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Cardiac design in lower vertebrates: what can phylogeny reveal about ontogeny?

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Summary. In very few instances can the cardiovascular systems of adult 'lower' vertebrates serve as *direct* models for development in 'higher' vertebrates, primarily because numerous evolutionary specializations for preferential distribution of cardiac output between systemic tissues and gas exchange organs occur in the highly derived circulation of most extant lower vertebrates. Yet, the extensive literature on the cardiovascular anatomy and physiology of aquatic and air breathing fishes, amphibians and reptiles offers important conceptual insights into both patterns and mechanisms of development in birds and mammals. The primary contribution of such studies to the student of developing bird and mammal circulations is the clear demonstration that surprisingly complex hemodynamic function can develop from supposedly 'simple' cardiovascular systems typified by incompletely divided heart chambers. Thus, the hemodynamics of embryonic bird and mammal circulations should be determined by measurement, rather than inferred from structure.
Key words. Heart; circulation; blood; lower vertebrate; embryology; evolution.

What can developmental biologists gain from studying lower vertebrates?

During the course of development, the heart of embryonic birds and mammals progresses through a series of complex and critical stages involving both morphological and physiological changes. While some of these developmental stages clearly resemble the cardiovascular systems of adults of various lower vertebrates, the classic notion of 'ontogeny recapitulates phylogeny' long ago fell out of favor due to excessively literal interpretations. It is certainly not the intent of this article to attempt to revive it on even a limited basis, nor to suggest as a corollary that phylogeny recapitulates ontogeny! Rather, it is important to emphasize that understanding cardiovascular form and function in lower vertebrates – and in particular recognizing how surprisingly complex hemodynamics can arise from anatomically undivided hearts – can facilitate our understanding of developing hearts in embryonic birds and mammals. This facilitation can occur on two levels.

Firstly, we can begin to understand comparatively simple, microscopic embryonic circulations still beyond the reach of our technology by studying similar systems in adult lower vertebrates. For example, there will be certain hemodynamic constraints and demands on a cardiac pump that consists of only a single ventricle, regardless of whether it is to be found in an adult fish or a 36-h chick embryo in which ventricular division has yet to occur. In either situation, the systemic tissues and the gas exchange circuits are located in series and the single cardiac pump must generate sufficient pressure to perfuse both circulations. Thus, by understanding the cardiovascular system of fishes (which in many instances is far more ammenable to experimental investigation than the cardiovascular system of early embryonic stages of birds or mammals) we may gain insights into embryonic systems at early stages of anatomical and functional complexity.

A second way in which the study of the circulations of adult lower vertebrates will add to our understanding of embryonic circulation in birds and mammals involves the general perception of 'complexity'. A closer examination of the purportedly 'simple' (or even 'inefficient') hearts of lower vertebrates in fact reveals generally unappreciated complexity and sophistication, with cardiac output and its distribution between the various body tissues often being highly regulated. If the hearts of lower vertebrates can function in unexpectedly complex fashions, then might we not reasonably expect the potential for similarly complex performance from the 'simple' hearts of early embryonic stages? Yes, of course, but unfortunately the comprehensive collection of data on cardiac structure and function in lower vertebrates has only infrequently been exploited for insights by investigators studying cardiac function in avian and mammalian embryos.

This article discusses the cardiac morphology and physiology of adult lower vertebrates. There is a large primary literature covering literally centuries of studies. Consequently, the reader will be referred to recent reviews of this literature wherever possible. My hope is that investigators of developing hearts in avian and mammalian embryos might come to a new appreciation for the amazing physiological potential of supposedly 'simple' cardiovascular systems, whether in embryos or adults.

Form and function in 'single ventricle' systems

There are many common misconceptions about the cardiac anatomy and physiology of lower vertebrates. Most prevalent is that all hearts fall more or less on a single continuum, with the heart of homeotherms at the end perceived as most 'complex and efficient'. Such views are not only unnecessarily restrictive, but are rarely based on fact. Certainly, there is no disputing that the most anatomically simple hearts are found in most fishes and some amphibians. However, the reptiles have a central cardiovascular complexity that rivals, if not surpasses that of birds and mammals. Moreover, as will become apparent below, there is far, far more intraclass variation in fishes, amphibians and reptiles than in birds or mammals. Consequently, it is inappropriate to refer to "the reptile heart" or "the fish heart" and then attempt to place this fictitious organ on an equally fictitious continuum.

Overall, then, the concept of a cardiac continuum serves little useful purpose because there are few vertebrates with hearts that, pending some small modification, would clearly resemble the hearts of adjacent animals on the continuum. Rather than attempt to follow such an approach, the ensuing discussion takes a primarily functional rather than systematic viewpoint. While a gross parallel of cardiac function with ascending phylogenetic position is revealed, there are numerous instances where the phyletically more primitive, rather than more derived, taxa have a more complex cardiac anatomy and physiology.

1) Water breathing fishes – the basic piscine pattern

Considerable variation exists in the general morphological arrangement of the hearts in extant fishes. Most notable are the variable numbers and arrangements of conal valves and the composition of the conus arteriosus.

Two common variations on the basic cardiac anatomy of aquatic fishes are shown in figure 1. In the more primitive condition, as evidenced by the cardiac morphology of not only elasmobranch fishes (sharks and rays) but also holosteian fishes and the Dipnoi (lungfishes), the conus arteriosus is thickly walled, contractile, and contains a variable number of conal valves [1, 14, 31, 37]. In the more derived teleosts (bony fishes), the valves have been lost and the bulbus arteriosus is devoid of both cardiac muscle and valves.

[Goodrich [14] argues that term 'conus arteriosus' be reserved for the most anterior chamber in adult fish and amphibians, while the term 'bulbus cordis' be reserved for this chamber in embryos of all craniates and the adults of those reptiles, birds and mammals that have this structure or its remnants. Unfortunately, present convention in the anatomy of fishes often interchangeably uses conus arteriosus and bulbus (see fig. 1). Goodrich [14] presents a discussion of the origin of still-persistent terminology variations.]

