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The Fusion of the Cardiac Anlages in the Duck, *Anas*.

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The Fusion of the Cardiac Anlages in the Duck, *Anas*.*

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Abstract

Blood-cells: extraembryonically it is found in very early stages, but it first appears in the heart, in the 13 - 14 somite stage. Pericardial cavity: the first sign of its appearance is indicated in the 2 somite stage. It commences simultaneously in the multiple foci, and thus formed intramesodermal spaces become confluent to form a single pericardial cavity. Angioblast: it is derived from the cardiogenic plate, and first appears in an embryo with 3 somites. It is fundamentally bilateral in origin, and at very early stage are the two sides anastomosed with each other in the cranial region. Myocardium: it is formed from paired cardiogenic folds. There can not be noted any distinct demarcation on the surface of the myocardial tubes before they are fused together. The folds are first fused at the middle part in the 7 - 8 somite stage, and the fusion is completed in the 12 somite stage. Mesocardium: the ventral mesocardium first appears in the embryo having 6 - 7 somites, and ruptures at its middle part in the 8 - 9 somite stage. Its remnant is observed in still later stages, namely, we can find it in an embryo with 12 somites. The dorsal mesocardium forms between the 9 and 10 somite stages, and rupture between the 15 and 18 somite stages. Endocardium: it is formed from angioblasts. It has the endothelial character in the 5 - 6 somite stages, and the paired tubes are derived from it in the 9 somite stage. The endothelial tubes unite in the bulbar and the ventricular region. From the network of angioblasts which are spread at the cranial portion of the embryonic shield from which a pair of aortic arches are derived. In the bulbar region, the right endothelial tube markedly exceeds in caliber that of the left side. The latter atrophies and disappears, and the former makes the genuine bulbus cordis. Caudally, on the contrary, the left tube exceeds that of the right and the former is situated sinistro-ventrally to the latter. In the 13 - 14 somite stages the fusion of the paired tubes is almost completed, and the ventricle and atrium may be distinguished by the atrioventricular constriction. In closing, I wish to express my cordial thanks to Prof. Dr. J. Shikunami for his kind criticism and advice.

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The Fusion of the Cardiac Anlages in the Duck, *Anas*.

By

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Introduction.

Previously the author undertook an investigation on the early stages of development of the birds under Prof. Dr. *Ŷ. Shikinami*'s direction for two years and published the result. This paper deals with the transformation of the bilateral anlages of the heart into a single median organ which is distinguished into two processes, namely the fusion of the myocardial mantles and the fusion of the endothelial tubes. Since these processes are very complicated, many investigators failed to reach the same conclusion hither-to-fore, and it is very important to investigate this subject further.

Review of Literature.

In the mammals the fusion of the cardiac anlages has been investigated relatively extensively, for example the cat by *Schulte*; guinea-pig, *Yoshinaga*; rabbit, *Bremer*; ferret, *Wang*; human, *Davis* and others.

Schulte showed in the cat that proximal to the interventricular union, the paired tubes fuse at the bulbo-ventricular isthmus and again in the region of the aortic bulb, and that between these points the left tube atrophies and disappears. *Davis* showed that a similar process takes place in the seven somite stage of human heart, where, between the fused halves of the bulbus cordis and aortic bulb, there exists a distinct unfused interval in which the right tube markedly exceeds that of the left side in caliber.

Wang C. noted that 'the fusion of the two endothelial tubes in the ferret takes place in the ventricular region, and the fused part lies clearly on the right side of the median plane.'

In the dog, it was pointed out by *Bonnet* and also in the ferret by *C. Wang*, that the primary divisions of the endothelial tubes into sinus venosus, atrium and ventricle, occur before they form a single tube

completely. In connection with the fusion of the two endothelial tubes, there is one more point which requires attention. That is, many investigators noted the asymmetry of the heart rudiments at this period of development. In the cat, *Schulte* pointed out that the definitive bulbus is derived chiefly from the left. In both of these cases one tube enlarges while the other diminishes in size and eventually interrupted in its continuity and is only partially utilized in the formation of the single cardiac tube.

In the birds, *Mollier* stated that in the 6 somite stage of the chick embryo, the endothelial tubes are approximated to each other, and in the 8 somite stage, they fuse on the median line. *Patten*, in the recent publication entitled, *Embryology of the Chick*, sums up the matter as follows: 'the endothelial primordia are a pair of delicate tubular structures, a single cell in thickness, lying between the entoderm and mesoderm. The endothelial tubes of the right and left side are brought toward each other beneath the newly completed floor of the foregut. In the 28 hour chick, the endocardial primordia are approximated to each other and by 29 hours they fuse in their mid region to form a single tube.'

On the development of the circulatory system in the duck, some publications are found. *Weber A.* studied on the development of blood-cells in the area opaca; *Evans* and *Rabl* examined the system of arteries and *Fischel*, in his text book of human embryology, printed the transverse sections of the two embryos having each 8 and 10 somites; *Evans*, in the German edition of the second volume of the *Keibel-Mall* Embryology represented the model of reconstruction of the heart of duck embryo having 13 somites. Recently, *Nishimura* studied on the development of the cardinal vein, the nephric vein and other venous system; *Manzawa* investigated the origin of blood-cells and blood-vessels, and indicated that their early development is like that of reptilia. The present author fails among those who have studied the cardiac development in the birds, to find any record on the mechanism of the fusion of the heart primordia in the duck.

Material used and Method of Procedure.

From many duck embryos, a series shown in table 1 was selected as the representative of the changes covering the period selected for study, and these embryos furnish the immediate basis for this paper. A large majority of the embryos were fixed in *Zenker's* fluid, and stained with borax-carmin. After subsequent treatment in the usual manner, these specimens were embedded in paraffin, trimmed and cut in transverse

serial sections with a thickness of 10 microns.

In a study of the development of the endothelial and the myocardial tubes, to show their successive developmental changes and their topographical relations, a number of models were prepared according to *Born's* method of wax-plate reconstruction. These were made at a magnification of 200 (a few, 100) diameters, with an aid of *Edinger's* projection apparatus, by superimposing the drawings of every section of the cranial part of the embryo. The thickness of the wax-plate was 2.0 mm and a few, 1.0 mm respectively.

Table 1.

Stages	Embryo No.	Somites	Thickness of sections	Magnification	Model No.
1	No. 25	First somite is just forming	10		
2	No. 4	2	♢	200	1
	No. 18	2	♢		
3	No. 24	3	♢	♢	2
4	No. 13	4	♢		
5	No. 21	5	♢		
6	No. 14	5 - 6	♢	200	3
7	No. 22	6 - 7	♢		4
8	No. 15	7 - 8	♢	200	5
					6
9	No. 1	8	♢	♢	7
	No. 12	9	♢		
10	No. 6	9 - 10	♢	100	8
11	No. 20	11	♢	200	9
12	No. 8	12	♢	♢	10
13	No. 27	13 - 14	♢	♢	11
14	No. 31	15	♢	100	12
15	No. 32	18	♢		

Experiments.

Stage 1, No. 25.

This embryo measured 3.0 mm after it has been cut. The first mesodermic somite was just forming but it was not sharply defined. Caudal to the primitive streak, a faint neural groove was present on the surface of the embryo. The notochord was tubular at its caudal end. In parts its ventral wall opened into the yolk-sac. Its caudal extremity was fused with the ectoderm at the cranial end of the primitive streak. Extra-embryonically there are so-called "blood-island", but they were not surrounded by any endothelium. Neither the coelomic space nor

the heart primordia was found in this specimen.

Stage 2, No. 18, Somites 2.

The total number of the sections were 317. The head fold was observed along 8 sections and the foregut just began to develop in the embryo; for the length of two sections, its lumina was invaginated upward into the head fold. There was a broad, shallow neural groove which narrowed cranially and disappeared caudally. The caudal end of the notochord was fused with the cranial part of the primitive streak. Extra-embryonically the clusters of blood-cells were found scattered over the large portion of the yolk-sac to which they were often adherent. The intra-embryonic coelomic space which may be regarded as the future pericardial cavity, considering its topographic position, showed simply a beginning of a very narrow cleavage in the lateral mesoderm of the cranial portion of the embryo. Fig. 1. shows the lateral view of the model of reconstruction of the right coelomic spaces. The coelomic cavity is formed from multiple foci and in the middle line of the embryo it was not found entirely. There was no heart rudiment as seen in Fig. 2.

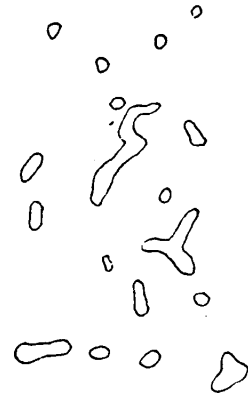


Fig. 1. Embryo, No. 18. Graphic reconstruction of pericardial cavity (right side) as viewed from the ventrum. $\times 200$.

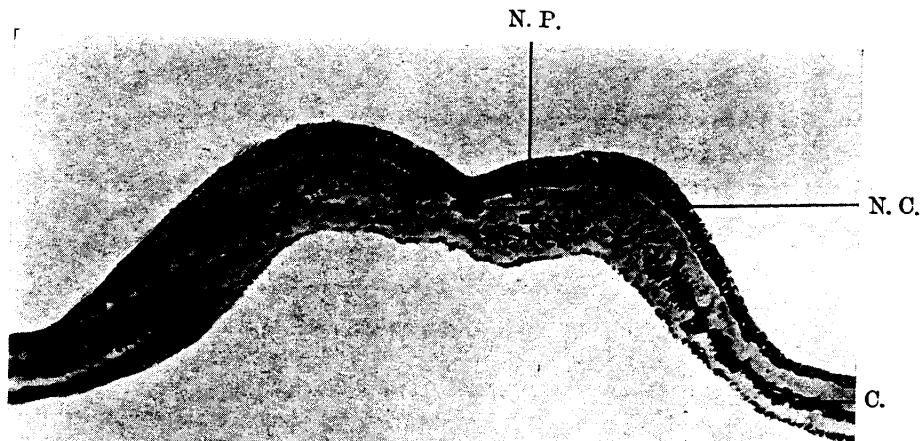


Fig. 2. No. 18. Transverse section through pericardial region. $\times 125$. C. = Coelom, N. C. = Notochord, N. P. = Neuralplate.

