

Resumen por G. Carl Huber, por el autor Tanzo Yoshinaga.

Contribución al estudio del desarrollo temprano del corazón de los mamíferos, con especial mención del del conejillo de Indias.

Las observaciones publicadas en el presente trabajo se basan en el estudio de una serie de estados muy próximos en el desarrollo de los embriones del conejillo de Indias, cortados en serie de 5 a 10 $\mu$  de espesor, tanto en el plano sagital como en el transverso. Los estados estudiados comprenden el periodo de desarrollo desde el duodécimo al décimo-quinto día después de la inseminación, o sea desde el momento en que aparecen por primera vez los angioblastos en la región más tarde ocupada por el corazón hasta el estado de corazón en forma de S. Los estados muy próximos han sido reconstruidos en placas de cera mediante el método de Born, y el estado más joven presenta el comienzo de la formación del espacio pericárdico.

Las pruebas obtenidas mediante este estudio son interpretadas por el autor como una demostración de que los angioblastos que han de formar el futuro corazón tubular endotelial derivan independientemente del mesodermo de la esplancopleura. El esbozo de los pliegues miocárdicos y su desarrollo progresivo son objeto de una discusión por parte del autor, quien presenta su desarrollo en una serie de figuras basadas en reconstrucciones.

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A CONTRIBUTION TO THE EARLY DEVELOPMENT  
OF THE HEART IN MAMMALIA, WITH SPECIAL  
REFERENCE TO THE GUINEA PIG

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TWENTY-THREE FIGURES

INTRODUCTION

Since the fundamental investigations on the development of the heart in mammals by His, Born, and others were published, many prominent investigators have contributed to our knowledge concerning the earlier stages of development of the heart and of the pericardial cavity in representatives of almost all classes of vertebrates. The earlier workers began their investigations after the developmental stage in which the embryonal heart tube had already assumed the complete S form. From the results of their work, however, only very general conclusions can be drawn. In many important details the literature shows contradictions, while each theory advanced has been supported by investigators of recognized ability.

The following work was undertaken at the suggestion of Professor Huber. I take this opportunity to express my hearty thanks to him for the use of his collection, which he placed at my disposal, and for his invaluable help. This work concerns itself chiefly with the origin of the endothelial tubes, the early development of the pericardial cavity, and the mode of confluence of the bilateral myocardial tubes, written with the hope of contributing something of interest to the early development of the heart.

The material for this study was obtained from many uninterrupted series of embryos of the guinea pig, from the embryological

collection in the Department of Anatomy of the University of Michigan, prepared by Professor Huber. The great majority of the embryos were fixed in Carnoy's fluid and all were cut into either cross or sagittal series at either  $5\mu$  or  $7\mu$  thickness, also a few at  $10\mu$  thickness.

In a study of the development of the endothelial and the myocardial tubes and of the pericardial cavity, to show their successive developmental changes and their relative topographical relations, a number of models were prepared according to Born's method of wax-plate reconstruction. These were made at a magnification of 300 diameters, with the aid of the projection apparatus, by superimposing the drawings of every section of the cranial part of the embryo. The thickness of the wax plates was changed in proportion to that of the sections from 1.5 to 2.1 and 3 mm., respectively. All the models described here are deposited in the Department of Anatomy at the University of Michigan.

All the figures of the sections presented here were drawn at relatively high magnifications with the aid of the camera lucida and subsequently reduced to the proper size in reproduction. The figures of the models were prepared according to the method described by Doctor Atwell. I am grateful to Doctor Guild and to Mr. Smith for help in preparing the wax plates.

#### LITERATURE REVIEW

Before considering the material studied in this investigation, I wish to refer to some of the theories held by earlier writers. I do not wish to give a complete résumé of the historical developments, but rather briefly to indicate the representative opinions directly concerning the subject.

In the embryonic shield of mammals at first there are present two pairs of longitudinal vessels, one of them situated near the middle line on the entoderm on each side representing the supra-intestinal longitudinal blood vessels, and the others are found primarily on the entoderm approaching the lateral margins of the embryonic shield and these representing the subintestinal longi-

tudinal blood vessels. In the cephalic portion of these latter the heart is formed, the walls of these vascular anlagen being peculiarly dilated and thickened. From this ontogenetical standpoint, the early development of the heart is merely a part of the development of the intra-embryonic vascular system.

In the survey of the literature on the subject of the origin of the intra-embryonic blood vessels in mammals, the theories held by the earlier investigators, and still maintained, can be divided into three categories.

The first theory was advanced by His, who made his observations of the flat embryonic shield of the chick. Hertwig, Kölliker, and others supported this theory. According to the *Einwachsungslehre* of His, 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 extra-embryonic vascular area. The ingrowth of the blood-vessel anlagen into the embryonic area takes place as solid, tenuous sprouts of the 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 of the blind end of the pharynx ventralward.

Türsting studied the development of the aortae in the rabbit and confirmed the conclusion of His, namely, that these vessels are

formed by a longitudinal anastomosis of the ingrown endothelial sprouts, derived from the extra embryonic area, through the space between the splanchnopleure and the entoderm. He noticed the early connection of the dorsal aortae with the vitelline plexus.

Vialleton and Evans studied the development of the intraembryonic blood vessels in birds and came to the conclusion that in birds the greater part of the descending aortae is developed by a conversion into a continuation of the aortae of the innermost strand of the capillary plexus, extended into the embryonic shield from the neighboring yolk sac.

Lewis investigated the intraembryonic blood vessels in rabbits from eight and one-half to thirteen days after insemination, and claims that from the network of vessels in the splanchnopleure of the yolk sac all intraembryonic vessels are apparently derived as offshoots. The network ends mesially in two aortae. With the formation of the pharynx, this net is so folded as to produce dorsal and ventral aortae with the connecting first aortic arch.

Bremer recently repeated the investigation of the same material and came to practically the same results. His summary is given as follows: "In the rabbit, the dorsal aorta, the first aortic arch, the conus arteriosus, and the lateral heart are all parts of an original network of angioblast cords, derived from the extraembryonic plexus of blood vessels. Those portions of the network which are mechanically favored in their position persist, the other portions disappear. Although dealing in this paper with the development as found in rabbit embryos, I have examined various other species, as the chick, pig, sheep, etc., and feel satisfied that in all essential points the story of the development of these primary vessels in other vertebrates will be found similar to that here described."

Many other investigators support this theory, while still others do not accept it. Ranvier seems to think that the *Einwachsungslehre* of His must be regarded as a simple hypothesis, and states: "Mais il est clair qu'aucun embryologiste n'a pu suivre ce développement continu par bourgeonnement dans le corps même de l'embryon; c'est là une simple hypothèse."

The second theory of embryonic vasculogenesis was first advanced by Rabl, who asserts that the first aortic arch in amphibia

is formed by the accretion and extension of the endothelial cells proliferated in the paired heart rudiments, when these endothelial tubes were developed. Moreover, he applies this possibility to other blood vessels of the embryo, in which they are formed by the extension of the preexisting endothelial cells, as, for instance, can be seen in the regeneration of the capillaries. His statement reads as follows: "Die Beobachtung, dass bei den Amphibien die ersten Aorten Bogen durch Auswachsen des Endothelsäckchen entstehen, legt uns aber noch die Frage nach, ob es vielleicht auch das Endothel aller anderen Gefäße in letzter Instanz auf die Zellen des Endothel-säckchens zurückzuführen sei, mit anderen Worten ob nicht vielleicht all Gefäße in derselben oder in ähnlicher Weise entstehen, wie die Capillaren."

Furthermore, in his later work, "The Theory of the Mesoderm," he repeated his assertion: "Ich habe die erste Entwicklung der Gefäße namentlich an den Aorten wiederholt genau verfolgt, und ist mir kein Fall erinnerlich, der mich an der Ueberzeugung irre gemacht hätte, dass neue Endothelen immer nur aus bereits bestehenden ihren Ursprung nehmen."

Rückert investigated the early development in the eggs of selachians, in which at first the subintestinal veins can be seen in the anterior embryonic shield. Here the endothelial cells are produced from the ventral angles of the lateral plates, detaching as free angioblasts, which subsequently assume forms of the cellular groups or chains between the splanchnopleure and entoderm. With regard to the anlage of the aortae in the anterior part of the embryonic shield, Rückert assumes still further that their origin is *in loco* and that here are anticipated the adjacent mesodermal somites, subordinately the dorsal wall of the gut comes under consideration.

P. Mayer discovered the transverse blood vessels in the eggs of the torpedo, connecting the subintestinal veins with the aortae, and he attributed their origin to the emigrated cells from the ventral parts of the mesoderm, wandering along the gut wall dorsalward. Rückert agreed with the opinion of Mayer. In the vascular development of the embryo he claimed that the angioblasts still arise *in loco*, for example, the blood vessels of the pronephros

from the visceral walls of the somite. The angioblasts of the mandibular blood vessels from the visceral plate of the second cranial somite as well as from the wall of the foregut.

Raffaele, Emmert, and others have accepted the local formation of the embryonic blood vessels and are in agreement with the idea of Rückert. After the first publication of his work in 1888, Rückert investigated all classes of vertebrates and confirms his claims to the local formation of the embryonic blood vessels. Concerning the development of the chick, he expressed himself as follows: "So findet also beim Huhn statt des Einwachsens der Gefässanlagen eine von der Peripherie des Keimwalles gegen den Embryo zu fortschreitende Differenzierung derselben aus dem Mesoderm statt, was mit den Beobachtungen an Selachieren übereinstimmt. Auch innerhalb des Embryo entstehen beim Huhn die Gefässanlagen durch locale Ausschaltung des Zellen Materials aus dem Mesoblast, wovon ich mich bei der Herz so wie der Aortenbildung überzeugt habe."

Mollier reached the same conclusion, confirming Rückert. After thoroughly studying the material of a wide scope, he concludes as follows: "Es lässt sich also für die Genese der embryonalen Gefässe der Amnioten zur Zeit ein Urteil dahin fassen, dass die Lehre von der localen Entstehung der Gefässzellen auch hier Geltung besitzt und dass die von His und Vailleton gegebenen Flächenbilder, ferner die Rekonstruktionsbilder von Türstig in dem Sinne zu deuten sind, dass die im Embryo sichtbaren ersten Gefässzellenstränge nicht als Sprossen ausserembryonaler Gefässanlagen entstanden sind, sondern vielmehr ihre Entstehung aus einzelnen, in loco entstandenen und netzförmig vereinigten Gefässzellen nehmen."

Sobatta not only supported the theory of Mayer and Rückert, but he also emphasized that those blood vessels found on the walls of the yolk sac were derived from the intraembryonic blood vessels as a result of their continuous outgrowth.

New light was shed on this contradictory evidence by a number of investigators who employed the methods of experimental embryology and were able to show that the yolk-sac angioblasts may be kept out of communication with the intraembryonic blood

vessels, thus lending evidence in favor of the local formation of the embryonal blood vessels.

The mechanical separation of the vessels of these two portions was employed by Gräper, Hahn, Miller and McWhorter. These workers have obtained endothelium on both sides of the chick embryo, in which one side was severed from the extraembryonic blastoderm. A further strengthening of the theory of the local formation is found in the recent experimental work by Reagan, who writes as follows: "In conclusion it is well to consider the following recently established facts which should share in defining our morphological interpretation. The yolk sac is not necessarily the site of formation of the earliest blood vessels. Intraembryonic blood vessels develop in situ when communication of the extraembryonic vessels with intraembryonic tissues is prevented by chemical or mechanical means."

The method of exposing the developing embryo to diluted anesthetics was employed by Stockard, who claimed as a result of his work that in *Fundulus* embryos the heart endothelium and the aorta arise in loco within the embryo, as the blood vessels, even the mesoderm are absent in the yolk sac in the cranial portion.

Among the investigators who have accepted the local formation of the intraembryonic blood vessels, opinions are still quite divergent at present as to from what part of the embryonal blastoderm the angioblasts are differentiated. I shall not again thoroughly discuss this point, as I have already done so in my previous paper on this subject. But I shall add that there are many authors who consider the origin of the angioblasts in mammals as derived from the entoderm. Martin figures a cross-section of a cat embryo 2 mm. long and in his prominent "*Lehrbuch der Anatomie der Haustiere*" states: Während man über die erste Entwicklung der Blutgefäße im Embryonaleib noch nicht im Klaren ist, kennt man die Bildung der peripheren Gefäße genau. Ihrer Abstammung nach sind die Innenwänd (endothelium) auch der embryonalen Gefäße wie die des Herzens entodermaler Natur, während die übrige Wand vom Mesoderm geliefert wird. Ich finde bei Katze die erste gefäßbildenden Zellen und Zellgruppen eininnigsten Zusammenhand mit dem Entoderm des Darmes, ja so



gar schon rundliche Spaltbildungen in Entoderm selbst. Auch die ausserembryonalen Blutgefäße sind nach Rabl entodermalen Ursprungs." In the figure depicted by Martin, angioblasts can be seen, connected with both the mesoderm and the entoderm.

In the study of a perameles embryo of 6.08 mm. in length, Miss Parker says with regard to the origin of the endothelial cells: "The evidence of this stage does not justify any definite statement with regard to the origin of the endothelium of the heart." And, moreover, she adds, that in an earlier stage the appearance by no means excludes the possibility of the entodermal origin of the endothelium.

After investigating the origin of the endothelial and blood cells in the embryo of the ferret, Wang comes to the following conclusion: "The facts revealed by the study of the early stages in the development of the ferret point to the conclusion that, whilst blood cells and vascular endothelium are closely related to each other and are formed invariably between the mesoderm and entoderm, there is evidence to show that, in the ferret, the origins of these two vascular elements are separate and distinct—the blood cells arising from the entoderm and the vascular endothelium from the mesoderm."

"If the biphyletic origin of the blood cells and vascular endothelium is to be accepted, two more points still remain to be solved, namely, how, when, and where the first blood cells enter the circulation. Unfortunately, the ferret embryos, at present worked upon, provide no definite evidence on this point, but it is quite clear that angioblast cells are formed outside the embryonic area, and that blood vessels are formed inside the embryonic area, and are at first devoid of blood corpuscles."

We shall now briefly review the data concerning the mode of the fusion of the bilateral heart anlagen; there are many divergent opinions.

Since Hensen first declared that in mammals the heart anlage is bilateral, one on each side of the embryo not far from the mid-sagittal plane and on the ventral aspect of the pericardial cavity, it was long believed that these two lateral heart anlagen ultimately came in contact and fused together in the middle ventral

surface of the embryonic shield, forming a single secondary heart tube. Even though the secondary fusion of the bilateral endothelial tubes is universally acknowledged, there are many contradictory theories concerning the formation of the single myocardial heart anlage together with the mode of fusion of the bilateral primitive pericardial cavity. In his work on comparative embryology, Balfour thus speaks: "In mammals the two tubes out of which the heart is formed, appear at the sides of the cephalic plates, opposite the region of the mid and hind brain. They arise at a time when the lateral folds which form the ventral walls of the heart, are only just becoming visible. On the formation of the lateral folds of the splanchnic walls, the two halves of the heart become carried inwards and downwards, and eventually meet on the ventral side of the throat. For a short time they here remain distinct, but soon coalesce into a single tube."

Minot writes: "In mammals by the bending down of the layers and the expansion of the coelom the vorderdarm is shut off and the lateral heartanlagen are brought together in the median line below the vorderdarm, and there fuse into a single thick tubular wall around the double endothelial heart; it is not long, however, before the endothelial tubes also fuse into one. As in the chick the two mesothelia, when the median heart arises, form a membrane (mesocardium) by which the heart is attached to the tissue above and below; both mesocardial membranes break through, putting the two coelematic cavities into communication and leaving the tubular heart suspended by its ends."

The theory of the fusion of the lateral folds of the splanchnic walls, enclosed within the laterally placed pericardial cavities at the ventral side of the foregut in mammals, as in amphibia or birds, as many investigators have asserted and now believe, is supported by the following authorities: Balfour ('81), Strahl and Carius ('89), Tandler ('12), Wilson ('14), Bryce ('08), Minot ('92) Bailey ('12), Schultze ('15), Dandy ('10), Martin ('02), and Arey ('17). From this it would seem that, as many of the above named authors claim, the heart must be provided, at least temporarily, with a ventral and a dorsal mesocardium.

