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Scanning and Light Microscope Studies of the Development of the Chick Embryo Semilunar Heart Valves

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Summary. The development of the semilunar valves of the great arteries was studied by light and scanning electron microscopy in the chick embryo. The results show that three distinct developmental periods can be distinguished. The formation of the anlage of the valves takes place in the first period (stages 26–29). These early anlage consist of three pyramidal shaped cusps formed by a core of loosely packed mesenchymal cells covered by a flattened endothelium. In the second period (stages 30–35) the cusps undergo excavation on their distal face. Morphological evidence is reported suggesting that this excavation process is produced by an initial solid ingrowth of the endothelium of the arterial face of the cusps which is immediately luminated by detachment of cells towards the bloodstream and by cell death. The histogenesis of the valves takes place in the third period (from stage 36 until hatching). It was observed that during this period some myocardial cells of the outflow tracts of the ventricles invade the valvular tissue and that in the upper part of the cusps a prominent fibrous layer is formed.

Key words: Chick embryo – Semilunar valves – Cell death – Heart morphogenesis.

Introduction

The development of the semilunar valves of the great arteries is one of the topics of heart morphogenesis which has received little attention from embryologists. As described in textbooks of embryology, the anlage of these valves arise from three pairs of swellings of mesenchymal tissue which

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grow at the proximal end of the truncus arteriosus. These swellings later undergo an excavation process on their distal (arterial) aspects to form the cusps of the semilunar valves (Romanoff, 1960; Boyd, 1965).

Attention has been focussed on the study of the origin of the mesenchymal swellings in different vertebrates (Shaner, 1962); and also on the modifications of the location and relationships of the valvular anlage determined by displacement of the truncus and conus of the embryonic heart (Kramer, 1942). However the morphogenesis (Shaner, 1963; De la Cruz et al., 1972) and the histogenesis (Hyams and Manion, 1968) of the valves which are essential steps in the understanding of their malformations, have been largely overlooked.

The purpose of this paper is to give a detailed study of the normal morphogenesis of the semilunar valves of the chick which might serve as a basis for future experimental analysis and for comparison in studies of aberrant development.

Material and Methods

Normal chick embryos ranging from 5 to 20 days of incubation were employed. The embryos were classified according to Hamburger and Hamilton (1951) stages and studied by the following techniques:

Light Microscopy

For light microscopy the hearts of embryos younger than 13 days of incubation were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) dehydrated in acetone and embedded in araldite. Frontal or transversal sections 1–2 μm thick were obtained and stained in 1% toluidine blue in borax. The embryos older than 13 days were fixed in Bouin's fluid, dehydrated in alcohol, cleared in xylol and embedded in paraffin wax. Frontal sections 7 μm thick were obtained and stained with Harris' hematoxylin and eosin.

Scanning Electron Microscopy (SEM)

Chick embryo hearts fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) were rinsed in cacodylate buffer alone in which they were microdissected to expose the semilunar valves. The specimens were then dehydrated in acetone, dried by the critical point method (Anderson, 1951) and gold sputtering coated. Observations were made using a Philips SEM 501, operated at 7.5 Kv.

Observations

Scanning Electron Microscopy

The development of the cusps of the semilunar valves begins at stage 26–27 by the growth of three pairs of swellings of mesenchymal tissue at the proximal end of the truncus arteriosus. As can be seen in Fig. 1, two of the swellings develop in close relation to the trunco-conal ridges and the other one on the lateral wall of the truncus (for details of these initial stages see: Tonge,

