SUMMARY. Sixty-two chick embryo hearts were studied at incremental stages of development (Hamburger-Hamilton stages 16 to 39) by scanning electron microscopy following 3% glutaraldehyde fixation and critical point drying. Early in cardiac development, the primitive ventricle becomes homogeneously trabeculated with highly organized sheets of myocytes lined by endocardial cells, with the trabeculae generally oriented in the dorsoventral direction. Coalescence of these trabecular sheets begins at stage 26, initially at the area of the bulboventricular flange, and later proceeding caudally toward the floor of the ventricle. The fusion process is finished by stage 30, resulting in a muscular ventricular septum that has now divided the primitive ventricle into right and left ventricles. Further growth of the ventricular septum is by continued fusion of the adjoining trabecular sheets. Remnants of the apposing trabecular sheets are found in the solidified muscular septum in the form of endocardial channels. We suggest that persistent patency of these channels results in muscular ventricular septal defects. (Circ Res 57: 759-766, 1985)

IT has been a traditional view that growth and development of the primitive ventricle of the early embryonic heart consist of a process of outpocketing of the ventricular free walls, resulting in a sponge-like mass of tissue that consists of disorganized trabeculations and tiny intracavitary spaces between these structures (Hochstetter, 1906; Patten, 1951; Goor and Lillehei, 1975). As the trabecular formations disappear at later stages of development, simultaneously with continuing enlargement of the heart, the cavity of the ventricular chambers becomes established.

The functional significance of the trabecular formations in the early stages of cardiac development is still unclear. Histological studies of the chick embryo heart have suggested that coalescence of the trabeculations results in formation of the muscular ventricular septum (Hochstetter, 1906; Patten, 1951; Morse, 1978). Other studies conducted in chick embryo hearts (Chang, 1932; Streeter, 1948; delaCruz, 1972; Goor and Lillehei, 1975) did not fully support the theory of trabecular coalescence, and instead suggested that the muscular ventricular septum originates from that part of the ventricular wall that is interposed between the expanding free walls (in response to the molding forces of the two intracardiac streams) of the future right and left ventricles. Thus, by this concept, the two ventricles may be viewed as developmentally distinct and separate, even at the earliest stages of cardiogenesis. The significance of the trabeculations within the developing ventricle was unclear to the proponents of the latter theory.

The present study was done to reexamine the morphogenesis of ventricular trabeculations and determine their role, if any, in the development of the chick heart.

Methods

Fixation and Preparation

Sixty-two chick embryo hearts were sectioned at different stages of development (Hamburger and Hamilton, 1951) ranging from stage 16 to 39. In all embryos, either the inferior vena cava or the omphalomesenteric vein or the sinus venosus were cannulated in situ, and the hearts were perfused with 3% glutaraldehyde in Tyrode's solution. The heart specimens were subsequently isolated and dissected at one of three planes: transverse, coronal, or sagittal (Fig. 1). The dissected specimens were fixed in 3% glutaraldehyde for an additional 15 minutes, and then stained with 2% osmium tetroxide (Carr and McGadey, 1974). After dehydration in alcohol, the hearts were placed in Freon TF as a transition fluid (Cohen et al., 1968) and were processed through the critical point in CO2 in a BOMAR Spc-900 dryer. Following gold sputtering (Boyde and Broers, 1971), the dehydrated specimens were examined with a scanning electron microscope (SEM). Photographs from the SEM were obtained on Polaroid 4 x 5 Land film type 55.

Photographs were also obtained of whole mount cardiac specimens after the initial fixation-perfusion of the heart, using an Olympus dissecting microscope and Polaroid Land film type 667. This technique was used only on those embryos up to stage 25. The whole mount photographs were taken both before and after dissection of the heart in one of the above-mentioned planes of dissection.
For orientation purposes, identification of right-sided and left-sided structures was based on a projection plane with the embryo viewed from its dorsal aspect.

**Nomenclature**

Terminology used was in accord with that of previous studies on cardiac development (Patten, 1951; Goor and Lillehei, 1975). However, some modifications of the usual nomenclature were necessary to highlight certain details of the developing ventricular structures.

