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Evolution of Cardiovascular Systems and Their Endothelial Linings

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The evolution of large, multicellular, complex metazoans from their simple unicellular animal ancestors comprises a rich history covering many hundreds of millions of years. Pieced together from fossil remains, DNA analysis, and comparative morphological studies of extant animals, biologists have described a staggering array of adaptations that have arisen (and in some cases been lost and arisen again) through the course of animal evolution. Describing the specific evolutionary pathways from unicellular organisms to human is, of course, well beyond the scope of this chapter. Rather, in this chapter, we focus specifically on the general evolutionary pathway leading to the cardiovascular systems of extant animals, especially highlighting the general principles that continue to shape cardiovascular form and function to this day. In doing so, we intend to create a backdrop and context for other chapters in this book that present more specific information on the vascular endothelium in specific groups of animals (e.g., fish, amphibians, reptiles, birds, and mammals).

Before delving into cardiovascular systems and their evolution, we first must address a semantic issue regarding our use of the term *endothelium*. The invertebrate literature is replete with discussions of "endothelium" – whether it exists, whether it is discontinuous or continuous, what functions it serves, and the like. Much less often, the vascular lining of blood spaces (where it occurs) in invertebrates is described more generically as a "vascular cellular lining." Of note, the position taken in this book is that "true" endothelium is found only in vertebrates, and it has distinguishing characteristics, such as being derived from a lateral plate mesoderm, possessing caveolae and Weibel-Palade bodies, and more. Although the vascular cellular linings in some invertebrates are analogous to the endothelium of vertebrates, these linings are not necessarily homologous. Consequently, in this chapter we carefully distinguish between the two tissues, although in fact their functions may overlap extensively. Future research directed specifically at a comparison and contrast of the cells that line vascular spaces is much anticipated.

Now, let us consider why animals have cardiovascular systems – lined or unlined.

WHY DID CARDIOVASCULAR SYSTEMS EVOLVE?

A key feature of almost all complex metazoans is that they have an internal system for the convection of body fluids – a cardiovascular system. Although enormous species diversity exists in cardiovascular form and function, fundamental components include a fluid pump (or pumps) that propel often specialized body fluids into a series of distribution, exchange, and collection vessels. Numerous variations occur on this basic circulatory plan of heart and vessels, ranging from the relatively low pressure, "open" circulation of many invertebrates to the typically higher pressure, closed system of vertebrates. Yet, the almost ubiquitous presence of a circulatory pumping of fluid through vessels begs the following questions: "*Why did cardiovascular systems evolve?*" The answer, as is often the case in biology, is to be found in chemistry and physics.

A feature common to both the earliest single-celled animals and today's 100-metric ton blue whale is that they both ultimately depend on diffusion to supply oxygen (O_2) and nutrients to, and remove carbon dioxide (CO_2) and wastes from, their intracellular sites for oxidative metabolism. Perhaps because physiologists often study diffusion over the ultrathin boundaries between blood and gas in the alveolus, or blood and tubular fluid in the nephron, we tend to attribute near-magical properties to the process of diffusion. In reality, diffusion is a highly imperfect physico-chemical process for the bulk movement of materiel. Although energetically inexpensive, diffusion is also a very slow process; a diffusing molecule takes a very long time to move by diffusion alone. Indeed, it is precisely because diffusion is so slow that the ultrathin boundaries of the alveolus, nephron, and other structures have evolved to reduce transmembranous diffusion times and thus maximize the bulk diffusion of molecules, ions, and other particles.

The transmembranous diffusion of molecules depends on several factors including the pathway length for diffusion, surface area across which diffusion occurs, molecular weight of the diffusion molecule, solubility of the substrate

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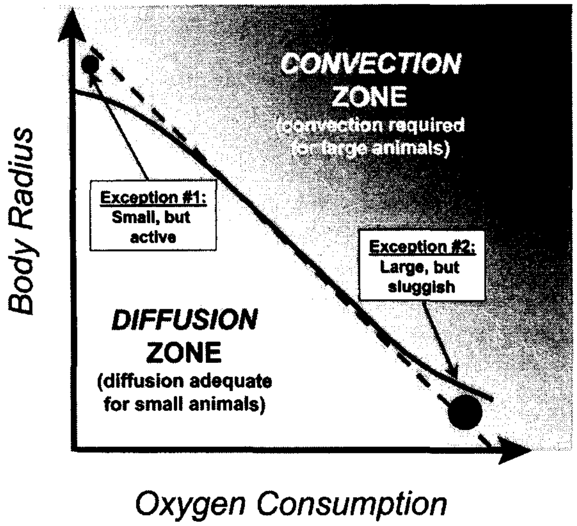

Figure 3.1. The role of diffusion and convection in the O_2 consumption of animals of different body size. Generally, small animals can thrive on diffusion alone, because the diffusion distances are small. The larger an animal's body radius, the more it depends on convection to support its O_2 consumption. However, very small yet active animals may require internal convection for delivery of O_2, whereas large but inactive animals may exist on diffusion alone.

for the diffusion molecule and, of course, the partial pressure or concentration gradient for the diffusion molecule. As a general rule of thumb, a hypothetical animal with a cylindrical shape, expressing a modest metabolic rate of about 100 μmol O_2/sec in a fully oxygenated environment, could survive on diffusion alone for its transport until it grew beyond a radius of about 2 mm (Figure 3.1) (1). Beyond this radius, diffusion alone cannot provide for substrate delivery and byproduct removal.[1] Some animals without internal circulations considerably exceed this size by having a very low metabolic rate, especially when combined with a large surface area-to-volume ratio–for example, the large, pancake-like marine Polyclads (Platyhelminthes) – as well as a modest internal convection system created by the muscular contractions of the animal's body wall during locomotion. Conversely, some animals with high metabolic rates, although near microscopic and thus possessing short diffusion distances, have evolved circulations for the internal transport of nutrients, wastes, and respiratory gases (e.g., small crustaceans) (see Figure 3.1).

Thus, the physico-chemical limitations of diffusive O_2 supply (together with the attendant limitations on the diffusion of CO_2, metabolic substrates, and nitrogenous and other wastes) have likely served as a strong selection pressure for the evolution of internal cardiovascular systems. Let us now consider the diversity of cardiovascular systems, beginning with relatively simple invertebrate systems and moving to the high-pressure systems used to perfuse large, complex vertebrate homeotherms.

CONVECTION OF INTERNAL BODY FLUIDS: DIVERSITY OF EVOLUTIONARY ADAPTATIONS

The mechanisms by which fluids are moved through a circulatory system evolved in loose association with both the complexity of the conduit system and particular species' metabolic demands. The advent of a "true" centralized muscular pump – that is, a heart that generates the pressure gradients used to move fluids (hemolymph or blood) through the circulatory system – is common in a variety of forms and to a broad array of animal taxa. Importantly, the evolutionary lineages of these pumps and their associated circulatory systems have multiple phylogenetic origins. Consequently, identifying homologous structures that cross phyla is difficult at best. It is, however, possible to identify analogous components that have arisen independently due to common selective pressures. Thus, distributed throughout the body and evident in a number of phyla are a variety of less familiar systems used to generate fluid pressures to facilitate blood movement and thus gas exchange.

INVERTEBRATE CIRCULATIONS

The evolution of the invertebrate cardiovascular system is not a continuum of homologous structures seen from taxa to taxa, but rather appears to have evolved independently in several phyla in response to limitations in diffusional gas exchange and high metabolic rates (for a thorough review of invertebrate cardiovascular systems see reference 2). Most invertebrates that have evolved a circulatory system do not show "vertebrate-like" circulatory complexity and lack sophisticated regulatory mechanisms. They also typically generate relatively low flows and pressure (Figure 3.2). Nonetheless, several examples occur in which invertebrates have evolved highly efficient, high-pressure, high-flow systems. These high-performance invertebrate cardiovascular systems show a number of convergent evolutionary[2] traits that are comparable to vertebrate taxa: muscular pumps capable of developing appropriate driving pressures and flows, cardiac regulatory mechanisms, a complex branching circulatory system and, of particular significance, a cell-lined vasculature, to list a few. A current misperception holds that the invertebrate cardiovascular system is sluggish, poorly regulated, and "open," wherein blood bathes the tissues directly as it moves through a system of ill-defined sinuses and/or lacunae, without an endothelial boundary (thus, in part explaining the use of the term *hemolymph* or a blood–lymph mixture versus "true" blood, which is

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[1] Interestingly, these same principles also apply to the sizes that tumors can achieve without additional circulation.

[2] *Convergent evolution* is the evolution of similar characteristics by taxonomically different organisms.

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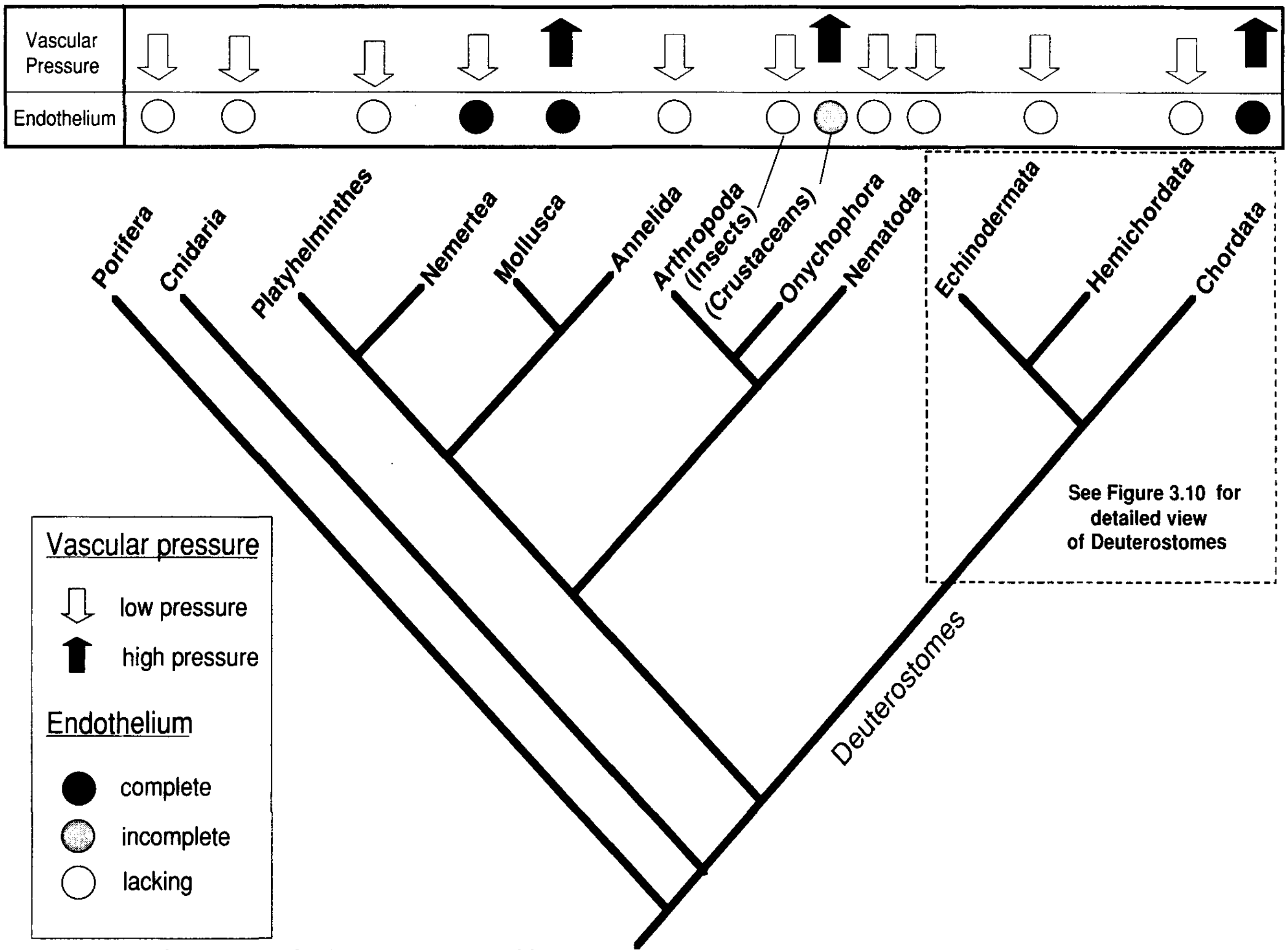

Figure 3.2. An evolutionary tree for showing the origins of the major invertebrate groupings and the appearance of the vertebrates. Also indicated is the presence of a complete or incomplete endothelial vascular lining. The arrows (*down* for low blood pressure, *up* for high pressure) indicate the taxon's blood pressure. (For comparison with vertebrates, see Figure 3.10).

typically contained within defined vessels and capillaries) (3). To the contrary, many invertebrates have evolved partially complete or even continuously cell-lined vessels (as revealed below). Why have both invertebrates and vertebrates coevolved these structures? What are the selective pressures behind the development of such systems? Several corollaries must be investigated, the most interesting being that animals having highly active lifestyles require an efficient pump and delivery system to maintain O_2 delivery (metabolic demand) and thus also require high-pressure, high-flow cardiovascular systems. In most examples of a high-pressure system, the vessels in that system are lined with an "endothelium." Why? The high vasculature pressures observed are the result of a well-developed muscular pump, with fluid contained within a robust arterial system followed by a capillary-like vascular bed to support gas exchange. Does a high-pressure cardiovascular system require the development of a cell-lined vasculature? A review of the literature in the area of invertebrate cardiovascular physiology, followed by a survey of the vertebrates, follows with a specific view toward supporting this hypothesis.

