PATTERNS OF FORM AND FUNCTION IN DEVELOPING HEARTS: CONTRIBUTIONS FROM NON-MAMMALIAN VERTEBRATES

WW Burggren, SJ Warburton

Department of Biological Sciences, University of Nevada, Las Vegas, 4505 Maryland Parkway, Las Vegas NV 89154, USA

Although most research on developmental cardiovascular physiology has focused on the bird embryo as a model for emulating developmental processes in mammals, there are increasingly compelling reasons to expand research to a variety of lower vertebrate systems. These reasons include circumventing inherent limitations of the avian embryo and identifying general vertebrate developmental patterns in the cardiovascular system. In this paper, we first review data from hemodynamic studies on amphibians and birds (and what little exists from fish and reptiles), to provide a background against which lower vertebrate development can be examined. We then describe non-mammalian, non-avian paradigms for studying developmental patterns of vertebrate hearts. Developmental spects of cardiovascular performance, especially heart rate, blood pressure and cardiac output and how they change with ontogeny, are described for several amphibians and a few reptiles, identifying, where possible, processes in common with birds and mammals. Finally, we indicate productive areas for future research with lower vertebrate cardiovascular systems, such as establishing "critical windows" for cardiovascular physiology during development, and determining the extent of developmental plasticity at the level of organ system physiology.

Key Words: development, hemodynamics, ontogeny

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Introduction

The *in vivo* study of both the morphology and hemodynamics of developing cardiovascular systems has flourished in recent years. In part this surge has been led by technological developments. For example, pulsed Doppler and servo-null micropressure systems now allow routine measurement of blood flow and blood pressure, respectively, in hearts and blood vessels of embryos or larvae that are at the edge of resolution by the human eye. Moreover, cardiovascular physiologists and clinicians alike realize that an understanding of normal and pathophysiological processes in the adult has its underpinnings in an understanding of the developmental events leading to the ontogenetic terminal point.

Until recently, most developmental physiology focused on the bird embryo in general, and specifically on the embryo of the chicken. This system undoubtedly will continue to be a useful model for emulating developmental processes in mammals. However, there are increasingly compelling reasons to expand research to a variety of lower vertebrate systems. These reasons include circumventing inherent limitations in the cardiovascular system of the avian embryo as a model for the mammal, identifying general patterns of vertebrate cardiovascular development, and determining the nature of cardiovascular specializations unique to specific vertebrate subgroups. This last will help us to understand better the nature of diversity in, and evolution of, the vertebrate cardiovascular system.

In this paper we will: 1) describe highly useful non-mammalian (and non-avian) paradigms for studying the development of vertebrate hearts; 2) review data from hemodynamic studies on amphibians and birds (and what little exists from fish and reptiles); and 3) indicate productive areas for future research, particularly in establishing "critical windows" for cardiovascular physiology during development, and determining the susceptibility of these systems to environmental challenges.

To understand commonalties and diversity in the development of the cardiovascular system in vertebrates, we must first explore briefly the diversity within the adults of different vertebrate taxa.

Adult cardiovascular systems.

Although all physiological systems are "important" and "vital", there can be no disputing the central role that the cardiovascular system plays in virtually every aspect of an animal's survival. As the transporter of the oxygen and nutrients required for metabolism and the carbon dioxide and other wastes produced as a consequence, the cardiovascular system is frequently cited as a limiting factor in cellular metabolism[1,2].

Given its central support role in metabolism, it is hardly surprising that the morphology and physiology of the cardiovascular system in vertebrates has been greatly molded by natural selection. The heart and central vessels in particular have become highly modified with the advent of major evolutionary events such as the development of bimodal breathing, separate pulmonary and systemic circulations, and a high pressure systemic circulation. Consequently, great variation exists in cardiac structure in adult vertebrates, ranging from the comparatively simple teleost heart to the most complex of vertebrate hearts evident in crocodilians. A variety of aspects of cardiac anatomy and physiology in adult non-mammalian vertebrates have been reviewed in the last decades, and the reader is referred to those articles for detailed information and for references that include more narrowly focused reviews on specific taxa or physiological processes[3-10].

