically indicated in Fig. 2.

The relations here are obviously peculiar; the peculiarity, however, does not consist in the continuity of the myo-epicardium with the parietal pericardium. Inasmuch as both the myo-epicardium and the parietal pericardium are developed from the mesothelium of the lateral plates in all vertebrates, this continuity is invariable. The peculiar feature in connection with the heart, in its present state, is that the anterior pole of the yolk in a sense replaces the posterior wall of the sinus venosus. Since the yolk is entirely naked, the vascular system, closed though it is, is not completely lined by vascular endothelium, as is very commonly the case in vertebrate embryos at a comparatively early stage of development.

The venous blood is returned to the heart through four veins, the jugulars and cardinals; also by means of a blood-sinus situate dorsal to the yolk, which may be called the supravittelline blood-sinus (or, for the sake of brevity, the supravittelline sinus). The supravittelline sinus is formed in the following way: The peritoneum has a ventral attachment on either side to the dorsal surface of the yolk; the peritoneal attachments involve almost the entire longitudinal extent of the yolk. Between the lines of attachment there is enclosed an arched tunnel, of which the floor is formed by the surface of the yolk,

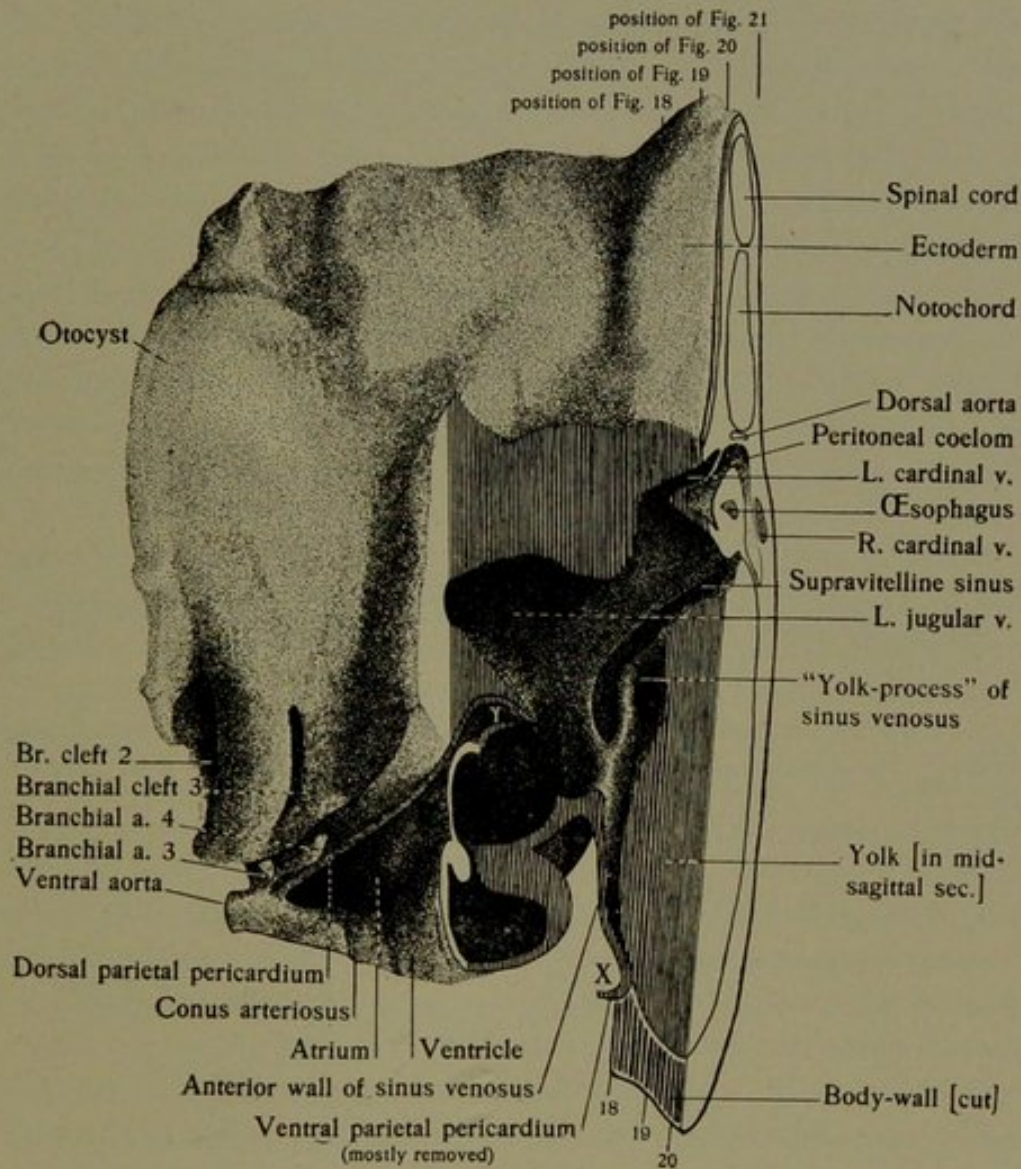


FIG. 1.—The left side of a reconstruction from the posterior gill region of a recently hatched shad, stage of 114 hours $\times 100$ diams.

Sufficient body-wall has been removed to open the pericardial coelom ventrally and laterally and to show the terminations of the left jugular and cardinal veins. The anterior pole of the yolk and the adjacent parts of the heart and pericardium are shown in mid-sagittal section.

and the roof by the ventral surface of the splanchnic mesoderm. The roof is entirely lined by vascular endothelium. The floor, having no endothelial lining, presents the naked periblast of the yolk to the blood-stream passing over it. Although the mid-sagittal plane on the embryo would fall (except near the anterior pole of the yolk, see Figs. 21 and 22) entirely within the tunnel, it would separate the latter into two unequal parts, of which the left would be greater than the right.

The supravittelline sinus, consisting of the tunnel described above, receives blood from the short *vena revehens* of the liver and discharges it into the, somewhat roomy, chamber embracing the anterior pole of the yolk. The liver, at this time, is situated dorsal to the posterior pole of the yolk (see Fig. 2). The chamber at the anterior

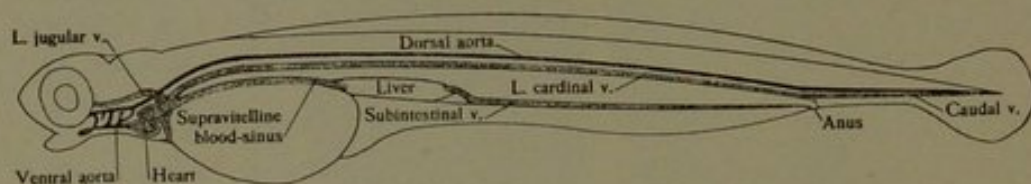


FIG. 2.—Diagram indicating the arrangement of the principal blood-channels of the recently hatched shad of which the heart is shown in Fig. 1. $\times 10$ diams.

Arteries black; veins, and supravittelline sinus, stippled.

pole of the yolk, which corresponds in position with the future sinus venosus, is bounded as follows: Posteriorly by the naked anterior pole of the yolk; elsewhere by the portion of the heart-wall immediately succeeding the atrium, which is later to form the anterior wall of the sinus venosus. Venous blood enters the chamber from the jugular and cardinal veins and from the supravittelline sinus, and leaves it by passing through the orifice leading into the atrium.

The relation of the yolk to the vascular system is somewhat as follows: While it converts the partially formed sinus venosus and the (subintestinal) supravittelline sinus into a closed passage, capable of retaining and transmitting blood, it, at the same time, delays the formation of the posterior wall of the sinus venosus and of the vessel later to be formed by the vascular endothelium lining the supravittelline sinus.

In order to identify the parts briefly described above with the structures found in the adult, it may be said that during the rapid dwindling of the yolk the piers of the arched supravitelline sinus approach each other and that between them, by a process of rearrangement of vascular endothelium, there is formed a vein, the hepatic vein of the adult. The portion of the heart-wall connecting the venous end of the atrium with the parietal pericardium forms not only the anterior wall of the sinus venosus, but also the pericardial surface of the pericardio-peritoneal septum. The posterior wall of the sinus venosus, together with the peritoneal surface of the pericardio-peritoneal septum, is furnished by the anterior part of the splanchnic peritoneum.

No vitelline vessels, other than the hepatic vein (derived from the vascular endothelium lining the roof of the supravitelline sinus) are ever developed.

Contrast drawn between embryos of the shad type and those in which a vitelline vessel-network occurs. Suggested classification of teleostean embryos into morphological types depending on the relation of the ventral vessel system to the yolk.

The entire absence of a network of vessels on the ventral and lateral surfaces of the yolk imparts to the egg of shad (and to other eggs of the shad type) an appearance strikingly different from that of the teleostean eggs (*e. g.*, those of *Salvelinus*) which for some time before hatching display a vitelline vessel-network filled with corpuscles. The type of egg to which shad belongs may be called Type 1 in contradistinction to the type in which a vitelline vessel-network occurs, which may be called Type 2.

Type 1 appears to be almost universal in pelagic eggs. *Uranoscopus scaber*¹ is the only pelagic teleost of which I find it recorded

¹The following are some examples of pelagic eggs in which a vitelline vessel-network is stated not to occur, or in which its absence has been inferred from figures depicting well-advanced stages: *Elacate canada*, *Gadus morrhua* (*callarias*), *Chaetodipterus faber*, *Scomberomorus maculatus* (Ryder '82, '84, and '87).

Hemipterus americanus, *Temnodon saltator*, *Lophius piscatorius*, *Ctenolabrus* (*Tautogalabrus*) *adpersus*, *Tautoga onitis*, *Pseudorhombus oblongatus*, *Motella argentea* (Agassiz and Whitman, '85).

Labrax lupus, *Serranus cabrilla*, *S. scriba*, *Sargus Rondeletii*, *Box vulgaris*,

that a vitelline network appears (Raffaele, '88). Type 1 is also commonly found in demersal eggs (among which the majority of the eggs belonging to Type 2 occur) including shad² itself; and, finally, although some of the viviparous eggs belong to Type 2 (*e. g.*, *Zoarces* and *Gambusia*) others³ occur which conform to Type 1.

Since the difference between the two types referred to can scarcely be said to exist in the earlier stages of development, it is well to define, as exactly as may be, what is considered to be the essential difference between them. In both types the heart pulsates prior to the appearance of free blood corpuscles, and the space between the yolk and the extra-embryonic ectoderm is occupied by circulating blood-plasma. In the type in which a vitelline network occurs (Type 2), the blood, which acquires corpuscles comparatively early, is, sooner or later, confined upon the yolk, as elsewhere, in actual vessels.⁴ In Type 1 no vessels are ever found upon the yolk, the

Scorpaena, *Lepidotrigla aspera*, *Callionymus*, *Mugil* (*capito?*), *Gadus minutus*, *Coris* (several species), *Merluccius vulgaris*, *Motella vulgaris*, *Solea* (several species), *Rhombus laevis*, *Arnoglossus*, *Clupea*, *Engraulis encrasicolus*, and several undetermined species (Raffaele, '88).

Hippoglossoides limatoides, *Rhombus* (*Psetta*) *maximus*, *Pleuronectes platesa*, *P. cynoglossus*, *P. microcephalus*, *P. flesus*, *P. limanda*, *Solea vulgaris*, *Molva vulgaris*, *Centronotus* (*Pholis*) *gunellus*, *Motella mustela*, *Gadus morrhua*, *G. aeglefinus*, *G. luscus*, *G. merlangus*, *G. pollachus*, *Lophius piscatorius*, *Trachinus*, *Clupea sprattus*, *Trigla gurnardus*, *Callionymus lyra* (McIntosh and Prince, '90).

Fierasfer dubius, *Stelaphorus ringens* (Eigenmann, '92).

²Also *Pomolobus vernalis* (*pseudoharengus*), *Roccus americanus*, *Osmerus* (Ryder, '84 and '87).

Typhlogobius californiensis (Eigenmann, '92).

Also *Pseudopleuronectes americanus* and, doubtless, many others.

³The examples found on record are *Sebastodes auriculatus* and *Cymatogaster aggregatus* (Eigenmann, '92 and '94). Probably a great many more of the viviparous perches also belong to Type 1. The absence of a vitelline vessel network in both the cases mentioned has been assumed from the figures alone. Through the courtesy of Dr. J. Percy Moore I have had an opportunity of verifying the type of *Cymatogaster aggregatus*.

⁴The arrangement of the vitelline vessels (which are invariably veins, Hochstetter, '87) varies considerably in different species, and these variations can be again classified into sub-types (see Ryder, '82, Wenckebach, '86, H. F. Ziegler, '87, Hochstetter, '87, Ziegenhagen, '94 and '96).

ventral surface of the splanchnic peritoneum is, however, lined by vascular endothelium which eventually forms the hepatic vein⁵

The hepatic vein then in Type 1, replaces the vitelline network of Type 2, and it might itself be considered a vitelline vessel, but for the fact that when it is fully formed the yolk is reduced to a very small size. The light in which the hepatic vein is regarded, however, does not affect the essential validity of the types: *Embryos of Type 2 differ from those of Type 1 in that they possess, at some period prior to the disappearance of the yolk, vitelline vessels lateral to the margin of the caelon.*

In all cases in which the site of origin of the blood corpuscles has been investigated in embryos of Type 2,⁶ it has been found to occur in the cardinal veins, which may be separate or partially conjoined (Stammvene). In embryos of Type 1⁷ the cardinal veins have invariably been found, when they first appear, to contain no corpuscles.

At the present time, although the information at our command is rather suggestive, it appears neither safe to assume that the blood anlage is *always* developed within the cardinal veins in embryos of Type 2, nor that this *never* happens in embryos of Type 1. It would seem that more information is needed on the entire subject of blood-formation in teleosts, before a generalization of this kind can be made with safety. I refer particularly to the fact that Marcus ('05) has recorded for *Gobius capito* (Type 2) that corpuscles arise in the tail as well as in the cardinal vein region;

⁵It is neither assumed nor implied that the formation of the hepatic vein in other embryos belonging to Type 1 is similar in mechanism to that later to be described for shad; the serial sections of other Type 1 eggs, mostly pelagic, in the possession of the writer do not cover all the stages necessary for the determination of this point.

⁶Zeigler, '82 and '87, *Salmo salar*; Wenckelbach, '85, *Perca fluviatilis*, '86 *Belone* and *Esox*; Felix, '97, salmon and trout; Swaen and Brachet, '00, trout, '02, *Leuciscus cephalus*, and *Exocoetus volitans*; Sobotta, '02, *Trutta fario*, *T. iridea* and *Salmo salvelinus*; Marcus, '05, *Gobius capito*.

⁷Wilson, '91, *Serranus atrarius* (*Centropomus striatus*, L.); Sween and Brachet, '02, *Clupea sprattus*, *Rhombus* (?), *Solea vulgaris*, *Pleuronectes microcephalus*, *Trachinus vipera*, *Caranx trachurus*, and *Callionymis lyra*; Derjugin, '02, *Lophius piscatorius*.

this is probably a fact of great importance. The blood anlage of shad (Type 1) arises as a cord of cells in the tail, which forms a direct continuation backwards of the, then, partially developed caudal aorta and caudal vein; part of the blood anlage of *Opsanus tau* (Type 2) also arises in a similar manner (the remainder arising in the cardinal veins). It is possible that the tail is the site of blood formation common to all teleosts, and that the cardinal vein blood anlage occurs as a further source of corpuscles in the forms which acquire numerous corpuscles at a comparatively early stage of development. Without assuming this actually to be the case, I would venture to suggest that, in connection with the origin of the blood corpuscles, the tail deserves thorough examination in all teleosts, whether or not, in the species under examination, corpuscles are found to arise in the cardinal veins. Reference to investigations setting forth the surface of the yolk as the source of blood corpuscles has been purposely omitted.

It has been my experience that the yolk in eggs of Type 1 is in excellent condition for cutting after it has been fixed in formalin; whereas formalin-fixation produces in eggs of Type 2 a yolk difficult to cut and sometimes of almost stony hardness. This is not due merely to difference in size, but seems to point to a difference in chemical composition between the yolks of the two types of egg.

It is well known that the differences in structure, and in the general processes of development which occur among teleostean embryos of different species bear little or no relation to the structure and affinities of the corresponding adults; since, therefore, the type of embryo cannot be inferred from the systematic position of the adult, it would seem advantageous to classify the embryos themselves according to their own structural peculiarities.

The division of embryonic teleosts into the morphological types indicated above appears to be warranted by the present state of our knowledge and, since it is applicable alike to pelagic, demersal, and viviparous eggs it may prove of some service as a starting point for classification. The demand for some such division into types is, I think, indicated by the not infrequent use in the literature of the terms pelagic and demersal in a morphological connection. The

following objections to the use of the words pelagic and demersal in a morphological sense will sufficiently indicate their unsuitability.