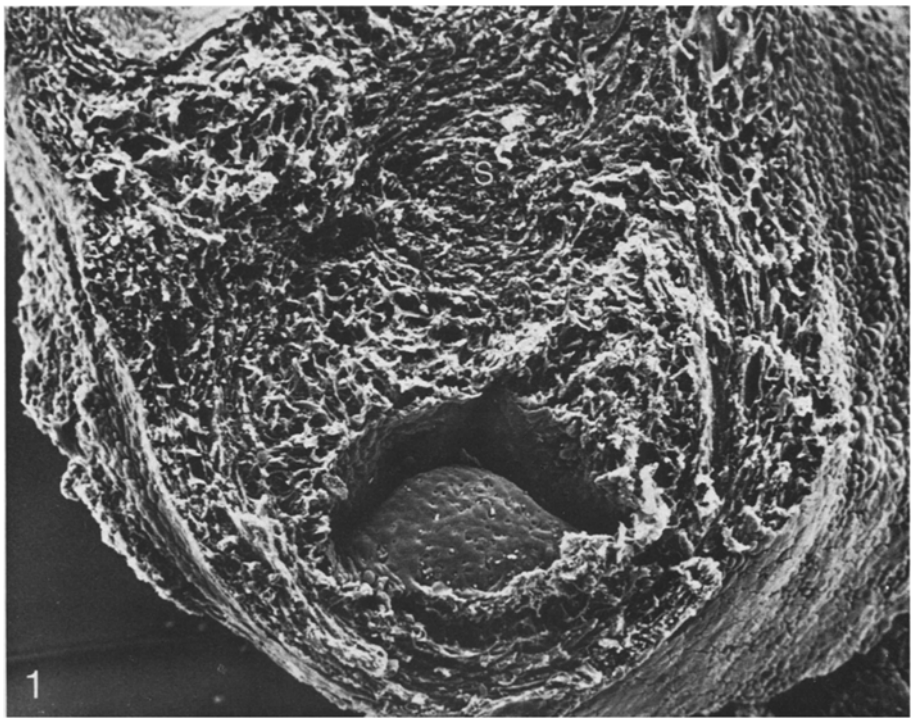


Fig. 1. SEM view of a transverse section of the truncus arteriosus at the level of the primordia of the semilunar valves. The cusps are pyramidal in shape and still do not occupy the whole lumen of the vessel. Aorto-pulmonary septum (*S*). (Stage 28). ×200

1869, and Shaner, 1962). The swellings are pyramidal in shape with the vertex being directed towards the lumen of the vessel (Fig. 1). By stage 29 the truncus has been divided into the roots of the aorta and main pulmonary artery and in the proximal ends of these vessels a definite anlage of the semilunar valves can be now observed (Fig. 2). The endothelial layer covering the cusps during these early stages, is formed by flattened cells with boundaries poorly established. As can be observed in Fig. 2, in the arterial face of the cusps the cells display considerable variations in size and microvillar density and frequently show cell extensions which protrude from the surface. The presence of large gaps or pits between the cells of this face of the cusps is also frequent.

By stage 30 the cusps have undergone considerable elongation along the longitudinal axis of the arterial trunk and an early stage of excavation of their arterial face can be detected (Fig. 3). The process of excavation is similar in the different cusps and progresses rapidly until stage 34–35 (Fig. 4). During these stages the cusps still consist of an inner mass of stellate mesenchymal cells covered by the endothelium (Fig. 5). While the mesenchymal tissue appears homogeneous, remarkable regional differences were noted between the endothelium covering the arterial (concave) and the ventricular (convex) faces of the cusps. The endothelium of the ventricular face and free margin of the cusps

is formed by rather polygonal flattened cells (Fig. 6). On the contrary the endothelial cells of the arterial face of the cusps (Fig. 7) are very irregular in size showing numerous rounded cell extensions and abundant microvilli. In some instances, cells showing small pits and holes in the surface, similar to the necrotic cells described by Hurle and Hinchliffe (1978), appear protruding towards the lumen, suggesting that they are dead cells in course of detachment.

As can be observed in Fig. 8, by stage 36 the cusps have apparently attained their final shape. In the following stages until hatching only differences in size of the cusps are observed (Fig. 9). During all these stages the ventricular surface of the cusps is formed by flattened endothelial cells which are very poor in microvilli while in the arterial face the cells are rounded up projecting from the surface and showing numerous microvilli and occasional blebs (Figs. 10, 11). Other differences, such as the presence of degenerating cells, present in previous stages, are no longer observed. The observation of longitudinal sections of the cusps reveals that each cusp consists of an upper thin region and a lower very thick region by means of which the cusp is attached to the arterial wall (Fig. 12). Although it is not possible with the SEM to ascertain the structural nature of the valvular tissue, observations reveal that in the upper part of the cusps the cells are tightly packed with abundant extracellular material while in the lower zone of the cusps the cells are loosely packed.

