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Cardiovascular development

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Introduction

The importance of knowledge of cardiovascular embryology has changed over the years. The field of embryology has been useful to the understanding of details of the anatomy of congenital malformations, although erroneous developmental explanations have hindered the understanding of congenital lesions.[1] Embryological–pathological correlations can be made only when both the development and the pathological anatomy have been adequately described.

Currently, the fields of fetal cardiology, genetics and molecular biology are developing fast. Much of this knowledge has been acquired from in vitro experiments using cell or tissue cultures, but in vivo studies on genetically manipulated mice have become gradually more important. Integration of the information should lead to full understanding of development within the embryo. In the present chapter, histological and gross anatomical features of the embryonic heart are discussed in an attempt to link them with prenatal cardiac function.

The description of the mature heart in normal individuals and in the case of congenital malformations has greatly benefited from the sequential segmental approach.[2] This approach is also useful for the description of consecutive developmental stages, because it highlights the course of the blood stream and, therefore, has functional implications. After a sequential description of development, some aspects will separately be highlighted.

Segments and intersegmental transitional zones in the embryonic heart

Prior to septation, which leads to the four-chambered organ, the heart is a simple tube in which the blood is pumped through a number of serially connected segments. Although the tube starts off as a straight one, arranged in a caudocranial direction in the embryonic body, the individual segments are best described when the looping process of the tube has taken place and the heart has adopted an S-shape. Extrapolation of the segmental organization to the straight heart tube stage is disputable, because at that stage not all components have yet been recognized using labeling experiments.[3] Distinct atrial and ventricular cell lineages have, however, been described before looping.[4] The most reliable criteria to distinguish the segments in the looped heart are unfortunately still based on general morphology, because specific markers for each segment, which would remain recognizable throughout development, have not yet become available. Several molecular markers are known to distinguish cardiac segments, but their specificity is restricted to limited periods of embryonic development.[5]

In the looped heart, cardiac segments can be distinguished by the presence of a series of constrictions in the myocardial tube and by the internal profile of the myocardium, which may be smooth or trabeculated, and in slightly advanced stages by the accumulation of endocardial cushion masses on the inside and subepicardial tissue externally. Following the blood stream from the venous to the arterial poles, the segments are the venous sinus, the atrium, the ventricular inlet segment and the ventricular outlet segment. The venous sinus collects all the embryonic veins, and the ventricular outlet segment is connected to the arterial trunk (aortic sac). Between these segments the intersegmental transitional zones are the sinuatrial, atrioventricular and primary interventricular transitions (Figure 1.1).

The venous sinus

The venous sinus is the most caudal segment, which is anchored within the transverse septum. Left and right