Both arrangements reflect the basic fish pattern of an S-folded cardiac tube with a dorsally lying atrium and a ventrally located ventricle with apex pointing posteriorly. All venous return is received at one end and all cardiac output is ejected out at the other. Valves are located at the sino-atrial, atrio-ventricular junctions and at the base of the conus arteriosus. The ventral aorta, from which the afferent circulation to the gills is derived, develops as the proximal extension of the conus or bulbus arteriosus. One notable difference between the ventricle of birds and mammals and that of fishes in particular and lower vertebrates in general is the trabeculate nature of the ventricle (only slightly evident in figure 1). Rather than the ventricle consisting of a discrete central lumen as in

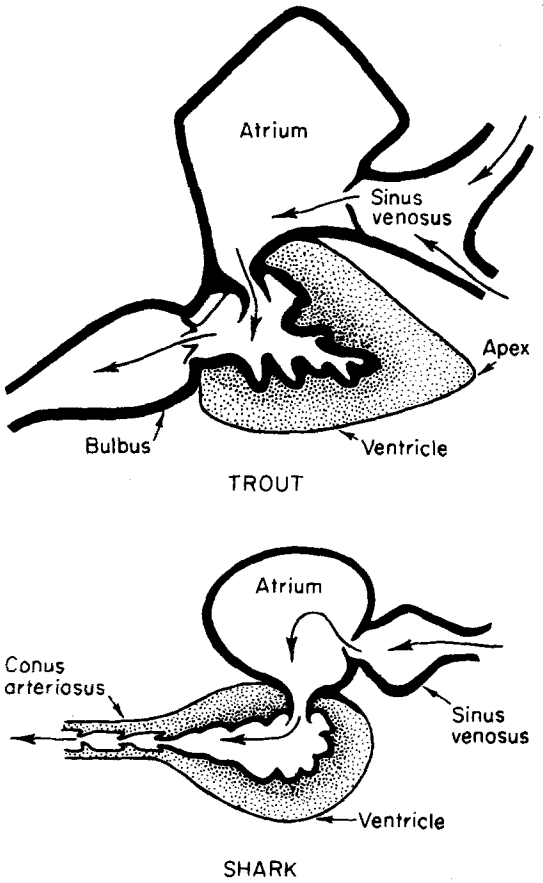

Figure 1. Basic cardiac anatomy of a representative Teleost (bony) fish, the trout, and a shark. Note that the primary difference lies in the region of outflow from the ventricle. (From Randall[28])

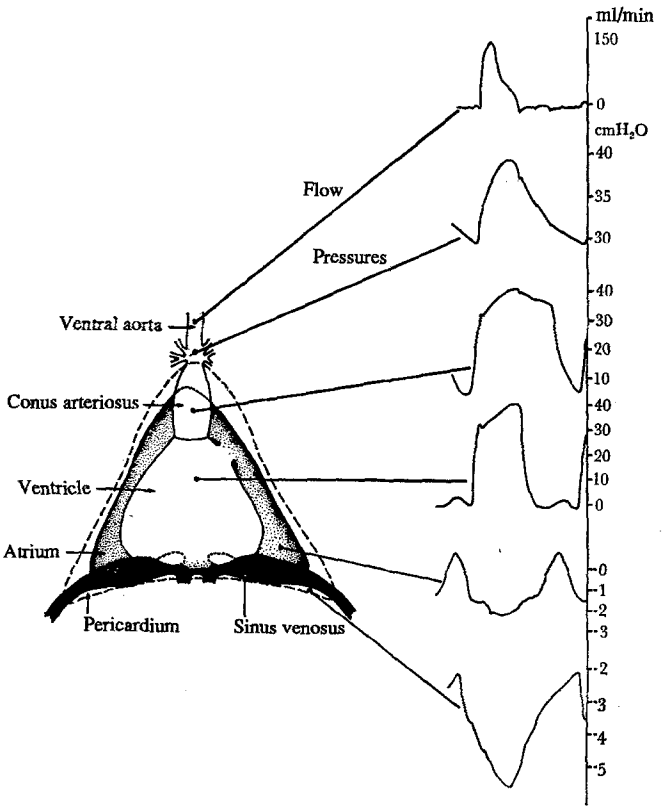

Figure 2. Heart structure and pressures within the pericardium, heart and ventral aorta of the Port Jackson shark, *Heterodontus portusjacksoni*. (From Satchell[31])

mammals, for example, there is a sponge-like muscular trabeculum surrounded by a thin layer of compact myocardium[22].

Intracardiac blood pressures and ventral aortic flow have been measured in many elasmobranchs and teleosts under a variety of acute and chronic conditions[21,22,28,31]. Clearly the ventricle provides the major impetus for ejection of blood from the heart (fig. 2). However, as early as 1839 Muller[27] proposed that the muscular conus arteriosus served as an auxiliary cardiac pump. Subsequent experiments have confirmed the role of the conus arteriosus in maintaining blood pressure and flow in the conus after the onset of ventricular relaxation[18,24,38]. In some sharks contraction of the conus arteriosus does not appear to maintain additional flow after ventricular relaxation, since flow in the ventral aorta falls rapidly with the onset of ventricular diastole (fig. 2). Nonetheless, contraction of the conus arteriosus is important in bringing together opposing walls bearing conal valves, and thus maintaining valve patency[22].

Ventricular systolic pressures in fishes generally range between 30 and 80 mm Hg, with sharks and rays generally showing lower pressures than teleosts[29,31]. The gills and systemic tissues are located in series without an intervening cardiac pump. This situation is similar to that in 4–5-day embryonic chick or 5–7-mm human embryo, in which the aortic arches intervene between the ventral and dorsal aorta[14]. Even though in fishes the entire cardiac output passes through the extensive vascular bed of the gills en route to the systemic tissues, passage through the branchial circulation reduces arterial pressure by only 25–40%, ensuring a sufficiently high dorsal aortic pressure for systemic tissue perfusion. Venous pressures in fishes range from slightly below atmospheric to as high as 10 mm Hg[29,31]. Aspiration of venous blood into the sinus venous is crucial to cardiac filling in fishes. The heart of sharks, in particular, resides in a tough, rigid pericardium. Contraction of the atrium and ventricle results in a sub-ambient intrapericardial pressure which is transmitted to the walls of the sinus venosus, causing aspiration of venous blood into the sinus venosus and atrium (fig. 2). In fishes, unlike in adult birds and mammals, atrial contraction plays a major role in ventricular filling[22].

The typical cardiovascular arrangement of aquatic fishes closely resembles the very early embryonic stages of birds or mammals. As will now become evident, however, the

cardiac anatomy of air breathing fishes, amphibians and reptiles shows increasing specialization and complexity.

2) Air breathing fishes – the origins of the divided circulation

In strictly aquatic fishes, the gills are the primary organs for gas exchange and there are few if any selection pressures that would lead to the evolution of specialization of the heart. In air breathing fishes, however, there are potentially several distinct sites for gas exchange, and numerous specializations appear which apparently are related to preferential distribution of blood between the various gas exchange organs and systemic tissues.

Cardiovascular specializations and gas exchange in air breathing fishes have been reviewed several times [8, 10, 19, 23, 30, 31]. In teleost fishes there are a myriad of vascular specializations for the perfusion of the potential sites for aerial gas exchange (skin, gills, branchial chamber, buccal cavity, swim bladder, gut). Importantly, however, in all teleost fishes oxygenated blood draining the air breathing organ is returned to the general venous circulation well before entering the heart. Thus, the heart essentially pumps mixed venous blood, and there is no opportunity for a separation within the heart of oxygenated and deoxygenated blood, as in early embryonic stages of birds and mammals prior to ventricular division.