In a similar way in his valuable work on the first heart anlage, Mollier says: "Das Mesocard, ventral oder medial gelegen, ist dem dorsalen der Anamnier zu vergleichen. Ein dem ventralen entsprechendes kann erst nach dem Zusammenstossen beider Pleuro-pericardialhöhlenwände gebildet werden. Beim Meer-schweinchen hingegen liegen die ersten Herzzellenstränge lateral von den Firsten der Darmfalten, und sie werden durch den Darm-schluss gar nicht unter die ventrale Darmwand verlagert, wie beim Kaninchen, sondern rücken, zwischen dorsaler and ventraler Mesocardfalte gelegen, einander näher, bis zur Berührung und endlich Verschmelzung. Doch erfolgt auch hier, wie aus der Figur ersichtlich, der Durchbruch des ventralem Herzgekröses zuerst."

Robinson has pointed out that the formation of the foregut is mainly attributed to the unproportionately rapid development of the embryo over the relatively stationary line between the embryonal and extraembryonal areas. If the idea were true, that lateral folds of the mesoderm converge ventrally until their entodermal covering has met together in the ventral middle line and both lateral pericardial cavities have fused together beneath the ventral walls of the foregut, then the heart is not only attached by the dorsal mesocardium to the foregut, but also by the ventral mesocardium to the ventral wall of the body. However, Robinson denies this generally accepted idea and the existence of the ventral mesocardium absolutely, at any time in the development of mammals, and he applies this fact to support his theory, that the separation of the foregut from the yolk sac is not due to the tenaciously held process of the tucking in of the margins of the embryonic area, but to the fact that the relatively slow-growing margin is demarcated between the embryonic and extraembryonic portions of the wall of the ovum, which rapidly increase their extent, expanding over the boundary margin. According to him, in mammals, the pericardial mesoderm is present in the pericardial portion of the embryonic area, and it is completely separated into somatic and splanchnic layers before the head fold appears. There is therefore a single pericardial cavity which extends from side to side along the anterior boundary of the embryonic shield. As the head fold is formed, the pericardial region

is reversed and it is carried into the ventral wall of the foregut, where it is present as a U-shaped tube, which communicates with the general coelom at each end. The rudiments of the heart are formed in the splanchnic layer of the pericardial mesoderm. Therefore, after the reversal of the pericardial area, they lie on the dorsal wall of the pericardial cavity, attached to the ventral wall of the foregut by the dorsal mesocardium.

Prior to Robinson, Ravn pointed out the correlation between the formation of the foregut and the forward growth of the embryonic shield. He also thoroughly described the mode of reversal in the primitive pericardial cavity.

On the other hand, many investigators believe that an active backward progression of the foregut opening occurs, in addition to the forward growth of the head fold. They deny the actual fusion of the lateral mesodermal folds in the ventral middle line. Rouviere agrees with Robinson as to the absence of the ventral mesocardium in mammals, while he does not dismiss the meaning of the forward growth of the head fold on the formation of the foregut. According to his account, in a rabbit embryo of 201 hours, both the pericardial cavities (*les deux cavités pariétales*) show the separated bilateral canal on each side, which has grown forward around the anterior end of the head fold and become fused together from a single continuous cavity in the embryo of 207 hours. The splanchnopleure forming the caudal wall of the pericardial cavity assumes a continuous fold, which Tourneux designated as the cardiac fold (*repli cardiaque*) and which he considered as growing backward automatically as a whole. As the free edge of the splanchnopleural fold has progressed always in advance of the primordial heart, no fusion of the splanchnopleure is involved and also no ventral mesocardium is formed.

In a description of the growing processes in the developing chick embryo, having kept them under direct observation while still alive, Gräper asserts that there is considerable evidence in support of the view that the margin of the foregut (*Darmpforte*) moves caudally, concurrently, with the forward growth of the head fold. Moreover, he marked out diagrammatically the mode of the backward progress of the foregut opening and a quite different manner of the closure of the foregut than that of the medullary canal.

Uskow also claims the automatic backward progress of the foregut opening, according to the increase of the pericardial cavity.

In her study of the early stages in the development of marsupials, Miss Parker declares that the forward growth of the head fold doubtless plays an important part in the initiation of the formation of the foregut and that the actual backward growth of the foregut opening, but not the fusion of the lateral folds, brings about the lengthening of the foregut.

In the study of the early development of the heart and cranial blood vessels in ferret embryos, Wang agrees with Miss Parker in the absence of the ventral mesocardium, there being no fusion of this part of the pleuropericardial wall, nor that any part of the gut closure is effected by the fusion of the lateral folds.

#### OBSERVATIONS

##### *Stage I*

The material for this stage consists of many specimens removed from the uterus of the guinea pig thirteen days and twelve hours or fourteen days and eleven hours, respectively, after insemination. Some of them were cut longitudinally and the others transversely.

A. The first embryonic shield which came under consideration, was removed from the uterus thirteen days and twelve hours after insemination and was in a cross-section having a  $7 \mu$  thickness; 233 sections fell to the embryonic shield. The head fold had not begun to develop. The shallow neural groove was present on the surface of the embryo. The primitive streak was well developed and terminated caudally in a shallow notched groove. The mesoderm was thickened in the caudal part of the embryonic area and indicated the allantoic mesoderm. In the notochord there was present the chordal canal at its caudal end, but in other parts it was spread out to form a chordal plate through dehiscence of the ventral wall. The caudal end of the notochord was fused with an area of ectodermal proliferation at the cranial end of the primitive streak.

In the mesoderm there was observed no evidence of mesodermic somites nor could the coelomic cavity be detected. The mesoderm consisted of two lateral wings on each side in the cross-section, separated completely in the middle line by the notochord, except in the cephalad end of the embryonic shield and in the region of the primitive streak. In the cranial end of the embryonic shield, that is, the part distal to the future pharyngeal membrane, in which the ectoderm and entoderm were coalesced, the lateral wings of the mesoderm were fused, continuing caudally into the lateral wings, but sharply terminated against the extraembryonic area cranially and laterally. In this portion of the mesoderm, namely, in the pericephalic mesoderm, it formed a thinner layer than anywhere else. In the primitive streak a large mass of undifferentiated mesodermal cells was fused with both lateral wings, obliterating the demarcation between the mesoderm and ectoderm. In the mesoderm two layers of the cell band could not be distinguished; the dorsal surface of the mesoderm was, in general, compact, its outline was clear-cut; here the spindle-shaped nuclei had a relatively regular arrangement.

Between the dorsal surface of the mesoderm and ectoderm, as well as between the ventral surface of the mesoderm and entoderm, there could be distinguished clear intervals, which could be attributed in large measure to shrinkage. The mesodermal cells were spread out in two or three layers and were spindle-shaped and connected with each other by short protoplasmic processes. In the ventral surface of the mesoderm a loosening of the cell band could be seen, characterized by an increased distance between the respective nuclei. Intercellular spaces became more distinct and wider, the spindle-shaped nuclei had no definite arrangement. In nearly all sections there could be demonstrated some free, isolated cells, detached from the cell band of the mesoderm, lying between the ventral surface of the mesoderm and entoderm, as shown in figure 1. According to His, these cells are identical with the angioblasts. In some sections the angioblasts are connected with the indented ventral margin of the mesoderm by broad protoplasmic bridges;

in some other sections the protoplasmic bridges are narrow, the cells are distinctly pedunculated; in still other sections they are joined to the mesoderm by faint fibrils. Frequently a mitotic figure can be recognized in the mesodermal cells, adjacent to the angioblasts. These findings show that there can be no doubt of a distinct proliferative activity of the mesodermal cells; furthermore, every transitional feature of the migration or the detachment of the mesodermal cells, which apparently are destined to become angioblasts, point out the fact that these angioblasts have originated in the ventral surface of the mesoderm.

Figure 1 was reproduced from the forty-ninth section, counting from the cephalic border of the embryonic shield, and

#### LEGEND LETTERS FOR ALL THE FIGURES

<i>A.</i> , atrium	<i>F.G.O.</i> , foregut opening
<i>Am.</i> , amnion	<i>I.M.S.</i> , intermesocardial space
<i>Ang.</i> , angioblast	<i>L.P.C.</i> , lateral pericardial cavity
<i>Ao.</i> , aorta	<i>M.C.</i> , myocardial cavity
<i>A.V.C.</i> , atrioventricular constriction	<i>Mes.</i> , mesoderm
<i>A.V.Ca.</i> , atrioventricular canal	<i>M.G.</i> , medullary groove
<i>B.</i> , bulbus cordis	<i>M.T.</i> , myocardial tube
<i>B.V.C.</i> , bulboventricular constriction	<i>N.</i> , notochord
<i>C.M.L.</i> , craniomedian limb of the pericardial cavity	<i>P.</i> , pericardium
<i>Co.</i> , coelom	<i>P.C.</i> , pericardial cavity
<i>D.M.</i> , dorsal mesocardium	<i>P.M.</i> , pharyngeal membrane
<i>D.W.P.</i> , dorsal wall of pericardium	<i>P.P.P.</i> , pleuropericardial passage
<i>Ect.</i> , ectoderm	<i>S.A.C.</i> , sino-atrial constriction
<i>End.</i> , endothelium	<i>Som.</i> , somatopleure
<i>Ent.</i> , entoderm	<i>Spl.</i> , splanchnopleure
<i>E.O.</i> , endothelial offshoot	<i>S.R.</i> , septum ridge
<i>E.T.</i> , endothelial tube	<i>S.V.</i> , sinus venosus
<i>F.G.</i> , foregut	<i>T.A.</i> , truncus arteriosus
	<i>V.W.F.</i> , ventral wall of the foregut

Fig. 1 The 49th section of a series of 233 cross-sections of  $7\ \mu$  thickness of an embryonic shield of the guinea pig, removed 13 days 12 hours after insemination. The early stage of the formation of the angioblasts from the ventral surface of the mesoderm.  $\times 150$ .

Fig. 2 The 41st section of a series of 237 cross-sections having a  $7\ \mu$  thickness, removed from the uterus of a guinea pig 14 days 11 hours after insemination. The ventral surface of the mesoderm becomes loosened and angioblasts are separated.  $\times 150$ .

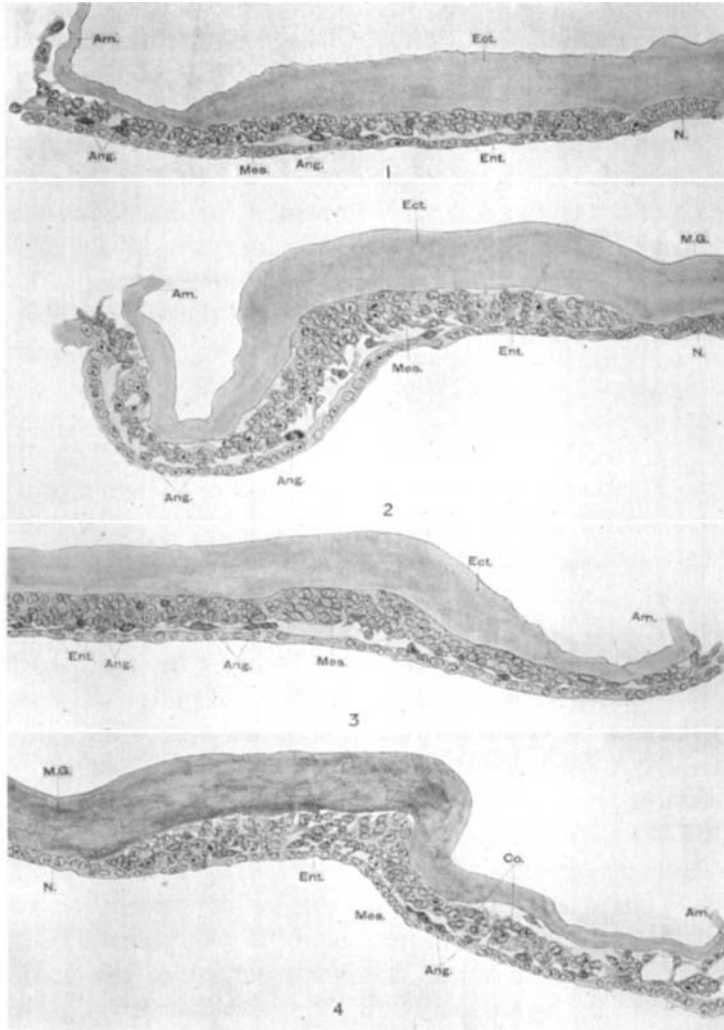


Fig. 3 The 40th section of a series of 162 sagittal sections having a  $7\ \mu$  thickness. This embryonic shield was removed from the uterus of a guinea pig 13 days 12 hours after insemination. The angioblasts form the cell strands between the splanchnopleura and entoderm.  $\times 150$ .

Fig. 4 The 63rd section of a series of 300 cross-sections of  $7\ \mu$  thickness of an embryonic shield of a guinea pig, removed 13 days 11 hours after insemination. In the lateral plate of mesoderm the discontinuous coelom is present.  $\times 150$ .



corresponds approximately to the future hindbrain region, in which region endothelial tubes had been differentiated from the angioblasts in the next stage. As the contour of the entoderm is distinctly demarcated from these cells in our specimens, as the figure shows, it may be excluded from direct participation in the formation of these cells.

B. The next embryonic shield selected for discussion was removed from the uterus of a guinea-pig fourteen days and eleven hours after insemination. It is cut in cross-sections, having a  $7 \mu$  thickness, and 237 sections fall to the embryonic area. The flat incipient head fold has begun to develop, marking definitely the cranial margin of the neural plate. In this portion the ectoderm has been elevated above the surrounding embryonic shield. A broad and shallow neural groove, which gradually narrows cephalad, is present on the surface of the embryo. The primitive streak is well distinguished on the embryonic surface as of a transitional portion from the cranial neural groove into the caudal primitive groove. The notochord has acquired a tubular form at its caudal end in some more anteriorly placed portions, but in the majority of the sections its ventral wall is opened into the yolk sac, to form the chordal plate. The caudal end of the notochord is fused to the cranial part of the primitive streak. No mesodermal somites are recognized nor can any indication of the coelomic cavity be detected.

Figure 2 was reproduced from the forty-first section, counting from the cephalic amnion attachment. The mesodermal layer is thicker than that found in the preceding specimen; this is due partly to the numerical increase of its component cells and partly due to a loosening of the arrangement of the cells. In the mesoderm no layers can be distinguished, the spindle-shaped cells having no definite arrangement. The loosening of the cell is more readily demonstrable on the ventral surface of the mesoderm; its ventral outline forms a zigzag contour, due to the shorter or longer protoplasmic processes which are seen extended from the ventral row of the mesodermal cells toward the underlying space. Moreover, as can be pointed out in the figure, some cells are projected into the underlying space beyond

their surrounding group of cells, while others present a mitotic figure and have their axis directed to the space under the mesoderm and are pedunculated into the underlying space, but remain connected with the mesodermal layer by means of their narrow protoplasmic bridge. There can be seen a few spindle-shaped cells, which appear completely detached from the mesoderm and lie scattered between the mesoderm and entoderm. The area of the distribution of these cells, which we regard as angioblasts, more numerous in this embryo, extends over a wider range than is observed in the previous embryo. A glance at the figure will prove that the origin of these cells is derived from the mesoderm of the splanchnopleure.

In figure 3 there is presented a drawing of a sagittal section of an embryonic shield of approximately the same stage of development as that described under figure 2.

This series belongs to an embryonic shield of a guinea pig, removed from the uterus thirteen days and twelve hours after insemination. It includes 162 sections, having a  $7 \mu$  thickness.

The figure was reproduced from a drawing of the fortieth section, counting from the lateral amnion attachment. This section passed through the flat head fold near its lateral margin. The line of sectioning in this series was almost parallel to the mid axis of the embryonic shield. A study of the series shows that the developmental stage is just prior to the formation of the first mesodermal somite, which is indicated but not completely formed. The primitive streak extends approximately a third of the length of the embryonic shield. The general finding of the mesoderm is similar to that of the foregoing specimen. In the midsagittal plane the cranial end of the chordal plate terminates insensibly in the entoderm, where the pharyngeal membrane will be recognized. Cephalad to this membrane the pericephalic mesoderm is observed; its cranial limit terminates freely at the cranial amnion attachment. This pericephalic mesoderm is continued into both mesodermal wings caudolaterally. The ventral surface of the mesoderm is loosened and presents a coarse appearance. Between the ventral surface of the mesoderm and the continuous layer of ectoderm angio-

blasts can be seen forming cell strands, ranging one after another, approximately parallel to the long axis. In the cross-section these cell strands may be shown as single cut cells. The faintly stained protoplasmic processes or slightly rotated, tenuous protoplasmic fibrils are given off from the surface of the cell strands. Some of these anastomose with each other and others are connected with the cells of the adjacent mesoderm. In this fashion their protoplasmic fibrils form a kind of feltwork between the mesoderm and entoderm. In brief, it is only a repetition of the processes which produce the angioblasts from the mesoderm of the splanchnopleure, as observed in the preceding embryo, but the angioblasts are a step further differentiated.

