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Development of the origin of the coronary arteries, a matter of ingrowth or outgrowth?

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Summary. Inconsistencies still exist with regard to the exact mode of development of proximal coronary arteries and coronary orifices. In this regard 15 quail embryos were investigated using a monoclonal anti-endothelium antibody, enabling a detailed study of the development of endothelium-lined vasculature. Coronary orifices emerged at 7–9 days of incubation (Zacchei stages 24–26) and were invariably present at 10 days of incubation (Zacchei stage 27).

We never observed more than 2 coronary orifices; these were always single in either of the facing sinuses of the aorta. A coronary orifice was always observed being connected to an already developed proximal coronary artery, which belonged to a peritruncal ring of coronary arterial vasculature. We did not find any coronary orifice without a connection to a proximal coronary artery. Moreover, at 7–9 days of incubation (Zacchei stages 24–26) we observed coronary arteries from the peritruncal ring penetrating the aortic media. In 2 specimen this coronary artery, with a lumen, was in contact with the still intact endothelial lining of the aorta.

We conclude that coronary arteries do not grow out of the aorta, but grow into the aorta from the peritruncal ring of coronary arterial vasculature. This throws new light on normal and abnormal development of proximal coronary arteries and coronary orifices.

Key words: Coronary artery development – Coronary orifice development – Peritruncal ring

Introduction

Recently, the existing theories on proximal coronary arterial development were shown to be insufficient for explanation of either the consistency of the normal coronary arterial pattern or its abnormal variations (Aikawa and Kawano 1982; Bogers et al. 1988a; Gittenberger-de Groot et al. 1988; Hirakow 1983; Hutchins et al. 1988; Ogden 1988).

New data strongly suggest the dual development of the main coronary arteries, consisting of a single coronary orifice developing in either sinus of the aorta facing the pulmonary artery (Bogers et al. 1988a; Corone et al. 1984; Gittenberger-de Groot et al. 1988; Heintzberger 1983; Hutchins et al. 1988; Ogden 1988).

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Until recently it was assumed that coronary arteries grew out of the aorta to connect with the coronary arterial bed in the peritruncal ring (Aikawa and Kawano 1982; Corone et al. 1984; Hirakow 1983; Hutchins et al. 1988; Ogden 1988). In earlier studies we already doubted this assumption because we never observed a coronary orifice without a proximal coronary artery, but instead could readily identify proximal coronary arteries without yet a coronary orifice (Bogers et al. 1988a; Gittenberger-de Groot et al. 1988).

To further substantiate these observations we studied proximal coronary artery development using a monoclonal anti-endothelial antibody directed against quail endothelium (Labastie et al. 1986; Péault 1987; Péault et al. 1983; Péault et al. 1988). In this way subtle steps in arterial development could be investigated.

Materials and methods

15 quail embryos (*Coturnix coturnix japonica*) were studied from 5 to 10 days of incubation. Embryonic stages were determined according to Zacchei (1961).

Embryos were fixed in periodate-lysine-paraformaldehyde, pH 6.2, containing 0.01 M sodium m-periodate (Merck, Darmstadt, FRG), 0.075 M l-lysine monohydrochloride (Merck), 2% paraformaldehyde (Merck) and 0.0375 M phosphate buffered saline (PBS), pH 7.4. Following fixation the embryos were dehydrated in ethanol and embedded in paraffin. Serial transverse sections 5 μm thick were mounted on slides, deparaffinized in xylene and rehydrated. After rinsing twice in PBS for 15 min and once in PBS with 0.05% Tween-20 (Sigma, St. Louis, MO, USA) for 15 min, the sections were incubated overnight at 21° C with 1:20000 diluted monoclonal IgM-anti-MB1. This monoclonal mouse-anti-quail serum was kindly provided by the Institut d'Embryologie du Centre National de Recherche Scientifique et du College de France, Nogent-sur-Marne, France. The preparation and the properties of this serum were exactly as described before (Labastie et al. 1986; Péault 1987; Péault et al. 1983; Péault et al. 1988).