Fishes. The most anatomically simple form of vertebrate heart is found in aquatic fishes. All adult gnathostome fish posses a heart with four chambers — sinus venosus, atrium, ventricle and bulbus arteriosus (sometimes called either the bulbus cordis or conus arteriosus in cartilaginous fishes). These four chambers are located in series, and are almost invariably molded into an S-shape with the sinus venosus dorsal-most and the bulbus arteriosus ventral-most. The fish heart is considered a "venous" heart since it pumps only systemic venous blood. Depending upon species, the coronary system is either absent or rudimentary, but the thick ventricular walls are highly trabeculate and this presumably aids the exchange of nutrients and waste between cardiac muscle and the blood being pumped through the heart.

Air breathing fishes. The advent of airbreathing, an event that has evolved several times in fishes, is associated with various degrees of modification of the typical piscine cardiac pattern. A great variety of air breathing structures have either arisen *de novo* or have resulted from modification of existing structures intended for other functions. Not surprisingly, the arrangement of central vessels reflects the location of the airbreathing organ, and the extent to which gills have been retained. In almost all adult airbreathing fishes, however, the heart is little modified, and the oxygenated blood draining the air breathing organ and the deoxygenated blood draining the systemic tissues merge at a central venous site before entering the heart. The major divergence from this pattern is in the Dipnoi (lungfishes), where a true pulmonary venous circulation has arisen. Systemic and pulmonary venous return arrive at the heart separately, which for the first time provides a strong selection pressure for the evolution of structures and processes maintaining the separation of these separate streams of blood as they flow through the chambers of the heart. Indeed, in all three extant genera of lungfishes the atrium, ventricle and bulbus cordis show partial division. This trend is most striking in the South American lungfish *Lepidosiren*, where the atrium is divided into left and right sides by the pulmonalis fold, which is a partial septum arising from a deformation of the atrial wall produced by the overlying pulmonary vein. The ventricle is also partially divided by a vertical septum arising from the dorsal and ventral ventricular walls. Depending on the species of lungfish, the bulbus cordis also has rows of small conal valves, a more complex spiral fold, or complete division.

Amphibians. The heart of almost all adult amphibians is characterized by complete atrial division. Completely separated oxygenated and deoxygenated streams enter the left and right atria, respectively. Both atria in turn pump into a single ventricle where partial separation of these streams is thought to be aided by the deep ventricular trabeculae. These small pockets fill during diastole and disgorge during systole. The large, contractile conus arteriosus contains a complex spiral valve that maintains a highly effective functional separation of blood entering from the ventricle. Some urodele amphibians (*Cryptobranchus alleganiensis, Siren intermedia* and *Necturus maculosus*) show partial division of the ventricle by a vertical septum of variable prominence. Also, a whole family of salamanders (Plethodontidae) completely lack lungs as adults. Pulmonary arteries and veins are completely absent and the atrium is undivided.

Reptiles. Of the five vertebrate classes, reptiles show by far the greatest diversity in cardiac form and function. An unfortunate tendency of earlier studies on the heart of adult squamate reptiles

(lizards, snakes) was to take a mammaliocentric view that the reptile ventricle "suffered" an interventricular septal defect. In fact the squamate heart (and that of the crocodilians, discussed below) is a highly derived condition that provides reptiles with the ability to regulate the intracardiac shunt to their advantage, depending on respiratory needs. Essentially, the squamate heart has completely separate left and right atrial chambers. The ventricle is most appropriately viewed as having three distinct chambers or cava. Pulmonary venous return passes from the left atrium into the cavum arteriosum. This chamber has no direct arterial output, and blood flows from it around a muscular septum into the cavum venosum. The cavum venosum is the main systemic pump, ejecting into a left and right aortic arch. The right margin of the cavum venosum also receives blood from the right atrium. Most of this blood flows around a vertical septum into the cavum pulmonale. This chamber, which receives blood only by way of the cavum venosum, ejects blood into the pulmonary artery. In most squamates the ventricle acts a single pressure pump. In the large varanid lizards, which are an unusual taxa in several respects, the cava venosum and pulmonale become functionally separated during systole, and the systemic circulation is consequently perfused at a much higher pressure than is the pulmonary circulation. Although the potential exists for severe intracardiac mixing of left and right atrial blood, the actual degree of intracardiac shunt is now known to be closely regulated. Very little intracardiac shunting occurs when the animal is breathing. However, a prolonged period of apnea can lead to a highly efficient, energy saving pulmonary bypass (large net right-to-left shunt), a feat unobtainable in diving birds or mammals.