(a) Their use, in this sense, is apt to entail the statement that a given egg is either demersal in habit and pelagic in structure, or *vice versa*, which is undesirable.

(b) Neither of these terms can be used to express the structure of a viviparous egg.

(c) These terms, as strictly applied, have no more morphological significance than has the term viviparous itself.

Statement of the object in investigating the development of the heart in shad, and of the scope of this investigation.

The development of the heart in embryos of Type 2 has received a large share of attention, particularly in *Salmo* and allied genera in which the process of heart-formation has been definitely made out.

The heart in embryos of Type 1 differs considerably from that in embryos of Type 2, particularly in its relation to the vascular system of the yolk. The development of the heart, however, appears to have received somewhat scant notice. Ryder has given a brief account of some of the changes undergone by the heart in *Gadus morrhua* (callarias), '82, and in *Clupea* (*Alosa*) *sapadissima*, '85; Boeke has described the early development of the heart in *Muraena* (endocardium particularly), '03. There are also some earlier investigations, dealing with the living embryo alone, which, necessarily, leave much to be desired.

It has been attempted here to give a consecutive account of the development of the heart in shad, as representing Type 1, from the earliest possible stage until the adult arrangement is recognizable.

MATERIAL AND METHODS.

The material investigated, the property of The Wistar Institute of Anatomy, Philadelphia, was collected during the seasons 1905, '06, and '07 at the hatchery of the Pennsylvania State Fish Commission, Torresdale, Pa. To the Commissioner, Mr. W. J. Meehan, I hereby tender my thanks for his many courtesies.

Prior to a period of development at which the embryo is capable of

self-extension after removal of the egg-membrane (so that it may be fixed in the extended position) the stages have been estimated by the number of somites. Stages have been designated in terms of length of embryo (in a straight line from end to end) from the time that the embryo is approximately straight until the, somewhat arbitrarily selected, period of hatching. After hatching the age is given as being the sole guide to the stage of development; the length of the embryo, unfortunately, does not convey the required information.

It is well known that development is relatively accelerated by a high water temperature. The period of development within the egg is actually shorter, however, in warm water, for the embryo is hatched in a progressively immature state in direct proportion as the water-temperature rises. To mention a few examples: My stage of 114 hours, 10.5 mm. (just hatched at a low-water temperature⁸) is much more advanced in development than the stage of 8.7 mm., 63 hours, (hatched about twenty hours in warmer⁹ water); the latter is only slightly more advanced than a stage of 8.3 mm., 107 hours (still in the egg, water-temperature⁸ low). Direct comparison, except in individual cases, is not easily made between the hatching stages in different water-temperatures because the embryos of any one batch do not hatch simultaneously but continue to hatch over a period lasting twenty-four hours or more; the hatching period must, therefore, be in any case somewhat arbitrarily determined. I have selected the stage of 114 hours as the just hatched stage (in preference to earlier "just-hatched" stages) because at all stages prior to this the length of the embryo, from the time it is capable of self-extension, accurately indicates the period of development.

Shad is anadromous; the egg, demersal and non-adhesive, is convenient for study on account of its transparency and because it is easily removed from its roomy capsule (diameter of egg proper is about 2 mm. capsule slightly under 4 mm.). Details of the spawning-habits and of the methods of rearing eggs and larvæ are given in "A Manual of Fish-Culture," published by the U. S. Fish Commission, revised edition, Washington, 1900.

⁸Average temperature 63° F.

⁹Average temperature 70° F.

All the methods of fixation in common use for teleosts were tried. That of Sumner (saturated corrosive sublimate containing 10 per cent glacial acetic; followed by 10 per cent. formalin; see Sumner, '00) proved the most satisfactory and was generally employed; all the figures were drawn from material fixed in this way except Fig. 8 (H. Virchow's method) and Figs. 10 and 11 (Pereny's fluid).¹⁰

The embryos were cut into serial paraffine sections ranging from 5 to 10 microns in thickness. Sections of the earlier stages were stained with iron hæmatoxylin, later stages were usually stained in toto with alcoholic carmine.

Eleven wax-plate reconstructions¹¹ were made, two hundred times larger than the originals after fixation (correction having been made for shrinkage in paraffine). In making the figures the reconstructions were photographed (natural size) and the outlines of the photograph traced. In finishing the drawings the irregularities due to the plates were omitted. All figures, representing reconstructions, have been reduced one-half in reproduction.

Below follows a list of the figures with data for identification of their sources. (*The numbers are from the catalogue of The Wistar Institute of Anatomy*):

FIG. 1.—Reconstruction 14504, from series 14275, sections 87 to 134.

FIG. 2.—Outline from embryo afterwards cut into series 14275.

FIG. 3.—From series 14556; 3A section 91, 3B 120, 3C 132, 3E 142, 3F 152, 3G 181.

FIG. 4.—Reconstruction 14534, from series 14533, sections 45 to 87; 4A section 53, 4B 57, 4C 62, 4E 71.

FIG. 5.—Reconstruction 14535, from series 14524, sections 91 to 151; 5B section 104, 5C 111, 5D 114, 5G 148.

FIG. 6. Reconstruction 14536, from series 14520, sections 63 to 110; 6B section 76, 6C 79, 6D 82, 6F 92.

FIG. 7.—Reconstruction 14537, from series 14532, sections 68 to 113; 7B section 82, 7C 86, 7D 89, 7G 113.

¹⁰Pereny's fluid, in which, unfortunately, all my material about the time of beginning heart-beat has been fixed, gives fair general results, but is extremely unfavorable for cytological study.

¹¹Shown at the Chicago meeting of the Association of American Anatomists, Christmas, 1907.

FIG. 8.—Series 14567, section 39.

FIG. 9.—From the reconstruction used for Fig. 12.

FIG. 10.—From series 14667; 10B section 70, 10D 79.

FIG. 11.—Reconstruction 15011, from series 14670, sections 68 to 110; 11C section 79.

FIG. 12.—Reconstruction 15012, from series 14668, sections 39 to 69; 12C section 54.

FIG. 13.—Reconstruction 15013, from series 14671, sections 21 to 80.

FIG. 14.—Diagram from the reconstruction used for Fig. 13

FIG. 15.—Diagram from reconstruction 15014, from series 14672, sections 28 to 61.

FIG. 16.—Reconstruction 15015, from series 14568, sections 20 to 56.

FIG. 17.—Fig. 1 repeated.

FIG. 18.—Series 14275, section 126.

FIG. 19.—Series 14275, section 130.

FIG. 20.—Series 14275, section 133.

FIG. 21.—Series 14275, section 136.

FIG. 22.—Reconstruction 14507, from series 14538, sections 82 to 136.

FIG. 23.—Series 14538, section 128.

FIG. 24.—Series 14538, section 132.

FIG. 25.—Series 14538, section 140.

FIG. 26.—Series 15002, section 138.

FIG. 27.—Series 15002, section 148.

DEVELOPMENT OF THE HEART.

The process of development of the heart in shad may conveniently be divided into four periods as follows:

1. Formation of the heart anlage.
2. Lasting until rhythmical contraction begins in the partially formed heart-tube.
3. In which the heart-tube is completed to form conus, ventricle and atrium, and assumes the adult position.
4. Formation of the sinus venosus and hepatic vein.

PERIOD I. FORMATION OF THE HEART ANLAGE.

The myo-epicardium and the parietal pericardium are developed from the lateral plates of the mesoderm. That the endocardium is derived from mesoderm, and from mesoderm alone, has already been



THE DEVELOPMENT OF THE HEART IN SHAD.

H. D. SENIOR.

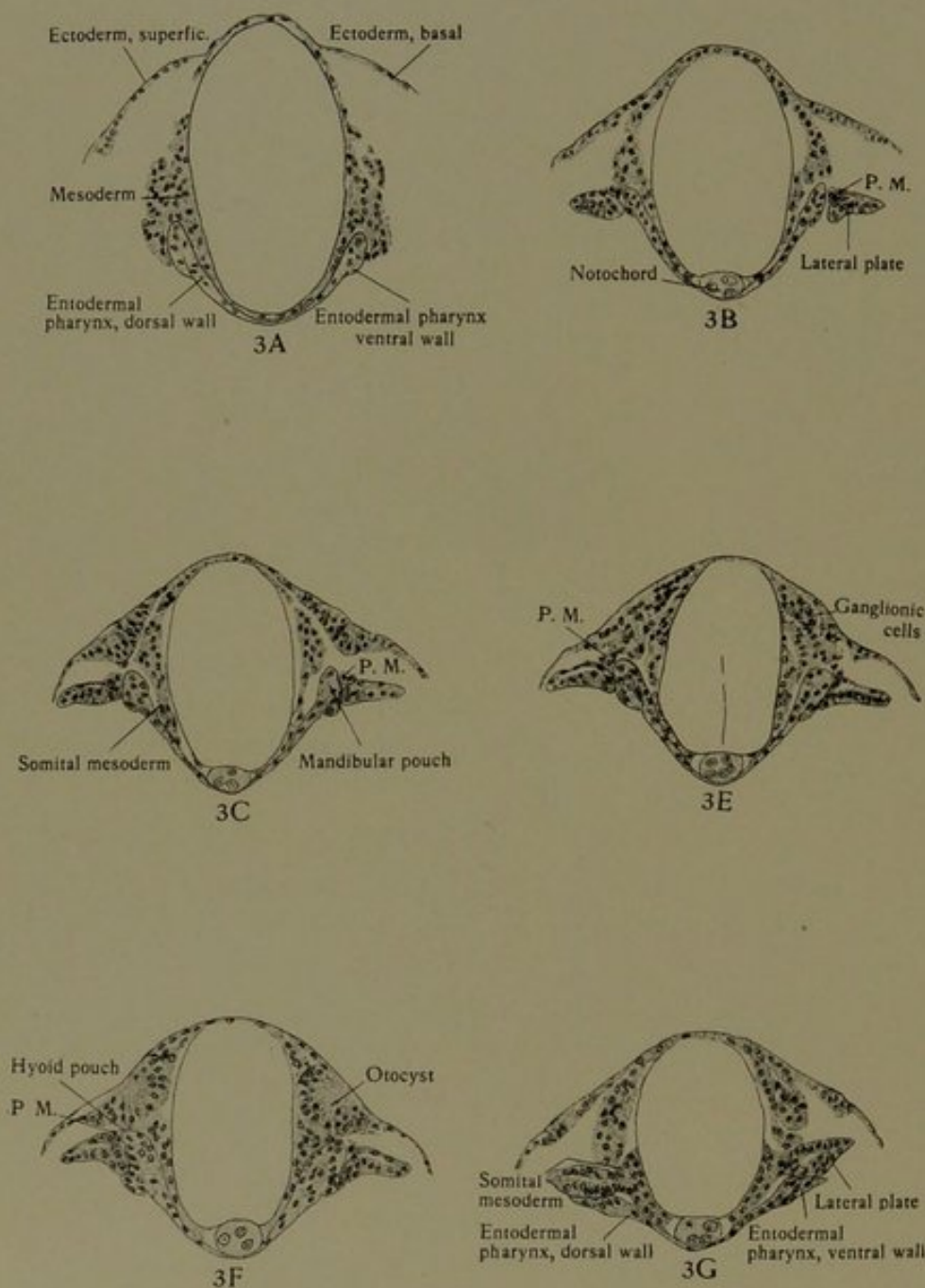


FIG. 3.—Transverse sections of shad, stage of 15 somites $\times 100$ diams. For situation of sections see footnote on opposite page.

shown in other teleosts by Oellacher, '73, H. E. Ziegler, '87, Swaen and Brachet, '00, and by Sobotta, '02. Nothing has been found in shad supporting the view that the endocardium arises from entoderm alone or from entoderm and mesoderm together.

The particular part of the mesoderm from which the endocardium is derived is a, bilaterally symmetrical, cord of cells on either side immediately adjacent to the medial borders of the lateral plates; of this Swaen and Brachet have given a careful description (as found in *Trutta fario*) and to it they have applied the term "*Portion moyenne du mésoblaste*." In shad at a stage of 15 somites the portion moyenne is recognizable throughout most of its, eventual, longitudinal extent; although comparison with earlier stages shows that it has been recognizable for some time but in a less advanced stage of development.

In order to gain a clear conception of the portion moyenne, as it occurs in shad at the stage of 15 somites, it is necessary to enquire into the cause of its distinctness from the remainder of the head mesoderm. There are, as far as I can see, no features in the cells composing the portion moyenne which distinguish them at this stage from other mesodermal cells. The portion moyenne, as a whole, is distinguishable from the lateral plate by reason of the orientation of the cells of the latter (where this has occurred) around the, now virtual, *cœlon*; see Figs.¹² 3B, 3C, 3E and 3F. The lateral plate,

¹²To facilitate comparison between the sections shown in figures 3, 4, 5, 6, 7, 10, 11, and 12 the same letter has been used throughout to indicate a certain region. Seven regions have been selected as follows:

A is near the, eventual, anterior limit of the lateral plates.

B is between A and the mandibular entodermal pouch.

C is through the mandibular entodermal pouch.

D is midway between C and the gill anlage spoken of in the text as the hyo-branchial.

E is a short distance in front of the hyo-branchial anlage.

F is through the hyo-branchial anlage and the anterior part of the developing otocyst.

G is some distance posterior to the branchial region of the pharynx.

In figures of sections P.M. indicates portion moyenne.

The anterior aspect of the section is always represented; the structures on the right side of the embryo will, therefore, appear on the left side of the figure, and *vice versa*.

which is now in progress of differentiation from behind forwards, is not yet clearly distinguishable at the site of Fig. 3A (q.v.) and here the portion moyenne (although it is later well developed, see Fig. 4A) is not clearly defined.

The distinction of the portion moyenne from the somital portion of the head mesoderm depends on an actual separation of cells from the lateral region of the somite; or (perhaps more correctly) on an isolation of the cells situated between the somite and the lateral plate. The position of the portion moyenne, where portion moyenne occurs, bears a definite relation to the lateral margin of the entodermal pharynx. In the region of the entodermal pouches the pharynx is wide and the portion moyenne is always situated lateral to its lateral margin (see Figs. 3B, 3C, 3E and 3F). Posterior to the region of the entodermal pouches the entodermal pharynx is much reduced in width, and here the portion moyenne does not occur at all, for the lateral plate is in contact with the somite (see Figs. 3G and 5G). The transition between the wide and narrow portions of the entodermal pharynx occurs, somewhat abruptly, on a level with the middle of the otocyst. In the region of the entodermal pouches, the prominent lateral margin of the pharynx tends to insinuate itself between the somital mesoderm and the portion moyenne. Posterior to the entodermal-pouch region the margin of the narrow entodermal pharynx tends to remain altogether ventral to the somital mesoderm (see Figs. 3G and 5G).

Swaen and Brachet suggest that the portion moyenne is separated from the somital mesoderm under the combined influence of the prominent margin of the entodermal pharynx on the one hand, and of the ectoderm and the anterior part of the otocyst on the other; the conditions found in shad are quite in accordance with this view. The appearances in Fig. 3F clearly suggests the influence of the wide hyoid pouch and of the otocyst in causing separation of the portion moyenne. In Figs. 3B, 3C and 3E the ectoderm would appear to be of little assistance in causing separation of the portion moyenne, but it is probable that the ectoderm is normally in contact with the mesoderm and that the separation of ectoderm from mesoderm, seen in the sections, is artificial and mainly due to the action of the fixative.

The portion moyenne appears as a cord of mesodermal cells on either side, intervening between the somital and lateral mesoderm, which tends to remain in contact with the lateral plate. It extends from the middle of the otocyst forward to the anterior end of the lateral plate (see Fig. 4A). Small posteriorly, the portion moyenne becomes larger anterior to the mandibular pouch; here and there it comes into immediate contact with the somital mesoderm. Where contact occurs at this stage, the distinction between portion moyenne and somital portion of the head mesoderm cannot be made out because there is, as yet, no differentiation between the cells belonging to these two parts of the mesoderm. In spite of the fact that in occasional sections the portion moyenne is not quite clearly defined, it forms, as a whole, a perfectly definite structure.

That the portion moyenne, as found in shad, is directly comparable to that described with such admirable distinctness in trout by Swaen and Brachet cannot, I think, be doubted; for this reason I have ventured to adopt the term employed by them rather than run the risk of confusion by the unnecessary introduction of another name.

It has been said that the endocardium is derived from the portion moyenne of the mesoderm; before proceeding to trace the formation of the endocardium it may be stated that special care has been taken to determine whether the entire longitudinal extent of the portion moyenne is involved in the production of endocardium. In order to settle this question (among others) a series of four wax-plate reconstructions has been made from stages during which the cells of the portion moyenne are undergoing migration and differentiation to form the endocardium. *The result indicates that the endocardium, together with the central aorta, is derived exclusively from that part of the portion moyenne originally situated anterior to the transverse plane passing through a point midway between the mandibular and hyoid entodermal pouches.* That the posterior part of the portion moyenne takes absolutely no share in the formation of the endocardium is an important point which will be referred to later.