After rinsing again twice in PBS for 15 min and once in PBS with 0.05% Tween-20 for 15 min the second incubation was performed with 1:300 diluted rabbit-anti-mouse peroxidase (Dakopatts, Glostrup, Denmark) for 2 h at 21° C.

After rinsing three times with PBS for 15 min, the sections were treated with diaminobenzidine (Sigma) for 10 min and rinsed again with PBS for 15 min.

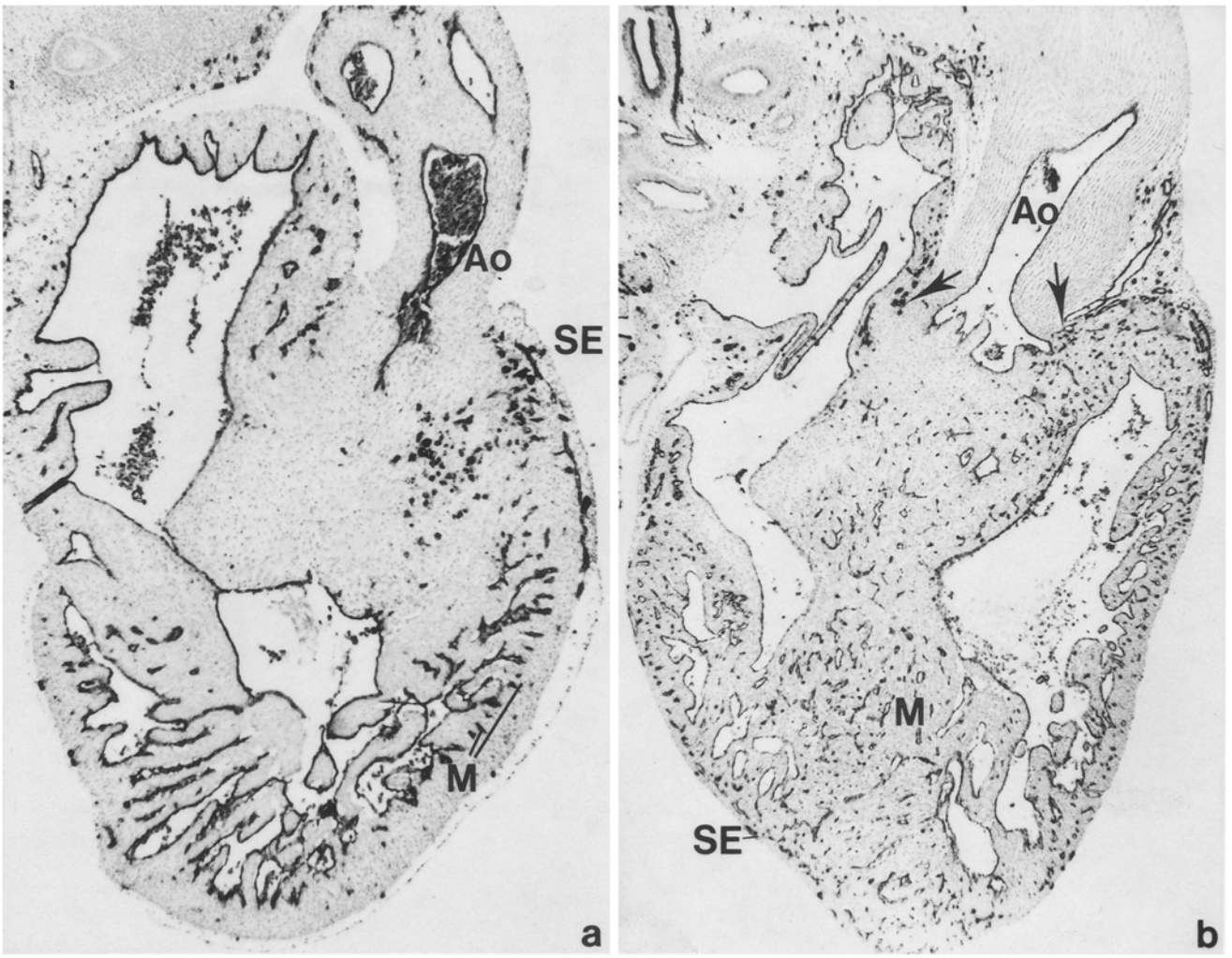

Fig. 1 a, b. Overview of embryonic quail hearts stained with monoclonal anti-MB1 antibody. The aorta (*Ao*) is marked. **a** Vascular primordia, both intramyocardial (*M*) and subepicardial (*SE*), are already present in the earliest investigated embryos. Quail embryo of 5 days of incubation. Transverse section. × 58. **b** In later stages the vascular primordia increase in density, vasculature develops and a peritruncal ring (*arrows*) becomes evident. Quail embryo of 8 days of incubation. Transverse section. × 58

Finally, the sections were stained for 10 s in Mayer's hematoxylin. After dehydration in ethanol the sections were covered and investigated by light microscopy.