Histology

Prior to the onset of the excavation process both semilunar valves appear in sections at the same level of the truncus arteriosus (Fig. 13). The mesenchymal core of the cusps is occupied by loosely packed cells forming a network with extensive intercellular spaces. In the following stages the location of the valves suffers some changes due to the displacement of the trunco-conal region of

Fig. 2. SEM view of a cusp of the aortic semilunar valve at stage 29. The endothelial cells are flattened displaying numerous microvilli on the surface. Numerous intercellular fenestrations are present (*arrows*). Note the lumen of the vessel virtually occluded by the cusps. ×450

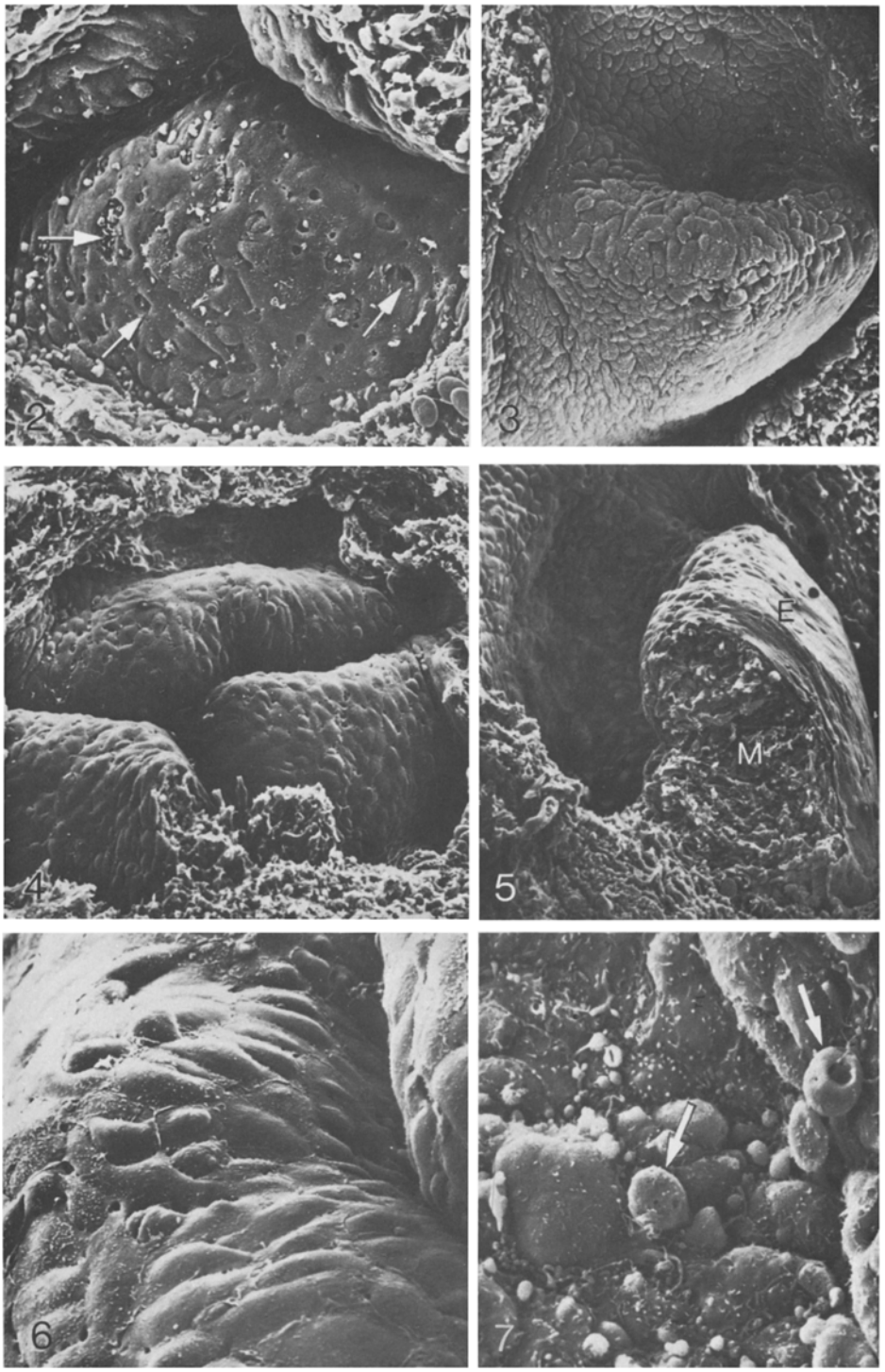
Fig. 3. Panoramic SEM view of a cusp of the aortic valve at stage 30. The cusp is still pyramidal in shape with the arterial face showing an early stage of excavation. ×280