As a consequence of the venous anatomy in teleost air breathing fishes, there appears to have been little selection pressure in air breathing species for specialization of the basic piscine cardiac pattern. One interesting exception is an air breathing teleost commonly called the 'snake head', *Channa argus* [17]. This fish appears distinct from all other bony fishes yet examined in that it lacks sino-atrial valves, has a particularly trabeculate myocardium, has a bulbus cordis with internal longitudinal muscular ridges, and has two ventral aortae emerging from the bulbus arteriosus (fig. 3). Blood gas analysis reveals that the anterior ventral aorta, which selectively perfuses the two anterior branchial arches and the air breathing organ, conveys blood with a consistently lower oxygen content than the posterior aorta, which selectively perfuses the dorsal aorta. While *Channa* (indeed, all bony fishes) are entirely removed from the main course of tetrapod evolution, the heart of *Channa* resembles a first hypothetical stage of cardiac specialization leading to division of the vertebrate ventricle.

The lungfishes (Dipnoi) show important qualitative differences in cardiac anatomy when compared with all other fishes [8, 10, 13]. As the name implies, the lungfish possess a 'true' lung, in that the pneumatic duct originates embryologically from a ventral evagination of the esophagus, and the pulmonary blood supply is derived from the sixth branchial arch. A crucial evolutionary step is the appearance of a discrete pulmonary vein which returns oxygenated pulmonary venous blood either into the sinus venosus (Australian lungfish, *Neoceratodus*) or directly into the left side of the atrial chamber (the South American lungfish, *Lepidosiren* and African lungfish, *Protopterus*). The existence of a discrete pulmonary vein provides for separation of oxygenated and deoxygenated venous blood at the point of entry to the heart, resembling early embryonic avian and mammalian stages in which ventricular separation is incomplete but discrete atrial chambers are well formed. With the venous arrangement evident in lungfishes, cardiac specializations would convey the ability to maintain this separation in passage through the heart. Indeed, the heart of lungfishes shows several important modifications not seen in any other fishes. (There are many structural differences between the three genera of lungfishes [8] and the present account will only describe the fundamental properties.) The single atrium is partially divided into a larger right and smaller left chamber in all three genera (fig. 4). The

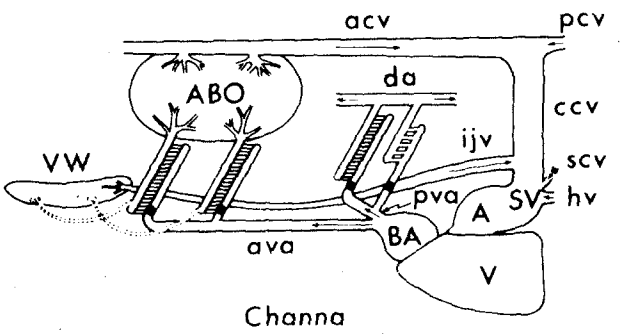

Figure 3. Central cardiovascular anatomy of the air breathing teleost fish *Channa argus*. Arrows in the vessels indicate the direction of blood flow. A, atrium; ABO, air breathing organ; BA, bulbus arteriosus; ccv, common cardinal vein; da, dorsal aorta; hv, hepatic vein; ijv, inferior jugular vein; pcv, posterior cardinal vein; pva, posterior ventral aorta; scv, subclavian vein; SV, sinus venosus; V, ventricle; va, ventral aorta; VW, vascular wall of the buccal cavity. (From Ishimatsu and Itazawa [17])

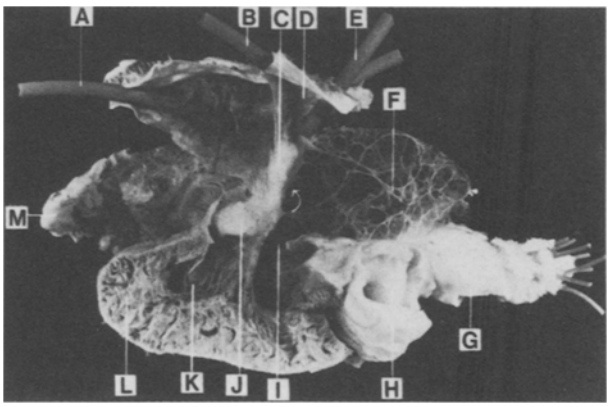

Figure 4. Sagittal section through the heart of the African lungfish *Protopterus aethiopicus*. The arrow shows where the vena pulmonalis ends behind the pulmonalis fold. A, probe in vena cava posterior; B, probe in vena pulmonalis; C, pulmonalis fold; D, oblique fold separating the apertures of the two ducti Cuvieri; E, probe in the right ductus Cuvieri; F, anterior unpaired part of the atrium; G, distal section of the bulbus cordis; H, spiral fold in the transverse section of the bulbus cordis, which has been opened; I, atrioventricular aperture; J, atrioventricular plug; K, ventricular septum; L, ventricular apex; M, left auricular lobe. (From Bugge [1])

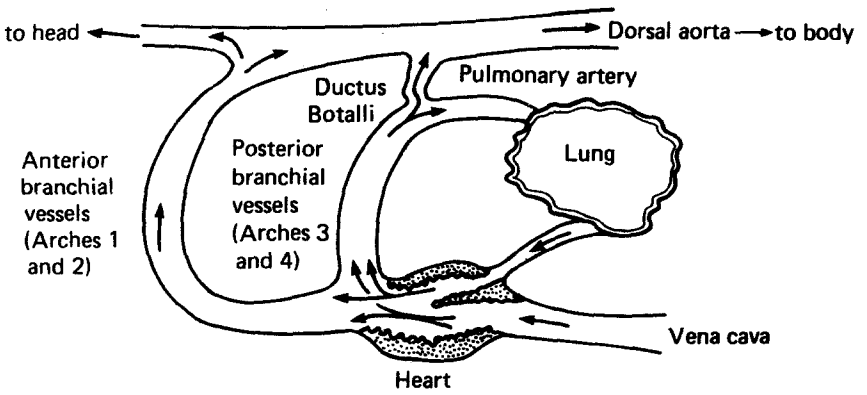

Figure 5. A highly schematic diagram of the central vascular circulation of the lungfish. (From Randall et al.[30], after Johansen et al., 1968)

division is achieved by the pulmonalis fold and a unique structure termed the atrioventricular 'cushion' or atrioventricular 'plug'. The pulmonary veins of *Lepidosiren* and *Protopterus* enter the left side of the atrium, while the sinus venosus enters to the right. *Lepidosiren* shows the greatest degree of atrial specialization, with almost complete atrial subdivision.