C. In figure 4 there is presented a cross-section drawing of an embryonic shield of a guinea pig, removed from the uterus thirteen days and eleven hours after insemination. This series includes 300 sections, having a  $7 \mu$  thickness. In actuality this embryo presents only a slight advance in development over that discussed under figures 2 and 3.

The first mesodermic somite is indicated, but not completely formed. The intraembryonic coelomic space, which may be regarded as the future primitive pericardial cavity, considering its topographic position, shows simply a beginning of a very narrow cleavage in the lateral mesoderm of the cranial portion of the embryo. In some sections the two layers of the lateral plate of the mesoderm are separated from each other, and there can be seen narrow, discontinuous clefts between the mesodermic layers, while in other sections the whole mesoderm remains apparently solid. As a transition of these two extremes, in still other sections the coelomic space is shown as little more than a lineal cleavage. In brief, the coelomic cavity is forming from multiple foci and is not connected with the extraembryonic coelom. In the pericephalic mesoderm no coelomic space can be seen.

The figure reproduces a drawing from the sixty-third section, in which there can be seen an irregularly outlined splanchnopleure, from a relatively clear-cut contour of the somatopleure, separated by an incipient slip of the coelomic space. The

splanchnopleure shows a slightly thicker layer of spindle-shaped cells; in some of them mitotic figures are present, indicating a proliferative activity. A number of angioblasts are scattered singly, while some others are grouped in a flat strand between the ventral surface of the mesoderm and entoderm.

Practically the same stage of development as that described in figure 4 can be seen in an embryonic shield, removed from the uterus fourteen days and four hours after insemination.

In figure five we see a drawing of the thirty-second section, counting from the lateral amnion attachment. This series, cut in the sagittal plane, includes 156 sections, having a 7  $\mu$  thickness. This section passes through the well upwardly projected head fold near its uplifted lateral margin. Under this head fold there can be recognized five discontinuous coelom spaces in the lateral plate of the mesoderm, each of which is interrupted by a substantial bridge. The mesodermal cell layer of the splanchnopleure is distinctly thickened and loosened. The spindle-shaped mesodermic cells assume a somewhat irregular arrangement. A number of them have disposed themselves in such a direction that their long axis is vertical to the ventral surface. A number of angioblasts are scattered under the mesoderm of the splanchnopleure and some of them are connected with this layer by their protoplasmic processes. Mitotic figures, seen in some of the mesodermal cells, show their proliferative activity. In some of the sections the discontinuous coelomic space can be seen in the pericephalic mesoderm, but it entirely disappears as it approaches the midsagittal plane, where the mesodermic layer has remained still in a solid condition, as can be found in figure 5B. In this respect this embryonic shield differs from that shown by Robinson and of several other workers. Robinson says that in mammals the mesodermic layer extends through the pericardial portion and is cleft into somatic and splanchnic layers before the head fold is formed. In our specimens the intraembryonic coelomic space is present discontinuously in the cranial region of the embryonic shield and totally absent as it approaches the middle plane of the pericephalic mesoderm, even though the head fold is already formed.

*Stage II*

The material on which the following description of stage II is based consists of several embryos, certain of which are cut transversely and others longitudinally.

A. This specimen was removed from the uterus of a guinea pig fourteen days and eleven hours after insemination. The series includes 307 cross-sections, having a  $7\ \mu$  thickness. A plastic reconstruction of the cranial portion was made with the Born method, and the whole shield, reconstructed for another purpose, was used for this study. This embryonic shield, as the model shows, presents a flat head fold, which is slightly more elevated than in the previous stage. The head fold can be divided into two primary parts. The cranial part is long and wide and projects over the cranial and lateral walls of the cranial body elevation. The caudal part is small and passes insensibly into the spinal portion. There is present a well-developed medullary groove in the cranial portion of the embryonic shield and its caudal end becomes gradually shallower until it disappears at the primitive streak in the caudal fourth of the embryonic shield. Its cranial end is terminated near the cranial extremity of the head fold. The deepest portion of the medullary groove corresponds to the region of the hindbrain plate. A well-marked anlage of the trigeminus is present as a thickening of the ectoderm. There are present three pairs

Fig. 5A The 32nd section of a series of 156 sagittal sections of  $7\ \mu$  thickness of an embryonic shield of a guinea pig, removed 14 days 4 hours after insemination. The mesoderm is thickened and loosened, a number of discontinuous coelomic spaces is present, angioblasts are being produced from the splanchnopleura.  $\times 150$ .

Fig. 5B The 77th section of the same series from which figure 5 was drawn, passing through practically parallel to the midsagittal line. The pericephalic mesoderm shows a relatively thinner layer than elsewhere and lies anterior to the primitive pharyngeal membrane, as the foregut has not yet developed. In accordance therewith, the reversal of the preumbilical region of the embryonic body does not occur.  $\times 150$ .

Fig. 6 The 87th section of a series of 307 cross-sections of  $7\ \mu$  thickness of an embryonic shield of a guinea pig, removed 14 days 11 hours after insemination. The pericardial cavity opens widely, under the thickened splanchnopleura the endothelial tube is first formed in this embryo.  $\times 150$ .

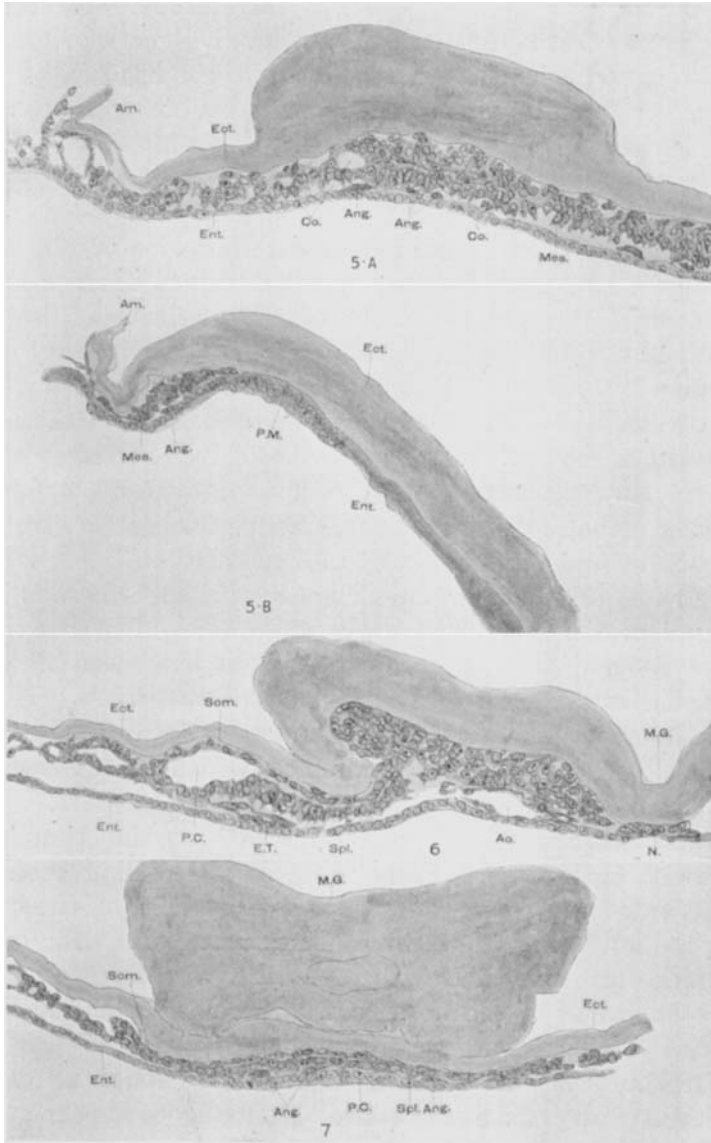


Fig. 7 The 27th section of the same series from which figure 6 was drawn, embracing the nearly anterior margin of the head fold. The pericephalic mesoderm separates into two layers, by the lineal coelom space, which continues into the lateral pericardial cavity.  $\times 150$ .

of mesodermic somites with a fourth pair forming. The foregut has begun to develop in the embryo; for the length of five sections its lumen is invaginated upward into the head fold.

Both the lateral primitive pericardial cavities are presented in the lateral plate of the mesoderm (fig. 6), which is completely separated into two lateral wings by the notochord, except in the cranial end of the embryonic shield, namely, beneath the cranial extremity of the head fold and in the region of the primitive streak. In these places two lateral wings come to fusion. In the mesoderm, which is produced by the fusion of both the lateral mesodermic wings in the middle line, beneath the cranial extremity of the head fold, in front of the pharyngeal membrane, a lineal cleavage can be recognized (fig. 7) by which the mesoderm is separated into two distinct layers. This coelomic space in the pericephalic mesoderm is formed by a forward extension of both the lateral primitive pericardial cavities into pericephalic mesoderm. These communicate with each other through this pericephalic coelomic space, which is now forming the craniomedian limb of the inverted U-shaped pleuropericardial cavity and may be accounted for as the essential future pericardial cavity. This cavity communicates freely with the future pleural cavity, which, in turn, passes into the peritoneal coelom. But this does not communicate with the extraembryonic coelom (fig. 8).

The reconstruction of the whole shield shows that the pleuropericardial cavity corresponds to a vague swelling presented by the ectodermal layer on the dorsal surface of the model along the lateral margins of the neural plate, and their cranial extremities are connected with each other directly beneath the the cephalic end of the head fold. Therefore, the cranial extremities of the primitive pericardial cavity and of the head fold fall practically in the same level. The caudal extremities of both lateral pericardial cavities gradually disappear at the level of the caudal termination of the neural plate. And this corresponds to the gradual diminution of the prominent ectodermic swelling on the surface of the model. In this fashion, therefore, the rhomboidal shaped head fold is surrounded by

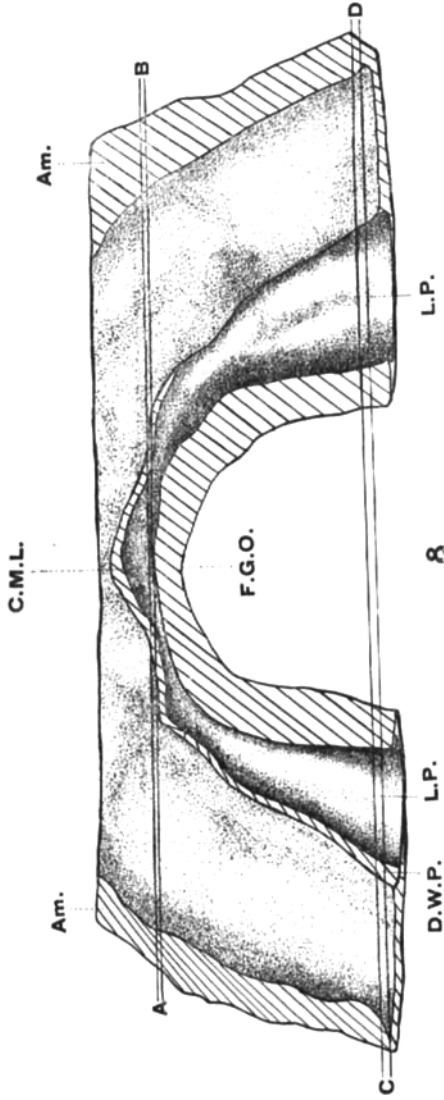


Fig. 8 Dorsal view of the reconstruction of the same embryonic shield (stage II, A), from which figures 6 and 7 were drawn. The dorsal wall of the pericardial cavity and the overhanging head fold have been removed. A-B indicates plane of section of figure 7, C-D indicates plane of section of figure 6. X 100.



the horseshoe-formed primitive pericardial cavity both laterally and cephalad.

In this stage the dimension of the craniomedian limb of the pericardial cavity is yet very narrow. Its ventrodorsal extent is not more than a lineal cleavage (fig. 7), while its craniocaudal length extends throughout five sections. In tracing the lateral limbs from the craniomedian limb, however, the pericardial cavity gradually increases in width until it reaches its maximum opposite to the hindbrain region (fig. 6), and then again a gradual reduction takes place behind this region until the coelomic cavity has completely disappeared in the region of the first mesodermic somite.

On the ventral surface of the model there can be seen the crescentic gut-groove of the entoderm at the cranial portion of the embryonic shield its apex being directed cranialward and deepened gradually until it reaches the opening of the fore-gut, which is invaginated cranialward between the ectodermal head fold and the craniomedian limb of the pericardial cavity, as a horizontal diverticulum of the yolk sac. The base of the crescentic gut-groove is directed caudalward, and gradually becomes shallower, until it has entirely disappeared at the level of the hindbrain. On both sides of the gut-groove and along the fore-gut opening there is a rounded ridge running from the latero-caudal to the craniomedian end; in both position and direction, this ridge corresponds to the horseshoe-shaped pericardial cavity.

In this stage a number of angioblasts are scattered under the considerably thickened mesoderm of the splanchnopleure throughout the full extent of the pericardial cavity. A few angioblasts can be seen in the portion of the narrowly opened craniomedian limb of the pericardial space in which the mesoderm of the splanchnopleure is in close contiguity with the underlying entoderm, and they increase in number toward the lateral limbs. In some sections in which the wide open pericardial cavity is present, endothelial tubes can be seen differentiated from the angioblasts lying under the mesoderm of the splanchnopleura, which is subsequently elevated from the entoderm and projected into the pericardial cavity as a prominent fold,

as depicted in figure 6. As the differentiation of the angioblasts into the endothelial tubes takes place irregularly, the distribution of both kinds of cells intermingles irregularly with reference to the level of sections; for example, even in the wide open pericardial region in some sections a tubular endothelium appears, while in the next succeeding section there are merely scattered angioblasts or cell strands of angioblasts. For this reason the invagination of the mesoderm of the splanchnopleura into the pericardial cavity as a prominent fold cannot be attributed simply to the development of the endothelial tubes, resulting in the increase of their volume. In this embryo, generally speaking, the angioblasts apparently predominate over the endothelium.

The dorsal aortae can be seen developed in the cephalad portion of the embryonic shield, while in many sections they are present as incompletely formed endothelial tubes resting on the entoderm on both sides of the notochordal plate (fig. 6), in their caudal extent they remain as cell strands of angioblasts.

In this embryo the foregut is present as a completely formed short entodermic tube, and the gut-groove, following caudally, is shown as a deep furrow. Not until a later stage is reached are the endothelial tubes completely developed. This corresponds apparently with the development of the human embryo, as described by Graf Spee. In the slightly younger embryonic shield the coelomic cavity in the pericephalic mesoderm is not yet formed in the median plane, where this is separated in many places by thin mesodermic bridges. But this condition is only temporary and both lateral pericardial cavities will communicate with each other, as is shown in this embryonic shield.