Crocodilian reptiles (crocodiles, alligators, caimans) are unique among reptiles in having a heart with four anatomically separate chambers. Although the two ventricles are completely separated, adult crocodilians retain a left aorta arising from the right ventricle. This aorta fuses distally with the right aorta, and provides a potential route for a right-to-left shunt. Although the explanation of the hemodynamics of this heart is beyond the scope of this paper, during periods of active lung ventilation all right ventricular blood enters the pulmonary artery rather than the left aorta, and the heart operates essentially as a bird or mammal heart. During prolonged periods of diving apnea, however, blood is shunted away from the lungs and into the left aorta (and on to the systemic circulation as a right-to-left shunt), thereby saving energy by not perfusing a lung that is nonventilated or storing significant amounts of oxygen.

Birds. At the gross anatomical and physiological level, the hearts of adult birds and mammals are identical, and constitute a distinct left pump perfusing the lungs at low pressure, and a right pump perfusing the body at high pressure. However, it is worth noting that Goodrich[11], in a detailed examination of the anatomy of vertebrate hearts, comments "...The resemblances of the "four-chambered" avian heart to that of the mammal are superficial and misleading, and the clue to its structure and origin must be sought in the crocodilian heart", a view which has received more recent support[7,11].

Clearly there is great structural and functional diversity among the hearts of adult vertebrates. However, all embryos start out with fusion of the paired endocardial tubes forming a single tubular peristaltic pump that then undergoes S-folding and eventually, septation. Many unanswered questions surround the hemodynamics of these embryonic hearts, especially the extent to which they show the diversity of form and function that is known and well understood for the adult form.

We now turn to consideration of heart development in non-mammalian vertebrates, to illustrate both common and distinctive features and how they resemble the adult forms of other lower vertebrates.

Development of cardiac form and function

The heart is unusual among organs in showing a high degree of developmental plasticity — that is, it is shaped during development by the very blood flow that it generates. In turn, its emerging form determines is function in the embryo and then ultimately in the adult. The development of such a system, with form and function as codeterminants, obviously must respond appropriately to both basic genetic instructions as well as hemodynamic influences that potentially alter its form (*e.g.* blood volume, blood pressure and peripheral resistance). Can the study of diversity among vertebrate embryos give us insights on how cardiac form and function is determined generally, and provide us with a set of common "rules" by which vertebrate hearts form?

What is the function of the embryonic heart? A hallmark of vertebrate ontogeny is the development of the cardiovascular system as the first functioning, regulated organ system. Rhythmic pulsations of the heart are visible very early in development in all vertebrate groups studied to date. In the skate (*Raja erinacea*) the heart begins to beat about 20% through the 5 month gestation[12]. In the direct-developing anuran amphibian (*Eleutherodactylus coqui*, which hatches as a miniature frog from its

egg, heart beats are first visible at stage 5, about 30% through the approximately 3 week development period[13]. In the well-studied embryonic chick system, the heart begins to beat on day 3 (14% of a 21 day incubation).

However, this heart activity in the earliest embryos may not be serving its adult function (or indeed, any transport function). Certainly, the oxygen transport role of the circulation is not critical to early embryonic development and growth. Exposure to carbon monoxide at levels sufficient to block Hb-O_2 binding has little apparent effect on early embryos of fish[14], amphibians (our unpublished observations), and birds[15]. An extreme case is seen in a "cardiac lethal" mutant of the salamander *Ambystoma tigrinum*, where a normal heart is formed, but never begins to beat. Surprisingly, even thus handicapped, these animals hatch and then swim for several days before dying[16]. Evidently, diffusion combined with a small amount of convection caused by myotome contractions and relaxations is adequate to transport materials between deep tissues and the environment surrounding the animal.