The duck embryo No. 4, having two pairs of mesodermic somites was almost at the same stage of development.

Stage 3, No. 24, Somites 3.

The total number of the sections of this embryo were 333. The lumina of the foregut was invaginated, for the length of 10 sections, upward into the head fold which was elevated 180 microns from the embryonic shield. There was a well-developed flat, V-shaped medullary groove in the cranial portion of the embryonic shield, and its cranial end became shallow gradually. The model of reconstruction of the right coelomic space is shown in figure 3 (a). The boundary of the coelomic space is, so to speak, a map of the earth. Cranially it was not converged in the median line and not fused with the opposite side like that of mammals, such as, the pig, the guinea-pig, the cat etc. Between the ventral surface of the mesoderm and the entoderm, there could be distinguished a clear interspace, and here and there some free, isolated cells, detached from the cell band of the mesoderm were found, as shown in figure 3 (b). Most workers regard these cells as the angio-

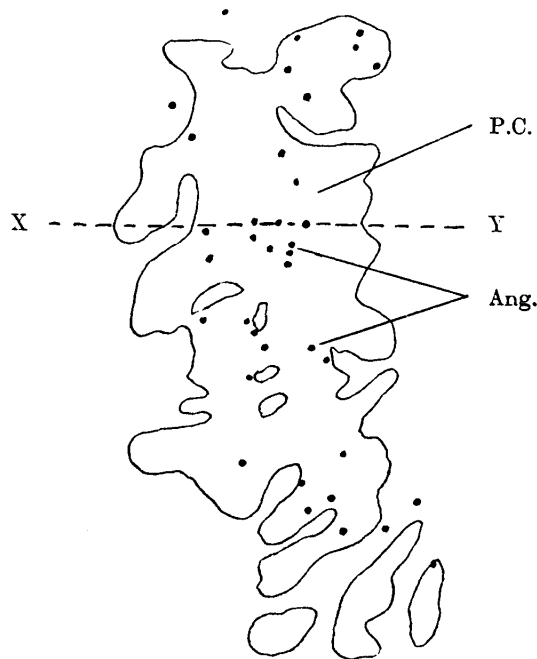


Fig. 3(a). Embryo, No. 24. Graphic reconstruction of pericardial region as viewed from the ventrum, to show relation of angioblasts to pericardial cavity (right side). X-Y indicates plane of section of No. 3. $\times 200$. Ang = Angioblasts, P. C. = Pericardial cavity.

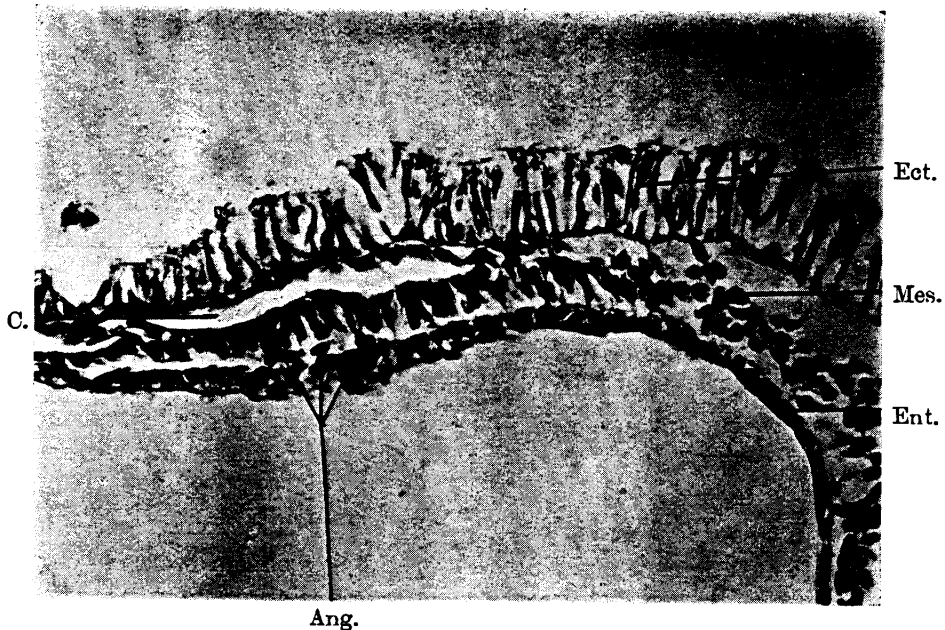


Fig. 3 (b). No. 24. Transverse section showing endothelial cells. $\times 290$.
 Ang. = Angioblast, C. = Coelom, Ect. = Ectoderm, Ent. = Entoderm,
 Mes. = Mesoderm.

blasts. In some sections, the angioblasts were joined to the adjacent mesoderm by faint fibrils. Frequently a mitotic figure was recognized in the mesodermal cells, adjacent to the angioblasts. These findings show that there can be no doubt about a distinct proliferative activity of the mesoderm cells; furthermore, as it was stated by *T. Yoshinaga*, every transitional feature of the migration or the detachment of the mesodermal cells, which apparently are destined to become angioblasts, pointed to the fact that these angioblasts are originated in the ventral surface of the mesoderm. Fig. 3 (b) was reproduced from the fiftieth section, counting from the cephalic border of the embryonic shield. The mesoderm is divided into two layers of cells, namely the parietal and the visceral plate, by the developing coelom. The parietal and the visceral plate is called the somato- and the splanchno-pleure respectively, and the latter shows a slightly thicker layer of spindle-shaped cells some of which the mitotic figures are present, indicating a proliferative activity. The angioblasts are scattered singly, not forming the string, between the ventral surface of the mesoderm and the entoderm.

Stage 4, No. 13, Somites 4.

This specimen was prepared from the egg of the duck 42 hours incubation. It was cut in a cross-section, having a 10 microns thickness,

and 343 sections were made to the embryonic area. The head was elevated 440 microns from the embryonic shield. The foregut was more elongated than that of the preceding specimen and measured 300 microns. There was present a well developed medullary groove in the cranial portion of the embryonic shield and its caudal end became gradually shallower until it disappeared at the primitive streak in the caudal third of the embryonic shield. In the heart region, the coelomic chamber which is called the pleuropelvic cavity was much wider than that of the previous embryo. The cardiogenic fold which is called the heart plate, was sharply defined and it was the thickest layer of the splanchnopleure. The number of angioblasts which were scattered in the space between the splanchnopleure and the entoderm, was increased and the distance of both cardiogenic folds (right & left) was decreased, see Fig. 4.

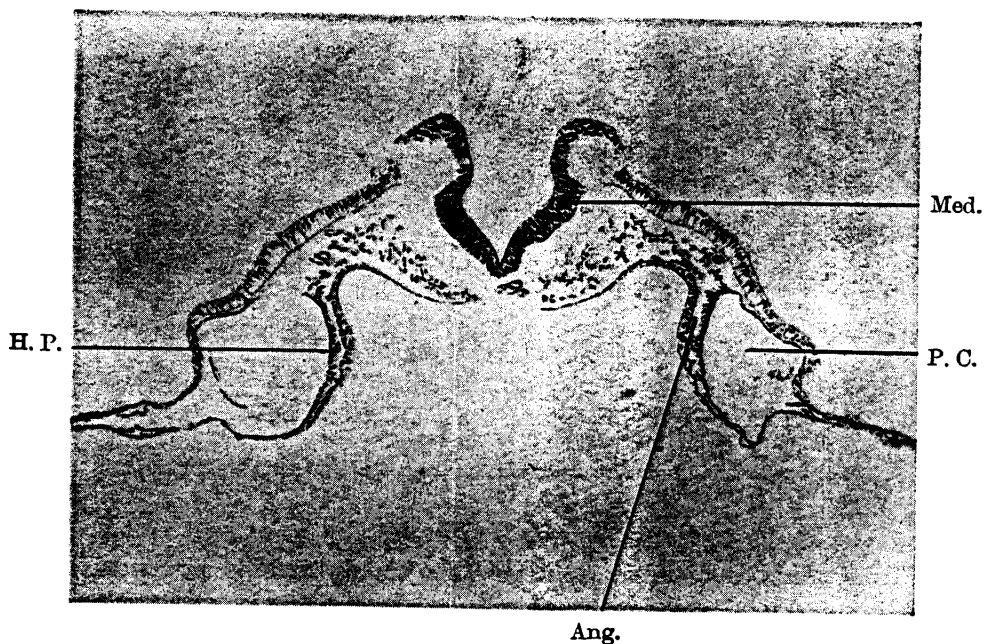


Fig. 4. Transverse section through heart region of duck embryo having 4 somites. $\times 80$. Ang. = Angioblast, H. P. = Heart plate, Med. = Medulla, P. C. = Pericardial cavity.

Stage 5, No. 21, Somites 5.

Total number of sections were 270; Incubation, 40 hours; Length of the head-elevation, 400 μ .

The neural groove was still widely opened. The foregut was more elongated and measured about 330 μ . Intra-embryonically, there was

no sign of the formation of blood-vessels. The cardiogenic fold was, as indicated in figure 5, the thickest wall of the pericardial coelom and its form was like a crescent. A number of angioblasts were scattered in the space between the heartplate and the entoderm, but there were no potential cavities among these cellgroups.