The primitive ventricular tube (PVT) is defined as that part of the cardiac tube that lies between the atria and the bulbus cordis prior to cardiac looping. After looping of the heart and the development of ventricular trabeculations, the PVT persists as a relatively smaller compartment with the same cranial, dorsal, and ventral walls. However, its floor consists of an imaginary surface formed by connecting all the cranial edges of the ventricular trabecular sheets.

The primitive ventricle is defined as that chamber that is interposed between the atrioventricular canal (AVC) and the truncal cushions. It contains the PVT in its cranial portion, and the ventricular trabecular sheets including intertrabecular spaces in its caudal portion.

Right and left ventricles are identified only after the formation of a solid interventricular muscular septum.

Major trabecular bundles are bands of trabeculae running, for the most part, in the dorsoventral direction. Minor trabecular bundles are single-band trabeculae that interconnect one major bundle to another from side to side. Trabecular sheets are lattice-like plates of trabeculae resulting from the confluence of multiple ventricular trabeculations. At certain stages of development, the cranial edges of the trabecular sheets become flat and wide, and this is referred to as trabecular plate.

**Results**

The following is a description of the morphogenetic events in the ventricle at incremental stages of development from stage 16 through stage 39. A description of the conotruncal events has purposely been omitted.

A: **Stage 16: (51–56 hours of development, 26–28 somites)**

At this stage, the heart is already looped, with the atria situated craniodorsally to the primitive ventricular tube (PVT). The atrioventricular canal (AVC) is still undivided and opens into the proximal end of the PVT. The inner surface of the PVT is smooth and untrabeculated, and lined by endocardial cells. The distal end of the PVT is continuous with the bulbus cordis. Both the atrioventricular and truncal cushions are emerging as small ridges at this stage of development.

B: **Stage 17: (52–64 hours of development, 29–32 somites)**

Ventricular looping is becoming more pronounced, with formation of the bulboventricular flange that is still relatively wide. The interior of the PVT shows the beginning of trabecular formation at the bulbar regions and at the distal part of the PVT. The rest of the PVT is still untrabeculated (Fig. 2A). The truncal and atrioventricular cushions are now more prominent.

C: **Stages 18 to 19: (3–3½ days of development, 30–40 somites)**

Looping of the PVT has progressed further, resulting in a narrow bulboventricular flange with an acute angle. The inner surface of the PVT reveals extensive trabeculations, distributed more or less uniformly throughout, and lining the PVT as far proximally as the AVC (Fig. 2B). The trabeculae consist of myocytes lined by endocardial cells. They are arranged in major bundles directed dorsoventrally, and cover the floor of the PVT as well as its dorsal and ventral walls (Fig. 2, B and C). Thinner and smaller trabeculae (minor trabecular bundles) extend from one major trabecular bundle to another from side-to-side (Fig. 2, B and C). Trabeculation is homogeneous throughout the PVT at this stage.

D: **Stages 20–22: (3½–4 days of development, 40 somites to tip of tail)**

The ventricular loop has become larger, and the bulbus cordis is now displaced ventrally and medially. The distal part of the ventricular loop is also displaced cranially. A groove forming on the outside of the ventricular loop identifies the border zone which demarcates the proximal part and the cranially displaced distal part of the ventricular loop. The proximal and distal segments are unequal in size, the proximal ventricular loop being larger and up to twice the size of the distal loop.

The external groove between the ventricular loops corresponds to an area of increased concentration of the major trabecular bundles inside the ventricle (Fig. 3A). Major trabecular bundles are also seen on
both sides of the border zone, covering the whole surface of the ventricular loop underneath the PVT (Fig. 3, A and B). The interior of the ventricular loop is now lined with trabecular sheets that have the appearance of pages in a book. The cranial borders of these sheets are oriented in so as to form an imaginary floor of the PVT. Their caudal ends originate from the floor of the ventricular loop (Fig. 3B). The axis of each trabecular sheet is approximately 90° to the axis of the PVT.

The trabecular sheets are oriented in a dorsoventral direction similar to that of the major trabecular bundles in previous stages. Each sheet resembles a net with large intertrabecular spaces and delicate trabecular strands (Fig. 3B). Minor trabecular bands extend from one trabecular sheet to another in transverse or right-to-left direction. Neither on coronal nor on transverse sections can any solid muscular ventricular septum be identified (Fig. 3C).