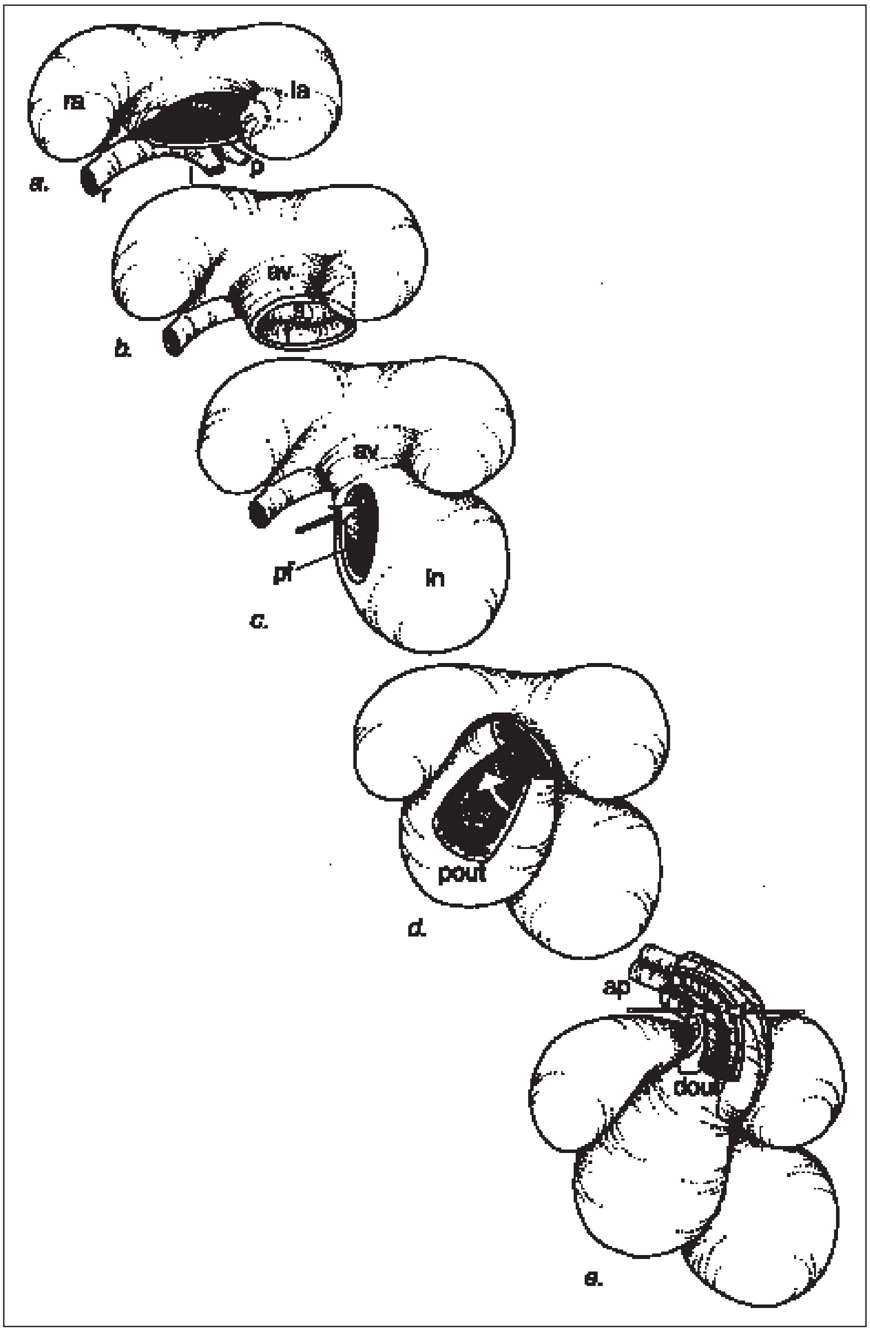

Figure 1.1
Schematic dissection of the embryonic heart after looping. (a) The venous sinus, which receives the systemic veins in its right (r) and left (l) sinus horns and the common pulmonary vein (p) separately, opens into the common atrium with its left (la) and right (ra) atrial appendages. (b) From the atrium, the blood continues into the atrioventricular canal (av) with superior (s) and inferior (i) endocardial cushions. (c) The atrioventricular canal (av) completely drains into the ventricular inlet segment (in). On its right side, the inlet segment shows an opening, the primary foramen, which is encircled by the primary fold (pf). Note the close proximity of the primary fold and the atrioventricular canal, which can be seen through the primary foramen (arrow). (d) The proximal outlet segment (pout) receives the blood from the primary foramen (arrow). (e) The most downstream portion of the ventricular mass is the distal outlet segment (dout) which has started to be septated by two endocardial outlet ridges (arrows). Within their core, these ridges contain the extremities of the aortopulmonary septum (ap) which are attached to both sides of the myocardial wall of the distal outlet segment.

sinus horns contribute to the segment and these collect all the veins of the left and right sides of the embryo. There has been considerable discussion about the relationship of the pulmonary veins with the venous sinus. According to classical descriptions the primitive common pulmonary vein develops by sprouting from the dorsal wall of the left atrium.[6] More recent accounts, using HNK-1 expression as a marker for the sinuatrial boundary in the chicken and the rat, describe the pulmonary vein connecting originally to the venous sinus.[7–9] Observations in the mouse embryo still claim the primary connection to be established beyond the venous sinus.[10] This controversy is a clear example of the need for distinct cardiac segmental markers in addition to more classical methods of distinguishing between the segments, as mentioned above.

