Mass Transport: Circulatory System with Emphasis on Nonendothermic Species

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ABSTRACT

Mass transport can be generally defined as movement of material matter. The circulatory system then is a biological example given its role in the movement in transporting gases, nutrients, wastes, and chemical signals. Comparative physiology has a long history of providing new insights and advancing our understanding of circulatory mass transport across a wide array of circulatory systems. Here we focus on circulatory function of nonmodel species. Invertebrates possess diverse convection systems; that at the most complex generate pressures and perform at a level comparable to vertebrates. Many invertebrates actively modulate cardiovascular function using neuronal, neurohormonal, and skeletal muscle activity. In vertebrates, our understanding of cardiac morphology, cardiomyocyte function, and contractile protein regulation by Ca^{2+} highlights a high degree of conservation, but differences between species exist and are coupled to variable environments and body temperatures. Key regulators of vertebrate cardiac function and systemic blood pressure include the autonomic nervous system, hormones, and ventricular filling. Further chemical factors regulating cardiovascular function include adenosine, natriuretic peptides, arginine vasotocin, endothelin 1, bradykinin, histamine, nitric oxide, and hydrogen sulfide, to name but a few. Diverse vascular morphologies and the regulation of blood flow in the coronary and cerebral circulations are also apparent in nonmammalian species. Dynamic adjustments of cardiovascular function are associated with exercise on land, flying at high altitude, prolonged dives by marine mammals, and unique morphology, such as the giraffe. Future studies should address limits of gas exchange and convective transport, the evolution of high arterial pressure across diverse taxa, and the importance of the cardiovascular system adaptations to extreme environments. © 2017 American Physiological Society. *Compr Physiol* 7:17-66, 2017.

Introduction: The Case for Mass Transport: Diffusion, Convection, and the Evolution of Complex Cardiovascular Systems

There is a wealth of literature on the evolution of cardiovascular systems. However, a comprehensive understanding of the evolution of mass transport by an internal circulation begins with understanding that an internal circulation creating a convective flow is not an inherent requirement for animals. Internal circulation developed in response to the need for convection only when simple diffusion no longer sufficed for the transport of respiratory gases. First, we will briefly consider the roles of both diffusion and convection in internal material transport, and how they have factored into the evolution of circulatory systems. Abbreviations used throughout this article can be found in Table 1.

Diffusion versus convection for material transport

All early animals achieved oxygen (O_2) delivery to the tissues, and carbon dioxide (CO_2) elimination to the environment, by diffusion down partial pressure gradients. Similarly, nutrients taken in from the environment and waste eliminated from cells moved in response to concentration gradients across the animal's surface. Far from being a highly efficient process for transport, diffusion was effective primarily because diffusion distances are typically very short. As evident in the Fick equation (186, 187), many factors contribute to diffusion, including: surface area across which diffusion occurs; the solubility of the diffusing molecule in the material forming the diffusion pathway; the molecular weight of the diffusing molecule; the partial pressure or concentration differences driving diffusion; and the distance along which the diffusing molecule must travel (i.e., the thickness of the diffusion

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Table 1 List of Acronyms and Definitions

Acronym	Definition	Acronym	Definition
5HT	5-Hydroxytryptamine	C_{max}	Maximum calcium-activated force
ATP	Adenosine triphosphate	T_{max}	Maximum isometric tension
R	Animal radius	MCHC	Mean cellular hemoglobin content
AV	Atrioventricular	mmHg s^{-1}	Millimeters of mercury per second
Q	Blood flow	nNOS	Neural nitric oxide synthase
BK	Bradykinin	NO	Nitric oxide
CGRP-IR	Calcitonin gene-related peptide	L-NA	Nitro-L-arginine
SERCA2a	Calcium ATPase of cardiac sarcoplasmic reticulum	NANC	Nonadrenergic noncholinergic
CICR	Calcium-induced calcium release	O_2	Oxygen
CO_2	Carbon dioxide	C_VO_2	Oxygen content of mixed venous blood
CO	Carbon monoxide	$C_{a\text{-}v}O_2$	Oxygen extraction
cMyBP-C	Cardiac myosin-binding protein C	Hb-O_2	Oxygenated hemoglobin
CNS	Central nervous system	CaO_2	Oxygen content of arterial blood
CBF	Cerebral blood flow	DPO_2	Partial pressure gradient of oxygen
$\Delta V/\Delta P$	Change in ventricular volume to the change in ventricular pressure	PCO_2	Partial pressure of carbon dioxide
		PO_2	Partial pressure of oxygen
P_{crit}	Critical oxygen tension	PLB	Phospholamban
U_{crit}	Critical swimming speed	PAP	Pulmonary arterial pressure
cAMP	Cyclic adenosine monophosphate	Q_{10}	Rate change per 10°C change
PKA	Cyclic AMP-dependent protein kinase	dP/dT	Rate of change in ventricular pressure
CSE	Cystathionine l lyase	$\dot{M}O_2$	Rate of oxygen consumption
°	Degrees Celsius	$\dot{V}O_2$	Rate of oxygen consumption
DHPR	Dihydropyridine receptor	RBC	Red blood cell
ET-1	Endothelin 1	RyR	Ryanodine receptor
eNOS	Epithelial nitric oxide synthase	SR	Sarcoplasmic reticulum
E-C	Excitation contraction	SV	Stroke volume
GTP	Guanosine triphosphate	K_m	Substrate concentration at which a reaction rate is half of the maximum velocity
HR	Heart rate		
H_2S	Hydrogen sulfide	T tubule	Transverse tubule
HPV	Hypoxic pulmonary vasoconstriction	TnC	Troponin C
HVC	Hypoxic vasoconstriction	TnI	Troponin I
iNOS	Inducible nitric oxide synthase	VEGF	Vascular endothelial growth factor
I_{Ca}	Inward calcium current	VIP	Vasoactive intestinal polypeptide
iCa^+	Ionized calcium	P_VO_2	Venous partial pressure of oxygen
KO_2	Krogh's diffusion constant		

barrier). Collectively, the values of most of the variables in the Fick equation do not lead to innately rapid diffusion of gases or other materials into or out of an animal but result from the thin diffusion distances. An extensive literature on gas-exchange organs is currently available (358, 359, 389). Diffusion across body surfaces, through multiple cell layers, and into the mitochondria, is limits the evolution of large size in animals lacking internal convection and has, in turn, driven the selection of more complex circulations as larger animals evolved.

The limitations of O_2 diffusion are illustrated in a simple model for inward diffusion of O_2 in a hypothetical animal

of spherical body shape (80). Based on the radius of the animal (R), is the partial pressure difference driving diffusion (ΔPO_2), the Krogh's diffusion constant for the material through which O_2 diffuses (KO_2), and $\dot{M}O_2$ is the rate of O_2 consumption.

$$R = \sqrt{\frac{\Delta PO_2}{(1/6) \times (1/KO_2) \times \dot{M}O_2}}$$

Substituting a maximum possible ΔPO_2 of ~20.00 kPa, a Krogh's diffusion constant of 0.05 μmol cm^{-1} s^{-1}, and a $\dot{M}O_2$ of 200 μmol s^{-1}, the maximum radius of our hypothetical animal becomes ~1.3 mm. Driving O_2 gradients could be smaller, as a result of increasing altitude or aquatic hypoxia; $\dot{M}O_2$ values could be higher, for example, induced by high environmental temperature. Either would further limit animal size.

In an evolutionary sense, newly evolved internal fluid circulation did not allow animals to grow larger, nor did already large animals eventually evolve an internal circulation. Instead, increasing animal size and internal circulation coevolved, each limiting and enabling the other. As animals become larger and more active, with higher metabolic rates, they develop increasingly complex and effective internal circulations because of the insufficiency of diffusion to supply O_2 and nutrients and eliminate CO_2 and waste (Fig. 1). Exceptions exist when very small animals are very active (e.g., the water flea *Daphnia*) or when much larger animals are dorsoventrally flattened and/or have a low rate of O_2 consumption (e.g., some large marine flatworms). Some complex animals that have an elaborate internal circulation system as adults may exist on bulk diffusion early in their development, before an effective convective circulation of blood is generated by the differentiating heart and vessels [e.g., larval fishes (323,446), amphibians (386), and avian embryos (77, 84, 622)].

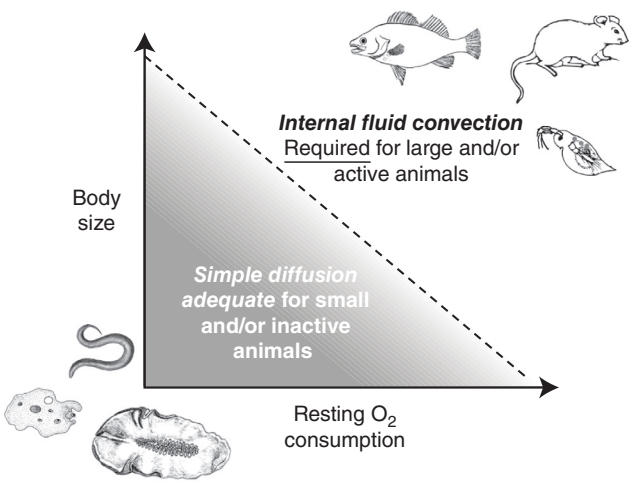

Figure 1 A conceptual view of the relationship between resting oxygen (O_2) consumption and body size, establishing two "zones." In the zone to the lower left below the dashed line, simple diffusion of gases, nutrients, and wastes is adequate for either very small organisms (e.g., protists and worms) or the exceptional larger, but relative inactive animals (flatworms). In the upper right zone, internal blood convection is required for larger animals (vertebrates) or the exceptional very active smaller animals (e.g., *Daphnia*).

Diffusion, convection, and development

A discussion of diffusion versus convection is relevant even in large animals with vigorous internal circulation, because all sexually reproducing animals begin development as a single microscopic cell, passing from the zone of diffusion to the zone of convection (depicted in Fig. 1) at some point in their ontogeny. Interestingly, this transition from diffusion to convection during development is not readily predicted. One might assume that the onset of heartbeat and actual movement of blood through the embryo/larvae signals that mass transport is being used for the delivery of O_2 and nutrients to the embryo's body, and for the removal of waste and CO_2. However, evidence indicates that the onset of convective blood flow is not required for continued growth and development in the early vertebrate embryo. For example, in early larval zebrafish *Danio rerio* (446) or larva of the African clawed frog *Xenopus* (586-588), elimination of Hb-O_2 by poisoning hemoglobin with CO or by lysing red blood cells (RBCs) does not interrupt highly O_2-dependent cardiac processes. It should be noted that adult anurans can maintain resting metabolism after hemolysis (262). Complete ligation of the outflow tract of the 3- to 4-day-old embryo of the chicken *Gallus domesticus* has no effect for 24 h on O_2 consumption or embryonic growth (77, 84, 622). Even more invasively, complete removal of the heart anlage, primordial cells that will ultimately develop into the heart, in the frog *Xenopus* results in a heartless larva that can grow and swim for several days postoperatively (234).

The findings described earlier indicate that cardiac-generated blood convection is not a requirement for O_2 uptake and growth in early vertebrate embryos. Why, then, does the energetically expensive process of cardiac production of pressure and flow occur in advance of the actual need for convective blood flow? Several studies [see (79)] have pointed to angiogenesis rather than bulk transport as the reason for this "early" onset of heartbeat, called *prosynchronotropy*, as opposed to onset of heartbeat precisely when convective O_2 supply is needed, called *synchronotropy*. Briefly, the stress and strain created by pulsatile pressure within the developing blood vessels has been hypothesized to stimulate angiogenesis through a mechanism mediated by vascular endothelial growth factor (VEGF) (Fig. 2).

Irrespective of its ultimate purpose or purposes, the early onset of heartbeat reflects the fact that, when smaller than a certain size, even embryos with quite high metabolic rates can exist on diffusion alone. Figure 3 shows calculations for several vertebrates (mouse, zebrafish, chicken, and three anuran amphibians), indicating that the embryonic heart in these taxa all start to beat at a radius far smaller than the size at which

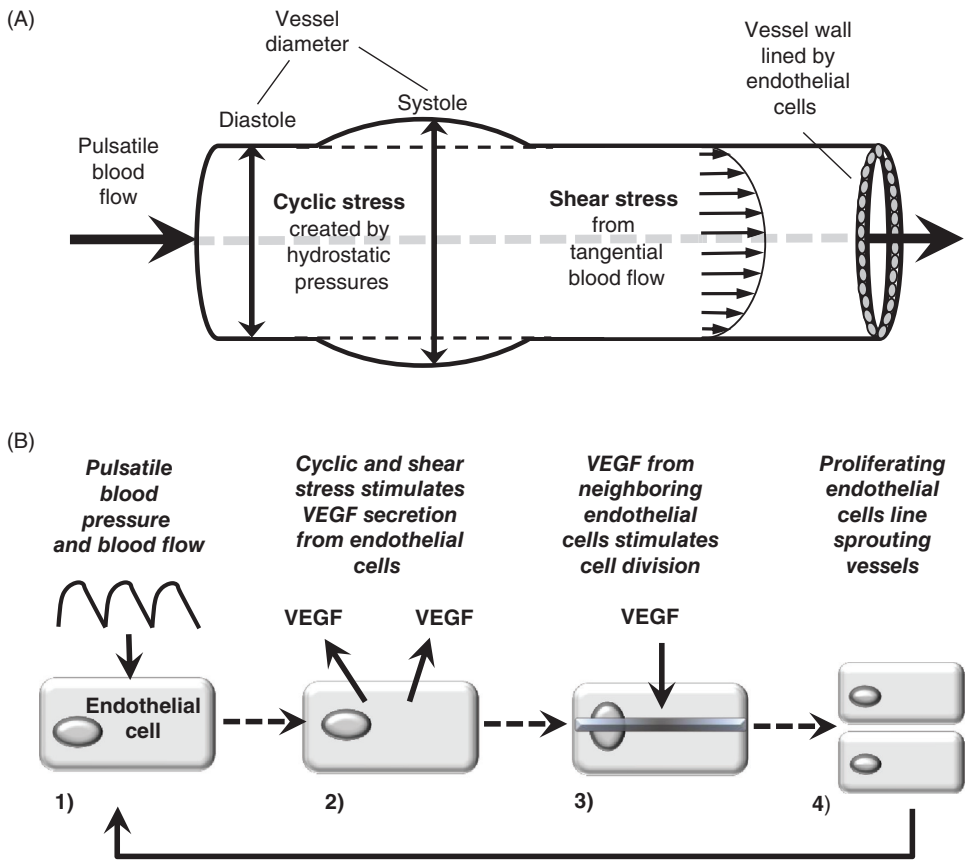

Figure 2 A model for pressure/flow stimulation of angiogenesis in the early vertebrate embryo. Panel A shows the hemodynamic factors of flow- and pressure-generating cyclic and shear stress on the endothelium-lined walls of the vasculature. Panel B shows the response of individual endothelial cells to hemodynamically generated stressors. Secretion of VEGF through a paracrine effect stimulates division of adjacent endothelial cells. Adapted, with permission, from (83).

diffusion becomes inadequate and convection is required. Interestingly, there appears to be a tight correlation between radius at onset of heartbeat and the maximum attainable radius based on diffusion alone (solid line in Fig. 3). However, neither endothermy nor body size appears to account for this relationship, which requires further exploration.

For developing animals and for adults, internal mass transport systems become a necessity in all but the smallest or most lethargic animals, leading to the evolution of complex internal circulatory systems. As we now consider the evolution of such mass transport systems, it is important to recognize that the selection pressures for these systems arise relatively early in development, not just in the adults, which are most often examined in the context of circulatory evolution.

Interestingly, most analyses of the adequacy of diffusion of respiratory gases have been carried out in animals at rest. Yet, under conditions of maximum gas-exchange needs (e.g., elevated temperature and rapid locomotion), diffusion across cutaneous surfaces may become inadequate as a means of gas exchange at even smaller body mass sizes. Using a variety of experimental conditions to reveal further limitations presented by diffusion as a means of gas exchange is warranted.

Evolution of Mass Transport Systems

Animals exhibit an amazing, and sometimes confusing, array of circulatory systems. The evolution of cardiorespiratory systems has been reviewed extensively, and these reviews provide an introduction to the voluminous literature on both invertebrates (74, 83, 248, 255, 381, 637) and vertebrates (49, 74, 78, 122, 127, 170, 292, 474). Indeed, the vast diversity of invertebrates, comprising some 30 phyla, and species diversity that outnumber vertebrates by an estimated 30 to 40 times, precludes schematic representation of their cardiovascular systems and their evolution. As many have observed, cardiovascular evolutionary change is driven by the need to enable a particular metabolic level in aquatic, semiterrestrial, or terrestrial environments rather than a series of progressive cardiovascular adaptations.

One aspect of cardiovascular form and function that transcends both vertebrate and invertebrate taxonomy, and which continues to generate new syntheses and debate, is the evolution of high pressure-high flow circulations. When did they arise, why did they arise, and what structures are necessary to enable them? Figure 4 presents a cladistics analysis of

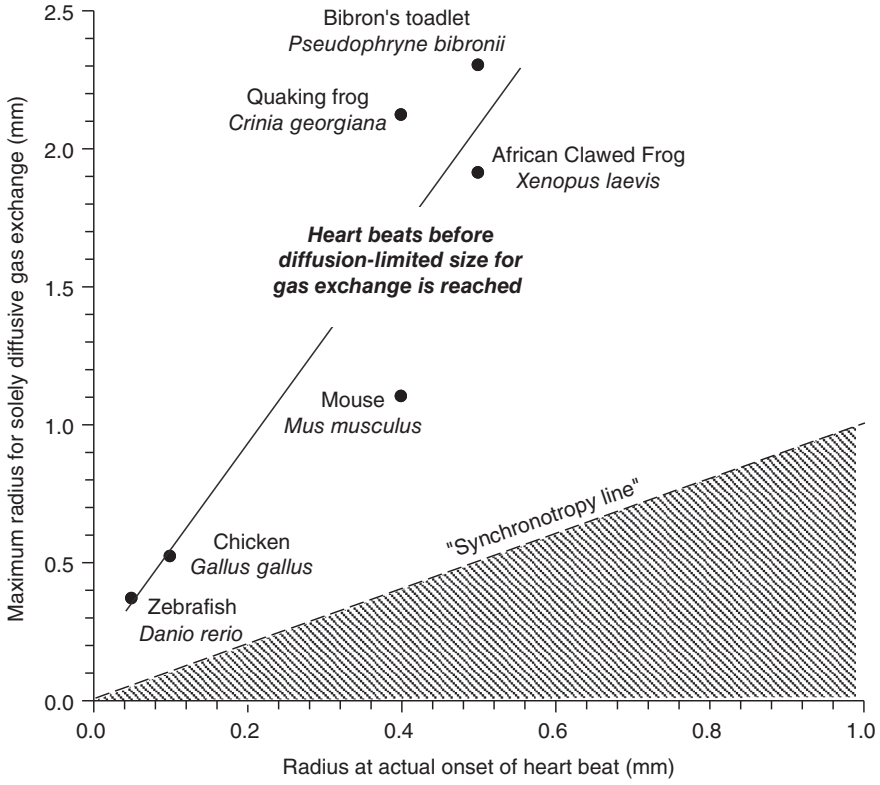

Figure 3 The relationship between embryo size (as radius) at the onset of convective blood flow (r_{onset}) and the calculated maximum embryo radius at which diffusion can serve O_2 uptake needs (r_{max}). Four vertebrate classes with highly varying rates of O_2 consumption are represented. The "synchronotropy line" (dashed line) indicates the point in this interrelationship at which there is a perfect match between the onset of blood flow and the development of the need for blood flow for O_2 transport. The solid line represents a regression through the plotted data points. See text and (80) for additional details of the calculations and their interpretation.

animal circulations with respect to pressure generation. High pressure, often assumed to be a feature of more advanced vertebrate circulations, has in essence appeared independently in the mollusks and arthropods (especially in crustaceans) as well as in the chordates. Importantly, there are some considerable correlations of higher arterial pressures with the appearance of an endothelial lining of the vasculature. Although an endothelial lining can occur in the absence of high pressure (e.g., in the Nemertea, or ribbon worms), any animal with relatively high pressure has at least a partial endothelial lining (Fig. 4). Moreover, high pressure is not in the single domain of the highly muscularized ventricles of the birds and mammals. Arterial pressures, with accompanying endothelial linings, can approach those of the systemic circulation of mammals and birds in animals as far ranging as varanid lizards (76) and the giant earthworm (293).

Evolution and Diversity of Patterns in Invertebrate Circulation

All large active invertebrates require internal convective mechanisms to meet their metabolic demands and to regulate gas transport, nutrient circulation, and waste elimination. 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 both limitations of diffusion for gas exchange and increasing metabolic demands. Most invertebrates that have evolved a circulatory system do not possess the complexity, regulatory mechanisms, and ability to generate high flows and pressures that vertebrates possess. Nonetheless, several invertebrates have evolved high-pressure flow systems. These high-performance invertebrate cardiovascular systems have a number of convergent evolutionary traits that are comparable to those of vertebrate taxa: muscular pumps, cardiac regulatory mechanisms, an endothelium-lined vascular system, and a complex circulatory system capable of meeting the active animal's metabolic demands.