Although gas transport functions of the early embryonic circulation appear minimal, we should not assume that early cardiac activity is without purpose. For example, even the small amount of blood flow generated during the initiation of cardiac activity will move dissolved materials between tissues regardless of whether transport is the primary goal. Early blood flow may also be important in angiogenesis and early cardiac development, given the well-established link between blood flow and cardiovascular morphogenesis[17].

Certainly, in later embryos experiencing burgeoning body mass, the establishment of a functional cardiovascular system becomes pivotal to future development. Nutrient supply and waste removal required for subsequent organogenesis can occur by convective processes in addition to purely diffusive processes, with a corresponding dramatic increase in efficiency of the exchange of materials within the embryo.

Ontogeny of heart rate. For years, comparative physiologists have been evaluating heart rate during development in many species. Observations are numerous, explanations few. An increase in heart rate very early in embryonic life appears to be nearly universal in all species[12,14,18-20]. An initial slow heart rate may be beneficial due to limitations in passive ventricular filling[21], but this remains to be tested in other embryos. Regional differences in electrophysiological and membrane characteristics like those in the adult heart are present in embryos and larvae, and the early rise in heart rate may be due to a pacemaker role provided by succeeding regions of the embryonic heart. That is, the presumptive ventricle begins to beat earliest and slowest, followed by the atria and finally, with the highest frequency, by the sinus venosus. There is also an increase in heart rate with maturity within each portion of the heart, as evident from the activity of explanted portions. Finally, nerves and hormones alter heart rate. As early as 1907, Babak and Boucek[22] used frog tadpoles to determine how early in development handling the gut would slow heart rate. Copenhaver[23] found that the heart of salamander embryos responded to vagal stimulation (due to pressure on the gills) almost as soon as the heart was innervated. This was early evidence that receptors are present and functional as soon as cardiac nerves reach the heart. More recent studies have verified the early appearance of both cholinergic and adrenergic receptors in heart tissue, and the presence of functional cardiac innervation[24].

An overview of current data on heart rate development allows for few generalizations. Patterns of heart rate development are as varied as the animals from which they are obtained (Figure 1). In fish, heart rate typically rises immediately after hatching and falls to a stable level in the weeks that follow[12,14,30-31]. Species differences in heart rate during development may be the most documented in amphibians. Many species have been studied, and there appears to be no common pattern[32]. Heart rate in developing bullfrogs (*R catesbeiana*) is precisely correlated with body mass (W Burggren, unpublished). Thus, at least in this species, organogenesis *per se* has a minimal effect on heart rate. In another anuran amphibian, *Eleutherodactylus coqui*, heart rate rises during development despite little change in embryo mass, then falls as body mass increases further. In *Alligator*, the only reptile measured to date, heart rate falls sharply at first, and then more gradually before hatching.

The pattern of heart rate change in birds seems to be most closely associated with whether the species is altricial or precocial. In precocial birds, which require little parental care after hatching, embryonic heart rate rises in a hyperbolic fashion. In some species (*e.g.* chicken) it then falls toward the end of incubation. Heart rate in the embryonic duck and goose similarly drops before external pipping. Turkey embryos show a similar pattern to chickens, but there is no decline in the last day or two as in the chicken (see reference 33 for references). Altricial birds, which require extensive parental attention, show a more or less progressive increase in embryonic heart rate during the last half of development, with no decline near pipping[33].