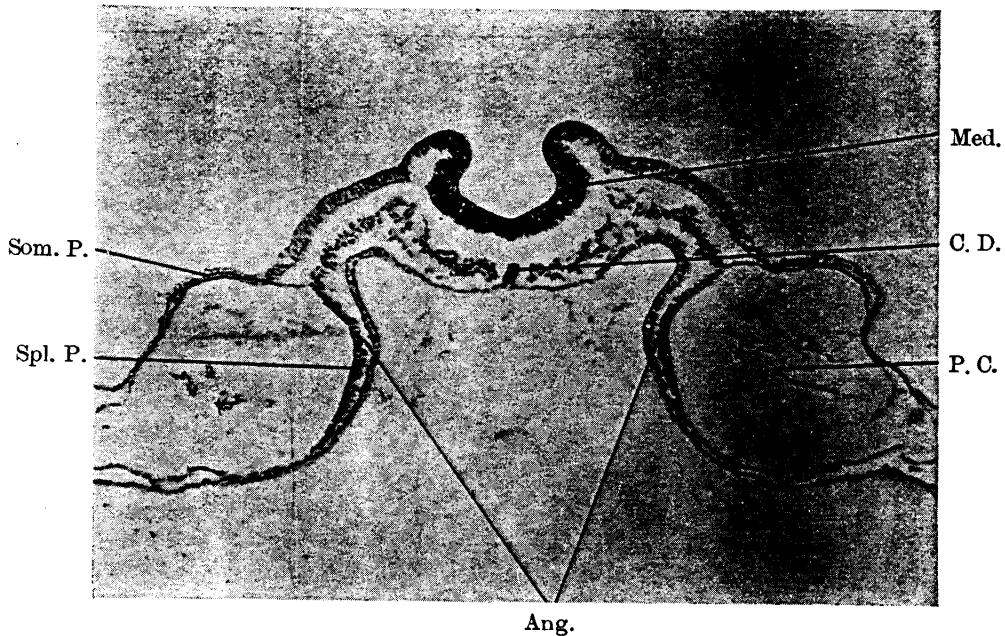


Fig. 5. Transverse section through heart region of duck embryo having 5 somites. $\times 80$. Ang. = Angioblast, C. D. = Chorda dorsalis, Med. = Medulla, P. C. = Pericardial cavity, Som. P. = Somatopleure, Spl. P. = Splanchnopleure (Heart plate).

Stage 6, No. 14, Somites 5-6.

Total number of sections were 386; Incubation, 42 hours; Head-elevation, $540\ \mu$; Foregut-invagination, $450\ \mu$.

The neural groove was deepest at the brain region, and caudally it was shallower. Cranially the notochord was entirely fused with the ectoderm. Extra-embryonically there was a large number of blood-cells on the yolksac surface. It was observed in a part of the heart primordia, the pleuro-pericardial cavity reached its maximum width in proportion to the myocardial and endothelial development. The cranial extremity of the pericardial cavity disappeared under the head fold slightly caudal to its cranial margin. Dorsal aortae were not yet formed, but in some sections a number of angioblasts was grouped in its premature stage. The heart itself consisted of a bilateral symmetrical myoepicardial

mantle enclosing an endothelial plexus. The myoepicardial mantle consisted of a pair of cardiogenic folds of which middle parts, situated about fifty microns cranially from the anterior intestinal portal, were drawn closely together, and diverged cranial- and caudal-ward gradually as shown in Fig. 7. The cardiogenic fold was much heavier than that of the previous specimen and its central part began to invaginate into the pericardial cavity, rising from the underlying entoderm. Structurally the endocardium was a flattened sheet of endothelial strands forming an angioblastic plexus. Many of the strands were solid but the others

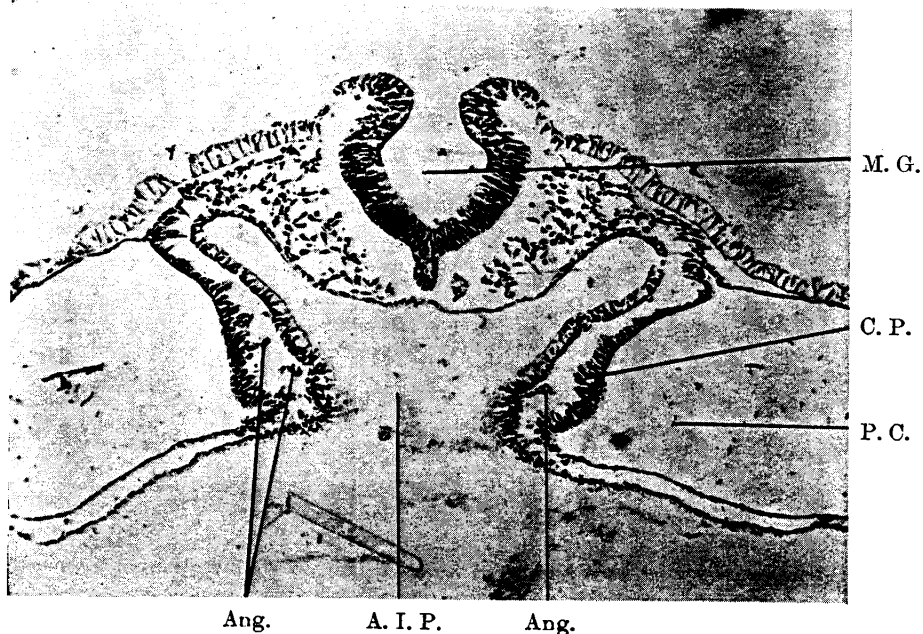


Fig. 6. Transverse section through heart region of duck embryo having 5-6 somites. $\times 110$. A. I. P. = Anterior intestinal portal, Ang. = Angioblast, C. P. = Cardiogenic plate, M. G. = Medullary groove, P. C. = Pericardial cavity.

possessed lumina as Fig. 6 indicates. The latter was found especially approaching the anterior intestinal portal. It is called the omphalomesenteric vein. The first appearance of the cavity in the endocardial strands in the duck was thus found in the embryo having 5-6 somites. It was hardly different from that of the pigeons heart which came to the present author's observation in the 7 somite stage of the pigeon. The endothelial plexus which was situated cranial-ward from the omphalomesenteric vein, represents the irregular configuration and anastomoses one another as in Fig. 8. It was impossible to verify the

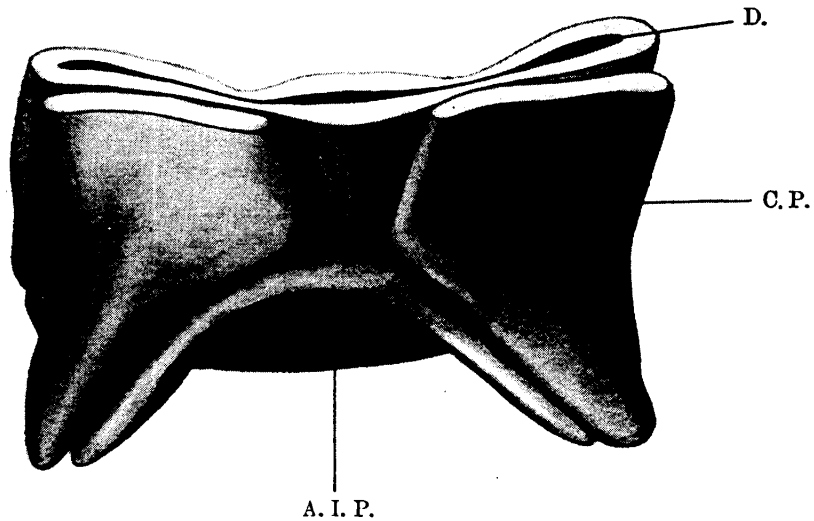


Fig. 7. Embryo No. 14, Ventral view of a wax model of the cardiogenic plate. $\times 200$. A. I. P. = Anterior intestinal portal, C. P. = Cardiogenic plate, D. = Fore-gut.

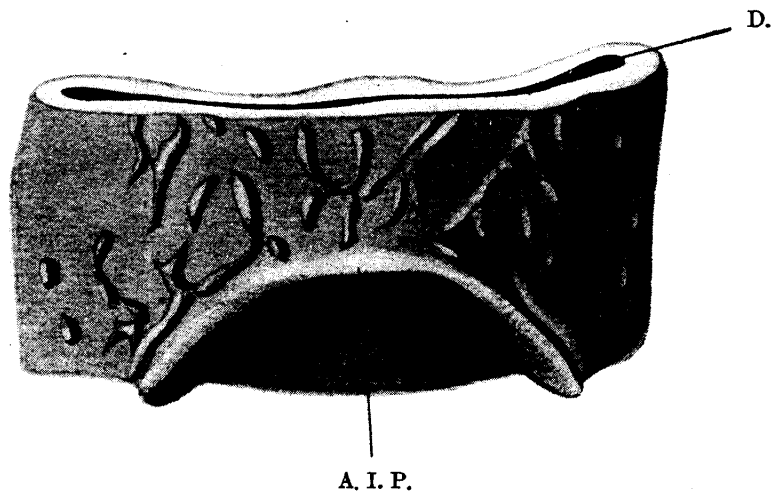


Fig. 8. Embryo No. 14. Reconstruction of the angioblast, anterior part of the embryo, seen from the ventrum. $\times 200$. A. I. P. = Anterior intestinal portal, D. = Fore-gut.

existence of the sharp demarcation among these bilateral anlages on the median line. From these observations, it may be stated that in the bird, especially in the duck, there are communications between the endothelial plexus of both sides in the very early stage of development.

Stage 7, No. 22, Somites 6 - 7.

Total number of sections were 360; Head-elevation, 480μ ; Foregut-invagination, 580μ .

No reconstruction was made at the time, but a study of the sections shows that the embryo in its general development was slightly in advance of the previous specimen. The closure of the medullary canal was seen until the point where 150 microns cranialward separated from the anterior intestinal portal. There are two rudiments of the dorsal aortae which run caudo-medially one on each side of the medullary groove. They were still more or less plexiform in character and they terminated blindly at cranial extremities some distance caudal to heart rudiment. Bilateral cardiogenic plates and pericardial cavities approached a little more than that of the preceding specimen. The ventral mesocardium which makes the wall between the pericardial cavities on both sides was built from two mesodermal layers as shown in Fig. 9. Cranially among these mesocardial layers, there was a small room which is an ectodermal nature and is called the mesodermal cyst. The development of the endocardium was more advanced, and found that there were vacuoles in the groups of angioblasts on the median line. But it was less defined than the next stage.