During development the venous sinus is incorporated into the atrium. Most of it contributes to the posterior wall of the mature right atrium, but a small portion forms the posterior left atrial wall and will contain the pulmonary venous orifices. A problem with nomenclature is apparent

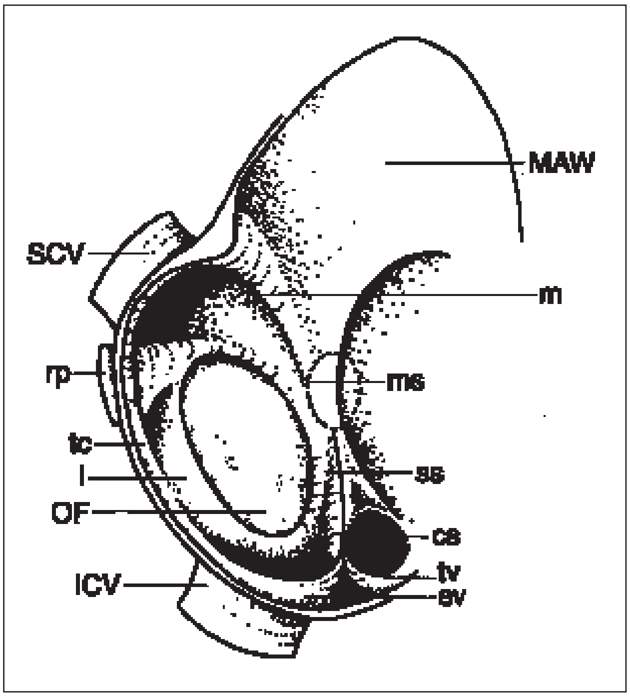

Figure 1.2
Diagram of the opened right atrium of the mature heart to show the embryonic derivation of the septal structures. The right side of the sinuatrial transition is almost circular. Starting at the membranous septum (ms), it is represented by a muscular band (m) between the atrial appendage (MAW) and the entrance of the superior caval vein (SCV), from where it continues first into the terminal crest (tc) and then into the valve (ev) of the inferior caval vein (ICV) and the valve (tv) of the coronary sinus (cs). Only the valve of the oval foramen (OF) and the limbus (l) of the oval fossa are of embryonic atrial origin (from reference 8).

in this description; what is known is the atrium in the mature heart is built from two distinct embryonic cardiac segments called the venous sinus and the atrium.

In the mature heart, those parts that are derived from the embryonic venous sinus are easily distinguished by their smooth internal wall, in contradistinction to the wall of the embryonic atrial compartment, which carries pectinate muscles. In particular on the right side, the two embryonic compartments can be distinguished by the presence of structures derived from the sinuatrial transitional zone (Figure 1.2).

The sinuatrial transitional zone

With the incorporation of the venous sinus into the atrium, the sinuatrial transition gradually becomes more apparent. Although its left side, which delimits the pulmonary venous orifice, is never very prominent,[7] the invagination into the right part of the atrium leads to the formation of large valves, the venous valves. The left venous valve is no longer discernible in the mature heart because it is incorporated into the atrial septal structures. The right venous valve, however, remains present as the valve of the inferior vena cava (Eustachian valve) and the valve of the coronary sinal orifice (Thebesian valve) (Figure 1.2). The Eustachian valve continues into the terminal crest, which courses along the posterior atrial wall to surround the entrance of the superior vena cava. Much of this intersegmental transitional myocardium is involved in the development of the conducting tissues. The described position at the entrance of the superior vena cava coincides with the sinus node. The terminal crest has been implicated in descriptions of "internodal tracts". The basal portion of this transition may be responsible for the formation of atrial inputs to the atrioventricular node.

Distinction of the venous sinus and the sinuatrial transition from the embryonic atrial myocardium is possible because of the HNK-1 expression, which is always absent from the atrium.[8]

The atrium

The atrium starts off as a relatively large unseptated cavity. Most of its remnants in the mature heart are represented by the left and right atrial appendages. These remain recognizable by the pectinate muscles. The relatively early development of these pectinate muscles in the embryonic atrial compartment may imply an early contractile function of the atria, just as has been described for the trabeculations in the embryonic ventricles.