The Sponges: Internal Circulation of Seawater

One of the simplest yet more interesting pressure-generating systems is that of the phylum Porifera.[3] Sponges have circulatory systems for distributing seawater internally, the architecture of which incorporates a diffuse yet highly efficient pumping and circulatory system (4–5). A brief description of this circulatory system begins where seawater enters the sponge through in-current dermal pores (ostia) spaced throughout the exterior covering of epithelial-like flattened cells (the pinacoderm) (Figure 3.3). Internally, seawater then moves through the in-current canals and into a series of interconnected flagellated or radial chambers (the spongocoel or atrium), then

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[3] Porifera, a phylum within the kingdom Animalia, consists of approximately 5,000 species of sponges. Sponges are among the simplest animals. It is believed that they evolved from the first multicellular organisms. Sponges are filter feeders and have no true digestive tract; instead, they digest food within cells. They lack the gastrula stage during development, and the three cell layers present are not homologous to the body layers of most animals.

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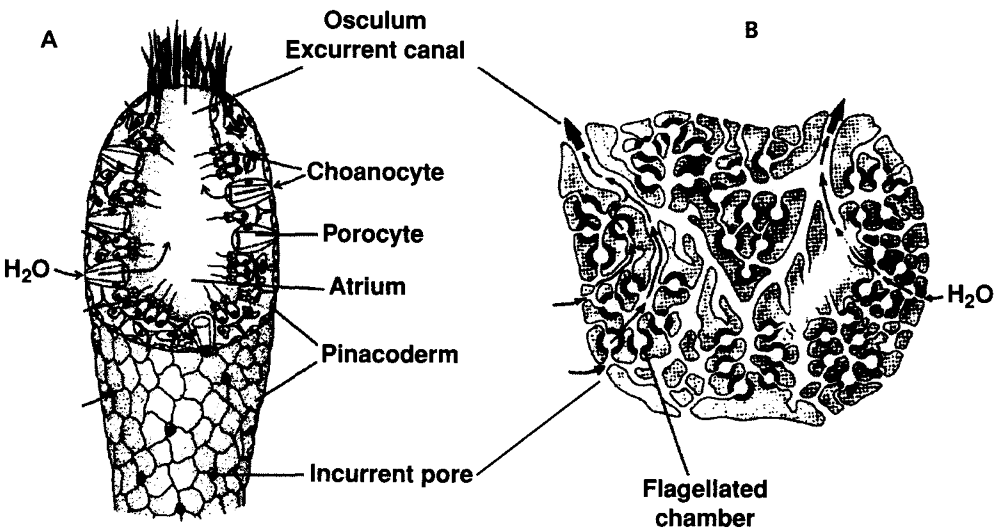

Figure 3.3. Circulatory system in sponges. The sponges are one of the simplest of multicellular animals, yet they have developed an internal circulatory system for the distribution of seawater. (**A**) The general architecture and cell types are illustrated in a simple Asconoid sponge, shown with arrows indicating the flow pattern of water into and out of the sponge. (**B**) The more complex pattern of water flow through a Leuconoid-type sponge. (Reproduced with permission from Ruppert EE, Barnes RD. *Invertebrate Zoology*, 6th ed. Fort Worth: Saunders, 1991.)

into major ex-current canals, and ultimately out of the sponge through the osculum (6–7).

Convective water movement through the sponge is generated by the beating action of the flagellated choanocytes that line the spongocoel or flagellated chambers. This noncentralized pump generates sufficient pressure differentials to move a substantial volume of water through the sponge, which facilitates not only filter feeding but also the more "typical" cardiovascular functions of O_2 uptake and waste removal (8–9). Although the historical view of sponge circulation was thought to be both simple and unregulated, it now appears that its pressure–flow dynamics are regulated through simple yet effective mechanisms. Pressures generated by flagellar beating may be started and stopped on a diurnal basis in some species (e.g., Demospongiae, Halichondria) and, in many sponges, changes in ambient water conditions may influence choanocyte flagellar activity. Moreover, flows and pressures can be regulated throughout the various chambers of the sponge by either opening or closing the in-current ostia or by increasing or decreasing the size of the osculum. This regulatory activity is accomplished through the action of specialized mesohyl cells (myocytes), which surround the osculum and act much like vascular smooth muscle cells with regard to contractility. Modulation of osculum radius has a tremendous effect on flow and pressure (10–12), just as modulation of arteriole diameter has profound effects in vertebrate cardiovascular systems.

The Cnidarian Gastrovascular Cavity

In many invertebrate phyla, "circulatory systems" consisting of muscular pumps generate pressure for the primary purpose of digestion, yet these invertebrates can and do use these systems secondarily for gas transport and waste removal (13–14). The internal pressure-generating systems of other invertebrates are used primarily for locomotion and are only secondarily adapted to generate pressures for the movement of internal body fluids or ingested food materials. Examples include the radial canals and gastrovascular cavity of the cnidarians[4] (Figure 3.4) and the highly convoluted gut of the Platyhelminthes (discussed in the next section). The primary function of these structures is to provide a large surface area for food digestion and absorption, as well as provide the structural elements for the animal's hydraulic skeleton. However, the disruption of internal boundary layers to maintain internal diffusion gradients could be an important positive functional consequence of internal convective fluid movement. In the cnidarians, the complex network of gastric pouches and blind-ending cavities of the gastrovascular system allows nutrient uptake by cells located some distance from the pharynx and "stomach" (14). Pressures in the gastrovascular cavity are generated to move food and fluids into and out of the gastrovascular system by the contraction of the locomotory muscles of the velum in more primitive species, by coronal muscles of the subumbrella in jellyfish and, in the more complex sea anemones, via the

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[4] Cnidaria, a phylum within kingdom Animalia, consists of hydras, jellyfish, sea anemones, and corals. This group has two key characteristics: The first is that all Cnidaria possess stinging or adhesive structures called *cnidae*, and each cnida resides in a cell called a *cnidocyte*; the most common example of which belongs to the nematocysts. The second characteristic most directly related to this chapter is that the mouth leads directly into the gastrovascular cavity (a digestive cavity). Because no anus is present, excess food and waste products must exit through the mouth; thus, the digestive system is considered incomplete. This gastrovascular cavity is filled with fluid that services internal cells, provides body support, and helps maintain structure.

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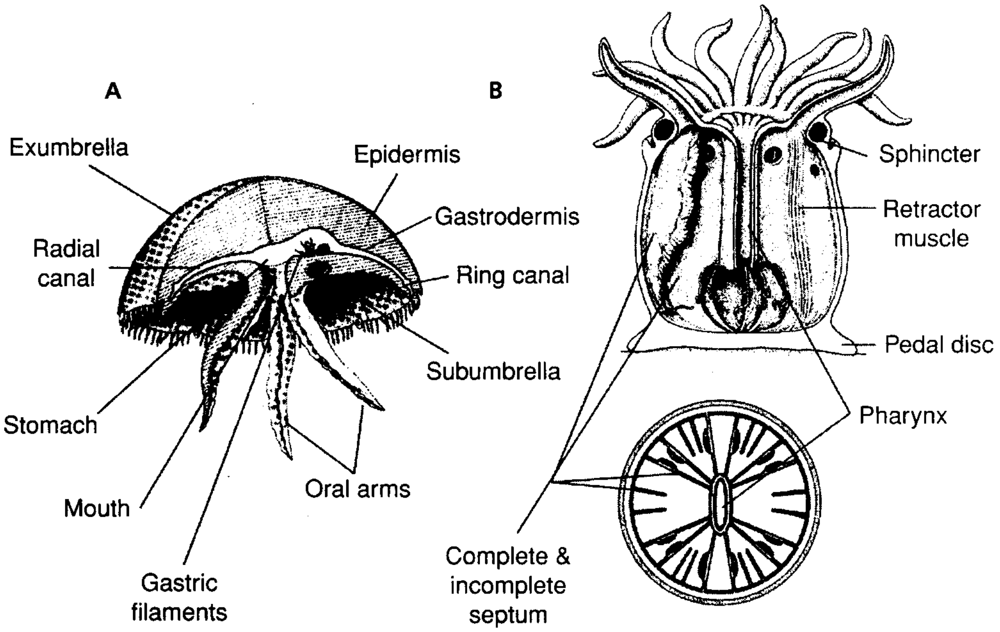

Figure 3.4. Circulatory system in cnidaria. The cnidaria are able to make dual use of their mouth, stomach, and gastrointestinal track for both ingestion and digestion but also to generate internal pressures and circulate fluids. (**A**) This panel illustrates an Aurelia jellyfish showing the mouth, stomach, gastrodermis, and radial canal. (**B**) The internal structure of a sea anemone shows a more complex series of internal compartments that are used to both increase the internal surface area for nutrient absorption as well as an internal pressure-generating system. (Reproduced with permission from Ruppert EE, Barnes RD. *Invertebrate Zoology*, 6th ed. Fort Worth: Saunders, 1991.)

digestive septal retractor muscles and pharynx (15–16). Given the size of some of the cnidarians and the complexity of the gastrovascular cavity, gas exchange is likely also facilitated by the pressure-driven movement of fluid throughout the body of these animals.

The Platyhelminth Gut

The phylum Platyhelminthes (flatworms),[5] and specifically the marine polyclads of the class Turbellaria, exhibit similarities to the cnidarians with the potential for their highly convoluted gut to facilitate both digestion and nutrient/gas exchange (Figure 3.5). Platyhelminths can be active predators that can grow to several centimeters in width and length. The larger size and active nature of these animals requires not only a well-developed gastrovascular system (gut) but also some form of internal fluid circulation to support nutrient, waste, and gas exchange, which may be seen in the pseudocoel of some turbellarians (17–20). It would appear that both the larger size of these animals and their more active behavior are in part possible because of their highly convoluted gut, which provides a very large surface area for the exchange of nutrients and other fluids.