Invertebrate internal convection systems are highly diverse. The least complex range from simple channels with water current generators to enhanced gastrovascular systems, used to bring the external environment into close proximity with internal tissues. These systems can be seen in the phyla Porifera, Cnidaria, and Platyhelminthes. More developed cardiovascular systems include tubal hearts and short aortic

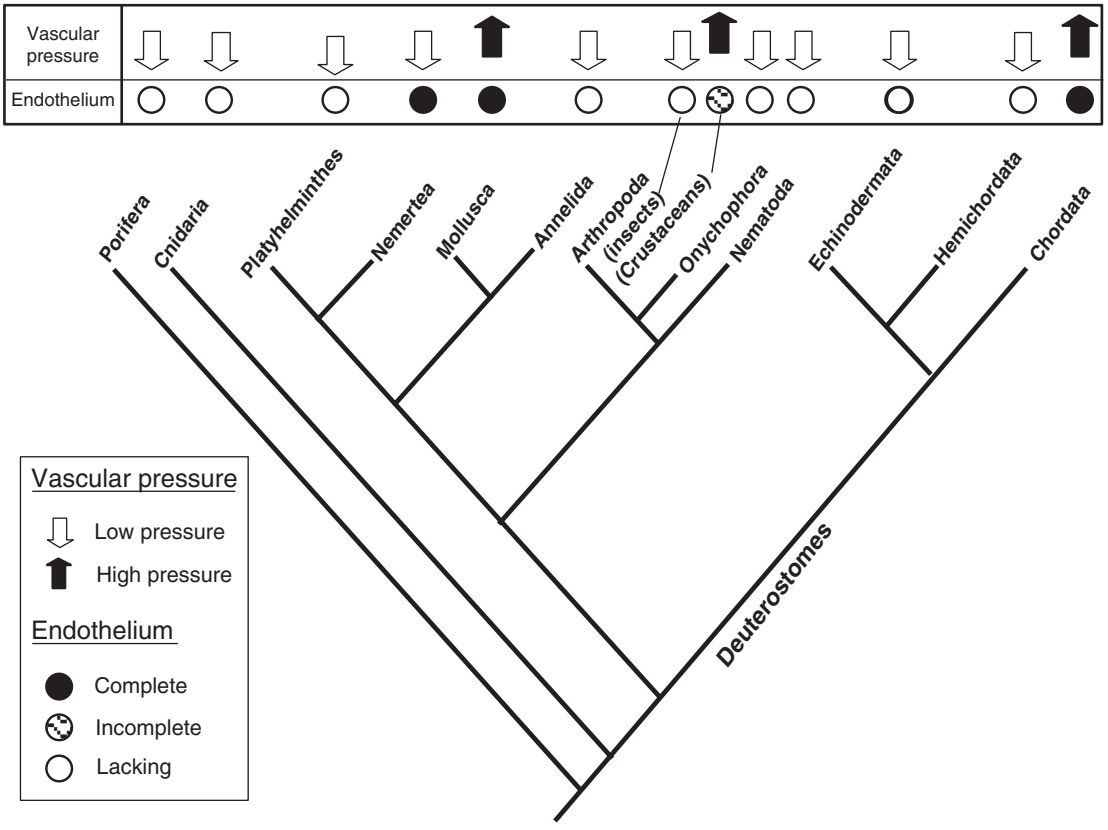

Figure 4 Endothelial vascular lining, blood pressure, and the major animal lineages. The appearance of a complete or incomplete endothelial vascular lining is shown, along with an indication of general blood pressures for each group. Downward arrow indicates low blood pressure; upward arrow indicates high blood pressure. Adapted, with permission, from (83).

delivery vessels, as seen in many of the lower worms such as nemerteans and annelids. Some of the most complex and highly regulated invertebrate cardiovascular systems include globular hearts, highly branched vascular beds, and integrated physiological control mechanisms, exemplified in taxa of the phyla Mollusca and Arthropoda, such as cephalopods and decapods.

The sponges: Internal circulation of seawater

One of the simplest pressure-generating systems is that of the phylum Porifera. Sponges have circulatory systems that distribute seawater internally, incorporating a diffuse yet functional pumping and circulatory system (139, 639). Seawater enters the sponge through dermal pores or ostia, propelled by the beating action of flagellated choanocytes that line the internal cavities of the sponge. The ostia are incurrent (or "inhalant") dermal pores spaced throughout the exterior covering of the pinacoderm, or epithelial-like flattened cells on the exterior of the sponge. Internally, seawater moves through the ostia into the spongocoel, or atrium, which is a series of interconnected flagellated or radial chambers. The flagella move seawater into major excurrent (or "exhalent") canals and ultimately out of the sponge through the osculum (339, 345).

Convective water movement through the sponge is generated by the beating action of the flagellated choanocytes that line the spongocoel. This beating action generates sufficient pressure to move a substantial volume of water through the sponge, which facilitates not only filter feeding but also the more typically cardiovascular functions of O_2 transport and waste removal (61, 480). Though the historical view of the sponge circulatory system was that it is unregulated, it appears that the pressure-flow dynamics are regulated by simple, yet effective mechanisms. Flagella activity may start and stop altering pressure on a diurnal basis in species, such as in the class Demospongiae and the genus *Halichondria*. In many sponges, changes in ambient water conditions may influence choanocyte activity. Moreover, flow and pressure are regulated throughout the chambers by incurrent ostia diameter and/or by the size of the osculum. Flow and pressure, in this group, are regulated by specialized mesohyl cells, called myocytes, which are contractile and surround the osculum. Modulating the radius of the osculum has a tremendous effect on flow and pressure (435,480,611), just as modulating 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, and these systems are used secondarily for gas transport and waste removal (216, 349, 500). In others, the internal pressure-generating system is primarily used for locomotion, and secondarily adapted to generate pressure for movement of internal body fluids or ingested food. Examples include the radial canals and gastrovascular cavity in Cnidaria, and the highly convoluted gut of Platyhelminthes. These systems provide the structural elements for the animal's hydraulic skeleton and a large surface area for food digestion and absorption. 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 gastrovascular cavity (216). Food and fluids move into and out of the gastrovascular system via different mechanisms: in primitive species, by the contraction of the locomotory muscles of the velum; in jellyfish, by the coronal muscles of the subumbrella; and, in the more complex sea anemones, via the digestive septal retractor muscles and pharynx (349). 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.

The platyhelminth gut

The phylum Platyhelminthes, comprised of flatworms, and specifically the marine polycladidas of the class Turbellaria, are similar to the cnidarians in the potential for their highly convoluted gut to facilitate both digestion and nutrient/gas exchange. Platyhelminths are active predators that can grow several centimeters wide and long, requiring internal fluid circulation to support exchange of nutrients, waste, and gases. This is evident in the pseudocoel of turbellarians, which possess a highly convoluted gut that provides a very large surface area for nutrient and gas exchange (286, 466, 483, 585).

Nemerteans: The first circulatory system

The first circulatory system evolved in the nemerteans, or ribbon worms (401, 529). Their relatively large body size and robust muscular body wall presents a significant barrier to diffusion, and therefore internal convective fluid flow is needed to facilitate gas exchange, nutrient cycling, and waste removal (495). Additionally, a rhynchocoel circulatory system perfuses the head and proboscis, and also functions as a hydrostat to evert the proboscis for feeding (108, 371, 604). In its simplest form, the nemertean circulatory system lacks a heart-like structure, instead having two lateral vessels that parallel the gut and anastomose at the anterior and posterior ends of the animal; more complex systems (e.g., in *Tubulanus* and *Amphiporus*) have an additional dorsal vessel with multiple connecting transverse vessels (605).

The vessels of the nemertean 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 (604, 605). The vessels are surrounded by circular and longitudinal muscles that facilitate vascular contractions, which, combined with contractions of the body wall, provide the pressure that moves blood within the circulatory system. The presence of a vascular cell lining is unique at this phylogenetic level and complexity within invertebrates, closely resembling the coelomic lining in invertebrates in phyla Mollusca, Annelida, Arthropoda, and Echinodermata.

It has been suggested that the nemertean circulatory system originated as specialized coelomic channels that became secondarily adapted to circulatory functions. Myoepithelial differentiation of these coelomic cells has given rise to the endothelial lining (317, 408, 495, 605). Equally interesting is the integration of the nemertean circulatory system and the protonephridia of their excretory system. It appears that in some species the protonephridia take advantage of significant circulatory pressures to aid in nephridial filtration for osmoregulation and potentially for nitrogen excretion (289). 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

Molluscans generally have developed extensive circulatory networks with highly efficient centralized pumps that function in an integrated fashion with other physiological systems. These true hearts are composed of cardiomyocytes and are capable of generating pressure and flow similar to that in some ectothermic vertebrate circulatory systems. At the most complex, mollusks have evolved a vasculature system able to generate pressure comparable to that of some of vertebrates (56, 408, 529).

The typical mollusk (Monoplacophora and Cephalopoda) has a robust muscular heart consisting of a ventricle that is supplied by two atria, or auricles, that drain the gills and sit within the coelomic cavity, or pericardial chamber (606). The circulatory system is functionally integrated with the gills through direct vascular connections and with the metanephridial systems through blood pressure-dependent filtration. Arterial O_2-rich hemolymph is pumped anteriorly by the contractile action of the muscular ventricle through a major aortic vessel, which can then branch into smaller vessels to supply defined tissues through hemolymph sinuses (243). Deoxygenated venous hemolymph then moves from the tissue sinuses into the gills via afferent vessels and the hemocoel.

Hemolymph flow through the gills 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 atrium, for recirculation (56, 363). Metanephridial or renal function is dependent on vascular pressures generated by the heart. Hemolymph filtration occurs through the walls of the atria 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 and into the renopericardial cavity, then into the nephrostome and on into the kidney tubules. Excretion ultimately occurs via the nephridopore in the mantle cavity (462).

The design of the molluscan cardiovascular system meets a variety of demands, as seen in the diversity of molluscan classes. The cephalopods show the most extensive specialization of circulatory function of the mollusks (56, 431). Effective ventricular contractility is possible due to intracellular features such as a high mitochondrial density, the presence of defined Z lines and T-tubules, and the abundance of sarcoplasmic reticulum (SR) cisternae between myofilaments. This makes possible an efficient calcium-induced calcium-release mechanism similar to vertebrates, with rates of calcium uptake in the SR comparable to those of rat cardiac tissue (11).

To sustain high O_2 uptake rates, paired branchial hearts have evolved to pump blood through the gills, after which the oxygenated blood flows to the ventricle, where it is pumped into the systemic circuit. Cephalopods have evolved multiple hearts 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 meet metabolic demands (543). The effective cardiac ultrastructure, the anatomical complexity of the cardiovascular system, the cell-lined, thin-walled, capillary-like exchange vessels, and the appropriate regulatory mechanisms appear to have all developed to facilitate increased activity patterns associated with predatory behavior, swimming, and jet propulsion (431).

The annelid blood-vascular system

The general pattern of circulation through an annelid is best seen in the polychaete worm. Its circulatory system starts with a dorsal vessel that runs just above the digestive tract. Blood moves anteriorly, 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 possesses a pair of parapodial blood vessels that arise from the ventral vessel. The segmental parapodial vessels supply the parapodia, the body wall or 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 returns to the dorsal vessel through a corresponding segmental pair of dorsal parapodial vessels. When gills are present and integrated with the blood-vascular system (instead of being perfused with coelomic fluid), they contain both afferent and efferent vessels (495). Pressures are generated by peristaltic waves of contractions 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 (71).

There are many anatomical variations in the annelid cardiovascular system, which appear to have evolved due to activity patterns, feeding behaviors, and environmental factors. The basic anatomical pattern in polychaetes consists of segmental vessels providing blood flow to well-developed integumental capillary beds. This system is maintained to support gas exchange across the skin. The pressure-generating system of oligochaetes is better developed than that of the polychaetes, with primary pressure generated by the contractile dorsal vessel. The hearts of the oligochaetes are 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 between oligochaete species: *Lumbricus* has five pairs of hearts, and *Tubifex* has only one heart (495). With the exception of the capillary bed in the integument, oligochaetes lack the high degree of tissue perfusion seen in more active animals, and their vasculature lacks any cell lining, which may reflect the generally low flow and pressure of the system and the relatively low metabolic rates and activity patterns of the animal.

The arthropod cardiovascular system: Basic anatomy

Arthropoda, by far the largest animal phylum, exhibits tremendous adaptive diversity. Of the major arthropod subphyla (Chelicerata, Crustacea, and Uniramia), only the crustaceans have evolved a complex cardiovascular system. Chelicerates have a relatively undifferentiated cardiovascular system, compared to the general arthropod model. The horseshoe crab (*Limulus polyphemux)* from the class Merostomata, for example, has a tubal heart, segmentally arranged ostia, and blind-ending segmental vessels that supply large sinuses. The arachnids are also part of the subphylum Chelicerata and 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 through the ventral sinus with hemolymph, which then returns to the heart by way of the pericardial sinus.

Insects within the subphylum Uniramia are an incredibly diverse group of arthropods, yet diversity has apparently come without major evolutionary advances in the heart or circulatory system from that of the Chelicerata. The success of insects can in part be attributed to the development of the tracheal system, which is a series of tubes that open to the

environment through spiracles located on the lateral edges of the abdomen. The tracheae then branch repeatedly, allowing for the diffusion of O_2 and CO_2 between the environment and cells. This diffusion can be augmented by muscular abdominal compressions. In insects, instead of a circulatory system carrying O_2 to the cells, this tracheal system replaces capillaries to meet gas-exchange demands (71, 413, 495).

The arthropods exhibit a segmental structure similar to that of the annelids, and in Crustacea, vestiges of segmentation can be seen in the anatomy of the heart and circulatory system. The heart of the crustacean ranges in structure from a primitive elongated tube, to a more highly evolved a globular, boxlike structure. The ventricle is located within a pericardial sinus, where it receives hemolymph directly from the hemocoel through three paired ostia (369). The ostia appear to be segmentally arranged in the more primitive tubal heart, but, in the globular hearts, the ostia form a boxlike structure, which developed as the cardiac muscle folded upon itself (634). In the more primitive crustaceans, segmental arteries are seen branching from the anterior and posterior vessels. In the more advanced decapods, the vasculature of the posterior aorta in the abdomen has collateral arteries that branch off the main artery at each segment.

Decapod crustaceans have evolved complex cardiovascular systems with a high level of anatomical complexity and physiological control. Typically, there are seven arteries exiting the heart of a decapod crustacean. These arteries consist of an anterior aorta, paired lateral arteries, paired hepatic arteries, a sterna artery, and a posterior aorta, each supplying a defined region or tissue. A single cardioarterial valve controls arterial flow into each vessel. The vessels branch up to three times before ending in either a hemolymph sinus or in what appears to be a true capillary bed (377). The anterior aorta, which supplies the esophageal ganglia, mouthparts, antenna, and eyes, possesses an accessory hemolymph pump, called *cor frontale.* This structure, composed of muscle surrounding the anterior aorta, 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, and then follows well-defined venous pathways to major inferior sinuses that supply the gills, or the branchiostegal tissue, which is used by terrestrial crabs as a lung (229), and then into the pericardial sinus and back to the heart for recirculation (377).

Hemolymph flow and pressure are relatively low $\leq$ 0.5 to 1.0 kPa in many of the lower crustaceans (379). However, in the highly active shrimps, crabs, lobster, and crayfish, metabolic demands are met through high hemolymph flow rates and relatively high driving pressures that can range from 1.5 to 7 or higher kPa (52, 82, 381, 478). The hearts of these animals are able to generate considerable pressure and flow during periods of brief and long-term exercise.

In many ways, the heart and extensive vasculature is analogous in decapods and cephalopods. The decapod cardiovascular system is highly regulated by both intrinsic and extrinsic mechanisms, and it responds to a variety of internal and external stresses (4, 374, 477, 633). The similarities with cephalopods continue at the level of the fine vessels. Although these vessels are larger than their counterparts in vertebrates, they appear to serve the same function (377). There have been many examinations of the fine structure of decapod vessels, but few conclusions have been made regarding the function of the cells that line the vasculature and the extent to which they contain smooth muscle. Several have observed an endothelial-like lining of both capillaries and hemolymph sinuses. This brings into question the definition of the decapod open vascular system as it has traditionally been described: may more accurately be described as a closed or partially open system.

Cardiac contraction in invertebrates

The heart of the decapod crustacean is a single-chambered boxlike structure with walls consisting of bands of cardiac muscle arranged in a three-dimensional network to maximize the ability to eject hemolymph upon contracting. This trabecular network of branching and anatomizing myocardial cells forms an avascular myocardium that acts as a functional syncytium to facilitate coordinated contraction (274). The ventricle is suspended within the pericardial cavity by elastic alary ligaments (369). The myocardium, alary ligaments, and pericardial cavity together comprise a functional hemolymph pump. Ventricular contraction results in hemolymph ejection into the arterial system. Contraction of the myocardium is stimulated by cardiac pacemaker cells found in the cardiac ganglion, which is located along the dorsal surface of the heart (Fig. 5A) and regulates heart rate and muscle contractile force (369, 379, 631). Some of the energy produced by the contraction is stored in the elastic alary ligaments attached to the nearby tissues, and this energy plays a role in the passive expansion of the ventricle, initiating diastolic filling. Venous pressure is aided by this passive ventricular expansion, allowing hemolymph to fill the heart through three paired ostia located on the heart dorsally, laterally, and ventrally. Once diastolic filling is complete, systolic contraction begins. Initial cardiac myocardial contraction places tension on the edges of the ostia, resulting in their closure and preventing backflow (Fig. 5B).

Control of cardiac function in crustaceans

The neurogenic myocardium of the crustacean heart contracts when stimulated by cardiac ganglion nerves. Overlying and embedded within the cardiac muscle, the cardiac ganglion serves as pacemaker and regulator of muscle contractile force (369, 380, 578). The cardiac ganglion is composed of a small number of neurons of two cell types. The cells are two different sizes and morphologically distinct, with functional differences (332, 378, 379). In the crayfish (*Procambarus clarkii*), for example, the cardiac ganglion is approximately 5 mm in length and contains eight small and eight large neurons. Normally, the smaller neurons act as a pacemaker by establishing

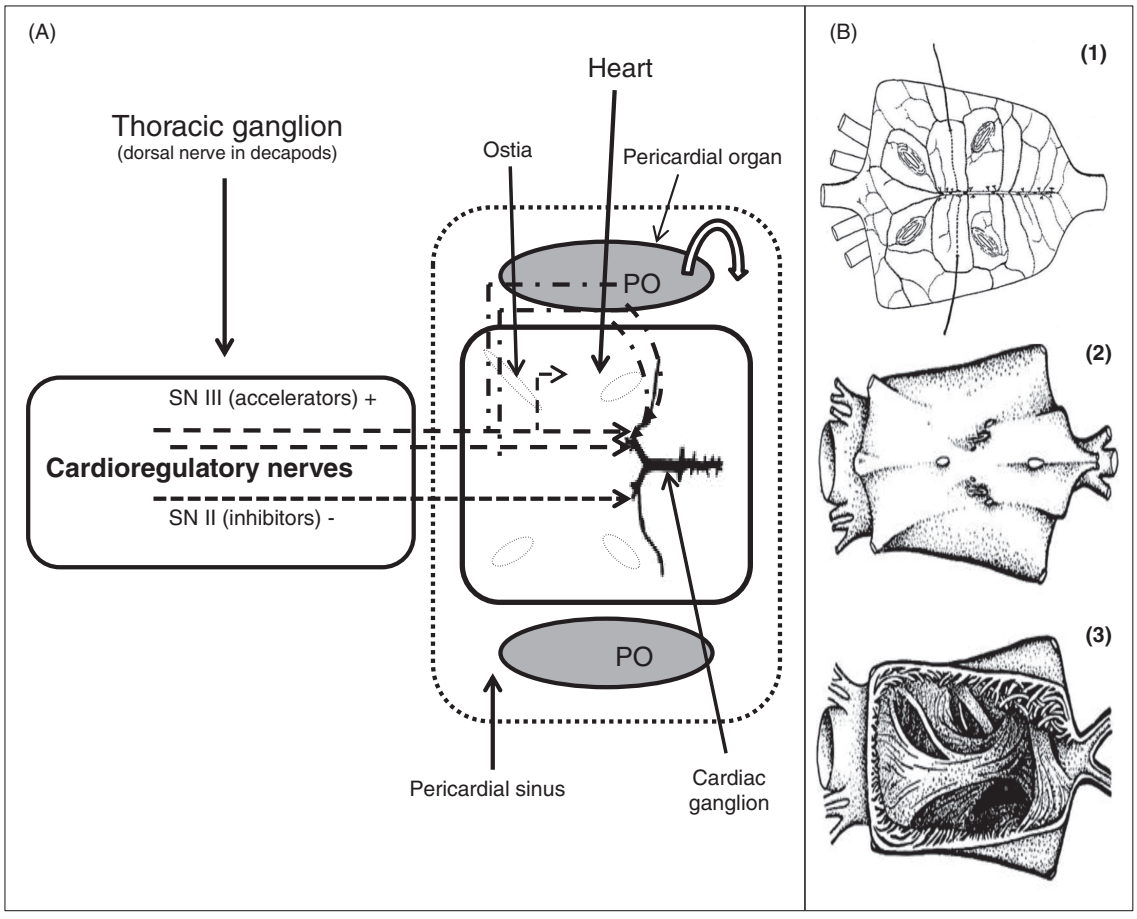

Figure 5 Mechanisms of cardiac regulation. (A). Intrinsic cardiac regulation: The neurogenic decapod heart contracts in response to pacemaker cells associated with the cardiac ganglion located in or on the dorsal aspect of the heart. These interneurons synapse with larger motoneurons that innervate cardiac muscle fibers directly and through extensive dendritic ramifications control both rate and strength of cardiac contractions. Extrinsic cardiac regulation: The cardiac ganglion, myocardia, ostia, suspensory ligaments, and arterial valve are all under extrinsic neuronal and neurohormonal control. The rate and force of cardiac contraction are modulated through cardioregulatory nervous input to both the cardiac ganglion and myocardia. Through the thoracic ganglion (dorsal nerve) two pair of cardio accelerator nerves (SN III) and one pair of cardio inhibitor nerves (SN II) influence the rate of depolarization of the pacemaker cells in the cardiac ganglion and force of myocardia contraction. Neurohormonal control of cardiac function is through the pericardial organ (PO), which releases an array of cardioactive substances into the paricardial sinus in response to dorsal nerve input via the neuroregulatory fibers. (B) Cardiac contraction is initiated by the cardiac ganglion, resulting in the generation of tension and myocardial contraction. The myocardia is composed of a three-dimensional array of fibers arranged around the lumen of the heart (B3) and forces hemolymph into multiple arterial systems through the arterial valves (B2). Both nervous regulate arterial and ostial valve tone and neurohormonal input to facilitate coordinated and functional contraction (B1). B1 adapted, with permission, from (6), B2 and B3 adapted, with permission, from (369).

burst frequency. Through chemical synapses, the smaller cells drive the larger neurons, which act as follower cells and innervate the myocardium directly. The rate of heart contraction is determined by the burst frequency of the small cells of the cardiac ganglion, and the force of the contraction is dependent on the spike frequency within each burst.