The atrioventricular canal

The atrioventricular canal is the transitional zone that connects the atrium with the ventricular portion of the heart loop. In preseptation stages this canal forms an important proportion of the total myocardium, but its growth is relatively slow and in advanced fetal stages the canal has become no more than the boundary between atrial and ventricular structures.[11]

Internally, the atrioventricular canal is invested with relatively large endocardial cushions which derive their cells from the covering endocardium by a process of endocardial–mesenchymal transformation.[12] Also, neural crest cells have been demonstrated to migrate into the cushions.[13] Because they are compressed by atrioventricular contraction, the cushion masses prevent regurgitation

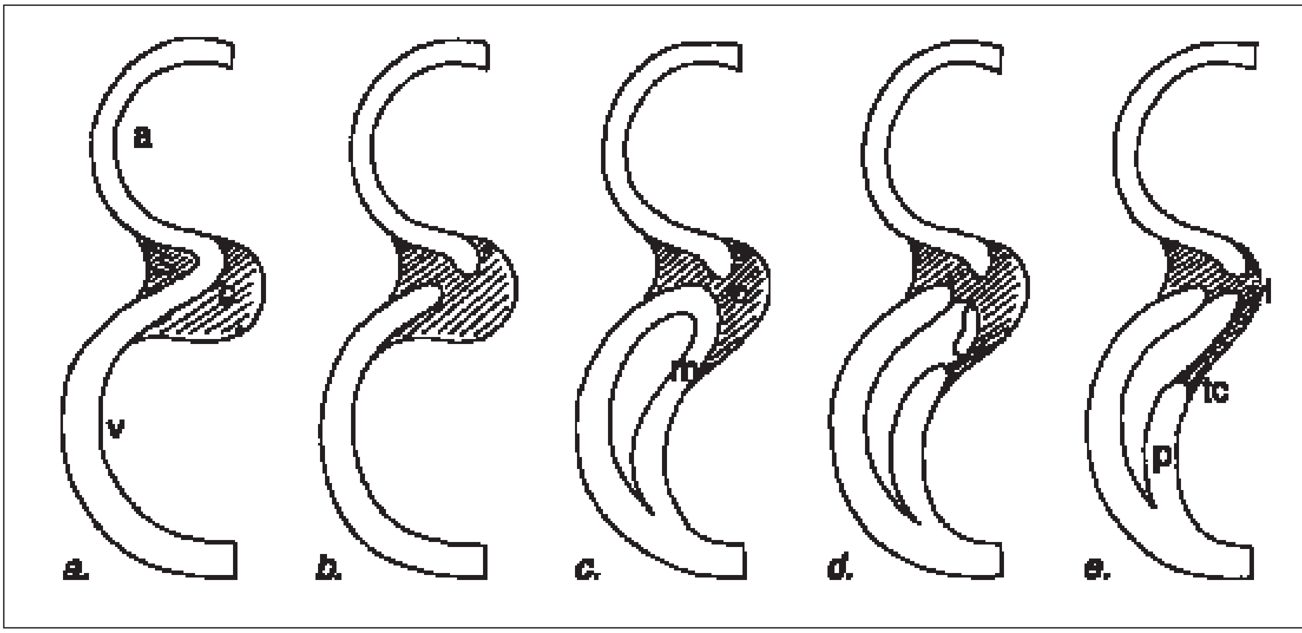

Figure 1.3
Diagrams to show the mechanism of atrioventricular valve formation. (a) In the early stages, the myocardium of the atrium (a) and ventricle (v) is continuous at the atrioventricular canal. Externally, the atrioventricular groove is filled with subepicardial sulcus tissue (s). Internally, the atrioventricular myocardium is covered by endocardial cushion tissue (c). (b) The continuity of atrial and ventricular myocardium is disrupted. (c) The inner layer of ventricular myocardium is delaminated, which process creates a primitive flap valve consisting of cushion tissue (c) on its atrial side and of myocardium (m) on its ventricular side. (d) The myocardial layer on the ventricular side of the valve gradually disappears and retracts towards the atrioventricular canal and towards the ventricular apex. (e) The myocardial layer of the valve has completely disappeared. The cushion tissue has thinned out to form the valve leaflet (vl) and the tendinous chords (tc). The remaining delaminated myocardium constitutes the papillary muscle (p).

and they are indeed the precursors of the atrioventricular valves.[14] Externally, the atrioventricular canal is represented by a groove, which becomes filled with subepicardial mesenchyme. The originally single atrioventricular canal is septated into two separate atrioventricular valve orifices. Fusion of the endocardial cushions in the center of the canal forms the basis of this septation process. Because the superior cushion is immediately related to the base of the atrial septum and the inferior cushion is attached to the developing ventricular inlet septum, the same process also brings atrial and ventricular septal structures into continuity. It is not probable that this septation process forms the basis for the creation of separate left and right blood streams. Such blood streams already exist in early preseptation stages[15] and they are necessary for the creation of the inlet portion of the mature right ventricle and the splitting off of the ventricular inlet septum from the primary fold (see below).