B. In figure 9 is represented a drawing of a midsagittal section of an embryonic shield of a guinea pig, removed from the uterus fourteen days and twenty-three hours after insemination. This series includes 172 sections, having a  $7 \mu$  thickness. The figure represents a drawing of the eighty-fifth section, and this passes through the embryo just parallel and just lateral to the midaxis. As reckoned by age, this embryonic shield is just slightly older than that discussed under figures 6, 7, and 8 but,

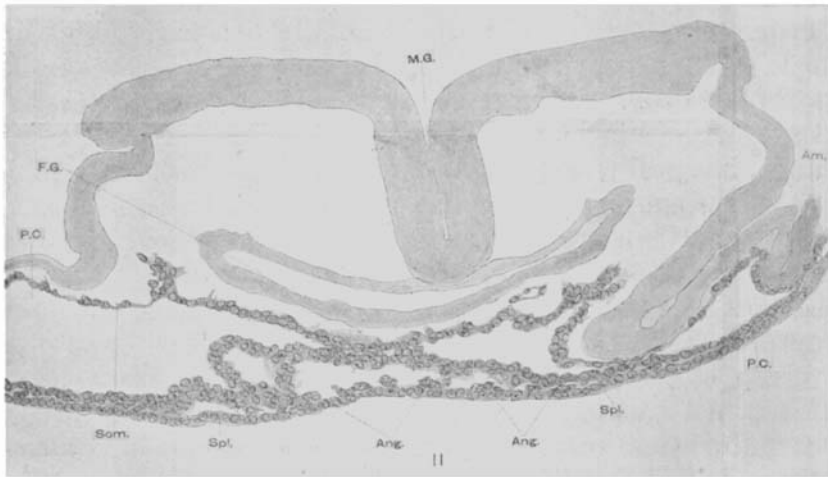
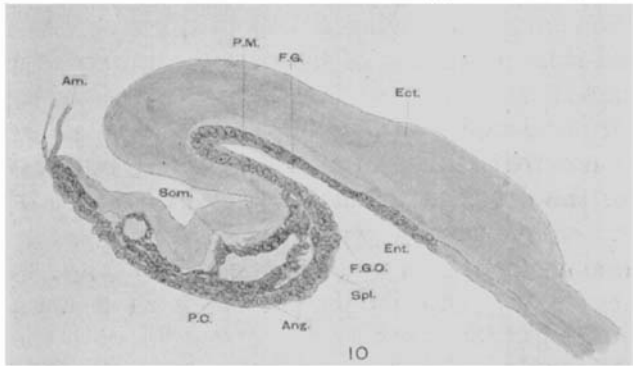
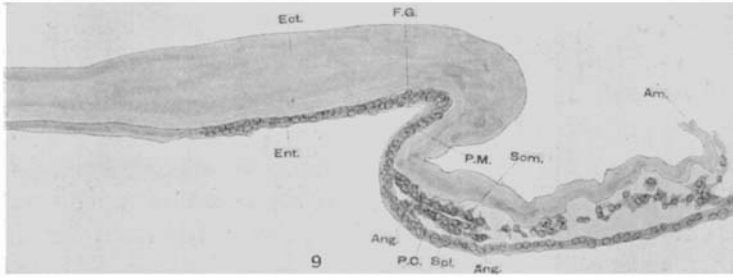
judged by the stage of general development, the same findings can be noted. Here it is noticed that the craniomedian limb of the pericardial cavity is not more than a lineal cleavage in the pericephalic mesoderm, which extends in this plane from the more anteriorly situated extraembryonic mesoderm up to the pharyngeal membrane, where the mesoderm is entirely absent and the ectoderm and entoderm are in close contiguity.

In the peripheral two-thirds of the mesoderm in this section the cells have no definite arrangement, but are loosely and irregularly scattered. This portion can be seen as a continuation of the extraembryonic mesoderm, and the same condition is shown in figure 5B in the younger stage. In the central third of its extent the two layers of the cell band can be distinguished, namely, the mesoderm of the splanchnopleura and the somatopleura, and between them lies a craniomedian limb of the pericardial cavity. Its craniocaudal extent is short and its cranial extremity is approximately on the same level with that of the head fold. Its sagittal long axis forms an obtuse angle with the sagittal long axis of the embryonic shield.

Fig. 9 The 85th section of a series of 172 sagittal sections, having a  $7\ \mu$  thickness. This embryonic shield was removed from the uterus of a guinea-pig 14 days and 23 hours after insemination. The pericephalic mesoderm separates into two layers by the lineal coelem space, through which the two lateral pericardial cavities communicate with each other. The foregut has just begun to develop.  $\times 150$ .

Fig. 10 The 83rd section of a series of 166 sagittal sections of a  $7\ \mu$  thickness of an embryonic shield of the guinea pig, removed 14 days 12 hours after insemination. The craniomedian limb of the pericardial cavity in the pericephalic mesoderm is wide open. The ventral wall of the pericardial cavity in figure 9 forms the caudal wall of it in this figure, as the reversing process occurs in the preumbilical region of the embryonic body, in conjunction with the development of the foregut, which in this embryo shows a longer lumen than in figure 9.  $\times 150$ .

Fig. 11 The 45th section of a series of 318 cross-sections, having a  $7\ \mu$  thickness of an embryonic shield of a guinea pig, removed from the uterus 15 days 14 hours after insemination. This section passes through the forebrain plate near its anterior margin. The craniomedian limb of the pericardial cavity is wide open. The splanchnopleural fold projects into the pericardial cavity, rising from the underlying entoderm. This is especially prominent on the left side of the figure. Between the splanchnopleura and entoderm a number of angioblasts are scattered.  $\times 150$ .



In the younger stage the pericephalic mesoderm is situated anterior to the pharyngeal membrane, approximately in the same horizontal plane with the embryonic shield (fig. 5B). But in this stage it is brought ventrally to the pharyngeal membrane, as the foregut has begun to develop; the reversal of the pre-umbilical portion of the embryonic body accompanies this development.

C. In figure 10 is presented a drawing of a midsagittal section of an embryonic shield of a guinea pig, removed from the uterus fourteen days and twelve hours after insemination. This series includes 166 sections, having a  $7 \mu$  thickness. The figure is reproduced from a drawing of the eighty third section, passing through almost exactly parallel to the midaxis of the embryonic shield. As measured by age, this embryonic shield is slightly younger than that discussed under figure 9, but the general findings of the development are slightly in advance of that of the latter.

The craniomedian limb of the pericardial cavity, which lies under the foregut, extends dorsocaudally in a cranioventral direction, and has increased its dimensions in both the ventrodorsal and craniocaudal directions (fig. 10). Compared with the foregoing embryo (fig. 9), its sagittal long axis forms an angle a little more acute with the longitudinal axis of the embryonic body. Its caudal half presents the crescentic coelom cleavage, directing its convexity caudoventralward, while its cranial half still remains as a lineal slit. The cranial extremity of the craniomedian limb of the pericardial cavity is practically situated on the same level with that of the head fold (fig. 10). It may be demonstrated, when we compare figures 9 and 10, that the backward movement of the foregut opening from the cranial extremity of the head fold is greater than the rate of progress of the head fold forward from a certain fixed point. This actual backward progress of the foregut opening brings about the lengthening of the foregut. The foregut in this embryo is longer than in the preceding embryo. Its cranial extremity is slightly caudad to that of the craniomedian limb of the pericardial cavity. The ventral wall of the foregut

coalesces with the ectoderm, indicating the pharyngeal membrane, while its dorsal wall corresponds to the cranial end of the notochord. The entoderm, which forms the dorsocaudal wall of the craniomedian limb of the pericardial cavity, is reflected from the foregut opening to the ventral wall of the craniomedian limb of the pericardial cavity, representing the so-called cardiac fold (repli cardiaque Tourneux). The cranial wall of the craniomedian limb of the pericardial cavity is formed by the ectoderm, which is reflected from the pharyngeal membrane to the proamnionic region.

A few cell strands of angioblasts can be seen between the mesoderm of the splanchnopleura and the underlying ectoderm. The splanchnopleura is present, its convexity turned caudoventrally, in accordance with the entodermic cardiac fold. Its central part has begun to invaginate into the pericardial cavity, rising from the underlying entoderm. Between these two layers the angioblasts are scattered.

### *Stage III*

The material on which the description of stage III is based consists of two embryos, one of which was cut transversely and the other longitudinally.

A. This specimen was removed from the uterus of a guinea-pig fifteen days and fourteen hours after insemination. This series includes 318 sections, having a  $7 \mu$  thickness. The plastic reconstruction of the cephalic portion of the embryo was made with wax plates, and the whole shield, reconstructed for another purpose, was used for this study.

The neural groove extends from the cranial end to the caudal amnion attachment. It is wide and shallow in the caudal portion, while it is narrow and deepens toward the head fold. The head fold is divided into two primary vesicles; the cranial vesicle is wide and long, projecting laterally and cranially over the cranial and lateral walls of the cranial body elevation. The caudal vesicle is small and passes insensibly into the spinal portion. The anlagen of the trigeminal ganglia, as well as the rudiment of the otic ganglia, are to be seen. Four somites

are completely segmented, besides in their cranial and caudal territory, a somite is in process of formation. The dorsal aortae and the first aortic arch are present, while the ventral aortae are not yet completely differentiated. In the region of their anlagen the angioblasts are irregularly distributed. The foregut extends throughout twenty-two sections, appearing first in the twenty-seventh section and continuing to the forty-ninth section, while the craniomedian limb of the pericardial cavity extends throughout sixteen sections, appearing in the twenty-third section and continuing to the thirty-ninth section. The cranial end of the head fold appears in the eighth section, on account of the forward progress of the head fold. This fact can be demonstrated in the dorsal surface of the whole reconstruction model, in which the prominent swelling of the dorsal surface of the pericardial cavity is much more distinct than that of the foregoing model. The cranial extremity of the pericardial cavity disappears under the head fold slightly caudad to its cranial margin.

In this embryo the mesoderm of the splanchnopleural projects into the pericardial cavity, forming prominent folds, already presented in the previous stage, but in this stage is well developed. These folds are converted on both sides into continuous myocardial tubes. Their dorsal surfaces approach the dorsal wall of the pericardial cavity, coming nearly into contact with it. These lateral myocardial tubes are best developed at the level of the hindbrain, where they are relatively dilated and contain the well-developed endothelial tubes. On tracing cranialward, both myocardial tubes gradually diminish in height and width, until they finally disappear opposite to the foregut opening on its left side, while on the right side the myocardial tube continues into the caudad portion of the craniomedian limb of the pericardial cavity, converting it into the prominent rounded ridge of the mesoderm of the splanchnopleura. In this region the thickened mesoderm of the splanchnopleura, present as a somewhat flattened fold, is elevated above the underlying entoderm. In the space between these two layers a number of angioblasts can be seen (fig. 11 and 12). In tracing still farther cranialward, the relatively thin layer of mesoderm

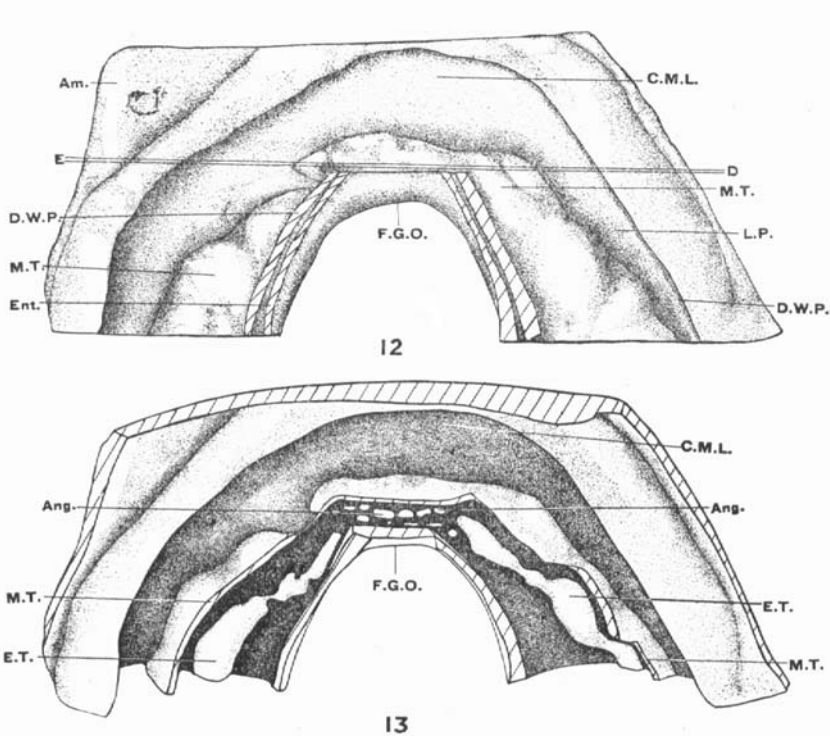


Fig. 12 Dorsal view of the reconstruction of the same embryonic shield (stage III, A) from which figure 11 was drawn. Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. At the caudal part of the craniomedian limb of the pericardial cavity the splanchnopleura projects into the pericardial cavity, forming the prominent fold, which is absent in front of the cranial extremity of the myocardial tube on the left side. *E-D* indicates plane of section of figure 11.  $\times 100$ .

Fig. 13 Dorsal view of the reconstruction of the same embryonic shield (stage III, A) from which figures 11 and 12 were drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to show the underlying endothelial tubes and angioblasts. Angioblasts are scattered under the splanchnopleural fold at the caudal part of the craniomedian limb of the pericardial cavity, almost connecting both cranial extremities of the lateral endothelial tubes. But angioblasts are absent in front of the cranial extremity of the lateral endothelial tube on the left side, where the splanchnopleura has not risen from the underlying entoderm.  $\times 100$ .



of the splanchnopleura remains attached to the underlying entoderm; between them no angioblasts can be seen (fig. 13).

It can be ascertained that the formation of the mesodermal splanchnopleural folds occurs in loco and progresses cranialward, until both cranial extremities of the myocardial tubes will ultimately unite and communicate with each other at the craniomedian limb of the pericardial cavity. The craniomedian limb of the pericardial cavity increases its dimensions both in the ventrodorsal and in the craniocaudal directions, while the formation of the myocardial anlage in this portion remains in its primitive condition. In the hindbrain region the pericardial cavity reaches its maximum width in proportion to the myocardial and endothelial development. But it shows here a rather narrower space in the ventrodorsal direction, on account of the dorsal expansion of the myocardial tubes. In tracing still further caudalward, the pericardial cavity gradually narrows until it entirely disappears in the somitic region, parallel with the gradually diminishing splanchnopleural folds and endothelial tubes.

The endothelial tubes are differentiated at great length, extending throughout nearly the whole extent of the lateral pericardial cavity. But in many places these tubes are irregularly interrupted, their continuity bridged by angioblast cords. The endothelial tubes terminate cranially opposite to the foregut opening on the right side and slightly caudad to it on the left side. In front of these terminations a number of angioblasts are scattered. Extending still farther craniomedially, by means of these angioblasts, the cranial extremities of the endothelial tubes are connected with each other through the middle plane underneath the flat splanchnopleural folds of the craniomedian limb of the pericardial cavity (fig. 13). There is a distinct significance in the fact that these angioblasts are directly derived from the mesoderm of the splanchnopleural cells in loco. Moreover, in some other parts the angioblasts or endothelial cells are undoubtedly connected with the thickened and indented mesoderm of the splanchnopleura. Therefore, it is conceivable that the productive activity of these cells from the mesoderm of the splanchnopleura is still continued in this embryo.

*Stage IV*

The material on which the following description of stage IV is based consists of two embryos, one of which was cut transversely and the other longitudinally.

A. This specimen was removed from the uterus of a guinea-pig fourteen days and eight hours after insemination. This series includes 566 sections, having a  $5\ \mu$  thickness, from the cranial margin of the head fold to the caudal end of the mesodermic thickening of the allantois. The plastic reconstruction of the cephalic portion of the embryonic body was made with wax plates.

The head fold has progressed cranial- and dorsalward. Its cephalic extremity is represented in that of the embryonic shield. There are present seven pairs of mesodermic somites, the first and last being small and indistinctly segmented. The neural groove extends from the cranial end to the caudal amnion attachment. In the hindbrain region the neural groove shows very narrow and deep as both the neural plates approach each other.

In the model it can be recognized that the craniomedian limb of the pericardial cavity increases its dimensions in the ventrodorsal direction, while its lateral and craniocaudal extent remains approximately unchanged in comparison with the previous stage III. The cranial extremity of this portion extends at its dorsal part into the mesodermic cavity of the mandibular region on both sides. While their outline gradually approaches the horizontal plane, the caudal extremity of this portion is continued into both the lateral pericardial cavities, which gradually diminish in width caudalward. In the region of the second somite they entirely disappear.

The formation of both the lateral myocardial tubes, which has been discussed in stage III, are considerably developed and have so far progressed cranialward, that their cranial portions have partially come into contact and been fused together. Through this portion the myocardial tubes communicate with each other. On the dorsal surface of this fused portion of the

lateral myocardial tubes the myocardial walls are reflected directly onto the dorsal wall of the pericardium, thus forming the dorsal mesocardium on both sides. Between the lateral mesocardial layers there is present an irregular triangular space, which we purpose to designate as the intermesocardial space and through which the endothelial offshoots come out from the myocardial cavity onto the space between the mesocardial layers and the floor of the foregut. Its apex is directed cranialward, where the lateral mesocardial layers come in contact, marking the cranial margin of the communicating myocardial cavity. Its basal portion is directed caudalward and corresponds to the foregut opening, by which the lateral mesocardium layers diverge from each other and continue farther caudalward along the lateral myocardial tubes (fig. 14). Between the above-mentioned adherent cranial margin of the mesocardium and the foregut opening, the lateral myocardial cavities communicate with each other across the middle plane to the extent of eight sections. From this communicating myocardial cavity are sent out two short cranial diverticula on either side, separated by a septal wall in the middle plane, corresponding to the cranial extremities of the lateral myocardial tubes. These diverticula are present as the rounded myocardial horns, directed cranialward and separated from each other by their own inner walls. These inner walls are caudally converted into a wedge-shaped prominent ridge, which continues into the communicating portion of the myocardial cavity and gradually diminishes caudalward (fig. 15). The communicating portions of the lateral myocardial tubes are directly continued into the lateral myocardial tubes caudolaterally on both sides and they are separated from each other by the foregut opening. The ventral wall of the communicating portion of the myocardial cavity is reflected onto the ventral wall of the pericardium and is recognized only in the caudal portion. The reflection points from the myocardium to the ventral pericardial wall are fused together at the cranial part, but at the caudal part the reflection points diverge from each other and a triangular space remains between them in just the same manner as can be seen in the dorsal wall.