The lungfish ventricle is highly trabeculate, more closely approximating the condition of amphibian myocardium. Unlike other fishes as well as amphibians, however, there is a vertical septum arising from the dorsal and ventral walls of the ventricle. This septum serves to divide much of the ventricular lumen, particularly towards the apex of the heart. Oxygenated blood from the pulmonary veins thus tends to enter the left portion of the ventricle from the atrium, while deoxygenated blood from the systemic tissues tends to enter the right region of the ventricle. The conus (bulbus) arteriosus, like that of many phyletically ancient fishes, is a complex structure containing several rows of conal valves. Once again, there are differences between genera with respect to the derivation of the branchial arches. Basically, however, the blood perfusing the two most anterior gill arches directly enters the dorsal aorta and ultimately perfuses the systemic tissues. Blood perfusing the two most posterior arches passes either into the pulmonary vein or passes via the ductus Botalli into the dorsal aorta (fig. 5). In *Lepidosiren* in particular, the anterior arches are largely devoid of gill filaments, and essentially serve as shunt vessels passing blood to the dorsal aorta.

The S-shaped curvature of the heart of lungfishes is more pronounced than other fishes, with the sinus venosus lying primarily dorsally to the ventricle, the apex of which extends posteriorly below it. The shape of the heart of lungfish thus resembles that of amphibians such as salamanders (see below).

Extensive measurements of blood gases, pressures and flows have been made by Johnsen and his colleagues in order to determine the hemodynamics of the lungfish heart[8, 19, 23, 25]. Oxygenated blood from the left side of the atrial chamber is preferentially distributed to the left side of the ventricle, while deoxygenated blood enters the right side. Assisted by the trabeculate nature of the myocardium as well as the vertical septum, this separation of blood streams is well maintained during both ventricular filling and subsequent ejection into the bulbus cordis. Oxygenated blood primarily perfuses the anterior arches, while deoxygenated blood primarily enters the posterior arches. Immediately following an air breath, when pulmonary O_2 levels are highest, as much as 70% of the total cardiac output perfuses the lungs[25]. Importantly, approximately 90% of the blood entering the dorsal aorta via the anterior branchial arches is derived exclusively from this oxygenated pulmonary venous blood. Lungfish, like almost all air breathing fish, only intermittently ventilate their lungs. Following prolonged breath holding the O_2 levels of the lung have fallen and the gas exchange potential of the lung may be lower. Continued high rates of perfusion during breath holding (apnea) might be regarded as disadvantageous under these conditions. Indeed, lungfishes are able to regulate finely the distribution of blood flow between the anterior and posterior arches and between the pulmonary artery and ductus Botalli[8, 13]. This regulation is achieved primarily by carefully orchestrated adjustment in the vascular resistance of the branchial circulation and the ductus Botalli. Thus, during apnea cardiac output is distributed away from the temporarily less effective lungs to the gills, which continue to be ventilated with water and to exchange gases.

The structural modifications of the heart of the lungfish thus appear highly effective in maintaining separation of oxygenated and deoxygenated blood flows through the heart. When combined with the central vascular capability for regulating the distribution of blood flow according to gas exchange requirements, the lungfish emerge as an important example of increasing structural and functional complexity in lower vertebrates. It is important to emphasize that the extant lungfishes are highly derived sarcoptyrigian fishes that are only distantly related to the ancestral terrestrial tetrapods[2], and thus should not be viewed as the evolutionary 'raw material' for the amphib-

ian heart. Indeed, as will now become evident, the heart of the lungfishes is quite different, but no less derived, than that of the amphibians.

3) Amphibian hearts – 'simple' anatomy yields complex function

Significant anatomical differences exist between the anurans (frogs and toads) and urodeles (salamanders and newts) – the hearts of the much less common apodan amphibians have not been extensively examined. As emphasized earlier, referring to a single 'amphibian heart' as descriptive for the class is clearly inappropriate.

The heart of adult anuran amphibians has two separate atrial chambers emptying into a common ventricle (fig. 6). In anurans in particular the S-shaped curvature of the heart is very pronounced. Consequently, the two atria are carried anteriorly in front of the ventricle and open posteriorly into it. The ventricle lacks any subdivision, and thus is unique among vertebrates that breathe with true lungs. The myocardium has many muscular trabeculae running predominantly in an anteroposterior direction. The many crypts and pockets have been suggested as a crucial characteristic for separation of blood flowing through the ventricle [33]. A bulbus arteriosus (also termed conus arteriosus) arises from the single ventricle. Three non-muscular, semi-lunar cusp valves lie at the base of the bulbus arteriosus and prevent reflux of blood back into the ventricle.

The bulbus cordis of anurans contains a longitudinal spiral valve which is attached to the inner bulbus wall along one edge, but is free along the other [14]. The bulbus arteriosus twists through nearly one rotation before branching into systemic and pulmocutaneous arches, each arising from different sides of the spiral valve (fig. 6).

Measurements of blood pressure and flow as well as analysis of blood gases have begun to elucidate the function of this complex system in anurans [22, 26, 33–35]. Oxygenated blood entering the ventricle from the left atrium during ventricular diastole is sequestered in the many small chambers of the left wall of the ventricle, while a parallel process occurs with deoxygenated blood entering the right side of the ventricle. During ventricular systole, there appears to be a laminar flow of blood from the trabeculae on the right and left side of the heart such that little blood admixture occurs. Oxygenated blood is directed primarily to the side of the spiral valve from which the systemic arches are derived, while deoxygenated blood is directed primarily to the side from which the pulmocutaneous arches originate. Blood gas analysis from pithed bullfrogs [39] (*Rana catesbeiana*) reveals that 48% of blood perfusing the gas exchange circuit (i.e. the pulmocutaneous arteries) comes from the right atrium. Thus, the so-called 'left-to-right shunt' (L-R shunt) is only 16%. The 'right-to-left' (R-L) shunt is even smaller, since 91% of the blood perfusing the systemic arteries comes from the left atrium. Thus, even under extreme experimental conditions there is highly effective separation of oxygenated and deoxygenated blood flowing through the single ventricle.