The general process of atrioventricular valve formation[14] (Figure 1.3) does not explain the detailed final morphology of these valves. It does explain the general architecture, with anular attachment, a fibrous veil and chords attached to papillary muscles, but the morphological differences between mitral and tricuspid valves are related to the morphology of the ventricles in which they develop. In the same line, many congenital malformations of the atrioventricular valves are not the result of defective valve formation but of pre-existing abnormalities of the underlying ventricular myocardium. Straddling valves and the common valve in atrioventricular septal defects belong in this category. On the other hand, congenital valve pathology based on deviation of the general developmental mechanism is extremely rare. Ebstein's anomaly is an example of this category.

The myocardium of the atrioventricular canal is involved in the formation of the atrioventricular node and the proximal His bundle. These structures have been recognized by their HNK-1 content, as in the sinuatrial transitional myocardium.[8] Any relationship between this immunohistochemical marker and the specific function of the conducting tissues has, however, not yet been elucidated.

The ventricular inlet segment

Before septation, all of the atrial blood is propelled into a single cavity, the ventricular inlet segment. The embryonic heart may be described to have a univentricular atrioventricular connection. Roughly, the inlet segment may be compared with the mature left ventricle, but the term “primitive left ventricle” is not used here, because not all of the tissues of the mature left ventricle are derived from this segment.

The future ascending aorta and pulmonary trunk are connected to the more distal outlet segment of the heart. This means that the process that brings the aorta above the mature left ventricle also makes the outlet segment contribute to the left ventricle. The transfer, therefore, concerns the subaortic outflow tract in addition to the aortic orifice.

The embryonic inlet segment has a sponge-like appearance because of its many trabeculations. These trabeculations are thought to be the main structures that cause ventricular systole.[16] Their degree of myofibril assembly is considerably advanced when compared to that of the compact free wall.[17] In early stages, the myocardial volume of the ventricular inlet segment is considerably larger than that of the outlet segment, making the inlet segment largely responsible for ventricular force.[11]

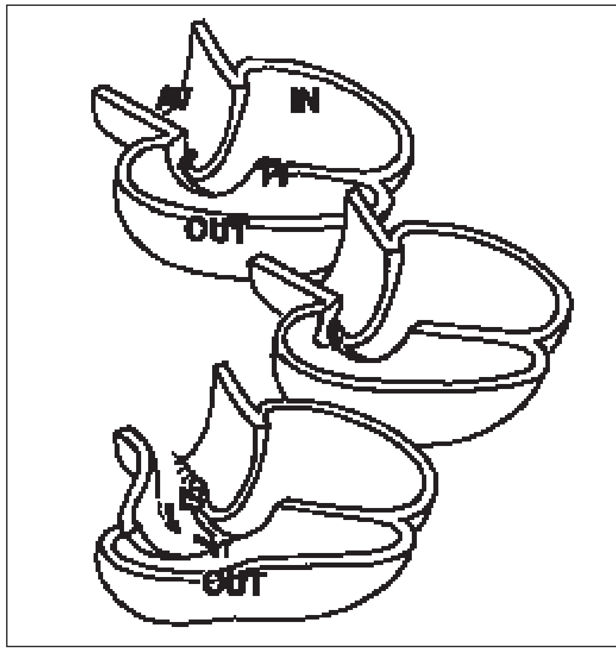

Figure 1.4
Diagram to show the development of the inlet portion of the right ventricle. Initially, the atrioventricular canal (AV) drains completely into the ventricular inlet segment (IN). The blood has to pass the primary fold (PF) to reach the ventricular outlet segment (OUT). The blood coming from the right part of the atrioventricular canal excavates a new cavity within the tissue of this fold which splits the fold into an inlet septum (IS) and the septomarginal trabeculation. The latter is pushed forward and has always to be passed by the blood stream to reach the outlet segment (second arrow in bottom figure) (from reference 20).