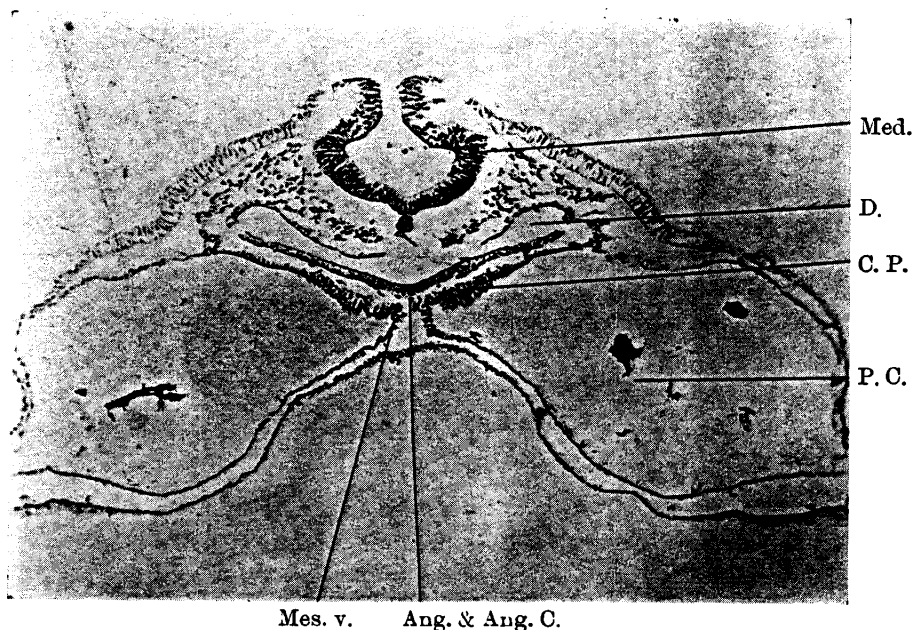


Fig. 9. No. 22. Transverse section (1-8-1) showing mesocardium ventrale. $\times 80$. Ang. & Ang. C. = Angioblast and angiocyst, C. P. = Cardiogenic plate, D. = Fore-gut, Med. = Medulla, Mes. v. = Ventral mesocardium, P. C. = Pericardial cavity.

Embryo No. 16, having 7 somites was scarcely different from No. 22, but the distance between the cardiac folds on both sides was a little narrower. Accordingly the ventral mesocardium increased in length and decreased in width. The dorsal mesocardium was not yet found in this stage. The first appearance of the ventral mesocardium in the bird was therefore almost at the same stage, namely, 8 somite stage in the chick, 7-8 in the pigeon and 6-7 in the duck.

Stage 8, No. 15, somites 7-8.

Total number of sections, 421; Head-elevation, 540 μ ; Foregut-invagination, 660 μ .

This specimen was removed from an egg of the duck, 42 hours incubation. The medullary canal was no more enclosed than the previous specimen. The dorsal aortae were found in the form of capillaries, but they were still more or less plexiform in character, and they terminated blindly at their cranial extremities some distance to the heart primordia. In the model it can be recognized that both the lateral cardiogenic folds were considerably developed, and their middle portions have partially come into contact and been almost fused together, as in Fig. 10. In this portion, the myocardium arched over the ventral surface of the pharynx, the floor being the roof and side walls of the pericardial cavity, formed by the ventral wall of the pharynx. The myocardium of which position neighbouring the foregut

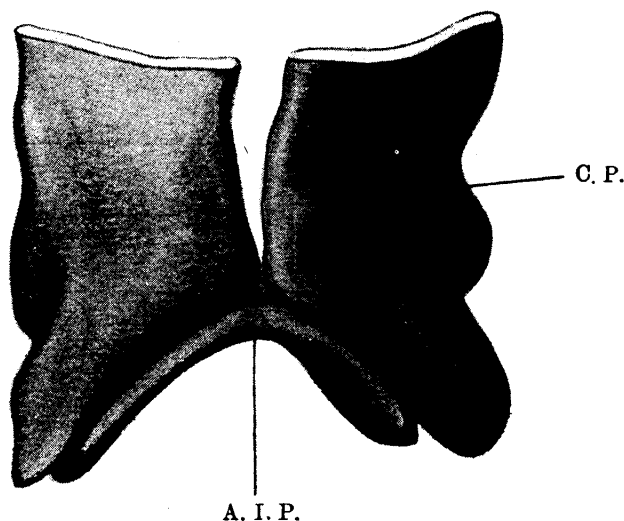


Fig. 10. Embryo No. 15. Ventral view of a wax model of cardiogenic plates, showing they approach each other at the middle part. $\times 200$.
A. I. P. = Anterior intestinal portal, C. P. = Cardiogenic plate.

opening, indicated that the thickest layer and the surface of the myocardial mantle were uneven at this portion. This characteristic was recognized more distinctly on the left side than on the right. The ventral mesocardium was much more developed and it was found throughout the heart primordia without a single exception. Accordingly the pericardial cavities on both sides were not yet communicated with each other. As it is found in the model, the network of the angioblast-cords was spread out on the ventral surface of the pharynx. They did not have the potential cavities except at a part which belongs to the omphalomesenteric vein and the middle portion which is destined to become the future bulbus cordis. Compared with the later stages, the angiocyst at the latter portion is perhaps belonging to the endothelial tube of the right side. The other angioblast cords were arranged irregularly as shown in Fig. 11.



Fig. 11. Embryo No. 15. Ventral view of a wax model reconstruction of the angioblast network. $\times 200$. B. = Bulb, O. V. = Omphalomesenteric vein, V. = Ventricle.

Stage 9.

The material of this stage consisted of two embryos, one having 8 somites and the other, 9 somites.

a) No. 12, Somites 9. This embryo was removed from an egg, 42 hours incubation. The head fold had progressed cranial and dorsalward. Its cephalic end was elevated about 560 microns from the embryonic shield. The foregut measured 830 microns. The medullary canal was closed to the point just caudalward to the foregut opening, and the cranial neuroporic aperture was widely open. Three partitions were observed at the cephalic part of the medullary tube, namely, the fore brain, mid brain and hind brain. The dorsal aorta was in a capillary form. Both the pericardial cavities approached and united with each

other, making a single room. Consequently the ventral mesocardium which was the boundary of the pericardial cavities, was ruptured from its center. From the model of the myocardium it is easily understood that the ventral mesocardium begins to rupture at the part which is about 50 microns away caudalward to the anterior intestinal portal, as Fig. 12 indicates. The myocardium consisted of a pair of cardiogenic folds which bear the same general relation to the foregut and the anterior intestinal portal as seen in the preceding specimen. Ventrally the paired cardiogenic folds completely fused with one another except in the region of the atria. But externally it seemed as if the fusion of the cardiogenic folds did not occur at all because there is a deep sulcus on the line of fusion, as seen in Fig. 13. The network of the angio-

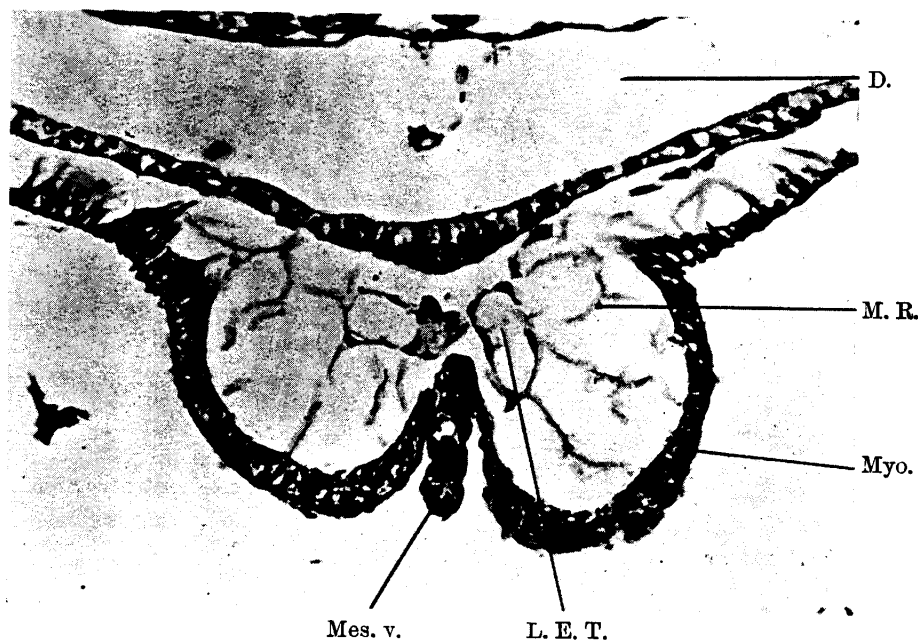


Fig. 12. Transverse section through heart region of duck embryo having 9 somites. $\times 290$. D. = Foregut, Z. E. T. = Left encardial tube, Mes. v. = Ventral mesocardium, M. R. = Myoendocardial Reticulum, Myo. = Myocardium.

blastocords which was spread over the space between the ventral wall of the pharynx and the cardiogenic folds, was now gathering in the middle line and was forming two principal canals which are running side by side. These endothelial canals are united at a part which is destined to become the bulbus cordis in some sections, and again at the proximal part of the ventricle in one section. In the unfused region of the bulbal

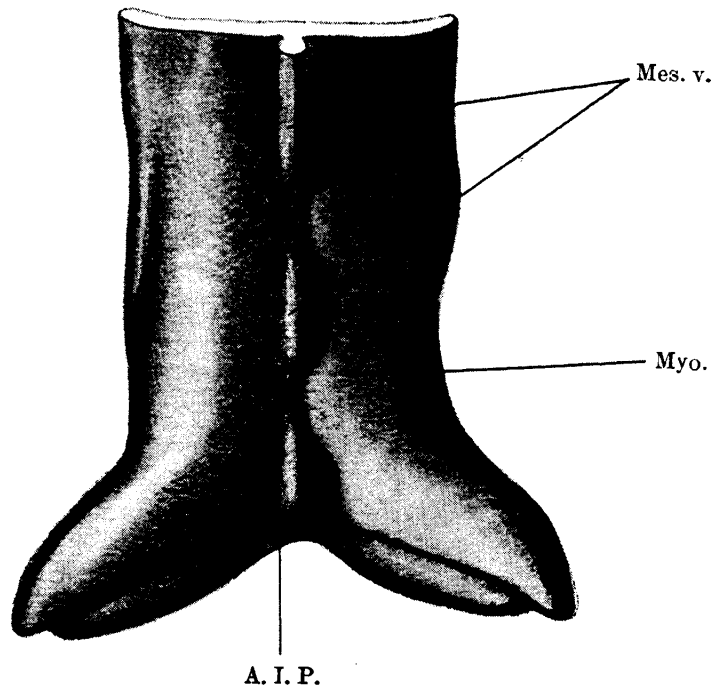


Fig. 13. Embryo No. 12. Ventral view of a wax model of the heart. $\times 200$. A. I. P. = Anterior intestinal portal, Mes. v. = Mesocardium ventrale, Myo. = Myocardium.

part, the right tube markedly exceeds in caliber that of the left side. But at caudalward, the left tube is, on the contrary, superior to the right, and here the former is situated more ventrally than the latter, as in Fig. 14.

b) No. 1, Somites 8. It was almost the same stage with No. 12. The cardiogenic fold was a little thicker than that of the preceding specimen and the surface of the caudal part of the left side was markedly uneven. The angioblast cords formed a network in the bulbal region, whence caudalward the condition was like the preceding specimen.