A coronary orifice was defined as an invagination of the arterial (aortic) wall with a discontinuation of the straight endothelial lining in the serial sections. Proximal coronary arteries were defined as subepicardial vascular structures lined by endothelium.

Results

Septation at the arterial orifice level was already complete in the investigated embryos. Subepicardial vascular primordia were seen in all embryos, increasing in density with age and concentrating with age in the atrioventricular ring, the pertruncal ring and at the subepicardial areas overlying the interventricular septum (Fig. 1 a, b).

The subepicardial vascular primordia developed along the sequence of single cells confluing to vascular structures that turn into the coronary network and concentrate in the above mentioned areas.

Table 1. Observed proximal coronary arteries (PCA) and coronary orifices (CO) in quail embryos investigated by monoclonal anti-endothelial antibody

Days of incubation	Zacchei stage	PCA		CO	
		Left	Right	Left	Right
5	20	–	–	–	–
5		–	–	–	–
5		–	–	–	–
6	22	–	–	–	–
6		–	–	–	–
7	24	–	+	–	–
7		+	+	–	–
7		+	+	–	+
8	25	+	+	+	–
8		+	+	+	–
9	26	+	?	+	–
9		+	+	+	–
9		+	+	+	+
10	27	+	+	+	+
10		+	+	+	+