As in almost all other intermittently air breathing vertebrates that have been examined, there is very close matching of perfusion to ventilation in frogs and toads. Thus, during and immediately following a breathing episode, pulmonary resistance falls and cardiac output is preferentially distributed to the lungs. During prolonged apnea blood flow is diverted away from the lungs and

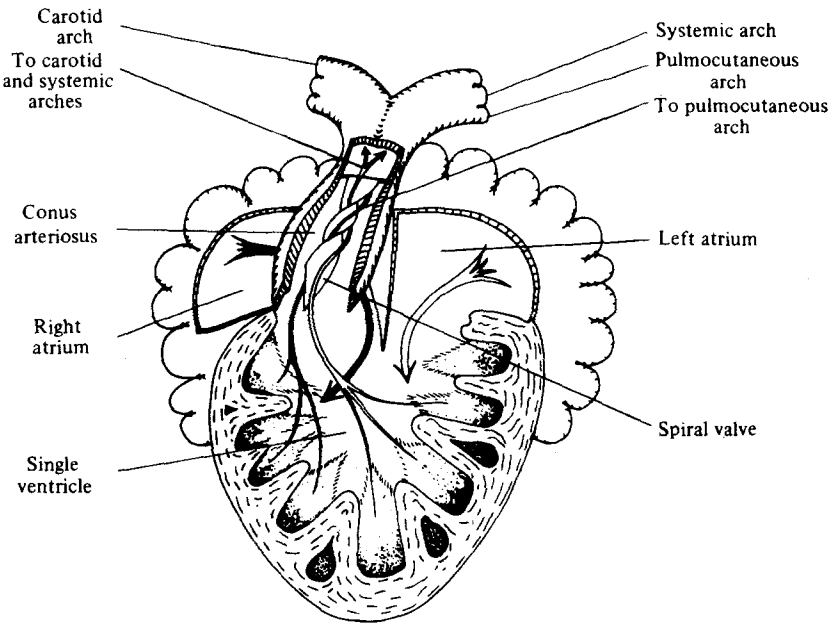

Figure 6. Diagrammatic representation of a typical amphibian heart from the ventral aspect. The flow of oxygenated blood is shown by the open arrows, while the flow of deoxygenated blood is shown by the black arrows. (From Shelton [33])

directed to the systemic tissues, and to the skin, which serves as an important accessory gas exchange organ [12]. In salamanders and apodan amphibians the septum separating the two atria may be very thin and perforated, while the spiral valve is reduced or non-existent [14]. Unlike in frogs, there is no pulmocutaneous artery giving rise to a distinct cutaneous artery. The pulmonary artery arises from the truncus arteriosus along with a variable number of systemic arches. While it is tempting to speculate on the basis of this apparently simpler anatomical arrangement than in anurans that considerable mixture of pulmonary venous and systemic venous blood is likely to occur in passage through the heart, this appears not be the case. In the urodele amphibian, *Amphiuma tridactylum* ('Congo eel'), for example, the dorsal aorta carries blood that is 80% derived from the pulmonary vein during and immediately following an air breath [40]. As apnea progresses, the extent of the R-L shunt increases, and the proportion of pulmonary venous blood in the dorsal aorta may fall to as low as 40%. Regardless of ventilatory state and the extent of the L-R shunt, however, the L-R shunt remained insignificantly small at all times.

Finally, it is of interest to describe briefly the cardiac anatomy of the Plethodontid salamanders. As adults they have no lungs (nor any vestiges of lungs) and subsist entirely on cutaneous gas exchange [12, 14]. There are no pulmonary veins or pulmonary arteries, and the interatrial septum is highly reduced. The conus arteriosus is much simplified. This anatomical condition represents a regression to a cardiac condition not dissimilar from aquatic fishes.

Reptilian hearts: complex ventricular divisions

A 'representative' reptilian heart is often placed adjacent to that of adult birds or mammals on the fictitious cardiac continuum. Two points must be emphasized. Firstly, there is no representative reptile heart. In some instances widely divergent taxa have very similar cardiac anatomy, while in other instances closely related taxa show surprising variation. Cardiac anatomy seems to reflect 'lifestyle' more closely than systematics. Secondly, the many different cardiac arrangements in various reptiles frequently are highly derived patterns that show few resemblances to the hypothetical hearts of ancestral birds and mammals. Indeed, in stark contrast to common belief, reptilian hearts *do not* have a hole in an 'interventricular septum' that, if closed, would immediately yield the heart of a bird or mammal.

Many different aspects of the anatomy and physiology of the heart and central circulation of a variety of reptiles have been intensively investigated [4, 5, 16, 22, 44, 46]. Three general anatomical and physiological patterns have emerged, represented by most squamates (snakes and lizards) and chelonians (turtles and tortoises) as one group, the lizards of the genus *Varanus* (monitor lizards) as the second, and by the crocodilian reptiles.

1) Chelonian and squamate reptiles – the 'five-chambered' heart

The basic cardiovascular arrangement to be seen in most chelonian and squamate reptiles [5, 36] represents a highly complex pattern that bears little resemblance to the hearts of other living amniotes, embryonic or adult. Figure 7 schematically depicts the general arrangement for adults. The atria retain the condition in anuran amphibians, being entirely separated by a common medial atrial wall. Large valves guard the arterio-ventricular orifices. The ventricle is complexly divided into three more or less distinct cava. The cava arteriosum is situated in a anteriolateral position near the base of the heart. This cavum receives all of its input as oxygenated blood from the left atrium. The cavum arteriosum has no direct arterial output, however, directing all blood within it into the largest cardiac cavum, the cavum venosum. The cavum venosum also receives all deoxygenated blood from the right atrium. During diastole and atrial systole, some proportion of blood from the cavum venosum passes around a muscular ridge into the third cardiac cavum,

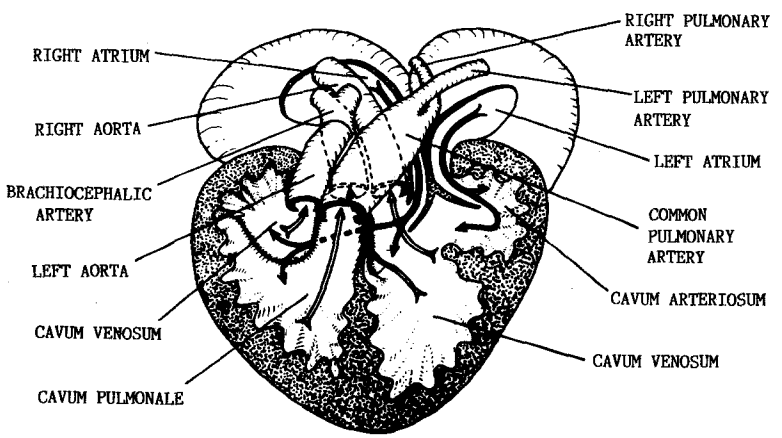

Figure 7. Diagrammatic illustration of the heart of the freshwater turtle *Pseudemys scripta*. Pathways for blood flow from the ventricular cava to the arterial arches are indicated by the solid arrows. (From Shelton and Burggren [36])