Nemerteans: The First Cell-Lined Circulatory System

The first true cell-lined "circulatory system" is seen in the nemerteans or ribbon worms (21–22). The relatively large body size and robust muscular body wall of ribbon worms presents a significant barrier to diffusion and thus requires internal convective fluid flow through the action of a blood circulatory system that facilitates gas exchange, nutrient cycling, and waste removal (23). Additionally, a second, rhynchocoelan circulatory system is used to perfuse the head and proboscis and also functions as a hydrostat to evert the proboscis for feeding (24–26). In its simplest form, the nemertean circulation consists of two lateral vessels that parallel the gut and anastomose at the anterior and posterior ends of the animal. More complex nemertean circulatory systems (e.g., as in *Tubulanus* and *Amphiporus*) have an additional dorsal vessel with multiple connecting transverse vessels (Figure 3.6) (27).

The vessels of this system are surprisingly complex, with a complete vascular cell lining that, in some species, consists of myoepithelial cells with cilia facing into the vessel lumen (26–27). The vessels are surrounded by both circular and longitudinal muscle to facilitate vascular contractions that, in combination

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[5] The phylum Platyhelminthes includes all flatworms in the kingdom Animalia. Platyhelminthes are bilaterally symmetrical, having an anterior, posterior, dorsal, and ventral end and two seemingly identical lateral surfaces. However, flatworms lack any type of body cavity or fluid-filled space located between the digestive tract and the body wall. In flatworms, this area is filled with tissue. Flatworms have primitive brains, comprised of clusters of nervous tissue and two long nerve cords that run the length of the body. These cords branch to form small nerves running throughout the body. Another hallmark of flatworms is the lack of a complete digestive tract. Flatworms take in food and excrete waste from one single opening.

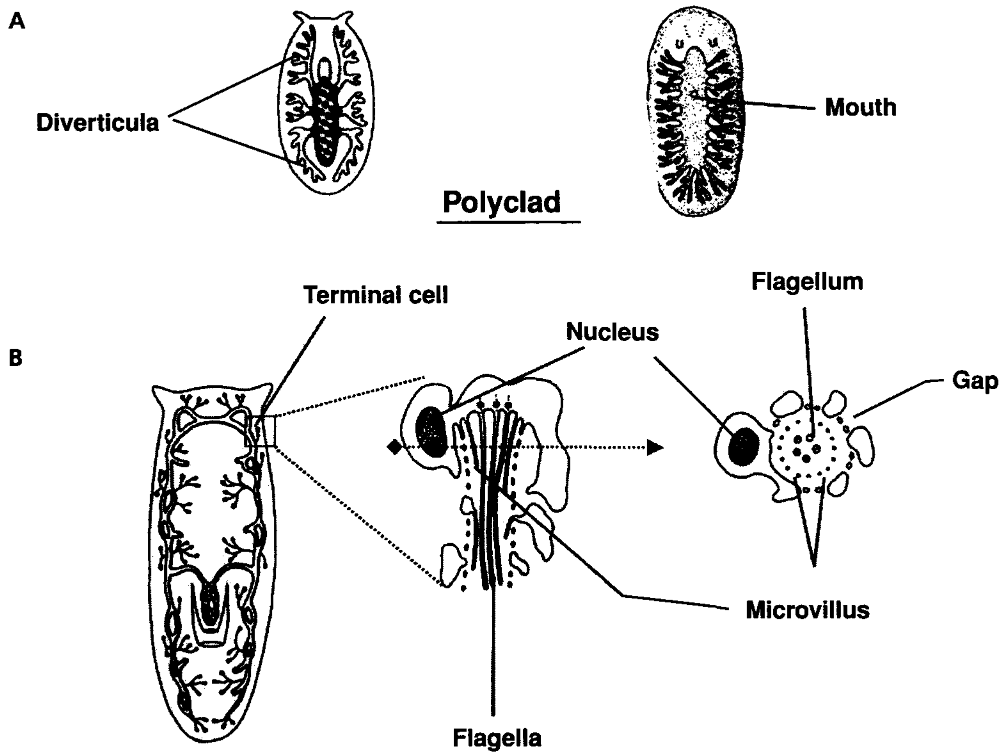

Figure 3.5. Circulatory system in platyhelminthes. The gut of the platyhelminths can show extensive diverticula, as is the case in the polyclads. These diverticula, which are small and vessel-like, serve to increase the absorptive surface area and distribute nutrients throughout the animal (**A**). An extensive network of complex anastomosing ducts interconnect the protonephridia (primitive renal structures) of the Turbellaria. The terminal cells of the protonephridia end within the tissues, where nitrogenous waste as well as other fluids are accumulated and then transported to exterior pores via ciliary action (**B**). (Reproduced with permission from Ruppert EE, Barnes RD. *Invertebrate Zoology*, 6th ed. Fort Worth: Saunders, 1991.)

with contractions of the body wall, provide the pressures to move blood within the circulatory system. The presence of a vascular cell lining is unique at this phylogenetic level and complexity within invertebrates, and it closely resembles the coelomic lining of other coelomate invertebrates. Thus, the nemertean circulatory system is proposed to have its evolutionary origins as specialized coelomic channels that have become secondarily adapted to perform circulatory functions. The myoepithelial differentiation of these coelomic cells has given rise to the endothelial lining (23,28–29). Debate exists as to the functional role of this "endothelium" or, more correctly stated, "vascular cell lining" with regard to its permeability, diffusional characteristics, and functional impact on hemodynamics, none of which has been investigated (29). Equally interesting is the tight integration between the nemertean circulatory system and the protonephridia of their excretory system. It would appear that, in some species, the protonephridia take advantage of significant circulatory pressures to aid in nephridial filtration for purposes of osmoregulation, and potentially, nitrogen excretion (29). An analogous situation exists in some platyhelminths, in which a proteonephridial system is integrated with the fluid-filled spaces of the pseudocoel. In the nemerteans, however, these two systems appear to be more functionally linked, with circulatory pressures driving filtration.

The Molluscan Cardiovascular System: High Efficiency Muscular Pumps

With the exception of a fluid circulation within the pseudocoel of a variety of coelomate worms (four minor phyla comprising fewer than 600 species – the Sipuncula, Echiura, Pogonophora, and Vestimentifera) and the lacunar system found in the epidermis of the acanthocephalans, which are not cell-lined systems and do not appear to take on a significant circulatory role in terms of pressure development or flow, few examples of circulatory systems and no examples of centralized pumps ("hearts") are seen until the advent of the molluscs[6] (23,30). Members of this phylum have developed extensive circulatory networks, with highly efficient centralized pumps (true hearts,

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[6] The phylum Mollusca is made up of over 150,000 diverse species. All molluscs have a muscular foot used for locomotion, as well as a mantle, an outgrowth that covers the animal. Molluscs have a coelom, which is developed from solid cell masses. Thus, all species in this phylum are protosomes. All organs are suspended in mesentery tissue within this mesodermic coelom, between the outer covering (ectoderm) and the digestive tube (endoderm) of the animal. The phylum Mollusca includes the following classes: Aplacophora (solenogasters), Monoplacophora, Polyplacophora (chitons), Gastropoda (snails and slugs), Bivalvia (clams and oysters), Scaphopoda (tusk shells), and Cephalopoda (nautilus, squids, cuttlefish, and octopi).

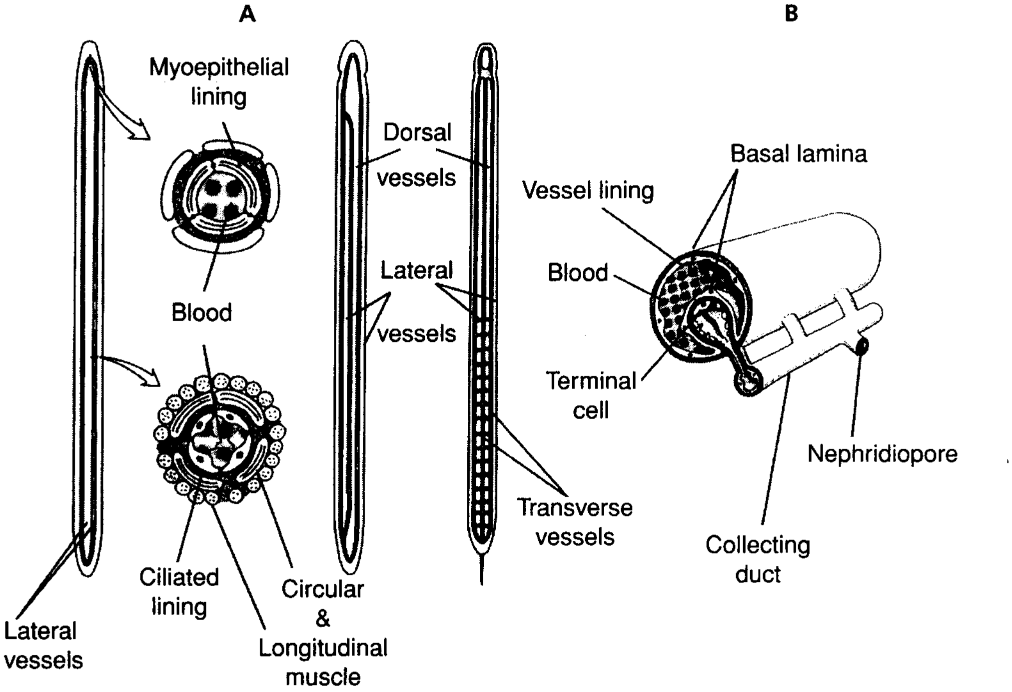

Figure 3.6. Circulatory system in nemerteans. The nemerteans show the first endothelial-lined circulatory system (**A**), which not only facilitates the movement of blood to promote gas exchange but also is integrated tightly with the excretory systems (**B**), which are pressure-dependent. (Reproduced with permission from Ruppert EE, Barnes RD. *Invertebrate Zoology*, 6th ed. Fort Worth: Saunders, 1991; and Turbeville JM. Nemertinea. In: Harrison FW, Bogitsh BJ, eds. *Microscopic Anatomy of Invertebrates*. New York: Wiley-Liss, 1991.)

composed of cardiomyocytes and capable of generating pressures and flows comparable to some lower vertebrates); these hearts function in an integrated fashion with a variety of other physiological systems (Figure 3.7). Moreover, the most complex molluscs (the highly active cephalopods) have evolved a cell-lined vascular system able to generate pressures and generally perform at a level comparable to some of the simpler vertebrates (3,22,29).

The generalized molluscan body plan has been extensively modified in both form and function to allow this group of animals great adaptive radiation (23,31–33). With specific regard to the heart and circulatory system, the typical mollusc (monoplacophorans and cephalopoda) has evolved a robust muscular heart consisting of a ventricle supplied by two atria or auricles, which drain the gills and sit within the coelomic cavity (the pericardial chamber) (34). The circulatory system is functionally integrated with both the respiratory structures (gills) and the metanephridial systems (kidneys) through both direct vascular connections and blood-pressure–dependent filtration, respectively. Arterial O_2-rich hemolymph (blood) is pumped anteriorly through a major aortic vessel by the contractile action of the muscular ventricle. This aortic vessel then branches into smaller vessels to supply defined tissues through hemolymph sinuses (32). Deoxygenated venous hemolymph then moves from the tissue sinuses into the gills via afferent vessels and the hemocoel. Hemolymph flow through the gills (branchial circulation) is countercurrent to mantle water flow, which, as in fish gills, maximizes gas exchange. Hemolymph then exits the gills through an efferent vessel and enters the atria or auricles for recirculation (3,35). Metanephridial, or renal, function depends on vascular pressures generated by the heart, with hemolymph filtration occurring through the walls of the atria (auricles) and their associated podocytes, which serve a similar function as podocytes of the visceral layer of the mammalian glomerular capsule. Pressure-driven filtrate moves through the walls of the atria (auricles) and into the pericardial cavity (renopericardial cavity), where it moves into the nephrostome and on into the kidney tubules. Excretion ultimately occurs via the nephridopore in the mantle cavity (36).

