Cardiology in the Young (2010), **20(Suppl. 3)**, 92–99
doi:10.1017/S1047951110001137

Original Article

Development of the outflow tracts with reference to aortopulmonary windows and aortoventricular tunnels

Robert H. Anderson,[1,2,3,4] Andrew Cook,[2] Nigel A. Brown,[3] Deborah J. Henderson,[4] Bill Chaudhry,[4] Timothy Mohun[5]

[1]*Division of Pediatric Cardiology, Department of Pediatrics, Medical University of South Carolina, Charleston, South Carolina, United States of America;* [2]*Cardiac Unit, Institute of Child Health, University College;* [3]*Division of Basic Medical Sciences, St George's Medical University, London;* [4]*Institute of Human Genetics, Newcastle University, Newcastle upon Tyne;* [5]*National Institute of Medical Research, London, United Kingdom*

Abstract Although malformations involving the ventricular outflow tracts are often described as conotruncal malformations, there is no consensus as to the lesions included in, or excluded from, this category, reflecting, in part, the current lack of precise definitions of the embryonic truncus and conus. Analysis of development of the outflow tract in terms of proximal, intermediate, and distal components greatly facilitates understanding of the morphology of the aortopulmonary window and aortoventricular tunnels. The aortopulmonary windows reflect failure to close the embryonic aortopulmonary foramen, the space between the distal end of the cushions that divide the lumen of the outflow tract itself and the dorsal wall of the aortic sac. The aortopulmonary tunnels are produced subsequent to abnormal development of the cushions themselves. The distal ends of these cushions excavate to produce the sinuses and leaflets of the arterial valves. The proximal parts of the cushions muscularise to form the subpulmonary infundibulum. The middle part of the cushion mass disappears to provide a tissue plane between the infundibulum and the aortic root. Abnormal formation of this area accounts for the various types of aortoventricular tunnel. In our brief review, we show how the anatomy of these lesions correlates with development of the outflow tract.

Keywords: Aortopulmonary window; aorto-left ventricular tunnels; aorto-right ventricular tunnels; cardiac development

MALFORMATIONS INVOLVING THE VENTRICULAR outflow tracts are still frequently described as conotruncal malformations. To date, however, there is no consensus about those lesions that should be included in, or excluded from, this category. Agreement about the precise definition of the "truncus" and "conus" is also lacking. These terms were introduced by Kramer[1] when describing the morphological changes occurring concomitant with embryological development of the ventricular outflow tracts in humans. He described how the developing outflow tract extending from the developing right ventricle, then considered to represent the bulbus, to the margins of the pericardial cavity had two obvious anatomical components. He defined the proximal part as the conus, and named the distal part as the truncus. He then described how, with further development, the arterial valves formed within the tissues at the junction of these two components, emphasising that during their development the valvar primordiums were enclosed within a muscular sleeve. He thus effectively considered the outflow tract to have three developmental components, describing also how the so-called intercalated cushions appeared in the intermediate component, albeit without ascribing a discrete name to this third part. At that time, it was believed that all the cells of the definitive heart were already present at the so-called "straight tube" stage of development. Within the past two decades, we have learned that this is far from the case. We now know that new material is added to the developing heart at both its arterial[2] and venous[3]

Correspondence to: Professor R. H. Anderson, BSc, MD, FRCPath, 60 Earlsfield Road, London SW18 3DN, United Kingdom. Tel: +00 44 20 8870 4368; E-mail: r.anderson@ich.ucl.ac.uk