Mammals similarly show a variety of heart rate

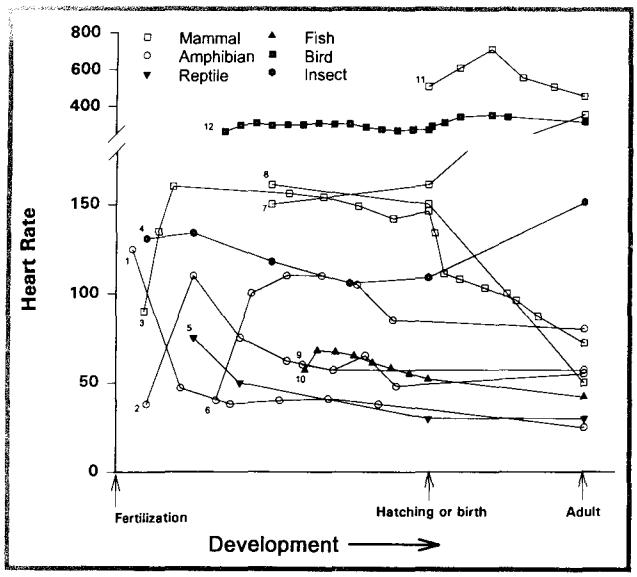

Figure 1. *Changes in resting heart rate as a function of development in vertebrates. Data have been "normalized" with respect to development to facilitate comparison between species with different lengths and patterns of development. Species key is as follows: 1) bullfrog, Rana catesbeiana[4]; 2) clawed toad, Xenopus laevis[36]; 3) human, Homo sapiens,[26]; 4) mosquito, Anopheles quadrimaculatus[20]; 5) alligator, Alligator mississippiensis (S Warburton, unpublished); 6) coqui, Eleutherodactylus coqui[13]; 7) domestic dog, Canis familiaris[26]; 8) cow, Bos taurus[26]; 9) rainbow trout, Onchorhyncus mykiss,[14]; 10) paradoxical frog, Pseudis paradoxsus[27]; 11) house mouse, Mus musculus[28]; 12) chicken, Gallus domesticus (Howe, Burggren, and Warburton, unpublished, and see references 26 and 29).*

changes during development. In humans, heart rate rises sharply up to about day 50 after conception, and then levels off in the fetus before declining again after birth[25], a pattern that is almost the opposite of that occurring in the domestic dog.

In summary, heart rate changes provide little information by themselves, given the multitude of factors that affect this variable. Changes in membrane permeability, the complex and possibly differential rate of development of cholinergic and adrenergic neural control, the onset of hormonal control, and developmental changes in the pacemaker regions may all may contribute to the complex patterns observed.

Ontogeny of blood pressure and cardiac output. Advances in technology now permit hemodynamic measurement not previously possible in embryos. Servo-null blood pressure systems, pulsed Doppler and laser blood flow systems, and microvideo assessment of cardiac output have been used to determine central hemodynamics in a variety of embryonic and larval vertebrates. In all vertebrates thus far studied, blood pressure rises many-fold during development. In the skate, mean arterial pressure rises from 1 mmHg with the initiation of heart beat to about 13 mmHg towards the end of incubation[12]. In the bullfrog, *Rana catesbeiana*, mean arterial pressure determined in fall/winter larvae rises linearly with larval body mass, increasing from 1 mmHg to about 8 mmHg while systolic pressure increases from 2 mmHg to 12 mmHg (systolic pressure = 3.98 body mass$^{0.61}$)[18]. There is a strong seasonal effect, however, with larvae measured in spring/summer at the same temperature as the fall/winter larvae yielding much higher pressures (systolic pressure = 10 body mass$^{0.15}$). The physiological basis for this seasonal effect on blood pressure in *Rana catesbeiana* (which can take three years to metamorphose in high latitudes) is unknown, but warrants closer scrutiny because so many lower vertebrates experience a similar protracted larval development over several seasons. Blood pressure also increases in bird embryos, beginning with near-zero pressure as the ventricle begins to beat and rising to 25 mmHg near hatching[20,34].