Fig. 4 is a diagrammatic representation of the ventral surface of a wax-plate reconstruction of the pericardial region of a shad's head. Stage of 18 somites (the embryo is one hour and a half older

than that used in the preceding figure). The parts shown¹³ are (normally) in immediate contact with the yolk, and represent the region extending from the tip of the notochord back nearly to the anterior limit of the first body somite. Formation of the ventral wall of the pharynx, by the folding ventralwards of the (originally) lateral region of the gut-entoderm on either side, is complete in the anterior region and is rapidly extending backwards. The lateral plates are following the medial margins of the ventral pharyngeal wall in their progress toward the mid-line.

The outline of the entodermal pharynx presents, on each side, three prominences which require some explanation. Of these the posterior is the hyoid ectodermal pouch which has now reached, and blended with, the (very shallow) ectodermal pouch; this, for reasons stated below,¹⁴ will be referred to as the hyo-branchial anlage. The middle prominence is the mandibular entodermal (solid) pouch; this blends with the ectoderm later but (as is well known) gives rise to no cleft; it undergoes disintegration soon after the perforation of

¹³Explanation of Figures 4, 5, 6, and 7.

Red, lateral plates, where these are covered ventrally by endocardium their outline is indicated by a red line.

Blue, endocardium.

Continuous black line indicates the position of the outline of the entodermal pharynx and adjacent part of the head-fold (see text); interruptions in this line indicate blending of pharyngeal entoderm, or of head-fold, with the basal layer of the surface ectoderm.

Broken black line, medial margin of (closing) ventral wall of pharynx.

Stipple, ventral wall of the entodermal pharynx and adjacent head-fold ectoderm.

Plain white, dorsal wall of (incomplete) pharynx.

Arrows on each side indicate the longitudinal limits of the "descent area" of portion moyenne.

"Although the (solid) hyoid pouch is alone present at this stage the branchial entodermal pouches (also solid) are about to be laid on, very rapidly, from before backward. The hyoid and branchial entodermal pouches all reach a common ectodermal anlage and are separated from one another by an extremely delicate partition of mesoderm so that, at any given time, a very careful examination is necessary to determine the exact number of pouches actually present; in order to avoid a repeated analysis of the condition, which is unnecessary for this investigation, the entire series of compactly grouped pouches has been looked upon as a single structure.

THE DEVELOPMENT OF THE HEART IN SHAD.

H. D. SENIOR.

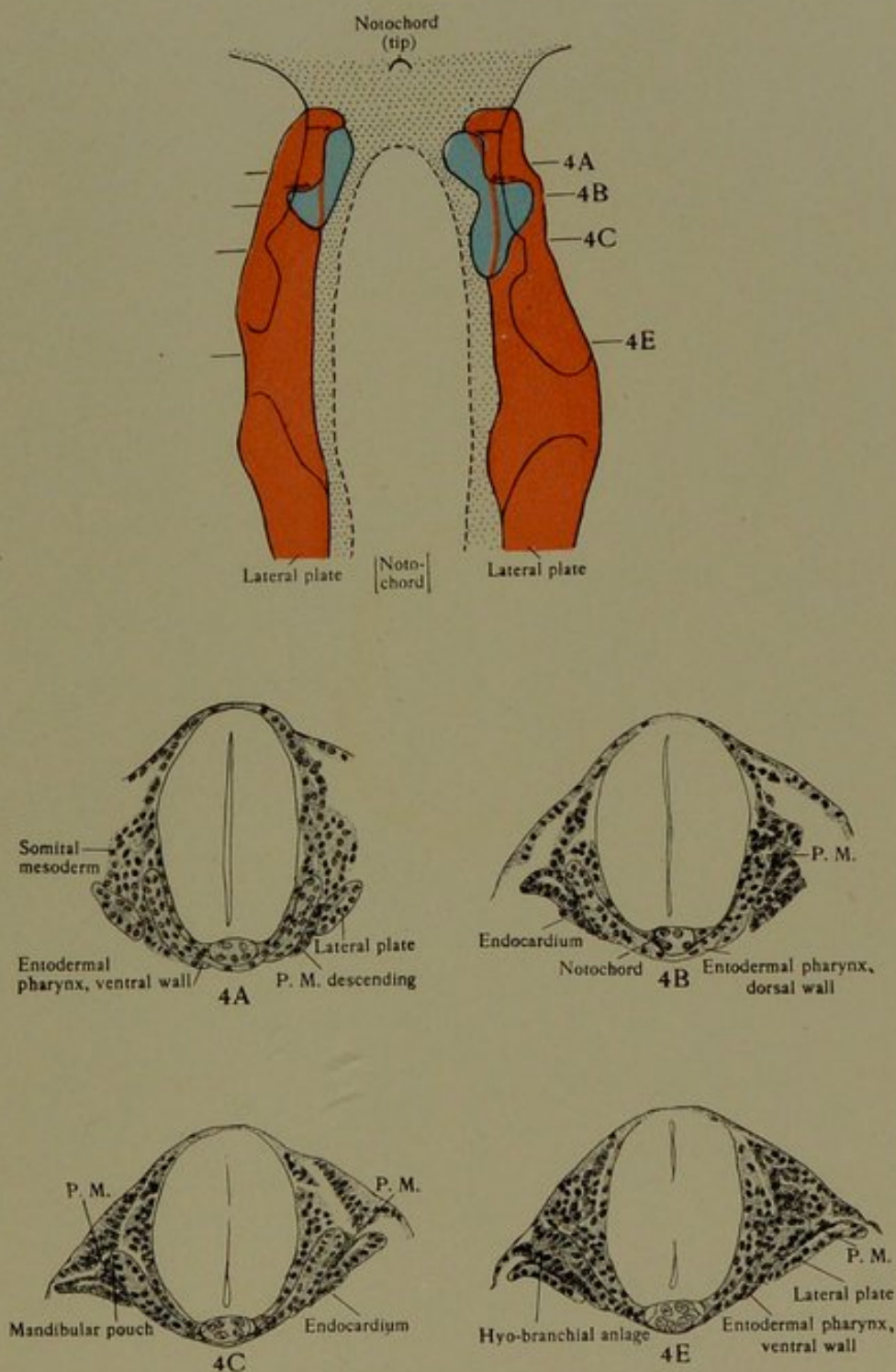


FIG. 4.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 18 somites $\times 100$ diams. See explanation of figures, footnote on opposite page.



the hyoid cleft. The anterior prominence is not strictly pharyngeal; it represents the posterior limit of the head-fold ectoderm,¹⁵ which, growing in from either side, blends with the anterior extremity of the closing entodermal pharynx. Inasmuch as it has been found difficult or impossible to distinguish the line of union between ectoderm and entoderm after blending has occurred, no attempt has been made to indicate this in Figs. 4, 5, 6 and 7; there is no doubt, however, that, although the lateral prominences consist of ectoderm, the axial region (as far as shown in the figures) is truly pharyngeal.

At 18 somites the anterior part of the portion moyenne is descending on either side, between the lateral plates and the, now closing, pharynx to gain a position ventral to these structures; the longitudinal area over which descent is occurring ("descent area") is indicated by the space between the arrows in the diagram. Fig. 4A is a section from this region. Sections from behind the descent area (Figs. 4B and 4C) show that the portion moyenne, which can be seen in them dorsal to the lateral plate, is not descending; descent is prevented, apparently, by the close contact between lateral plate and pharyngeal entoderm. It will be seen that the portion moyenne is

¹⁵The "optic region" of the head (anterior to the parts reconstructed) is separated from the yolk by a double layer of ectoderm which grows in, in this situation, from the basal ectoderm around the anterior periphery of the head. This ectoderm, which performs several functions, corresponds, in teleosts, to the head-fold of other vertebrates (see Friorip, '05). The formation of head-fold from this double layer of ectoderm occurs literally, in shad, only as far back as the hypophysial region, anterior to which no gut-entoderm occurs. There is a region, extending from some point (approximately) ventral to the hypophysis back to a point slightly posterior to the tip of the notochord, throughout which the ingrowing ectoderm encounters the anterior extremity of the pharyngeal entoderm; the ectoderm in this region, although its origin is intimately connected with that of the head-fold, has an entirely different subsequent history, briefly indicated as follows: Until the head of the embryo begins to arise from the yolk the double-layered ectoderm of the region in question forms a bond of union between the anterior end of the pharynx and the surface of the embryo. As the head rises from the yolk the layers of the head-fold ectoderm proper become separated to cover the contiguous portions of the head and yolk. Shortly before perforation in the oral plate occurs, the anterior cul-de-sac of the pharynx becomes widely dilated, and the ectoderm connecting the lateral margins of the anterior end of the entodermal pharynx with the basal ectoderm of the surface undergoes, very rapid, disintegration.

in process of an entire alteration in its distribution; it now consists, on either side, of three parts: One part still remains dorsal to the lateral plate, a second is on the ventral surface of this structure, and a third forms an isthmus between the other two and occupies the descent area. From now on it will be convenient to speak of the part ventral to the lateral plate as endocardium (for such it really is) and to retain the original term for the part which is still dorsal to the lateral plate and for the isthmus. The endocardium now appears as two patches (colored blue in Fig. 4) ventral to the entodermal pharynx and the lateral plates. These patches are not limited to the descent area, but are spreading backwards (more so on the left side of the embryo than on the right, see Fig. 4B and 4C). The backward (caudad) movement of the endocardium, which is now beginning, is soon to become very pronounced. The asymmetry seen in this reconstruction is thought to be due to unequal growth of the embryo, rather than to faulty building up of the plates, the entire right side of the head appears to be in a less advanced stage of development than is the left.

In an embryo of 22 somites (Fig. 5, one hour and a half older than the preceding stage) there has been considerable advance in development. Ventral closure of the pharynx has progressed rapidly from before backwards, and is also beginning in another place posterior to the gill-region. The medial margins of the lateral plates are approaching one another, and the notochord, slightly longer than before, is now fully formed as far as its anterior end is concerned. The endocardium has travelled back to a point posterior to the mandibular pouch (Fig. 5D), and the portions arising from each side have met across the mid-line. Fig. 5B (as compared with 4B) shows that the descent area has extended considerably backwards, but that descent is not yet occurring opposite the apex of the mandibular pouch is shown on the left side of Fig. 5C (right side of embryo).

At a stage of 26 somites (Fig. 6, one hour and a half later than the preceding stage) the ventral closure of the pharynx is approaching completion; rapidly, however, as closure of the pharynx is taking place, it has been overtaken by the backward growth of the endo-

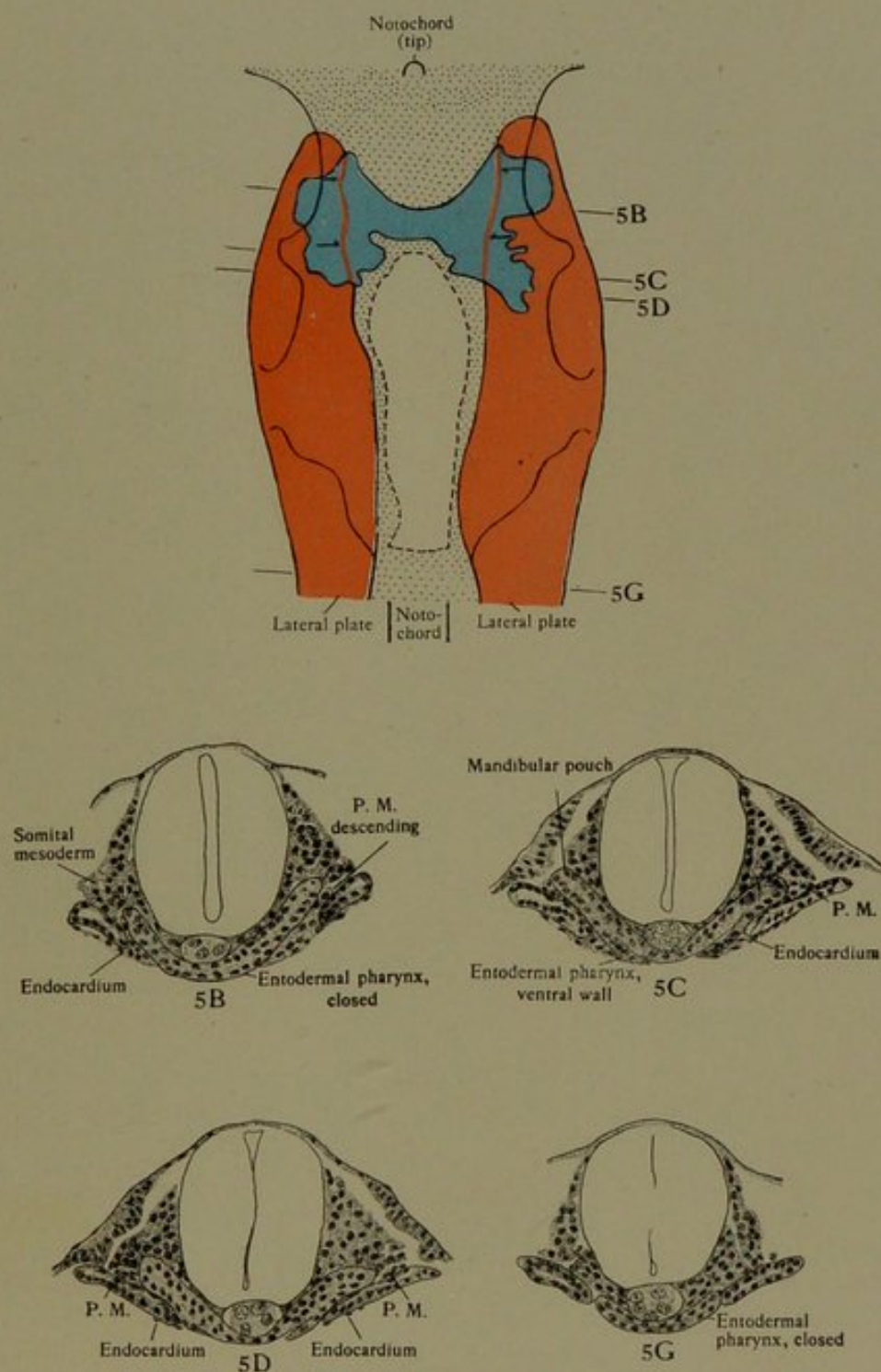


FIG. 5.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 22 somites 100 diams. See explanation of figures, footnote on page 226.

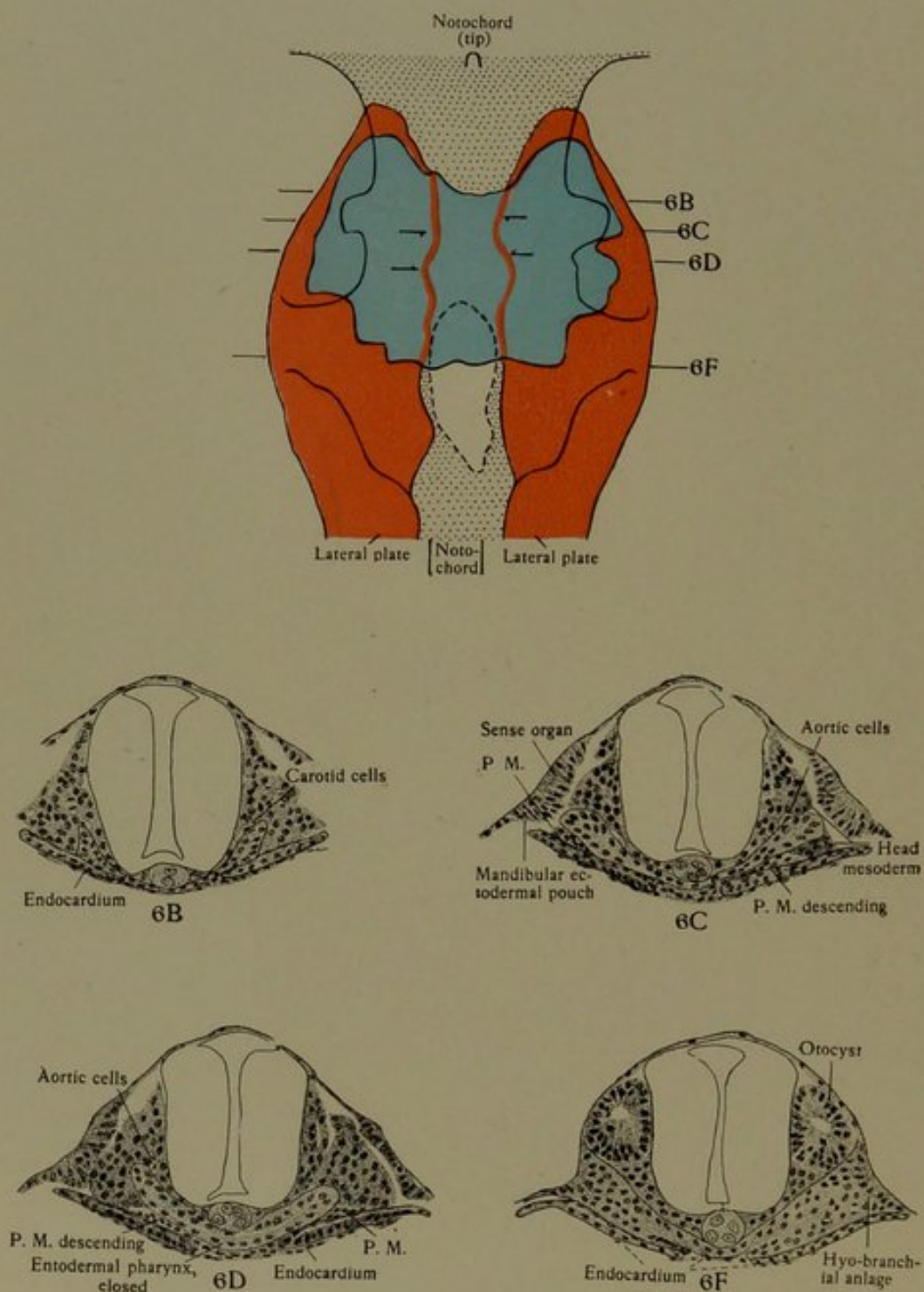


FIG. 6.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 26 somites $\times 100$ diams. See explanation of figures, footnote on page 226.