This ventral triangular space is covered by the entoderm cephalad to the foregut opening, while the dorsal intermesocardial space is covered by the foregut floor. Ventrally to the communicating myocardial cavity, the pericardial cavity passes from side to side, because of the absence of the ventral mesocardium. Along the ventral mesodermic reflection the anlage of the septum transversum of His will be presented in the future development, and the mesodermic reflection may be erroneously taken for

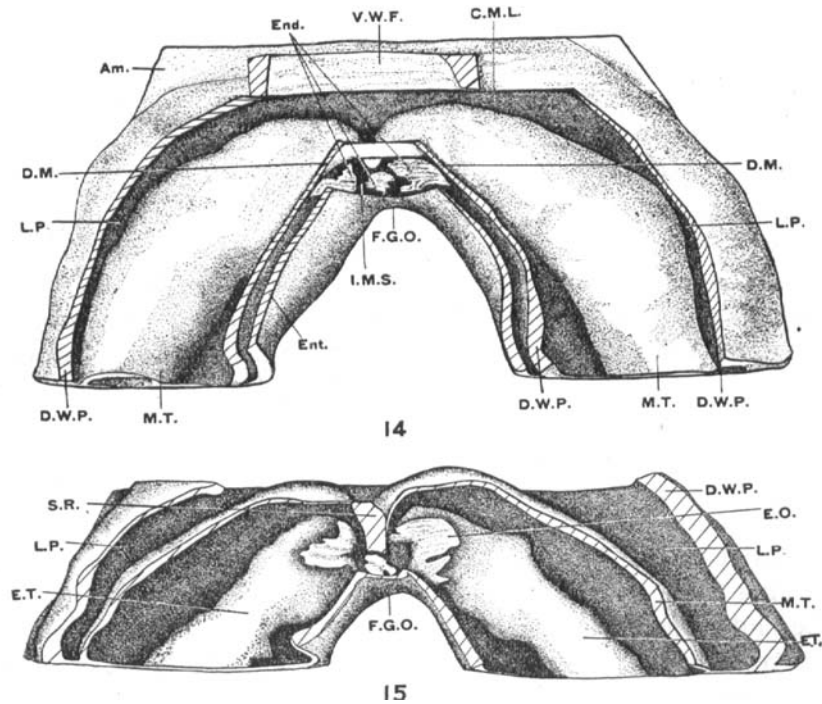


Fig. 14 Dorsal view of the reconstruction of an embryonic shield (stage IV). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. The two lateral myocardial tubes are partially confluent, slightly cephalad to the foregut opening. The two short cranial horns of the myocardial tubes are directed toward the top of the page.  $\times 100$ .

Fig. 15 Dorsal view of the reconstruction of the same embryonic shield (stage IV) from which figure 14 was drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to expose the underlying endothelial tubes. The two lateral endothelial tubes approach most closely to each other at the confluent myocardial portion, where independent endothelial cells are interposed between the two tubes.  $\times 100$ .

the ventral mesocardium, if a single section of this portion should be examined, as many workers claim the existence of the ventral mesocardium in mammals.

The endothelial tubes are well developed and are enclosed within the myocardial cavities on both sides. Their lumina are patent throughout their cranial extent, their cranial extremities terminate blindly opposite to the cranial extremities of the myocardial tubes, where the myocardial tubes are projected into the pericardial cavity, like the rounded lateral horns on either side, which contain the cranial extremities of the myocardial cavity. In tracing caudally, the endothelial tubes reduce their calibers gradually and they are irregularly interrupted in their continuity by angioblasts. They entirely disappear in the somitic portion, where the lateral pericardial cavities assume a narrow and horizontal space and the splanchnopleural folds have entirely disappeared. The lateral endothelial tubes are most remarkably dilated and considerably approximated to each other at the communicating myocardial cavity, where the independent intermediate endothelial cells can be seen between the endothelial tubes. Throughout many sections in the communicating myocardial cavity, the endothelial tubes give off their endothelial offshoots from their dorsomedian aspects, coursing dorsolateralward, between the dorsal mesocardium and the floor of the foregut. These offshoots may be considered the future truncus arteriosus.

Owing to the gradual transition from these dilated endothelial tubes into the portion of the vitelline veins caudalward, their demarcation cannot be pointed out on the endothelial tubes nor on the myocardial tubes.

The dorsal aortae and the first aortic arch are completely developed, while the ventral aortae are incompletely differentiated. In their anlagen a number of angioblasts are distributed irregularly.

#### *Stage V*

The material on which the following description of stage V is based consists of two embryonic shields, which were cut trans-

versely. The partial plastic reconstruction of the cephalic portion and the reconstruction of the whole embryonic shield, made for another purpose, were used for this study.

A. This specimen was removed from the uterus of a guinea pig fourteen days and eight hours after insemination. The series includes 612 sections, having a  $5 \mu$  thickness. As measured by age, this embryonic shield is slightly younger than that discussed in stage IV. As judged by the stage of general development, it is slightly more advanced, indicated by the facts that eight somites are present and that the medullary groove is much deeper and narrower in the hindbrain region, so that to a great extent both neural plates are in contact; here it passes insensibly into the spinal region. The forebrain plate still remains wide open, projecting cranially and laterally over the cranial and lateral wall of the anterior body elevation. It is, moreover, bent considerably ventralward. In the model it can easily be recognized that, owing to the fact that this embryonic shield is considerably folded off from the yolk sac, it is in general thicker in the ventrodorsal diameter and narrower in the lateral diameter than that in stage IV. In accordance therewith, the cranial extremities of the lateral myocardial tubes are forming a more acute angle than that of the previous stage. The craniomedian limb of the pericardial cavity increases its ventrodorsal and craniocaudal dimensions, while its lateral diameter diminishes on comparison with the embryo of stage IV, in proportion to the rounded outline of this embryo. The craniomedian limb of the pericardial cavity is elongated at its dorsal part into the mandibular mesoderm. In coursing caudalward, the lateral pericardial cavities gradually diminish their width, and at the same time their outline approaches the horizontal plane as a whole. They disappear entirely opposite to the fourth somite. The direction of the lateral myocardial tubes tends to their running parallel to each other. The lateral myocardial tubes are considerably dilated at their cranial portion, where they abruptly become voluminous in comparison with their caudal portion. The transitional points of these two different portions are situated a little caudad to the foregut opening on both sides,

where the myocardial tubes mark slight indentations. These indentations indicate the future atrioventricular constriction (fig. 16).

The cranial extremities of the lateral myocardial tubes become more voluminous as compared with those of the previous stage and are projected cephalad into the pericardial cavity as large lateral rounded horns. They are separated from each other by their own inner walls, which fuse caudally into one septal wall and, as we trace still farther caudally, we find them converted into the prominent wedge-shaped ridge which projects into the communicating myocardial cavity. This ridge gradually diminishes in height caudalward until it has entirely disappeared in the middle of the communicating cavity (fig. 17).

The dorsal wall of the fused myocardial tubes is reflected directly onto the dorsal wall of the pericardium; forming the dorsal mesocardium on both sides. These lateral mesocardial layers come to fusion in a region a little cephalad to the foregut opening, where it makes the cranial margin of the communicating myocardial cavity. On the dorsal surface of the fused myocardial portion an irregular intermesocardial space can be seen, covered by the floor of the foregut. Its apex is directed cephalad, corresponding to the point where the lateral mesocardium layers are fused together. Its base is directed caudad, corresponding to the foregut opening. Its sides are formed by the lateral mesocardial layers. Corresponding to this intermesocardial space, the lateral myocardial tubes communicate with each other through the median plane throughout the extent of nine sections. In a similar way, the ventral wall of the fused myocardial tube is reflected onto the ventral wall of the pericardium, but only in its caudal portion. Between these two lines of the mesodermal reflection there remains a narrow space free from the mesoderm and covered directly by the entoderm. However, these lines of reflection on the ventral wall are disposed in a rather transverse direction and are located only for a short extent in the caudal part of the communicating myocardial tube, while on the dorsal surface the lines of reflection of the mesocardium are directed rather longitudinally and extend

throughout the whole length of the communicating myocardial tube. The communicating portion of the myocardial cavity terminates blindly in the cranial diverticulum cephalad, cor-

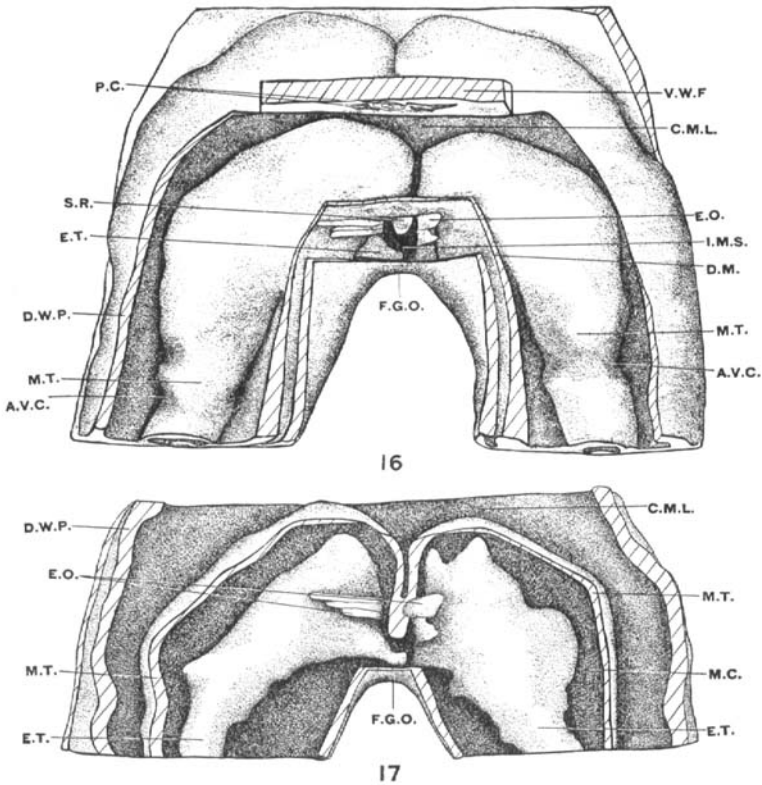


Fig. 16 Dorsal view of the reconstruction of an embryonic shield (stage V). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. The two lateral myocardial tubes become quite voluminous, especially at their cranial portions, which continue farther caudalward, gradually diminishing in size. The atrioventricular constriction is marked on the surface of the myocardial tubes, caudad to the foregut opening on both sides. The two cranial horns of the myocardial tubes become enlarged, and they are directed toward the top of the page.  $\times 100$ .

Fig. 17 Dorsal view of the reconstruction of the same embryonic shield (stage V) from which figure 16 was drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to expose the underlying endothelial tubes, which are apparently enlarged at their cranial portions, where they most nearly approach each other.  $\times 100$ .



responding to the cranial extremities of the lateral myocardial tubes, while caudally it is elongated into the lateral myocardial tubes, which are diverged by the foregut opening, and their lumina are gradually diminished toward the somitic region.

In general, the endothelial tubes are much more developed than those of the previous stage, since they have become deeper and wider. At the widest portion of the endothelial tubes, corresponding to the communicating myocardial cavity, the endothelial tubes approach each other so that they come nearly into contact. On these portions, throughout many sections, the endothelial tubes give off a number of endothelial offshoots from their dorsomedian surface into the space between the dorsal mesocardium and the foregut floor. From these portions they become gradually narrower, toward both the cranial and caudal directions. The cranial extremities of the endothelial tubes terminate blindly opposite to the cranial myocardial extremities, while caudally they continue into the portion of the vitelline veins. The endothelial tubes assume the distinctly narrow calibers opposite to the atrioventricular constriction. The endothelial tubes sprout out into innumerable tenuous fibrils, often forming a feltwork, which occupies the wide space between the myocardium and the endothelium.

The dorsal aortae and the first aortic arch are developed, while the ventral aortae are not completely formed, as in their anlagen a number of angioblasts are scattered.

### *Stage VI*

The material on which the following description of stage VI is based consists of one embryo, cut transversely. The plastic reconstruction of the cephalic portion of the embryo was made with wax plates.

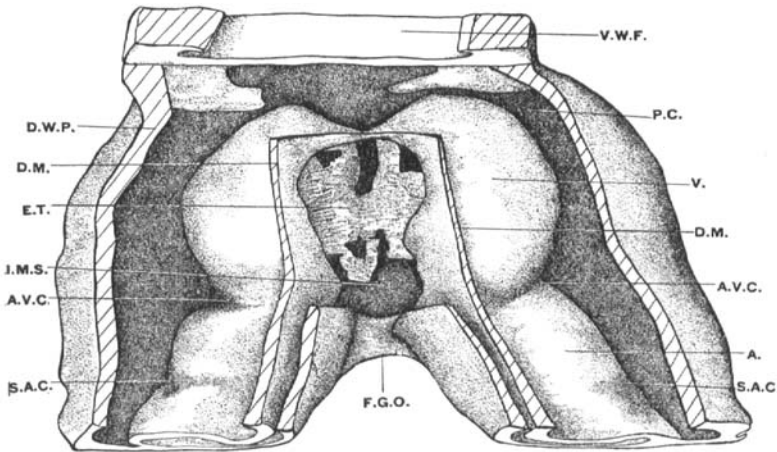
This specimen was removed from the uterus of a guinea pig fourteen days and eight hours after insemination. The series includes 582 sections, having a  $5\ \mu$  thickness. As measured by age, this embryonic shield is the same as that of the previous embryo. As reckoned by general development and special de-

velopment of the heart, it is considerably advanced over the preceding embryo. Eight well-segmented somites are present. The medullary groove, extending from the cranial end to the caudal amnion attachment, is as deep and narrow as in the previous embryo. The form of the embryonic shield is, in general, more rounded in comparison than with the foregoing embryo, as the ventrodorsal diameter of this embryo is apparently increased while its lateral diameter has remained unchanged. The first visceral pouch and the oral pit are developed; in these places the entoderm coalesces intimately with the ectoderm.

The reconstruction shows that at this stage of development the craniomedian limb of the pericardial cavity increases considerably in the craniocaudal dimension and in the ventrodorsal dimension. The craniomedian limb of the pericardial cavity communicates caudally with the lateral pericardial cavities. On coursing caudally, these become gradually narrower, until they disappear entirely opposite to the sixth somite. The craniomedian limb of the pericardial cavity is elongated cranially at its dorsal part into the mandibular portion, lying under the foregut floor. The caudal half of the ventral surface of the craniomedian limb of the pericardial cavity is covered by the yolk sac, while its cranial half and all other surfaces are covered by the amnion.

In this stage the fused portion of the lateral myocardial tubes increases remarkably throughout its craniocaudal extent. In accordance therewith, the cranial bilateral myocardial horns, which correspond to the cranial extremities of the lateral myocardial tubes, and predominate in the craniomedian limb of the pericardial cavity in the previous stage, apparently diminish their dimensions in this embryo, and show only their rudiments. They assume only short and wide bilateral processes, divided by a shallow and wide intervening groove. In the previous stage this groove was present as a narrow and deep sulcus. Subsequently, the inner walls of these horns diverged markedly from each other (fig. 18). The wedge-shaped ridge which, in the previous stage, projected into the communicating myocardial cavity at its middle cranial wall, as a caudal continuation

of the converted septum walls of the cranial bilateral myocardial horns, is considerably retired cranialward in this embryo. Therefore, the cranial wall of the communicating myocardial cavity approaches in such a manner toward the cranial wall of the pericardium as to come nearly into contact with it and, simultaneously, the communicating myocardial cavity is elongated cranialward. The fused portion of the myocardial tube



18

Fig. 18 Dorsal view of the reconstruction of an embryonic shield (stage VI). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardial tubes. The confluent portion of the two lateral myocardial tubes is considerably elongated in the craniocaudal direction. The two cranial horns of the myocardial tubes diminish to short rudiments, as their septal wall retires cranialward. They are directed toward the top of the page.  $\times 100$ .

becomes distinctly narrower and thicker in comparison with that of the previous stage. In two of the same magnified models ( $\times 300$ ) the widest lateral diameter of this portion is calculated as 14.5 cm. in this embryo, instead of 19 cm. of the previous embryo, while the ventrodorsal diameter of this portion presents 5.1 cm. in this embryo and 3 cm. in the former embryo.