**E: Stages 23–26: (4–5 days of development)**

Externally, there is a further increase in heart size and further displacement of the bulbus cordis ventrally. The truncus arteriosus is now positioned immediately ventral to the cranial part of the ventricular loop, impinging upon the latter and on the two atria.

Internally, the AVC has descended more caudally and is now positioned close to the horizontal plane. The two atrioventricular cushions are very prominent and close to one another, but are not yet fused. One of the most striking internal features is concerned with the architecture of the trabecular sheets.

![Figure 2](image-url)
In the proximal portion of the ventricular loop, the sheets are oriented in a dorsoventral direction as previously noted (Fig. 4, A and B). However, they fan out in a somewhat semi-circular manner in the distal ventricular loop, with their dorsal ends held close together but with their ventral ends spread widely apart (Fig. 4C). The trabecular sheets in the proximal ventricular loop are also changed in shape—they consist of a wide flat cranial portion, the trabecular plate, and a wall of trabecular strands and spaces more caudally (Fig. 4, A and B). In the ventricular loop, however, no trabecular plate can be identified, and the trabecular sheets consist of a network of strands and spaces only. Minor trabecular bands are scantier and more delicate structurally.

By the end of stage 26, the trabecular sheets which have increased in number at the border zone begin to coalesce at their trabecular plates (Fig. 5A). However, the caudal extensions of the sheets are still separate and apart from each other (Fig. 5B). The coalescing trabecular plates are lined by the endocardial cells of the PVT.

**F: Stages 28–30: (5½–7 days of development)**

By these stages, there are no further major changes in the external shape of the ventricular tube, aside from an increase in size. Internally, coalescence of the trabecular sheets in the border zone is completed by late stage 28 to 29. The coalescence results in formation of a thin muscular ventricular septum (Fig. 6). The cranial crest of the muscular ventricular septum is continuous with the superior ventricular septum that originates from the atrioventricular cushion and forms the floor of the left ventricular outflow tract (Fig. 6). The superior ventricular septum has thus divided the PVT.

In stage 30, the two ventricles are still full of trabecular sheets that are directed dorsoventrally in the left ventricle and appear to fan out in semicircular fashion in the right ventricle. Midway between these structures lie the muscular ventricular septum (Fig. 7).

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**FIGURE 3.** Formation of early trabecular sheets. Panel A: stage 20, whole mount, coronal section, ventral wall removed, ventral view. Ventricular loop consists of a larger proximal part (P) and a smaller distal part (D). Note dense concentration of trabeculations between the loops (arrow) in contrast to the homogenous distribution in Figure 2B. Panel B: scanning electron micrograph of same specimen as in panel A, coronal section, ventral wall removed and viewed from behind. Note beginning trabecular sheet formation (white arrowheads). Magnification (90×). AVO, atrioventricular orifice. Panel C: stage 22, scanning electron micrograph, transverse section, view toward apex. Note extensive trabecular sheets, but there is no solid muscular septum. Magnification (100×).
From stage 30 and on, further development of the muscular ventricular septum consists of continued thickening of the septum and decreasing size of the trabecular spaces within it (Fig. 7). The trabecular sheets inside both ventricular chambers decrease relative to the progressively increasing intracavitary volumes of both ventricles and most of the sheets become gradually plastered against the ventricular free walls (Fig. 8).

Within the fused muscular septum from stage 30 and on, endocardial-lined channels are identified that are oriented in craniocaudal as well as left-right directions (Fig. 9). These channels appear to be remnants of the trabecular endocardial surface which have yet to become apposed through coalescence of the trabecular sheets.

**Discussion**

Previous studies, consisting mainly of reconstructions of histological sections of chick embryo hearts (Hochstetter, 1906; Bremer, 1925; Patten, 1951; delaCruz, 1972) or of human embryo hearts (Davis, 1927; Streeter, 1948; Goor and Lillehei, 1975), have suggested that the right and left ventricles are morphogenetically separate structures. The development of each ventricle was considered to consist initially of outpocketing of the ventricular loop at two predominant sites, with concomitant formation of trabecular structures appearing as sponge-like masses with no particular organization or apparent function. By this concept, the ventricular wall interposed between the two expanding or outpocketing sites, and adjoining trabecular, would eventually develop into a solid muscular interventricular septum.
Our work in the chick embryo heart has demonstrated that both ventricles arise as a result of intrinsic division of a common primitive and already highly trabeculated ventricular chamber through progressive coalescence of trabeculae rather than through wall outpocketing. We also demonstrated that ventricular trabeculation is a very complex but highly organized developmental process that is constantly changing at every stage of development. The orientation and geometric pattern of the trabecular formations differ in the proximal and distal ventricular loops, and this may account for the distinctive differences in the morphological landmarks of the right and left ventricles in the mature and fully formed heart.
The architecture of the ventricular trabeculations in the developing heart has not been appropriately emphasized in previous investigations. This could be ascribed to the techniques used. Most of the previous studies have consisted, for the most part, of histological reconstructions that utilized viscous embedding media and regular dehydrating techniques which tend to deform the fragile structure of the trabecular network. In our study, the combination of critical point drying of the tissue with scanning electron microscopy enabled preservation of fine structural details and their display in a relatively undisturbed manner.