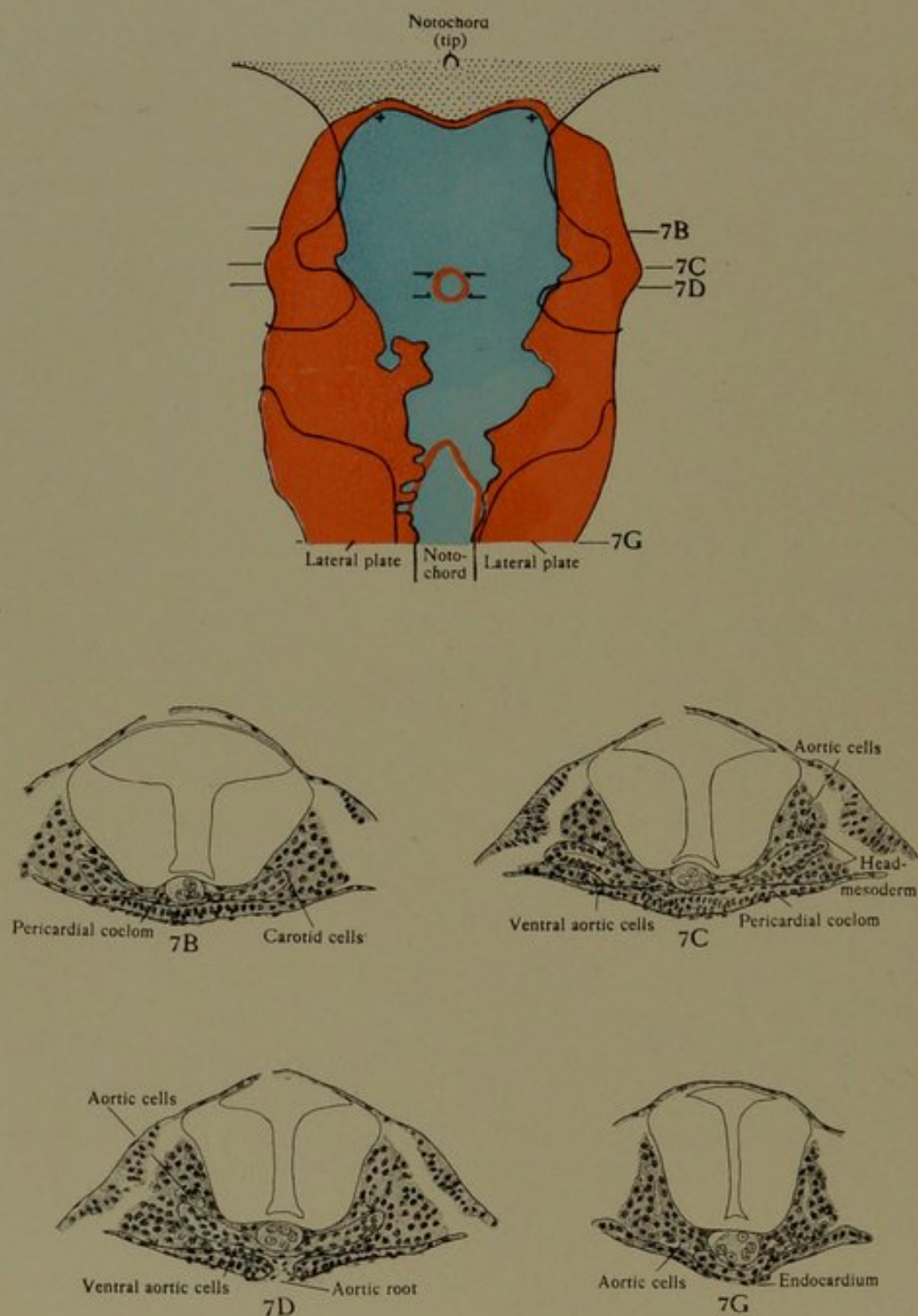


FIG. 7.—Diagram of the ventral surface of a reconstruction of the pericardial region of shad, stage of 32 somites \times 100 diams. See explanation of figures, footnote on page 226.

cardium. The endocardium, which now covers a considerable area, not only lies over (ventral to) the closing edges of the entodermal pharynx, but partially covers the ventral surface of the future roof as well (see Fig. 6 and 6F). The portion *moyenne* is no longer descending in the region anterior to the mandibular pouch, for here it has, apparently, been exhausted (see Fig. 6B). The descent area on the left side (right in Fig. 6C) is situated toward the back of the mandibular pouch, and on the right (left in Fig. 6D) is altogether behind this structure; here again there is slight asymmetry. The lateral plates, which are now much increased in width, are approaching the mid-line; the notch in the medial border of each foreshadows the point at which the actual borders never meet, but at which they are about to embrace the root of the ventral aorta.

The lateral margins of the somital portion of the head mesoderm are, at this stage, spreading ventrally around the lateral margins of the entodermal pharynx in the region between the mandibular and hyo-branchial pouches (see Figs. 6C and 6D). The cells from the somital mesoderm, which now partially embrace the gut ventrally, will form the muscle and supporting framework of the hyoid arch. There is no difficulty *now* in distinguishing the head mesoderm proper from the undescended portion *moyenne*, with which it is, in many places, in close contact. The cells of the portion *moyenne* (like the endocardial cells ventral to the entodermal pharynx) are becoming plainly endothelial; they differ from the other cells of mesodermal origin in that the nuclei appear, in transverse sections of the embryo, to be small and rather flat and to be surrounded by a comparatively large amount of cytoplasm.

At a stage of 32 somites (Fig. 7, three hours later than the preceding stage¹⁶) the medial margins of the lateral plates have met and blended throughout the anterior three-fourths of the pericardial region except at one place foreshadowed in the preceding stage. The medial margins of the lateral plates, where they fail to

¹⁶In my stage of 30 somites (exactly intermediate in time between the stage of Fig. 6 and that of Fig. 7) the conditions in the heart-region are practically indistinguishable from those found at 32 somites (Fig. 7); the later stage has been used for reconstruction on account of its superior preservation.

blend, enclose a circular area which contains the cells about to form the root of the aorta (see Fig. 7D). Where blending has occurred (Figs. 7B and 7C) continuity is established between the somatic layers of the right and left lateral plates; the splanchnic mesoderm of the two sides becomes continuous across the mid-line in a similar manner. In the process of blending the coelom becomes continuous across the mid-line by the apparent loss of the medial margin of each lateral plate; the medial margins together constitute the *dorsal mesocardium* which is, thus, early lost. The entire coelom, paired or unpaired, occurring in Fig. 7 is pericardial. The site of discharge of the jugular veins (which later determines the points of separation, on either side of the embryo, between the pericardial and peritoneal regions of the original coelom) will occur slightly behind the site of Fig. 7G.

The endocardium has undergone very rapid growth backwards (see Fig. 7G), and has now reached the anterior limit of the first body somite. The interval between the first body somite and the head is some little distance behind (caudad from) the posterior limit of the reconstruction and the future site of discharge of the jugular veins. The ventral surface of the entodermal pharynx and of the adjacent region of the (peritoneal) splanchnic mesoderm is, therefore, in the head region posterior to the reconstruction, covered ventrally by the endocardium. As far as has been ascertained, the endocardium does not encroach upon the region ventral to the first body somite itself; a re-investigation of this difficult point will form a part of a study of the origin of the body-vessels to be undertaken at a later date.

The descent area of the *portion moyenne* has moved slightly backwards since the preceding stage; it has narrowed considerably (in the antero-posterior dimension), and now consists of only a narrow cord of cells on each side. The term "descent area" which has hitherto been used to designate cellular connection between the endocardium ventral to the lateral plates and the *portion moyenne* dorsal to them is no longer applicable, for *descent has ceased*; the cells between the entodermal pharynx and lateral plate (seen on each side in Fig. 7D) represent the first (transverse) part of the ventral aorta, and the *portion moyenne*, as such, has ceased to exist.

The entodermal pharynx is now closed throughout, forming a flat tube with a horizontal (virtual) lumen; its ventral surface scarcely appears in Fig. 7 since this is almost entirely hidden by the peri- and endocardium.

The heart anlage is now complete, and, although it is quite flat, its component parts can be (by comparison with later stages) already recognized. If an isosceles triangle be described, the base of which corresponds to a straight line connecting the two crosses near the top of Fig. 7, and whose, truncated, apex skirts rather closely round the (red) circle which embraces the aortic root, the area of splanchnic

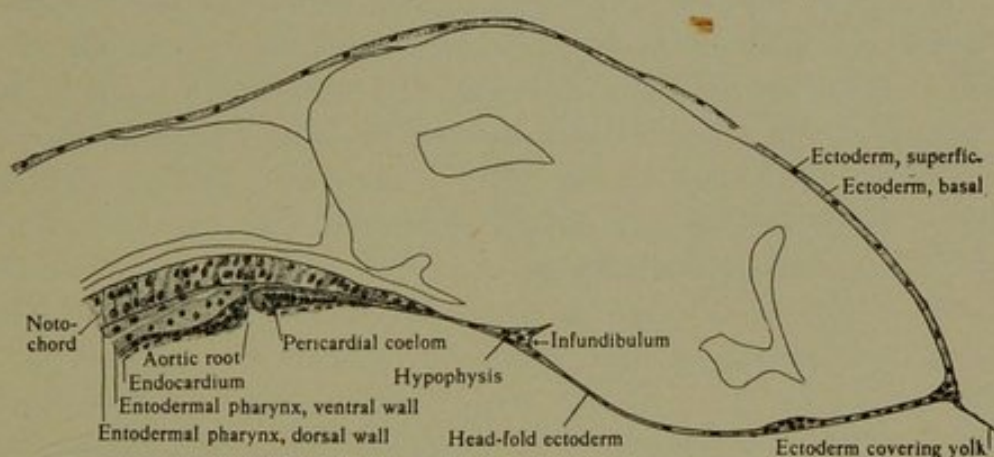


FIG. 8.—Mid-sagittal section through the head of shad, 30-31 somites $\times 100$ diams.

Owing to slight obliquity the section passes through the brain mainly to the right of the ventricular cavity. Fixation by Virchow's method.

mesoderm contained within the triangle will represent conus, ventricle and atrium (in the order named, from behind forwards). The (pericardial) splanchnic mesoderm not included in the triangle will form the anterior wall of the sinus venosus and of the pericardio-peritoneal septum. All the somatic mesoderm anterior to the (future) site of discharge of the jugular veins will become parietal pericardium. The heart anlage in mid-sagittal section is shown in Fig. 8. The embryo of Fig. 8 is slightly younger (30 to 31 somites) than that of Fig. 7 (32 somites).

Two points in regard to the descent of the endocardial cells have been brought out by the use of the plastic method of reconstruction

which, it appears from the literature, have previously escaped notice. Firstly: The endocardial cells do not descend in a hap-hazard fashion; descent proceeds, in a perfectly orderly manner, continuously from before backwards; the last cells to descend (*i. e.*, those in the region of the future aorta) are arrested, as it were, in the act of descent to form the first part of the central aorta. Secondly: In the region posterior to the aortic root descent of endocardial cells does not occur at all; the endocardium in this region is exclusively furnished by cells descending in, and derived from, the region anterior to the root of the aorta.

The part of the portion moyenne, which at 15 somites was easily recognizable in the region behind the (future) aortic root, extending back as far as the middle of the otocyst(see Figs. 3E and 3F), has disappeared long before the completion of the heart anlage, apparently by blending with the adjacent somital mesoderm. It is still recognizable at 18 somites (see right side of Fig. 4E), but posterior to this it has already disappeared or is very indefinite, see left side of Fig. 4E (through their hyo-branchial anlage). At 22 somites the portion moyenne, in this region, has entirely disappeared.

Review of the evidence bearing on the relation of the endocardium to the vascular endothelium of the head in general.

The development of the pericardial cœlom, including in this term the future myo-epicardium, may be looked upon as a subject practically complete in itself which can be considered independently of that of other structures. The case of the endocardium is entirely different; the fact alone that part (at least) of the ventral aorta arises, in common with the endocardium, from the portion moyenne is quite sufficient to indicate that the endocardium cannot be considered as an independent structure. It is only reasonable to suppose that the origin of the aorta is essentially similar throughout the head, so that separation of the aorta from the endocardium, in this connection, would be artificial and, therefore, not conducive to a clear conception of the origin and relations of the latter. During the study of the process of formation of the heart anlage several facts bearing on the origin of the vascular endothelium became

evident; mention of these, since they had no direct bearing on the subject in hand, has, hitherto, been omitted. In the following notes the facts alluded to are briefly reviewed, and an attempt made to estimate their significance.

The portion of the head shown in Fig. 7 may be divided transversely into three regions: anterior, middle and posterior; of these the middle region extends from the back of the red circle, indicating the aortic root, back to the place at which the entodermal pharynx becomes narrow behind the hyo-branchial anlage; the anterior region corresponds to the pericardial area in front of the middle region, and the posterior to the corresponding area behind it.

In the embryo of 15 somites, from which sections are shown in Fig. 3, the cells immediately adjacent to the medial borders of the lateral plates are isolated from the remainder of the mesoderm to form the *portion moyenne* of Swaen and Brachet; this isolation of the *portion moyenne* occurs only in the anterior and middle regions (comparing the stages of 15 and 32 somites approximately) and not in the posterior. The endocardium, as seen in Fig. 7, is derived exclusively from the *portion moyenne* of the anterior region. Having recalled these points regarding the *portion moyenne* and endocardium, the occurrences bearing on the vascular endothelium belonging to the three regions mentioned will, as far as possible, be considered separately.

The anterior region may be examined first. In Fig. 7D the lateral, now pericardial, plate is not in contact with the ventral surface of the entodermal pharynx, as is the case elsewhere (save in the two neighboring sections), contact being prevented by a cord of cells on either side. The cells of these cords were derived from the *portion moyenne*, and, like other cells known to come from the same source, they are at this stage easily distinguishable from the ordinary mesoderm.

Fig. 9 is from a reconstruction of the aorta at the earliest stage in which it appears as a well defined vessel throughout the head (42 somites; the ventral surface of this reconstruction is shown as Fig. 12). The cells between the entodermal pharynx and pericardial plate in Fig. 7D correspond exactly in position with the first (or transverse) part of the ventral aorta.