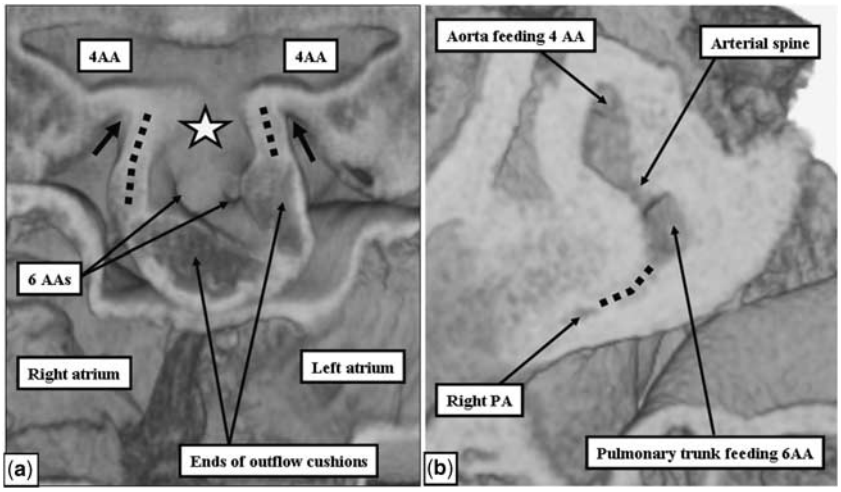

Figure 1.
*The panels show high-resolution episcopic images from frontal erosions of reconstructed mouse hearts early (**a**) and late (**b**) during embryonic day 11.5. (**a**) The initially symmetrical arrangement of the arteries running through the fourth (4AA) and sixth (6AA) branchial arches. These arteries arise from the aortic sac, which is confluent with the common lumen of the outflow tract at the margins of the pericardial cavity (arrows). At this stage, it is the dorsal wall of the sac (star) that represents the effective aortopulmonary septum. The distal outflow tract itself is being separated into separate aortic and pulmonary lumens by the formation within it if opposing endocardial cushions, the ends of which have been transected by the erosion. Already the distal parietal walls of what will become the intrapericardial aorta and pulmonary trunk have grown into the pericardial cavity from the pharyngeal mesenchyme (black dotted lines in **a**). By the end of day 11.5, marked morphological changes have taken place at the transition from the outflow tract to the aortic sac (**b**). The dorsal wall of the sac has now protruded into the outflow tract as an arterial spine, with the aorta now feeding the cranial and rightward part of the sac, and the pulmonary trunk the leftward and caudal part. The aorta feeds the fourth arches, which become the systemic pathways. The developing pulmonary arteries arise from the caudal part of the sac (black dotted line in **b**). With disappearance of the right sixth arch artery, these pulmonary arteries will be in communication exclusively with the pulmonary trunk and the left sixth arch artery, which will become the arterial duct.*

poles. We also know that, concomitant with the addition of the new material, the cells making up the original distal part of the outflow tract are shifted towards the heart. These new cells, which form the walls of the intrapericardial arterial trunks, having entered the pericardial cavity, occupy the anatomical area initially designated by Kramer as the truncus.[1]

The paediatric cardiologist may well question the relevance of this information to malformations of the intrapericardial outflow tracts. It is our belief that knowledge of the changes occurring during early cardiac development can now provide the basis for understanding the morphology of two important lesions involving the outflow tracts, namely aortopulmonary window and aortoventricular tunnels. In this review, therefore, we summarise in brief our recent findings about development of the ventricular outflow tracts, showing how it can be interpreted to provide concepts for understanding the morphogenesis of these lesions.

Development of the outflow tracts

Subsequent to looping of the cardiac tube, the outflow tract of the heart extends from the roof of the developing right ventricle to the margins of the pericardial cavity.[4] At this early stage, the outflow tract has a common lumen within its myocardial walls, which, at the margins of the pericardial cavity, becomes confluent with an extrapericardial manifold known as the aortic sac. Anatomical changes occurring in the configuration of the outflow tract are now shown with exquisite accuracy using high-resolution episcopic microscopy.[5] This technique permits serially sectioned developing hearts to be reconstructed in three dimensions, the accuracy achieved rivalling that produced by scanning electron microscopy. Preliminary examination of hearts from embryonic mice at 11.5 days of development in this manner has revealed morphological changes that occur with great rapidity, albeit that we have still to conclude our full investigation. Our pilot studies show that, at this stage of development, and as we have described previously,[4] the arteries having an origin from the extrapericardial manifold, and extending through the mesenchyme of the fourth and sixth branchial arches to reach the descending aorta, are bilaterally symmetrical (Fig 1a). Very rapidly, marked rotation occurs at the margins of the pericardial cavity, so that by the end of the 11th day of development, the developing aorta connects with the rightward and cranial component of the aortic sac, whereas the developing intrapericardial pulmonary trunk feeds the leftward and caudal component (Fig 1b). The right and