The larger cells of the cardiac ganglion innervate the myocardium directly and show mechanoreceptor-like sensitivity (369). In the lobster (*Panulirus japonicas)*, for example, distention of the cardiac ganglia occurs when the heart is exposed to high filling pressure; heart frequency is then determined by the bursting pattern of the large nerves (116,328,330). When the heart is exposed to neurohormones, such as serotonin, heart frequency is set by the smaller neurons. Although the smaller cells act as primary pacemaker, driving the larger cells, modulation of heart frequency can depend on both cell types.

Cardiac ganglion neurons are influenced by three cardioregulatory nerves (Fig. 5A), one pair of inhibitory fibers (SN 11), and two pairs of acceleratory fibers (SN 111) (379, 631). The nerves originate in the anterior ventral nerve cord and the subesophageal ganglion. The three nerves come together at the lateral pericardial plexus and enter the pericardial sinus. Here they separate, and they terminate in a variety of regions. SN 111 sends out axons that innervate the pericardial organ, the cardiac ganglion, the dorsal muscles of the

myocardium, and the alary ligaments. Cardiac accelerator and inhibitor fibers have been found to fire tonically with intermittent bursts of higher-frequency spikes (379,578,631,633).

Central neural integration of crayfish heart function originates in the command interneurons found in the circumesophageal ganglion. Interneurons have been identified that cause tachycardia, bradycardia, or cardiac arrest (379). The impulse frequency of cardioinhibitory fibers has been found to vary linearly with stimulation of specific interneurons. It is interesting to note that when these interneurons are stimulated or sensory stimulus is given, the cardioaccelerator nerve and inhibitor nerve act reciprocally, such that when one increases firing frequency, the other decreases its rate. The circulation of Crustaceans is also under hormonal control. Cardioacceleratory (cardioactive) peptide, proctolin, calcitonin-like diuretic hormone, FMRFamid-related peptides and a variety of other compounds are known to influence the amount and distribution of heart rate and blood flow in crustaceans (354,411,636). However, relatively few measurements of circulating levels of cardioactive hormones have been made, and this remains an area ripe for exploration.

Regulation of cardiac output

Cardiac output has been measured in a variety of Crustaceans, predominantly from larger species such as American lobster *Homarus americanus* and the larger crabs. Cardiac outputs vary across species. For example, cardiac output at 12°C is ~14±2 mL kg^{-1} min^{-1} in the crab *Maja squinado* and a slightly higher estimated value of about 20 mL kg^{-1} min^{-1} in the crab *Cancer magister* (199). Cardiac output in *Cancer productus* ranges from 103 to 275 mL kg^{-1} min^{-1} (82) in the lobster *Homaraus americanus* is ~94 mL kg^{-1} min^{-1} at 10°C (477) and the crayfish *Procambarus clarkia* ~250 mL kg^{-1} min^{-1} at warmer temperatures of 25°C (477). These crustacean cardiac outputs (especially when corrected for much lower body temperatures) illustrate the crusteacn circulation is capable of high output values.

Not only are crustacean cardiac outputs relatively high, but the total cardiac output as well as its distribution are highly regulated. There are four broad mechanisms that regulate vascular hemolymph flow in decapods: cardioarterial valves, peripheral valves, skeletal muscle contractions, and vascular contraction, though the last is speculative (Fig. 6). As we have previously discussed, at the interface between the heart and each arterial system is a muscular cardioarterial valve that is under neuronal and neurohormonal control (329,331,422). As the tonus of these valves change, flow to each peripheral arterial system can be modulated to control bulk arterial flow. In lobsters, peripheral valves located distal to the cardioarterial valves actively regulate resistance, and therefore hemolymph flow, to individual vessels (128, 632, 636). Contractility and elasticity has been observed in lobster arteries and actin and myosin have been identified, which suggests the capacity to change vessel diameter (100, 635) and meet system-specific metabolic requirements.

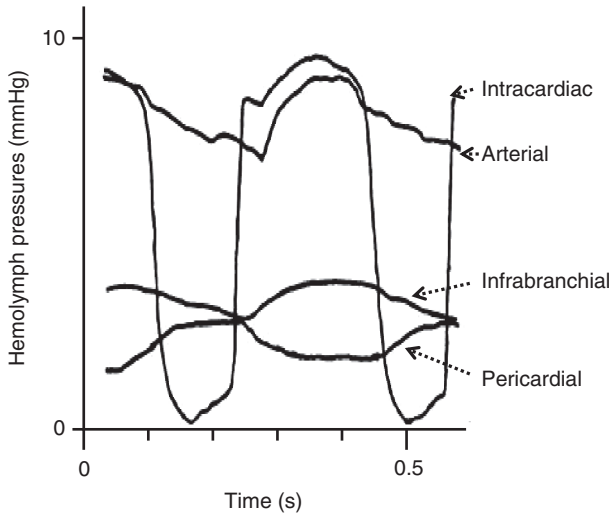

Figure 6 The functional linkage between the actions of the cardiac ganglion and the generation of hemolymph pressure to drive blood through the circulatory system is myocardial contraction. The decapod (*Procambarus clarkii*) cardiac cycle begins with systole, isovolumic contraction (muscle fiber depolarization with the generation of pressure or tension) as seen by the rapid rise in intracardiac pressure which rapidly goes from close to zero to approximately 1.33 kPa. A notch similar to the dicrotic notch seen in mammalian ventricular pressure profiles is observed as the multiple arterial valves open due to the ventricular-arterial pressure difference. Intracardiac and arterial pressures equalize once the arterial valves are open and hemolymph is propelled out of the ventricle into the arteries during the ejection phase. Arterial pressures are maintained above intracardiac pressure once the arterial valves close and the heart goes into isovolumic relaxation. Arterial hemolymph pressure diminishes slowly due to the passive elastic properties of the major vessels leaving the heart. Diastole or ventricular filling occurs as the result of a pericardial-intracardiac pressure difference. During diastole, pericardial pressure continues to increase as hemolymph moves from the infrabranchial sinus through the gills and into the pericardial region. Open ostial valves allow the pressure difference to drive hemolymph into the ventrical during the filling phase, which ends with onset of the next systolic contrition (478).

The third mechanism by which decapods, specifically macrurans, modulate arterial resistance is contraction of major skeletal muscle groups. Although this mechanism is not inherent to the vascular system, major changes in resistance and flow have been demonstrated during activity (477). Flow is substantially altered by tail flexion in both lobsters and crayfish. Contraction of the abdominal muscles during tail flexion substantially increases stroke volume and causes a redistribution of cardiac output, with no effect on heart rate. Cardiac output is redistributed during tail flexion, increasing blood supply to the abdominal muscles, and thereby accommodating the increased metabolic demand during activity. Blood supply also increases to the limbs, the mouthparts, and the muscles supplying the scaphognathites (gill bailers).

Hypoxia, salinity, and cardiac function in crustaceans

Modulation of cardiac function and regional redistribution of blood flow facilitates delivery of oxygen, nutrients, and

hormones to the tissues, and enables the subsequent removal of waste. As such, cardiovascular mechanisms are closely coupled with respiratory and metabolic processes. Crustaceans encounter permanently or temporarily hypoxic aquatic environments as well as in some species environments of variable salinity. This has resulted in the evolution of an array of molecular, physiological, and behavioral responses (35, 379). The physical characteristics of water as compared to air, including low oxygen-carrying capacity, high viscosity, and slow diffusion rates, contribute to the stress of an animal exposed to hypoxia. Freshwater and estuarine habitats are also characterized by variable salinity, and crustaceans thus must develop mechanisms to deal with this stress (600). The diversity of crustacean habitats has led to physiological responses to hypoxia and salinity that are often profound but difficult to categorize across species. For example, when exposed to water with a PO_2 of ~4.7 kPa, the crab *Cancer magister* exhibits a bradycardia with heart rate declining from ~70 to 55 bpm (375, 376). Yet, when exposed to 25% seawater this species shows a tachycardia, with heart rate increasing from ~70 bpm to nearly 80 bpm—an effect lasting hours behind the 6-h exposure period. Additionally, prandial state can significantly mute hypoxic bradycardiac response (375, 376). These examples illustrated the possible diversity of circulatory responses across crustaceans.

Animals are classically divided into two mutually exclusive groups: oxygen regulators, which maintain O_2 consumption ($\dot{M}O_2$) independent of environmental O_2 tension (PO_2); and oxygen conformers, which vary $\dot{M}O_2$ in proportion to PO_2. This division is used for easy categorization rather than to fit observed responses (362).

A reduction in O_2 partial pressure elicits a wide range of physiological responses, with O_2 regulation and O_2 conforming at opposite ends of the spectrum. The basic problem of maintaining O_2 delivery to metabolically active tissues with low hypoxic tolerance is central to these physiological responses. Distinguishing oxygen regulators from oxygen conformers is not always simple, many animals will regulate $\dot{M}O_2$ independent of water PO_2 down to some critical level, below which they become oxygen conformers. This critical point is represented as P_{crit}, and it is used when comparing hypoxic tolerance in organisms. P_{crit} for crustaceans varies widely. Species such as burrow-dwelling Thalassinidean shrimps have P_{crit} values as low as 1.3 kPa, and similarly low vales of 1 to 3 are found in a variety of Mysid shrimps, copepods, and ostracods that live in highly hypoxic environments (105). At the other extreme, Pcrit values of 5 to 8 kPa are common in intertidal and freshwater crabs and crayfish (580). P_{crit} is not even constant for a given species, with a variety of physical and physiological parameters influence the point at which an individual animal will switch from O_2 regulation to conformation (254, 369, 580). P_{crit} must be interpreted as an integrated homeostatic balance point changing with internal and external conditions. The interpretation of P_{crit} remains under debate. If P_{crit} is considered a relative value that will be influenced by physical conditions, such as temperature, salinity, etc. as well as physiological parameters, such as respiratory system, cardiovascular system, metabolic rate, etc., then one can predict how a given parameter will influence this critical point (254, 362).

Conclusions and future directions

Complex invertebrates have existed on earth for well over 500 million years and have expanded into a variety of ecological niche over that time. Their cardiovascular evolutionary history predates that of the vertebrates and shows complexities that have allowed the more advanced and active invertebrates to occupy environments equal to many vertebrate taxa. This success has been largely due to the ability to meet metabolic demands through evolutionary advances in cardiac, circulatory, and respiratory complexity at both the anatomical and physiological levels. Our understanding of many fundamental physioregulatory mechanisms is superficial or incomplete to the extent that we do not understand the basic evolutionary challenges that resulted in the levels of cardiovascular complexity in invertebrates we see today. We also have an incomplete understanding of the molecular and cellular machinery of most regulatory mechanisms and the homeostatic feedback control required for complex invertebrates to survive the challenges presented to them.

Cardiac Excitation and Contraction in Vertebrates

This section focuses on the cellular signals, structures, and mechanisms that define excitation-contraction (E-C) coupling in vertebrate striated muscle. A summary of E-C coupling in vertebrate cardiac muscle is shown in Figure 7. Cytosolic Ca^{2+} is the trigger for muscle contraction, and classic experiments (484) demonstrated for the first time that the frog heart required extracellular Ca^{2+} for muscle contraction. This observation contrasts with observations of skeletal muscle, which contract for several minutes in the absence of extracellular Ca^{2+} (17). Myocyte contraction in the vertebrate heart is initiated by a rise in cytosolic Ca^{2+} in response to an action potential. Action potentials can differ between cardiac myocytes within a given heart (atrial vs. ventricular) with, up to a point, an action potential of longer duration results in greater inward Ca^{2+} current (I_{Ca}) and a greater increase in force development. The action potential is followed by a brief increase in cytoplasmic Ca^{2+} known as the Ca^{2+} transient. The amplitude of the Ca^{2+} transient depends on intracellular buffering of Ca^{2+} and both the amplitude and the source of activator Ca^{2+} (extracellular vs. the SR) may vary between species (205,527). Cytoplasmic Ca^{2+} then contributes to force of contraction.

Overall, hearts from ectothermic vertebrates display greater changes in contractility in response to changes of extracellular Ca^{2+} compared with mammalian hearts (412).

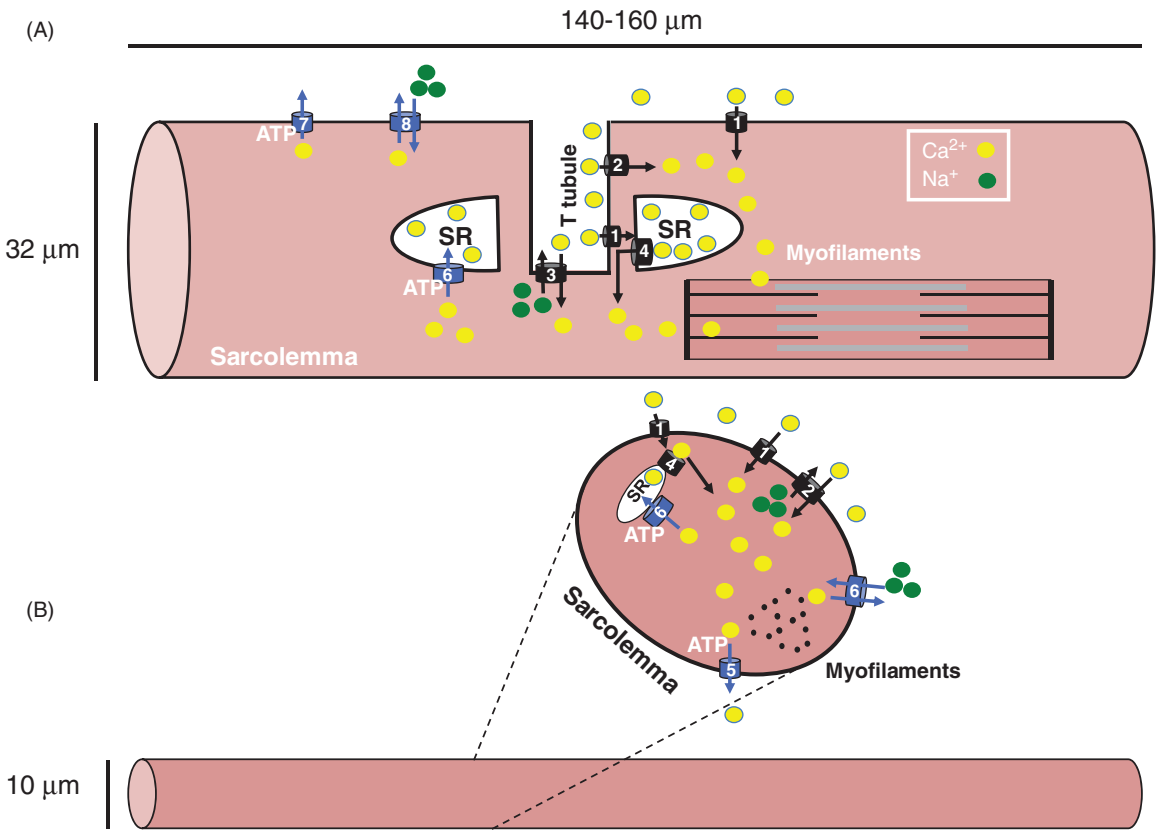

Figure 7 A comparative scheme of the sources of activator Ca^{2+} during excitation-contraction coupling in mammalian (A) and teleost (B) ventricular myocytes. Both cardiomyocytes are approximately the same length, but the teleost cell is narrower, elliptical in cross section, lacks transverse tubules, has a less well-developed SR, and has a much higher surface area to volume ratio. Contractile activity in all vertebrate hearts depends, in large part, on how much Ca^{2+} is delivered, to myofilaments. In the mammalian cardiomyocyte, activator Ca^{2+} comes from extracellular Ca^{2+} entering the cell through Ca^{2+} channels (DHPR, #1 and 2) and Na^+/Ca^{2+} exchangers (reverse mode, #3) on the sarcolemma and transverse tubules (T tubules). Ca^{2+} influx also triggers Ca^{2+} release from the SR (intracellular Ca^{2+} store) by the RyR (#4). Relaxation occurs by SR Ca^{2+} pumps (SERCA, #6), sarcolemmal Ca^{2+} pumps (#7), and Na^+/Ca^{2+} exchangers (#8). The primary source of Ca^{2+} for contraction in the teleost cardiomyocyte is extracellular with transsarcolemmal influx through Ca^{2+} channels (#1) or Na^+/Ca^{2+} exchangers (#2) in reverse mode. Depending on the fish species and environmental conditions, SR Ca^{2+} may also be important (#4). For relaxation, teleosts rely on sarcolemmal Ca^{2+} pumps (#5), Na^+/Ca^{2+} exchangers (#6), and possibly SR Ca^{2+} pumps (#7). Based on information in references (40, 528, 593). See text for additional details.

Given its importance in function, the concentration of ionized calcium (iCa) in the extracellular fluid is regulated precisely in all vertebrates (494). For example, the plasma calcium concentration in humans ranges from 2.3 to 2.6 mmol/L, of which 1.2 mmol/L is ionized (189). The concentration of extracellular Ca^{2+} in mammals is approximately 10,000 times greater than the cytosolic Ca^{2+} concentration of the resting heart during diastole (100-200 nmol/L), and this high concentration sets up a large transsarcolemmal gradient in favor of Ca^{2+} entry. Similar values are seen for free (1.3 mmol/L) and total (2.3 mmol/L) blood calcium in rainbow trout, *Oncorhynchus mykiss,* under control conditions (16). Neither sustained exercise, hypercapnic acidosis, nor hypoxia affects Ca^{2+} concentrations in rainbow trout. However, plasma calcium increases abruptly in many vertebrates as a result of intense bouts of exercise (115,265,494) and pH change will alter plasma Ca^{2+} level in fish, but these changes are 1/3 to 1/6th those in terrestrial vertebrates (16,62,240). Interestingly, there are extreme examples, as in the turtle *Chrysemus picta bellii,* in which total plasma calcium ranges from 3 to >100 mmol/L during prolonged dives (281). The corresponding values for free Ca^{2+} and effects of increasing plasma calcium on cardiac function in the turtle during dives are not known.

There are two generalized models for E-C coupling in vertebrate hearts. One involves storage and significant release of Ca^{2+} from the SR; the other requires influx of Ca^{2+} through calcium channels in the sarcolemma. The primary source of regulatory Ca^{2+} for cardiac contraction in nonmammalian vertebrates is predominantly extracellular. A general feature of mammalian ventricular cardiac muscle is that transverse tubules (T-tubules) and junctional SR are arranged to reduce the radius for diffusion of Ca^{2+}. A well-developed T-tubule network in mammalian ventricular myocytes facilitates spatially homogeneous Ca^{2+} entry across the width of the cell (63). The sarcolemma on the surface of the myocyte is continuous with the membrane of the T-tubule. The T-tubule is

the functional bridge between the sarcolemma and SR, bringing sarcolemmal L-type Ca^{2+} channels, or dihydropyridine receptors (DHPRs), close to the SR Ca^{2+}-release channels, or ryanodine receptors (RyRs) (630). A well-developed T-tubule network ensures uniform Ca^{2+} entry across the myocyte (63). In contrast to mammalian models, the influx of extracellular Ca^{2+} across the sarcolemma of fishes and amphibians is largely responsible for the Ca^{2+} binding to troponin C (TnC) and activation of myocyte contraction. In the rainbow trout, trans-sarcolemmal influx of Ca^{2+} is the major (~90%) source of Ca^{2+} for cardiac power production between 12°C and 22°C (523). Generally, in most fishes, it is assumed that there is very little contribution by SR Ca^{2+} to the contraction-relaxation cycle (615). However, ultrastructural and functional studies in rainbow trout provide evidence for a physiological role of the SR in cycling Ca^{2+} during E-C coupling (2,273,501,521,524). Differences in the ventricular cardiomyocyte dimensions may be an important contributing factor to a greater dependence on transarcolemma Ca^{2+} flux in ectotherms.

Relative to skeletal muscle, cardiac myocytes are smaller (diameters < 20 μm), contain larger (diameter 200 nm) T-tubules associated with a diminished SR component, consistent with a significant dependence on extracellular Ca^{2+}. Diameters of cardiac myocytes in fish, frog, and lizard hearts are smaller than mammalian ventricles, while fish, amphibian, reptilian, and bird hearts lack T-tubules entirely (54, 55, 433, 501). In the absence of a T-tubular system, diffusion distance from the cardiac sarcolemma to the innermost myofilament is minimized in fishes and amphibians because of the small myocyte diameter (174, 433, 501). Fish cardiac myocytes can be elliptical in cross section (109) and contain myofibrils located around the periphery of the cell, with mitochondria in a more central location (109, 643). Based on cell dimensions, the predicted surface-area-to-volume ratio of fish cardiomyocytes is five to 10 times greater than in mammalian cardiomyocytes (594). This large surface-area-to-volume ratio allows sarcolemmal Ca^{2+} flux alone to provide adequate intracellular Ca^{2+} for activation of myofilaments.

A large transsarcolemmal Ca^{2+} gradient in cardiac myocytes requires that control of the L-type Ca^{2+} channel be central to the I_{Ca} and the rise in cytosolic Ca^{2+}. L-type I_{Ca} appears in all mammalian cardiac myocytes. The L-type classification, with the *L* signifying *long-lasting,* includes the following criteria for cardiac L-type channel identification: activation by strong depolarizations, high sensitivity to dihydropyridine agonists and antagonists, relatively slow activation kinetics, Ca^{2+}-dependent inactivation with little voltage-dependent inactivation (i.e., *long-lasting*), and large single-channel conductance (353, 421). The transsarcolemmal I_{Ca} density is apparently quite similar for amphibian and mammalian species (373, 594). It is important to note that while useful for grouping, classifications of Ca^{2+} channel types are oversimplifications; there are substantial differences in the activation and inactivation kinetics among L-type channels (40). Sarcolemmal L-type Ca^{2+} channels are rapidly activated by depolarization and regulated by intrinsic G proteins as well as intracellular cyclic adenosine monophosphate (cAMP) levels in mammalian, amphibian, and fish hearts (304, 481). β-Adrenergic activation of Ca^{2+} channels and cardiac inotropism is mediated through a classic signaling pathway involving occupation of the β-adrenergic receptor by an agonist, activation of a guanosine triphosphate binding protein, stimulation of adenylyl cyclase, increased production of cAMP, dissociation of the regulatory and catalytic subunits of the cAMP-dependent protein kinase A, and phosphorylation of several proteins including the L-type Ca^{2+} channel. β-Adrenergic stimulation increases I_{Ca} twofold to threefold in fish hearts (273, 613) and sevenfold to tenfold in frogs (188). Channel phosphorylation increasing the probability that the channel will be open, increasing I_{Ca} (70, 95).