Fig. 4. Cranial SEM view of a semilunar valve at stage 34. The cusps are now clearly excavated on their arterial face. ×200

Fig. 5. SEM view of a sectioned cusp of the pulmonary valve at stage 34. The cusp consists of a core of mesenchymal tissue (*M*) covered by an endothelium (*E*). ×230

Fig. 6. High magnification SEM view of the free margin of a cusp at stage 35. The endothelial cells are flattened and rather polygonal in shape. ×930

Fig. 7. High magnification SEM view of the arterial face of a cusp at stage 34. The endothelial cells are very irregular in shape showing abundant microvilli and blebs. Note two cell profiles showing a pitted surface (*arrows*). ×1,600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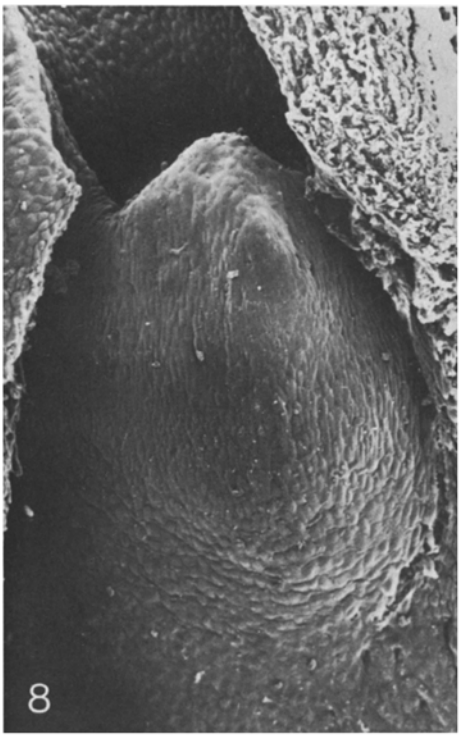
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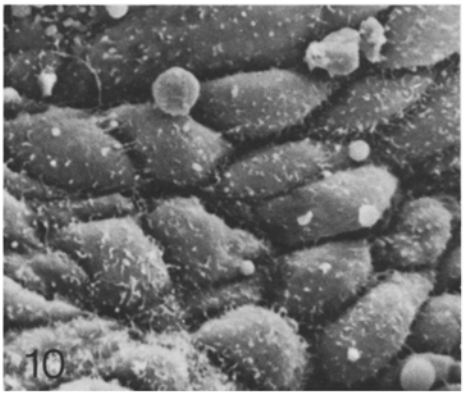
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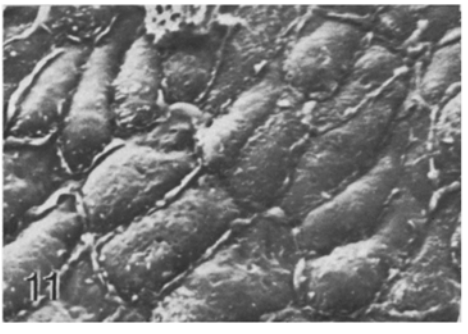
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the heart (Kramer, 1942). Although some minor differences can be found between the different cusps, the general development appears similar in all of them, therefore in the following description no distinction will be made between the different cusps.