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press

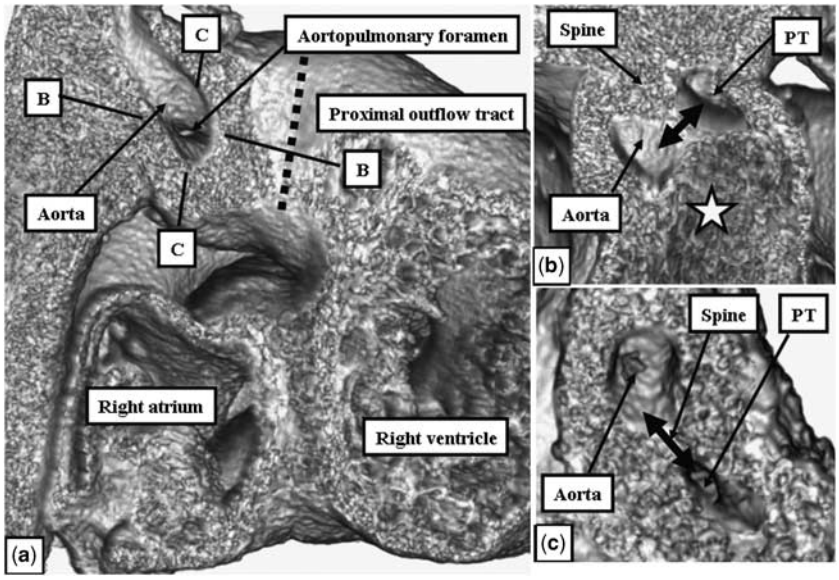

Figure 2.
*(**a**) An erosion from the right and anterior of a reconstructed mouse heart late on embryonic day 11.5. It shows how the outflow tract extends from the right ventricle to the margins of the pericardial cavity, divided by a dog-leg bend (dotted line) into proximal and distal parts. The arterial valve will form in an intermediate part of the outflow tract around the level of the dog-leg. The erosion shows how the intrapericardial arterial trunks are being formed in the distal part of the outflow tract, designated by Kramer[1] as the truncus, the erosion having cut away the parietal walls of the developing aorta. The embryonic aortopulmonary foramen is seen towards the margins of the pericardial cavity. It is failure to close this aortopulmonary foramen that produces aortopulmonary windows. (**b** and **c**) Further erosions prepared along the planes B-B and C-C in (**a**). (**b**) The view from above, illustrating how the aortopulmonary foramen (double-headed arrow) is between the arterial spine, itself in continuity with the pharyngeal mesenchyme, and the distal ends of the outflow cushions (star). (**c**) The short-axis cut seen from the front, and how the arterial spine forms the dorsal margin of the foramen (double-headed arrow). PT – pulmonary trunk.*

left pulmonary arteries are already formed at this stage, arising from this caudal part of the extrapericardial manifold.[4] Concomitant with these rotational changes, the dorsal wall of the aortic sac, effectively functioning as the initial aortopulmonary septum, has protruded into the intrapericardial outflow tract. The reconstructions show that the effect is to produce an arterial spine, which moves towards the distal ends of cushions, the cushions themselves having compacted within the outflow tract from the endocardial jelly initially lining the entirety of the common lumen. The cushions are located distally so as, subsequent to fusion along their facing surfaces, to divide the common lumen into right-sided aortic and left-sided pulmonary channels. The episcopic reconstructions show that, at this stage, a foramen exists towards the margins of the pericardial cavity between the leading edge of the arterial spine and the distal ends of the cushions. The hole provides a communication between the developing intrapericardial aortic and pulmonary channels, and is the embryonic aortopulmonary foramen (Fig 2).

During embryonic day 12.5 in the mouse, the aortopulmonary foramen is closed concomitant to the fusion of the distal cushions with each other, the spine itself fusing with the distal ends of the cushions. The closure of the aortopulmonary foramen separates the developing aortic and pulmonary circulations, placing the intrapericardial aorta in communication exclusively with the systemic arteries derived from the third and fourth arches, and leaving the intrapericardial pulmonary trunk feeding the artery of the left sixth arch, which in turn feeds the pulmonary arteries. Essential to the separation of the circuits is disappearance of the right sixth arch, as only subsequent to the regression of this channel is communication disrupted between the caudal extent of the aortic sac and the descending aorta.[4] Failure to close the foramen will result in its persistence as an aortopulmonary window, the adjacency during development of the foramen to the developing right pulmonary artery accounting for the frequent finding of aortic origin of the right pulmonary artery in the setting of an aortopulmonary window (see below).