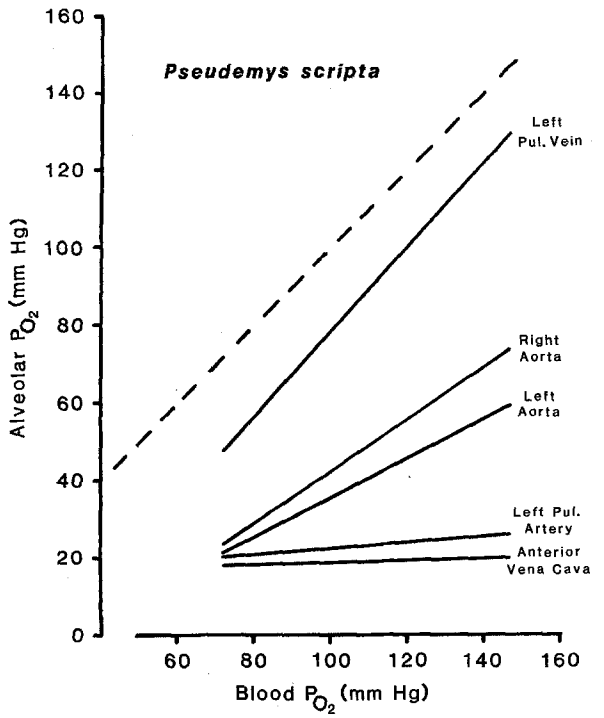

Figure 8. The relationship between O_2 partial pressure in blood from central arteries and veins and the O_2 partial pressure of alveolar gas in nine freely diving freshwater turtles, *Pseudemys scripta*. High alveolar PO_2s correspond to periods of time during and shortly after air breathing, while low alveolar PO_2s develop during breath holding (i.e. diving). (Modified from Burggren and Shelton [9])

the cavum pulmonale. The cavum pulmonale receives no direct atrial input, depending entirely upon blood from the cavum venosum for filling. Upon contraction of the cavum pulmonale, blood is ejected into the common pulmonary artery. Contraction of the cavum venosum ejects blood into the base of the two aortic arches. Depending upon species, a separate brachiocephalic arch arises from the right aorta very close to the heart and thus also receives blood from the cavum venosum. The bases of all aortic arches are guarded by large flap valves. Clearly, the ventricular anatomy of turtles, snakes and lizards bears little resemblance to that of birds and mammals.

Measurements of intracardiac blood pressure in the non-crocodilian reptiles have generally revealed that during both systole and diastole the three cardiac chambers remained in complete communication [5]. Contraction of the chelonian heart is analogous to simultaneously squeezing the fingers of a water-filled rubber glove. In spite of a complex subdivision of the glove, there is equal water pressure throughout as each finger empties. Thus, the ventricle of most snakes, turtles and lizards appears to function as a single pressure pump.

Oxygenated and deoxygenated blood from the left and right atrium, respectively, must undergo considerable redistribution within the three ventricular cava before ejection into the arteries can occur. Because the ventricular cava and the blood within them remain in functional contact throughout this process, there is a potential for both L-R and R-L intracardiac shunting during both diastole and systole [4, 5, 44]. Many chelonian reptiles are intermittent breathers and so, as for amphibians, the extent of intracardiac admixture should be placed in the context of whether the animal is breathing or apneic. Figure 8 illustrates how central vascular O_2 partial pressures change during intermittent breathing in the freshwater turtle, *Pseudemys scripta*. Blood PO_2s are plotted against alveolar gas PO_2, with the highest alveolar PO_2s corresponding to periods of air breathing and the lowest alveolar PO_2s corresponding to prolonged diving. Regardless of breathing state, the L-R shunt remains very small, with left pulmonary artery PO_2 being only slightly elevated over that in the anterior vena cava. When alveolar PO_2 is high during and shortly after breathing, the PO_2 of blood in the two aortae is markedly greater than in the pulmonary artery, but also lower than in the pulmonary vein, revealing the presence of a significant R-L shunt. The slightly lower left aortic PO_2 reflects the closer proximity to the cavum pulmonale of the origin of the left aorta. Although significant intracardiac shunting occurs, there is nonetheless substantial separation of oxygenated and deoxygenated blood when alveolar PO_2 is high.

During diving, alveolar PO_2 falls and the R-L shunt increases in magnitude, as evident by the decreasing PO_2 difference between systemic arterial and pulmonary arterial blood. The typical view of investigators most familiar with avian or mammalian circulations would be that large shunt fractions in either direction represent an 'inefficient' cardiac system unable to maintain blood separation. However, it is crucial to realize that the benefits of pulmonary perfusion decrease during progressive apnea. Not only does alveolar PO_2 continue to fall, reducing gas exchange efficacy in the lungs, but fluid accumulation of the lung may begin to develop during prolonged high rates of pulmonary blood flow [35]. Because R-L shunting can occur, reptiles with a cardiac arrangement where the lungs and body are located in parallel rather than in series have the potential to bypass partially the pulmonary circuit and direct that proportion of the cardiac output that would have perfused the lungs directly to the systemic circulation (i.e. R-L shunt). In fact, because the lungs and systemic tissues are located in parallel, rates of pulmonary and systemic perfusion are somewhat independent of each other. Systemic perfusion (e.g. blood flow to the brain, kidney, muscles) can be fully maintained even when pulmonary perfusion has decreased or even stopped. This anatomical and physiological arrangement represents a far greater cardiovascular flexibility than is evident in adult birds or mammals.

2) Varanid lizards – dual pressures from an anatomically undivided ventricle

Varanid lizards ('monitor' lizards) represent a fascinating variation on the typical non-crocodilian cardiac pat-

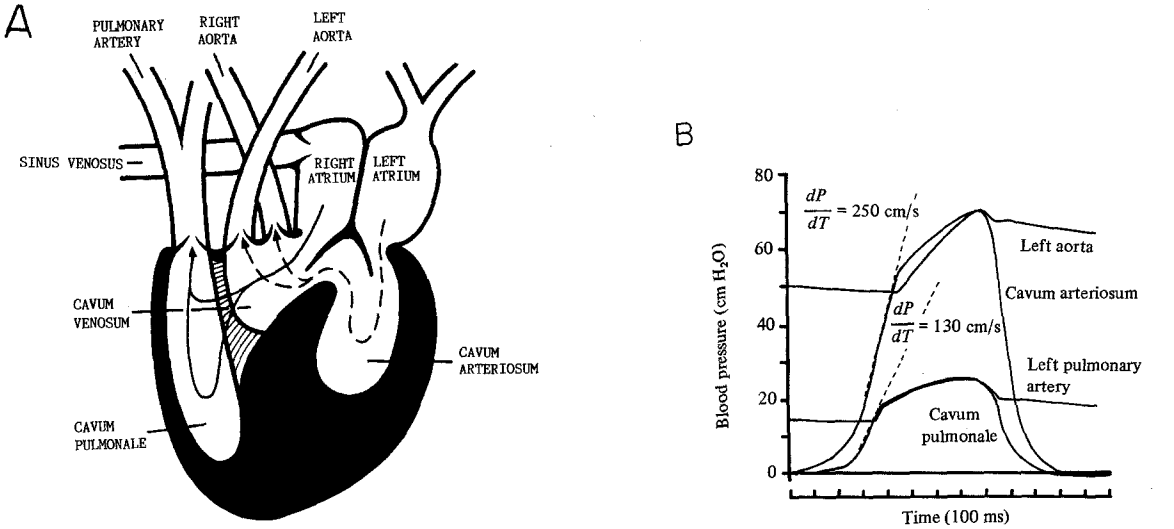

Figure 9. *A* Diagrammatic illustration of the heart of a varanid lizard. The muscular ridge (striped area) between cavum venosum and cavum pulmonale is projected onto the outer heart wall for clarity. (From Heisler et al.[15]). *B* Intracardiac and arterial blood pressures measured simultaneously in an anesthetized savannah monitor lizard, *Varanus exanthematicus*. (From Burggren and Johansen[7])