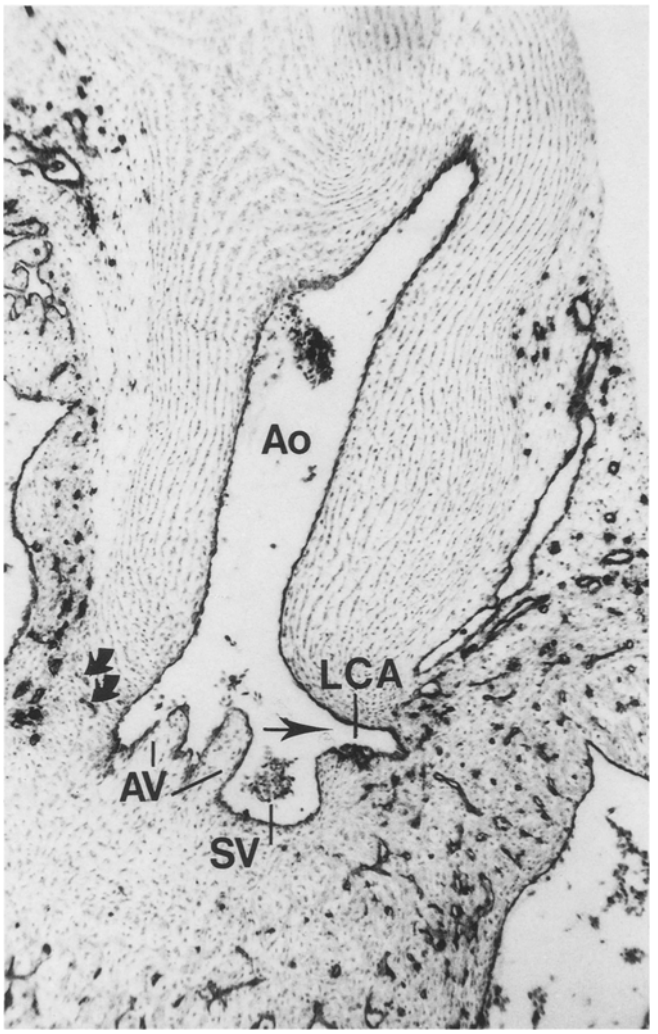

Fig. 2. Detail of Fig. 1b showing a well developed left coronary artery (*LCA*) and coronary orifice (*arrow*) in the sinus of Valsalva (*SV*), the developing aortic valve leaflets (*AV*) are shown. In the opposite wall of the aorta (*Ao*) a small number of endothelial cells (*curved arrows*) are the early indication of the developing right coronary artery. Quail embryo of 8 days after fertilization. Transverse section. ×150

Coronary orifices (Table 1, Fig. 2) were variably identified at 7–9 days of incubation (Zacchei stages 24–26) and invariably present at 10 days of incubation (Zacchei stage 27). We never observed more than 1 coronary orifice in an aortic sinus. The aortic sinuses involved were always facing the pulmonary artery.

In the stage of variable identification of coronary orifices, a peritruncal ring of vascular structures, including proximal coronary arteries, was already clearly present (Table 1, Fig. 1a, b).

In serial sections of quail embryos at 7–9 days of incubation (Zacchei stages 24–26), proximal coronary arteries with a lumen were observed to be extending through the aortic adventitia into the aortic media. In 2 specimen a coronary artery was in contact with the still intact endothelial lining of the aorta (Fig. 3a–f). An isolated coronary orifice was not observed in any of the specimen. Coronary orifices were always in continuity with proximal coronary arteries in the peritruncal ring.

Discussion

Until recently it was assumed (but not actually proven) that coronary arteries grew out of the aorta to contact the coronary arteries in the peritruncal coronary arterial ring (Abrikosoff 1911; Aikawa and Kawano 1982; Corone et al. 1984; Hackensellner 1956; Heintzberger 1983; Hirakow 1983; Hutchins et al. 1988; Ogden 1988). Using conventional techniques of investigation, this assumption was made because it allowed a theoretical explanation of congenital coronary arterial abormalities (Abrikosoff 1911; Corone et al. 1984; Hackensellner 1956; Ogden 1988) and because the process of contacting was regarded to be difficult to investigate in view of the short time span it takes (Hutchins et al. 1988).

Our previous investigations in human and rat embryos made this assumption questionable because we never observed a coronary orifice without a proximal coronary artery, but instead readily identified proximal coronary arteries without a coronary orifice (Bogers et al. 1988a; Gittenberger-de Groot et al. 1988). However, conventional histological methods were considered to be inadequate to solve this problem (Bogers et al. 1988a). In this regard, studies using injection techniques, like perfusion with ink (Dbaly et al. 1968; Zuber and Wortmann 1984) and corrosion-casting (Aikawa and Kawano 1982), are even principally inadequate in dealing with questions of aortic ingrowth or outgrowth of coronary primordia, because these methods depend on flow from the aorta into the coronary arteries and are therefore methodologically biased.

A more detailed study of the mode of development of proximal coronary arteries and coronary orifices was possible, using quail embryos. We chose this species because of the availability of a monoclonal antiserum against quail endothelium, identifying vascular endothelium in all stages (Labastie et al. 1986; Péault 1987; Péault et al. 1983; Péault et al. 1988). Because of the excellent endothelium-staining properties of this antiserum, misinterpretation of endothelial folds, both artificial as well as those associated with semilunar valve development, could be avoided (Bogers et al. 1988a; Hutchins et al. 1988) in studies using conventional techniques (Zuber and Wortmann 1984; Dbaly et al. 1968; Aikawa and Kawano 1982; Heintzberger 1983).