The primary fold

The subdivision of the ventricular mass in the looped heart is made by an external groove, which is called the primary groove. It corresponds to an internal myocardial profile, the primary fold, which delimits the communication between inlet and outlet segments. The communication has been described as an “interventricular foramen”.[18,19] This term might cause confusion, because any interventricular communication would logically be closed by the ventricular septation process, which is not the case for this foramen. In the present account, the communication between the two segments within the ventricular mass is called the primary foramen. To guarantee the continuity of the blood stream from the inlet segment into the outlet segment and the great arteries, the primary foramen will never close.

Most of the primary fold will form the muscular ventricular septum. In the inner curvature of the heart loop, the fold establishes the boundary between the arterial and atrioventricular regions of the ventricular mass (also known as the ventriculoinfundibular fold).

A special process is involved in the formation of the ventricular inlet septum, i.e. that part of the ventricular septum which separates the blood streams coming from the two mature atrioventricular orifices.[20] Initially, no other cavity than the outlet segment is present to the right of the primary fold. Then, the right atrioventricular blood stream seems to create an excavation in the posterior part of the fold and to split off a separate inlet septum to its left side. This excavation will enlarge and is present in the mature heart as the inlet portion of the right ventricle. The enlargement of this inlet portion increasingly separates the right part of the primary fold from the inlet septum. This part of the primary fold will continue to indicate the boundary between inlet and outlet segments, and the displaced portion is found in the mature heart as the septomarginal trabeculation between inlet and outlet parts of the right ventricle (Figure 1.4).

The ventricular outlet segment

The right side of the ventricular part of the heart loop is initially constituted by only the ventricular outlet segment. The term “primitive right ventricle” should be avoided, because rather complex processes still have to lead to the formation of the mature right ventricle. These processes involve the excavation of a right-sided inlet cavity and the splitting of the primary fold into an inlet septum and a septomarginal trabeculation (see above). The ventricular outlet segment is composed of two subdivisions: the proximal outlet segment and the distal outlet segment.

The proximal outlet segment looks very much like the ventricular inlet segment, having initially a thin compact myocardial wall and showing many trabeculations. Before septation, this segment is considerably smaller than the inlet segment.[11] Hemodynamically, in these early stages the heart might be compared to the congenital lesion called univentricular atrioventricular connection to the left ventricle, in which a comparatively small right ventricle is usually present. It may be that the ventricular inlet segment in these stages propels the blood directly into the next portion of the heart, the distal outlet segment.

The distal outlet segment has a smooth myocardial wall and is invested internally with thick endocardial ridges, which contribute to septal structures and to the arterial valves. The cells within these ridges have originated from the covering endocardium by endocardial–mesenchymal transformation.[12] Another source of cells in these cushions is the neural crest.[13] Before the presence of the arterial valves, the myocardium of the distal outlet segment compresses the endocardial ridges to prevent regurgitation. This myocardium is precociously differentiated and has particular functional properties.[17,21] As the atrioventricular canal, the distal outlet segment shows only temporary growth, and its proportional volume and length diminish gradually.[11,22]

The tissues of the embryonic heart

The heart tube, which develops as a specialized portion of the vascular system, consists of three layers of different cellular composition. The main constituent is the myocardium, which is lined internally by the endocardium and externally by the epicardium.

The myocardium

The myocardial constituent of the embryonic heart is derived from the splanchnic mesoderm, which forms a single horseshoe-shaped structure in front of the buccopharyngeal membrane.[23] The limbs of this horseshoe contribute to the venous pole of the heart, its central portion giving rise to the ventricular structures.

The endocardium

The splanchnic mesoderm also gives rise to a single endothelial plexus, which forms the internal endocardial tube.[23,24] Initially, the space between the endocardium and myocardium is occupied by the cardiac jelly.[25] This jelly becomes invaded by mesenchymal cells, which are derived from the endocardium.[12] The invasion leads to the formation of local masses of endocardial cushion tissue which are restricted to the atrioventricular canal and the distal outlet segment.