At this stage, the newly formed intrapericardial arterial trunks occupy the position within the pericardial cavity initially taken up by the so-called "truncus" as defined by Kramer.[1] Bartelings and Gittenberger-de Groot[6] showed that the parietal walls of these vessels are formed by the ingrowth of the pharyngeal mesenchyme, thus producing a fish-mouth appearance of the distal margins of the persisting myocardial walls. They also described the

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press

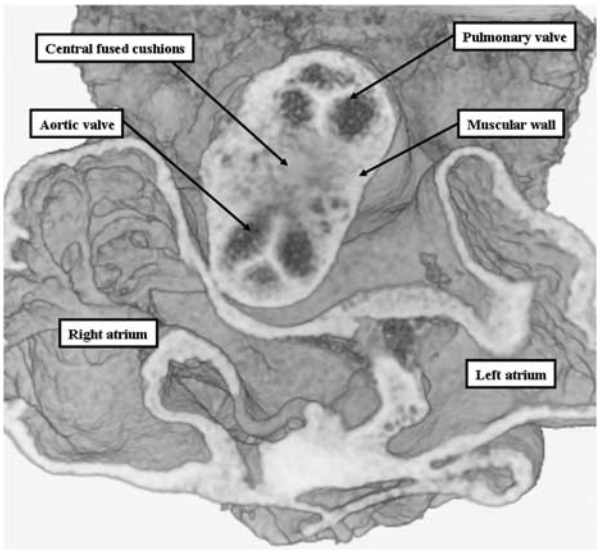

Figure 3.
This episcopic image comes from a frontal erosion of a reconstructed mouse heart early in embryonic day 12.5. It is cut through the middle part of the developing outflow tract, and shows how the endocardial cushions have fused with each other centrally, producing a mesenchymal mass, but remain unfused peripherally. Formation of intercalated cushions opposite these unfused peripheral part established the trifoliate arrangement that will set the scene for formation of the aortic and pulmonary valves. Note that the cushions themselves remain encased within the persisting muscular walls of this part of the outflow tract. The reconstruction also shows the intrapericardial parts of the arterial trunks now extending beyond the region of formation of the valves to the margins of the pericardial cavity.

rapid change that occurred in the developing human heart as the myocardial border effectively regressed to the level of the distal margins of the developing arterial valves, which Kramer[1] had shown to appear in the intermediate component of the outflow tract. Our current findings indicate that the distal parts of the adjacent walls of the intrapericardial trunks, towards the pericardial margins relative to the aortopulmonary foramen, are formed from the opposite sides of the arterial spine. Subsequent to closure of the aortopulmonary foramen, our findings also suggest that the most proximal parts of the adjacent walls are formed from the outflow cushions, this notion being supported by the morphological structure of the trunks in the setting of the aortopulmonary windows (see below). The mechanics of these changes, however, remain to be elucidated. Subsequent to the formation of the intrapericardial trunks, nonetheless, the intermediate and proximal parts of the outflow tract retain their muscular walls, with the arterial valves (Fig 3) and the ventricular outflow tracts (Figs 4 and 5) forming from these components.

By the time that the aortopulmonary foramen has closed distally, the cushions within the intermediate