In Fig. 7C the entodermal pharynx and pericardium are in contact, and here cells descending from the portion *moyenne* have been arrested *lateral to the line of contact* between the two; reference to Fig. 9 will show that these cells correspond in position to the second (or oblique) part of the ventral aorta. This is not all; in Fig. 7C (also 7B and 7D) just dorsal to the entodermal pharynx there are one or two cells on either side which differ markedly from the surrounding mesodermal cells; the cells in question (which can be dis-

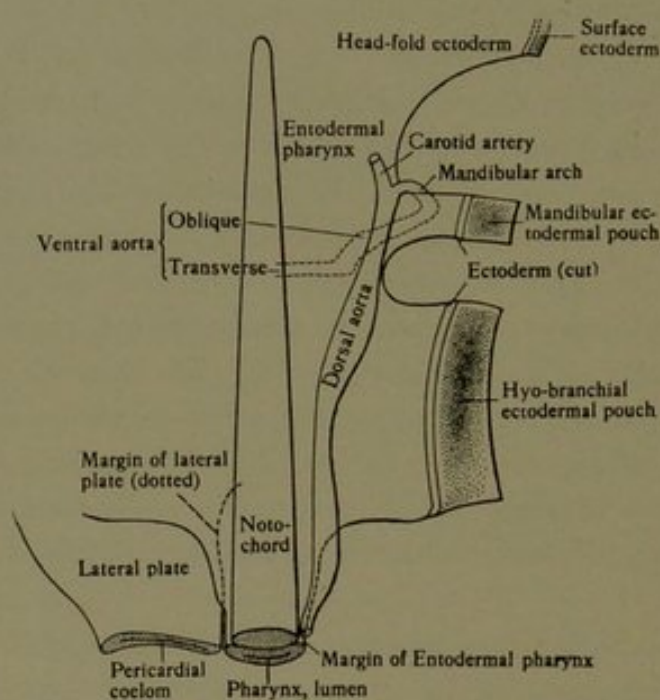


FIG. 9.—Diagram showing the course taken by the right aorta throughout the pericardial region of shad, stage 42 somites $\times 100$ diams.

The dorsal surface of the entodermal pharynx is shown on the right side, and the dorsal surface of part of the lateral plate on the left.

tinguished in practically every section used in the reconstruction) are found (see Fig. 9) to invariably occupy the line of the dorsal aorta and are, without doubt, aortic endothelium. It is hoped that the position of the aortic cells has been sufficiently indicated in the figures referred to; there are rarely more than two dorsal aortic cells on each side of a section, the differentiation of these will be illustrated in a future study of the aorta itself.

The aortic cells do not appear suddenly. At 26 somites (Figs. 6B, 6D, etc.) a break is apparent, in most sections, in the line of the future dorsal aorta, (the breaks together forming a tunnel) the cells about the break have small nuclei and a large amount of cytoplasm; at 32 somites the aortic cells are not only clearly endothelial but tend to line the tunnel. In the light of later stages the break referred to can be distinguished in many sections at 22 somites (Fig. 5), but it is questionable whether the cells about it have undergone much differentiation. The differentiation of the cells of the dorsal aorta thus goes hand-in-hand with that of the cells known to be derived from the portion *moyenne*, and the dorsal aorta is recognizable before the ventral aortic cells have taken up their definitive position. Finally, the history of the carotid artery is similar to that of the dorsal aorta, and, at 32 somites, the cells of the ventral and dorsal aorta are continuous around the front of the mandibular pouch through the mandibular aortic arch.

This entire chain of circumstances suggests very strongly that the dorsal aorta and carotid of the anterior region, like the ventral aorta and endocardium, arise from the portion *moyenne*.

In the middle region (of the three before mentioned) there is also isolation of the cells adjacent to the medial borders of the lateral plates to form the portion *moyenne*. Soon after the portion *moyenne* (which is here less bulky than in the anterior region) ceases to be recognizable the differentiation of the cells about to form the dorsal aorta can be followed in exactly the same manner as in the anterior region. Here are two sets of facts, the disappearance of the portion *moyenne*, and the gradual differentiation of the dorsal aorta nearer to the mid-line; they are, of course, not necessarily dependent on one another, but considered in connection with the circumstances in the anterior region they may be looked upon as suggestive.

By reference to Fig. 9 it will be seen that the dorsal aorta in the anterior and middle regions is always situate *medial* to the line of the lateral margin of the entodermal pharynx (usually very much so). Figs. 3B, 3C, 3E and 3F show that the portion *moyenne* is always in a position lateral to the above mentioned line. From these two facts it follows that although the conditions in the anterior

and middle regions suggest the origin of the dorsal aorta from the portion moyenne, any attempt to prove this supposition is met with the difficulty that the aortic cells are known to differentiate *in situ*. To trace a few undifferentiated aortic cells in their migration through mesodermal cells which they closely resemble would scarcely be possible by ordinary embryological methods.

In the posterior region the cells adjacent to the medial borders of the lateral plates are not isolated, as in the anterior and middle regions, to form portion moyenne. Fig. 9 shows that the aorta in the posterior region is situate immediately dorsal to the lateral margin of the entodermal pharynx, and, in the posterior part of this region, just dorsal to the medial margin of the lateral plate as well (sections from this part of the posterior region are shown in Figs. 3G and 5G; 7G is slightly anterior to it). The aorta is thus placed exactly in the line of the mesodermal cells adjacent to the medial borders of the lateral plates *apparently arises directly from them*.

In the foregoing notes an attempt has been made to bring together some evidence bearing on the development of the vascular endothelium of the head in order to arrive at a conception of the nature and relations of the endocardium. The evidence in question appears to justify the statement that the endocardium in shad arises from mesodermal cells which are found, after differentiation of the lateral plates, to be placed in the region bordering on the medial margins of the latter; further, that the mesodermal cells in this situation appear to be given up exclusively to the formation of vascular endothelial cells of which the endocardium only forms a part.

A few words may be added by way of re-examination of the "Portion moyenne du mésoblaste" of Swaen and Brachet. In the anterior pericardial region the cells which later form the endocardium must necessarily be separated from the somital portion of the mesoderm, for the former eventually take their place as endocardium ventral to the lateral plates, while the latter retains its position dorsal to them. In the middle and posterior pericardial regions, separation of the cells bordering on the lateral plates is not a necessity, for neither region produces endocardium. Nevertheless, separation occurs in the middle region but not in the posterior; the dif-

ference in behaviour of the cells in question does not necessarily depend on the nature of the cells themselves, but appears to be due to conditions occurring in the middle region which are not found in the posterior. It may be said that the portion *moyenne* appears to consist in the main of cells which will later form vascular endothelium, but it is difficult or impossible to show that in the anterior region it includes all of these, or that in the middle region it does not include more. In other words, although the portion *moyenne* forms a well defined group of cells which is of great assistance in following the movements of the endocardium, it does not appear, in itself, to be a structure of real morphological importance.

Greil, in his recent paper on the origin of the blood and blood-vessels, '08, traces the origin of the endocardium in *Ceratodus*, and in some amphibia and selachii, from two sources which he distinguishes as the Angioscleroblast and Angiohæmoblast. Greil does not refer to teleosts in this connection, but his statement gives additional interest to the description by Boeke, '03, of a two-fold origin of the endocardium in *Muraena*. Boeke describes the major part of the endocardium as arising from cells developed in the head-region, but traces the origin of some of the cells lining the venous end of the heart from the region of the closing blastopore (the latter cells would seem to correspond to those described by Greil as emanating from the hæmangioblast). I have looked carefully for cells corresponding to those described by Boeke as arising from the region of the blastopore without result, and believe that any cells which may migrate forward from this region in shad must be arrested posterior to the junction of the first body somite with the head.

It is intended to re-investigate the origin of the aorta in the head, and to look for additional evidence regarding the origin of the jugular veins, and afterward to study the origin of the vascular endothelium of the body vessels.

PERIOD 2. LASTING UNTIL RHYTHMICAL CONTRACTION BEGINS
IN THE PARTIALLY FORMED HEART-TUBE.

Between the stages of 30 and 32 somites, the heart anlage has undergone little change; at 33 somites the beginning of progress

becomes apparent. In mapping out the heart anlage (see page 231) it has been shown that the splanchnic mesoderm contained within a triangle drawn with its apex at the aortic region and its base near the anterior end of the pericardial plate would correspond to the future conus ventriculi and atrium. By referring to Figs. 7B, 7C and 7D it will be seen that the splanchnic mesothelial cells included in this triangle are columnar in shape, while those lateral to the triangle are much flatter. Passing forward from the region 7B, the cells within the triangular area diminish in height until they become cubical, and finally, near the anterior limit of the pericardial plate, quite flat.

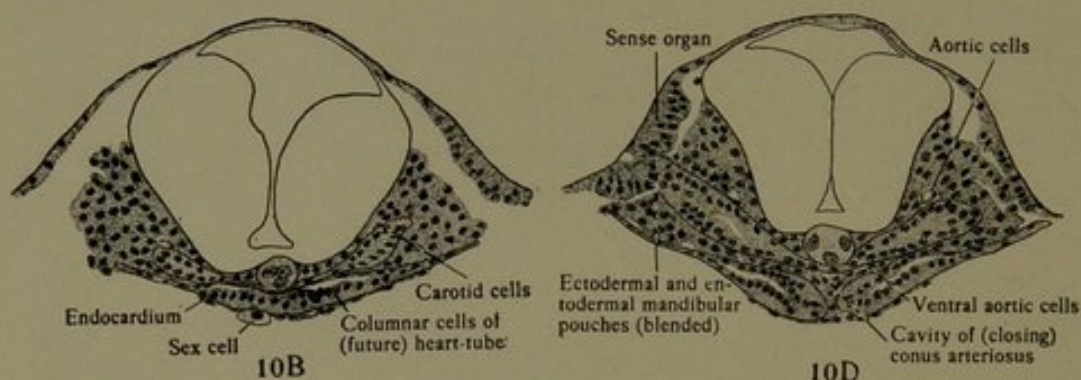


FIG. 10.—Two transverse sections of shad, stage of 34-35 somites $\times 100$ diams. 10B passes through the mid-sagittal plane of the embryo anterior to the mandibular pouch, 10D midway between the mandibular and hyoid pouches. The left side of the figures represents a region anterior to that seen on the right. Fixation by Pereny's method.

The splanchnic mesoderm included within the triangular area is soon to form a tube, which may provisionally be called the heart-tube; the columnar mesothelial cells will form the myo-epicardium.

Before formation of the heart-tube begins, the triangular area spoken of undergoes a migration toward the left, carrying with it the underlying endocardium; this is the movement which begins at 33 somites. The sinistral movement of the "columnar area" of the splanchnic mesoderm and of the endocardium, which begins at 33 somites, is very pronounced an hour later at the stage of 34-35 somites from which Fig. 10B is taken. Reference to Fig. 10D shows that in the aortic region there has been no sinistral movement; this

movement takes place around the aorta as a center, and becomes progressively greater as the base of the triangle referred to is reached. In the region of Fig. 10B (anterior to the mandibular pouch) the columnar area of the splanchnic mesoderm, being about two-thirds to the left of the mid-line, has accomplished its migration. The movement of the columnar area of the splanchnic mesoderm is not quite simple; it is accompanied, and partly brought about, by a slighter movement to the left of the entire pericardial plate (see Fig. 10B). The slighter sinistral movement of the entire pericardial plate began as early as the stage of 32 somites (see Fig. 7B).

In Fig. 10D the initiation of heart-tube formation can be recognized. Union is about to take place between the splanchnic mesoderm in the region of the original medial margins of the right and left lateral plates. This union takes place around the lateral and posterior circumference of the aortic root. The splanchnic mesoderm to the right and left of the aortic root is seen in Fig. 10 D to be somewhat prominent ventrally, so that, by uniting in the mid-line (which it is just about to do), it will enclose a small chamber, the conus arteriosus; the latter contains a small amount of endocardium directly continuous with the endothelium of the aorta.

At a stage of 36 somites (see Fig. 11, one hour older than the preceding stage) the formation of the heart-tube has made considerable progress. The arterial extremity of the heart-tube has been formed by the blending of the splanchnic mesoderm about the circumference of the aortic root symmetrically in the mid-line. The remainder of the heart-tube is also undergoing formation by the blending of splanchnic mesoderm on either side. The axis about which the further blending of splanchnic mesoderm occurs corresponds to a straight line connecting the middle of the base of the triangle spoken of with its apex. The apex of the triangle corresponds in position with the aortic root; the middle of the base is now placed (owing to rotation of the triangle to the left around its apex) quite near to the left side of the anterior margin of the pericardial plate. The axis, then, along which union of the splanchnic mesoderm is proceeding, extends from the (medially placed) aortic region forward; as it passes forward it diverges to the left so as to form an acute angle with the mid-sagittal plane of the embryo.

In the formation of the heart-tube anterior to the aortic region, the splanchnic mesoderm of the right side undergoes active movement, while that of the left remains comparatively passive. The splanchnic mesoderm to the right of the heart-tube axis arises abruptly from the somatic layer to form a crest which moves over to the left; this crest becomes imminent and falls to the left somewhat in the manner of a wave breaking upon the shore (see Fig. 11C). The splanchnic mesoderm to the left of the axis rises slightly to meet the splanchnic mesoderm from the other side as the latter falls; between the two a tube of splanchnic mesoderm is formed of which the ventral wall is derived mainly from the right side, and the dorsal wall mainly from the left.

Fig. 11 shows the heart-tube in process of formation, as indicated by a reconstruction of the stage of 36 somites; contact of the two sides has occurred at the posterior (arterial) end. The splanchnic mesoderm on each side (right particularly), for some little distance anterior to the contact area, shows evidence of preparation for bending in the manner described above (Fig. 11C). Heart-tube formation is now in rapid progress; the posterior (arterial) end now being complete, the venous end will be progressively formed, from behind forward, along an axis deviating to the left.

The irregular black line in Fig. 11 indicates the outline of the endocardium; a small quantity of the latter has been included in the heart-tube.

The heart shown in Fig. 11 (such as it is) is contracting rhythmically, and has been doing so for some 10 or 15 minutes. The heart which was quiescent at the stage of 35 somites began beating (after very little preliminary oscillation) at a rate of 52 beats per minute, about 15 minutes before the thirty-sixth somite was completely marked off.¹⁷

It may be questioned whether the rhythmical contraction of the

¹⁷Water temperature 62° F. (July 11, 1907). Some evidence has been obtained which suggests that in higher water temperatures the heart begins to beat at an earlier stage of development (as estimated by the number of somites). In order to exclude the possibility of a miscount of somites the data on which this evidence rests require to be controlled by comparison with the results of further observations, preferably made on eggs of another species.

heart anlage is of assistance in the folding over of the splanchnic mesoderm to form a tube; that it is not essential to this process is

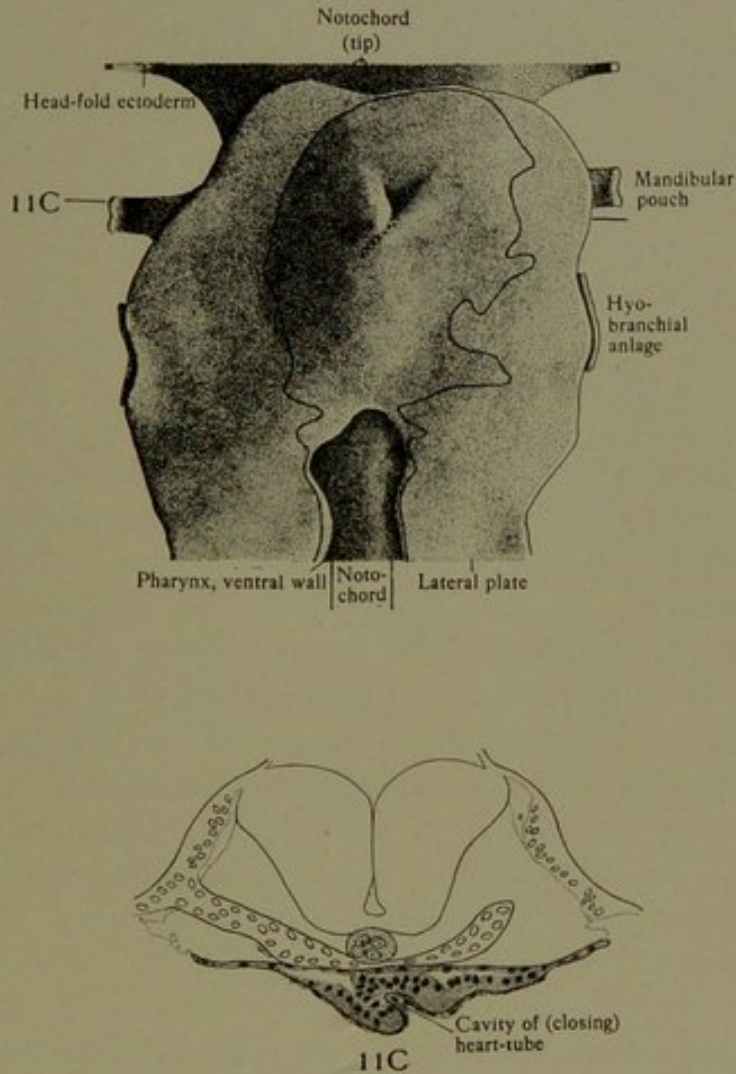


FIG. 11.—Ventral surface of a reconstruction of the pericardial region of shad, stage of 36 somites $\times 100$ diams.

The heart-tube is in process of formation, contact of the two sides has occurred along the broken line. The continuous black line encloses the area covered by endocardium. Fixation by Pereny's method.

indicated by the fact that a stage of 35 somites (half an hour earlier) the folding over has already commenced, although it is not so advanced as at 36 somites.