Stage 10, No. 6, Somites 9-10.

This embryo was removed from the egg, 48 hours of incubation. The head was elevated 530 microns from the embryonic shield. The neural canal was closed to the fifth somite region, and the anterior neuropore was now open. The pharynx measured 850 microns. The well developed dorsal aorta which ran along the dorsal wall of the pharynx, formed a continuous endothelial tube. The anterior intestinal portal was fairly removed caudalward, and it was situated in the first somite region. The ventral mesocardium was ruptured a little more

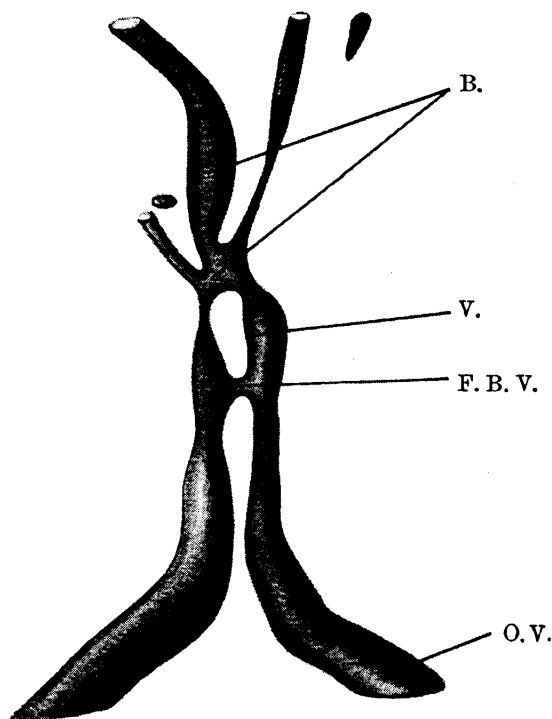


Fig. 14. Reconstruction of endothelial heart tubes of a duck embryo of 9 somites. $\times 200$. B. = Bulb, F. B. V. = Fusion between ventricle, O. V. = Omphalomesenteric vein, V. = Ventricle.

than that of the previous specimen. Paired cardiogenic folds were now completely fused across the median plane of the embryo, but there was yet a deep depression on the line of fusion. The remnant of the ventral mesocardium was now found at the cranial part of the ventral surface of the myoepicardial mantle. In the region of the ventricle, the dorsal wall of both cardiogenic folds which were drawn near in the median line, was reflected directly into the dorsal wall of the pericardium, forming the dorsal mesocardium on both sides. In a part of the aortic bulb, both endothelial tubes united with each other and indicated a single tube through the length of nine sections. A distance of six sections distalward to this portion, the paired tubes have come actually in contact with each other and further more three sections distalward, the second union is noted along the length of four sections as in Fig. 15. The third union is seen through the length of nine sections, and the interval between the second and the third union was 40 microns. The caudal extremities of the endothelial tubes continue into the portion of the vitelline veins, while cranially they run latero-cranially towards the lateral margins of the foregut and then parallel with these margins, as

shown in Fig. 16. They loop round the cranial end of the foregut, join the dorsal aortae, and thus constitute the first aortic arches. From the right side of the aortic bulb a small vessel runs laterally making an angle with the aortic arch of the right side, and terminates blindly in a short distance. Perhaps it belongs to the ventral portion of the future, second aortic arch. On the left side, far from the aortic bulb, there are a number of scattered angioblasts, but impossible to determine whether they are destined to become the second aortic arch or not. The length of the heart primordia, measured along the original mid-dorsal line of the heart tube from the point of bifurcation of the aortic roots to the point of convergence of the omphalomesenteric veins, was 39 mm (in model).

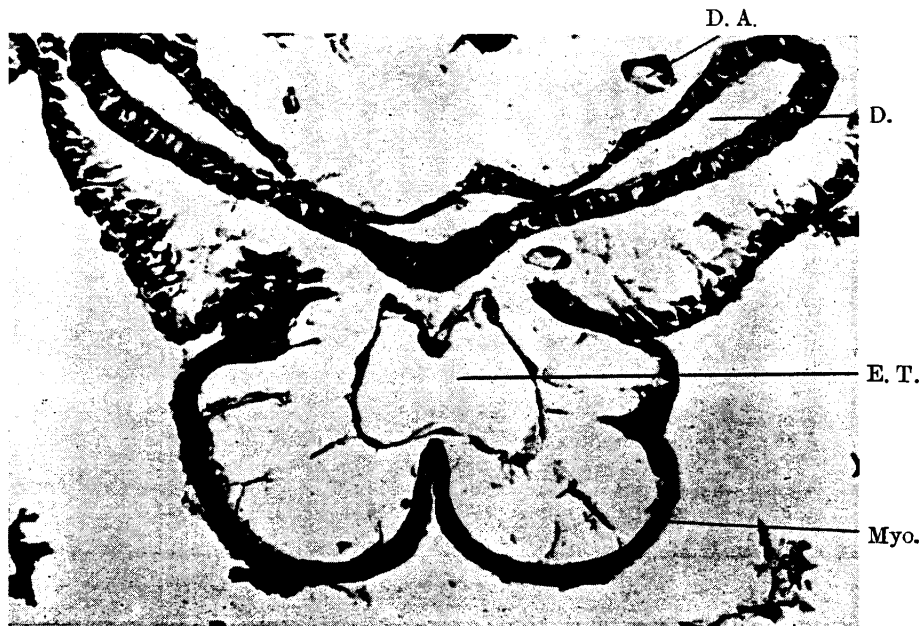


Fig. 15. Transverse section through fused ventricular region of duck embryo (No. 6) having 9 - 10 somites. $\times 290$. D. = Foregut, D. A. = Dorsal aorta, E. T. = Endocardial tube, Myo. = Myocardium.

Stage 11, No. 20, Somites 11.

Head-elevation, $540\ \mu$; Foregut-invagination, $940\ \mu$.

As judged by the stage of general development, the embryonic shield was slightly more advanced than that discussed in stage 10, indicated by the fact that eleven somites are present and that the medullary canal is closed at the last somite region. The first visceral pouch is now on the way of development. The dorsal aortae were well

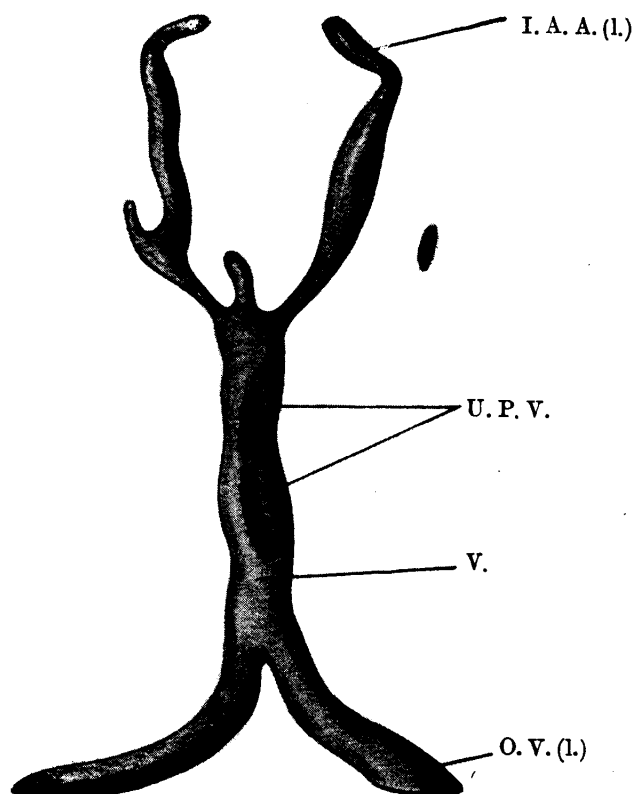


Fig. 16. Reconstruction of endothelial heart tubes of a duck embryo of 9 - 10 somites. $\times 100$. I. A. A. (l.) = 1st aortic arch (left), O. V. (l.) = Omphalomesenteric vein (left), U. P. V. = Unfused part of ventricle, V. = Ventricle.

developed in this specimen. At the distal end of the atria the fusion of the cardiogenic folds was not yet completed. There was a sulcus running along the line of fusion of the myoepicardial mantle. It is the deepest at the cranial part of the ventricle. The leavings of the ventral mesocardium were found in some sections at the cranial part of the heart. The fusion of the endothelial tubes was a little more advanced, and at the region of the aortic bulb the left one disappeared. In the ventricle region the fusion was accomplished, but cranialward to the inter-ventricular union there existed a distinct unfused interval in which the left tube markedly exceeds in caliber that of the right side, see Fig. 17. The length of the heart primordia, measured in the same manner with the previous specimen, was 66 mm (in model).

Stage 12, No. 8, Somites 12.

Number of sections were 451; Head-elevation, 630μ ; Foregut-

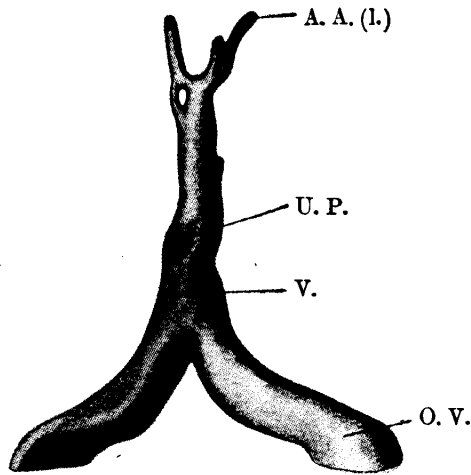


Fig. 17. Reconstruction of endothelial heart tubes of a duck embryo of eleven pairs of somites. $\times 50$. A. A. (l.) = Aortic arch (left), O. V. = Omphalomesenteric vein, U. P. = Unfused part, V. = Ventricle.

invagination, $1020\ \mu$.