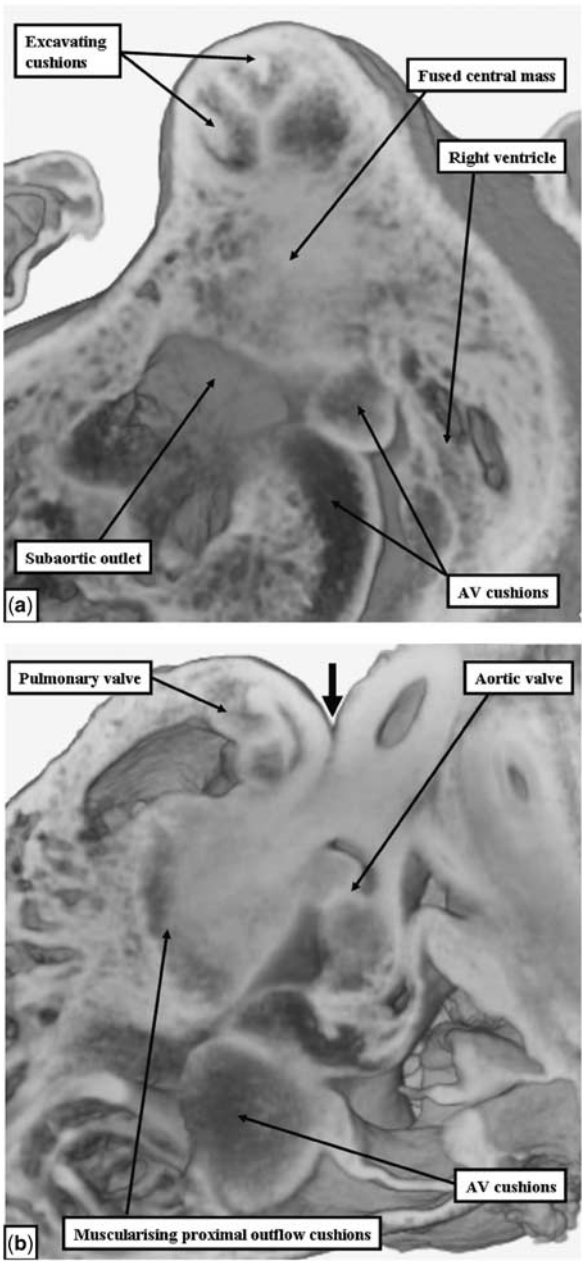

Figure 4.
*These images are prepared by eroding a reconstruction made from episcopic sections of a mouse heart late on embryonic day 12.5. (**a**) A short-axis section across the ventricular mass viewed from above, illustrating the excavating leaflets of the pulmonary valve. Note that the fused central part of the outflow cushions is fusing with the atrioventricular cushions and the developing muscular ventricular septum to close the embryonic interventricular foramen (see also Fig 5). (**b**) It has been prepared to replicate the parasternal long-axis echocardiographic section, showing how the proximal part of the centrally fused cushion mass is muscularising to form the subpulmonary infundibulum, and how the peripheral parts of the cushions are excavating to form the arterial valvar primordiums. Note that the intrapericardial trunks are already separated one from the other (arrow). A similar tissue plane will eventually develop within the central cushion mass to separate the subpulmonary infundibulum from the aortic root (see Fig 5). AV – atrioventricular.*

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press

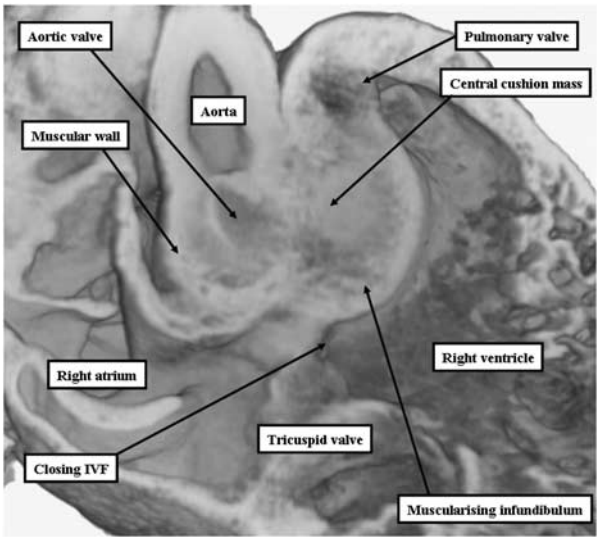

Figure 5.
The image shows erosion of the reconstruction shown in Figure 4 from the right side, replicating the oblique subcostal echocardiographic cut of the right ventricle. Note that the proximal outflow cushions have now muscularised on their surface, with fusing to the atrioventricular cushions and the muscular ventricular septum almost closing the embryonic interventricular foramen. The margins of the central mass have still to develop so as to produce the adjacent sinuses of the aortic and pulmonary roots. As these sinuses develop, so a tissue plane will develop between the aortic root and the muscular subpulmonary infundibulum. It is an abnormal development within this central cushion mass that produces the aortoventricular tunnels. IVF – interventricular foramen.

part of the outflow tract have also fused along the larger parts of their facing surfaces. As was also initially described by Kramer,[1] by this time new intercalated cushions have been formed opposite the unfused peripheral parts of the cushions (Fig 3). Pits now appear in the distal margins of the intercalated cushions, at the same time cavitating the distal margins of the unfused peripheral parts of the central cushions (Fig 4). The process of cavitation is such that the parts of each cushion adjacent to the muscular covering or the central fused part form the developing valvar sinuses, whereas the parts adjacent to the lumens form the valvar leaflets, thus producing the pulmonary and aortic valves (Fig 4). More proximally, towards the cavity of the heart itself, the cushions were initially unfused as the valves began their process of excavation. By the end of embryonic day 12.5 in the mouse, the cushions have also fused proximally, with the cushion mass thus formed also fusing with the crest of the muscular ventricular septum, in this way placing the aorta into communication with the left ventricle, but leaving the pulmonary trunk arising from the right ventricle (Fig 5). Muscularisation of the facing surface of this proximal muscle mass will produce the subpulmonary infundibulum, whereas the disappearance of the central core will produce the tissue plane that eventually separates the free-standing subpulmonary infundibulum from the aortic root. It is the abnormal excavation of the distal margins of the cushions, and abnormal maturation of the central part of the fused cushion mass, that provides the explanation for the existence of the aortoventricular tunnels (see below).