Sections of the valves during the excavation process (stages 30–35) reveal conspicuous morphological changes occurring both in the endothelium and in the mesenchymal tissue. At the beginning of the excavation process (stage 30) the cusps appear slightly depressed on their arterial face, and the endothelium of this zone appears thickened (Fig. 14). This zone consists of three or four layers of rounded endothelial cells and shows abundant mitosis. In the next stages the depression of the arterial face of the cusps progressively increases forming the sinus of Valsalva. During these stages numerous endothelial cells appear detached towards the bloodstream and as can be observed in Fig. 15 some endothelial cells undergo degeneration. The mesenchymal tissue at these stages appears more condensed than in the preceding ones and shows abundant mitosis and dying cells. The number of those degenerating cells is always small and necrotic areas like those reported in other regions of the developing heart (Pexieder, 1972; Hurle and Ojeda, 1978) are not observed.

At stage 36 the endothelial changes described above are no longer observed and in the mesenchymal tissue two distinct regions can be distinguished. The upper region, located under the endothelium of the arterial face of the cusp, is formed by a condensed tissue. The lower region (similar to the layer *spongiosa* of the adult human valve, see Gross and Kugel, 1931) still shows the morphology of an undifferentiated mesenchymal tissue. A striking feature of this lower region is the presence of myocardial cell strands which extend from the myocardial layer of the outflow tracts of the ventricles towards the core of the cusps. As can be observed in Fig. 16, by stage 38 these morphological characters of the cusps appear accentuated. The upper zone of the cusp has now the appearance of a fibrous layer and the number of myocardial cell fibers invading the valvular tissue appears increased. In the following stages the fibrous layer

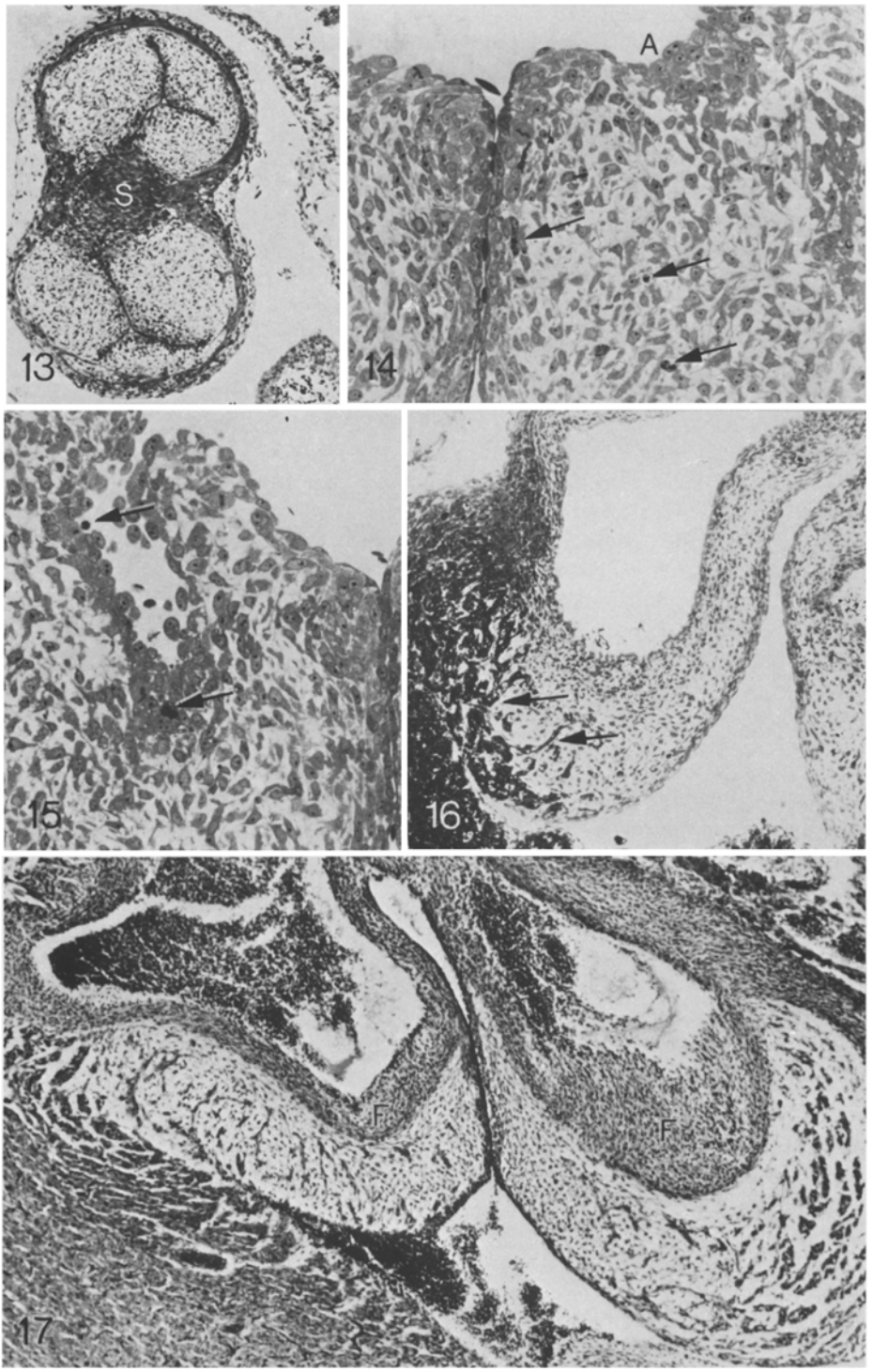
Fig. 8. Panoramic SEM view of a cusp of the pulmonary valve at stage 36. At this stage the cusps have almost attained their definitive appearance. ×170