tern[5,7,15,41]. As in other lizards, the ventricular cava are anatomically patent. However, the relative proportions of these cava are quite distinctive (fig. 9A). The cavum venosum has become reduced to the extent that it serves primarily as a channel for communication between the cavum pulmonale and the cavum arteriosum. The cavum arteriosum is much enlarged, occupying a considerable proportion of the right side of the heart. Contraction of the cavum arteriosum moves blood completely through the reduced cavum venosum and into the systemic vessels. Thus, the cavum arteriosum rather than the cavum venosum should be regarded as the main systemic cardiac pump. As in other squamate reptiles, redistribution of inflowing atrial blood between ventricular cava in varanid lizards must occur prior to ejection into the systemic and pulmonary arteries, and so intracardiac shunting potentially can occur during diastole. During systole, however, the cavum arteriosum and cavum venosum become functionally separate, as revealed by direct measurement of intracardiac pressures[7] and X-ray cinematography[20]. Figure 9B shows simultaneously recorded intracardiac and arterial pressures in the savannah monitor lizard *Varanus exanthematicus*. During diastole identical pressures are recorded in the ventricular cava. As systole begins, however, pressure in the cavum venosum rises first, faster and to higher levels than in the cavum venosum, clearly indicating functional separation of the ventricular chambers. Systolic pressures in the cavum arteriosum can rise to 120 mm Hg with attendant systolic pressures in the cavum pulmonale reaching only 20–50 mm Hg. Consequently, the systemic circulation is perfused at considerably higher pressures than the pulmonary circuit (fig. 9B) in *Varanus*, reflecting a hemodynamic performance resembling that of the heart of adult birds and mammals. Intracardiac shunting in varanid lizards, as in other reptiles, appears variable in magnitude[7,15]. During continuous lung ventilation, admixture of oxygenated and deoxygenated blood can be almost negligible[7] and blood perfusing the systemic tissues is at least 75% oxygen saturated and often much higher.

The varanid heart provides an important lesson in the study of cardiovascular form and function. Not only does it become evident that lack of functional separation within the heart does not necessarily follow from anatomical patency between chambers, but also that a particular hemodynamic pattern (e.g. high systemic and low pulmonary arterial pressures) can be achieved in ways other than with the completely divided avian or mammalian ventricular arrangement.

3) Crocodilian reptiles – the 'perfect' vertebrate circulation?

Of all lower vertebrates, the heart of crocodiles and alligators (the 'crocodilian reptiles') most resembles the heart of adult birds and mammals. There is strong bilateral symmetry, and for the first time the use of 'left' and 'right' as cardiac descriptors becomes appropriate. Not only are the two atrial chambers completely separate, but the larger and more thickly walled left ventricle is anatomically completely separate from the smaller right ventricle (fig. 10A). Thus, with respect to the cardiac chambers, the crocodilian heart is qualitatively identical to that of adult birds and mammals. Unlike in higher vertebrates, however, crocodilians retain both left and right aortic arches throughout development. Whereas the right aorta is perfused directly from the left ventricle, the left aorta arises from the right side of the vertical septum separating the ventricular chambers. The two aortae coalesce peripherally, and are also connected proximally by a narrow connecting channel termed the 'foramen paniz-

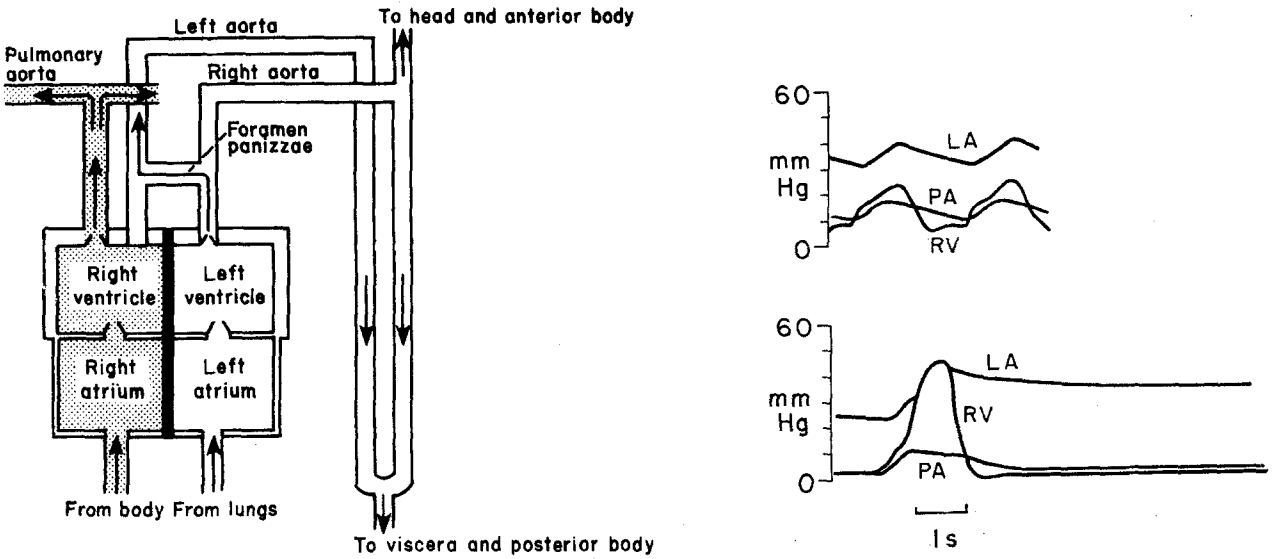

Figure 10. The crocodilian circulation. *A* Diagrammatic representation of the cardiac chambers and greater vessels of the crocodilian heart. The shaded area represents deoxygenated blood, while possible pathways for blood through the central circulation are indicated by arrows. (From Johansen and Burggren[22], after White[43]) *B* Simultaneously recorded pressures recorded in an unanesthetized alligator during lung ventilation (top) and after 12 min of apnea (bottom). LA, left aortic arch; PA, pulmonary artery; RV, right ventricle. (From White[42,43])

zae'. Blood ejected from the right ventricle can potentially enter both the pulmonary artery and the left aorta. When right ventricular blood enters the left aorta, then a R-L shunt is in operation. It is interesting to note that although the developing bird circulation has two systemic arches at early embryonic stages, there is no embryonic stage that transiently resembles the crocodilian situation with one systemic arch arising from the right ventricle[14].