At 7–9 days of incubation (Zacchei stages 24–26) the quail embryos showed coronary arteries, with a lumen, that were connected with the peritruncal ring and extended into the aortic wall. In 2 specimen the coronary artery penetrated the aortic medial layers up to the intimal lining of the aorta. In these specimen the aortic intima was still completely intact. Because coronary outgrowth would be dependant on a coronary artery with a lumen growing out of the aorta to the peritruncal coronary artery, the assumption of coronary outgrowth cannot be maintained. In contrast, because the aortic intima was still intact while the proximal coronary artery was already histologically connected to the aortic wall, we assume that proximal coronary arteries grow into the aorta, inducing a coronary orifice at the site of contact (Fig. 4a–f). This would explain the exclusive presence of a coronary orifice in connection with a proximal coronary artery. In both normal and abnormal hearts coronary orifices are almost exclusively positioned in one of the sinuses of the aorta that face the pulmonary artery (Bogers et al.1988a; Gittenberger-de Groot et al. 1983; Gittenberger-de Groot et al. 1988; Quaegebeur et al. 1986).

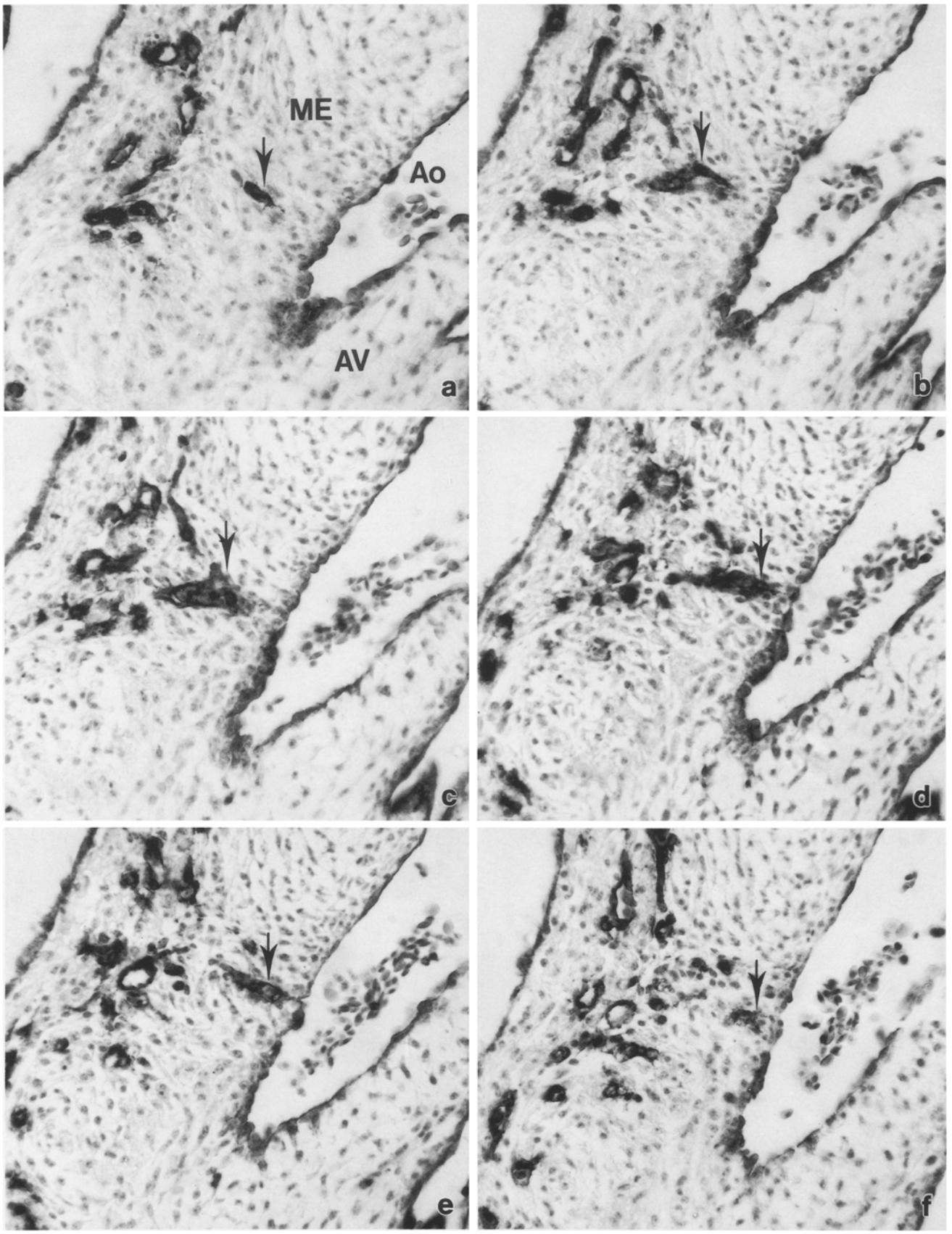

Fig. 3a–f. Detail of Fig. 1b showing an intermediate stage in proximal right coronary artery development; the right coronary orifice has not yet developed. Sequential sections of 5 μm thickness in a quail embryo of 8 days of inucubation. Transverse sections. ×360. **a–d** The right coronary artery (*arrow*) is crossing the media (*ME*) of the aorta (*Ao*). The aortic valve (*AV*) is shown as well. Pale grey erythrocytes are present in the lumen of the artery. **e** The proximal right coronary artery reaches the still intact intimal lining of the aorta. **f** The developing right coronary artery is nearly passed

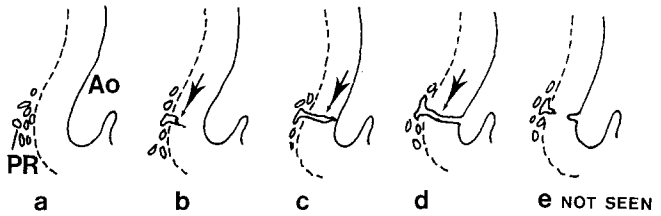