In mammalian cardiac muscle, the junctional gap between the inner surface of the sarcolemma and the SR is just 10 to 15 nm, and the junctional SR is typically situated less than 1 μm from the I-band of muscle filaments. DHPRs appear to be concentrated at sarcolemmal junctions with the SR (198). These junctional processes are defined most clearly in birds and mammals that have very fast heart rates (e.g., mice, bats, and passerine birds) (547) and rapid cycling of intracellular Ca^{2+} levels. In cardiac myocytes from nonmammalian vertebrate, junctional processes are either absent or more difficult to find than in mammals (40). In mammals, the RyR/DHPR ratio correlates with SR dependence for contractile activation (44). The density of RyRs and the RyR/DHPR ratio are significantly higher in the rat ventricle compared with fish (burbot, carp, dogfish, hagfish, and trout), indicating limited importance of Ca^{2+}-induced Ca^{2+} release from the SR (CICR) during E-C coupling in the fish heart (590, 595). The percentage of cell volume occupied by SR is more than 10 times higher in the ventricular myocytes in rodent hearts (6.9% in the mouse; 3.5% in the rat) compared with fish (0.5%), frog (0.4%-0.5%), and lizard (0.7%) myocytes (54, 190, 432, 433, 501). Results from studies of the frog heart show the absence of CICR, suggesting that the cardiac E-C coupling mechanism in this group of ectothermic vertebrates relies primarily upon direct activation of the myofilaments by simple diffusion of Ca^{2+} from the sarcolemma, and that the SR is of little or no importance as a Ca^{2+} storage site or amplifying mechanism (164).

For all species studied, the degree of contractile activation is determined in part by the amount of Ca^{2+} binding at the single, low-affinity binding site on thin-filament protein TnC. Ca^{2+} binding in vertebrate striated muscle triggers a cascade of protein conformational changes culminating in cross-bridge cycling between actin and myosin filaments. The complex consists of three proteins: TnC, the Ca^{2+} binding protein; troponin I, which inhibits the myosin head from binding to the actin filament; and troponin T, which is associated with tropomyosin. In contrast to findings for mammalian and avian species, intraspecific and interspecific differences in cardiac TnC have not been demonstrated in fishes (212, 214). The binding of Ca^{2+} to the regulatory TnC initiates cross-bridge formation between the myofilaments and myocyte contraction. As intracellular Ca^{2+} rises, the TnC binding site has to

compete with other Ca^{2+}-binding sites on the inner sarcolemmal surface, mitochondria, SR, and in the cytoplasm. Studies of myofilament sensitivity to Ca^{2+} in intact ventricular muscle demonstrate high affinity with a Michaelis constant (K_m) of ~600 nmol/L and cooperativity (Hill coefficient = 4-6) (25, 209, 644). These data also suggest a steep force dependence on Ca^{2+} between 300 and 800 nmol/L. In mammalian ventricular muscle, addition of ~60 μmol Ca^{2+}/L cytosol will activate a normal twitch (40). The affinity of the contractile elements for Ca^{2+} can also change with temperature.

Temperature sensitivity of vertebrate cardiomyocytes

Many ectotherms are exposed to wide fluctuations in temperature on a daily or seasonal basis. Temperature has a profound effect on the maximum Ca^{2+}-activated force (C_{max}) in amphibian and mammalian cardiomyocytes (245-247, 569). As cardiac temperature decreases, the Ca^{2+} affinity of the contractile elements diminishes dramatically, and cardiac function becomes impaired (106, 245, 247). Normal temperature (5°C) for eurythermal fishes, such as the rainbow trout would be cardioplegic for the mammalian heart. When rabbit cardiac tissue is cooled from 36°C to 1°C, C_{max} is reduced by 89% and the Ca^{2+} required for half-maximal contraction increases (sensitivity decreases) more than fivefold (from 3.4 to 18.6 μmol/L, 310). In the frog *Rana pipiens*, C_{max} can change almost fivefold over the temperature range of 1°C to 22°C (247). When compared to cells from rat and rabbit (106) at the same temperatures, skinned ventricular fibers from trout hearts required 10 times less Ca^{2+} to generate the same measure of twitch force. The decrease in Ca^{2+}-activated force that occurs at lower temperature is due to a reduction in Ca^{2+} affinity of cardiac TnC and a decrease in the maximal velocity of actomyosin ATPase in both cardiac and skeletal muscle (212). Functional differences also exist between trout and mammalian cardiac RyRs. Namely, the trout RyR appears less sensitive to cold temperatures than the mammalian RyR; this feature might help maintain SR Ca^{2+} release and cycling over a wide temperature range (273, 525, 534). Decreasing temperature may increase Ca^{2+} availability by either prolonging action potentials or depressing Na^+/K^+ ATPase activity, increasing intracellular Na^+ and activation of reverse mode Na^+/Ca^{2+} exchange in the sarcolemma (148).

Ion flow through cardiac L-type Ca^{2+} channels in both mammals and rainbow trout is extremely temperature sensitive, with rate change with a 10°C change in temperature (Q_{10}) values of 1.8 to 2.1 for acute temperature change *in vitro* (315,522). Trout sensitivity to ryanodine, which inhibits function of the SR Ca^{2+} release channel, was insignificant at 5°C to 10°C, but well developed at 20°C to 25°C (272). This suggests diminished importance of the SR to E-C coupling at reduced temperatures, which agrees with similar studies of mammalian ventricular muscle between 22°C and 37°C (518). The SR Ca^{2+} release channel is functional at warmer temperatures (18°C) for rainbow trout hearts, but not at cold temperatures (8°C) and routine physiological heart rates (>0.6 Hz). Acclimation of the rainbow trout to low temperature results in a greater capacity of the SR to store releasable Ca^{2+}, or an increase in the amount of Ca^{2+} that is in a releasable form (305). An increase in adrenergic sensitivity may be an important compensatory mechanism for maintenance of transsarcolemmal Ca^{2+} influx through L-type Ca^{2+} channels during acute exposure of the fish heart to cold temperatures (524), although not for thermal acclimation (613). Cold acclimation shortens action potential duration through changes in repolarizing potassium ion (K^+) currents: the inward rectifier is depressed, and the delayed rectifier is increased. However, further investigations are needed to fully understand the mechanisms involved in cardiac cold acclimation and the degree the capacity to acclimated myocyte function to cold exposure is conserved across species.

Relaxation of the vertebrate cardiomyocytes

For relaxation to occur, Ca^{2+} must dissociate from TnC and removed from the cytoplasm to lower Ca^{2+} concentration back to diastolic values. One option for the reduction of cytosolic Ca^{2+} is activation of the Ca^{2+} ATPase of the cardiac SR (SERCA2a). SERCA2a pumps Ca^{2+} from the cytoplasm of myocyte to the lumen of the SR using adenosine triphosphate (ATP) hydrolysis (385). To sustain active Ca^{2+} transport, SERCA2a alternates between at least two states with different affinities for Ca^{2+} and opposing orientation of the Ca^{2+}-binding sites on the plane of the membrane (574). Mammalian cardiac muscle has lower Ca^{2+} ATPase activity than fast-twitch muscle, due in part to the presence of integral protein phospholamban (PLB) in the SR (573). Studies of the ATP dependence of ATP hydrolysis show that the K_m for ATP is similar in cardiac and skeletal muscle preparations. Under β-adrenergic stimulation, PLB is phosphorylated by cyclic AMP-dependent protein kinase (PKA). The phosphorylation of PLB, either by PKA or by Ca^{2+}/calmodulin-dependent protein kinase, leads to an increase in both the maximal rate of Ca^{2+} uptake by the Ca^{2+} ATPase and in its affinity for Ca^{2+}. Relaxation can also be enhanced by phosphorylation of TnI results in a decrease in myofilament Ca^{2+} sensitivity and enhanced off-loading of Ca^{2+} from TnI (503).

A second option for cardiac muscle relaxation involves the sarcolemmal Ca^{2+} transport systems. The amount of Ca^{2+} entry via L-type Ca^{2+} channels must be extruded from the myocyte through the sarcolemma by the end of each cardiac cycle to maintain a steady state. Sarcolemmal Na^+/Ca^{2+} exchange can move Ca^{2+} either into or out of the cardiomyocyte, depending on membrane potential and the Na^+ and Ca^{2+} gradients (8). Intracellular Ca^{2+} exerts a positive influence on Na^+/Ca^{2+} exchange, and the Na^+/Ca^{2+} exchange functions at a rate comparable to twitch relaxation. Na^+/Ca^{2+} exchange appears to be the major mechanism for relaxation in frog and trout myocardia (102, 594). In contrast, in rabbit ventricular myocytes, reuptake of Ca^{2+} by the SERCA2a contributes 68% to relaxation, and Na^+/Ca^{2+} exchange at the sarcolemma contributes 30% to relaxation (43). For a

more focused discussion of mammalian E-C coupling, consult (40, 42, 275, 403).

The heart functions as a syncytium with the electrical activation of relaxed cardiac myocytes resulting in a twitch contraction with an amplitude proportional to that of the Ca^{2+} transient (41, 303, 340, 429). As such, E-C coupling is a key determinant of cardiac mechanical activity and the degree of activation. Similar to skeletal muscle, Ca^{2+} regulates the interaction between myosin and actin filaments in cardiac muscle. The amount of tension, regulated by the number of filament cross bridges formed, varies with the concentration of intracellular available Ca^{2+}. However, unlike with skeletal muscle, contraction of cardiac muscle is graded. Increasing free iCa^{2+} in mammalian cardiac muscle from resting diastolic values (~260 nmol/L) to an elevated level (~10 μmol/L) results in a progressive increase of tension development to maximal values (209, 364). Half-maximal activation of contraction requires an intracellular Ca^{2+} concentration of about two to three times resting values [600 nmol/L (41)]. In addition to the importance of Ca^{2+} development of tension in cardiac muscle, physiological and physical parameters affect cardiac mechanical activity.

Cardiac structural considerations

The function of mammalian cardiac muscle is considered in detail in other sections of *Comprehensive Physiology* volumes 1 through 5, and in previous chapters in the *Handbook of Physiology* series (75, 251). For detailed reviews and discussions on the important topic of fundamental myocardial mechanics as described by force-velocity-length relationships, see (9, 60, 72). The vertebrate ventricle is anisotropic and is comprised of a complex arrangement of extracellular matrix and cardiomyocytes. The muscular structure and geometry of the ventricle varies considerably across species (211), especially among fishes (598). The structural architecture is an important feature that determines differences in the ventricular filling, contractility, ejection fraction, and the ability to generate pressure. Cardiac mass scales isometrically to body mass in fish (463), birds (357), and mammals (119). It appears that relative heart mass is a good indicator of adaptive specialization for prolonged locomotor activities in birds and mammals (48). Active fishes also have relatively larger hearts (180). A larger heart has increased stroke volume and cardiac output, and it provides the convective means to elevate maximal rates of oxygen consumption. Ventricle mass in amphibians is positively correlated with aerobic capacity (318) and enhanced dehydration tolerance (264), allowing the animal to maintain cardiac output and cardiovascular performance.

With few exceptions, cardiovascular performance of ectothermic vertebrates is significantly lower than that of birds or mammals of similar body size. This may be explained in part by species differences in cardiomyocyte morphometrics, E-C coupling, reductions in heart rate, and cardiac power output (75). Small spindle-shaped cardiomyocytes from nonmammalian vertebrates have an extended length-to-width ratio, lack a transverse tubular system, and rely more on transsarcolemmal transport of extracellular Ca^{2+} for E-C coupling than on intracellular Ca^{2+} stores (433, 501, 528, 593). Given this dependence on transsarcolemmal Ca^{2+}, it is logical that the contractile elements, or myofibrils, of nonmammailian cardiomyocytes are located at the periphery of the cell (109, 613). Together with the more irregular lattice structure (485), this may reduce diffusional gradients and allow for greater adjustments in stroke volume over a wide range of environmental conditions. Studies of some fish and amphibians suggest that this architecture of cardiac myocytes from these vertebrates can develop force and perform work over a greater range of sarcomere lengths than mammalian cardiomyocytes can, conveying an advantage to these ectothermic vertebrates (440, 528).

Diastole: Filling the ventricle

Lusitropy describes the ability of the heart to fill at any diastolic pressure. Lusitropy is influenced by passive viscoelastic properties of cardiac tissue and by dynamic interactions between cardiac contractile proteins (506). The passive tension, or stiffness, of cardiac muscle plays a critical role in ventricular filling, and it is determined by the extracellular matrix and by the giant sarcomeric protein titin, which is also called connectin (12, 307). For the mammalian heart, another key determinant of ventricular filling and rapid relaxation is β-adrenergic stimulation, which desensitizes the contractile proteins to Ca^{2+} and increases the Ca^{2+} sensitivity of the SR Ca^{2+} ATPase (302). Basically, sympathetic stimulation promotes more rapid relaxation and elastic recoil of the ventricle, leading to a longer diastolic filling period and increased stroke volume of the next beat (104, 437, 564).

Relative to skeletal muscle, cardiac muscle is very stiff. As a result, passive tension of the myocardium plays an important role in the Frank-Starling mechanism (or Frank-Starling law of the heart) by affecting ventricle filling (528). This intrinsic mechanism, in which increased diastolic volume leads to increased systolic contraction, applies to all vertebrates and coordinates changes in stroke volume (40, 527). The degree of ventricular filling is determined by ventricular compliance (or stiffness). Compliance is the ratio of change in ventricular volume to the change in ventricular diastolic pressure ($\Delta V/\Delta P$); ventricular stiffness is the inverse [change in pressure for a given change in volume ($\Delta P/\Delta V$)] (348). Molecular studies highlight the importance of titin to passive tension of the sarcomere length-tension relationship (306). Titin binding to cardiac myosin-binding protein C (cMyBP-C) may also modulate passive stiffness (434), and titin allows the actomyosin cross bridge to sense stretch (201).

In the mammalian heart, ventricular filling occurs in response to a pressure differences from central venous and atrial. It begins with a rapid-filling phase followed by a slower-filling phase, also called diastasis. Normally, most ventricular filling occurs during early diastole. Atrial contraction accounts for less that 1/3 of ventricular filling at rest.

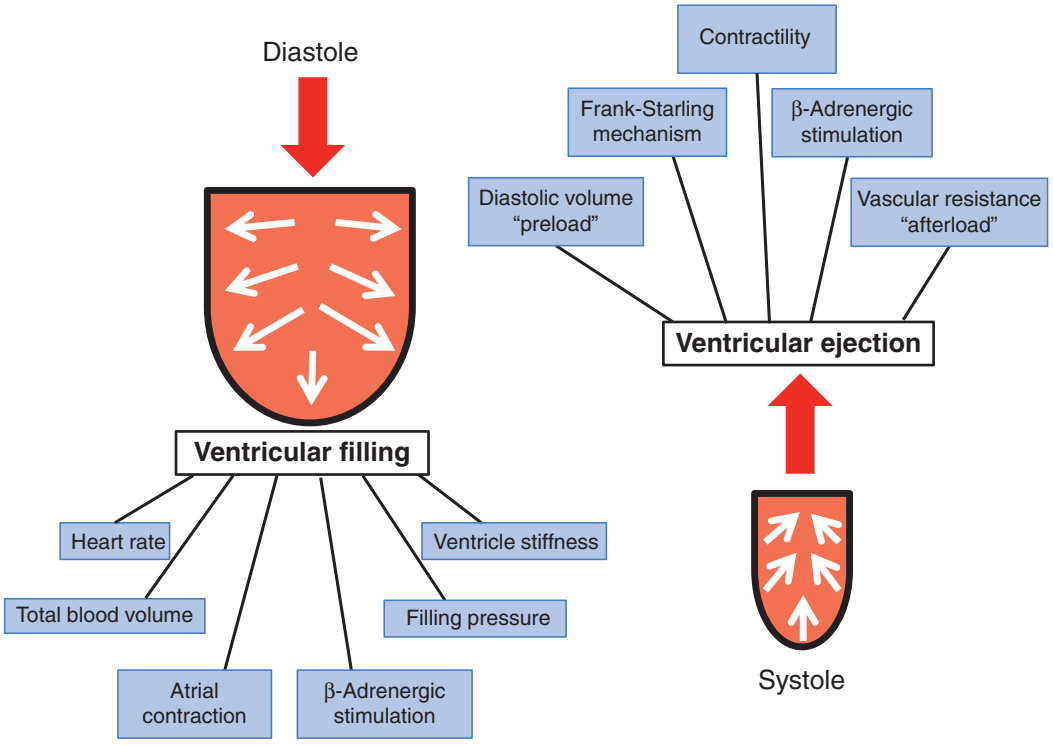

Figure 8 A list of known regulators of ventricular function during a cardiac cycle in vertebrates. Although blood is shown as red, reflecting complete oxygenation, the vertebrate ventricle may pump different degrees of oxygenated or deoxygenated blood. See text for details.

A summary of major determinants of ventricular filling and contraction is shown in Figure 8. During exercise with high heart rates, diastole is shortened proportionately more than systole, and atrial contraction can make a 40% greater contribution to ventricular filling (97). Adequate filling of the ventricles also occurs, despite a very brief diastole, because sympathetic activity increases the rate of ventricular relaxation, as explained earlier. In fishes, amphibians, and reptiles, atrial contraction plays a prominent role in ventricular filling, apparently due to the presence of valves or structures just upstream of the atria (291). For most teleosts, the volume of the atrium is equal to or greater than the volume of the ventricle (191), and atrial contraction is the sole determinant of ventricular filling (117, 180, 292, 471). However, similar to mammals, some elasmobranchs and teleosts have biphasic filling patterns with early ventricular filling followed by atrial systole (124, 335, 336, 338).

Most vertebrates exhibit *vis-a-tergo* (literally, force from behind) filling of the heart, in which central venous pressure is the primary determinant of both atrial and ventricular filling. However, atrial filling in fishes occurs via both *vis-a-fronte* (literally, force from in front) and *vis-a-tergo* mechanisms (180). Although rainbow trout have a semirigid pericardium, venous (*vis-a-tergo*) pressure and active participation of veins appears to be the primary determinants of venous return and cardiac output (392). With *vis-a-fronte* filling, ventricular contraction in elasmobranchs and certain active fishes creates a subambient, or suction, pressure within the pericardium, distends the atrium, and assists in atrial filling. Venous capacitance is an important means of regulating venous return and cardiac performance in elasmobranches (499). Regardless of the filling mechanism and extent, diastolic ventricular distension sets up the Frank-Starling mechanism, which results in a direct correlation between changes in venous return and stroke volume, and regulates cardiac output.

Systole: Ventricular contraction

Sufficient blood pressure must be developed within the ventricle to eject blood through the valve and into the circulatory system. During ventricular contraction, atrial pressure is lower than ventricular pressure and the atrioventricular valves remain closed. Dimensional changes in the ventricle due to slight alterations in myocyte length during isovolumetric contraction raise ventricular pressure without changing ventricular volume until it reaches the level of aortic diastolic pressure. Once ventricular pressure exceeds aortic pressure, ending the isovolumetric phase of systole, the aortic valve opens, the ventricular myocardium shortens isotonically, and cardiac ejection begins.

Cardiac contractility describes the change in developed force at a given preload and afterload, defining the intrinsic performance of cardiac muscle. Preload, is the stretching of cardiac muscle by blood filling the ventricle prior to contraction, while afterload is the tension in the ventricle generated when blood is ejected during systolic contraction, against

vascular resistance (118). Contractility, which equals inotropic state (the ability of the tissue to contract at a given preload and afterload) can be measured as maximum or peak isometric tension in isolated cardiac muscle, or by a wide spectrum of approaches developed using the intact mammalian heart (118). Developed force is directly related to the Ca^{2+} concentrations at the myofilaments, independent of its source (164). An increase in heart rate is associated with the contractility of the mammalian heart, in both the atrial and ventricular muscle. Increased heart rate is often associated with increased sympathetic stimulation, which increases noradrenaline and adrenaline, and activates intracellular adenylate cyclase. This, in turn, increases the production of cAMP, which activates PKA, and ultimately causes an increased influx of Ca^{2+} through L-type calcium channels (601). This positive force-frequency relationship is discussed in the section "Effects of Heart Rate and Contraction Frequency on Contractility."

The rate of change in pressure (dP/dt) during isovolumic contraction has been used as an index of ventricular contractility, and it varies widely among vertebrates. For tuna, dP/dt values are roughly five times higher than values for other teleost fishes [49.0-64.0 kPa s^{-1} (132)], while values for sharks [3.3-4.8 kPa s^{-1} (338)], hagfish *Myxine glutinosa* and *Eptatretus cirratus* [~3 kPa s^{-1} (132)] are even lower. Although hagfish are capable of resting cardiac output similar to that of less active teleosts (8-15 mL min^{-1} kg body $mass^{-1}$), they have the lowest blood pressure of any vertebrate [0.7 kPa (290)]. Intermediate values of dP/dt are seen in the varanid lizard *Varanus exanthematicus* [12.7-24.0 kPa s^{-1} (76)], Western painted turtles *Chrysemys picta bellii* [~10.7 kPa s^{-1} (623)], and anuran amphibians *Rana catesbeiana* [8.0 kPa s^{-1} (265)], and *Bufo marinus* [14.7 kPa s^{-1} (265)]. The cellular and mechanical basis for this variability in contractility across vertebrates is unknown, but likely involves differences in the structural architecture of cardiac tissue and myocardial energy metabolism.