The fundamental design of the molluscan cardiovascular system has been functionally modified to meet a variety of demands, as is seen in the diversity of molluscan classes. The cephalopods show the most extensive evolution and specialization of the cardiovascular system, with blood driven at high pressures by the heart through an endothelial-lined (i.e., more closed than open) circulatory system that contributes to the maintenance of metabolic rates almost equivalent to some vertebrates (3,37). To sustain such high O_2 uptake rates, paired branchial hearts have evolved to pump blood through the gills, after which the now-oxygenated blood flows to the ventricle, where it is pumped into the systemic circuit. Functionally, the

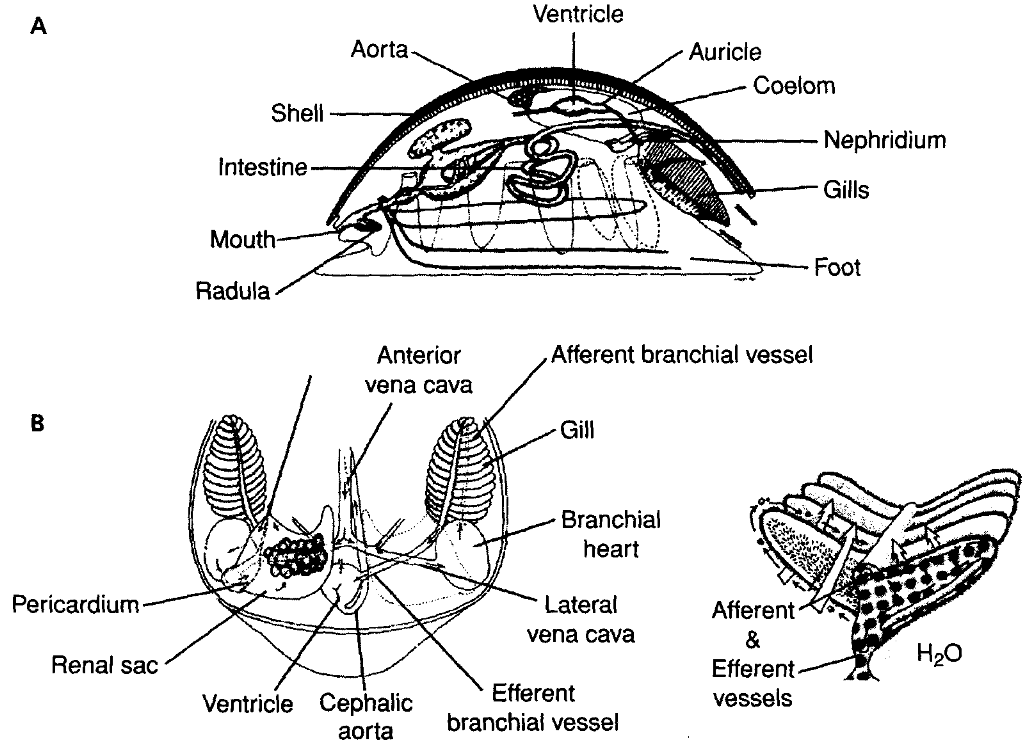

Figure 3.7. Circulatory system in molluscs. The generalized molluscs (**A**) have a well developed heart and circulatory system. The cephalopods have evolved highly efficient hearts, pumping blood at high pressure through both a branchial and systemic circuit (**B**). The inset illustrates the complex branchial circulation. The vessels of the cephalopods are lined with endothelium. (Panel **A** reproduced with permission from Ruppert EE, Barnes RD. *Invertebrate Zoology*, 6th ed. Fort Worth: Saunders Press, 1991; panel **B** reproduced with permission from Packard A. Cephalopods and fish: The limits of convergence. *Biol Rev.* 1972;47:241–307.)

cephalopods have evolved a multichambered heart capable of maintaining separation between venous and arterial blood and regulating branchial and systemic circulations. Additionally, this group of animals has developed the cardiorespiratory regulatory mechanisms needed to integrate cardiovascular and ventilatory performance with metabolic demands (38). The anatomical complexity of the cardiovascular system, along with the development of cell-lined, thin-walled, capillary-like exchange vessels and the appropriate regulatory mechanisms, appears to have been selected for in this group by increased activity patterns associated with predatory behavior, swimming, and jet propulsion (37).

The larger and more active cephalopods, such as the nautilus and the squid (the giant squid being the largest of any invertebrates), swim using jet propulsion. The use of this system does have some significant cardiorespiratory implications, in that a large volume of water must be moved through the funnel at some minimal acceleration to propel the animal. This results in excess O_2-carrying capacity in the ventilatory stream or more water flowing over the gills than is necessary for purely ventilatory purposes. O_2 uptake must be independent of jet volume to prevent postexercise mismatches in ventilatory volume and the repayment of an O_2 debt (39). The nature of the jet propulsion system requires that blood delivery to both the gills and tissues be at relatively high rates of flow and pressure. Ultrastructural examination of the vasculature of these very active cephalopods reveals a complete endothelial vascular lining, with highly branched "capillary-like" vessels that perfuse metabolically active tissues (3,40,41). Octopi are less active than squid, because they have reverted secondarily to a more benthic (i.e., bottom dwelling) existence. Octopi are restricted in the use of jet propulsion because the mantle edges have fused dorsally and laterally to the head, which limits water movement into the mantle cavity. Thus, their primary means of locomotion is crawling, through the use of their arms; they use their jet propulsion system only for shorter distances and when startled (23). The cardiovascular systems of these two cephalopods with different activity levels are similar, but discrepancies arise in reports as to the nature of the endothelia lining the vessels and the degree of tissue perfusion.

The inner cell lining of the cephalopod vasculature (like that of the nemerteans) appears quite permeable. It may not serve as a selectively permeable barrier, as it does in the vertebrates, which would make the cephalopod vascular system more "open" than "closed" (3,30,40,41). This leads to the question: Why have cephalopods evolved a cell-lined vascular system? It can be hypothesized that the invertebrate endothelia has evolved for reasons more to do with hemodynamics and the maintenance of laminar flow than with the array of functions ascribed to the vertebrate vascular endothelia. It appears the embryonic origin of the invertebrate vascular system and the associated vascular cell lining found in some taxa is derived from the coelom. Those taxa that have evolved such a vascular cell lining appear to be more active, with higher metabolic demands, and also have well-developed centralized pumps, an extensively branched vasculature with "capillary-like" vessels, and relatively high blood pressures (see the section on crustacean circulatory system for further details).

Laminar flow is required to minimize the energy needed to move blood through these complex vascular systems. Laminar flow through a cylindrical tube can be predicted based on vessel diameter, mean blood velocity, and blood density and viscosity (Reynold's number).[7] However, turbulent flow can result if sudden spatial variations occur in vessel diameter or irregularities appear along the vessels walls. In turbulent flow, a significantly greater pressure is required to move a fluid through the vessels, as compared to laminar flow. This is best exemplified by the fact that, in turbulent flow, the pressure drop is approximately proportional to the square of the flow rate, as opposed to laminar flow, in which the pressure drop is proportional to the first power of the flow rate (42–43). It would require a more robust heart and would be energetically inefficient to move blood in a turbulent pattern through a vasculature that changes shape abruptly and where the interiors of the vessels are not smooth, as seen in many lacunar systems. Thus, a vascular cell lining may have evolved to facilitate laminar flow to minimize the energy required to move blood through the cephalopod circulatory system.

The Annelid Blood–Vascular System

Members of the phylum Annelida[8] contain some of the largest examples of wormlike invertebrates. Thus, in many ways, these animals should be comparable to the molluscs with regard to cardiovascular development. However, because annelids tend to be sluggish, with relatively low metabolic rates, the high rates of flow and pressure characteristic of molluscans, for example, generally are not seen in annelids (Oligochaeta are an exception, as discussed in later paragraphs) (44–46). The segmented annelids have evolved several mechanisms by which to enhance internal transport between compartments, the most primitive being the development of a coelom and coelomic circulation. They also have evolved intracellular, iron-based O_2-binding pigment (hemoglobins). The most advanced annelid system is a fairly well-developed blood–vascular system (47–48). In the smaller annelids, few cardiorespiratory adaptations exist, however; in the larger more active worms (Oligochaeta), such as the giant Australian earthworm, the heart and vasculature are well developed (49).

The general pattern of circulation through an annelid is best seen in the polychaete worms, and starts with a dorsal vessel that runs just above the digestive tract. Blood moves anteriorly at the point where the dorsal vessel anastomoses with a ventral vessel either directly or by several parallel connecting vessels. The ventral vessel runs under the digestive tract and moves blood posteriorly. Each segment of the animal receives a pair of parapodial blood vessels that arise from the ventral vessel. The segmental parapodial vessels supply the parapodia, the body wall (integument), and the nephridia, and give rise to intestinal vessels that supply the gut. Blood moves from the ventral vessel through the parapodial system, and it returns to the dorsal vessel through a corresponding segmental pair of dorsal parapodial vessels (Figure 3.8). When gills are present and integrated with the blood vascular system (as apposed to being perfused with coelomic fluid), they contain both afferent and efferent vessels (23). Pressures are generated by contractile peristaltic waves through the dorsal vessels. These blood vessels and their associated blood sinuses lack an endothelium and are lined by only the basal lamina of overlying cells (33).

Although many of the anatomical variations observed in the cardiovascular system of annelids appear to have evolved due to activity patterns, feeding behaviors, and environment, the most complex systems are seen in the class Oligochaeta. The basic anatomical pattern seen in polychaetes is maintained: segmental vessels providing blood flow to well-developed integumental capillary beds, to support gas exchange across the skin. The pressure-generating system of oligochaetes is better developed than that in the polychaetes, with primary pressure generation coming from the contractile dorsal vessel. The "hearts" of the oligochaetes are actually robust contractile vessels that connect the major dorsal and ventral blood vessels and act as accessory organs to aid in blood movement. These hearts, along with the other major blood vessels, contain folds in their walls that act as one-way rectifier valves for blood flow. The number of accessory hearts varies among Oligochaeta species, with *Lumbricus* having five pairs of hearts and *Tubifex* only one (23).

The blood vascular system of the annelids is complex in its architecture, yet has not evolved to the level seen in the cephalopod molluscs. The cardiovascular physiology of annelids is one of low pressures and flows designed to support the relatively low metabolic rates of slow-moving or benthic animals. With the exception of the capillary bed in the integument, oligochaetes lack the high degree of tissue perfusion seen in more active animals. The vasculature lacks any cell lining, which may reflect the generally low flows and pressures observed in these systems and the animals' relatively low metabolic rates and activity patterns.

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[7] $N_R = 7745.8\, u\, d_h / v$, where, N_R = Reynolds number (nondimensional) u = velocity (ft/s); d_h = hydraulic diameter (in); v = kinematic viscosity (cSt) (1 cSt = 10^{-6} m^2/s). The Reynold's number (N_R) is used to predict the occurrence of turbulent versus laminar flow based on the ration of inertial to viscous forces.

[8] The phylum Annelida is made up of approximately 15,000 species of segmented worms. Body segmentation, a key characteristic of annelids, was a major step in the evolution of animals. Classes include Polychaeta (sand-, tube-, and clamworms), Oligochaeta (earth- and freshwater worms), Myzostomida (flattened, oval, and/or aberrant annelids), and Hirudinida (leeches).