PERIOD 3. IN WHICH THE HEART-TUBE IS COMPLETED TO FORM
CONUS, VENTRICLE AND ATRIUM, AND ASSUMES THE
ADULT POSITION.

Stage of 42 somites.

Fig. 12 is from a reconstruction of the ventral surface of the pericardial region of an embryo of 42 somites. The ventral wall of the pericardial coelom is very thin and to some extent moulded upon the heart-tube, so that the outlines of the latter are clearly indicated. A portion of the splanchnic mesoderm forming the ventral wall of the pericardial coelom has been removed over an area mainly to the right of the mid-line. In the area referred to a portion of the heart wall and of the somatic mesoderm are seen near the mid-line. Toward the lateral region of this area the somatic mesoderm and some of the mesoderm of the pharyngeal floor are represented as having been removed in order to uncover the ventral aspect of the entodermal pharynx and of the ventral aorta.

The heart-tube, which has only just been completed, is cone-shaped; the (venous) base of the cone is directed anteriorly and to the left. There is, as yet no external indication of separation into conus, ventricle and atrium, but the wall becomes progressively thinner in passing from the arterial to the venous end.

The splanchnic mesoderm, not included in the heart-tube, forms the ventral wall of the pericardial coelom; it becomes continuous with the wall of the heart-tube at the venous end of the latter. In closing, the two sides of the heart-tube do not appear to blend where the splanchnic mesoderm first meets; some further adjustment occurs in order to bring the columnar area of the latter (and the endocardium in contact with this) within the limits of the tube. Completion of the heart-tube is effected by the blending of splanchnic mesoderm of the right and left sides, and continuity of the ventral wall of the pericardial coelom is maintained by a similar process; the two processes together entail loss of the ventral mesocardium.

Comparison of Figs. 11 and 12 shows there is a large amount of endocardium not included in the heart-tube; also that, after closure of the latter, the endocardium tends to move over to the left side.

Before passing from the stage of 42 somites to that of 6.2 mm. (the next one to be examined) it will be necessary to make a digres-

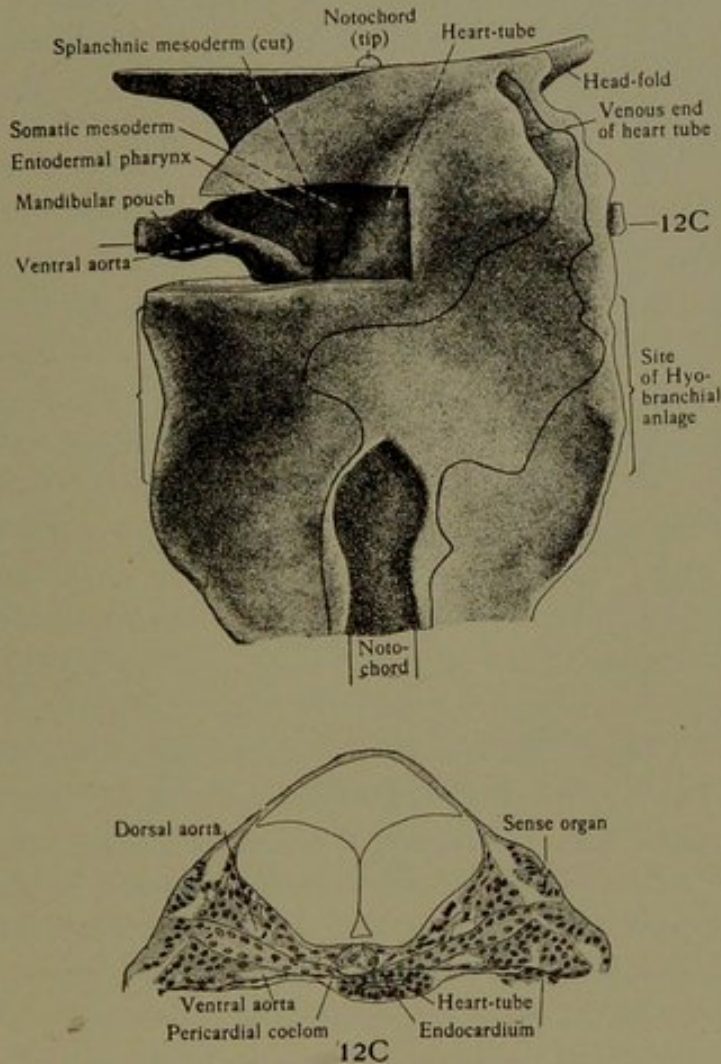


FIG. 12.—Ventral surface of a reconstruction of the pericardial region of shad, stage of 42 somites $\times 100$ diams.

Over an area mainly to the right of the mid-line the ventral wall of the pericardial coelom has been removed to show heart-tube and somatopleure; also, laterally (where somatopleure has been removed), the pharynx and ventral aorta. The continuous black line encloses the area covered by endocardium.

sion; firstly, to correlate the stages as estimated by the number of somites with those designated by the length in millimeters; secondly, to mention some points in connection with the general circulation.

Correlation of stages.

The embryo becomes sufficiently straight to yield a satisfactory end-to-end measurement when it has about 51 somites; its length is then 3.6 mm. From this time on the stages are designated by the length in millimeters; it may be mentioned that the last somite (59th or 60th) is formed at the stage of 5.2 mm.

Mechanism of the circulation at different stages of development.

Prior to the stage of 42 somites there has been no true circulation, because the aorta opposite the first three somites, late in being formed, is still wanting. At 42 somites the aorta is practically complete as far back as the anus; posteriorly it bifurcates, and the two vessels form a loop encircling the gut and join the subintestinal vein. The blood plasma (for there are no corpuscles) flows from the heart into the aorta, turns forward near the anus, into the subintestinal vein, which, in turn, discharges it on to the posterior pole of the yolk. The plasma flows ventral and lateral to the yolk in a wide channel between the ectoderm and the yolk-periblast and, following the contour of the yolk, enters the venous end of the heart-tube. The plasma is not in contact with the periblast ventral to the peritoneum, for it is excluded from this situation by the attachment of the lateral margins of the latter to the yolk; as far as can be determined the periblast elsewhere is bathed in plasma. The caudal aorta and vein are as yet unformed, but a cord of cells ventral to the notochord in the tail represents these vessels together with the blood-anlage. The jugular and cardinal veins are not yet developed; there is no liver.

The jugular veins have reached completion at a stage of 4.4 mm., and discharge their blood upon the yolk immediately posterior to the vagus ganglia. It may be mentioned that the endothelial cells of the jugular veins have been recognizable for some time prior to the complete formation of the veins themselves, certainly as early as the stage of 42 somites. The place of termination of the jugular veins is a point of great interest, for here the coelom is separated into its pericardial and its peritoneal portions. The orifices of discharge of the jugular veins are placed laterally to the lateral

plates in a situation approximately corresponding to the lower end of Fig. 12. The cœlom anterior to the orifices become pericardial; posterior to them it becomes peritoneal; the cœlom between the two orifices ceases to exist.¹⁸ Since separation of the pericardial from the peritoneal portion of the cœlom occurs at a situation in which the lateral plates are still separate, the posterior end of the pericardium extends farther backward on each side than in the mid-line.

At the stage of 6.2 mm. the caudal aorta and caudal vein replace the anterior end of the cord of cells found in the tail at 42 somites, and the blood contains a very few corpuscles. Blood passes from the dorsal aorta through the short caudal aorta and then forward through the caudal vein. The caudal vein meets the cardinals¹⁹ near the anus, and from the point of junction two veins pass ventrad (embracing the gut) to join the subintestinal vein; through these two veins most (or all) of the blood from the caudal vein enters the subintestinal.

The subintestinal vein is now involved in the rapidly growing liver; its extreme anterior end (*vena revehens* of liver) is free, and discharges its blood ventral to the peritoneum. Blood is now retained in the space ventral to the peritoneum (supravittelline sinus) by the very agency which formerly prevented its flowing there, *i. e.*, by the lateral attachment of the peritoneum to yolk. At the site of discharge of the jugular veins the blood from these meets that flowing from the supravittelline sinus; and the blood from these two sources enters the venous end of the heart. There appears to be no special mechanism for retaining the blood in the space between the ventral wall of the pericardial cœlom and the yolk, for the pericardial plate is attached peripherally to the ectoderm and not to yolk.

The condition of *the circulation in the stage described in the introduction* differs from that in the stage just described in that the cardinal veins are fully formed. The blood from the, now practi-

¹⁸Conditions at the site of discharge of the jugular veins are much complicated by the fact that the mesenchyme of the pectoral fins is arising from the somatic mesoderm in this region. A more thorough study will be undertaken later in connection with the veins themselves.

¹⁹The cardinal veins, at this stage extend only from the anus as far as the anterior end of the liver. I am unable to determine their functions in connection with the general circulation.

cally complete, caudal vein is received entirely by the cardinals. The subintestinal vein has lost all connection with the caudal and the cardinals, and is now the portal vein.

Stage of 6.2 mm.

Fig. 13 represents the ventral surface of a reconstruction of the pericardial region of an embryo of 6.2 mm.; the reconstruction extends further forward than those shown in the previous figures; it, in fact, includes the whole of the anterior part of the head which remains at this time in contact with the yolk. The ventral wall of the pericardial coelom has been partially removed to show the condition of the heart-tube. The conus, ventricle and atrium are now quite distinct, but there is no prominence on the ventricle corresponding to its future apex. There is evidence, at this stage, that the ventral wall of the pericardial coelom is attached, rather extensively, to the yolk just to the right of the venous orifice of the atrium. Over the area of attachment no endocardium is present; the exact distribution of endocardium over the remainder of the ventral wall of the pericardial coelom is difficult to make out owing to the extreme tenuity of the latter.

The venous end of the atrium has moved forward and is now placed ventral to the posterior half of the left eye (it was altogether posterior to the eye in the preceding stage); the anterior end of the pericardial plate has moved forward even more than the atrium and now *appears* to have reached the anterior limit of the head-fold. There are several changes going on, however, which tend to complicate matters by shifting former landmarks; these changes can be partially appreciated by reference to Figs. 13 and 14. In the first place the head is rising from the yolk: this is accomplished by forward growth of the head, by shrinking of the yolk, and by a horizontal separation of the head-fold into its original two layers. The dorsal and ventral layers of the head-fold are now being added to the surface ectoderm (basal layer) of the continuous regions of head and yolk respectively. In the second place the, now separating, head-fold is moving bodily backwards so that it approaches the mandibular pouch; the latter shows evidence of antero-posterior com-

pression and is, in its turn, crowded back so as to approach the hyomandibular anlage. The backward movement of the above mentioned structures produces an effect of advance in the tip of the notochord, this is relative only and not actual.

Having found that the heart-tube represents conus, ventricle and atrium, it remains to be seen how the different parts of the wall of the pericardial cœlom attain their definitive positions; this can be most conveniently studied from the left side.

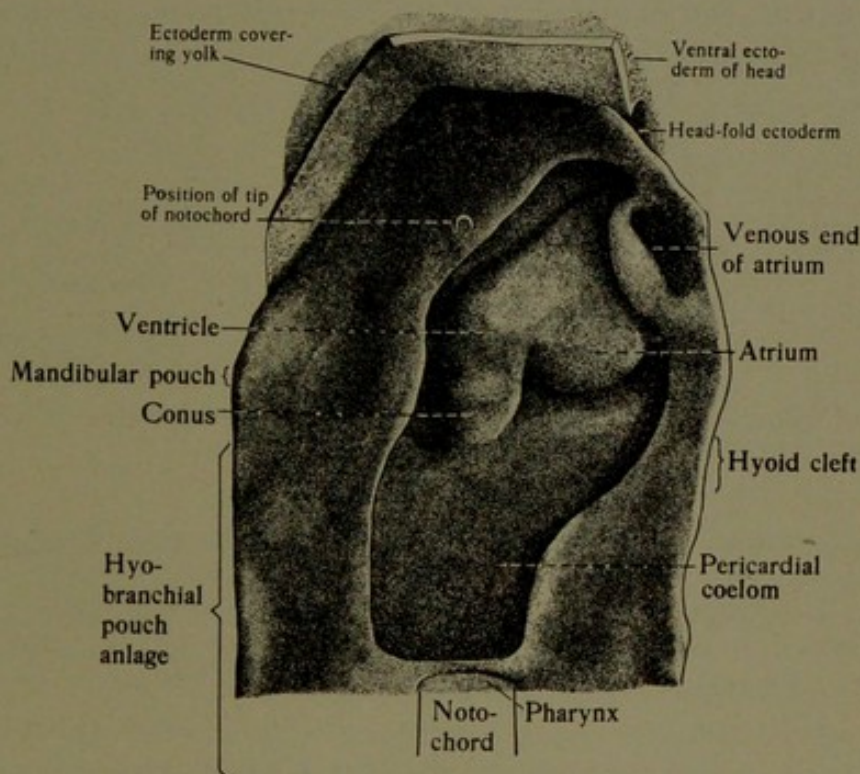


FIG. 13.—Ventral surface of a reconstruction of the pericardial region of shad, stage of 6.2 mm. $\times 100$ diams.

Part of the ventral wall of the pericardial cœlom has been removed to uncover the conus, ventricle, and atrium.

To make a satisfactory drawing of the heart at this, and the following, stage, from the left side would be very difficult; a complete view of the organ is obstructed partly by the dorsal and partly by the ventral wall of the pericardial cœlom. The parts considered essential to the elucidation of the changes about to occur are, therefore, shown diagrammatically and the less important parts eliminated altogether.

In Figs. 14 and 15 the heart has been almost entirely omitted, the position of the venous orifice of the atrium (as it lies near the surface of the yolk) is indicated by a broken line; the other structures shown are indicated as they would appear in a mid-sagittal section of the embryo.

Fig. 14 is a diagram made from the reconstruction used for Fig. 13, the left side is represented. The relations of the head-fold ectoderm to the surface have been approximately determined by comparison with earlier stages, and are indicated in the diagram. The line of separation of head from yolk is indicated externally, in the

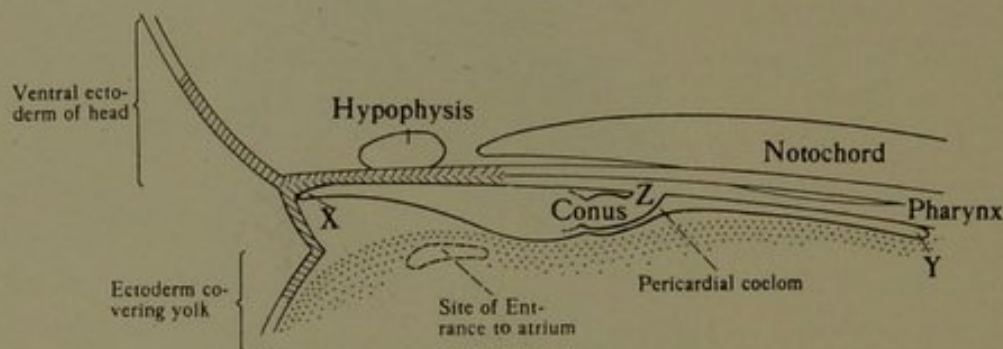


FIG. 14.—Diagram made from the reconstruction shown in Fig. 13 as seen from the left side, $\times 100$ diams.

The venous end of the atrium is indicated by a broken line; other structures are represented as cut in the mid-sagittal plane. Ectoderm belonging to the original head fold is shaded. The yolk is stippled.

embryo, by a U-shaped groove; the anterior part of this groove (corresponding to the cross-piece of the U) appears in section in Fig. 14. The anterior margin of the pericardial plate is attached to the head-fold ectoderm and is, at present, dorsal to the groove referred to (see Fig. 14); the lateral margins of the pericardial plate are attached to the surface ectoderm just ventral to the groove. The pericardial coelom is, as in previous stages, dorso-ventrally compressed; three points in its wall will require to be noticed in the following stages. The points referred to are: the anterior end, the posterior end and the aortic root (apex of conus arteriosus); these are indicated, respectively, by the letters X, Y and Z.

Stage of 7.3 mm.

Fig. 15 is a diagram, made in the same way as Fig. 14, from a reconstruction of the pericardial region of an embryo of 7.3 mm.