The rapid increase in blood pressure in vertebrate embryos occurs in the face of equally rapid declines in peripheral resistance. In the chick embryo, peripheral resistance decreases by 25-fold over several days while arterial pressure is increasing[35]. In larvae of the African clawed toad *Xenopus laevis*, peripheral resistance decreases 1000-fold from hatching at about 1 mg body mass to 1 gram larvae[36]. In many cases, embryonic or larval development entails the formation of abundant exchange vessels (*e.g.* the placenta, the chorioallantois, the gills and even the skin). These vessels add parallel resistances, which would be expected to decrease total peripheral resistance. (If simple addition of parallel resistances were to occur in an adult, blood pressure would fall unless cardiac output increased).

The mechanisms affecting and controlling blood pressure in early larval development are unknown. If Frank-Starling relationships are functioning, then preload must increase while the total vascular volume is presumably also increasing. Pelster and Burggren[18] have shown increased preload (although minimal) during development in bullfrog larvae, as has Clark in chick embryos[34]. In chick embryos, cardiac hypertrophy can result from increased ventricular pressure[37] as occurs in adult animals (*e.g.* right heart hypertrophy from pulmonary hypertension). Hemodynamics in the embryo is far more difficult to assess than in the adult, however, because many factors are changing rapidly. For example, the heart is growing while blood pressure is rising, even against a continually

decreasing resistance. At the same time, blood flow increases enormously during development in both chick[35] and amphibian[38] embryos, and appears to match equally dramatic increases in body mass and its attendant vasculature. Again, which of the above parameters — blood flow, blood pressure, blood volume, vascular architecture, etc. — is controlled, remains to be discovered.

Physiological changes at hatching/birth. There are many examples of changes in structure and process in nonmammalian hearts at hatching, birth or metamorphosis that parallel those at birth in mammals. For example, just as the ductus arteriosus in mammals closes within a few days of birth, the ductus arteriosus (probably homologous to the mammalian ductus arteriosus) in a salamander constricts with metamorphosis (reference 39, cited in 40). This constriction causes a high percentage of pulmonary blood flow to pass thorough a "pulmonary plexus" of uncertain origin and function[40]. As in mammalian embryos, chick embryos shunt blood away from the lungs into the systemic circulation through a ductus arteriosus[34]. This vessel closes after hatching. Chicks also exhibit a right-to-left shunt between the atria, not through a foramen ovale, but with numerous smaller perforations[41]. Finally, blood flowing to the gas exchange organs (either placenta or chorioallantois) arises from the same arterial blood supply that perfuses the lower half of the embryo. As in mammals, birth or hatching represents a challenge. Among other aspects, it requires reorganization (and often anatomical simplification) of a cardiovascular system that in the embryo is topologically specialized for gas exchange with chorioallantoic/placental structures. Using microspheres, Rahn *et al* .[42] demonstrated an interruption of flow to the chorioallantoic circulation concomitant with internal pipping (*i.e.* beginning of lung function) and increasing flow to the lungs. Hemodynamic changes in reptile embryos, including those associated with hatching or birth, have not been investigated. In *Alligator mississippiensis*, however, there is an apparent increase in chorioallantoic vascular reactivity near hatching that may indicate impending vascular constriction and eventual shutdown of chorioallantoic vessels (S Warburton, unpublished). In some reptile species, the ductus arteriosus does not close following hatching. The relative anatomical positions of heart, ductus arteriosus and carotids in a given species may be the determining factor in maintaining a patent ductus[43]. Perhaps such a location favors continued flow through a patent ductus, resulting in its retention. If true, this has intriguing implications for flow/pressure involvement in retention of embryonic vessels. The lack of knowledge of hemodynamic changes in reptilian embryos should provoke investigation, especially given the diversity of adult cardiac morphology and function.

Changes in cardiac performance associated with hatching have not been extensively examined in any lower vertebrate, but the response of the heart to this probable stress deserves more attention. In the neotropical frog *Eleutherodactylus coqui* heart rate is elevated by about 20-30% within ten minutes of hatching, returning to prehatch values after several hours[13]. This suggests that, while the act of hatching is associated with a presumed greater cardiac output, there does not seem to be a fundamental change in heart rate associated with hatching and the transition to air breathing.