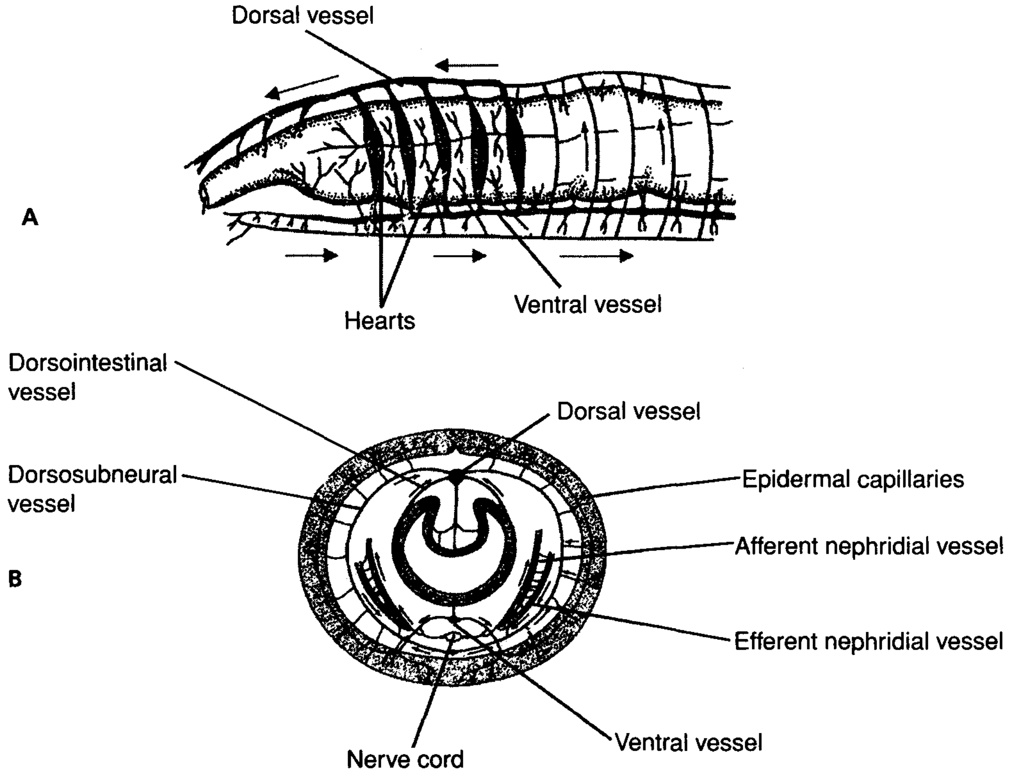

Figure 3.8. Circulatory system in annelids. The annelid circulatory system, although extensive, is a low-pressure system with contractile vessels for pumps. It lacks an endothelial lining. (**A**) Longitudinal section and (**B**) cross-section of the earthworm (*Lumbricus*). Reproduced with permission from Brusca, RC, Brusca GJ. *Invertebrates.* Sunderland, MA: Sinauer, 1990.)

The Arthropod Cardiovascular System

The arthropods[9] are by far the largest animal phylum, and they exhibit tremendous adaptive diversity. Of the major arthropod subphyla – Chelicerata, Crustacea, and Uniramia – only the crustaceans have evolved a complex cardiovascular system. The chelicerates have a relatively undifferentiated cardiovascular system when compared with the general arthropod model. The class Merostomata is best exemplified by the horseshoe crab (*Limulus*), which has a tubal heart, segmentally arranged ostia, and segmental vessels that are blind ending and supply large sinuses. The Arachnids are also part of this subphylum, and they have a similar cardiovascular system, with a tubal heart that supplies major vessels to the anterior and posterior of the animal. A unique structure found in this class is the *book lung*, which is a modified gill open to air via spiracles. This structure is perfused with hemolymph through the ventral sinus, which then returns to the heart by way of the pericardial sinus.

The insects (Uniramia) are an incredibly diverse group of arthropods, yet this diversity has apparently come without major evolutionary advances in the heart or circulatory system, which shows very little change from the Chelicerata. The success of the insects in part can be attributed to the development of the tracheal system. The trachea are a series of tubes that open to the environment through spiracles located on the lateral edges of the abdomen. The trachea then branch repeatedly, allowing for the diffusion of O_2 and CO_2 between the environment and cells that can be augmented by convective mechanisms using muscular abdominal compressions. Thus, instead of a circulatory system carrying O_2 to the cells, a tracheal system replaces capillaries to meet gas exchange demands (23,33,50).

The arthropods exhibit a segmental structure similar to that of the annelids and, in the Crustacea, vestiges of segmentation can be seen in the anatomy of the heart and circulatory system. The heart (primary pressure pump) of the crustacean in its primitive form is an elongated tube and, in the more highly evolved examples, is a globular, box-like structure.

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[9] The phylum Arthropoda is by far the largest, with estimates of over 50 million species in existence. Arthropods range in size from less than 1 mm in length to over 4 m (the Japanese spider crabs), and show tremendous diversity in body form. Primary characteristics include bilateral symmetry; a tough, regularly shed chitinous exoskeleton; a coelom reduced to portions of the reproductive and excretory systems and the main body cavity an open hemocoel; a muscular dorsal heart with lateral ostia for blood return; and a complete gut with a well-developed nervous system. Major extant subphyla include Cheliceriformes, with classes including Merostomata (horseshoe crabs) and Arachnida (spiders, scorpions, mites, and ticks); Uriramia, with classes including the Myriapoda (centipedes, millipedes, insects) and Insecta (insects); Crustacea, with classes including the Branchiopoda (fairy, brine, and tadpole shrimp and cladocerans) and Malacostraca (true crabs: crabs, shrimp, lobster, crayfish, and pillbugs).

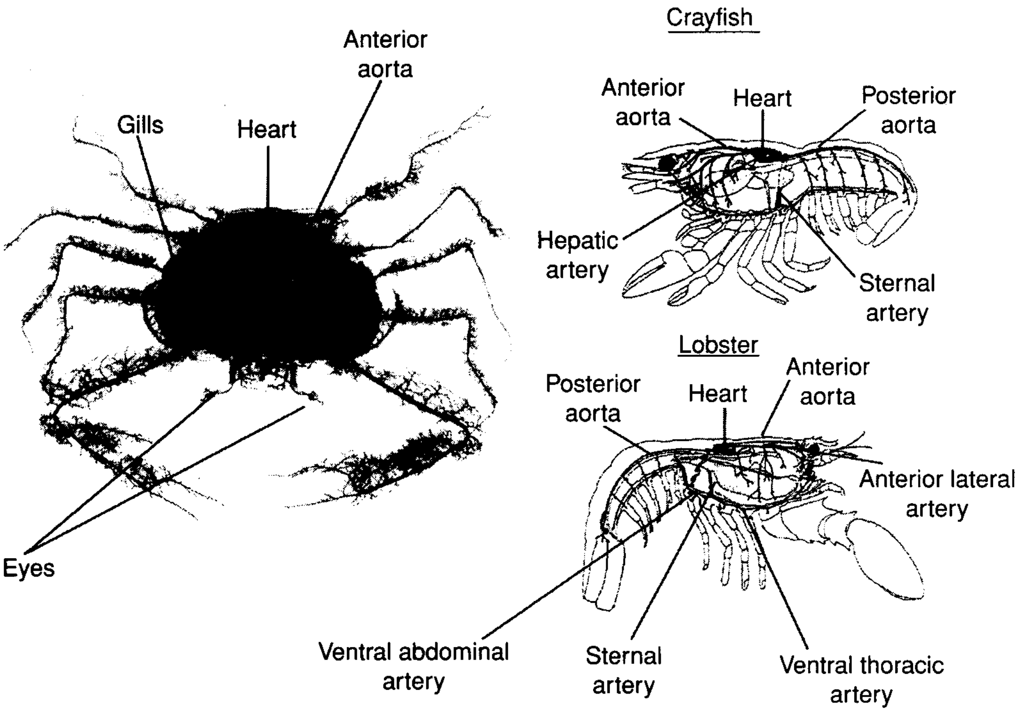

Figure 3.9. Circulatory system in crustaceans. The cardiovascular system of decapod crustaceans is highly developed, with a globular heart capable of delivering hemolymph at relatively high pressures and capillary-like vessel flows supplying metabolically active tissues. This distribution system is dramatically illustrated (*left*) in a corrosion cast of the blue crab's circulatory system and in the schematics of the crayfish and lobster. (Blue crab circulatory system reproduced with permission from McGaw IJ, Reiber CL. Cardiovascular system of the blue crab (*Callinectes sapidus*). *J Morph.* 2002;251:1–21; crayfish and lobster schematic reproduced with permission from Reiber CL, McMahon BR, Burggren WW. Cardiovascular functions in two macruran decapod crustaceans (*Procambarus clarkii* and *Homarus americanus*) during periods of inactivity, tail flexion and cardiorespiratory pauses. *J Exp Biol.* 1997;200:1103–1113.)

The ventricle is located within a pericardial sinus, where it receives hemolymph directly from the hemocoel through three paired ostia (51). In the more primitive tubal heart, the ostia appear to be segmentally arranged; in the globular hearts, however, these ostia have migrated, as the cardiac muscle has folded upon itself to form a box-like structure (52). Additionally, in the more primitive crustaceans, segmental arteries are seen branching from the anterior and posterior vessels. In the more advanced decapods, the development of the cephalothorax hides external evidence of segmentation, yet the vasculature specific to the posterior aorta in the abdomen has collateral arteries that branch off the main artery at each segment (Figure 3.9).

Typically, seven arteries leave the heart of a decapod crustacean. Arterial flow into each vessel is controlled by a single cardioarterial valve. The seven arteries consist of an anterior aorta, paired lateral arteries, paired hepatic arteries, a sternal artery, and a posterior aorta, each supplying a defined region or tissue. These vessels branch up to three times before ending in either a hemolymph sinus or in what appears to be true capillary beds (53) (see Fig. 3.9A). In the specific case of the anterior aorta, which supplies the esophageal ganglia, mouth parts, antenna, and eyes, an accessory hemolymph pump is present, the cor frontale. This structure is composed of muscle surrounding the anterior aorta, and aids in maintaining pressure and flow through the complex series of capillary-like blood vessels in the anterior regions of the animal. Hemolymph passes through the sinuses and capillaries, where gas exchange occurs, then follows well-defined venous pathways to major inferior sinuses that supply the gills or the branchiostegal tissue (used by terrestrial crabs as a lung) (54). Once hemolymph has moved through the gills or the branchiostegal sinus, it flows into the pericardial sinus and back into the heart for recirculation (53).

Hemolymph flows and pressures are relatively low in many of the lower crustaceans (55). However, in the highly active shrimps, crabs, lobster, and crayfish, metabolic demands are met through high hemolymph flow rates and relatively high driving pressures (55–57). Intracardiac pressure has been measured previously in a number of decapod crustaceans, with mean pressures ranging from 36.3 to 6.1 mm Hg during systole to 16.2 to 1.2 mm Hg during diastole (57,60,61). The hearts of these animals are able to generate considerable pressures and flows during periods of both brief and long-term exercise (swimming). In many ways, the decapods are analogous to the cephalopods in their development of an extensive vasculature

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and heart. The decapod cardiovascular system has been shown to be highly regulated through both intrinsic and extrinsic mechanisms, and it responds to a variety of internal and external stresses (56–59). The similarities with cephalopods continue at the level of the fine vessels. Although these vessels are larger than those of their vertebrate counterparts, they appear to serve the same function (53). Many examinations have been performed of the fine structure of decapod vessels, but few conclusions have been reached as to the nature of the cells that line the vasculature. Several authors have observed an endothelial-like lining of both capillaries and hemolymph sinuses. Clearly, the walls of decapod vessels do not contain muscle; thus, vasodilatation and contraction cannot be used to regulate hemolymph flow. A systematic examination of the vasculature for the presence of a cellular lining in sedentary versus very active decapod crustaceans would be interesting, and could support the hypothesis that active high-pressure systems will develop a vascular endothelia. Additionally, the nature of this "endothelium" must be examined to determine the permeability of the vasculature. In other words, is this system "open" as previously thought, or is it more appropriately viewed as "closed"?

VERTEBRATE CIRCULATIONS

Vertebrate circulations generally are characterized by having:

- A single muscular pump for propelling body fluids at relatively high arterial pressures
- A series of closed vessels leading from arteries to arterioles to capillaries to venules to veins
- Vasculature with a complete endothelial lining and walls invested with both smooth muscle and sympathetic and parasympathetic innervation
- Blood with both a fluid matrix and a cellular component consisting of several different cell types

A few fascinating discrepancies exist, either because they represent extremely primitive lineages (e.g., hagfish with multiple hearts) or they represent extreme adaptations in more advance groups (e.g., lack of red blood cells in the Antarctic icefish). Despite some common design features, great variation occurs in vertebrate cardiovascular systems. Consequently, it would be inaccurate to leave the reader with the sense that a heart structure or vascular layout is common to all vertebrates. With that in mind, let us now consider the taxonomic variation in vertebrate cardiovascular systems.