The head fold proper no longer exists, having been absorbed into the basal ectoderm of the surface. The pharynx is dilated, and the oral plate is soon to be perforated. Antero-posterior compression of the head is still more marked than in the preceding stage, and is accompanied by the formation of a head-bend of the mid-brain

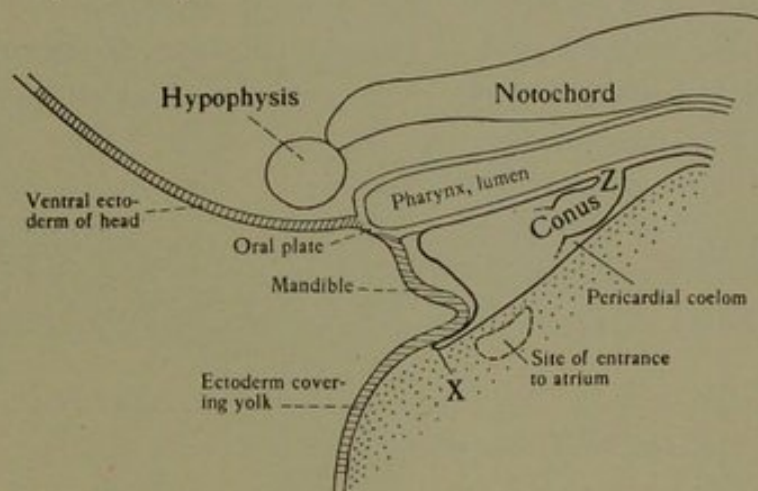


FIG. 15.—Diagram made from a reconstruction of the pericardial region of shad, stage of 7.3 mm. $\times 100$ diams.

The left side is shown, structures indicated as in the preceding figure.

and of a curve, in the ventral direction, of the anterior end of the notochord. There is an ectodermal band connecting the lateral margin of the anterior end of the entodermal pharynx, on either side, with the basal ectoderm of the surface. The ectodermal bands referred to represent the posterior margin of the original head-fold ectoderm, and have a developmental history similar to that of the head-fold proper (see footnote on page 227.) These ectodermal bands and the mandibular anlage (combined entodermal and ectodermal mandibular pouches) are now undergoing disintegration.

The heart differs from that of the preceding stage in that the apex of the ventricle is well-marked.

Fig. 15 shows (compare with Fig. 14) that the ventral layer of the head-fold, in completely separating from the dorsal layer, has

carried with it the (attached) anterior periphery of the pericardial cœlom. The point X has, therefore, moved in a ventral direction, and is now placed between ectoderm and yolk some distance below the head of the embryo. (The point Y, which has not altered its position, is not shown.) The groove between the head and yolk is now deep and, anteriorly, very narrow. The ectoderm bounding the groove impinges upon the dorsal wall of the pericardial cœlom and constricts it. Accompanying this constriction there is a diminution in the area of the ventral wall of the pericardial cœlom, and the venous orifice of the atrium is brought nearer to the mid-line. The, somewhat dome-shaped, part of the pericardial cœlom dorsal to the constriction (and, therefore, on the side of the groove towards the head) contains the conus and ventricle. The lateral periphery of the pericardial cœlom, together with the venous orifice of the atrium, is ventral to the constriction (and, therefore, on the side of the groove towards the yolk).

Stage of 8.75 mm., and a comparison of the heart with that of the stage described in the introduction.

Fig. 16 represents a reconstruction from an embryo of 8.75 mm. The reconstruction, which is shown from the left side, was made from sagittal sections. The last section (on the left side) passes through the yolk on a level with the left margin of the pericardial cœlom, and fails to complete the left wall of the pharynx.

In the preceding stage the groove between the head and yolk was narrow and nearly horizontal; the periphery only of the pericardial cœlom was ventral to it. The groove in question at this stage has become, by reason of the recession of yolk from head, oblique and much wider. Practically the entire pericardial cœlom is now on the yolk side of the main axis of the groove, and radical changes have taken place in the arrangement of its walls. The point X has moved so far back that the somatic mesoderm between X and Z has been stripped from the ventral wall of the pharynx and will form the ventral parietal pericardium (compare Figs. 16 and 17). The somatic mesoderm between the points Z and Y has remained stationary and will form the dorsal parietal pericardium. The

splanchnic mesoderm between the points X and Y, which has hitherto formed the ventral wall of the pericardial coelom, has undergone considerable contraction and is stouter than before; it will form the anterior wall of the sinus venosus (compare Figs. 16 and 17).

Between the stage of 8.75 mm. and that of 114 hours, noticed in the introduction (see Fig. 17), the heart is brought into the adult position; the arterial end is fixed (by the ventral aorta) to the floor of the pharynx, while the venous end follows the retreating yolk.

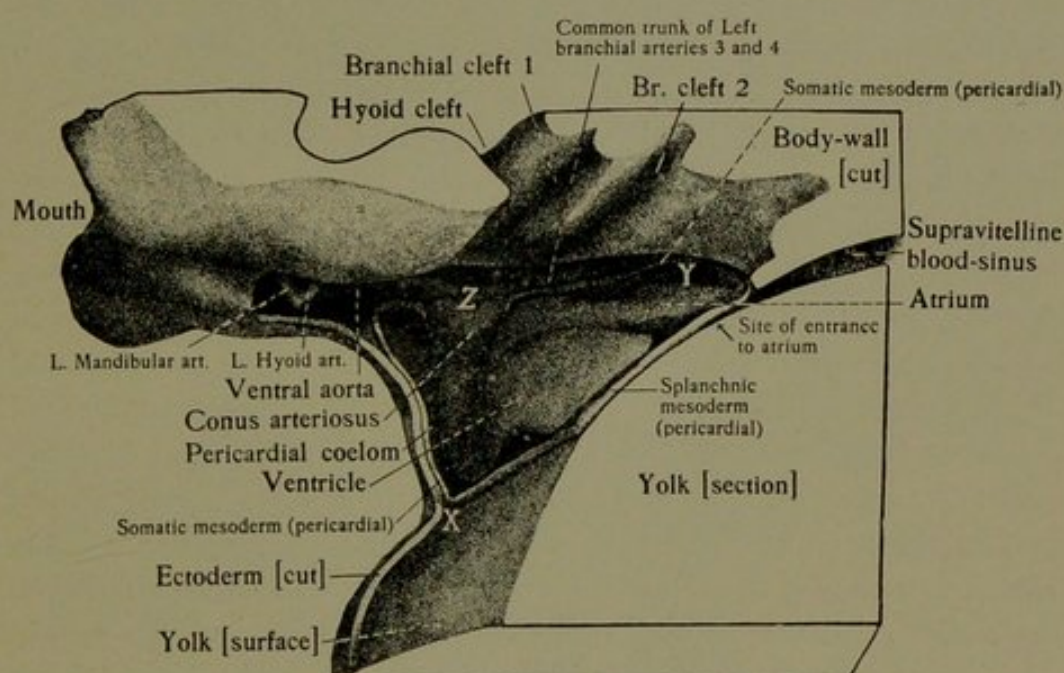


FIG. 16.—The left side of a reconstruction from the gill region of shad, stage of 8.75 mm., $\times 100$ diams.

The pericardial coelom and adjacent parts have been laid open to the left of the mid-sagittal plane.

The main axis of the ventricle (approximately transverse at 8.75 mm.) becomes longitudinal; the venous orifice of the atrium moves from left to right until it reaches the mid-sagittal plane of the embryo.

On comparing the parts of the pericardium and heart, as described in the introduction (Fig. 17), with those of the original heart anlage (Fig. 7), it will be seen that the entire parietal pericardium has been derived from somatic, and the myo-epicardium from splanchnic

mesoderm. Part of the original splanchnic mesoderm was employed in the formation of the tube which has become the conus, ventricle and atrium; the remainder (hitherto referred to as the ventral wall of the pericardial cœlom) is now about to take part in the formation of the sinus venosus.

PERIOD 4. FORMATION OF SINUS VENOSUS AND HEPATIC VEIN.

Fig. 17 is from an embryo 10.67 mm. in length, designated (for reasons already stated) the stage of 114 hours. The structures shown have been briefly mentioned in the introduction; it is now necessary to describe them more fully.

The portion of the reconstruction posterior to the venous orifice of the atrium may be divided into two regions by a vertical transverse plane passing through the orifices of discharge of the cardinal veins. The region anterior to the plane mentioned is the site of the future sinus venosus and may be called the sinus-venosus region; the region posterior to it corresponds to the anterior end of the supravittelline sinus and may be called the hepatic-vein region.

In the sinus-venosus region the peritoneal cœlom has extended forwards dorsal to the pericardial cœlom. The peritoneal cœlom is here small (and remains so) being practically confined to the region dorsal to the gut. The splanchnic mesoderm extending between the points X and Y is to form the anterior wall of the sinus venosus. The part of this immediately to the right of the venous orifice of the atrium is known to have been attached to the yolk since the stage of 6.2 mm. The anterior wall of the sinus venosus is now, at the site of attachment, drawn out into a long process referred to below as the "yolk-process" of the sinus venosus. The posterior surface of the anterior wall of the sinus venosus is in contact with (and possibly attached to) the yolk around its ventral and lateral periphery. Endocardium lines the posterior surface of the anterior wall of the sinus venosus where the latter is not in contact with yolk, and, at this stage, is beginning to migrate from the yolk process on to the yolk itself (see Fig. 18). Elsewhere the yolk is entirely destitute of endocardium (see Figs. 19, 20, 21 and 22). The yolk process, extending obliquely up-

ward from the anterior wall of the sinus venosus to a projection on the yolk (dorsal to the anterior pole of the latter) forms an

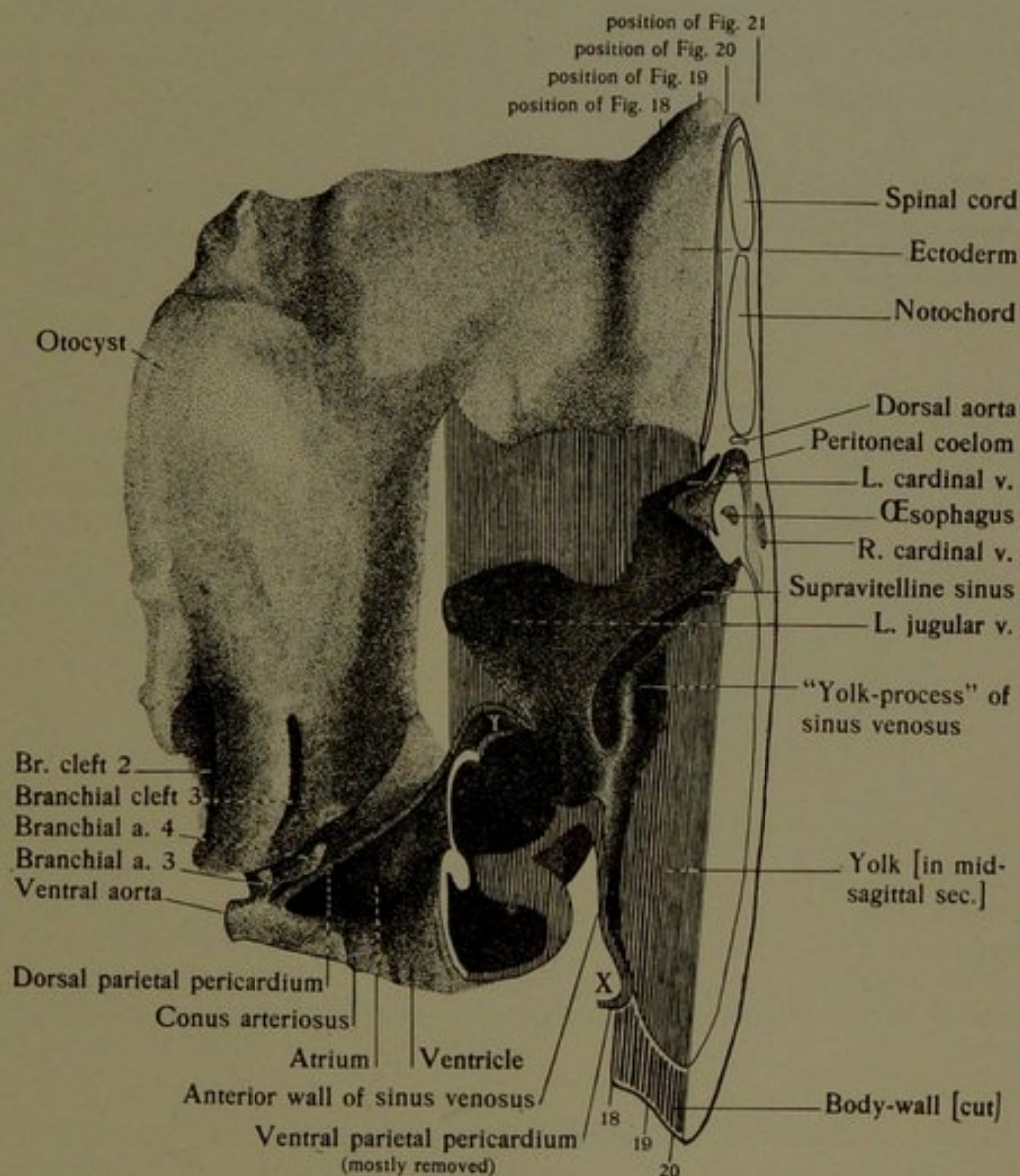


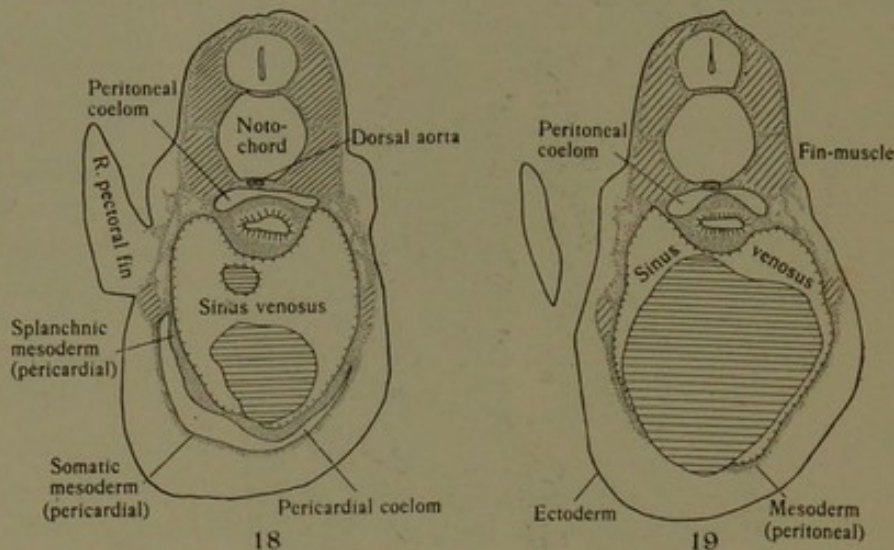
FIG. 17.—The left side of a reconstruction from the posterior gill region of a shad, stage of 114 hours. $\times 100$ diams.

Explanation as in Fig. 1. NOTE.—The warping of the posterior plates of this reconstruction (concavity forwards) was not noticed before drawing; otherwise it would have been rectified. The warping has to be taken into consideration in estimating the correct positions of Figs. 18, 19 and 20.

incomplete septum between the right and left sides of the sinus venosus. The two sides of the sinus venosus are in communica-

tion both anterior (dorsally) and posterior (ventrally) to the yolk process.

Conditions in the hepatic-vein region become more readily intelligible after preliminary examination of a section from 0.02 mm. posterior to the reconstructed part of the embryo. Here (Fig. 21) the peritoneal mesoderm (in which the coelom is to a large extent virtual) covers the yolk laterally and dorsally and is generally in contact with it. Contact between the yolk and peritoneum is interrupted on the left side by the interposition of



FIGS. 18 and 19.—Two transverse sections of which the positions are marked on Fig. 17. $\times 50$ diams.

Yolk horizontally shaded, voluntary muscle obliquely shaded. The distribution of vascular endothelium, including endocardium, is indicated by large dots.

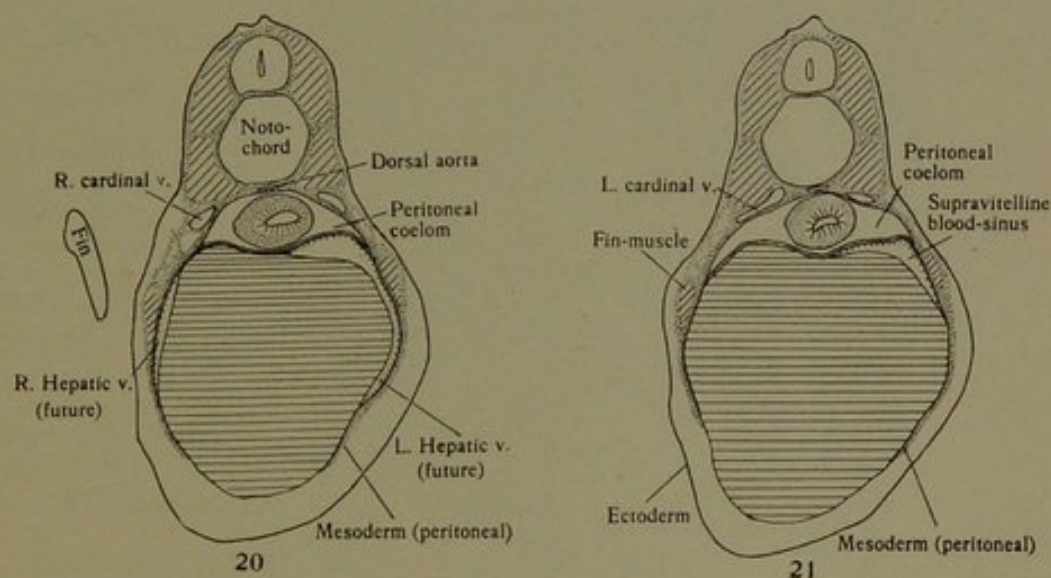
the supravittelline blood-sinus (see introduction). In passing tailward in the series from the position of Fig. 21, the yolk increases in size and the supravittelline sinus becomes more dorsally placed with regard to the yolk, but is always mainly to the left of the mid-sagittal plane.