Septation of the primitive ventricle and conotruncus has been attributed to the molding effects of the blood streams inside the developing heart (Spitzer, 1923; Bremer, 1932; Goerttler, 1955; Barthel, 1960; DeVries et al., 1962; Jaffe, 1962; Jaffe, 1965, 1966, 1967; Harh et al., 1973; Clark and Rosenquist, 1975; Sweeney, 1981). Recent work in our laboratory consisting of methylene blue microinjection into the peripheral vitelline veins of chick embryos, studied in ovo at the early stages of development prior to and during beginning cardiac septation, has demonstrated two intracardiac streams that ran more or less in parallel, with one stream anterior to the other within the ventricle and conotruncus. Because of the unusual flow pattern of these streams, which differ significantly from those of the fully developed heart, we questioned the propriety of the flow-molding theory as it pertains to the role played by intracardiac streaming upon ventricular, bulbar, and aorticopulmonary septation (Yoshida et al., 1983). The orientation of the intracardiac streams, as demonstrated in that microangiographic study, is approximately at 90° angle to the axial orientation of the developing trabecular sheets which eventually form the muscular interventricular septum. Thus, there appears to be even less support for the traditional concept that the blood streams within the primitive heart tube play a major role in the initial processes leading to cardiac septation. Our findings again are consistent with the suggestion that these initial processes are intrinsic or programmed events (Yoshida et al., 1983). Nevertheless, the molding effects per se of the blood streams upon the developing cardiac chambers cannot be ignored, based on hydrodynamic principles. Thus, we suggest that ventricular development is influenced by its changing preload in the form of increasing venous return at incremental stages of development. Clark and Hu (1982) have demonstrated progressive increase of aortic flow and decreasing systemic vascular resistance at increasing stages of cardiac development in chick embryos. It is conceivable that a corresponding increase in ventricular compliance also occurs, but documentation of this assumption is difficult if not impossible.

Harh and Paul (1975) have also suggested trabecular coaptation as the mechanism for muscular septal formation based on progressive shortening of the distances between labeled myocardial cells during heart growth of chick embryos. In most, if not all, of the studies referred to earlier where coalescence of the ventricular trabeculations has been described as a mechanism leading to muscular septal formation, the presence of an intrinsic muscular structure between the two expanding and develop-
ing ventricles has been assumed. Our work has clearly demonstrated: (1) the absence of any solid preformed muscular septum during the early stages of cardiac development, and (2) the occurrence of a well-organized process of coalescence of the trabecular sheets, starting cranially at the bulboventricular region and proceeding caudally, leading to formation of the muscular septum.

Based on our observations, we suggest that muscular ventricular septal defects are due to continued patency of the endocardial channels in the muscular ventricular septum of the fully developed heart. This is presumably due to abnormal or incomplete trabecular coalescence. Furthermore, we would like to speculate that the highly organized ventricular trabeculations in the developing heart play not only an important role in ventricular septal formation but may also regulate the systolic and diastolic functions of the primitive ventricle.

Supported by National Institutes of Health Grant HL-13831.
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Received May 24, 1984; accepted for publication August 20, 1985

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INDEX TERMS: Ventricular trabecular formations • Muscular ventricular septum • Cardiogenesis • Scanning electron microscopy • Primitive ventricle • Chick embryo heart
Ventricular trabeculations in the chick embryo heart and their contribution to ventricular and muscular septal development.
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Circ Res. 1985;57:759-766
doi: 10.1161/01.RES.57.5.759

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