In addition to the endocardial–mesenchymal transformation, other sources contribute to the cellular composition of the cushions. It should be realized that the endocardial tube forms part of the continuous system of endothelial structures forming the veins as well as the arteries. At the same time, the myocardial wall of the heart develops as part of the coelomic wall which shows reflections into the pericardium at the venous and arterial poles. Therefore, the space between the myocardial and endocardial layers can be invaded by cells from extracardiac sources. A main source of extracardiac contribution is the embryonic neural crest.[26] Indeed, neural crest cells have been demonstrated to invade the endocardial cushions in the distal outlet segment and to contribute to outflow tract septation.[27] Neural crest cells also invade the inflow tract.[13]

A second extracardiac source of cushion cells is the epicardium, which has been demonstrated to send its derivatives into the atrioventricular cushions.[28] They also form the substrate for the smooth muscle cells and fibroblasts of the coronary vasculature.[29]

The epicardium

As described above, the coelomic epithelium forms the myocardium and the pericardium, and these tissues are continuous at the venous and arterial poles. The epicardium is of secondary origin. At the site of the transverse septum the epicardial organ starts to protrude into the pericardial cavity and sends out vesicles. These gradually cover the entire myocardial mantle, where they give rise to the epicardial lining and the subepicardial mesenchyme, including the early subepicardial vasculature.[30–33] The epicardial vesicles have been described to carry mesenchymal cells from within the transverse septum.[34]

Septation and valve formation

Complex processes transform the looped heart tube into the four-chambered organ with valves at the various transitions. All segments and intersegmental transitional zones are septated into left and right portions, although the processes and the tissues involved are variable. Evidently, all septal structures have to fuse to guarantee continuous left and right blood streams. For this reason, it is impractical to describe the septation processes individually for each separate segment. In the text to follow, the septation processes will be dealt with in an integrated fashion, only subheading the venous and arterial portions of the heart separately.

Septation and valve formation at the venous pole

Because most of the venous sinus is incorporated into the atrium, it is vital to analyse the mature atrial septal structures to find out which of them belong to the atrium proper and which are of sinus or sinuatrial origin. It appears that only a very small portion of the mature atrial septum, contained within the rims of the oval fossa, is of embryonic atrial origin, all other structures being derived from the sinuatrial transitional zone.[8] The invagination of the venous sinus into the embryonic atrium leads to the formation of several distinct profiles. The sinus part receiving the systemic veins invaginates deeply into the right portion of the atrium, which process creates prominent double structures called the venous valves. These flaps can be distinguished to consist of sinal and atrial layers. Towards the pulmonary portion of the sinus, the invaginated profiles gradually diminish and the pulmonary venous orifice is only temporarily bordered by distinct elevations. It is this less well developed part of the sinuatrial transitional zone that is cut off from its right-sided counterpart by the atrial septum primum. This muscular septum is completely contained within the embryonic atrium, but when it descends it compresses the sinuatrial junction just to the right of the pulmonary vein.[7] The posterior portion of its lower rim fuses with the inferior atrioventricular endocardial cushion, which is also the site where the inferior parts of the venous valves become fixed. As a result, the systemic part of the venous sinus is kept to the right of the atrial septum primum. To the left of the left venous valve, between it and the atrial septum primum, the atrial wall still invaginates somewhat to form an internal profile which is called the atrial septum secundum. This structure never becomes a full septum, but forms a semicircular rim that eventually fuses with the left venous valve. At birth, it forms the material to which the flap of the atrial septum primum is pressed to close the interatrial communication.

Septation and valve formation at the arterial pole

Septation of the outflow tract takes place in immediate continuity with the septation of the aortic sac[35] (Figure 1.1e). The extracardiac mesenchyme, which descends from the region between the fourth and sixth pharyngeal arch arteries, not only forms the aortopulmonary septum between the ascending aorta and pulmonary trunk, but also sends two extensions toward the myocardial wall of the distal outlet segment. These extensions are completely covered by the endocardial outlet ridges. During expansion of the outflow tract the parts of its myocardial wall that are attached to the aortopulmonary septum seem to be kept together and the attached myocytes will later invade the outflow tract septum which initially consisted of mesenchymal tissues only. The downstream portions of the endocardial outlet ridges form the semilunar valves.[36]

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