The fused myocardial tube is reflected directly onto the dorsal wall of the pericardium, and thus forms the dorsal mesocardium on both sides. Between the lateral mesocardial layers there can be seen a long rectangular intermesocardial space; its

plane is approximately parallel with the horizontal. Its cranial margin is formed by the fused portion of the mesocardial layers in the middle line; its caudal margin corresponds to the foregut opening, while both lateral margins are represented by the lateral mesocardial layers, which continue farther caudalward, diverted by the foregut opening. In accordance with this mesocardial space, both myocardial tubes communicate freely with each other through the median plane, and thus form

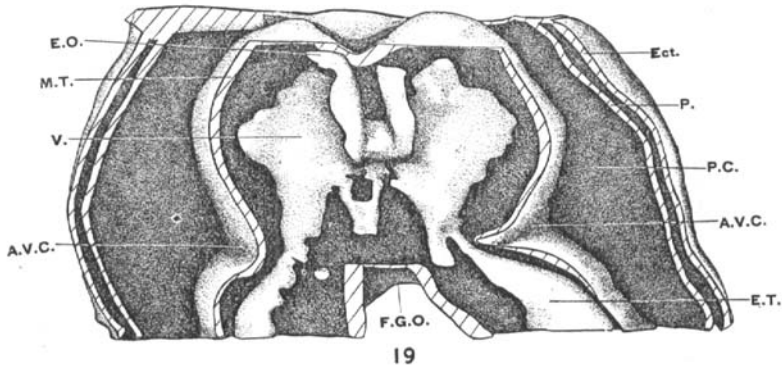


Fig. 19 Dorsal view of the reconstruction of the same embryonic shield (stage VI) from which figure 18 was drawn. Dorsal wall of the pericardium and of the myocardial tubes have been removed to expose the endothelial tubes. The two lateral endothelial tubes have fused and communicate with each other at a middle third of the ventricle, where they most closely approach each other in figure 17. The lateral endothelial tubes apparently diminish their size opposite to the atrioventricular constriction.  $\times 100$ .

the craniomedian limb of the myocardial cavity. This limb of the myocardial cavity is bifurcated caudally into the lateral myocardial tubes, which are diverged from each other by the foregut opening and in which the vitelline veins are enclosed, leading cranially into the craniomedian limb of the myocardial cavity (fig. 19). In brief, the myocardial anlage presents cranially two short rudimentary horns, which terminate blindly as the cranial myocardial extremities, while caudally there are two lateral myocardial prolongations, into which the vitelline veins enter. Between these four extremities the myocardial wall is relatively considerably expanded dorsal, lateral, ventralward and

contains the widest portions of the endothelial tubes, which are united in the communicating cavity. This region corresponds to the future ventricle region. On the midsagittal line of the ventral surface of this fused myocardial portion a shallow longitudinal groove can be seen.

On the ventral aspect of the fused myocardial portion the ventral myocardial layer is reflected onto the ventral wall of the pericardium, but this is limited to a short length, extending only to the caudal part of this portion.

The transition from the cranial expanding ventricle to the caudal myocardial prolongations is indicated by an annular constriction, which is produced by the infolding of the whole myocardial wall, a little deeper on the right side than on the left. This indicates the atrioventricular constriction and is situated at the level slightly cephalad to the foregut opening on both sides.

Proceeding caudally from this constriction, the lateral myocardial tubes reduce their calibers abruptly and diverge from each other. On the surface of these caudal prolongations of the myocardial tubes there are present indefinite, shallow indentations at the level slightly caudad to the foregut opening, and these constrictions have been regarded as the future sinoatrial construction.

The lateral endothelial tubes are partially fused and their lumina communicate with each other, for their inner walls have been absorbed throughout seven sections. This portion is situated in the middle third of the bulging ventricle anlage, where in the previous stage both endothelial tubes were closely approximated and presented the greatest dilation and where in this embryo also the endothelial tube is greatly expanded.

From this fused portion of the endothelial tube the two cranial horns and two caudal prolongations are given off. The bilateral cranial horns are short and gradually diminish in size cranialward, until they terminate in a pointed apex, opposite to the cranial extremities of the myocardial horns. From the dorsomedian part of these cranial endothelial horns a number of endothelial branches are given off. These endothelial

branches are connected with the ventral aortae through the intermesocardial space.

Bilateral caudal prolongations are given off on both sides from the caudal aspect of the fused endothelial tube. Continuing caudalward, both endothelial tubes gradually diminish their calibers, until the lumina have entirely disappeared at the atrioventricular constriction. Still further caudalward from this constriction, again they begin to dilate their calibers gradually and continue into the endothelial vitelline veins without any indication at their transitional point. At the atrioventricular constriction the endothelial tubes closely approach the infolding of the myocardial wall, while in the ventricle the intervening space between the myocardium and endothelium is relatively wide.

From the caudal aspect of the fused endothelial tube another intermediate endothelial branch is given off caudally. This branch is situated between the endothelial prolongations and terminates at the atrioventricular constriction.

The dorsal aortae and the first aortic arch are developed, while, in many sections, the ventral aortae are interrupted by angioblasts.

#### *Stage VII*

The material on which the following description of stage VII is based consists of one embryonic shield, which is cut transversely. The plastic reconstruction of the cephalic portion of the embryo was made from wax plates.

The specimen was removed from the uterus of a guinea-pig fourteen days and eleven hours after insemination. The series includes 418 sections, having a  $7 \mu$  thickness, from the cephalic end of the head fold to the end of the mesodermic thickening of the allantois. Nine pairs of well-segmented somites were found, each somite showing a thick wall and enclosing a uniform cavity with a compact arrangement of cells, except two caudal somites, which contained no cavity nor presented the regular arrangement of the cells.

The medullary canal is closed from the second somite to the last, but elsewhere the medullary plates remain open. The notochordal plate is separated from the entoderm throughout from the second somite to the last, but elsewhere it is still connected with the entoderm. The first and second visceral pouches are developed, in which the ectoderm and entoderm have tightly coalesced. The oral pit is formed and the pharyngeal membrane becomes quite thin.

In this embryo the pericardial cavity is closed in all directions, forming a sac, except at the dorsal part of its caudal extremities, where the pleuropericardial passages are opened on each side. These passages are represented by the narrow coelomic space, which continues farther caudalward into the peritoneal cavity and they are situated dorsomedian to the myocardial coat of the vitelline veins. From the dorsal part of the cranial extremity of the pericardial cavity a slit-like space is elongated into the mandibular region.

The surface of the pericardium is covered by the yolk sac ventrally, while laterally, cranially, and dorsally it is covered by the amnion. The pericardial cavity shows a wide space around the myocardium. Between the cranial extremity of the myocardium and the cranial wall of the pericardium there remains a wider interval than that of the embryo of stage VI.

The myocardium presents cranially an undivided cranial extremity, which expands considerably in all directions and assumes a sac form, while caudally this myocardial sac is bifurcated into two rather slender myocardial prolongations, in which the endothelial vitelline veins are enclosed on both sides. The transition from the cranial myocardial sac to the bilateral myocardial tubes is indicated by the deep atrioventricular constriction, at the level slightly cephalad to the foregut opening. This constriction is produced by the infolding of the whole myocardial wall and shows apparently deeper on the right side than on the left. On the ventral surface of the ventricle there can be seen a shallow groove in the midsagittal line at its caudal half, and in accordance therewith the whole thickness of the myocardial wall is slightly infolded into the myocardial cavity.

This superficial groove and the infolding of the myocardial wall are located in the caudal half of the ventricle, for they gradually disappear toward its cranial extremity, which is of conical form. The ventral myocardial layer is reflected onto the ventral wall of the pericardium, but is confined to the atrial region to a short length opposite to the foregut opening.

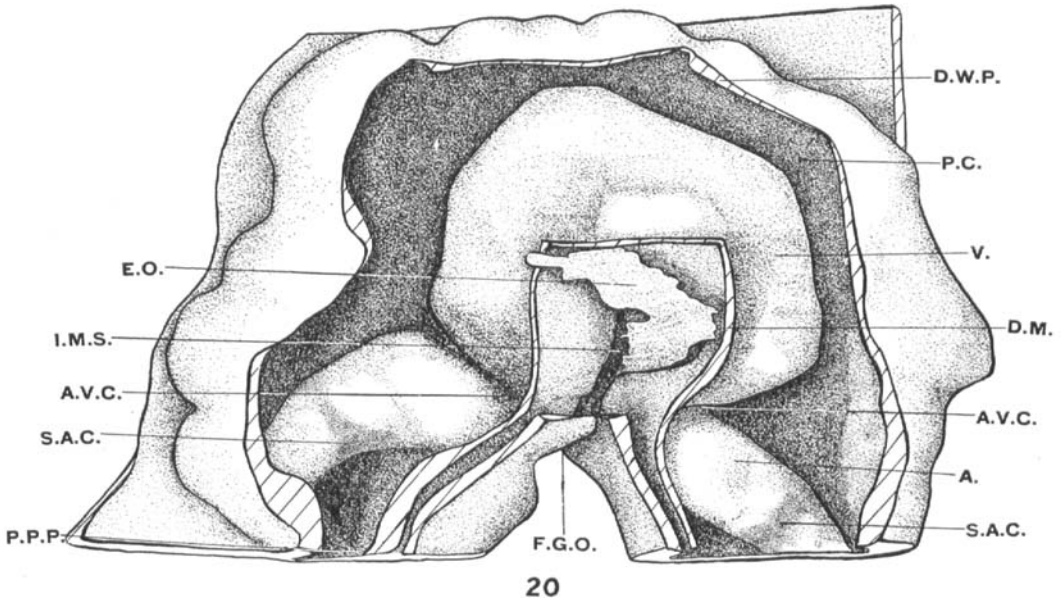


Fig. 20 Dorsal view of the reconstruction of an embryonic shield (stage VII). Dorsal wall of the pericardium has been removed to show the pericardial cavity and the myocardial tubes. The myocardium shows cranially a single sac-formed ventricle, which bifurcates into two slender myocardial tubes caudalward. The transition between them is marked by the atrioventricular constriction cephalad to the foregut opening. A single cranial extremity of the ventricle is directed toward the top of the page.  $\times 100$ .

On the dorsal aspect the myocardial wall is reflected onto the dorsal wall of the pericardium and forms the dorsal mesocardium on both sides. The cranial extremity of the dorsal mesocardial attachment corresponds to a middle third of the ventricle, and from this point it continues farther caudalward. Consequently, the cranial half of the ventricle is free from the mesocardium (fig. 20). Between the lateral mesocardial layers



there can be seen an irregular triangular intermesocardial space. Its plane is directed caudodorsalward on account of the abrupt dorsal expansion of the ventricle. Its apex is, therefore, situated caudoventrally and is formed by the lateral mesocardial layers, approaching contact, opposite to the atrioventricular constriction, while its basal portion is directed craniodorsally and corresponds to the portion where the lateral mesocardial layers come to fusion and mark their cranial extremities.

On the caudal surface of the ventricle the right half assumes an apparently wider dimension than the left. This is attributed partly to the exceeding expansion of the myocardial wall in the laterocaudal direction on the right half and partly to the deeper infolding of the atrioventricular constriction on the right side. On this bulging portion of the caudal extremity of the ventricle at the right half the caudal extremity of the right ventricle will be developed, and this is shown distinctly in the next stage.

The lateral myocardial tubes of the atrium are diverged from each other by the foregut opening. The right atrium is practically beginning just opposite to the foregut opening, while the left one begins slightly cephalad to it, for in comparison with the right side, the left atrioventricular constriction is shallower and situated slightly cephalad.

On the myocardial tubes of the atrial portion indefinite indentations, indicated as the sino-atrial constriction, can be recognized. These are between the atrioventricular constriction and the level of the pleuropericardial passages. This is especially noticeable on the left side.

The lateral endothelial tubes are fused together throughout the cranial two-thirds of the ventricle. Its cranial extremity terminates as a single conical apex opposite to the cranial extremity of the myocardial ventricle. At this fused portion the endothelial cavities communicate with each other and show considerable dilation. In tracing caudalward from this united portion, the tubes are separated from each other, even though they appear to approach each other. At the atrioventricular constriction the endothelial tubes present their smallest size and

simultaneously they approach closely to each other. Proceeding still farther caudally from this portion, they are diverged from each other by the foregut opening and again assume a gradual enlargement of their calibers. At the caudal part of the ventricle, where the endothelial tubes are separated, they present an asymmetrical size, for the right one is extraordinarily de-

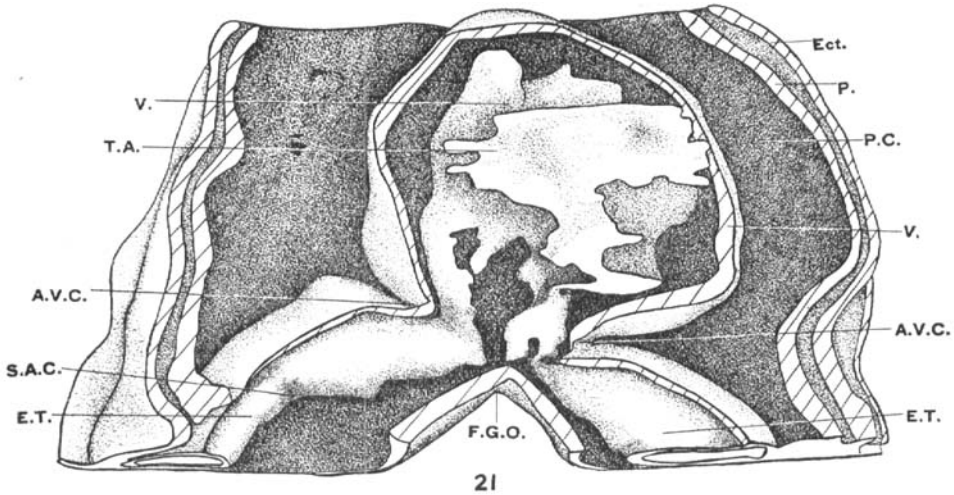


Fig. 21 Dorsal view of the reconstruction of the same embryonic shield (stage VII) from which figure 20 was drawn. Dorsal walls of the pericardium and of the myocardium have been removed to expose the endothelial tubes. The two lateral endothelial tubes have fused and communicate with each other throughout the cranial two-thirds of the ventricle. The ventricular endothelial tubes show a distinct asymmetry, due to the extraordinary enlargement of the right side, regardless of the fused or non-fused portion. The endothelial tube is elongated dorsalward from the dorsal surface of the enlarged right endothelial tube, passing through the intermesocardial space. This endothelial elongation is bifurcated into the two lateral branches, which are continuous into the ventral aortae.  $\times 100$ .

veloped and expended in the lateral and caudal directions, forming a curvature whose convexity is turned laterocaudalward. At this endothelial portion the endothelial tube is elongated vertically dorsalward and comes out from the myocardial cavity onto the foregut floor through the intermesocardial space. The cranial part of this endothelial elongation is bifurcated into two later branches which connect it cranially with the corresponding

ventral aortae. The asymmetrical development of the endothelial ventricle corresponds to the myocardial asymmetry in the ventricle, which has been mentioned above. In this part of the ventricle the most important change will be noted in the next stage, here developing, namely, the right limb of the ventricle. And this change is initiated in this embryo as a considerable asymmetrical expansion of the caudal extremity of the ventricle on the right side.

At the ventricle the endothelial tubes are separated from the myocardial wall by a wide intervening space, but they gradually approach each other in the direction of the atrio-ventricular constriction caudalward, as in the caudal part of the atrium no more intervening space can be pointed out between the myocardial wall and the endothelium (fig. 21).