The primary optic vesicle on each side was well represented by a slight bulge of the lateral wall of the anterior portion of the prosencephalon of the corresponding side. The otic vesicle was represented by a thickened plate of epithelium which showed a shallow invagination, and situated about 180 microns cranialward to the first pair of somites. The medullary canal was closed except at its cranial and caudal ends. The first visceral pouch did not reach the ectoderm. Wolf's ducts were found at the last somite region. The fusion of the cardiogenic folds was almost completed, and we can find scarcely the line of fusion on the ventral surface of the myocardium as shown in Fig. 18 & 19. The ventral mesocardium was ruptured, and the dorsal mesocardium was now developed in the ventricle region. The endocardium was divided into several chambers in the part of the aortic bulb. When it was traced a little caudalward from this portion, there existed a distinct unfused part, in which the left tube exceeded in caliber that of the right side and the former was situated sinistro-ventralward to the latter. In the atrial region, the endothelial tube was divided into the right and the left tube which continue the right and the left omphalomesenteric vein respectively.

No. 29. with twelve somites was almost at the same stage with No. 8. The lumen of the endocardium which is situated between the aortic bulb and the ventricle was very narrow. It was similar to that of No. 31.

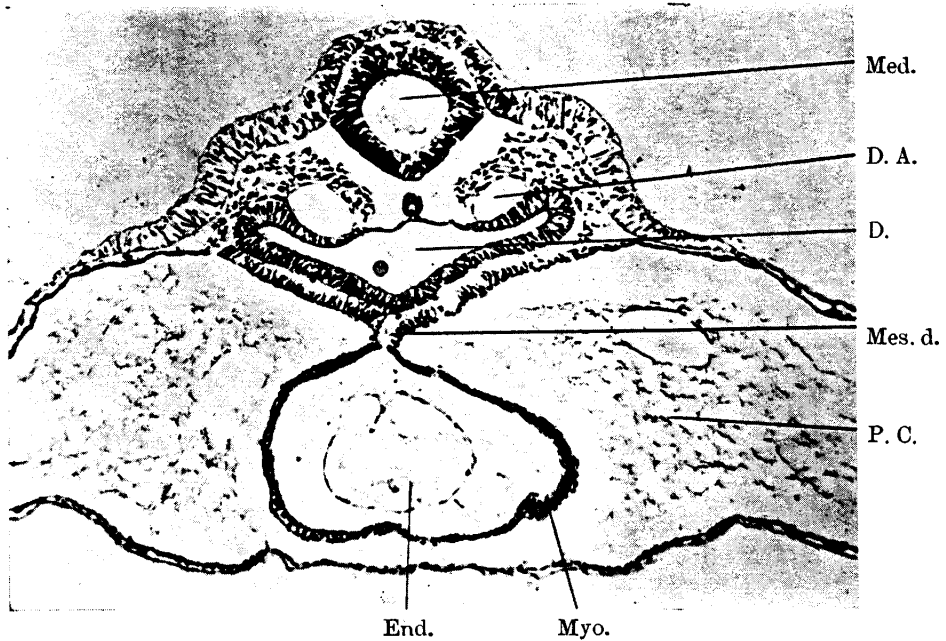


Fig. 18. Transverse section through ventricular region of duck embryo (No. 8) showing mesocardium dorsale. $\times 110$. D. = Foregut, D. A. = Dorsal aorta, End. = Endocardium, Med. = Medullary tube, Mes. d. = Dorsal mesocardium, P. C. = Pericardial cavity.

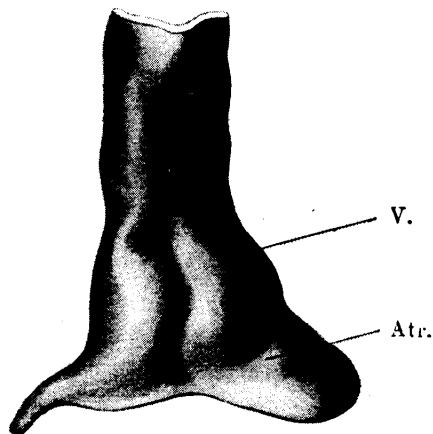


Fig. 19. No. 8. Ventral view of a wax model of the heart. $\times 50$. Atr. = Atrium, V. = Ventricle.

Stage 13, No. 27, Somites 13-14.

Number of sections were 397; Head-elevation, 450μ ; Foregut-invagination, 1000μ .

The medullary tube was closed except at its cranial and caudal

ends, where the anterior and posterior neuroporic apertures are present. The auditory pit showed a shallow invagination. The optic vesicle was more prominent than that of the preceding specimen. The first visceral pouch reached the ectoderm, but the second did not. The rudiment of the nephritic body was first seen opposite the 7th somite, and thereafter it was scarcely separated from the paraxial and lateral mesoderm. The ventral mesocardium was ruptured entirely, but the dorsal mesocardium was well developed throughout the heart without any interruption.

On the ventral surface of the myoepicardial mantle there was no residual sign of the sulcus which was present distinctly in the preceding specimen. The axis of the heart primordia was almost in a straight line of which direction was longitudinal. The endocardial tube was also running in the longitudinal direction as in Fig. 20. In the region of

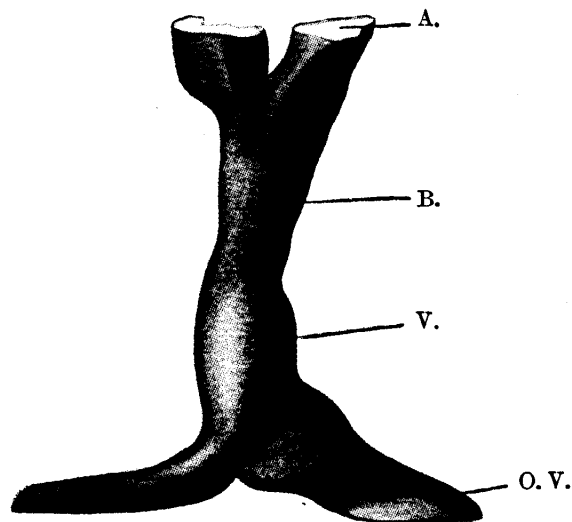


Fig. 20. Reconstruction of endothelial heart tubes of a duck embryo of 13-14 somites. $\times 50$. A. = Aorta, B. = Bulb, O. V. = Omphalomesenteric vein, V. = Ventricle.

the aortic bulb, the endocardial tube was divided into a number of small chambers. A similar process was found in the pigeon's heart of nine somite stage. In the atrial portion, two endothelial tubes were lying side by side. From the anterior intestinal portal, they diverge one another and turn into the omphalomesenteric veins. The endocardial tube had the minimal width in the part between the bulb and the ventricle and had the maximal extension in the atrial region, that is, the heart was suspended in the systolic period. The constriction between the atrium and the ventricle was marked off, on the left side,

but not on the right. A few blood cells were seen in the heart. The length of the heart was 90 mm (in model).

Stage 14, No. 31, Somites 15.

The length of the head-elevation and the foregut-invagination was 680 and 900 mm respectively. The upper end of the embryo was covered by the amnion to the part of the mesencephalon. The anterior neuropore was closed entirely, but the posterior one was now open. The optic vesicle was more prominent. The auditory pit was shallow. The first visceral pouch reached the ectoderm, but the second did not. When the whole series of sections were examined, the form of the myocardium was elliptic in the bulbar region and circular in the ventricle. The dorsal mesocardium was in the highest development, and it had the maximal length in the middle ventricular portion, where its rupture was just going on as in Fig. 21. The endocardium indicates a single cavity except in the atrial region where the endothelial septum was now present, and the union of the endocardial tubes was not yet completed. In the region of the aortic bulb, the endocardial lumen lied

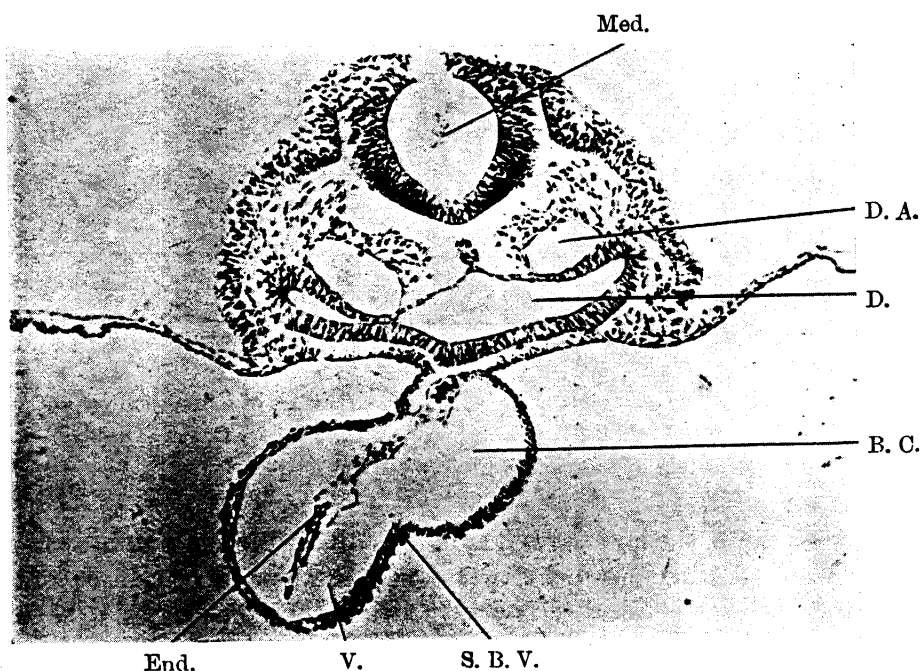


Fig. 21 (a). Transverse section through bulbo-ventricular constriction of duckembryo having 15 somites (No. 31). $\times 110$. B. C. = Bulbus cordis, D. = Foregut, D. A. = Dorsal aorta, End. = Endocardium, Med. = Medullary tube, S. B. V. = Sulcus bulbo-ventricularis, V. = Ventricle.