Protochordates

The Protochordates (Hemichordates, Urochordates, Cephalochordates) are an informal grouping of marine animals that are pelagic (i.e., free swimming) as larvae and largely benthic as adults. They are best described as occupying an evolutionary position between the ancestral invertebrates leading to the vertebrates and the true vertebrates (see Figure 3.2) (62–64).

The phylum Hemichordata (acorn worms) are worm-like filter feeders. Their blood contains amebocytes (leukocyte-like cells showing amoeboid motion) and hemoglobin in solution, which is synthesized from epithelial tissue lining the coelomic cavity (64). Blood is pumped by the peristaltic actions of a dorsal and ventral blood vessel through a combination of distinct vessels and large, open sinuses (65). The dorsal vessel, having a dilation often referred to as the "heart," pumps blood into the glomerulus, presumably for the excretion of wastes through filtration. Blood draining the glomerulus flows into the ventral vessel and on to the digestive tract and other organs. A cell lining is lacking in both vessels and sinuses.

The phylum Urochordata (tunicates) comprises three classes best recognized by the class Ascidiacea or sea squirts. The cardiovascular system of ascidians has been examined fairly carefully because of the pivotal position held by the Urochordates on the proposed evolutionary pathway from echinoderms through hemichordates to urochordates and cephalochordates to vertebrates (Figure 3.10). Most recent research has taken a cellular or molecular approach toward the differentiation of cardiac mesoderm, with a view to identifying homologies with vertebrate hearts (see, for example, 66–67). Previous anatomical and physiological studies reveal a defined heart, formed of a single layer of muscle cells, that lies within a fluid-filled pericardium (68). Major aortas convey blood from both cephalic and caudal ends of the heart into a series of interconnected sinuses. Like the hemichordates, no recognizable endothelial linings are present in the heart, vessels, or sinuses of urochordates (69–72).

The phylum Cephalochordata or lancelets more closely resembles the primitive living fishes, in part because they are free-living and have a very clear bilateral symmetry, with distinct head and tail regions. Their developmental biology has been investigated extensively (see 70). Much less is know about the cardiovascular morphology and physiology of cephalochordates, but several general accounts of their cardiovascular layout have been offered (62,68,70,72). Briefly, one of the main distinguishing features of *Amphioxus* (*Branchiostoma*) is that it lacks a heart. Instead; blood is propelled through the branchial and systemic circulations by three contractile vessels. Superficially, the circulation resembles that of fishes, in that a distinct branchial circulation serves some 50 gill bars used in filter feeding, and a systemic circulation receives blood from the efferent branchial arteries and distributes it via vascular spaces and sinuses to the body tissues. However, the circulation of cephalochordates lacks a complete cellular lining (22,69,73), although an incomplete (discontinuous) lining has been described recently (74). Moreover, the gill bars comprise only 4% of the animal's total gas-exchange surface, compared to 84% for the lining of the coelomic cavity in which the gills reside. Thus, the function of the gills appears to be almost entirely as mechanical structures for feeding, and the circulation of blood has little gas-exchange function (74–76). Circulation times are approximately 1 minute, as is the interval

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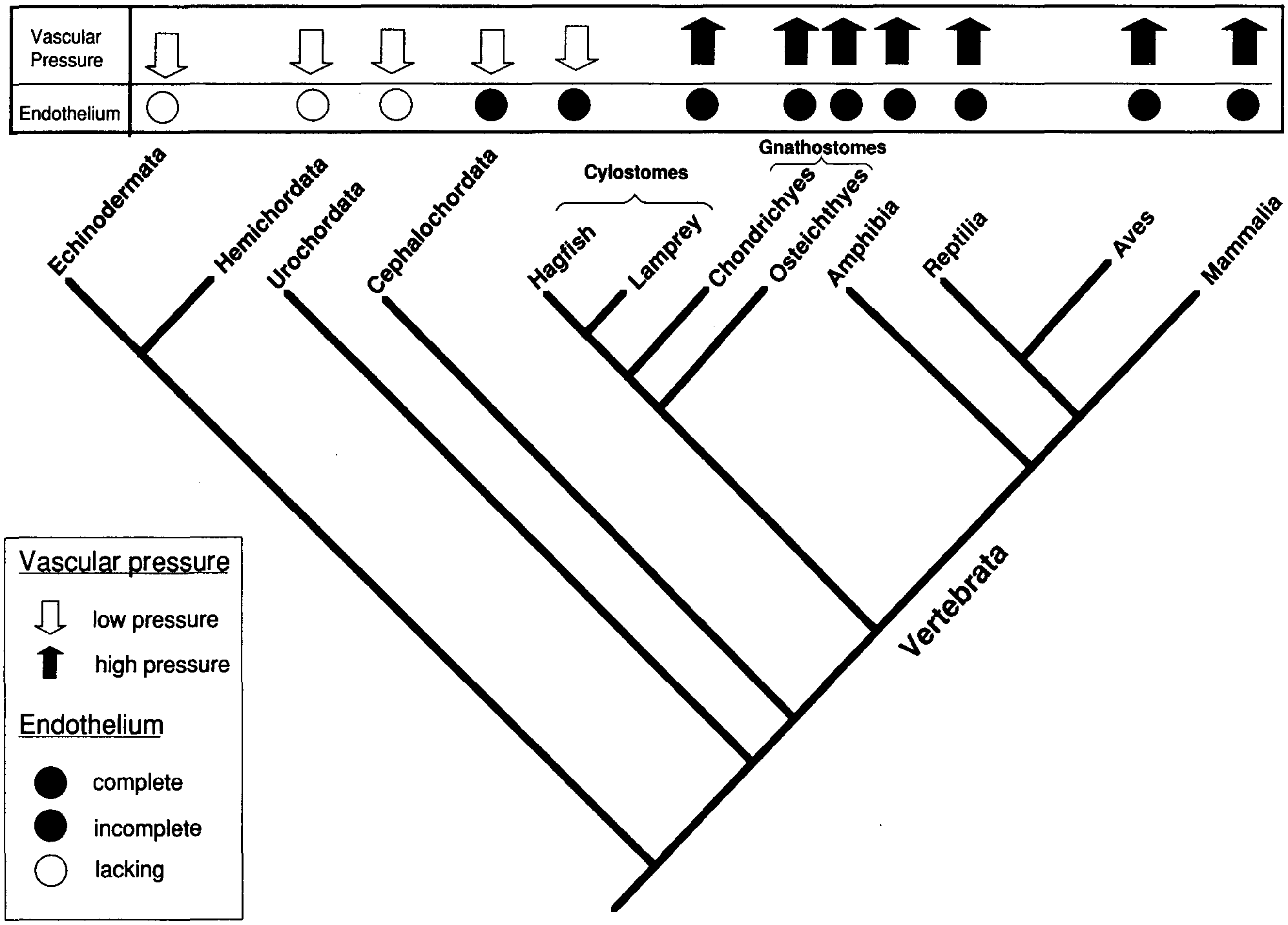

Figure 3.10. An evolutionary scheme for the protovertebrates and vertebrates. Also indicated is the presence of a complete or incomplete endothelial vascular lining. The arrows (*down* for low blood pressure, *up* for high pressure) indicate the taxon's blood pressure. The hatched perpendicular bar indicates the ancestral onset of endothelium, whereas the solid perpendicular bar indicates the ancestral onset of higher blood pressures.

between pulsatile vessel contractions (68). Intravascular pressures are thus presumed to be very low, although they have yet to be measured.

Cyclostomes (The Agnathan Fishes, Hagfish, and Lampreys)

The most primitive fishes are the Cyclostomes, represented today by the suborders Myxinoidea (hagfishes) and Petromyzontia (lampreys). The hagfish (*Myxine*) exhibits a cardiovascular system that, in many respects, resembles that of elasmobranch and teleost fishes (see Chapter 6). A heart homologous with that of more highly evolved fishes sits centrally and ventrally, and propels blood into a ventral aorta that carries blood to the gills via the branchial vasculature. From there, a dorsal aorta carries blood to the systemic tissues. However, this heart has several unusual features, including a lack of autonomic innervation and a relative insensitivity to extracellular calcium (Ca^{2+}) (see 77–78). Supplementing the "systemic heart" is a series of "accessory hearts" serving the posterior of the body (caudal heart), the abdominal cavity (portal heart), and the anterior region of the body (cardinal heart). Entrance into each of these hearts, which pump venous blood, is guarded by one-way valves. Although the peripheral circulation of the hagfish superficially resembles that of gnathostome fishes, it has large open sinuses devoid of a continuous endothelial lining (77–79). Mean blood pressures in the hagfishes *Myxine* and *Eptatretus* are on the order of 5 to 10 mm Hg, placing them among the lowest recorded for any vertebrate (see 80 for references).

The lampreys appear to occupy a morphological and functional position intermediate between hagfishes and the gnathostome fishes (78,83). The lamprey cardiorespiratory physiology has been extensively investigated (see 81–85). Unlike the hagfishes, lampreys have only a single ventrally located heart. Although the heart is innervated, vagal stimulation and acetylcholine enhance rather than inhibit cardiac performance (86). Mean arterial blood pressure is about 25 to 35 mm Hg, well within the range of many gnathostome fishes. Endothelium lines their capillaries (22,87–88).

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Gnathostome Fishes (Cartilaginous and Bony Fishes)

The cartilaginous fishes (Chondrichthians) and bony fishes (Osteicthyians) generally show a common piscine cardiovascular layout, characterized by a single heart and a series of completely endothelial-lined blood vessels comprising the typical vertebrate vascular bed: arteries, arterioles, capillaries, venules, veins (see reviews 78,89–92) (also see Chapter 5). Deoxygenated blood returning from the systemic tissues enters into a large atrial chamber. Deoxygenated blood pumped from the atrium enters the single ventricle. Subsequent ventricular contraction creates mean arterial pressures typically in the 15 to 30 mm Hg range, although tunas and other similarly highly active fishes may exhibit mean pressures as high as 50 to 100 mm Hg and, remarkably, may generate peak systolic pressures in excess of 150 mm Hg (for references, see 78,93). Deoxygenated blood ejected from the heart traverses a short ventral aorta, then enters the branchial circulation and perfuses the lamellae of the gill arches. After gaining O_2 and losing CO_2 and ammonia, oxygenated blood enters the dorsal aorta for distribution to the systemic tissues. The branchial capillary network represents a significant resistance to blood flow; consequently, blood pressure in the dorsal aorta may be reduced by 20% to 50% compared with that in the ventral aorta.

Air breathing has independently evolved several times in fishes (for review see 62,81,94–95). Because the circulation plays a fundamental role in gas exchange, whether aquatic or aerial, it is thus not surprising that the introduction of an additional gas-exchange organ in the form of a gas bladder (e.g., *Lepisosteus*, the gar pike) or a true lung (the lungfishes, *Lepidosiren*, *Protopterus*, and *Neoceratodus*) has resulted in major modifications to the basic piscine circulatory pattern. To operate at highest efficiency with maximal air-to-blood O_2 diffusion gradients, an air-breathing organ must preferentially receive deoxygenated blood from the tissues via a newly evolved, dedicated arterial pathway, while oxygenated blood draining the air-breathing organ must be preferentially directed towards the tissues. Because most air-breathing fishes retain reasonably well developed gills (even if just for osmoregulation), the dilemma exists that O_2 picked up during air breathing might actually be lost from the gill surfaces into hypoxic water surrounding the gills. Thus, in many air breathing fishes, some or all of the gill arches are modified to present a much reduced surface area to the water flowing over them. This is particularly true in the lungfishes, in which oxygenated blood leaving the air-breathing organ enters the left atrium by a single pathway – the pulmonary artery (78). Concurrently, oxygenated blood from the systemic veins enters a right atrium. Although the left and right atria empty into a single, muscular ventricle, the trabecular nature of the ventricular lining, as well as the hemodynamic blood flow patterns through the ventricle, results in partially maintained separation of these two streams of arterial blood as they are ejected into the ventral aorta. A spiral valve that runs longitudinally down the internal length of the ventral aorta rotates the streams of blood such that deoxygenated blood is primarily directed into the last two pairs of gill arches (from which are derived the pulmonary arteries), while oxygenated blood is preferentially directed into the first pair of gill arches (from which arises the dorsal aorta leading to the main systemic circulation). The anterior-most arches tend to be small and poorly developed, with lower surface area. These arches act more as shunt vessels that allow oxygenated blood to transit the branchial circulation without diffusing back out into the potentially hypoxic water irrigating the gills.