The transition from the atrial endothelial tubes into the sinus portions can be pointed out by the abrupt decrease of the endothelial caliber on the left side, corresponding to the relatively distinct sino-atrial constriction of the myocardium. But on the right side the atrial endothelial tube continues farther caudalward without any demarcation, in accordance with the relatively indistinct myocardial constriction.

On both sides the ductus cuvieri can be seen opening into the sinus venosus. The dorsal aortae and first aortic arch are well developed, while in many places the ventral aortae still retain the plexus form.

#### *Stage VIII*

The material on which the following description of stage VIII is based consists of one embryonic shield, which is cut transversely. The plastic reconstruction of the cephalic portion of the embryo was made; the reconstruction of the whole embryonic shield, made for another purpose, was used for this study.

This embryo was removed from the uterus of a guinea pig fourteen days and twelve hours after insemination. The series includes 408 sections, having a 10  $\mu$  thickness, from the cephalic end of the head fold to near the caudal extremity of the allantoic mesodermic thickening. Nine pairs of the well-seg-

mented somites are present and the tenth is in process of formation; each somite shows the thick wall and encloses a uniform cavity. The neural canal is closed from the region of the hindbrain to the region of the last somite, though in the fore- and midbrain region it still remains open. The cranial flexure is shown in the region of the midbrain and that of the forebrain is bent downward and forward, bringing it to a plane parallel with the long axis of the hindbrain. The foregut is closed to the first somitic region. The first visceral pouch is found in the region of the midbrain with the entoderm and ectoderm coalesced, while the second visceral pouch is in process of formation also in the region of the hindbrain, in which region between the entoderm and ectoderm a thinner layer of the mesoderm than elsewhere is found interposes. The oral pit is well formed, the pharyngeal membrane is present as a thin single layer of cells. On the ventral surface of the model the edge of the foregut opening is elevated by two prominent limbs, which on each side are confluent into an extensive ventral bulging cranially to the foregut opening. In position and direction this corresponds to the pericardial cavity, containing the voluminous heart.

The pericardial sac is closed except at the dorsomedian part of its caudal extremity, where the pleuropericardial passages are found. In the region of the sinus venosus each one of the bilateral pericardial cavities is divided into a median and a lateral part by the myocardial fold and in the region of the pleuropericardial passages the lateral parts of the bilateral pericardial cavities terminate blindly caudalward, so that only their median portions are continued caudally into the peritoneal cavity. Accordingly, these passages are represented merely by narrow, crescentic coelomic spaces, dorsomedian to the vitelline veins, proceeding caudally and mesially, crossing with the vitelline veins, which run cranially and mesially.

On account of the considerable enlargement of the muscular heart, the pericardial space is, in general, proportionately reduced, especially in the well-developed ventricular portion a simple narrow space surrounds the muscular sac of the ventricle.

In the region of the atria and the sinus venosus a relatively wide space intervenes between the rather flat muscular tubes and the pericardial wall (fig. 22). There are present two distinct constrictions on the tubular muscular heart, infolding the whole thickness of the myocardial wall, one of which represents the atrioventricular constriction and the other the sino-atrial constriction.

The atrioventricular constriction is present asymmetrically on both sides; on the right side it is marked more deeply and situated slightly caudad, while on the left side it is shallower and lies a little cephalad. Therefore, this constriction forms an oblique angle with the long axis of the muscular heart.

On the contrary, the sino-atrial constriction is marked more deeply on the left side and is situated slightly cephalad to the foregut opening, while on the right side it is less deeply constricted and is situated slightly caudad, just opposite to the foregut opening.

The ventricle can be divided into two lateral limbs by a ventral and a dorsal longitudinal sulcus. On the dorsal surface it is marked along the attachment line of the dorsal mesocardium and terminates caudally opposite to the right margin of the atrioventricular constriction, while its cranial extremity gradually disappears at the portion where the *bulbus cordis* is differentiated from the dorsal wall of the ventricle. On the ventral surface the longitudinal sulcus extends to a caudal third of the ventricle.

At the caudal part of the ventricle, for a short length, both lateral limbs are divided into two completely independent cavities by the septal wall. The caudal extremity of the right ventricle is shown as the conical process, projecting caudolaterally and terminating blindly, while at the caudal extremity of the left ventricle the atrioventricular canal opens, which is formed by the infolding of the muscular wall, corresponding to the atrioventricular constriction.

The septal wall between the two lateral limbs at the caudal part of the ventricle is farther continued cranialward and is converted into wedge-shaped prominent ridges at the inner sur-

face of the ventral and dorsal wall of the ventricle, in relation with longitudinal sulci on the external surface. These ridges have gradually disappeared within a caudal third of the ventricle (fig. 23). These prominent ridges show their anlage only

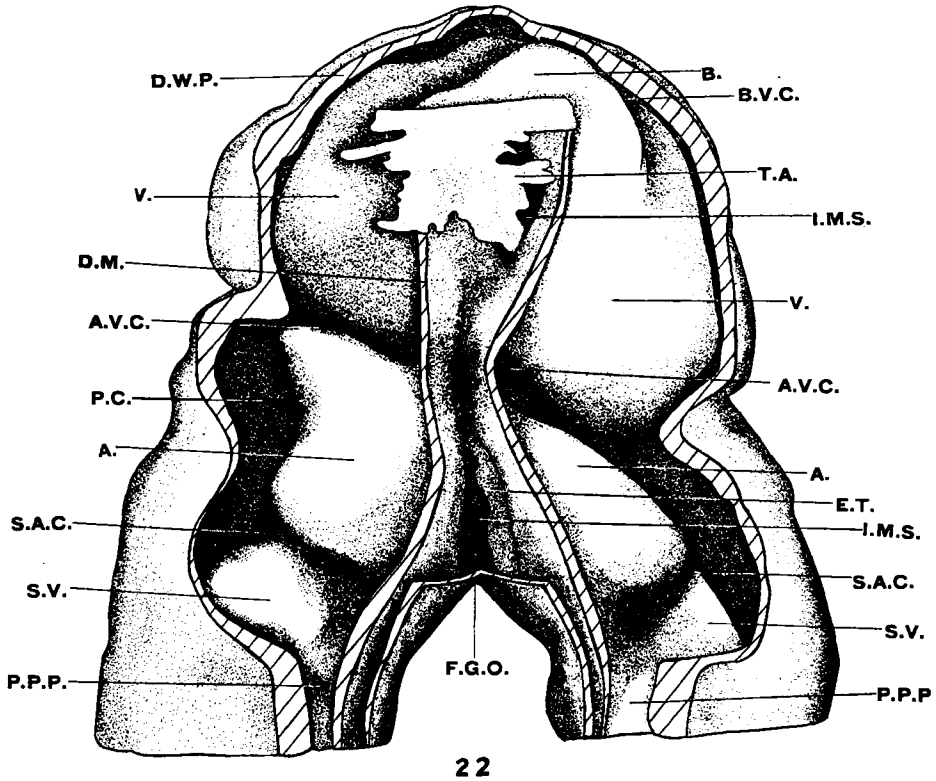


Fig. 22 Dorsal view of the reconstruction of an embryonic shield (stage VIII). Dorsal wall of the pericardium has been removed to show the pericardial cavity and myocardium, which is subdivided into several individual portions (bulbus cordis, ventricle, atrium, sinus venosus, etc.) by the distinct bulbo-ventricular atrioventricular, sino-atrial constrictions.  $\times 100$ .

on the ventral wall of the ventricle, near its caudal end, as seen in the previous stage. Consequently, there can be but little doubt that these folds cannot be regarded as the remnants of the primitive cardiac septum.

The bulbus cordis is differentiated from the dorsal wall of the right ventricle near its cranial end, bulging out its wall cranial-dorsal and laterally. Its ventral wall is distinctly separated from the dorsal wall of the right ventricle at its cranial portion, projecting cranialward as an independent muscular sac, while in its caudal portion there can be noted no distinct demarcation between the wall of the bulbus cordis and that of the right ventricle. At the left and cranial sides of the bulboventricular junction, a deep external furrow can be seen, accompanied by a consequent infolding of the muscular wall. On the right side the bulboventricular furrow is indefinitely marked only its cranial part, while in its caudal part it has disappeared entirely and insensibly continues into the dorsal wall of the right ventricle. On this account the bend of the heart tube at the bulboventricular junction is effected toward the right side, turning its concavity to the left side, beneath the left layer of the dorsal mesocardium.

On the dorsal surface of the bulbus cordis there can be seen a triangular intermesocardial space, its plane is directed dorsally and slightly to the left. Its apex is directed caudally and at a lower level, where the demarcation between the bulbus cordis and ventricle wall show indefinitely. Its base is situated cranially and at a higher level, where the wall of the bulbus cordis is distinctly demarcated from that of the ventricle, and there marks the cranial termination of the dorsal mesocardium. Both at the apical and basal portions the lateral mesocardial layers come to fusion.

A single myocardial tube of the atria begins at the atrioventricular constriction cranially and continues into the sinus venosus caudally, demarcated by the sino-atrial constriction. This muscular tube shows a marked asymmetry on both sides, for the left side, being decidedly expanded in all directions in comparison with the right side, just contrary to the ventricle, in which the right side is apparently more voluminous than the left side and bulges considerably caudolaterally. Moreover, this opposing asymmetry must be attributed partly to the normal oblique direction of the atrioventricular constriction.

In consequence of this asymmetrical relation, the atrial tube forms a typical curvature with the ventricular limb at the atrio-ventricular junction, so that its convexity is directed toward the left side in the horizontal plane and ventralward in the vertical plane.

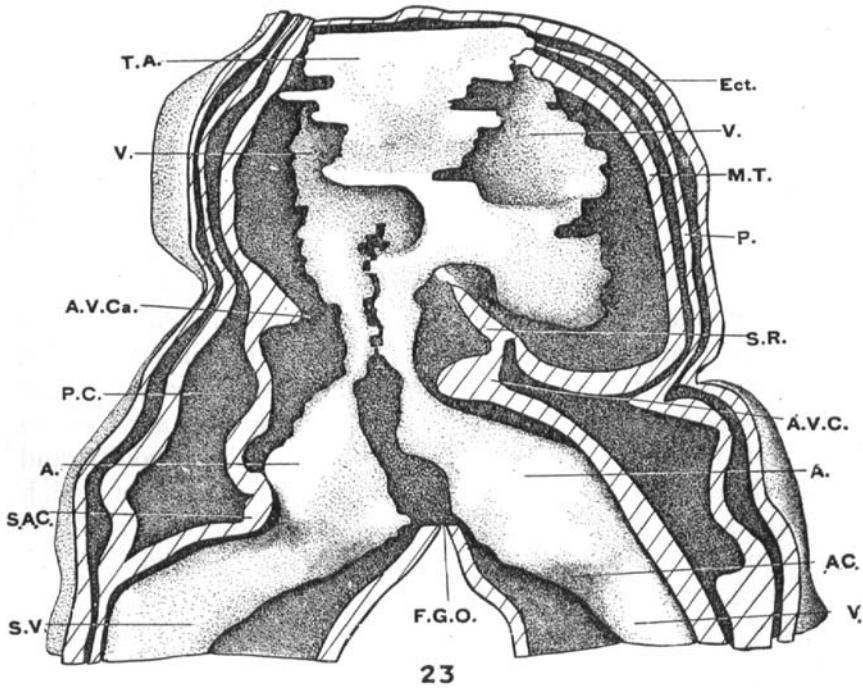


Fig. 23 Dorsal view of the reconstruction of the same embryonic shield (stage VIII) from which figure 22 was drawn. Dorsal wall of the pericardium and of the myocardium have been removed to expose the endothelium, which is subdivided into several individual portions in conformity with the myocardial subdivision. The two lateral endothelial tubes have fused and now communicate with each other throughout a middle third of the ventricle, but elsewhere they are separated.  $\times 100$ .

The cranial extremity of the atria opens into the left ventricle through the atrioventricular canal, which is situated on the left side from the midsagittal line, for the right atrioventricular constriction is apparently more deeply infolded and proportionately the prominent ridge at the inner wall is strongly pro-



jected into the canal on the right side. The caudal extremity of the atria is continued into the sinus venosus, which diverge from each other into the two lateral myocardial tubes in relation with the foregut opening. The demarcation of these different portions is indicated by the sino-atrial constriction, of which on the left side the myocardial wall is more deeply infolded than on the right side.

The two lateral layers of the dorsal mesocardium are fused together from a middle third of the ventricle to the cranial part of the atria; they are in close contiguity at the atrioventricular constriction, in which region the dorsal mesocardium is beginning to disappear in an embryo slightly older than that of this stage. But at the portion of the bulbus cordis and the caudal part of the atria, the layers of the dorsal mesocardium have not come in contact. The arterial opening is disposed nearly vertically, but slightly to the left side, through which the endothelial tube comes from the myocardial cavity, while the venous opening is disposed dorsocaudally and assumes an irregular triangular space. Its base is situated caudally and ventrally opposite to the foregut opening, as a result of which the layers of the dorsal mesocardium divert together with the corresponding muscular tubes, which continue caudally. Its apex of the venous opening is directed cranialward and lies at the higher horizontal level. Through this intermesocardial space the enclosed endothelial tube can be seen.

On the ventral surface the myocardium is reflected onto the ventral wall of the pericardium at the sinus venosus. Here it can be observed that the mesodermal cells have proliferated to form an appreciable thickening around the endothelial tubes, indicating the future septum transversum.

The lateral endothelial tubes are fused in the middle third of the ventricle to the extent of fifteen sections. In this part they communicate with each other and the endothelial cavity is considerably dilated (fig. 23). This craniomedian part of the endothelial tube is bifurcated into the two cranial horns and two caudal prolongations. The cranial horns extend symmetrically from the cranial wall for a short distance on both sides

and terminate blindly opposite to the cranial myocardial extremity of the ventricle, which is of conical form.

The right caudal prolongation is given off from the right side of its caudal wall and terminates as a short conical projection; its apical terminus is directed opposite to the caudal extremity of the right ventricle, conforming with it. The left caudal prolongation is given off from the left side of its caudal wall and caudally connects with the endothelial tubes of the atrium. These separate from each other and continue farther caudalward. From the origin of this left caudal prolongation caudally to the atrioventricular canal, that is, in the caudal part of the left ventricle, the two endothelial tubes show their smallest size and are very close in contact in some places, while in others they separate into two quite independent tubes with complete walls. Opposite to the atrioventricular canal the two endothelial tubes above mentioned begin definitely to separate into two lateral atrial endothelial tubes, increasing their calibers gradually caudalward, while they lie approximately in a parallel direction.

The caudal extremities of the atrial endothelial tubes continue immediately into the endothelial tubes of the sinus venosus and then into those of the vitelline veins. The transition from the atrial endothelial tubes into those of the sinus venosus is marked by a sudden diminution in their diameter together with the general decrease of their calibers and the abrupt lateral divergence of their course, due to the intervention of the foregut opening. These transitional points correspond to the external groove of the sino-atrial constriction.

In the ventricle the endothelial tubes are separated from the myocardial wall by a wide intervening space. In the atrium the intervening space becomes considerably narrower, and finally in the sinus venosus the endothelial tubes are enclosed intimately by their own independent myocardial wall, so that no appreciable space can be seen.

The endothelial tube of the bulbus cordis extends from the dorsal surface of the right ventricular endothelium as its continuous prolongation. This endothelial tube proceeds at first

dorsocranially and then slightly toward the left side. This is enclosed by the corresponding myocardial wall of the bulbus cordis, which is closed cranially, but caudally opens and communicates with the ventricular cavity, as already mentioned. The right ventricular endothelium, which gives off the endothelial tube of the bulbus cordis, is fused together with the left one, but the left part of the fused ventricular endothelium participates in no way with the bulbus cordis.

The endothelium of the truncus arteriosus continues farther dorsally and slightly toward the left side from the bulbus cordis and passes through the above-mentioned arterial opening, and then bifurcates into lateral symmetrical branches, which are located between the foregut floor and the lateral dorsal mesocardial layers and continue farther cranially into the ventral aortae.

The first aortic arch and the ventral aortae are completely formed and the dorsal aortae are considerably elongated caudalward.

#### SUMMARY AND CONCLUSION

In our observations the first sign of the formation of angioblasts is shown in stage I, embryo A, in which neither the head fold nor the anlage of the pericardial cavity has yet appeared.