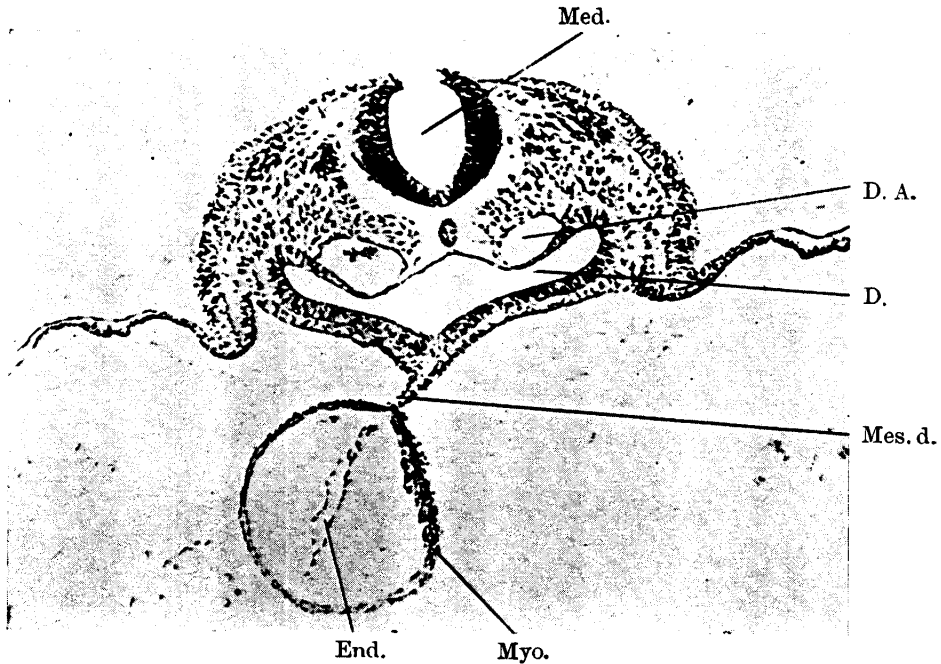


Fig. 21 (b). No. 31. Transverse section through ventricular region. The dorsal mesocardium is well developed and is now breaking. $\times 95$. D. = Foregut, D. A. = Dorsal aorta, End. = Endocardium, Med. = Medullary tube, Mes. d. = Dorsal mesocardium, Myo. = Myocardium.

clearly on the right side of the median plane having a slit like space, and the surface of the endocardial tube was directed sinistroversally as shown in Fig. 22. Its lumen had the maximal breadth in the atrial portion where a large quantity of blood-cells was found there.

Stage 15, No. 32, 18 somites.

The embryo was covered with the amnion toward the hindbrain region. The head was turned to the right. The auditory pit was much deeper than that of the previous specimen. The primary optic vesicle was well developed, but there was yet no lens thickening to be detected. The medullary canal was closed to the last few sections. The first visceral pouch reached the ectoderm, and the second one was just touching it. As the rate at which the heart tube is outgrowing the pericardial chamber in length exaggerated at this time, and the distance between the attached cephalic and caudal ends of the heart is actually being shortened by the process of flexion in the embryo, the cardiac loop is twisted in the shape of a U with its upright limbs attached to the body of the embryo. The fusion of the heart primordia was ac-

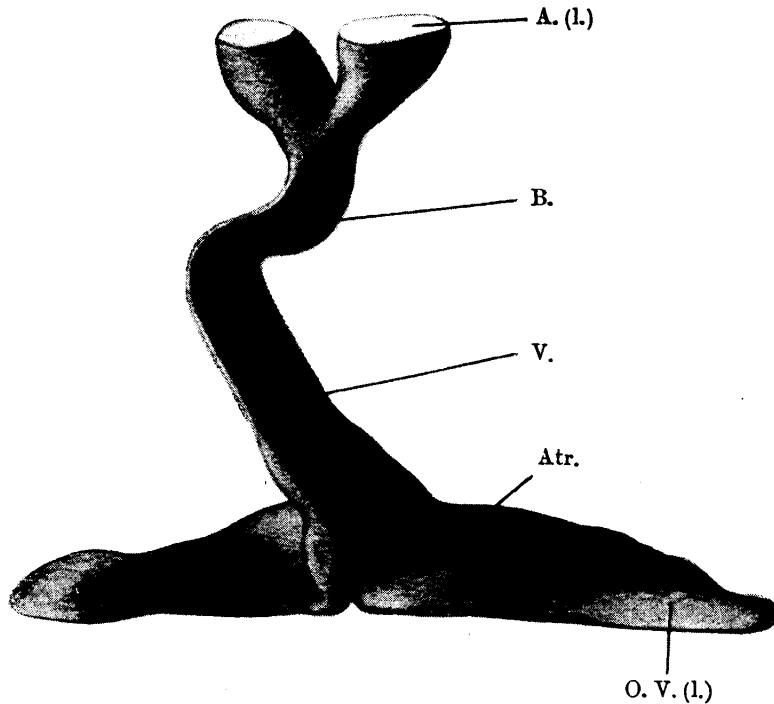


Fig. 22. Reconstruction of endothelial heart tubes of a duck embryo of fifteen pairs of somites. $\times 100$. A. (l.) = Aorta (left), Atr. = Atrium, B. = Bulb, O. V. (l.) = Omphalomesenteric vein (left), V. = Ventricle.

completed, and the auricular canal was not yet found in this embryo, see Fig. 23.

Discussion.

In our specimens, stage 1 shows no sign of coelomic spaces. In stage 2, the discontinuous formation of the intra-mesodermal spaces is noted in the cranial portion of the embryonic shield. These small intra-mesodermal spaces are the source of the pericardial cavity. In the mammals, the formation of the pericardial cavity by fusion of small vesicular intra-mesodermal spaces has long been recognized. The work of *Yoshinaga* on the guinea-pig confirms that of *Strahl* and *Carius*, regarding the nature of the formation of the pericardial cavity, but further shows that the lateral wings are the first to appear, whence the coelom develops craniomedially. *Streeter* showed a similar sequence of development in the pig, and *C. L. Davis* in the human embryo. *Yoshinaga* stated that the pericardial cavity commences simultaneously in the multiple foci, separating irregularly by mesodermal bridges

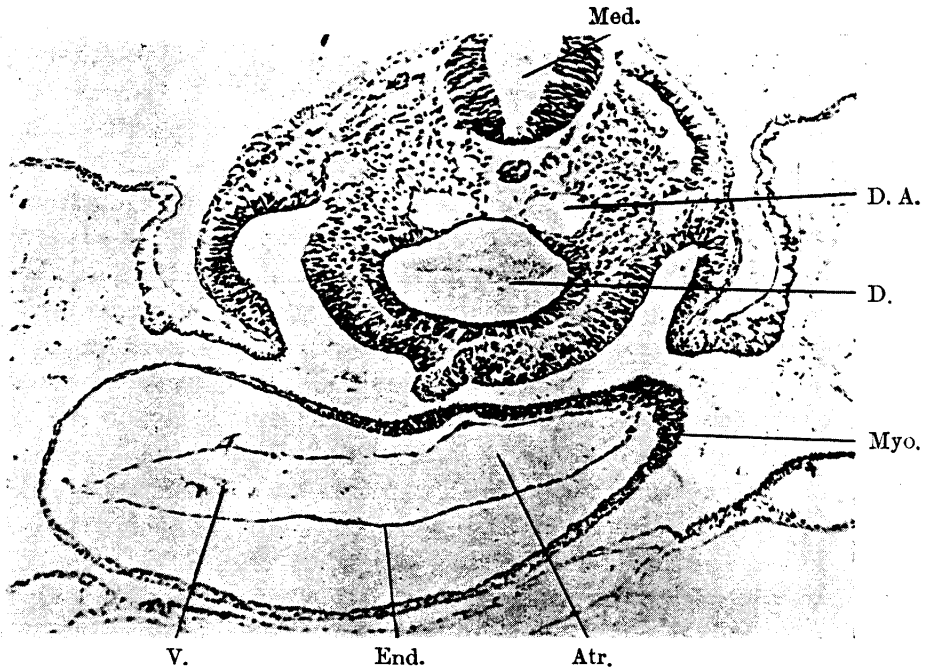


Fig. 23. No. 32. Transverse section through atrio-ventricular region. $\times 95$.
 Atr. = Atrium, D. = Fore-gut, D. A. = Dorsal aorta, End. = Endocardium, Med. = Medullary tube, Myo. = Myocardium, V. = Ventricle.

throughout the lateral plate in the cranial portion of the embryonic shield and in the pericephalic mesoderm. These multiple coelomic spaces become confluent to form a single pericardial cavity, having an inverted-U shaped, when the mesodermal bridges at the middle line of the pericephalic mesoderm have ultimately disappeared, and in consequence, the bilateral pericardial cavities already widely confluent, communicate at this time from side to side. The present author has observed in the pigeon embryo that the origin of the pericardial cavity is like that of the mammals, in the early stage the bilateral pericardial cavities do not communicate with each other, but they are running cranio-caudally aside from the median plane.

In stage 3, in which the first sign of the formation of angioblasts was observed, the intra-mesodermal coelomic space spreads out cranially into the pericephalic mesoderm. Both of pericardial cavities are never communicated with each other in such an early period. It is the difference between the birds and mammals in the development of the coelomic space.

As to the origin of the angioblast, there are two theories held by the earlier investigators, namely, intra- and extra-embryonic origin theory. The extraembryonic theory was advanced by *His* who made his

observation on the flat embryonic shield of the chick. It is supported by many eminent workers such as *Hertwig*, *Kölliker*, *Minot* and others. According to *His'* *Einwachsungslehre* with which the theory of the parablast was first connected, the early blood-vessels of the embryonic shield are formed by a sprouting or ingrowth of the preexisting endothelial lining of the blood-vessels which had previously developed in the extraembryonic vascular area. The ingrowth of the blood-vessel anlagen into the embryonic area takes place as the solid, tenuous sprouts of cells which are primarily found to be anastomosed with each other, to form a close network in the area pellucida. These sprouts of cells enter into the embryonic area through the space between the splanchnopleure and entoderm, until they reach the somitic region where they anastomose and become canalized to form hollow vessels. Ultimately, this network of the endothelial lining forms the dorsal aortae, uniting in a longitudinal direction. The ingrowth of the endothelial sprouts is not limited to the somitic portion, but takes place also in the cranial part, entering from the lateral margin of the embryonic shield, through the space between the splanchnopleure and the entoderm, until they reach the heart anlage where they develop the endocardium. The endothelial sprouts spread out, forming the ventral aortae and the blood-vessels of the cranial region. The former blood-vessels will be connected with the dorsal aortae which are prolonged cranialward by the sprouting and in the same way by turning over the blind end of the pharynx ventralward. *Bremer* in the rabbit derives the angioblast from which the aortic arches and lateral heart primordia are formed from an extra-embryonic source and furthermore states his belief that this is a universal process in vertebrates. *Vialleton* and *Evans* studied the development of the intraembryonic blood-vessels in the birds and came to the conclusion that in the birds the greater part of the descending aortae is developed by a conversion into a continuation of the innermost strand of the capillary plexus, extended into the embryonic shield from the neighbouring yolk sac. Many other investigators support this theory while some others do not accept it.