Although the lungfish has evolved a relatively specialized circulation, fishes using nonpulmonary bladders, labyrinth organs, or modified guts for air breathing tend to be plumbed into the circulation in ways broadly similar to those described for lungfishes (62,81,89,96). However, only the lungfishes have evolved a pulmonary artery leading to a distinct left atrium (62,81). Consequently, oxygenated blood from a nonpulmonary air-breathing organ typically re-enters the general systemic venous drainage. Thus, the presence of an air-breathing organ that is not homologous to a lung leads to a general elevation of blood O_2 levels in the fish, but does not lead to the peak efficiencies of gas exchange to be found in the lungfishes.

It is tempting to view the three living genera of lungfishes as on a direct evolutionary pathway to more terrestrial amphibians, simply because they have functional cardiorespiratory features intermediate between fish and tetrapods. However, the modern-day tetrapods, beginning with the amphibians, emerged from a vertebrate ancestor, which the lungfishes share with tetrapods. Not surprisingly, then, the amphibian circulation has its own evolutionary innovations – and limitations – as we will now explore.

Amphibians

Amphibians typically have two distinct atria but only a single, muscular ventricle generating mean arterial pressures generally in the range of 10 to 30 mm Hg (see 78,97–98 for reviews). Like the lungfishes, amphibians have a well-developed spiral valve in the single larger arterial outflow tract leaving from the heart. This valve maintains partial separation of oxygenated and deoxygenated blood streams derived from left and right atrium, respectively. In anuran amphibians (frogs and toads), oxygenated blood preferentially enters the systemic arches, while deoxygenated blood is directed primarily into paired "pulmocutaneous" arches. Each pulmocutaneous arch immediately splits into a pulmonary artery serving the simple, nonalveolar lungs, and a cutaneous artery, which perfuses the thin, highly vascularized skin (Figure 3.11). Amphibians are skin as well as lung breathers, often deriving more than 50% of their O_2 from cutaneous O_2 uptake under resting, normoxic conditions (99–100) (see Chapter 9). Noteworthy, however, is that the skin also receives a blood supply from the normal systemic circulation. Thus, any given patch of skin is likely to receive both deoxygenated blood via the cutaneous artery and oxygenated blood via the systemic circulation. Oxygenated

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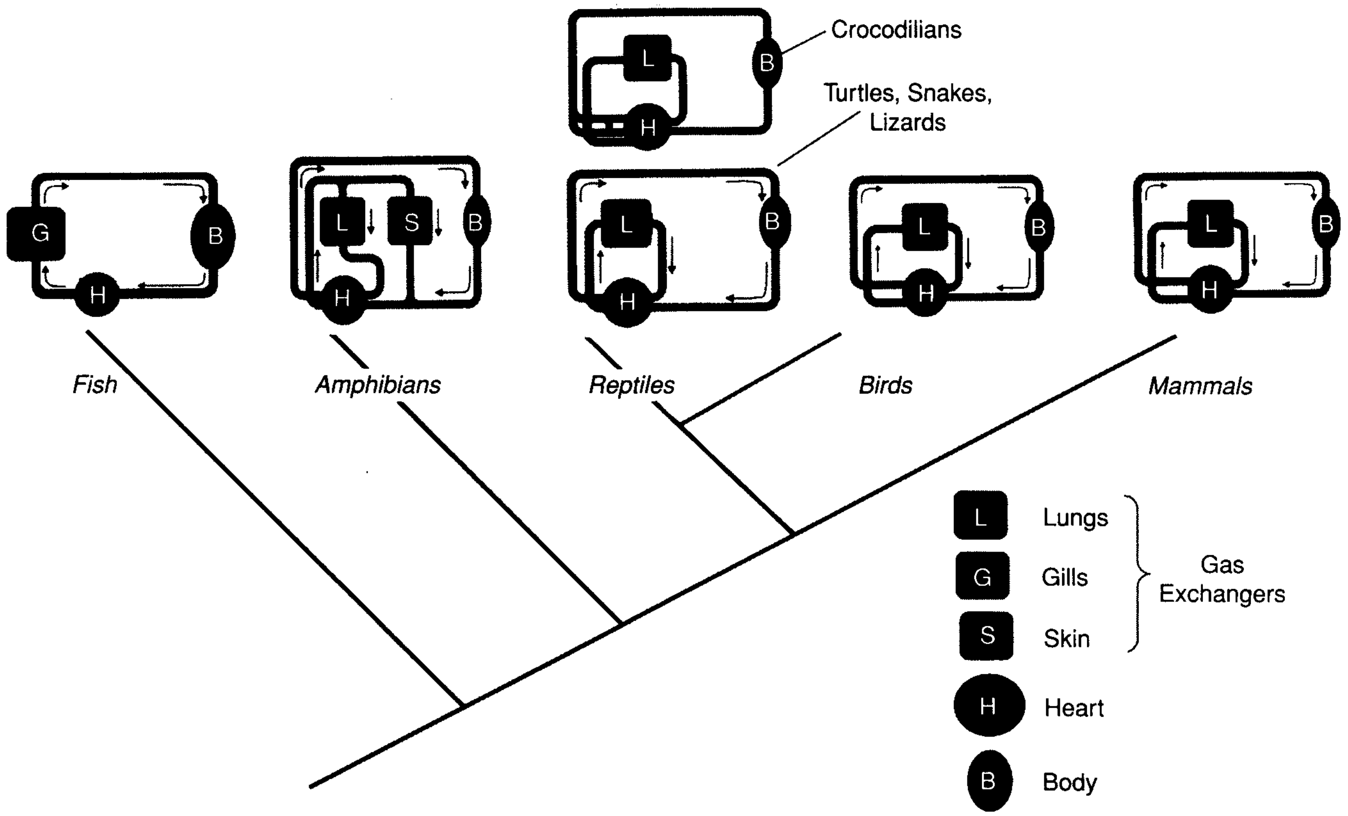

Figure 3.11. A schematic of vertebrate phylogenetic relationships and the basic cardiovascular layout of the vertebrate classes. Note that no smooth continuum of evolutionary improvement exists. Rather, each cardiovascular system is well designed to provide gas exchange at rates and in patterns appropriate for each group's environment and lifestyle. See text for further description. (Modified with permission from Hicks JW. The physiological and evolutionary significance of cardiovascular shunting patterns in reptiles. *News Physiol Sci.* 2002;17:241–245.)

blood draining the lungs returns to the distinct left atrium via pulmonary arteries. Oxygenated blood draining the skin, however, enters the general systemic venous circulation and eventually enters the right atrium.

Salamanders (Urodeles) differ from anurans in that they lack the distinct cutaneous artery of anurans, with the skin being served only by the systemic circulation typical of other vertebrates. Nonetheless, the skin still provides a significant boost to O_2 uptake. An interesting evolutionary oddity in the urodeles are the terrestrial Plethodontid salamanders, which have secondarily lost their lungs and subsist entirely on cutaneous gas exchange (100). These animals also have lost pulmonary arteries and veins, and retain only a vestigial left atrium. Some salamander groups have evolved a partially divided ventricle (e.g., *Siren*, 101). Although the physiological performance of these unusual amphibians has not been evaluated, improved separation of deoxygenated and deoxygenated blood would be expected on a purely morphological basis.

Reptiles

Reptiles are a highly polyphyletic group (that is, the group includes various distantly related taxa) and so, not surprisingly, as a vertebrate class, they show three highly distinctive patterns, each characterized by profound differences in heart structure and vascular layout (62). The reptiles show some of the most complex cardiovascular patterns of all vertebrates. Indeed, the notion that the "reptile heart" is a three-chambered heart somehow awaiting evolutionary repair to strive for the heights of the superior avian or mammalian heart is now a historical view that has been replaced by a considerable appreciation of the sophistication of the various patterns that evolved in the reptiles.

Chelonians (turtles and tortoises) and squamates (snakes and lizards) have two distinct atria that pump blood into a complex ventricle that – rather than being viewed as a single ventricle, as in amphibians – is best regarded as containing three distinct chambers (see 102–103 for reviews). The left atrium pumps oxygenated blood into a cavum arteriosum, which has no direct arterial outlet. The right ventricle empties deoxygenated blood into a large cavum venosum, separating the cava arteriosum and pulmonale. The major systemic arches arise from the cavum venosum. Clearly, the function of this heart depends on highly complex patterns of flow through this partially but complexly divided ventricle. The hemodynamics and, especially, the patterns of blood flow of the chelonian heart are highly complex and involve both spatial and

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temporal separation. Their description is beyond the scope of this chapter. Suffice it to say that the anatomically distinct chambers are functionally interconnected and, with each systole, blood traverses prominent intraventricular muscular ridges separating the cava. Mean arterial pressures are generally in the range of 25 to 40 mm Hg in chelonians (78), with a single pressure measured throughout the ventricle during systole despite the anatomical complexity of chambers within the ventricle.

One of the key features of chelonians is that they are typically intermittent breathers (breathing is of course suspended during diving in aquatic turtles, but breath-holding is also characteristic of terrestrial tortoises). Gas Po_2 and Pco_2 levels in the modestly alveolar and subdivided turtle lungs vary with the breathing–diving cycle; so too does the gas exchange performance of the lungs (see numerous authors in 98,104–105). To match ventilation to perfusion, chelonian reptiles are able to control potentially large intraventricular blood shunts by creating large changes in relative resistance between the systemic and pulmonary circulation (see 78,102–103,106 for reviews). Thus, during breath-holding, pulmonary resistance increases greatly due to constriction of a sphincter at the base of the pulmonary artery and vasoconstriction in the pulmonary vasculature. Because blood leaving the heart ultimately follows literally the path of least resistance, pulmonary vasoconstriction creates a large net right-to-left shunt leading to reduced pulmonary blood flow that, in extreme situations, is nearly eliminated, even as systemic blood flow continues. At the termination of the dive, the right-to-left shunt is reversed, and a modest left-to-right shunt appears to facilitate not only rapid reoxygenation of arterial blood, but also an elimination of CO_2 sequestered in tissues during the breath-holding event (102–103). Thus, turtles and tortoises have evolved a highly flexible circulation that is admirably adapted to a lifestyle characterized by breath-holding and low metabolic rates.

Squamate reptiles (lizards and snakes) tend to show cardiovascular morphology and performance similar to chelonians. However, fascinating exceptions exist that show that pressure separation within the complex ventricle of reptiles has independently evolved several times within the reptile group. The varanid lizards (probably best known by the Komodo dragon, *Varanus komodoensis*, although numerous smaller and less frightening species inhabit this genus), are capable of high levels of metabolism and brief periods of great activity, such as predation (107). Supporting this atypical reptilian performance is a modified ventricular structure (108). Although the three ventricular chambers – cava arteriosum, venosum, and pulmonale – are still in evidence, their relative sizes and interrelationships have been modified to allow pressure separation during ventricular systole. The net effect is that the pulmonary circulation of varanids is perfused at a mean pressure of about 10 to 15 mm Hg, while the systemic circulation is perfused at mean pressures that can exceed 60 to 70 mm Hg. This decidedly mammalian-appearing separation of the circulation into a high-pressure systemic and low-pressure pulmonary circulation represents an intermediary position on a functional continuum between the heart of lower vertebrates and mammals (109). The snake, *Python molurus*, also is capable of producing a functional separation of ventricular chambers during systole, leading to markedly different systemic and pulmonary perfusion pressures (110). However, it bears emphasis that the varanid lizards and pythons are, in an evolutionary sense, cardiovascular oddities that are in no way on the main pathway to the endothermic vertebrates (birds and mammals). However, that such circulations *can* evolve serves as evidence of an "evolutionary feasibility study" (111). The existence of these circulatory patterns has potentially profound implications as we ponder cardiovascular performance, metabolic rates, and attendant behaviors of those reptiles that do represent a more mainstream evolutionary pathway – the dinosaurs (112–113).