Fig. 9. SEM view of a cusp of the pulmonary valve at stage 41. At this stage the shape of the cusp is similar to that of the earlier stages (see Fig. 8) but a considerable increase in size can be noted. ×90

Fig. 10. High magnification SEM view of the arterial surface of an aortic cusp at stage 42. The endothelial cells are rouneded-up and project noticeably from the surface. Numerous villi can be seen as white spots on the surface of the cells. Some blebs are also noticeable. ×1,800

Fig. 11. High magnification SEM view of the ventricular face of an aortic cusp at stage 42. The endothelial cells are flattened with scarce microvilli. Note the presence of marginal folds at the cell boundaries. ×2,000

Fig. 12. SEM view of a sectioned semilunar valve at stage 41. Two distinct regions can be distinguish in each cusp: An upper region which is formed by closely packed tissue (*arrow*) and a lower one of loosely packed tissue (*B*). ×65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undergoes progressive maturation and the myocardial fibers tend to adopt a radial arrangement from the area of implantation of the cusp towards the fibrous layer (Fig. 17). Occasional thin blood vessels can be also observed within the valvular tissue at these stages.

Discussion

The results of this study show that three definite periods can be distinguished in the formation of the semilunar valves of the chick. The first period was not covered in detail in this study since it has been previously studied by Shaner (1962). During this period the formation of the first anlage of the valves takes place by the growth of three paris of ridges in the proximal end of the truncus arteriosus. This process is produced by the emigration and condensation of mesenchymal cells (Fitzharris, 1978) and begins at stage 26–27 ending by stage 29. The main feature of the second period is the excavation of the distal aspect of the cusps, and takes place from stage 30 to 35. The histological maturation of the valves takes place in a third period which extends from stage 36 until hatching and our unpublished observations suggest that this period continues for some days in the postnatal period.

Although experimental confirmation is lacking, the process of excavation of the cusps is usually thought to be a consequence of the turbulence of the blood flow. However the modifications of the cusps at the cellular level have not been analyzed. In our study we have observed morphological features, occurring both in the mesenchymal tissue and in the endothelium, which might account for the process of excavation. Both the condensation of the mesenchymal tissue and the presence of mesenchymal cell death observed in this study could be factors involved in the shaping mechanism of the cusps since they play

Fig. 13. Transverse section of the truncus arteriosus showing the primordia of the semilunar valves at stage 29. Each cusp consists of a mesenchymal core covered by the endothelium. The aorto-pulmonary septum is clearly observable between both valves (*S*). Toluidine blue. ×75

Fig. 14. Frontal section of a pulmonary valve at stage 30. The endothelium appears thickened at the arterial face of the cusp (*A*). In the mesenchymal tissue some necrotic cells can be recognized (*arrows*). Toluidine blue. ×240

Fig. 15. Section of a pulmonary cusp at stage 31. The sinus of Valsalva is now recognizable. Note numerous endothelial cells detached towards the sinus. Arrows show dead mesenchymal cells. Toluidine blue. ×260