Fig. 4a–e. Schematic representation of proximal coronary artery develoment. **a** Half aortic root (*Ao*) with no coronary orifices. The peritruncal ring (*PR*) is already present. **b** Half aortic root with coronary artery (*arrow*) penetrating the aortic wall out of the peritruncal ring. **c** Half aortic root with coronary artery penetrating the complete aortic wall, except for the aortic intimal lining. **d** Half aortic root with completely developed coronary artery. **e** A fully developed coronary orifice was never observed without a proximal coronary artery

This allows speculation on the processes controlling coronary ingrowth. In part, ingrowth might be dependant on the configuration of the arterial pole of the developing heart (Bogers et al. 1988a; Gittenberger-de Groot et al. 1988), in particular on the construction of the facing aortic sinuses (Hutchins et al. 1988). The facing aortic sinuses were found to have a saddle-shaped configuration and not, like the remaining aortic sinus and pulmonary sinuses, an outwardly convex shape (Hutchins et al. 1988). This configuration might become particularly pronounced regarding the angled and twisted position of the aortic and pulmonary orifices, implying a rather long pulmonary outlet and a rather short aortic outlet (Bartelings and Gittenberger-de Groot 1988). The overall result allows to speculate on the predisposition of the facing aortic sinuses for coronary orifice formation because of the close vicinity of the peritruncal ring.

The concept of coronary ingrowth allows a better understanding of the variation in the course of proximal coronary arteries and of the consistency of the location of the coronary orifices in the facing sinuses of the aorta. Coronary abnormalities, e.g., the left coronary artery arising from the pulmonary artery, known as the Bland-White-Garland syndrome (Bland et al. 1933; Bogers et al. 1988b), would in this concept be explained by abnormal ingrowth in the arterial pole. Consequently, from the embryological point of view the terminology should be changed into left coronary artery connected to the pulmonary artery.

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