Morphology of the aortopulmonary windows

The key to the understanding of the aortopulmonary fenestrations is that they provide communications between the cavities of the intrapericardial arterial trunks, but in the presence of the separate aortic and pulmonary valves (Fig 6a). The lesions can be found with atresia of one or the other arterial valve, then providing access to the otherwise blind-ending circulation, but these lesions imply foetal blockage of a previously formed ventricular outflow tract. The presence of separate aortic and pulmonary roots distinguishes the aortopulmonary windows from common arterial trunk, and also from the solitary aortic trunk, that latter entity being found when there is complete absence of the intrapericardial pulmonary arteries.[7] The best explanation for the morphogenesis of the lesions is failure to close the embryonic aortopulmonary foramen. The separate nature of the walls of the intrapericardial arterial trunks means that it is incorrect to describe the lesions as "aortopulmonary septal defects". At no stage during development is there a complete septum formed between the cavities of the intrapericardial arterial trunks.[8,9] As already discussed, our findings indicate that, subsequent to closure of the embryonic foramen, the adjacent walls of the distal trunks are moulded in part from the sides of the arterial spine, but also proximally from the adjacent sides of the most distal parts of the fused central cushions. Always, in the presence of aortopulmonary windows, it is possible to find discrete and separate walls for the aorta and pulmonary trunk. These walls cannot have been derived from the arterial spine, which forms the arterial walls distal to the window. The most likely source of the separate walls seen proximal to the window, extending to the level of the sinutubular junctions (Fig 6), are the distal ends of the fused central cushions. This notion, however, has still to be proved by further examination of our embryological material.

Although the arterial trunks always have separate walls proximal to the windows, the windows themselves can be found relatively close to the sinutubular junctions, more distally towards the margins of the pericardial cavity (Fig 6a), or occupying the larger part of the adjacent area between the arterial trunks.[10] In the

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press

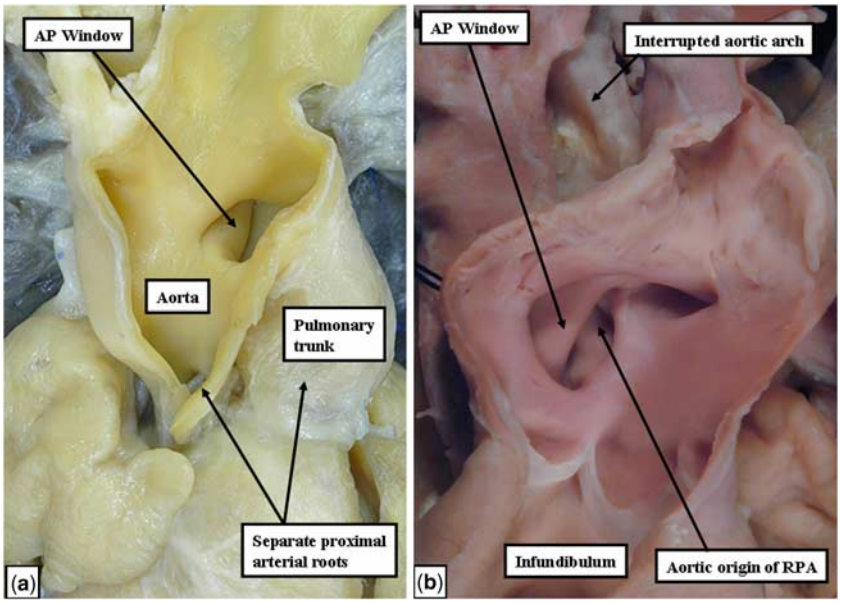

Figure 6.
*The illustrations show two aortopulmonary windows. (**a**) A small window towards the margins of the pericardial cavity. Note the presence of the separate arterial roots, and the separate walls of the intrapericardial trunks distal to the sinutubular junctions. (**b**) Frequent associated anomalies, namely interruption of the aortic arch, with the descending aorta fed through a persistently patent arterial duct, and aortic origin of the right pulmonary artery. The heart in (**a**) is from the Idriss Archive of Chicago Children's Memorial Hospital, and was photographed by Diane Spicer. We thank Diane, and Carl Backer, for permission to reproduce this illustration.*