Fig. 16. Panoramic view of a pulmonary valve at stage 38. The upper part of the mesenchymal tissue is being transformed into a fibrous tissue and numerous myocardial cells are present in the layer *spongiosa* of the cusp (*arrows*). Note that the endothelium of the arterial face is formed by a single layer of cells. Toluidine blue. ×90

Fig. 17. Light micrograph of a frontal section of the aortic valve at stage 43 to show the morphology of the valves before hatching. The fibrous layer appears well differentiated (*F*) and numerous myocardial fibers are observed in the layer *spongiosa*. Hematoxylin-eosin. ×100

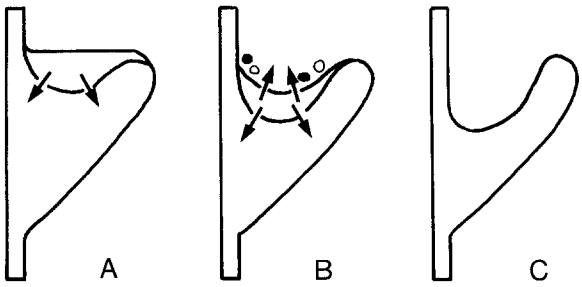

Fig. 18A–C. Schematic drawings of a semilunar cusp showing the endothelial modfications during ohe excavation process. In the initial stages of the excavatioon the endothelium of the arterial face grows as indicated by the arrows forming a solid epithelial cord **A**. In the following stages the endothelium still grows towards the mesenchymal tissue but numerous endothelial cells are removed to form the sinus of Valsalva **B**. The final shape of the cusp is showed in **C**

such a role in the morphogenesis of other embryonic organs (Ede et al., 1971; Glücksmann, 1951; Pexieder, 1975; Saunders, 1966; Thorogood and Hinchliffe, 1975). However neither of these mesenchymal features seem to be conspicuous enough to explain by themselves the process of excavation, and probably they are related rather to the histogenesis of the valves. On the contrary the changes observed in the endothelium appear precisely located in the zone of the excavation of the cusps and they are only observed during the stages in which the excavation takes place. Our results are consistent with previous observations of Shaner (1963) and suggest that the excavation of the cusps takes place by an initial solid ingrowth of the endothelium which is next luminated forming the sinus of Valsalva (Fig.18). It is not possible to ascertain from the present observations whether the process of lumen formation in the endothelium takes place mainly by cell death or by detachment of healthy endothelial cells. Our unpublished ultrastructural observations suggest that the detaching cells are in fact cells in an early stage of degeneration. This shaping mechanism of the semilunar valves differs from other apparently similar embryonic processes (Yamada, 1977) and it is very similar to the process of formation of new blood vessels (see: Eriksson and Zarem, 1977).

While the study of the structure of the adult semilunar valves of man is receiving increased attention because of its importance in the elaboration of prosthetic devices (Clark and Finke, 1974; Missirlis and Armeniades, 1977), little attention has been focussed in the semilunar valves of other vertebrates. The structure of the semilunar valves of the postnatal chick differs from the human ones by the presence of a thickened base containing cardiac muscle cells. Our study shows that both the differentiation of the fibrous layer and the invasion of myocardial cells take place very late in the development. These observations are in agreement with the idea that the structural development of the heart does not stop after the full anatomic differentiation of the cardiac chambers (Hyams and Manion, 1968; Shakibi and Diehl, 1972; Shakibi et al., 1977) and it is a factor which should be taken in mind to understand the malformations of the semilunar valves.

Our observations suggest that the fibrous layer is formed from the mesenchymal tissue of the valvular anlage. On the contrary, the myocardial cells seem to invade the mesenchymal tissue of the cusps from the myocardial layer of the conus. However, the possibility of a transformation of the mesenchymal tissue into myocardial cells can not be ruled out without ultrastructural studies, since this process has been described during the development of the mouse embryo heart (Viragh and Challice, 1973).

Finally the differences in shape observed during the later stages between the endothelial cells of the arterial face of the cusps and the ones of the ventricular face, might well be a consequence of differences in the blood flow characteristics between the two sides of the cusps since it has been demonstrated that the shape of the endothelial cells depends on the character of the blood flow (Flaherty et al., 1972).

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