Increasing ventricular stroke volume requires increased preload and or increased cardiac contractility by either intrinsic or extrinsic mechanisms. Intrinsic cardiac length-force characteristics are the defining aspect of the Frank-Starling mechanism (550). This mechanism describes how an increase in muscle length increases contractility (7). At a given intracellular concentration of Ca^{2+}, initial fiber length determines the number of active force-generating sites in the cardiomyocytes and changes in the lattice spacing between thick and thin filaments defines the probability of myosin binding to act. However, contractile state can be regulated independent of changes in sarcomere length. Factors such as cardiac temperature, intracellular pH, inorganic phosphate, ionic strength, and a number of inotropic agents can affect myofilament Ca^{2+} sensitivity and, therefore, the relationship between Ca^{2+} concentration and force generation (40). In the mammalian heart, the Frank-Starling mechanism also ensures a long-term balance between the output from the right and left ventricles (85): Systemic venous return must match the systemic cardiac output. If systemic venous return to the mammalian heart is increased, right ventricular preload increases, increasing stroke volume and pulmonary blood flow. Elevated pulmonary blood flow and venous return to the left atrium leads to increased preload of the left ventricle, which ultimately increases left ventricular stroke volume and cardiac output (317).

Ejection fraction, which is the ratio of stroke volume to the end diastolic volume, provides another measure of myocardial contractility. However, factors other than contractility, such as preload, afterload, and heart rate, can affect ejection fraction. Average left ventricular ejection fraction averages 0.51 (159) to 0.67 (308) for humans. Depending on the experimental conditions, values range 0.45 to 0.70 for canines (602), and ejection fraction decreases when heart rate increases (162). For fish, end systolic volume of the ventricle is much lower, resulting in much higher ejection fractions in fishes than in mammals. Rainbow trout ejection fraction approaches 1.00 (197); and Lai et al. (338) reported a ventricular ejection fraction of 0.80 in leopard shark *Triakis semifasciata.*

The sympathetic system enables the heart to increase or maintain stroke volume against higher pressure, independent of diastolic filling and the degree of stretching of the myocardium. This increase of contractility is called *positive inotropy*. It is also established that vascular resistance, or afterload, against which the ventricles contract, influences ventricular ejection significantly (355, 548). Similar to the process in mammals, homeometric regulation of stroke volume in the rainbow trout ventricle occurs by comparable increases in end diastolic and end systolic volume (197). Homeometric regulation can also be described as intrinsic length-independent regulation of inotropic state. As a group, fishes can exert adrenergic control of the heart via endogenous catecholamine stores (seen in cyclostomes and dipnoans), exogenous catecholamine release from chromaffin tissue (seen in elasmobranchs and teleosts), and direct adrenergic innervation (seen in holosteans and teleosts) (180, 416).

Regulation of cardiac output: Heart rate versus stroke volume

Cardiac output varies with changes in heart rate and stroke volume, and is dictated by rate, force of contraction, afterload, and preload. Fishes and some reptiles have lower heart rates than mammals do and use very modest increases in heart rate to increase cardiac output. Unlike mammals, fishes respond to different hemodynamic loads by increasing stroke volume rather than heart rate (468,599) which in cyclostomes, elasmobranchs, and teleosts, can equal as much as a twofold increase during exercise (75). Overall, fish appear to rely more on volume regulation of the heart than frequency modulation for increasing cardiac output during aerobic exercise (178,180). Based on a study of enforced exercise, amphibians

appear to have a limited capacity for increased heart rate (1.8-fold) and stroke volume (–7%) to accommodate the observed increase of oxygen consumption (8.3-fold) above resting values. Instead, there is a marked increase in arterial PO_2 and decreased venous PO_2, enhancing the arteriovenous blood O_2 difference during activity (639). However based on the species diversity evident in fishes and amphibians, investigations of the role of stroke volume to increase cardiac output should continue to be explored.

Effects of heart rate and contraction frequency on contractility

The force-frequency relationship describes the ability to develop force or tension at different contraction frequencies. Basically, with an increase in heart rate there is an increase in the contractile state of the heart. Measurements of Ca^{2+} transients during a single twitch suggest that the iCa^{2+} is not sufficient to saturate TnC (158), and that a positive staircase is due to an increase in available Ca^{2+}, allowing for a greater number of force-generating cross bridges to bind and increase tension development (641). Underlying the positive staircase in mammalian hearts is an increase in SR Ca^{2+} content and release (43). In ventricular tissue from mammals, frogs (60), and several species of elasmobranchs (147), and in atrial tissue from skipjack tuna (304), there is an additional positive relationship between heart rate and contractility at low pacing frequencies.

The effect of stimulation frequency on active tension, or maximum isometric tension, has been measured in a wide variety of fish species (526). The majority of fish hearts exhibit a negative force-frequency relationship, or negative staircase, with contractile force decreasing as contraction frequency increases. A negative force-frequency response limits the ability to increase cardiac output via heart rate, explaining why most fish vary stroke volume to meet increasing cardiac demand (180).

Effect of β-adrenergic stimulation on contractility

β-Adrenergic stimulation of frog and fish cardiac muscle increases maximum contractile force production by several times (19,211,304,612) via increasing cardiac myocyte influx of Ca^{2+} through L-type Ca^{2+} channels (273,613). In the frog *Rana esculenta*, rainbow trout *Oncorhynchus mykiss*, and flounder *Platichthys flesus*, the degree of positive inotropy induced by β-adrenergic stimulation is species-specific and dependent on contraction frequency and temperature (19). Under sympathetic stimulation, the decrease in contraction force associated with temperature-dependent tachycardia can be more pronounced than in controls. Contractility of the rainbow trout heart can be maintained at cold temperature (7°C) due to increased sensitivity to adrenaline (524). β-Adrenoreceptor agonists increase cardiac isometric force production by increasing the open probability of L-type Ca^{2+} channels in amphibian and mammal hearts (481, 526). However, in atrial tissue from skipjack tuna, *Katsuwonus pelamis*, adrenaline increases force production and the duration of contraction with no change in the rates of contraction or relaxation (304).

Effects of temperature on heart rate and contractility

Ectotherms such as amphibians, fish, and reptiles can have variable body temperatures, and their hearts must function over a range of environmental conditions. The firing rate of the pacemaker cells will be reduced significantly at cold temperature, potentially leading to significant reduction in cardiac output. Cold temperature can reduce contractility and stroke volume by reducing myofilament Ca^{2+} sensitivity (213). However, a slower heart rate increases diastolic filling time and end-diastolic volume during exposure to cold temperatures, thus increasing myocardial stretch, contractility, and stroke volume through the Frank-Starling mechanism (528). Maximum Ca^{2+}-activated force can increase almost fivefold between 1°C and 22°C in chemically skinned cardiomyocytes from frogs and mammals illustrating the importance of temperature on cardiac contractility (245, 247).

Given adequate time for temperature acclimation (3-8 weeks), the hearts of some eurythermic species (e.g., rainbow trout, striped bass, and perch) can compensate for the effects of cold temperatures to adjust contractility (2, 146, 487). For example, relative ventricular mass can nearly double with cold acclimation in rainbow trout (226). Heart rate and force production increases, while the duration of contraction and refractoriness of the heart decreases (2,146). Action potentials are shorter in cold-acclimated fish, and this may be the underlying mechanism for decreased refractoriness and higher heart rates in the cold. Prolonged twitch duration at cold temperatures ultimately allows more complete recruitment of cross bridges and alleviates the increased Ca^{2+} requirements of less-sensitive myofilaments (614).

In contrast to the effects of cold temperature, an increase in temperature of cold-acclimated eurythermic animals increases heart rate and velocity of contraction, while the force of contraction is reduced (272, 398, 612). Action potential duration and time to maximum tension (T_{max}) decrease with a Q_{10} of ~2.0 in electrically paced ventricle strips from ranid frogs and rainbow trout (26, 75). Heart rate, both in vitro and in vivo, also increases with temperature, with a similar Q_{10} value (2.0-2.3) (75) and relaxation time is also shorter with increasing temperature. For ectotherms, warm body temperatures may be problematic for volume modulation of cardiac output, because warm temperatures cause decreased diastolic filling times and high Ca^{2+} sensitivity of myofibrils, which limits off-loading of Ca^{2+} (593). Given that most vertebrate hearts use aerobic metabolism for ATP production, myocardial O_2 supply and O_2 consumption may not keep pace with elevated myocardial energy demands at

higher body temperatures. Overall, the pumping capacity of the fish heart is optimal at the physiological body temperature (521, 526), although phenotypic plasticity involving cardiac structure and function can enable such eurythermal teleosts as rainbow trout to remain active over a wide temperature range (1°C-25°C).

Conclusions and future directions

Given the wide range of physical activity capabilities and oxygenation requirements, it is not surprising that the vertebrate heart exhibits considerable structural and functional diversity. Although our understanding of the vertebrate cardiovascular system is due in large part to studies on mammals, ectotherms highlight functional capabilities over a wider range of environmental conditions and exhibit pronounced plasticity in both structure and function. This section provided a brief overview of vertebrate cardiac structure, myocyte ultrastructure, activator calcium, and EC coupling for the contractile process. A common theme for all vertebrate hearts is selective regulation of protein channels, molecular motors, ion pumps, and the use of signaling pathways to modulate cardiac contraction and relaxation. Regulation of cardiac filling and contraction is complex, varies between taxa, and is ultimately determined by extrinsic and intrinsic factors. Future comparative studies of cardiovascular responses will likely enhance our understanding of ontogeny, physiological adaptability, and pathology. In particular, these efforts should link molecular mechanisms with functional characteristics at the subcellular, cellular, and tissue levels, with an overarching challenge to define the limits of cardiovascular homeostasis in the face of environmental stressors.

Peripheral Circulation and Hemodynamic Regulation

Cardiovascular homeostasis and maintenance of convective transport are reliant on numerous regulatory systems. These systems can be loosely grouped into three categories: tonic central nervous system (CNS) control; reflexive CNS control; and humoral control. Tonic CNS control can be defined as continuous efferent motor output to the heart and vasculature maintaining function within an operational range. Reflexive CNS control is an alteration in motor output in response to transient deviation of a parameter from a set point, and it is relayed via changes in afferent information from a sensory structure. Humoral control is used as a generalization, encompassing both local and systemically released cardioactive and vasoactive compounds. Variations in these systems alter cardiovascular function to meet periods of elevated oxygen demand. We will provide a general overview of cardiovascular regulators and elaborate on recent findings that advance our understanding of cardiovascular regulation in vertebrates. We do not present an exhaustive review of the advances in the 15 years since the most recent edition of the *Handbook of Physiology,* as these advances have been partially reviewed previously (581,582), but we have emphasized highly relevant studies during this time (81).

Tonic central nervous system regulation of function

Cardiovascular physiologists have classically utilized pharmacological tools to assess the relative contribution of both the parasympathetic and sympathetic limbs of the autonomic nervous system to maintain cardiovascular function. These "baseline tones" as well as the anatomical descriptions of the CNS and cardiovascular connections, have been previously summarized for representatives of all vertebrate taxa (414, 416, 498). Although the list of vertebrates that have been investigated is limited to a handful in relation to overall species diversity, it includes representatives of each vertebrate taxa, and we can make general statements regarding the contribution of the parasympathetic and sympathetic nervous systems.

Parasympathetic or tonic vagal control of the adult heart is evident in the elasmobranchs, with myxinoids (hagfish) lacking vagal innervation and lampetroids displaying a unique nicotinic-receptor-mediated cardiac excitation (581, 583). Despite the unique nature of the stimulatory cholinergic cardiac response in lampetroids, it remains to be determined if a stimulatory vagal tone is present in this group. In the elasmobranchs, vagal tone is evident and it exhibits the typical inhibition of heart rate, a pattern that is found in the majority of both aquatic and terrestrial vertebrates with varying degrees of intensity (581).

An exception to the universal presence of a cardiac vagal tonus among teleost fishes and terrestrial vertebrates is its absence in the African lungfish *Protopterus aethiopicus* (294). Although vagal tone has been primarily studied in relation to its effects on heart rate, vagal efferent innervation has been identified in the pulmonary vessels of lungfish (582), the pulmonary artery in anuran amphibians (341, 541, 619), in chelonians, (80, 256, 391, 450), and in squamate reptiles (351, 582). In addition, cholinergic control of the pulmonary cogwheel valves, and possibly also the pulmonary vessels, has been identified in crocodilian reptiles (361, 571). In each study, vagal stimulation or application of acetylcholine (Ach) resulted in an increase in pulmonary vascular resistance (80, 256, 341, 351, 361, 391, 450, 541, 571, 579, 619). These studies include both anatomical and pharmacological assessments of the innervation as well as cholinergic-receptor-mediated responsiveness; however, limited information is available on the tonic contribution to pulmonary vascular tone.

Unlike the relatively exclusive chronotropic effects of vagal tone, the sympathetic division of the autonomic nervous system potentially influences cardiac contractile frequency and strength, and vascular resistance. Cyclostomes

and elasmobranchs lack sympathetic innervation of the heart; however, catecholamines are tonically released from cardiac chromaffin tissue in the genus *Myxine*, or hagfish (23), and possibly from the sinus venosus in elasmobranchs (497). The function of this tissue has yet to be established, and therefore, it remains unclear how the cardiac chromaffin tissue contributes to adrenergic tone on heart rate (470). Sympathetic innervation of the heart appears in bony fishes, with the exception of the dipnoans and sturgeon (29, 344), and it is retained in all terrestrial vertebrates (582). Sympathetic innervation is accompanied by an adrenergic tonus on the heart due to presynaptic release that varies in intensity depending on the species. Sympathetic and adrenergic tone on the heart is functional in most species, but there are exceptions, such as the pacu, *Piaractus mesopotamicus,* which lacks adrenergic tonus on the heart, though sympathetic innervation is present (579, 582). It is important to note that *in vivo* studies require surgical procedures and experimental manipulations that undoubtedly alter the relative intensity of the sympathetic, as well as vagal, tone on the heart. Therefore, relative differences in sympathetic tone between species may reflect both phylogenetic differences and methodological differences between studies.

The existence of a vascular tone dependent on adrenergic receptors has been demonstrated on the systemic and gas-exchange vasculature across vertebrates, and it is classified based on the types of adrenergic receptor located on the tissue: α-adrenoceptors and β-adrenoceptors. However, the source of this adrenergic tone, from either sympathetic neurons or chromaffin tissue, has only been identified in few species. Hagfish responds to catecholamine injections with an increase in vascular resistance, although the tonic contribution of catecholamines to vascular resistance has not been determined for hagfish or lampreys (23). Elasmobranchs have an α-adrenergic dependent vasoconstriction on the systemic vasculature (451,502) and skates possess adrenergic receptors in the branchial vasculature that may be stimulated by circulating catecholamines (126). Teleost fishes are the most extensively studied ectothermic vertebrate in regards to adrenergic vascular tonus, and the origin of this tone has been identified (416). In general, a pronounced adrenergic tone is evident in teleost somatic, gas-exchange, and gastrointestinal circulations. In the Atlantic cod *Gadus morhua*, a sympathetic originating α-adrenoceptor tonic constriction on the dorsal aorta is present (472, 542), with a systemic β-adrenoceptor tonic dilation (416). In several fish species, the branchial vasculature possesses functional adrenergic innervation, which results in vasoconstriction upon stimulation, however the prominent regulator may be circulating catecholamines (418). Tonic α-adrenergic constriction of gastrointestinal circulation has been reported in two teleosts: rainbow trout and Atlantic cod (24,472,512). In Atlantic cod, adrenergic tone is derived from both sympathetic as well as chromaffin-tissue-released catecholamines, which are augmented during exercise and attenuated during feeding (24).

Humoral regulation of cardiovascular function

Numerous systemic and local mechanisms modulate cardiovascular function to ensure arterial pressure is maintained within an effective range. Although nonadrenergic noncholinergic (NANC) factors have been extensively studied in mammals (551), their role in most vertebrate classes is poorly understood. Some important regulatory factors are described below, along with their effects on cardiovascular function in vertebrates; in addition, where data are available, differences in responses within major vertebrate classes are outlined.

Adenosine

The endogenously produced nucleoside adenosine is produced during periods of decreased O_2 availability and increased metabolic demand in numerous mammalian tissues (33, 404). This product of cellular metabolism alters mammalian cardiovascular function by increasing coronary artery conductance, causing acute bradycardia, and a vascular bed-dependent vasoconstriction or dilation (404, 572). As with most regulatory compounds, studies of adenosine have mostly focused on mammalian models, but its basic function in other vertebrate groups has also been investigated.

Given the diversity of fish species, it is not surprising that the function of adenosine differs across fish taxa. It is conceivable that natural hypoxic conditions experienced by hagfish, and the lack of autonomic nervous system innervation of the heart, might augment the functional significance of NANC factors such as adenosine. However, under normoxic conditions, adenosine strictly modulates vascular tone, resulting in a reduction in both gill and systemic resistance, without altering heart rate (23). In elasmobranchs, the typical bradycardic adenosine response seen in mammals is evident, coupled with a reduction in both ventral and dorsal aortic arterial pressure, which suggests a reduction in vascular resistance (558). An adenosinergic tone elevates both ventral and dorsal aortic pressure in anaesthetized sharks (558). In perfused shark cephalic preparations, adenosine produces a bimodal response with an increase in brachial vascular resistance at low concentrations, and a decrease in brachial vascular resistance at high concentrations (38, 445). These in vitro data, in combination with in vivo work, suggest a branchial, and possibly systemic, vasoconstrictive adenosinergic tone that can be overridden by acute adenosine-induced dilation in elasmobranchs.

In bony fishes, adenosine induces a pronounced bradycardia in all species studied to date (3, 565, 566). In addition, a resting inhibitory adenosinergic tone on heart rate has been reported in the crucian carp, *Carassius carassius* (615). Perfused head preparations of the European eel *Anguilla anguilla* illustrate an initial branchial vascular constriction followed by a dilation in response to increasing concentrations of adenosine (445). In the bald notothen, *Pagothenia borchgrevinki,* adenosine produces a biphasic response in the branchial circulation first decreasing and then increasing vascular resistance

(565). In the common carp, an adenosine-mediated tonic vasoconstriction of the ventral aorta is present at low temperatures (555). In trout, the action of adenosine has been isolated to specific regions of the branchial circulation, resulting in an increase in filament vascular resistance and a decrease in systemic resistance (566). This suggests that species, as well as resting versus active state, may dictate the role of adenosine in cardiovascular homeostasis of bony fishes.

Cursory studies have been conducted on the function of purinoceptors in amphibian cardiovascular homeostasis. Initial studies of isolated atria and ventricle preparations identified a negative inotropic and positive chronotropic adenosine function in both anuran and urodele amphibians (86, 382). This differs from data gathered using isolated perfused anuran hearts, in which a noted negative chronotropic and inotropic action of adenosine has been observed (347). These conflicting findings likely can be attributed to methodological differences in the studies. Isolated anuran amphibian aorta show decreased tension in response to exogenous adenosine if preconstricted with adrenaline, suggesting that this nucleoside contributes to a decrease in vascular resistance systemically, as in fish (327).

Cardiovascular actions of adenosine have also been investigated in reptiles, but studies are limited, and the results cannot be generalized across this large polyphyletic group. Initial investigations of isolated aortic ring preparations taken from agamid lizards and the garter snake *Thamnophis sirtalis* documented adenosine-mediated decreases in vascular tension similar to those reported in anuran amphibians (319, 320). At rest, heart rate is inhibited by an adenosinergic tone in the red-eared slider turtle, *Trachemys scripta*; however, when this tone is blocked, compensatory systems return heart rate to baseline values (554).

Endothelin 1

Endothelin 1 (ET-1), a potent vasoconstrictor, is the most extensively investigated of the endothelin family peptides across vertebrate classes. A number of cardiovascular responses to this peptide have been documented in fish. In both freshwater and saltwater fish, ET-1 increases resistance in the systemic and branchial vessels (194). In hagfish ET-1 causes a dose-dependent increase in vascular ring tension with the greatest effect on the venous ring preparations (194). The intensity of the change in vascular tension in the venous side suggests an important regulatory function, but the mechanism of release, as well as the intact cardiovascular response, must be delineated. Endothelin is a potent vasoconstrictor of the gill vasculature in teleost fishes (424), increasing gill vascular resistance in a dose-dependent fashion (267). Bolus injections of native ET-1 in trout produce a dose-dependent increase in pressure, with the exception of the dorsal aorta, which exhibits a triphasic increase-decrease-increase in arterial pressure (267). However, the Atlantic cod lacks this complex response to injections of ET-1, indicating that its function is species specific (557).

Isolated aortic arch rings of the grass frog *Rana pipiens* respond to ET-1 with a dose-dependent increase in tension (453). In the anesthetized red-eared slider turtle, *Trachemys scripta*, ET-1 causes a dose-dependent systemic vasodilation without affecting the pulmonary vasculature or heart rate (536). ET-1 receptors are found in the aorta of snakes *Oxyrhopus guibei* and *Bothrops jararaca* (388), and ET-1 causes either hypertension or a biphasic response consisting of a decrease followed by an increase in arterial pressure (53, 388). Anesthetized American alligators, *Alligator mississippiensis*, injected with native ET-1 exhibit a dose-dependent biphasic response consisting of an initial decrease followed by an increase in arterial pressure (452). Interestingly, the initial decrease is not mediated by secondary release of nitric oxide (NO) (452). ET-1 constricts isolated pulmonary arteries of the American alligator in a dose-dependent manner (537), indicating that ET-1 increases pulmonary vascular resistance and contributes to hypoxic vasoconstriction of pulmonary arteries (537). Further studies must be conducted to determine the significance of the differing response to ET-1 in nonmammalian vertebrates.