On the ventral surface of the mesoderm of the splanchnopleura of the cranial portion, cell bands first begin to separate, which separation is more advanced in the embryos B and C. These cell bands are regarded as angioblasts and they are frequently found to adhere to the indented and loosened mesoderm of the splanchnopleura by broader or narrower protoplasmic bridges. It has frequently been pointed out that mitotic figures are found in the mesoderm of the splanchnopleura in the neighborhood of angioblasts. Furthermore, in many cases where the angioblasts are in close contact with the mesoderm of the splanchnopleura, it is impossible to discriminate the angioblasts from the mesodermal cells of the splanchnopleura, as concerns their sizes, forms, staining reaction, and the form of the nuclei, while a great difference can readily be recognized between the angio-

blasts and the adjacent entodermal cells. These findings show that in the genetic origin the angioblasts for the future endocardium are derived directly from the mesoderm of the splanchnopleura. The origin of the angioblasts from the mesodermal cells continues until a later stage, in which the greater part of the endothelial tubes are already differentiated from the angioblasts in the anterior portion of the embryo, but the origin of the angioblasts can be recognized in the posterior part of the embryo, as is shown in the embryo of stage III.

In their well-known work on pericardial development, Strahl and Carius found it impossible to decide whether the embryonic coelom in the guinea pig appears at first in the region of the heart anlage, proceeding forward into the pericephalic mesoderm, or whether it begins first in the pericephalic mesoderm and then spreads out caudally. They speak as follows: "Doch können wir augenblicklich eine ganz sichere Entscheidung nicht geben." The cause of this ambiguity is that they began their investigation of the origin of the intraembryonic coelom at too late a stage.

For the dog, Bonnet states that, concerning the origin of the intraembryonic coelomic space, that the lateral pleuropericardial cavities, having already distinctly appeared, anticipate the formation of the pericephalic space. To quote Bonnet directly: "Eine ebensolche Spaltung des Mesoderms führt in VIII5 gleichzeitig im Bereiche des Herzwulstes zur Bildung der Pleuro-Pericardialhöhle. Ihre zuerst paarig angelegten spalten vergrössern sich, vereinigen sich nach vorne und bilden so ein nachhinten offenes Hufeisen; den pericephalen und lateralen Teil der Pleuro-Pericardialhöhle."

In our specimens, embryo C, stage I, shows the discontinuous formation of the intraembryonic coelomic spaces in the cranial portion of the embryonic shield, as also in the pericephalic mesoderm, these spaces beginning as multiple foci. But they are primarily absent in the middle portion of the pericephalic mesoderm.

In stage II, in which the head fold of the embryo begins to separate from the surrounding blastoderm and the foregut has

just begun to develop, the intraembryonic coelomic space spreads out cranially into the pericephalic mesoderm, cleaving the mesodermal layer in such a way, that the lateral primitive pericardial cavities communicate with each other. In a just slightly younger embryonic shield than this, each lateral pericardial cavity has progressed cranially into the pericephalic mesoderm, showing in this place a slit-like space, which however, is divided by a thin mesodermic bridge in the middle line.

The pericardial cavity, therefore, commences simultaneously in the multiple foci, separating irregularly by mesodermal bridges 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 shape, when the mesodermal bridges at the middle line of the pericephalic mesoderm have ultimately disappeared and, in consequence, at this time the bilateral pericardial cavities, already widely confluent, communicate from side to side (stage II, A). In this embryonic shield relatively wide endothelial tubes are differentiated only in the region of the hindbrain plate, where the pericardial cavity is wide open and the mesoderm of the splanchnopleura is thickened, projecting into the pericardial cavity as a prominent fold. In the pericephalic portion of the mesoderm, however, the pericardial cavity is seen merely as a lineal cleavage. Here a few angioblasts are scattered between the slightly thicker mesoderm of the splanchnopleura and the underlying entoderm.

In stage III the bilateral myocardial folds become quite prominent, so that in the region of the hindbrain plate they have been almost converted into the myocardial tubes, and enclose the well-developed endothelial tubes.

The formation of these myocardial folds has progressed cranialward opposite to the foregut opening on the left side. At the same time the endothelial tube becomes gradually thinner cranialward and terminates slightly caudad to the foregut opening. On the right side the formation of the myocardial folds proceeds still farther cranially into the caudal part of the craniomedian limb of the pericardial cavity, where the thicker mesoderm of

the splanchnopleura is raised from the underlying entoderm and in the space between them a number of angioblasts are distributed. On the right side the endothelial tube terminates cranially just opposite to the foregut opening. In front of the cranial termination of the lateral endothelial tubes a number of angioblasts are scattered, so that the cranial extremities of the endothelial tubes are nearly connected with each other through these angioblasts.

The cranial extremities of the lateral myocardial folds have not yet come to complete confluence, as the mesoderm of the splanchnopleura, slightly cephalad to the cranial extremity of the left myocardial fold, remains still in loose contact with the underlying entoderm. If this portion of the mesoderm of the splanchnopleura were completely raised from the underlying entoderm, forming the myocardial fold, then the myocardial anlagen would come into confluence.

The most prevalent opinions with regard to the mode of the formation of the unilateral myocardial heart anlage from the bilateral myocardial tubes agree that, as above described, the bilateral myocardial tubes, at first independently, come to actual fusion with each other, and then the septal wall between them is absorbed secondarily, thus forming a single myocardial cavity. Our specimens show that the formation of the myocardial folds does not occur synchronously throughout the pericardial cavity, as in the region of the hindbrain plate they first appeared and developed considerably, while in the craniomedian limb of the pericardial cavity the formation of the myocardial folds was just starting and rising slightly from the underlying entoderm. However, the communication of the lateral myocardial tubes is accomplished when the formation of the myocardial folds is completed in the craniomedian limb of the pericardial cavity, in which region the formation of these folds occurs last. For this reason, the cranial prolongation of the lateral myocardial tubes has not been brought about by the direct extension of the first part of the myocardial tubes, but by the continuous progressive differentiation into the craniomedian limb of the pericardial cavity. Therefore, the confluence of

the myocardial tubes into a single myocardial cavity is not accomplished by the actual fusion of the bilateral myocardial tubes, followed by absorption of the septal walls.

In stage IV the formation of the myocardial fold is completely accomplished in the craniomedian limb of the pericardial cavity, elevating the mesoderm of the splanchnopleura from the underlying entoderm and projecting into the pericardial cavity. Both lateral myocardial tubes communicate with each other in this region.

Both the myocardial tubes are quite voluminous. They dilate in all directions, especially in their cranial portions, and they gradually reduce their dimensions caudalward. On the surface of these transitions no distinct demarcation can be noted.

The two lateral endothelial tubes are well developed, lying side by side together in the portion of the confluent myocardial cavity, and here independent, intermediate endothelial cells are scattered between the lateral endothelial tubes.

In stage V the cranial portions of the two lateral myocardial tubes show much more dilatation and elongation, as their extent from the foregut opening to the cranial extremities considerably increases. But their confluent part still remains short. The transition from the cranial dilated myocardial tubes to their caudal slender portions is marked by a distinct annular atrioventricular constriction, which appears clearly first in this embryo.

A number of workers declare 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, '99; in the cat, Martin, '02; in the rabbit, Kölliker, '84. Kölliker states, "Ein Herz aus diesem Stadium ist sehr verschieden von dem primitiven Herzen eines Hühnerembryo, was einfach darin begründet ist, dass, wie bemerkt, bei Säugethieren schon vor der Verschmelzung der beiden Herzhälften die drei Herzabschnitte angelegt sind."

Mollier declares in similar language: "An den beiden Herzhöhren ist aber kurz vor ihrer Vereinigung schon eine Gliederung bemerkbar."

In our specimens this embryo shows first the atrioventricular constriction, even though in the previous embryo two myocardial tubes were already confluent into a single myocardial cavity.

The two lateral endothelial tubes are considerably developed, especially in the confluent portion of the myocardial cavity, where they approach each other almost to contact. From the atrioventricular constriction caudalward the two endothelial tubes assume an abruptly narrow caliber, thus distinguishing the larger cranial ventricular from the smaller caudal atrial portion. In tracing farther caudally, no demarcation can be detected on them.

In stage VI the confluent myocardial cavity increases especially in the craniocaudal extent. The cause of this may be attributed partly to the retirement of the wedge-shaped septal ridge cranialward, which had projected into the confluent myocardial cavity, as the conversion of the inner walls of the cranial lateral myocardial extremities, and partly to the active backward progress of the foregut opening. Approximately, in the whole extent of the ventricle and in the cranial part of the atria the two lateral myocardial cavities communicate with each other and these two myocardial portions are demarcated sharply by the atrioventricular constriction. In the middle third of the ventricle the two lateral endothelial tubes are actually fused and communicate with each other for a short distance. At the atrioventricular constriction the calibers of the two endothelial tubes are considerably reduced and, as we proceed still farther caudally, they are again gradually increased in diameter.

In an embryo of *Perameles nasuta* having fifteen to sixteen somites, Miss Parker pointed out that the fusion of the lateral endothelial tubes first took place. The fused portion already extends throughout about eighteen sections. She states that from this portion the *bulbus cordis* is derived.

Wang reports concerning a ferret embryo, having thirteen to fourteen somites, that the two endothelial tubes had united in a part of their extent. The fused portion, extending throughout about sixteen sections, appeared to be the ventricular part.



In our specimens this embryo shows first the fusion of the two lateral endothelial tubes throughout only seven sections, having a  $5\ \mu$  thickness, and this united part corresponds to a middle third of the ventricle and lies precisely on the midsagittal plane. In the guinea pig the fusion of the lateral endothelial tubes takes place at a relatively early stage of development—a stage in which in the myocardial and endothelial tubes there can be distinguished simply the ventricular and atrial portions. In the above-mentioned animals investigated by other authors, the fusion of the lateral endothelial tubes was first noted in the relatively older embryo, in which the different parts of the myocardial and endothelial tubes are already definitely subdivided. Moreover, their embryos show that the fused portion is considerably extended in comparison with this embryonic shield.

The factors which are generally accepted as the cause of the loop formation of the endothelial tubes depend on the fact that the rate of growth of the two endothelial tubes exceeds that of the pleuropericardial cavity. Bonnet depicts a dog embryo in which the primary subdivision of the endothelial tubes into sinus venosus, atrium, and ventricle has occurred before they have fused to form a single myocardial cavity.

Wang pointed out the loop formation with the subdivision of the heart (atrium, ventricle, bulbus, etc.) in the ferret embryo, before the endothelial tubes had become fused.

But in our specimens 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, even though the ventricle and atrium may be roughly distinguished by their difference in size.

Contrary to the above-mentioned assumption that the loop formation of the endothelial tubes has been brought about, the moment of fusion of the two lateral endothelial tubes shows quite other facts in the guinea pig. In the embryonic shield at this stage of development the confluent part of the myocardial tube grows excessively in the craniocaudal direction and decreases its lateral width in comparison with the embryo of stage V, as measured and compared on both reconstruction

models which had been magnified to the same degree. Consequently, the two endothelial tubes are brought together in the median plane, where they come to fusion, by the extreme longitudinal stretching of that part of the myocardial tube in which the endothelial tubes are enclosed. Concurrently, the active dilatation of the two endothelial tubes plays a part in bringing about the fusion, which takes place first in the most dilated portions.

In stage VII the myocardium presents cranially a single expanded craniomedian extremity, assuming a sac form, and here represents the ventricle, while caudally this myocardial sac is bifurcated into two rather slender myocardial prolongations. Their demarcation is indicated by the well-developed atrioventricular constriction slightly cephalad to the foregut opening. The transition from the atrium into the sinus venosus is marked by an indefinite indentation on the bilateral myocardial tubes caudad to the foregut opening and slightly more distinct on the left side. Corresponding to these myocardial constrictions, there can be pointed out a similar indentation on the endothelial tube on the left side.

The two lateral endothelial tubes are fused together in the cranial two-thirds of the ventricle, and its cranial extremity is terminated as a single conical apex opposite to the cranial end of the myocardial ventricle. In tracing farther caudalward from this united portion, the two endothelial tubes are separated.

In this stage of the development the myocardial and endothelial heart anlagen of the ventricle present a considerable asymmetry, due to the unequal growth of the individual parts, despite the fact that, in the embryonic shields prior to this stage of development, the heart anlagen are shown as a practically symmetrical development on both sides, even after the fusion of the lateral endothelial tubes has already been accomplished.

Miss Parker describes the heart of the *Perameles obesula* stage V as follows: "In the ventricular region of the heart, the right and left endothelial tubes are approximately equal in size, but where there is an inequality the right is the larger."

In the ferret embryo Doctor Wang says: "It has been found that the two tubes, prior to fusion, appear to have been shifted as a whole toward the right side and that they remain in this position even after partial fusion has taken place."

In our specimens the myocardial asymmetry of the ventricle is attributed partly to the extraordinary bulging of its right wall dorsally, laterally, and slightly caudadly, and partly to the deeper infolding of the myocardial wall of the atrioventricular constriction of the right side. In the ventricle the endothelial tubes are much more dilated on the right side than on the left throughout its whole length, regardless of the fused or non-fused portions, and it expands considerably dorsally, laterally, and caudadly. Consequently, they deviate from the middle plane toward the right side and are situated more to the right side of the myocardial ventricle. The right endothelial tube is elongated dorsally at the caudal third of the ventricle, where the two lateral endothelial tubes, being separated, pass through the intermesocardial space vertically. From this portion the bulbus cordis develops in a later stage; its termination is cranially bifurcated into two lateral endothelial branches, which continue farther cranialward into the ventral aortae.

In stage VIII there are present three distinct constrictions on the tubular myocardial surface, infolding the whole thickness of the myocardial wall into the myocardial cavity. Consequently, the myocardium can be subdivided by very distinct demarcations into the bulbus cordis, bulboventricular constriction, ventricle, atrioventricular constriction, atrium, sino-atrial constriction, and sinus venosus in the craniocaudal succession.

The bulbus cordis is demarcated from the dorsal wall of the expanded right ventricle at its cranial end by the horizontal bulboventricular constriction. The bulboventricular constriction shows considerable asymmetry, making a deeper furrow on the external surface of the myocardium at the left and cranial sides, while at the right side it is present as a shallow depression on the external surface, diminishing imperceptibly caudalward, until it has entirely disappeared at the part of the right ventricle. Thus neither external furrow

nor infolding of the myocardium is shown along the caudal boundary of the bulboventricular junction, and here the wall of the bulbus is directly continuous into that of the right ventricle. Corresponding to the external view, the deep infolding of the myocardial wall as the inner prominent ridge is shown at the left and cranial sides of the bulboventricular canal, while on the right side it can be recognized only cranially and disappears insensibly caudalward. In this fashion the curvature of the myocardium at the bulboventricular junction is effected in such a way that its convexity is turned toward the right side. On the dorsal aspect of the bulbus cordis there is a triangular intermesocardial space disposed vertically and slightly toward the left side, through which the truncus arteriosus passes out from the myocardial cavity up to the floor of the foregut.

The atrioventricular canal is well marked and is disposed approximately in the vertical plane. It is produced by the infolding of the myocardial wall, which is deeper and more caudad on the right side than on the left. Consequently, the opening of this canal is situated on the left side of the middle plane. In this relation the myocardial tube forms a marked curvature at the atrioventricular junction, turning its convexity toward the left side and ventralward, with the result that the ventricular portion lies at the right side and slightly ventrally to the atrial portion. This curvature is remarkably accentuated in the next stage of the development, in which, for a short extent, the dorsal mesocardium disappears at the atrioventricular junction and herewith the ventricle comes to the ventral surface of the atrium, being free from the restriction of the dorsal mesocardium.

The ventricle can be divided incompletely into two limbs by the ventral and dorsal longitudinal sulci. At the caudal part of the ventricle, for a short distance, the two limbs are divided into two completely independent cavities by the septal wall. The caudal extremity of the right ventricle terminates blindly as a conical process and it projects caudolaterally.

In the literature I could not find a description of this. On first observation it seemed to me that the septal wall and its conversion into prominent ridges at the inner surface of the ventral and dorsal myocardial wall were produced by the actual fusion of the two lateral myocardial tubes. Therefore, this may account for the remnant of the primitive myocardial septum. But in the embryos of stages VI and VII there was present no septal wall nor prominent ridge similar to this in their single ventricle, in which they would be more distinctly present if they accounted for the production of the actual fusion of the lateral myocardial tubes and the remnant of the primitive cardiac septum.

Accordingly, it appears to be due to the fact that the caudal surface of the myocardial ventricle on the right side is projected actively backward by the unequally excessive rate of growth in this portion, while in the middle plane a part of the myocardial wall does not proportionately accompany this active backward growth, but remains as the septal wall.

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