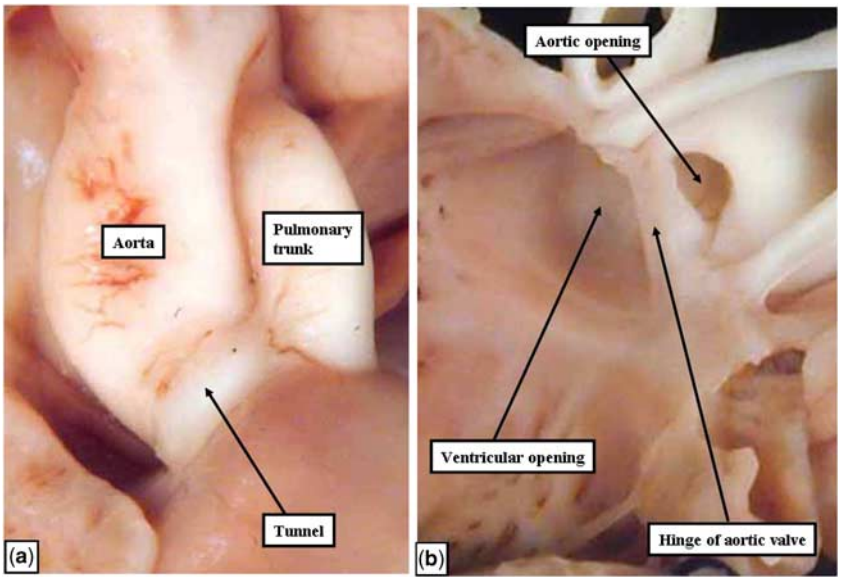

Figure 7.
*(**a**) The external appearance of a typical aorto-left ventricular tunnel. It is closely related to the right coronary aortic sinus, although the tunnels can also involve the left coronary aortic sinus. They very rarely involve the non-coronary aortic sinus. As is shown in (**b**), the essence of the lesion is separation of the hinge of the aortic valvar leaflet from the supporting valvar sinus.*

latter setting, it is also frequent to find the aortic origin of the right pulmonary artery, often in association with the interruption of the aortic arch (Fig 6b). Association with the origin of the left coronary artery from the pulmonary trunk is also frequent.

Aortoventricular tunnels

These fascinating lesions usually produce communications from the aortic root to the left ventricle, bypassing the hinges of the aortic valvar leaflets (Fig 7), but

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press

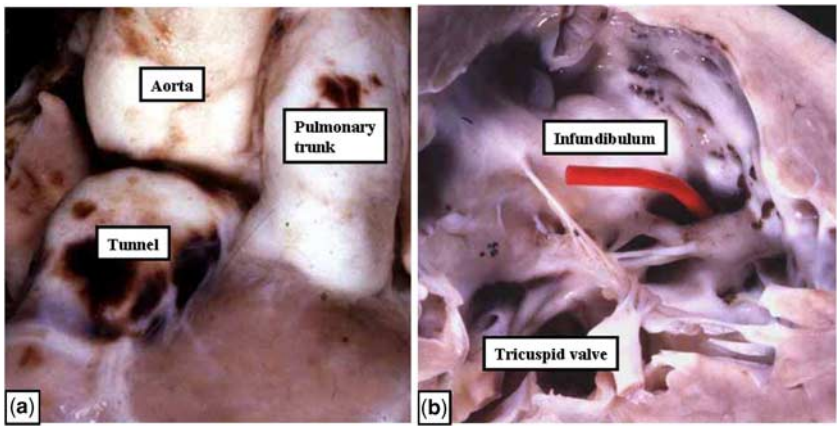

Figure 8.
*(**a**) An aorto-right ventricular tunnel. As with the left ventricular tunnel, it involves the right coronary aortic valvar sinus. In this instance, however, as shown in (**b**), probing shows that the origin of the tunnel is the right rather than the left ventricle.*