The Crocodilia – crocodiles and reptiles – exhibit a third pattern, representing a highly adapted cardiovascular performance that permits great efficiency (for reviews see 78,102–103,106,114,115). With respect to their heart structure, crocodilians have the same completely divided, highly muscular left ventricle and somewhat less muscular right ventricle as do birds and mammals. The fascinating adaptation in crocodilians comes from the numbers and positions of arterial arches. Crocodilians have a left arch that arises from the left ventricle and carries blood to the descending aorta, as in the homeotherms. However, crocodilians have retained a right aortic arch that derives from the right ventricle, as does the pulmonary arch. The bases of these two aortic arches are connected by a very short foramen of Panizza. How does this apparent oddity, which at first glance conjures up images of congenital pathologies in human patients, actually function? During active air breathing, high systolic pressures on the left side of the heart not only provide high driving pressures for systemic perfusion, but also transmit high pressure through the foramen of Panizza into the base of the right aorta, keeping shut the valve at the base of the right aortic arch. During air breathing, the low pressures generated by the right ventricle are enough to open the valve at the base of the pulmonary artery and perfuse the lungs, but not high enough to allow blood from the right ventricle to enter the base of the right aorta. Thus, during active air breathing, the crocodilian circulation is functionally indistinguishable from the circulation of homeotherms, sending equivalent amounts of blood under low pressure to the lungs and, under high pressure, to the systemic circulation. During what can be extended periods of breath-holding during diving, however, the same physiological degradation of pulmonary gas-exchange efficiency that occurs in diving turtles also occurs in crocodilians. Pressures generated by the left ventricle (which had been in the range of 60 to 80 mm Hg or higher), decrease greatly due to an as-yet poorly understood mechanism involving a decrease in left ventricular contractility. No longer held closed by high left aortic arch pressures, the valve at the base of the right aortic arch opens with each systole, and a proportion of right ventricular blood potentially bound for the lungs actually is

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diverted back into the systemic circulation via the right aortic arch. By virtue of this extraventricular right-to-left shunt via the right aortic arch, crocodilians, like turtles, can bypass the lungs and save the considerable energy that would have been expended on their continuing perfusion during a dive. Diving physiologists, in many respects, view the crocodilian circulation as the pinnacle of efficiency for an intermittent breather.

Birds and Mammals

The circulation of birds and mammals has been extensively investigated for literally centuries, and it is not our intent to repeat the efforts of the many authors who have recently reviewed the circulation of homeothermic vertebrates. Suffice it to say that, although we often lump the avian and mammalian circulation together, actually fairly major quantitative differences are present in their structure (e.g., relative size and wall thickness of the ventricles show considerable species diversity) (see Chapter 10). Another difference involves the developmental physiology of avian and mammalian cardiovascular systems. Unlike the placental arrangement supporting the mammalian fetus, the avian embryo develops its own extraembryonic chorioallantoic membrane (CAM). These anatomical distinctions in early development lead to both qualitative and quantitative differences in cardiovascular performance at all developmental stages, and especially during the transition from pulmonary breathing associated with hatching or birth. (For reviews of avian embryonic circulations, see 116.)

EVOLUTION OF CELLULAR LININGS IN INVERTEBRATE AND VERTEBRATE HIGH-PRESSURE CIRCULATIONS

As evident from the earlier discussions, cardiovascular systems have followed a series of complex and diverse pathways during their evolution. Against this backdrop of cardiovascular evolution, it is interesting to speculate on *why* endothelial linings evolved in cardiovascular systems. These speculations can be formed as a series of hypotheses.

The "Endothelium as Barrier" Hypothesis

Although our observations are simply correlative rather than causative at this point, it is interesting to note that, in the invertebrates, most of the cardiovascular systems that generate and experience relatively high pressures have evolved a continuous cellular vascular lining, as have all the high-pressure systems in vertebrates (see Figure 3.10). Of course, relatively high pressures generated by a heart or hearts in a circulation provide a hydrostatic gradient for moving blood longitudinally through the vessels. However, the generation of pressures within a vessel also generates a hydrostatic pressure gradient *across* the vascular walls. Whereas in some structures (nephridia of invertebrates, nephrons of vertebrates), this is a useful – indeed, necessary – phenomenon, another by-product of high intravascular pressure is the ultrafiltration of fluid from vascular into nonvascular fluid compartments in nonexcretory body tissues. Thus, this particular hypothesis holds, a cellular vascular lining may have evolved to provide some degree of control over the loss of fluids from across the vascular walls, presumably by decreasing the filtration coefficient, Kf, in the Starling equation for transcapillary fluid flux. Once endothelium as a physical barrier had evolved, secondary functions of endothelium could have evolved subsequently.

Morphological support for this thesis comes from an examination of the few but very significant appearances of cellular vascular lining in invertebrates. Shigei and colleagues (22) propose that a cellular vascular lining (endothelium or otherwise) is essentially a vertebrate adaptation. In citing numerous examples of studies that have failed to find a cellular vascular lining, these authors acknowledge but pay little attention to the fact that a cellular lining for the vasculature has indeed evolved independently from vertebrates in the Nemertea, cephalopods, and crustaceans. That these three groups of invertebrates also have a high blood pressure lends credence to the notion that the appearance of a vascular lining is in some way associated with higher sustained blood pressures. Additional evidence comes from the lack of continuous endothelium in *Amphioxus.* Although physiological examinations of this species are largely lacking, at least the morphological arrangement of the cardiovascular systems suggests a low-pressure circulation – certainly in comparison to even the most primitive of fishes. Then, in the hagfishes (with atypically low blood pressures for a vertebrate), the endothelial lining of the circulation is incomplete, being interrupted by unlined sinuses. However, speaking against the notion that endothelium serves as some form of barrier in high-pressure systems, Chapter 5 of this volume describes how the endothelium in fishes is generally a poor barrier against ultrafiltration (except in the gills and brain, where vascular permeability is much reduced).

Alternative Hypotheses for Evolution of Cellular Vascular Linings

Other equally valid hypotheses for the evolution of a cellular vascular lining, if not outright endothelium, relate to the many demonstrated functions of endothelium, including hormone secretion, leukocyte trafficking, control of blood clotting, and control of vascular tone. It is beyond the scope of this chapter on cardiovascular evolution to delve into these hypotheses. However, one bears consideration as an example. Muñoz-Chápuli and colleagues (29) present a "nonhemodynamic" hypothesis for the evolution of endothelium. They suggest that the epithelium may have first evolved as a system for immunological cooperation of "protoendiothelial cells" and, in a good example of an "evo–devo" approach, these authors present both ontogenetic and evolutionary evidence.

Although presenting a useful summary of the cell lineages lining the coelom of various invertebrates, these researchers (29) do not recognize the existence of cellular vascular lining in some cephalopods. Thus, their hypothesis focuses more narrowly on the invertebrate–vertebrate transition of the cardiovascular system. In so doing, they suggest a more hypothetical linear transition from invertebrate to higher vertebrate evolution than the widely accepted messy, nonlinear transition with numerous false-starts. Indeed, the presence of a cellular vascular lining in the cephalopods and other invertebrates remains a stumbling block to attributing the evolution of such linings strictly to the invertebrate–vertebrate transition.

The search for *the* hypothesis to explain all data for the evolution of vascular cellular linings is likely to be frustrated by the fact that such linings have so many critical functions, and these functions are highly likely to have evolved concurrently rather than in sequence.

Future Directions

Although the general anatomical layout of the cardiovascular system of most extant phyla is well documented, we know very little about the cardiovascular performance of the vast majority of invertebrates. In fact, we estimate that not even a single species in more than half of the phyla possessing internal circulatory systems has been assessed for blood pressure, blood flow, or even whether the circulation functions in respiration. We are discovering more and more evidence for sophisticated physiological regulation in "open" circulations (formerly viewed as inferior by physiologists, incidentally) and in lower vertebrates. Thus, we also should outline experiments that look at these same higher vertebrate-like functions that occur in invertebrate and protochordate vasculature. We also should explore how endothelial linings – wherever they exist – contribute to the regulation of blood or hemolymph flow at the tissue level, and whether this involves autoregulatory mechanisms, nitric oxide, adenosine, adenosine diphosphate, adenosine monophosphate, and the like.

We also know very little about even basic processes in the cardiovascular development of most invertebrates (e.g., the relative roles of vasculogenesis and angiogenesis in formation of the circulation), and even a surprising number of chordates. This leaves the currently popular and effective evo–devo approach to understanding physiology (117–118) waiting on the sidelines for want of even basic data for analysis. Not until we have a more comprehensive understanding of how cardiovascular systems develop and perform across development and taxa can we begin to piece together the selection pressures leading to particular suites of cardiovascular characteristics (e.g., regulated distribution of blood to selective vascular beds, efficient matching of blood or hemolymph perfusion to gas-exchanger ventilation).

In summary, taking a broad evolutionary perspective, we have learned a great deal about the cardiovascular systems of only a very small number of extant animals. A more sophisticated understanding of internal body-fluid circulation system evolution will require a more synthetic, integrative, "systems biology" approach that spans the fascinating diversity of living animals.

KEY POINTS

- Cardiovascular systems evolved when the combination of increasing animal size and increasing metabolic rate necessitated the internal convection of body fluids for gas exchange, metabolic substrate delivery, and waste removal.
- Many invertebrates have relatively low-pressure systems that slowly direct either ambient water or hemolymph through vascular spaces rather than through the *endothelium*-lined vessels characteristic of vertebrates. Contrary to historical perspectives, however, certain invertebrates (e.g., cephalopods and some crustaceans) have relatively high-pressure cardiovascular systems capable of the regulated preferential direction of hemolymph through cell-lined vessels.
- Evolution has led to a wide variety of cardiovascular patterns in nonmammalian vertebrates.
  - The relatively simple system of aquatic fishes becomes more complicated when the capability for air-breathing evolves, whether by appearance of a bladder-like, air-breathing organ or a true lung.
  - Amphibians have developed a dual atrial system and, in some taxa, possess specialized circulation for cutaneous respiration.
  - Reptiles show three distinct cardiovascular patterns. Chelonian circulations are well adapted to intermittent breathing by creating pulmonary bypass shunts during diving, when pulmonary respiration is less effective. Varanid lizards, with their functionally separated ventricle and high-system/low-pulmonary pressures, represent an evolutionary side track but an interesting indication that an ancestral intermediate step between typical reptiles and birds could occur. Crocodilian reptiles represent the most highly evolved reptilian system, allowing mammalian-like performance during air breathing, but the energy-saving performance of chelonians during diving.
- A correlation exists between the evolution of relatively high pressures and the presence of a cellular vascular lining. Importantly, a cellular vascular lining associated with elevated vascular pressures appears to have evolved independently multiple times (e.g., cephalopod molluscs, crustaceans, vertebrates). One (of many) possible hypotheses is that ECs might have

evolved as a barrier to the ultrafiltration of plasma in systems with high transmural vascular pressures. However, no single hypothesis is likely to account for the evolution of cellular vascular lining.

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Taken from Endothelial Biomedicine (edited by William C. Baird, ©Cambridge University Press 2007