Bradykinin

Bradykinin (BK) promotes vasorelaxation in preconstricted mesenteric vascular rings from hagfish, via a non-NO-dependent mechanism (185). In elasmobranchs, BK increases arterial pressure mediated by vasoconstriction of the branchial, mesenteric, and coeliac arteries, in part by an α-adrenergic mechanism (126). In an actinopterygian fish, BK causes a dose-dependent decrease in systemic arterial pressure, suggesting a transition in its role as a regulator of cardiovascular function (350). Bowfin, a holostean fish, responds to BK with an immediate drop in arterial pressure followed by a dose-dependent increase, indicating that the response to this peptide has become more complex during fish evolution (112). BK also affects cardiovascular function in the African lungfish, *Protopterus annectens*, a sarcopterygian fish, producing a dose-dependent increase in arterial pressure and an increase in heart rate at a higher dose (31). In teleost fish, BK causes dose-dependent dilation in preconstricted coeliac arteries of the cod *Gadus morhua* (517); and, in vivo, BK causes an increase in arterial pressure and heart rate attributed to catecholamine release (449). In the rainbow trout, *Oncorhynchus mykiss*, BK injections in the conscious animal produce a triphasic constriction-dilation-constriction response that is adrenergic-receptor dependent (425). Therefore, although the fish cardiovascular system responds to BK, the result of this stimulation is dependent on factors including phylogeny and habitat.

The BK system is apparently absent in amphibians (113), but present in reptiles. In the red-eared slider turtle, BK alters cardiovascular function, causing a rapid vasodilation (111). In the anesthetized South American rattlesnake, *Crotalus durissus*, BK produces a biphasic response composed of an initial NO-dependent reduction in systemic pressure and a final hypertensive tachycardia that is mediated in part by the release

of catecholamines (203). A similar NO-dependent response has been reported for isolated basilar arteries of the pit viper *Trimeresurus flavoviridis* (643). In the python *Python regius*, BK also causes a dose-dependent increase in arterial pressure and heart rate that is partially mediated by catecholamine release (618).

Gaseous regulators of cardiovascular function

NO is a key endogenously produced modulator of cardiovascular function in vertebrates. Specifics of NO production and its mode of action have been extensively characterized in numerous vertebrate models, including mammals, birds, reptiles, amphibians, and fish (121, 123, 204, 399, 452, 535). Several extensive reviews of NO cardiovascular action have been published (30, 426). Although the mechanisms of production of NO are similar, the site of production differs across vertebrate groups.

Three constitutive enzymes produce NO: nNOS, located in autonomic nitrergic neurons; eNOS, located in the endothelium of the vasculature (402); and iNOS, an inducible form expressed primarily in response to immune stimulation (5, 193). Modulation of vascular resistance is achieved primarily via nNOS and eNOS. In mammals, nNOS is primarily located in the parasympathetic system (402). Comparatively, nNOS has been localized to nitrergic neurons in multiple fishes, amphibians, reptiles, birds, and mammals (68, 283-285, 368, 402).

In contrast to nNOS, the presence of eNOS differs significantly across vertebrate taxa. Specifically, eNOS is absent from the endothelium of cyclostomes (185), elasmobranchs (145), and various bony fishes, including the lungfish *Neoceratodus forsteri* (283-285). Histological studies have localized nNOS in perivascular neurons of bony fishes, and this may be the source of NO vascular tone (283, 284). A similar mechanism is present in the marine toad *Rhinella marina*, in which NO is released by nitrergic neurons (69). In squamate reptiles, multiple analytical approaches suggest that endothelial-derived NO is functional (121, 123, 144, 452, 535). However, the actions may be dependent on species or vascular bed, as basilar arteries taken from the yellow-spotted pit viper, *Trimeresurus flavoviridis*, lack endothelial-derived NO (121, 123, 144, 452, 535, 643). The systemic arteries of the estuarine crocodile, *Crocodylus porosus,* and the common pigeon, *Columba livia*, rely in part on NO release from both nitrergic neurons and the endothelium (68, 284, 285, 288). Collectively, the absence of endothelial-derived NO in basal vertebrates, including amphibians, fish, and lungfish, and its presence in reptiles, birds, and mammals, suggests that vertebrate endothelial-derived NO first became functional in the common ancestor of extant amniotes, an assertion supported by molecular phylogenetic analysis (15).

The contribution of NO to vasomotor tone has been exhaustively investigated in mammals (119, 367, 390, 419, 620). The response in other taxa, however, differs from that documented in mammals. In hagfish, NO increases tension in ventral aortic rings; in lampreys, NO causes a bimodal reaction, increasing first and then decreasing tension (163). In elasmobranchs, NO has been reported to increase tension of vascular rings from systemic as well as branchial vessels and to have no effect (145, 162, 445). A limited number of nonbony fishes have been studied, and therefore definitive statements about the prevalence of the NO constriction cannot be made; however, findings suggest a fundamental difference in the intracellular mechanism compared to that of other vertebrates. While vascular ring preparations indicate a NO-mediated constriction, an in vivo study of the spiny dogfish, *Squalus acanthias*, suggests a limited NO dilatory tone on the systemic vasculature, which is augmented during acute hypoxia (570). Bony fishes, including lungfish, exhibit a characteristic systemic vasodilation in response to NO, with limited branchial vascular responses (163, 283, 596). However, the endothelium of large systemic vessels lack eNOS, and adults appear to lack NO tone on the vasculature (144, 283, 424, 426). A study on embryonic zebrafish, *Danio rerio*, documented a NO dilatory tone on the systemic vasculature (200), and a NO tone has also been reported in the embryonic/larval brown trout, *Salmo trutta*, as indicated by the tachycardiac response to blockade of NO production (150). Therefore, NO may tonically function in the microvascular circulation and/or during early windows of fish ontogeny. The presence of a NO tone in fish may be dependent on age and vascular bed; further studies are needed.

Multiple studies have examined NO tone in terrestrial ectothermic vertebrates, and different contributions have been documented (596). A NO dilatory tone on the systemic vasculature has been identified in turtles, varanid lizards, python snakes, and crocodilians, with limited or no tone on the pulmonary circulation (123, 204, 475, 535). Examples of NO tone on various divisions of the vascular tree have been documented in four of the major reptile orders, but its functional significance in surgically recovered animals is yet to be ascertained.

Hydrogen sulfide gas (H_2S)

In mammals, hydrogen sulfide gas (H_2S) is the product of cysteine metabolism in numerous tissues, a reaction that is facilitated by the enzyme cystathionine λ-lyase (CSE) (395, 790). Gene expression for CSE has been identified in vascular and cardiac tissue (617), and H_2S reduces vascular tone via a mechanism that involves both endothelium and smooth muscle cells of vessels working together with NO (202, 645, 646). The mechanism of H_2S function has been investigated almost exclusively in mammals, though response studies have been conducted in other vertebrate classes.

The vascular response of fish is dependent on taxonomic group as well as location of the vascular bed. In both hagfish and lamprey, H_2S increases vascular ring tension, which is enhanced by reduction in O_2 in the dorsal aorta and the efferent branchial arteries, but not in the ventral aorta or the afferent vessels (427). In the limited number of elasmobranchs studied,

vascular ring tension decreases independently of constrictive state of the tissue (143). In steelhead trout *Oncorhynchus mykiss*, a teleost fish, H_2S produces a triphasic response in conductive vessels, consisting of an initial weak dilation, followed by constriction and then dilation (142, 143).

The function of H_2S in amphibians has been investigated in a single species, the marine toad *Rhinella marina*. The H_2S response is variable, with the aorta constricting in response to the gas while the response of the pulmonary artery ranges from solely constriction to a triphasic response involving constriction-dilation-constriction as the gas increases in concentration (143). Thus, the functional role of H_2S in amphibian cardiovascular homeostasis remains unclear.

H_2S contribution to vascular tone in reptiles has been investigated in both in vitro and in vivo studies. In red-eared slider turtles, H_2S produces a general vasoconstriction in both the pulmonary and systemic vascular beds (553). In addition, H_2S appears to exhibit a tone on the cardiovascular system that is dependent on temperature and O_2 condition; a decrease in either factor up regulates the vascular tone associated with the gas (553). Pulmonary and mesenteric arterial rings respond to anoxia partially via a H_2S-induced vasoconstriction (553). These findings agree with investigations of aortic and pulmonary arterial rings in American alligators, *Alligator mississippiensis*, which respond to H_2S with a dose-dependent increase in tension (143). It may, therefore, be that reptiles respond to the gas exclusively with a vasoconstriction, which is different from the function reported in mammals.

The Coronary and the Cerebral Circulations

Coronary circulation

The heart has a significant O_2 requirement to maintain levels of transport functions that varies between species and between myocardial layers. Depending on the species, supply of O_2 and exogenous substrates for ATP production are provided via luminal and/or coronary circulations. The presence, pattern, and degree of coronary vascular support are quite diverse. The majority of vertebrate species lack coronary circulation because most fishes do not have coronary vessels. Adult avian and mammalian hearts receive O_2 exclusively via coronary circulation, whereas fish, amphibian, and reptile hearts receive O_2 from oxygenated arterial blood and/or deoxygenated venous blood, to support myocardial function.

The human heart represents only 0.5% of body weight, but at rest, it consumes 10% of the body's O_2. The human heart creates and consumes about 35 kg of ATP each day, more than 100 times its own weight (575). The heart's capacity to perform work depends on O_2 delivery to heart muscle because energy demands exceed the catalytic potential to generate ATP via anaerobic glycolysis (428). In contrast to mammals, the O_2 cost of cardiac pumping in fish can range from just 0.5% to 5% of total O_2 consumption (180, 251). Power output varies considerably between fish species, and yet the capacity to produce ATP anaerobically is not as widely varied. Anaerobic metabolism can contribute 25% of the ATP required during hypoxia in perfused sea raven (*Hemitripterus americanus*) hearts (21, 180), and most of the ATP demand of the hagfish (*Eptatretus cirrhatus*) branchial hearts, which have an extremely low cardiac power output (191, 192). The presence of the coronary circulation is related to the level of cardiac work and myocardial O_2 demand in fishes. The resulting capillary supply is extensive, decreasing diffusion distance and facilitating a higher metabolic rate for some fish species.

In adult birds and mammals, the heart receives O_2 and nutrients via a well-developed coronary circulation. Quantitative morphometric analyses have provided valuable insights into the structural characteristics of the coronary vasculature and microvasculature of the mammalian heart (39). During early development, cardiac support consists of luminal, venous blood moving through the heart chambers, with some delivery of O_2 at the epicardial surface as well (597). The coronary vasculature develops early during primary cardiac morphogenesis, as the ventricular wall thickens (228). The course and distribution of the major coronary arteries in all mammals is remarkably similar, from rodents to whales (103, 138, 228, 469).

The hearts of ectotherms are aerobic, and most cannot function without adequate myocardial O_2 supply. The cardiac blood supply is similar to that found during early cardiac development in mammals. However, the routes for myocardial O_2 supply in ectothermic vertebrates are more complex than those in mammalian hearts. O_2 is supplied to the myocardium by two sources, luminal and coronary. Ventricles and atria of cyclostomes, most teleost fishes, and possibly all amphibians consist of spongiosa muscle, which is also variously referred to as the inner, spongy, trabecular, or trabeculated layer. The avascular spongiosa consists of interlacing muscle bundles, called trabeculae, which are supported by luminal-deoxygenated blood. In all other ectothermic vertebrates, including elasmobranchs, certain teleosts, and all reptiles, a second layer of myocardial tissue, called *compacta*, surrounds the ventricular spongiosa. The ventricular compacta has variable thickness in ectothermic vertebrates, but represents almost the entire ventricle in birds and mammals. Cardiac blood supply in nonmammalian or nonavian vertebrates is predominantly through venous luminal blood delivery to a trabecular endocardium. The presence of a coronary circulation is not associated exclusively with either air breathing or terrestrial life (75).

Air-breathing fishes, such as bowfin, *Amia calva*, have a coronary circulation, but the Australian lungfish *Neoceratodus forsteri* and amphibians do not. The coronary circulation has two possible sites of origin for O_2-rich arterial blood. There is always an anterior source, also called the *cranial* or *cephalic* source: either the postbranchial vessels in fishes, or the systemic aorta close to the heart in amphibians, reptiles,

birds, and mammals. Because a greater proportion of compacta corresponds to a more eleborate coronary circulation, generalizations can be made about the coronary circulation by inference based on the measurements of compacta. In fish, the ventricular compacta has variable thickness; it may represent as little as 5% of total ventricular mass, as in chimaerid fish, or as much as 66%, as in skipjack tuna, *Katsuwonus pelamis* (180). In salmonid fishes, the compacta appears early in development and increases disproportionately with growth in juveniles, reaching a relatively fixed proportion of ventricular mass in adult fish (174, 316, 463). The compacta typically represents 20% to 40% of ventricular mass when present in ectothermic vertebrates. The compacta also selectively increases during sexual maturation in male salmonids (109, 227), reaching 70% to 75% of total ventricular mass (109). Interestingly embryologically, mammalian hearts are trabecular, and coronary vessels become incorporated as the heart is modified during development (228).

The anatomy of the coronary circulation in ectothermic vertebrates has been characterized in detail for many species (130, 195, 228, 238, 356, 436, 501, 598). The coronary artery is functionally analogous in mammals and fish; however, the coronary circulation in fish appears to provide only a supplemental supply of O_2 to the myocardium. Diverse cardiovascular architecture is displayed by agnathans, including hagfish and lamprey; by chondrichthyans, which are cartilaginous fishes, including sharks, rays and ratfish; and by bony teleost fishes. While coronary circulations are present in some fish species, to date only a third of all teleosts have been reported to possess a coronary circulation.

The presence or absence of coronary vessels and features of the spongiosa and compacta have been used as characters use to define different types of fish hearts (130,598). The presence of the compacta is always associated with a coronary circulation. However, the coronary circulation, when present, is not necessary confined to the compacta. Cardiomyocytes are more densely packed in the compacta than in the spongiosa, but myocyte size is similar in both layers (109). A coronary circulation can be found in active teleosts; in chondrosteans; and in all elasmobranchs, including chimaerids, sharks, rays, and skates. Some dipnoans have a coronary circulation, but with varying distribution patterns. Coronary vessels can reach the spongiosa and atrium in all elasmobranchs and in several active fishes: tuna and marlin, for example (130,180,501,598). These species possess a ventricle that falls into one of four categories. The majority of fish species have avascular hearts with no coronary artery support or microcirculation (Type I). However, if coronary vessels are present they fall into three categories, Types II, III, and IV, all of which have spongiosa and compacta tissue plus capillaries within the myocardium. Type II ventricles are characterized by coronary vessels and capillaries only in the compacta. The coronary circulation supplies both the spongiosa and compacta in the Type III heart. Type IV ventricles have a larger relative compacta and coronary vessels in the atrium. In view of this anatomical diversity, it is likely that the coronary circulation evolved more than once (130). It has been postulated that the uniform presence of coronary vessels in hearts of elasmobranchs may be due to an absence of a swim bladder, higher swimming costs, and a continuous mode of swimming that may lower venous, or luminal, O_2 supply to the heart (501). In other fishes, environmental hypoxia and elevated cardiac work levels appear to be important selection pressures favoring the development of a compact epicardium and coronary circulation (130). In teleosts, delivery of blood from the gills to the heart occurs via a single cranial coronary artery, which runs either along the dorsal surface of the ventral aorta, as seen in skipjack tuna, for example, or along the ventral surface of the ventral aorta, as seen in rainbow trout. Coronary blood pressure is roughly equivalent to that of the dorsal aortic pressure. In some fishes and reptiles, there is an additional pectoral or caudal origin that usually goes to the apex of the ventricle.

Urodeles and anurans have a single coronary artery that arises at the base of the ventricle or near the right carotid artery subdivision. Apodans do not have a coronary artery (196). Further in urodeles and anurans, no compacta is found in the ventricle (44,226,238,356), and the coronary vessels are confined to the outer connective tissues of the heart and not to the myocardium *per se*. The *bulbus cordis* has compacta and coronary vessels (196). Thus, it appears that mixed venous blood in the lumen of the heart provides an adequate supply of O_2 and nutrients for low cardiac work and myocardial O_2 demand of the amphibian heart.

The reptilian coronary circulation has been described in detail (356), and are largely in restricted to the ventricle. Typically, reptiles possess either one or two anterior coronary arteries, derived from the right, or sometimes from the left, trunk at various locations near the heart. Some reptiles have a posterior coronary supply from the coeliacomesenteric artery, which reaches the apical region of the ventricle via the gubernaculum cordis; although a gubernaculum cordis is found in most reptiles, it does not always contain a coronary artery. In the tortoise *Emys orbicularis*, coronaries are confined to the compacta (300), but this has not been confirmed for other reptiles.

The extent of coronary development in reptiles appears to be related to the level of cardiac work and myocardial O_2 demand. For example, in squamate lizards, fine coronary vessels are found on the ventricle; in varanid lizards, the ventricle generates higher aortic blood pressures, and has larger coronary vessels and more compacta (356). The crocodilian heart is anatomically equivalent to that of birds and mammals, with separate atria and completely separated right and left ventricles. The left ventricle, like that of mammals, is more thickly walled than the right and generates higher blood pressure. Similarly, the coronary circulation is more extensive on the left side of the heart. The absence of atrial coronary arteries may be directly related to the lower O_2 demand of the atrium. In varanid lizards, coronary vessels are located in the right, but not the left atrium, suggesting that the systemic, but not pulmonary, venous return may become limiting in terms of luminal O_2 supply. Coronary circulation to the atria may

become more important when reptiles grow to a large size and atrial wall thickness increases.

Determinants of coronary blood flow

In fish, the extent to which the coronary circulation supplements the luminal O_2 supply is not entirely clear (130). The presence of a coronary circulation in three diverse groups of fishes (in all cartilaginous fishes, in hypoxia-tolerant teleosts, and in teleosts capable of prolonged swimming activity) suggests that coronary circulation provides a selective advantage under hypoxic conditions and during prolonged swimming (556). In coho salmon, *Oncorhynchus kisutch*, coronary blood flow during spontaneous activity during resting, normoxic conditions, is 1.1% of cardiac output (20 mL 100 g ventricle^{-1} min^{-1}) (22). Additional estimates of coronary blood flow for rainbow trout were 38 mL 100 g ventricle^{-1} min^{-1} (1.5% of cardiac output), and for skipjack tuna, 67 mL 100 g ventricle^{-1} min^{-1} (1.9% of cardiac output) (173, 177).

In rainbow trout, which possess a Type II heart predominantly consisting of spongiosa, the coronary circulation is not essential for survival in captivity (180), and coronary artery ablation does not affect resting cardiovascular variables (206). *In vitro* studies on hearts of the dogfish, *Squalus acanthias*, and the eel *Anguilla australis*, a hypoxia-tolerant teleost, demonstrate that maximum cardiac output during normoxia is not dependent on coronary perfusion (130). Calculations for fish hearts suggest that, of the O_2 normally available in luminal venous blood, the entire myocardial O_2 demand would deplete luminal content by just 1% to 10%, even during exercise (180). Luminal blood may provide sufficient oxygenation during resting conditions, but this may not hold for extremely active fishes, such as skipjack tuna, even under aerobic conditions (173). Skipjack tuna hearts have a ventricular wall similar to that in mammals, generating high ventral aortic pressure. It appears that this high pressure requires O_2 via the coronary supply route to maintain functional diffusion distances through the thicker walled ventricle of the skipjack tuna heart. In the single study of reptiles, the physiological importance of the coronary circulation of the South American rattlesnake (*Crotalus durissus*) at rest and during enforced activity has also been questioned (236).

Coronary blood flow can increase several fold during hypoxic exposure and exercise in salmonid fishes (22, 207, 208, 556), and this increase appears to be important in ventricular generation of normal arterial blood pressures during hypoxia (556). In exercising and hypoxic fishes, venous PO_2 decreases and coronary flow increases (22); adequate coronary perfusion and myocardial oxygen consumption are necessary for maximum cardiac performance (131,175,182,183). In rainbow trout, during sustained, maximal swimming activity, cardiac output increases threefold, ventral aortic pressure increases by 50% (310), and myocardial oxygen consumption should increase fourfold (180). Because pressure in the dorsal aorta downstream from the gills is well regulated and rarely changes by more than 30% during exercise or hypoxia, pressure-dependent changes in coronary flow are not sufficient to account for the twofold increase in coronary blood flow observed in hypoxic coho salmon (22) and rainbow trout (208). This implies that a large coronary vasodilatory reserve is of primary importance in increasing coronary blood flow in salmonid fishes. During submaximal swimming in normoxic water, a 110% increase in cardiac power output was matched by an identical increase in coronary blood flow (208). In this case, the increase in coronary blood flow resulted from decreased resistance (~40%) in the coronary artery and increased pressure (~25%) in the dorsal aorta. The modulation of coronary flow during hypoxia or swimming suggests neurohumoral and/or local regulation of resistance in this vascular bed (1).

Information on blood flow and vasoactivity of coronary vessels in ectotherms is largely limited to studies on fish [see reviews in (130,178,180)], the tortoise *Emys orbicularis* (300) and the anesthetized American alligator, *Alligator mississippiensis* (287). The coronary arteries in fish hearts are derived from postbranchial arteries and have significantly lower blood pressure than ventricular values (81, 180). As a result, coronary blood flow in fish hearts does not have a direct relationship with blood pressure. The importance of perfusion pressure to coronary blood flow in the reptilian circulation is likely (81). Overall, this information suggests that there are differences in the hemodynamic properties, control, and role of the coronary circulation in ectothermic vertebrates compared with mammals.

Under resting conditions, there is tonic vasoconstriction of the coronary circulation of the fish heart, which can be reduced to increase coronary flow. A number of studies in fishes have identified α-adrenergic, β-adrenergic, cholinergic, and purinergic vasoactivities in the entire coronary circulation or the main coronary artery (34, 129, 173, 177, 179, 539). Receptor subtypes, their relative density, and their location are quite variable in fish species. This diversity makes a general model of vasoactive mechanisms in fishes challenging and renders our understanding of vasodilatory reserve incomplete. In some species, including Atlantic salmon, *Salmo salar*; rainbow trout; and marlin, *Makaira nigricans*, α-adrenoceptors, which are involved with constriction, outnumber β-adrenoceptors, which are involved with dilation. In salmonids, α-adrenoceptors dominate in arterioles, whereas β-adrenoceptors dominate in the main coronary artery (180). In the conger eel, β-adrenergic stimulation induces relaxation and acetylcholine causes contraction in the coronary circulation (34). However, extensive studies are still needed to develop a more complete picture of coronary blood flow regulation in fishes.