the tunnels can also extend between the aortic root and the cavity of the right ventricle (Fig 8). The majority of tunnels reported thus far have produced connections with the left ventricle, with only about one-eighth of the reported cases involving the right ventricle.[11] It is well established that the histological appearance of the aortic end of the tunnel differs markedly from the ventricular component.[12] This is hardly surprising, since the essence of the tunnels is that, when communicating with the left ventricle, they bypass the hinge of the aortic valve. Explanations for the morphogenesis of the lesions are legion.[11] Recognition that moulding of the intermediate part of the developing outflow tract not only produces the arterial valvar sinuses, but also the valvar leaflets, and that the outflow cushions also muscularise to produce the subpulmonary infundibulum, when coupled with analysis of the structure of the lesions, shows that they represent an abnormal development of the central cushion mass. It cannot be coincidental that all the tunnels reported thus far have involved the aortic sinuses adjacent to the pulmonary trunk.[11] It has often been suggested that the tunnels involve the infundibular, or muscular outlet, septum. Since such a septum barely exists in the normal heart,[8] explanations involving such a "septum" cannot be correct. As it matures, however, the central cushion mass becomes converted not only into the free-standing muscular subpulmonary infundibulum, but also the plane of space that interposes between the infundibulum and the aortic root. It is this plane of space that is occupied by the tunnels, irrespective of whether they open to the right or the left ventricle. Although most frequently called tunnels, this is a less-than-satisfactory descriptor, albeit one that is unlikely to disappear from the paediatric cardiological lexicon. Irrespective of what they are called, the lesions represent abnormal formation of the arterial valvar sinuses and leaflets, those emptying to the left ventricle being no more than the separation of the valvar leaflet from the supporting sinus. The involvement of the adjacent aortic valvar sinuses in their formation also explains why so many of the tunnels also involve the coronary arteries as part of the malformation.

Conclusions

In the past, one of us questioned the value of embryological investigations in explaining the origin of congenital cardiac malformations.[13] The huge advances made over the recent decades in understanding the mechanics of cardiac development have shown that opinion to be grossly misplaced. Our current account hopefully shows how these advances provide rational explanations for the origins and morphology of aortopulmonary windows and aortoventricular tunnels.

References

1. Kramer TC. The partitioning of the truncus and conus and the formation of the membranous portion of the interventricular septum in the human heart. Anat Rec 1942; 71: 343–370.
2. Moorman AFM, Christoffels VM, Anderson RH, van den Hoff MJB. The heart-forming fields: one or multiple? Phil Trans Roy Soc Biol 2007; 362: 1257–1265.
3. Goddeeris MM, Rho S, Petiet A, et al. Intracardiac septation requires hedgehog-dependent cellular contributions from outside the heart. Development 2008; 135: 1887–1895.
4. Anderson RH, Webb S, Brown NA, Lamers W, Moorman A. Development of the heart: (3) Formation of the ventricular outflow tracts, arterial valves, and intrapericardial arterial trunks. Heart 2003; 89: 1110–1118.
5. Geyer SH, Mohun TJ, Weninger WJ. Visualizing vertebrate embryos with episcopic 3D imaging techniques. Scientific World Journal 2009; 16: 1423–1437.
6. Bartelings MM, Gittenberger-de Groot AC. The outflow tract of the heart – embryologic and morphologic correlations. Int J Cardiol 1989; 22: 289–300.

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press

7. Anderson RH, Thiene G. Categorization and description of hearts with common arterial trunk. Eur J Cardiothorac Surg 1989; 3: 481–487.
8. Webb S, Qayyum SR, Anderson RH, Lamers WH, Richardson MK. Septation and separation within the outflow tract of the developing heart. J Anat 2003; 202: 327–342.
9. Anderson RH, Brown N, Webb S, Henderson D. Lessons learnt with regard to aortopulmonary window. Cardiol Young 2008; 18: 451–457.
10. Ho SY, Gerlis LM, Anderson C, Devine WA, Smith A. The morphology of aortopulmonary windows with regard to their classification and morphogenesis. Cardiol Young 1994; 4: 146–155.
11. McKay R, Anderson RH, Cook AC. The aorto-ventricular tunnels. Cardiol Young 2002; 12: 563–580.
12. Levy MJ, Lillehei CW, Anderson RC, Amplatz K, Edwards JE. Aortico-left ventricular tunnel. Circulation 1963; 27: 841–853.
13. Becker AE, Anderson RH. Cardiac embryology: a help or hindrance in understanding congenital heart disease? In: Nora JJ and Takao A (eds). Congenital Heart Disease: Causes and Processes. Futura Publishing Co., Mount Kisco, New York, 1984, 339–358.

https://doi.org/10.1017/S1047951110001137 Published online by Cambridge University Press