Development and hypoxia tolerance. Hearts of newly hatched fishes are more tolerant of hypoxia than are adults. Rainbow trout exposed to 5% carbon monoxide survive less than 30 minutes as adults, whereas larvae survive over 3 hours[14]. While very early amphibian embryos are highly resistant to anoxia, hypoxic sensitivity quickly develops[44]. At least in *Xenopus laevis*, the larvae are less tolerant of hypoxia than adults (D Hastings, personal communication). Hypoxic tolerance, of course, depends on many physiological factors in addition to cardiovascular performance. Direct investigation of the developmental changes in cardiocyte metabolism *in vivo* should help determine potential cardiac limitations on metabolism.

The occurrence of embryonic hypoxemia in mammals, birds and reptiles could suggest that it is a condition dictated by design constraints. However, hypoxia may play a beneficial role in embryonic development. Low arterial PO_2 in mammalian embryos is viewed as pathological, arising from imperfect placental gas exchange. However, in chick embryos (and possibly reptile embryos), blood leaving the gas exchange organ is highly saturated with oxygen. The hypoxemia results from central venous admixture of oxygenated blood with systemic deoxygenated venous blood. A marked desaturation results. The concept of "evolutionary feasibility" submits that a hypothetical morphological system could have evolved if selection pressures have led to a similar system in extant animals[45]. Using this concept, it is entirely possible that a circulatory system could provide the equivalent of the adult separation of flow through the heart, which would shunt "pulmonary artery" blood to the chorioallantois. Certainly the great variability in central vessels seen in adults of various vertebrate taxa would suggest that such a system could have evolved if suitable selection pressures existed.

Why, then, is embryonic hypoxemia so prevalent

among vertebrates? One possibility is that adult oxygen levels may be dangerous to a developing embryo, especially if the embryo has not yet developed enzymes for scavenging oxygen radicals[46]. Another possibility is that maintaining low tissue oxygen may stimulate angiogenesis, which must keep pace with the explosive growth in embryonic tissues. Certainly, incubation of chick embryos in moderate hypoxia results in greatly increased vascular conductivity of maximally dilated vascular beds, suggesting enhanced vascularization[47]. Both birds and reptiles have little ability to develop aerobic capacity from activity while in the egg, so hypoxemia *in ovo* may lead to the high level of vascular and mitochondrial density required to support immediate post-hatch aerobic activity. In other words, embryonic hypoxemia may provide for embryos the stimulus for expanded aerobic scope that in adults is provided by exercise.

Critical windows in development. A central tenet in developmental biology is the existence of "critical windows" — periods when a particular organ or organ system is especially vulnerable to perturbing effects of the environment. The heart and cardiovascular system is no exception, but the extent to which external events such as hypoxia shape cardiac development in vertebrate embryos is largely unexplored. Establishing critical windows can provide important insights into developmental processes that might otherwise remain obscure. For example, our understanding of development of the palate has been advanced by the use of teratogens applied to alligators in critical windows. Development of the lower jaw is prevented, and the developing animal subsequently exhibits a condition that mimics human cleft palate. A similar approach using known cardiovascular teratogens (*e.g.* catecholamines) has long been employed[48-49]. However, a systematic approach to establishing cardiovascular critical windows across a broad spectrum of taxa may reveal developmental "rules" that constrain ontogeny — for example, must blood pressure rise during specific periods of angiogenesis or are these events merely coincidentally related? Also unknown at this time is whether critical windows can be shifted forward or backward in development or time.

What we can learn from developmental studies of lower vertebrates?

Cardiology and comparative methods. As adults, different vertebrate taxa have highly distinctive cardiovascular systems — the uniqueness of their morphological patterns is often evident even in quite early embryonic stages. Yet, despite these morphological differences, there appears to be a basic suite of physiological commonalties. Stated another way, very immature stages of different vertebrates are physiologically far more similar than different. Heart rate, central arterial blood pressure and cardiac output rise while peripheral resistance falls during the early phases of development in all vertebrate classes examined to date.