In Fig. 20, from the hepatic-vein region of the reconstruction, contact between the peritoneum and yolk is interrupted on both right and left sides. The interruption on the right of the figure (left of embryo) corresponds to the future left hepatic vein and

is a direct continuation forward of the main supra-vitelline sinus. The interruption on the left side of the figure (right of embryo) corresponds to the future (smaller) right hepatic vein, which is not at present in direct communication with the supravitelline sinus. The future right hepatic vein communicates anteriorly with the future sinus and posteriorly ends blindly.

The dorsal mesentery in this stage, as in the adult, is absent in the region anterior to the stomach.

Fig. 22 is a left lateral view of a reconstruction of the posterior



FIGS. 20 and 21.—Two transverse sections of which the positions are marked on Fig. 17. $\times 50$ diams.

Yolk and voluntary muscle shaded and vascular endothelium dotted as in the preceding figure.

gill region of a shad of 166 hours (9.46 mm. in length). At this stage the peritoneal coelom in the hepatic-vein region is so large that it can be opened by merely removing the body-wall; the anterior pole of the yolk is shown *in situ*. The plane of separation between sinus-venosus and hepatic-vein regions is indicated, as before, by the point of discharge of the left cardinal vein.

It will be convenient to consider the hepatic-vein region first. In this region the peritoneal coelom has undergone great expansion by reason of the extensive separation of splanchnic from somatic meso-

derm (compare Figs. 20 and 24). The shrinkage of the yolk has had an effect on the shape of the future hepatic veins; the latter are now semilunar rather than crescentic in section. The ventral

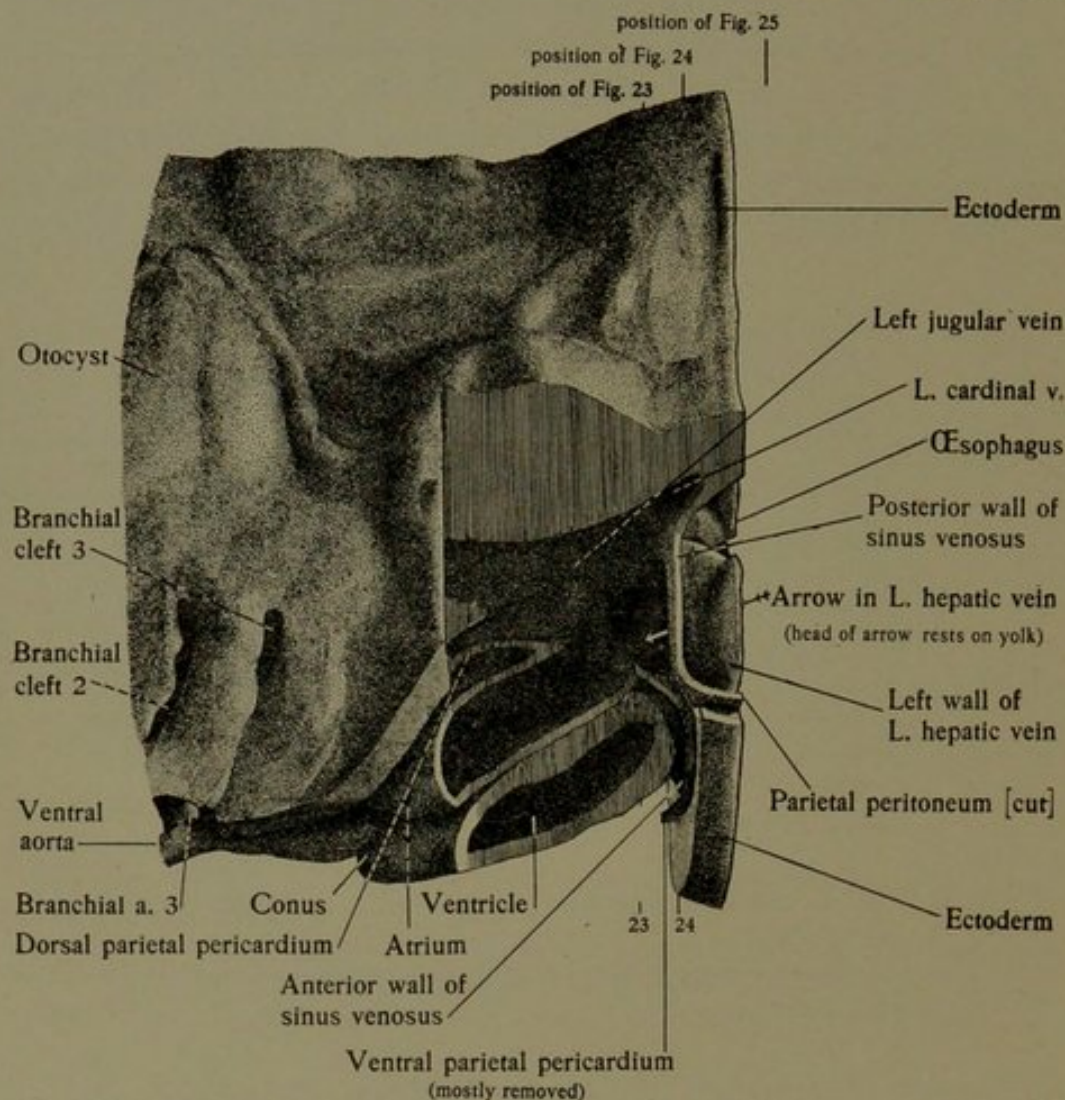


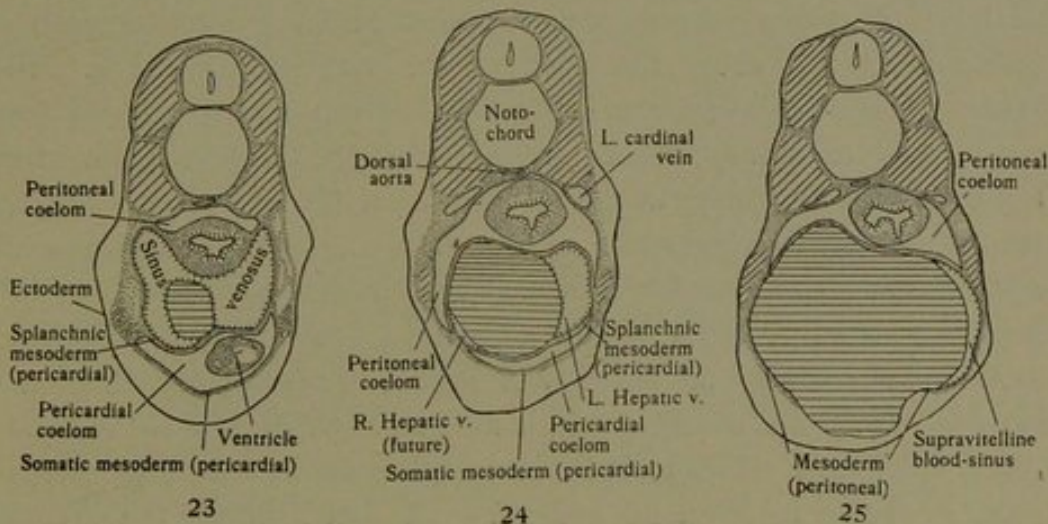
FIG. 22.—The left side of a reconstruction from the posterior gill region of shad, stage of 166 hours. $\times 100$ diams.

Sufficient body-wall has been removed to open the pericardial coelom laterally and ventrally and the peritoneal coelom laterally. The anterior pole of the yolk is intact, the ventricle and atrium have been opened. The vein immediately ventral to the left jugular is the left inferior jugular.

part of the pericardial coelom has extended backward, so that the endocardium lining the anterior wall of the sinus venosus assists in the formation of the hepatic veins (see Fig. 24). The expansion

of the peritoneal coelom affects only the hepatic-vein region (Fig. 22). It ends so abruptly that the splanchnic peritoneum, passing peripherally from the anterior ends of the hepatic veins to become somatic, forms a partition between sinus-venosus and hepatic-vein regions. This partition of splanchnic peritoneum represents the posterior wall of the sinus venosus, and has been labelled accordingly in Fig. 22.

In the sinus-venosus region shrinkage of the yolk has brought about marked diminution in the vertical dimension of this part of



FIGS. 23, 24, and 25.—Three transverse sections of which the positions are marked on Figs. 22. $\times 50$ diams.

Yolk, voluntary muscle, and vascular endothelium indicated as in Figs. 18 and 19.

the embryo (compare Figs. 22 and 17). The yolk-process has become thicker and much shorter; the space between its posterior surface and the yolk (which constituted the posterior communication between the right and left sides of the sinus venosus) is now obliterated.

The most striking change taking place at this stage occurs, alike, in the sinus-venosus and hepatic-vein regions; it is a process of rearrangement of the vascular endothelium. The vascular endothelium is rapidly spreading from the splanchnic mesoderm, both pericardial and peritoneal, on to the yolk so as to exclude the latter from the

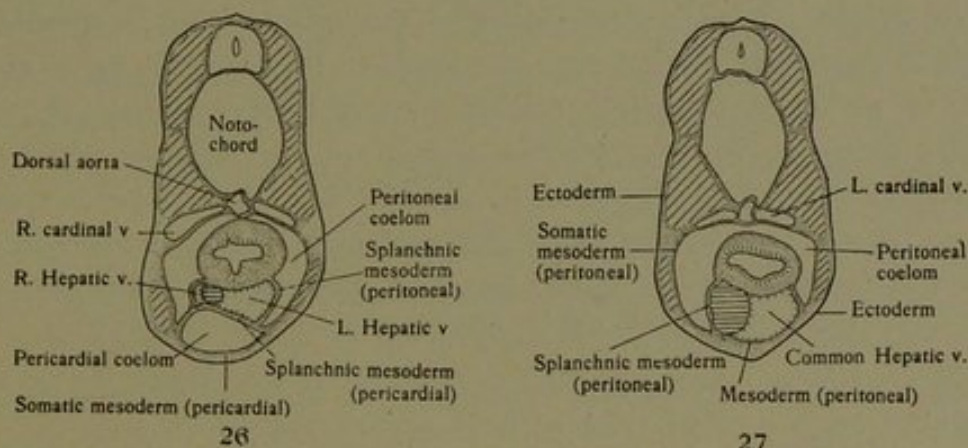
vascular system (see Figs. 23 and 24). This process has already progressed so far that the sinus venosus has a complete lining of vascular endothelium, which is true also of the adjacent part of the left hepatic vein. The yolk is still uncovered in the region of Fig. 25.

The sinus venosus, since the yolk has been consigned to a position definitely external to it, may now be looked upon as a complete structure in which the achievement of the adult condition is merely a matter of detail. The layers of splanchnic mesoderm, pericardial and peritoneal, which have been called the anterior and posterior walls of the sinus venosus are, to some extent, in mutual contact just behind and above the apex of the ventricle (see Fig. 22). Where these two layers are in contact the terms anterior and posterior wall of sinus venosus are not strictly applicable, for they together form the anlage of the pericardio-peritoneal septum. During the process of disappearance of the yolk, the pericardio-peritoneal septum undergoes further increase, and later growth produces the extensive structure of the adult.

A few words may be added with regard to the completion of the hepatic vein. The conditions indicated in Figs. 24 and 25 are tending in a direction which ends in the complete formation of the hepatic vein, as shown in Figs. 26 and 27. The latter figures come from sections of an embryo eight days old, and correspond in position to Figs. 24 and 25. In order to understand the condition of the hepatic vein at eight days it is necessary to appreciate the following facts: The yolk is not only smaller at its equator but the distance between the poles is also much diminished. The liver, which has grown forward so as to keep pace with the posterior pole of the yolk, is now very much nearer the heart than before. Redistribution of vascular endothelium, excluding the yolk from the vascular system (see Figs. 26 and 27), has now occurred throughout the entire extent of the yolk (*i. e.*, from heart to liver).

The hepatic vein is, at this time, rather a long vessel and consists of a main stem bifurcating anteriorly into short right and left branches; both stem and branches replace the original supravittelline blood sinus. The left branch formerly transmitted all the blood

passing from the liver to the sinus venosus; this function is now shared by the right branch. At eleven days the yolk has entirely disappeared; the final disappearance of the (latterly torpedo-shaped) yolk leaves the anterior pole of the liver still some distance posterior to the pericardio-peritoneal septum. The liver now grows forward (somewhat slowly) into the space formerly occupied by the yolk and, therefore, receives its peritoneal covering from the splanchnic mesoderm formerly enveloping the yolk and supravittelline sinus. The advancing liver receives into itself the entire main hepatic stem and the adjacent parts of the right and left branches. Finally, the anterior pole of the liver reaches the posterior surface of the peri-



FIGS. 26 and 27.—Transverse sections of shad, stage of 8 days. $\times 50$ diams. The transverse diameter of the sections is diminished from a tendency of the notochord to collapse. Yolk, voluntary muscle, and vascular endothelium as in Figs. 18 and 19.

cardio-peritoneal septum and protruding from its anterior end are the right and left hepatic veins of the adult which transmit blood into the sinus venosus.

The vascular endothelium lining the heart and the hepatic veins has been derived from two sources: Firstly, from the vascular endothelium (of which the origin has been described in the section dealing with the formation of the heart anlage) which has been referred to as endocardium. Secondly, from the vascular endothelium originally lining the roof of the supravittelline sinus, of which the origin has not been studied.

The endocardium originally lined the ventral surface of the splanchnic mesoderm anterior to the junction of the head with the first body somite. Of the splanchnic mesoderm lined ventrally by endocardium the (larger) part anterior to the site of discharge of the jugular veins became pericardial and the (smaller) part between the site of discharge of the jugulars and the first body somite became peritoneal. The disturbance of the original relations between the structures in the posterior head region which occurred between the stage of 7.2 mm. and that of 166 hours has been so extensive that it is very difficult to determine the exact amount of splanchnic peritoneum which was originally situated anterior to the line of junction of the first body somite with the head; nevertheless the account given below of the eventual distribution of the endocardium is probably not very far from the truth. The endocardium lining the ventral surface of the pericardial splanchnic mesoderm was partially included in the conus, ventricle and atrium to line these cavities; the remainder formed the lining of the anterior wall of the sinus venosus. The anterior wall of the sinus venosus assists in the formation of the anterior ends of the right and left hepatic veins (see Fig. 24). The vascular endothelial cells, migrating from the anterior wall of the sinus venosus on to the yolk to furnish the ventral lining of the hepatic veins, are, thus, derived from endocardium.

The splanchnic peritoneum forming the posterior wall of the sinus venosus is so close to the site of discharge of the jugular veins that the vascular endothelial cells lining the posterior wall of the sinus venosus are undoubtedly (like those lining the remainder of the heart) endocardial in origin. Since the ends of the hepatic veins immediately adjoining the sinus venosus are lined ventrally by endocardial cells migrating from the anterior wall of the sinus venosus, there is a great probability that the cells migrating from the splanchnic peritoneum to provide their dorsal lining (see Fig. 24), are also endocardial in origin.

The main stem of the hepatic vein is composed of vascular endothelium which originally lined the roof of the supravittelline sinus, and it is not at all unlikely that the adjoining roots of the right and left hepatic veins (eventually contained within the liver) are of similar origin.

There seems to be nothing unusual in the method of development of the heart valves. The sinu-atrial valves (two, right and left) are developed very late. I have serial sections of an embryo of 114 hours (No. 14,994) in which the heart has apparently ceased to beat during atrial systole. In this specimen the (valveless) venous orifice of the atrium is tightly contracted as by a sphincter. There is little doubt that this specimen indicates the normal mechanism of atrial systole prior to the formation of the sinu-atrial valves.

The true circulation does not begin with the initiation of rhythmical contraction in the, partially formed, heart-tube (36 somites), but with the completion of the aorta (42 somites), which occurs at a time when the heart-tube has been completely formed. Prior to the division of the primitive heart-tube into conus ventricle and atrium, the mechanism of systole of the entire tube is, in all probability, similar to that described for the atrium.

Syracuse, N. Y., November 2, 1908.

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