An important difference in coronary vasoactivity between mammals and ectothermic vertebrates is the coronary response to adrenaline. Both adrenaline and noradrenaline vasoconstrict the trout coronary system (410). In salmonids

and the tortoise, adrenaline increases coronary vascular resistance and reduces coronary flow (22, 177, 300). Opposite effects of adrenaline are observed in the mammalian coronary circulation, which might be due to the predominance of β-adrenoceptors versus more numerous greater α-adrenoceptors in ectothermic vertebrates. Hypoxia, adenosine, and NO also appear to be important modulators of coronary vasoactivity in fishes (1, 409, 410, 539). NO mediates the vasodilatory effects of serotonin, acetylcholine, and adenosine on the coronary system of trout (1). However, in contrast to mammals, NO does not appear to have an endothelial origin in fish blood vessels (426). In mammals (169) and fishes (288), evidence suggests that release of ATP from erythrocytes may affect blood flow in the coronary circulation. Other vasoactive agents in the coronary circulation of trout include thromboxanes, prostaglandins, cortisol, and 17-β estradiol (1, 288). The physiological importance of these compounds and their interactions await further elucidation.

Adaptability and remodeling of the coronary circulation

Unlike adult mammalian cardiac myocytes, thin elongated fish myocytes display both hyperplasia and hypertrophy during isometric ventricular growth (109, 174). Disproportionate ventricular remodeling, or enlargement, occurs in rainbow trout during cold acclimation (227, 439), during sexual maturation of males (28, 107, 133, 197, 227), and in response to chronic anemia (530). The ventricular enlargement associated with cold acclimation reduces coronary capillary density (151), which may be related to a proportionately smaller compact myocardium, reduced cardiac workload, and lower O_2 demand (531). During sexual maturation there is proportional growth of myocytes and the coronary microvasculature to maintain compacta diffusion distances and myocyte oxygenation (107). Ventricular remodeling during phenylhydrazine-induced anemia produces a disproportionate increase in coronary capillarity (530). Expansion of the coronary vascular volume may represent structural compensation for reduced arterial O_2 content during chronic anemia. Collectively, the ventricular mass and the coronary vasculature are dynamic in salmonid fishes, and vascular remodeling does not necessarily parallel ventricular enlargement.

Cerebral circulation

Cerebral blood flow (CBF) is dependent on arterial perfusion pressure as well as autoregulatory mechanisms (46). Given the clear implications for human health, mammalian cerebral vascular control systems have been extensively characterized. The driving force behind efforts to understand the critical regulatory factors that mediate changes in vascular perfusion has been the relative intolerance of the mammalian CNS to limits of convective transport. However, species that routinely experience periods of environmental hypoxia or even anoxia may have increased reliance on these autocrine systems.

In the Pacific lamprey, *Entosphenus tridentatus*, histological evidence indicates that serotonergic and catecholaminergic neurons are located in the adventia of the vasculature (278). However, acetylcholine esterase-containing neurons are absent, suggesting limited cholinergic regulation of the CBF in this species (278). Assessment of cerebral circulatory control in elasmobranchs has focused on a single hypoxic-tolerant species, the epaulette shark, *Hemiscyllium ocellatum*. During bouts of hypoxic exposure, CBF is maintained during a reduction in systemic arterial pressure in the anesthetized shark, indicating a hypoxic vasodilation (545) and implying functional cerebral circulatory control in this species. Interestingly, adenosine does not play a role in the hypoxic-induced vasodilation in this shark species; however, direct application of adenosine increases CBF (545).

Studies of CBF in bony fishes have been restricted to anesthetized preparations due to technical limitations of quantifying this parameter. Histological analysis illustrates that both adrenergic and cholinergic neurons innervate sections of the cerebral circulation (365). In the crucian carp, *C. carassius*, anoxia results in an increase in CBF, which is mediated in part by adenosine but does not involve the release of NO (276,414). In the same species, NO induces dilation and is released in response to cholinergic stimulation (276). Cerebral circulation, as represented by surface vessels on the optic lobes of rainbow trout, dilates via an oxytocin-mediated NO mechanism (241). This preparation has also been used to identify a marked vasoconstriction of the tectal artery in response to endothelin in the trout brain, which is a response similar to that seen in mammalian models. This similarity indicates that this peptide plays an intricate role in CNS vascular regulation in vertebrates (486).

Histological studies of the cerebral vasculature of bullfrogs, *Lithobates catesbeianus,* illustrate that cholinergic and adrenergic receptors regulate perfusion in an anuran amphibian brain (114, 365, 576). Immunohistochemistry has been used to identify peptidergic neurons, which contain both neuropeptide Y and vasoactive intestinal peptide, associated with the cerebrovasculature of Japanese newts, *Cynops pyrrhogaster*. These findings indicate that a similar pattern may be present in both anuran and urodele amphibians (13). In response to anoxia, superficial brain vasculature of the leopard frog, *Lithobates pipiens,* vasodilates, though the exact mechanism remains in question (546). While adenosine produces a relative vasodilation of superficial brain vasculature, it is not involved in the anoxic-induced dilation in anesthetized leopard frogs (546). Adenosine also maintains a tonic dilation of the cerebral vasculature in tadpoles of the clawed frog, *Xenopus levis* (282). As with cerebral vascular regulation in fish, further studies are needed in amphibians.

Microsphere distribution methods have been employed in numerous studies to establish relative changes in blood flow

to vascular beds that are technically challenging to assess via direct flow measurements due to their small size. Relative CBF in the red-eared slider turtle increases during bouts of anoxia, augmented by the addition of hypercapnic exposure (46, 134, 552). The capacity to increase or maintain CBF is independent of α-adrenergic-receptor-mediated influences due to the relative insensitivity of the cerebral vascular bed to α-adrenergic stimulation (552). The basilar artery, a vessel that runs along the medulla oblongata of the yellow-spotted pit viper, *Trimeresurus flavoviridis*, constricts in response to both noradrenaline (via an α-adrenergic stimulation) and serotonin receptor stimulation (643). Histological examinations of the cerebral vasculature in the Japanese four-lined rat snake, *Elaphe quadrivirgata,* and three chelonians (*Geoclemys reevesii*, *Testudo horsfieldi*, and *Trionyx chinensis*) indicate both cholinergic and adrenergic innervation plexus, suggesting that these plexi are required to achieve nervous regulation of cerebral perfusion (277, 279, 365). In the anesthetized estuarine crocodile *Crocodylus porosus*, acute anoxia causes vasodilation of the cerebellar surface vessels independent of both adenosine and NO production (544); but vascular regulation could have been impaired in the study because aminophylline (nonspecific purinergic blocker) or L-NA (NO-production inhibitor) failed to elicit a change in systemic arterial pressure (544).

The capacity to maintain convective transport to the CNS in birds is intriguing, given the challenge experienced by species that are active at high elevations where the partial pressure respiratory gases are low. Arterial partial pressure of CO_2 ($PaCO_2$) is of particular interest, as it is a major determinant of CBF in mammals, with a negative correlation between $PaCO_2$ and cerebral vascular resistance (253). For example Bar-headed geese, *Anser indicus*, respond to both hypoxic and hypocapnic conditions with an increase in CBF (166, 168). This indicates that the hypoxemic vasodilation in the cerebral vasculature of birds is not attenuated by the hypocapnic vasoconstriction seen in mammals (89). Pharmacological assessments of vascular rings taken from the medulla oblongata basilar artery of broiler chickens have been used to characterize a tonic NO-mediated dilation, and to demonstrate both a histamine-induced dilation and a 5-hydroxytryptamine receptor (5HT)-induced constriction (423). Histological evidence suggests that vasoactive intestinal polypeptide and cholinergic neurons are connected to the cerebral vasculature of the Japanese quail, *Coturnix coturnix japonica*, and that they potentially regulate perfusion (14). The presence of acetylcholine esterase-containing neurons in multiple avian species has been histologically verified in the cerebral vasculature (334). The presence of substance P as well as calcitonin gene-related peptide immunoreactive innervation has been identified in the cerebral vasculature of Japanese quail, suggesting an unknown regulatory role of these peptides (333). Studies of functional control of cerebral vascular perfusion in the pigeon, *Columbia livia,* suggest neural regulation via vagal outflow, which may contribute to variation in flow (441).

Cardiovascular Function in Challenging Environments

Cardiovascular adjustments to aerobic exercise

On average, endothermic and ectothermic vertebrates maintain resting rates of aerobic metabolism at just 10% of maximum rates (36, 37). Aerobic exercise increases the demand placed on the internal O_2 convection system, requiring increased blood flow to meet the greatly increased metabolic needs of contracting skeletal muscle. Ultimately, the cardiovascular system is rate limiting to other physiological processes and yet highly responsive and adaptable. The physiological basis for increasing O_2 delivery to contracting muscle is important to defining aerobic scope and the range of activity that animals can sustain. The active lifestyles of birds and mammals are facilitated by large aerobic scopes and fast response rates during aerobic transitions from rest to exercise (326). Despite more than a century of investigation, there is still intense debate regarding the major mechanisms responsible for controlling the respiratory and cardiovascular systems during exercise. This is true for endothermic and ectothermic vertebrates.

Whether it is central factors and O_2 delivery or peripheral factors that define the limits of maximal O_2 uptake and exercise performance has been debated for more than 80 years, since the sentinel research on the topic (260). The Fick equation defining convective O_2 transport is

$$\dot{M}O_2 = HR \times SV(CaO_2 - CvO_2)$$

In this equation, $\dot{M}O_2$ is whole body O_2 uptake per unit time; HR is heart rate; SV is cardiac stroke volume; CaO_2 is O_2 content of arterial blood; and CvO_2 is O_2 content of mixed venous blood. As seen in the Fick equation, an increase in cardiac output (HR × SV) is a key component for increasing the convective transport of RBCs, O_2 delivery, and O_2 consumption. The relative importance of increasing heart rate, stroke volume, or O_2 extraction varies among animals, but many rely equally on cardiac output and O_2 extraction during maximal activity. O_2 consumption can increase by 10 to 20 times in active mammals and in endurance-trained humans, but changes in cardiac output can be much lower because tissue extraction also increases.

Cardiac output increases linearly with organismal $\dot{M}O_2$ in mammals, birds, and fishes (366). Cardiac work rate, which is the product of stroke volume, arterial blood pressure, and heart rate, scales with the same exponent as metabolic rate [~0.75 (513)]. The majority of vertebrates increase cardiac output in exercise by 1.3 to 3.3 times over resting values [see Table 4.1 in (81)]. Known exceptions include endurance-trained human athletes, thoroughbred horses (*Equus caballus)*, canines, and birds (460). In humans, cardiac output increases immediately at the onset of exercise to increase O_2 delivery (160). Numerous studies of human and canine subjects in normoxic conditions show increases in heart rate and blood volume with

increasing work intensity, with little or no change in left ventricular stroke volume (45, 492).

Because tachycardia decreases the duration for diastolic filling, mean blood flow rate through the mitral valve must increase during exercise to maintain or augment left ventricular stroke volume. To increase cardiac output, the product of stroke volume and heart rate during exercise must increase. With a reduction in stroke volume, a greater proportional increase in heart rate is required to maintain cardiac output. In dogs and humans, the rise in cardiac output during dynamic exercise is usually accompanied by increases in cardiac filling pressures as measured as central venous pressure, right atrial pressure, and also indirectly as end-diastolic volume (101, 476, 520). However, with atrial pacing at higher heart rates than normal during maximal exercise, reductions in stroke volume and limitations of human cardiac output can occur during maximal exercise (405). Potential factors that might reduce stroke volume in this case include the lower ventricular filling time, reduced preload, and alterations of the Frank-Starling mechanisms. Other limiting factors for maximal rates of O_2 consumption include the concentration of hemoglobin, peripheral distribution of blood flow, and transport conductance between capillary and mitochondria (493). Ultimately, all components of the O_2 transport system may limit $\dot{M}O_2$ max, but the system appears most sensitive to changes in circulatory function (140). Mechanisms that regulate cardiac output, its distribution, and vascular conductance in mammals during exercise are discussed in detail in a *Handbook of Physiology* [Chapters 15-17 of (491)].

Exercising ectotherms

Information on the cardiovascular responses of exercising nonmammalian or avian vertebrates is limited, but studies describe a variety of different strategies. Most available information is on fish under laboratory conditions [see reviews in (86,180,311,473,504)], and, to a lesser extent, on reptiles and amphibians (217, 252, 639). Across diverse taxa, increases in cardiac output can result from an increase in heart rate or stroke volume.

Fish cardiovascular performance during exercise

Fish vary considerably in their capacity to perform exercise, and relatively few species, mostly salmonids, have been studied. For those species, cardiovascular responses to exercise are surprisingly variable, making generalizations difficult. Swimming has been arbitrarily defined as *sustained,* if maintained for more than 200 min without fatigue; *prolonged,* if lasting 20 to 200 min and ending in fatigue; and *burst*, or brief sprinting lasting less than 20 s. Critical swimming velocity (U_{crit}) indicates the maximum speed that can be maintained during a given time period, often 1 h (504). Exercise leads to greater O_2 uptake, which increases exponentially with swimming speed. Increasing swimming speeds generally lead to an increase in cardiac output and systemic blood pressure, and a decrease in systemic resistance. During prolonged swimming, cardiac output increases by 47% in Atlantic cod; 64% in the sea raven, *Hemitripterus americanus;* and 70% in the leopard shark, *Triakis semifasciata* (21, 24, 337). From rest to U_{crit}, $\dot{M}O_2$ increases in rainbow trout from 26 to 194 μmol kg^{-1} min^{-1}, with a corresponding threefold increase in cardiac output (from 18 to 53 mL kg^{-1} min^{-1}) (310). With sustained exercise, increases in cardiac output and O_2 extraction ($Ca\text{-}vO_2$) contribute about equally to internal O_2 convection (178, 298, 310).

During swimming, increased cardiac output is achieved in most fish via increases in both heart rate and stroke volume, with the increase in stroke volume of primary importance (180). For rainbow trout, the exercise-induced increase in cardiac output is achieved predominantly with an increase in stroke volume (from 0.46 to 1.03 mL kg^{-1}) but not heart rate (from 38 to just 51 bpm). Cardiac acceleration does not occur in teleosts when swimming at speeds above 50% U_{crit} (465). Tachycardia appears to be a fundamental limitation for ectothermic vertebrates, with a few exceptions. The Antarctic fish *Pagothenia borchgrevinki* and possibly tuna are unusual in this regard, relying more on tachycardia than stroke volume to increase cardiac output (20, 64).

Fish hearts are particularly responsive to the Frank-Starling mechanism, which enables the heart to increase output in response to a volume load. This might explain the reliance of most fish on stroke volume for increasing cardiac output. Just 0.13 kPa increase in the filling pressure of the heart of the sea raven (from 0.00 to 0.13 kPa) increased cardiac output fourfold (176,181). In rainbow trout, mean ventral aortic pressure perfusing gills increases from a resting value of 5.20 to 8.27 kPa during exercise. However, mean systemic blood pressure in the dorsal aorta of rainbow trout is only elevated 0.40 to 0.53 kPa after 1 h of steady state swimming (310). Tuna achieve remarkable levels of swimming performance, which require elevated cardiovascular performance and O_2 delivery to contracting skeletal muscle. In addition to sustained aerobic swimming, the high aerobic capacity of tuna has also been attributed to accommodating multiple, elevated, metabolic components such as standard metabolic rate, specific dynamic action, growth, reproduction, and O_2 debt recovery for anaerobic white muscle (325). Blood pressure in tuna is similar to birds and mammals, and cardiac output is almost exclusively regulated by heart rate (64, 172). Scombrid fishes achieve remarkable levels of swimming performance. It has been estimated that $\dot{M}O_2$ max in skipjack tuna is 2.5 times the maximum measured in other teleosts (86). Additional measurements of O_2 consumption in juvenile Pacific Bluefin (*Thunnus maccoyii*) and yellowfin tuna (*Thunnus albacares)* confirm the high metabolic rates of swimming tuna and highlight species differences in metabolic rates (bluefin > yellowfin) and differential impacts of water temperature on O_2 consumption of these species (51). O_2 transport in tuna is enhanced by high hemoglobin content due to increased hematocrit, and by increased mean cellular hemoglobin

content (MCHC) compared with other teleosts (64). Blood O_2 carrying capacities of tuna are comparable to those of mammals [18-20 vol% (64, 225, 296)]. With an elevated MCHC, O_2 delivery to contracting muscle can increase without an excessive increase in blood viscosity and cardiac work.

The change in regional blood flow distribution with exercise has been measured in several species of fishes. As in mammals, the distribution of systemic blood flow changes during exercise, compared to resting conditions. The axial musculature of fishes powers swimming activity and exists in two forms: superficial red muscle fibers and dominant white muscle fibers. Red muscle supports sustained swimming activity. It is red because it has a rich blood supply, myoglobin, and a significant mitochondria component. White muscle lacks myoglobin, receives limited circulatory support, and provides propulsive power for brief, high-intensity swimming. Blood flow to oxidative red muscle increases from 9% and 0.57% of cardiac output at rest to 42% and 13.2% during exercise in rainbow trout and large scale sucker, *Catostomus macrocheilus*, respectively (321, 472). Rainbow trout swimming at the limit of sustainable exercise increase mass-specific blood flow to red muscle by almost 14 times compared to resting blood flow values, up to values exceeding 120 mL min^{-1} 100 g^{-1} (584). In this one study, despite a 3.5-fold increase in cardiac output, exercise did not alter blood flow to white muscle or to any other tissue examined. Other studies have reported pronounced reductions in blood flow to visceral organs during exercise: a 30% reduction in Atlantic cod (24) and a 70% reduction in Chinook salmon (591). Because systemic resistance is reduced, the effect of skeletal muscle vasodilation must be greater than that of visceral vasoconstriction.

Frog, lizard, and turtle during exercise

There have been several studies on the cardiovascular performance of amphibians and reptiles during exercise (58,91,218, 252, 265, 628, 639). Most data is limited to frogs and lizards, but they appear to increase cardiovascular function during exercise, similar to that of other vertebrates. Determining blood flow from the amphibian heart is complicated because three arteries (carotid; aortic, or systemic; and pulmocutaneous) conduct blood from an undivided ventricle. In resting *Rhinella marina* toads, 90% to 95% of total cardiac output is distributed between the systemic and pulmocutaneous arteries (628). Independent of body temperature (10-30°C), enforced activity increases heart rate by twofold and increases stroke volume 1.6- to 2.2-fold (252). Both systemic and pulmocutaneous blood flow increases with activity; however, pulmocutaneous blood flow changes proportionately more. At 20°C, the scope for cardiac output is maximized with cardiac output increases 4.4-fold from rest to exercise, from 81 to 358 mL min^{-1} kg^{-1}; and pulmocutaneous blood flow was augmented sevenfold, from 32 to 227 mL min^{-1} kg^{-1}; however, systemic blood flow increases just 2.6-fold, from 49 to 131 mL min^{-1} kg^{-1}. This reflects a net left-to-right shunt in which systemic blood flow becomes a smaller fraction of total cardiac output. The large reductions (by 24 times) in pulmocutaneous resistance may result from vagal inhibition, given the vagus acts to increase contriction on this vessel (627), increased circulating adrenaline and sympathetic tone which may act to dilate the gas exchange vasculature (603) and/or vascular compliance differences (638). There is also an exercise-induced hemoconcentration for toads during exercise, which appears to result from osmotic fluid uptake by active muscle fibers (57), fluid pressure-mediated ultrafiltration to the interstitial space (265), and inhibition of lymphatic return (360), all of which could affect systemic resistance.

Comparative studies of exercising lizards report that *Varanus exanthematius* and *Iguana iguana* increase their cardiac output and O_2 extraction during treadmill activity (218). A higher $\dot{M}O_2$ max in *Varanus* relative to the iguana is due to the greater scope of both cardiac output and O_2 extraction from the blood. In iguanas, a twofold increase in heart rate is responsible for virtually all of the increase in cardiac output during strenuous activity. In *Varanus* heart rate elevation accounts for 80% of the increase in cardiac output, while an increase in stroke volume contributes 20%. Given that lizard ventricles lack complete septation, separation of oxygenated and deoxygenated blood is maintained by flow patterns within the ventricle. As a result, changes in stroke volume during activity might alter blood flow and limit effective ventricular shunting of oxygenated and deoxygenated blood. Some exercising amphibians and reptiles even show slight decreases in stroke volume associated with increases in heart rate (218, 263). There is also evidence in iguanas and *Varanus* (but not in the American alligator, *Alligator mississippiensis*) that locomotion and lateral undulations increase intra-abdominal pressure and constrain venous return, cardiac output, and O_2 delivery during exercise (171, 406, 407). The cardiovascular response to activity in marine turtles has also been investigated. Green sea turtles, *Chelonia mydas,* swimming at their maximum sustainable swimming speed elevated their O_2 uptake by 2.8 times and heart rate by 1.4 times, compared to resting values (91). Like squamate reptiles, the complexly structured, but incompletely divided ventricle of the turtle allows blood to be shunted away from or into the pulmonary circulation in a right-to-left or left to right shunt respectively. During periods of breath holding, there an increase in pulmonary vascular resistance shunting blood away from the pulmonary circulation (519). However, both pulmonary and systemic circulations received continuous blood flow during exercise. Overall, our understanding of how cardiac output is regionally distributed during activity in amphibians and reptiles is still very limited.