By employing a comparative approach to embryonic cardiology, in which similarities and differences in different vertebrate taxa are compared and contrasted, we will come to understand: 1) the basic molecular, cellular, tissue and organ events that lead to these similarities in the earliest embryos; and 2) the ontogenetic events and mechanisms that then lead to their divergence with further embryonic development. However, the most valid deployment of comparative methodologies consists of more than simply comparing two different species. If a goal is to understand the evolutionary origins of a structure or process, then close attention to systematics is necessary[45]. At the broadest level, reptiles have a closer phylogenetic relationship to birds than birds do to mammals. Consequently, carefully structured studies that compare, for example, the hearts of crocodiles and turtles with those of pigeons and chickens will, in the long term, be more informative than ones that simply compare directly the hearts of frogs with those of mammals. Thus, experiments should be based on solid phylogenetics rather than convenience of working with a particular embryonic system.

Productive areas for future research. Several aspects of cardiac development in lower vertebrates remain enigmatic, and demand further investigation:

1) Although a Frank-Starling relationship has been described in the heart of chick embryos[50] and larval bullfrogs[19], developmental changes in intrinsic regulatory mechanisms are unknown. The relative importance of intrinsic versus extrinsic (neural, hormonal) mechanisms in regulating embryonic cardiac output is also unknown, and needs to be elucidated.

2) Among fishes, amphibians, and reptiles can be found examples of closely related species that are viviparous (giving live birth), oviparous (egg laying) and ovoviviparous (laying eggs that hatch and develop in the ovary, before being birthed). Viviparous and ovoviviparous embryos depend to varying extents upon extraembryonic structures, analogous to the mammalian placenta, for exchange of respiratory gases, nutrients and waste. The regulation of blood flow through these structures, and the impact that they may have on the central hemodynamics of the embryo are unknown.

3) The identification of "critical windows" in cardiovascular development is largely unexplored in vertebrate embryos. The influence both of naturally occurring environmental stimuli (*e.g.* hypoxia, pH, temperature) and experimental stimuli (*e.g.* catecholamines and other potential teratogens) on cardiac form and function deserves attention, especially if we are to attempt to understand developmental trajectories and the molecular and cellular events that control them.

4) Experimental manipulation of normal events will reveal the potential plasticity of the cardiovascular system. The degree of developmental plasticity may determine the ability of the embryo to adapt to its environment which may be particularly important in free-living larvae. In addition to elucidating the extent of developmental plasticity *per se*, it is also important to determine the ability of the embryo to adapt in its immediate environment without compromising the future adult system.

5) The immediate environment in which the heart develops may have an important influence on cardiac differentiation and development of cardiac function. Hearts from the cardiac lethal salamander mutants mentioned earlier beat when explanted to a normally developing, non-mutant embryo, while genetically normal hearts cease to function when explanted into a mutant[16]. These data suggest that the lethal mutation was at a gene that controlled the immediate (ionic?) environment of the heart. Further studies employing genetic variants and modifications to tissue culture environments are needed to help understand cardiac differentiation and the beginnings of blood pumping.

6) Intraspecific variation in the pattern of change in heart rate during development occurs in all species. However, recent experiments on altricial bird embryos indicates that for a given species the developmental patterns in heart rate are much more similar between siblings than between nonsiblings[33]. This suggests either that specific patterns of physiological development are heritable traits, or that physiological processes in the embryo are subject to a significant maternal affect (*i.e.* "cytoplasmic inheritance"). Whatever the origin of this sibling effect, data from developmental studies of physiology need to be collected in a manner that can help solve this "nature/nuture" argument.

7) The large, invariable increase in arterial blood pressure during development may entail increased extravasation of fluid from alteration in the Starling forces at capillaries. Information is needed on the other variables of Starling's equations, *i.e.* intra- and extra-vascular oncotic pressure, and how these affect lymph formation and how that fluid is handled by the development of the lymphatic system.

These are but a few questions dealing with fundamental aspects of the development of cardiac form and function that remain poorly understood.

Address for correspondence:

Dr. Warren Burggren

Department of Biological Sciences
University of Nevada, Las Vegas

4505 Maryland Parkway
Las Vegas NV 89154
USA

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