The intraembryonic theory of vasculogenesis, first advanced by *Rabl*, has associated with it the name of *Rückert* whose investigations established the intraembryonic origin of blood-vessels in all the classes of vertebrates. Experimentally, the name of *Hahn*, *Miller* and *McWhorter*, *Reagen* and *Stackard* must be included among these supporting the intraembryonic origin of vascular tissue, the last named specifically stated that the heart endothelium of *Fundulus* arises in situ. More recently, the work of *Yoshinaga* demonstrated the origin of the heart primordia from the cardiogenic plate is essentially the manner seen in the human. *Davis* supported the intraembryonic origin theory, but he

states that it is difficult, in the face of the facts above stated, to share *Evans'* opinion regarding a strong possibility of their extraembryonic origin.

It can be stated with certainty that no connection between the well differentiated angioblastic tissue of the yolk-sac and that of the heart. It is noted that the yolk-sac circumscribing the cephalic end of the embryonic disk is conspicuously lacking in vascular tissue. Also the mesoderm, lateral to the forming pericardial coelom, is closely applied to the entoderm and there is not a slightest evidence of migration of extraembryonic mesenchym through this barrier. Lastly, the angioblasts are joined to the mesoderm in which mitotic figures can be recognized, that is, remotely removed from an extraembryonic source of origin.

The first sign of appearance of the angioblasts is found in the embryo having three somites. It is found almost in the same stage with the chick, the goose and the pigeon.

In stage 4 and 5, the coelomic space is much enlarged especially in the heart region. It is called the pericardial cavity. Cardiogenic plates are much more defined and heavier than that of preceding specimen. The angioblast increases its number and groups here and there, but the potential lumen is not found in the group of angioblasts.

In stage 6, the endocardium is a flattened sheet of endothelial strands forming an angioblastic plexus. Many of the strands are solid and situated on the ventral surface of the pharynx. They are arranged in an irregular manner, and we can not find the marked line in the median plane, but the symmetric arrangement of these strands is found in the region distalward to the anterior intestinal portal. Some of these endothelial strands become canalized to form hollow vessels, that is, they have the character of the endothelium. The mode of formation of the endocardium is like that of the pericardial cavity, and it commences simultaneously in the multiple foci. These findings were already proved by *Minot*, *Davis* and others. In the pigeon heart the canalization phenomena are first found in the six somite stage, and in the duck in the five to six somite stage.

In stage 7, the first sign of appearance of the ventral mesocardium is indicated. In the chick, *Funccius* found it in an embryo having seven somites, *Duval* in the eight somite stage and the present author has proved in the pigeon embryo having seven to eight somites. It is found in the duck embryo with six to seven somites, so the first appearance of the ventral mesocardium, in the birds, occurs almost in the same time, namely, six to eight somites.

The rupture of the ventral mesocardium, in other words, the coalescence of both pericardial cavities, occurs at first in the middle

portion of it at the eight to nine somite stages in the duck. *Funccius*, *Patten* and others have proved the same thing in the chick. My observations upon the first sign of destruction of the mesocardium ventrale in the duck is identical with that of the chick, but the remnant of the ventral mesocardium is found still at later stages in the bulbar region, and even in the twelve somites stage it can be found distinctly.

In stage 8, both the lateral cardiogenic folds have come into contact at the middle part which is situated through some sections proximalward to the anterior intestinal portal, and is now uniting with each other. Some workers claim that the lateral myocardial tubes are subdivided into many individual portions by the demarcations prior to the fusion of the lateral myocardial tubes; in the chick, *Duval*; in mammals, *His*, *Martin*, *Kölliker*, *Mollier*, etc. *Mollier* declares, "An den beiden Herzröhren ist aber kurz vor ihrer Vereinigung schon eine Gliederung bemerkbar." In our specimens, likewise in the pigeon, there can not be noted any distinct demarcation on the surface of the myocardial tubes.

The network of angioblastocords which spreads widely on the ventral surface of the pharynx, is now gathered in the median plane. The angiocyst which is running in the middle line, perhaps belongs to the bulbar portion. Compared with the next stage, this most prominent angioblastocord belongs to the endocardial tube of the right side.

In stage 9, both the pericardial cavities approached and united with each other in the middle portion, consequently the ventral mesocardium is ruptured in this portion. Although, externally, a deep sulcus is found in the median line of the myoepicardial mantle, paired cardiogenic folds have completely fused with one another except in the atrial region. On the ventral wall of the pharynx, there are two endothelial tubes running side by side. In the middle part, they touch and fuse with each other in the proximal part of the ventricle. In the bulbar region the endothelial tube of the right side markedly exceeds in caliber that of the left side. Compared with a little younger embryos, it can be stated that from the network of angioblastocords two principal canals are developed and the leavings of angioblastocords atrophy and disappear. On the contrary, compared with later stages, the fusion occurs at first in the bulbar and bulbo-ventricular region, whence cranially the delicate left tube atrophies and disappears, and the right tube becomes the genuine bulbus ordis. Asymmetry of the heart tubes, either before or after the fusion takes place, has been noted in the ferret embryos as in the *Perameles obesula* of Miss *Parker* and likewise in the 5.7 mm dog embryo of *Bonnet*. There seems to be a tendency for the two endothelial tubes, even before the fusion, to curve and to be shifted distinctly as a whole to the right side with the concavity of the bend facing to the left. *C. Wang* noticed that the ventricular portion of the heart is the first to

fuse, and situated on the right side of the embryo (a ferret embryo 3.14 mm in length with 13-14 somites). In the guinea-pig, the fusion of the lateral endocardial tubes takes place at a relatively early stage of development and the first united part corresponds to a middle third of the ventricle and lies precisely on the midsagittal plane. In an embryo of *Perameles nasuta* having 15-16 somites, Miss *Parker* pointed out that the fusion of the lateral endothelial tubes took place first in the region of the bulb. In the cat (*Schulte*), and in the human (*Davis*) it was proved that in addition to the interventricular union the paired endothelial tubes fuse at the bulbo-ventricular isthmus and again in the region of the aortic bulb, and that between these points the left tube atrophies and disappears. In the birds, the subject was less studied, namely, *Minot*, *Duval* and others indicated only the fact that the fusion of paired endothelial tubes took place first at their middle portions. Recently, *Patten* claimed "In the 28 hour chick, the endocardial primordia are approximated to each other and by 29 hours they fuse in their mid region to form a single tube." In the duck the fusion of the two endothelial tubes occurs simultaneously both in the region of the bulb and the proximal ventricular portion, and between these points the left tube markedly exceeds in caliber that of the right side, and before long the latter atrophies and disappears. *Wang* pointed out the loop formation with the subdivision of the heart (atrium, ventricle, bulbus, etc.) in the ferret embryo, before the endothelial tubes are fused. But in our specimen there is no loop formation, nor can the subdivision of the heart be marked out on either of the endothelial tubes before they are fused together.

In stage 10, the dorsal mesocardium first appeared. The fusion of the two endothelial tubes has been progressed, and we can find that besides the bulbar union the fusion occurs at three points in the ventricular portion.

In stage 11, there is a distinct unfused part between the bulbar and the ventricular union.

In stage 12, the fusion of the cardiogenic folds is almost completed, but in the atrial region the endothelial tube is divided into two tubes.

In stage 13, the ventral mesocardium has been ruptured entirely. The heart tube is almost straight, and the union of the endothelial tubes is not yet completed. Blood-cells are first seen in the heart, especially in the atrium and the sinus venosus.

In stage 14, the dorsal mesocardium has the maximal length in the ventricular region and it is just ruptured in its middle point.

In stage 15, the union of the cardiac anlages has been completed and the cardiac loop is twisted in the shape of a U.

Summary and Conclusions.

Blood-cells: extraembryonically it is found in very early stages, but it first appears in the heart, in the 13–14 somite stage.

Pericardial cavity: the first sign of its appearance is indicated in the 2 somite stage. It commences simultaneously in the multiple foci, and thus formed intramesodermal spaces become confluent to form a single pericardial cavity.

Angioblast: it is derived from the cardiogenic plate, and first appears in an embryo with 3 somites. It is fundamentally bilateral in origin, and at very early stage are the two sides anastomosed with each other in the cranial region.

Myocardium: it is formed from paired cardiogenic folds. There can not be noted any distinct demarcation on the surface of the myocardial tubes before they are fused together. The folds are first fused at the middle part in the 7–8 somite stage, and the fusion is completed in the 12 somite stage.

Mesocardium: the ventral mesocardium first appears in the embryo having 6–7 somites, and ruptures at its middle part in the 8–9 somite stage. Its remnant is observed in still later stages, namely, we can find it in an embryo with 12 somites. The dorsal mesocardium forms between the 9 and 10 somite stages, and rupture between the 15 and 18 somite stages.

Endocardium: it is formed from angioblasts. It has the endothelial character in the 5–6 somite stages, and the paired tubes are derived from it in the 9 somite stage. The endothelial tubes unite in the bulbar and the ventricular region. From the network of angioblasts which are spread at the cranial portion of the embryonic shield from which a pair of aortic arches are derived. In the bulbar region, the right endothelial tube markedly exceeds in caliber that of the left side. The latter atrophies and disappears, and the former makes the genuine bulbus cordis. Caudally, on the contrary, the left tube exceeds that of the right and the former is situated sinistro-ventrally to the latter. In the 13–14 somite stages the fusion of the paired tubes is almost completed, and the ventricle and atrium may be distinguished by the atrio-ventricular constriction.

In closing, I wish to express my cordial thanks to Prof. Dr. *J. Shikunami* for his kind criticism and advice.

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