Cardiovascular function in crocodilians is highly dependent upon ventilatory state (fig. 10 B). During periods of lung ventilation, systolic pressure in the left ventricle is 20–30 mm Hg greater than in the right ventricle[43]. The resulting higher arterial systolic pressure is transmitted to the left aorta via the foramen panizzae and the distal confluence of the arches. As a consequence, blood pressure at the base of the left aorta exceeds that immediately across the valves in the right ventricle at all stages of the cardiac cycle. The lunar valves at the base of the left aorta remain closed, and all blood ejected from the right ventricle enters the pulmonary artery. Thus, even though an anatomical pathway exists for a R-L bypass of the lungs, *functionally* the crocodilian heart during lung ventilation operates as in birds and mammals. During diving however, pulmonary arterial impedance rises relative to systemic arterial impedance. Systolic pressure in the right but not the left ventricle rises considerably (fig. 10 B). At the point when systolic pressures in the left and right ventricles become identical, the pressure gradient which formerly had kept closed the valves at the base of the left aorta disappears. Blood ejected from the right ventricle tends to enter the systemic circuit via the left aorta, rather than enter the high impedance pulmonary circuit via the pulmonary artery. Thus, a redistribution of right ventricular cardiac output into the systemic circulation (i.e. a R-L shunt) occurs even though the heart itself is completely divided. The physiological rationale for reducing pulmonary flow without necessarily reducing systemic flow is essentially the same for crocodilian reptiles as described above for squamates. The crocodilian circulation thus emerges as one of extraordinary flexibility, showing the zero-shunt condition of bird and mammal heart during active lung ventilation, and the pulmonary bypass of other reptiles and amphibians when diving.

In summary, reptiles show three very different patterns of cardiovascular form and function. It should now be abundantly clear that these patterns *do not* reflect direct intermediate conditions leading directly to the hearts of birds and mammals. However, they do show that a variety of hypothetical intermediate stages which could lead to the development of the four-chambered heart have arisen during the course of tetrapod evolution.

Conclusions

To repose a question asked at the outset, what can an understanding of the cardiovascular system of adult fishes, amphibians and reptiles tell us about ontogenetic processes in the circulation? The cardiovascular systems of lower vertebrates, which in many superficial ways anatomically resemble embryonic stages in birds and mammals, show amazing morphological and functional diversity. In many instances, surprisingly complex and unexpected patterns of blood flow and pressure have been

revealed through recent studies of cardiovascular systems that *on the basis of anatomy alone* historically have been regarded as 'primitive' or 'inefficient'. Similar biases appear to have pervaded the study of mammalian and avian embryonic circulations. For example, it is common for the incompletely divided embryonic heart of a bird or mammal to be viewed of as 'merely adequate' for transport functions pending 'improvement' upon maturation, rather than as a system – highly refined through evolution – that has the critical potential for the intracardiac shunting so necessary for embryonic or fetal life.

Whether there are direct comparisons to be made between characters in adult lower vertebrates and embryonic birds and mammals probably has to be assessed on a case by case basis, though no doubt some comparisons are valid. For example, hemodynamics in the simple S-folded heart of an adult fish may very well serve as a model for hemodynamics in the similarly structured heart of an early embryonic stage of birds and mammals. Perhaps more importantly, however, the value to embryologists in studying lower vertebrate cardiovascular systems lies in their providing a constant affront to the notion that simple functions must arise from simple structures.

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A molecular view of cardiogenesis

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Summary. Cardiac development involves a complex integration of subcellular processes into multicellular and, finally, whole organ effects. Until recently it has been difficult to investigate the genetic control of this organ level differentiation of the heart. The proliferation of molecular biology methodologies has provided mechanisms to directly investigate the control of these processes. This article focuses on molecular lines of research on two key areas in cardiac development: the regulation of expression of sarcomeric contractile and regulatory proteins, and atrial natriuretic factor. Molecular approaches are described which have allowed investigators to begin to determine the tissue and stage-specific expression of genes, to locate those genes in the genome, determine their sequences, and to directly investigate the mechanisms controlling their expression.
Key words. Cardiac development; embryogenesis; gene expression; complementary DNA; molecular methodologies; myocardial contractility; myosin; atrial natriuretic factor.

Introduction

Regulation of cardiac development involves a complex integration of subcellular processes into cellular and multicellular effects. The complex organ-level processes of cardiac looping, myocardial differentiation, septation, trabeculation, and valve formation must be controlled on the genetic level. Until recently it has been difficult to investigate the genetic control of these whole organ level processes. The proliferation of molecular biology methodologies has provided mechanisms to directly investigate the control of these processes. In recent years these methodologies have begun to be applied to developmental questions in higher vertebrates. However, because of their complex methodologies, these approaches have not been as extensively integrated into the research repertoire of cardiac developmental biologists as they could be.

This article will focus on molecular lines of research in two key areas in cardiac development: the regulation of expression of sarcomeric contractile and regulatory proteins, and the regulation of expression of atrial natriuretic factor, a major secretory product of cardiac myocytes which is involved in regulation of cardiovascular homeostasis. First, however, the major strategies in the molecular field will be outlined to provide the uninitiated cardiac developmental biologist with a primer for understanding molecular terminology, methodologies, and most importantly, the types of questions which can be answered with these approaches. It is not the purpose here to describe protocols; for this the interested reader is referred to excellent reference texts from which much of this outline is derived [2, 20, 23, 24, 40].

Molecular approaches allow investigators to determine the tissue and stage-specific expression of genes, to locate those genes in the genome, and determine their sequences. The newest approaches allow direct investigation of the mechanisms controlling the expression (or repression) of genes via action on regulatory portions of the gene. Using these methodologies, investigators can directly test which putative epigenic (extrinsic) factors are capable of regulating expression of specific genes, and how they accomplish this interaction with the genome. By building on such findings on a number of different cardiac-specific gene programs, we may be able to construct a pattern which translates to whole-organ effects.

Outline of molecular strategies

Molecular strategies yield results on structure and function on the gene, RNA, and protein product level. Approaches used to identify unique gene products on the protein level have been employed for some time. They include biochemical identification of the isolated protein on the basis of its molecular weight (MW) and charge differences in its native configuration, or MW alone (as determined by native and denaturing protein gel electrophoresis, respectively). Gross differences in amino acid sequence can be detected from the size of fragments