Exercising endotherms

Horses are elite athletic animals with remarkably high aerobic capacities, and they serve as unique models of cardiorespiratory adaptation and function. The $\dot{M}O_2$ max/body mass value of standard bred racehorses is 3-times the predicted value

for a mammal of their size (577). Racehorses can increase their $\dot{M}O_2$ from 10-fold to more than 20-fold in response to endurance exercise, and between 60- and 90-fold during high-intensity racing. Thoroughbred horses are exceptional in that a doubling of stroke volume and a tripling of heart rate produce up to a sixfold increase in cardiac output (589). During treadmill running, a 22-fold increase in O_2 delivery from rest to $\dot{M}O_2$ max was due to a 5.1-fold increase in cardiac output and a 3.9-fold increase in O_2 extraction (299). A considerable amount (38%) of the increase in Ca-vO_2 was due to an increase in the concentration of hemoglobin, and hematocrit increased from 35% at rest to 55% at $\dot{M}O_2$ max. At $\dot{M}O_2$ max, average muscle blood flow in 450 kg standard bred horses was 116 mL min^{-1} 100 g^{-1}, and total muscle blood flow was estimated at 226 L min^{-1}, which was 78% of total cardiac output (18). Trotting horses during maximal efforts have heart rates between 230 and 255 bpm (156) and stroke volumes over 1400 mL [3.1 mL kg^{-1} (299)]. Compared to cows of similar body mass, horses had higher cardiac outputs as a result of larger heart size, facilitating increased stroke volume; a higher mean arterial pressure; and a larger capillary bed in skeletal muscle, facilitating decreased systemic vascular resistance. At the extreme, racehorses experience pulmonary hemorrhage due to high cardiac outputs, high left atrial (>9.3 kPa) and high pulmonary arterial pressures (16.0 kPa), and transmural pressures (47, 626). The extremely high left atrial pressures may be necessary to achieve high cardiac outputs.

Avian flight and hind limb locomotion

Most birds have two independent locomotor systems: the wings that are used predominantly for flight; and the hind limbs, which are used for walking, running, swimming, and diving (88). In general, birds have larger hearts, lower resting heart rates, higher blood pressures, and higher body temperatures than similarly sized mammals (233, 342, 513). Maximum O_2 uptake in running or swimming birds is similar to that of mammals of similar body mass (87,438), but less than half the minimum O_2 uptake required for level flight. A 1.7-fold increase in cardiac output in homing pigeons, *Columba livia* L., may facilitate a 1.5-fold higher maximum O_2 uptake during flight than similar size, athletic mammals attain during running (447). When flying in a wind tunnel at 10 or 18.4 m s^{-1}, O_2 uptake of pigeons increases 10 or 17.4 times above resting values, respectively (92, 447) with an impressive sixfold increase in heart rate (from 115 to 670 bpm) and minimal change in stroke volume. Heart mass of pigeons represents 1.23% of body mass (447), 4.4 times greater than heart mass in a similar-size, nontrained white rat (219). When arterial O_2 content in flying pigeons is maintained at resting values, the O_2 content of mixed venous blood decreases by approximately 50% (447). Overall, higher heart rates and greater stroke volumes during exercise promote greater blood O_2 convection to working flight muscles in pigeons compared with running mammals.

Walking or running birds demonstrate a linear increase in O_2 consumption with increased velocity, as also seen in mammals. When emus, *Dromaius novaehollandiae*, run on a treadmill at 1.33 m s^{-1} and a 6° incline, O_2 uptake is 11 times the resting value (233). In this case, contributions to the enhanced delivery of O_2 to exercising muscles includes an increase in heart rate (from 46 to 180 bpm), wider arterial mixed venous O_2 content, and an increase in stroke volume (from 57 to 103 mL). It is noteworthy that mean arterial blood pressure does not change in either the pigeon or emu during exercise, and, as predicted, total peripheral resistance decreases by the same proportion as cardiac output increased.

Pekin ducks, *Anas platyrhynchos domesticus*, running at a speed of 0.4 m s^{-1} on a treadmill increase cardiac output by 60% and heart rate by 80% (from 174 to 328 bpm) (32). Corresponding stroke volume during exercise shows an insignificant decrease compared with zero speed values (2.77 versus 3.13 mL kg^{-1}). The increase in $\dot{M}O_2$ 2.6-fold, was supported by increases in cardiac output (55%) and the arteriovenous O_2 content difference (45%). Exercise increased blood flow by 3.7 times in the sciatic artery and by 2.3 times in the carotid artery. During swimming, $\dot{M}O_2$ of tufted ducks, *Aythya fuligula*, does not change between speeds of 0.2 and 0.5 m s^{-1}, but increases at higher speeds (93). At the maximum sustainable swimming speed, 0.78 m s^{-1}, $\dot{M}O_2$ increases 3.8-fold above resting values, and heart rate doubles to 235 bpm. This heart rate is less than half of the maximum recorded for this species (93), and probably reflects the smaller mass of the leg muscles utilized during swimming compared with the pectoral muscles, which support flight (464).

The role of the pericardium during exercise

A major function of the pericardium is to provide a mechanical constraint to acute variations in heart size (270). Maximal cardiac output and ventricular end diastolic volume is limited by the pericardium, suggesting that the Frank-Starling mechanism can be more fully utilized by removal of the pericardium. Pericardiectomy increases left ventricle stroke volume, cardiac output, and/or maximum O_2 delivery in various mammals. After receiving a pericardiectomy, pigs, *Sus scrofa,* demonstrated a 31% increase in maximum O_2 consumption, a 29% increase in maximum cardiac output, and 33% increase in end diastolic volume, compared to controls receiving a thoracotomy (239). In untrained dogs, pericardiectomy also increased maximal O_2 consumption and maximal stroke volume without any change in maximal heart rate or mean arterial pressure during maximal exercise (563). However, preventing ventricular chamber enlargement is advantageous because the laws of Laplace predict that dilatation of the ventricle increases wall tension and may decrease mechanical efficiency of contracting cardiac myocytes (352). An exercise-induced acute increase in end diastolic volume, may stretch the pericardium to a point at which its elastic properties restrict further cardiac dilation and utilization of the

Table 2 Comparative Aspects of Cardiovascular Responses to Low to Moderate Intensity Exercise in Ectothermic versus Endothermic Vertebrates

Similarities	Differences
All vertebrates use aerobic energy metabolism for ATP production.	Birds and mammals have higher levels of oxygen consumption and aerobic scope than terrestrial and aquatic ectotherms.
Cardiac output increases and therefore increases convective transport of oxygen and fuels to active tissues. As a result, cardiac output increases linearly with organismal oxygen consumption.	Birds and mammals have larger, faster hearts and exhibit higher cardiac outputs than ectotherms during exercise. Some reptiles selectively increase heart rate to elevate cardiac output. Fishes primarily increase cardiac output by increasing stroke volume.
Vertebrate hearts are responsive to the Frank-Starling mechanism during exercise.	The pericardium may limit cardiac filing and output in mammals and yet facilitate cardiac filling and output in fishes.
There are changes in regional blood flow distribution in favor of contracting skeletal muscle.	Systemic blood pressure can increase or remain relatively constant during exercise, depending on the species.
Splenic contraction and release of stored RBCs in the circulation occurs in select mammals and fishes.	Cold environmental temperature places significant constraints on cardiac muscle energy metabolism and performance in ectotherms.
Both endotherms and ectotherms can demonstrate cardiovascular plasticity and modify exercise performance.	

Frank-Starling mechanism. Limitations in ventricule volume due to a finite pericardial cavity also occur in fish (180).

Unlike in mammals, a twofold to threefold increase in stroke volume is common in fish. As stroke volume increases in elasmobranchs, fluid is removed from the pericardial cavity to the peritoneal cavity (337, 516); the ventricle can, therefore, occupy a greater proportion of the pericardial volume. Teleosts use variable pericardial volume, the upstream reservoirs of the sinus venosus and the atrium, and adjust the volumes of the sinus venosus and atrium inversely with the ventricle. The maximal end diastolic volumes of the sinus and atrium in teleosts are larger than the ventricle and remain high under low stroke volume conditions (178). Overall, the morphological and functional characteristics of the pericardium are diverse in fishes, and, unlike in the mammalian heart, the pericardium may facilitate cardiac filling and increase cardiac output in fishes.

Splenic delivery of erythrocytes during exercise

Internal convection of O_2 transport can be enhanced during exercise by the release of stored RBCs. In dogs and horses, the spleen constricts and releases large quantities of RBCs into the circulation during exercise (496) which is under sympathetic control (295). From rest to heavy exercise, hematocrit in normal foxhounds increases by approximately 40% at rest to 55% during heavy exercise and in 500 kg horse a splenic contraction in a increases the concentration of hemoglobin from ~14 to 22 g/dL, and increases the blood volume by 12 L. Splenectomy reduces $\dot{M}O_2$max, reduces maximal cardiac output by 20%, reduces total blood volume by 10 L, and consequently promotes lower cardiac filling pressures. In fish, the spleen also serves as a reservoir of plasma and RBCs that can affect exercise performance. The spleen is surrounded by a capsule containing smooth muscle and contracts strongly during exercise (307, 642), under the influence of circulating catecholamines and autonomic nerves (90, 417). In carp exercised for 1 hour, 33% of the erythrocytes in the blood were released from the spleen (306). Severe exercise for 15 min decreases spleen blood volume in the rainbow trout by 40% (559). Splenectomy impairs both aerobic swim performance and reduces blood hemoglobin concentration at exhaustion by ~20% in this species (442). In contrast to most temperate fishes, which use a α-adrenoceptor-mediated mechanism for splenic contraction and erythrocyte release, Atlantic cod and Antarctic fish *Pagothenia borchgrvinki* use a cholinergic component (136).

Overall the vertebrate cardiovascular response to exercise varies with intensity and vertebrates exhibit some similarities regardless of species (Table 2). The key similarities are an increase in cardiac output to meet metabolic demands, preferentially blood perfusion of skeletal muscle, and increase in hematocrit.

Thermal constraints of cardiovascular function

In ectotherms, cold temperature presents a significant challenge to normal contractile function in cardiac and skeletal muscle. Reduced catalytic potential of enzymes and slower diffusion of substrates present significant kinetic constraints on energy metabolism. Resting and maximal heart rates of fishes are significantly reduced by decreases in body temperature (148). However, if given adequate time to acclimate to cold temperatures, many eurythermal fishes maintain locomotory function relatively independent of temperature (489, 533). Some fishes compensate for decreased cardiac performance at low temperatures by enlargement of the ventricle (224, 227, 266, 309, 487), which can increase stroke volume and cardiac output at reduced heart rates. With increasing water temperature, there may also be cardiovascular

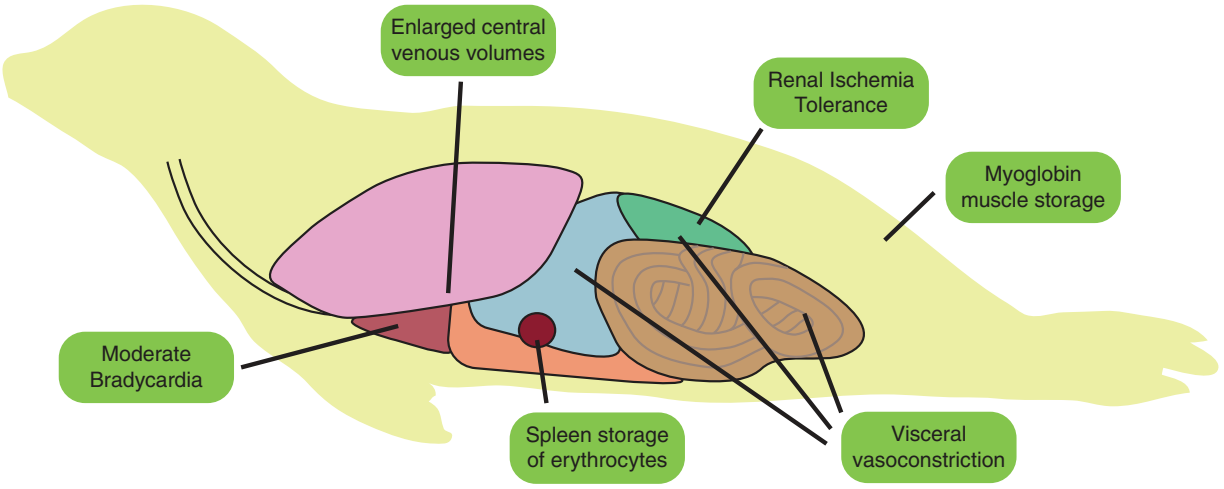

Figure 9 Summary of diving specializations in a generic seal species.

limitations that compromise O_2 transport and swimming performance of active fishes (461).

During acute heat stress, the primary cardiovascular response in fish is an increase in cardiac output, mediated by an elevated heart rate; stroke volume does not change (110, 223, 387, 560) or it decreases (65, 499). O_2 extraction remains unchanged or increases due to (i) elevated CaO_2 due to greater gill ventilation and/or increasing circulating erythrocytes following splenic contraction; or (ii) a reduction in CvO_2 due to greater tissue extraction. In fishes, cardiac contractility decreases with increasing contraction frequency and elevated temperature (272, 526). During an acute water temperature change from 13°C to 25°C, wild Chinook salmon experience cardiac arrhythmias, possibly due to low venous partial pressure of oxygen (PvO_2) and limitations on cardiac function, gill perfusion, and O_2 supply to tissues. One study proposed that high temperature tolerance decreases with body mass (110). As fish approach their critical thermal maximum, aerobic scope is negligible, and locomotion is compromised.

Cardiovascular adaptations to diving: Seals as a case study

The evolutionary return to the aquatic environment provided vast and unexploited foraging opportunities for numerous species of reptiles, birds, and mammals. Meeting the challenge of activity without lung ventilation involved adopting novel strategies to economize O_2 during inevitable apneic foraging periods, to increase tolerance to the physical effects of pressure at depth, and to allow for suitable thermal regulation in the cold and cooling water environment. The absence of lung ventilation and blood oxygenation while diving is resolved by a combination of strategies that reduce metabolism, increase O_2 storage capacity, and divert blood flow to O_2-craving organs. The degree to which these anatomical and physiological responses are able to sustain diving is seen in its maximal expression in pinnipeds and cetaceans, and this has been elegantly shown by phylogenetic reconstruction studies on the myoglobin storage in muscles in relation to the surface charge of the myoglobin molecule (393).

Recent research efforts in diving physiology have been devoted to the analysis of O_2 storage management in natural diving species (372, 383, 384, 393, 455, 567) and will not be reviewed here. Instead, the focus will be on the anatomical and physiological changes in the cardiovascular system geared toward the regulation of cardiac output reduction, blood flow redistribution to different vascular beds, venous return in a hypervolemic state, and splenic regulation of hematocrit. These adjustments have been better studied in seals than in any other group and are graphically depicted in Figure 9. Direct measurements of cardiac output during diving have not been done, but thermodilution measurements in pinnipeds swimming under water have shown that heart rate is a suitable surrogate indicator and upon submersion is due to a reflex reduction in heart rate without changes in stroke volume (458, 459). Control of the response is primarily under vagal control (153), but it fluxuates while swimming and foraging (420). In addition, there is evidence that the vagal mediated bradycardia can be learned (482), with an anticipatory change in heart rate to submersion and reemersion (98, 297), imply that it is cortically modulated. Maximal bradycardia is seen during forced diving, in what is now regarded as the classical dive response (280, 503). Extreme bradycardia is also seen in very long voluntary anaerobic dives, but in the vast majority of routine dives bradycardia is moderate (184, 322).

Concurrent with the drop in cardiac output during diving, there is a compensatory increase in peripheral resistance that keeps blood pressure constant (280). The intensity of the vasoconstrictory response seems to be maximal during forced diving experiments with angiographic evidence of extensive constriction of the renal, splanchnic, and muscle circulation (66). An anatomical arrangement with increased sympathetic innervation in proximal and distal arteries and a deeper

penetration of sympathetic fibers to the media of the vascular wall could explain the blood stagnation in specific vascular beds during diving (629). Further inulin clearance measurements during voluntary dives indicate that renal blood flow drops markedly during long dives only (135), so the ischemic tolerance of seal kidneys is instrumental in maintaining kidney function despite the alterations in kidney perfusion during diving (237). Alternatively, liver perfusion is not affected during long dives (135,490) where overall hepatic perfusion can be maintained through the hepatic portal vein and allow continuous glucose delivery during diving (490).

The cardiovascular system of divers must respond to a remarkable increase in blood volume. Greatest blood volumes have been measured in Weddell seals, elephant seals, and sperm whales, all with blood volumes exceeding 0.2 L blood per kg body mass (457, 532, 538). This is instrumental in augmenting O_2 storage capabilities, but requires an enlarged venous capacity which is evident in the central veins of these animals that are easily distensible, thin walled, and do not have valves (244). Seals also have large hepatic sinuses and an enlarged posterior *vena cava* that can accommodate up to 20% of total blood volume (157). Further, venous return from the abdomen and trunk can be actively regulated by the caval sphincter (244), a muscular ring in the posterior *vena cava* at the level of the diaphragm that completely blocks venous return during forced diving (155), but not during spontaneous apneas (456). It has been postulated that a tonic control of the sphincter could be instrumental in metering O_2 delivery to the heart while diving, but the evidence provided so far is only circumstantial (157).

Seals increase hematocrit during diving by spleen contraction as seen in exercising horses, which was proposed as a mechanism to increase diving time (268). Estimates of spleen size obtained from CT scans in anesthetized seals (454) and in postmortem examinations (467) yield a spleen mass of 0.8% to 3%, greater than the 0.6% to 1.9% estimated in sheep, dogs, horses, and goats [summarized in (467)]. Although larger spleen masses have been indirectly estimated for Weddell seals (14%), northern elephant seals (7%-11%), and harbor seals (4%) (99), such values have not been confirmed with direct measurements. The spleen is extensively innervated (507) and contraction is dependent on α-adrenergic sympathetic fibers (94). Upon contraction, the spleen is able to expel 80% of its volume, roughly 3 to 4 L of blood, equivalent to 11% to 13% of the total blood volume (94, 592), but the actual effect on lengthening dive time is rather modest and would not exceed 2 min (94). Alternatively, contraction of the spleen could increase O_2-carrying capacity and shorten surfacing time during repeated diving (99, 454), given that hematocrit increases slowly following contraction; contraction takes 2 to 3 min, and hematocrit increases over a 15-min period (592). It could also be argued that spleen contraction, together with splanchnic vasoconstriction, contributes to offset hypotension and maintains cardiac filling pressures during reflex bradycardia, as is proposed in dogs (96). Other possible interpretations emphasize the beneficial effects of blood storage in the spleen to reduce blood viscosity at rest (154) instead of the benefits of spleen contraction *per se*.

Cardiovascular adaptations to high altitudes: Bar-headed geese as a case study

Several features of the cardiovascular system allow animals to deal with the low partial pressures of O_2 at high altitudes. These cardiovascular adaptations work in concert with other adaptive traits of the respiratory system and the blood to facilitate O_2 delivery, nutrient delivery, as well as CO_2 removal. There is a clear phenotypic difference between species genetically adapted to high altitudes and lowland species acclimatized to high altitudes, so-called "phenotypically adapted" (400). This short overview will only consider species genetically adapted to high altitudes. A physiological syndrome of birds and mammals genetically adapted to altitude has been historically recognized (73). Generally, this syndrome includes an increase in O_2 affinity, a reduction in organic phosphates in the blood, the absence of polycythemia, and an absent or lessened hypoxic pulmonary vasoconstriction (HPV). These responses are opposite to the typical acclimatization responses to high altitude displayed by lowland species [reviewed in (562)].

The champions of the high-altitude environment are birds that manage sustained flights in these conditions, evidenced by anecdotal written accounts of the collision of a vulture with an airplane at 11.3 km (346) and the radar localization of flying swans above 8 km (152,561). The most iconic high-altitude phenotype is the bar-headed goose, which migrates twice a year over the Himalaya mountain range to overwintering grounds in India and back to the high plateaus of central Asia (568). Although some accounts have reported bar-headed geese flying over the highest mountaintops above 8000 m, we now know that the common migration route involves traveling through the valleys at altitudes below 5500 m (249). Researchers recording 38 individual migrating geese using GPS tags determined their common cruising altitude and reported that the geese mainly travel during night and early morning; they use an indirect route that is 112 km, or 3.6% longer than the direct path; and they do not take advantage of tailwinds. When flying over the Tibetan plateau, which is the highest sustained elevation in the route, birds do so at an average distance of 62 m from the ground, suggesting an attempt to minimize overall altitude (249). Although less glamorous than previous anecdotal accounts (568), these data do not minimize the achievement: namely, a 3000 km migration over a high mountain range in 47 days, climbing 4000 to 6000 m in less than 1 day (249,250), at very low temperatures, with estimated metabolic costs 12 times higher than basal metabolic rates (621), while vocalizing along the way (568).

Bar-headed geese can defy the low-O_2 partial pressures of altitude using a combination of three important features. First, ventilatory structural and functional adjustments that prevent hypocapnic hypoventilation and maximize alveolar PO_2 and

lung gas exchange [reviewed in (510)]; second, increased blood O_2 affinity via hemoglobin mutations (624) and magnification of organic phosphate effects (488) to improve O_2 saturation in the lungs; and third, cardiovascular adjustments that adequately supply and redistribute blood to key organs, such as the lungs, the brain, the heart, and the flight muscles. Capillary density is higher in flight muscles of bar-headed geese than in other species and also has an increased proportion of oxidative fibers (508). At the subcellular level, mitochondria are redistributed toward subsarcolemmal locations and into closer proximity to capillaries. This results in reduced diffusion distances and can improve O_2 transport in hypoxic conditions (508). Because these structural specializations are observed in bar-headed geese raised at sea level, they must be canalized adaptations to sustain flight at high altitudes (562). Indeed, bar-headed geese flying in a wind tunnel have an estimated 25% greater O_2 consumption than barnacle geese at heart rates of 450 min^{-1} [estimated from Fig. 3 from (621)]. This is likely due to improved oxygenation related to increased capillarization. An alternative explanation is that bar-headed geese have a larger stroke volume, but this is unlikely, because the heart of bar-headed geese is similar in size to that of lowland anatids (0.65% of body mass) (511).

Cardiac output increases during flight, as much as 4.2-fold from resting values with the assumption that heart rate and cardiac output are linearly related (621). At the same time, O_2 consumption increases 12-fold, meaning that arteriovenous difference should account for the remaining 2.8-fold, well in line with results in exercising ducks and pigeons (232). These values are quite similar in barnacle geese (621), and it would be remarkable if bar-headed geese could maintain or preserve cardiac output at altitude, which is something that humans acclimatizing to altitude cannot accomplish (616). Two features of the bar-headed geese that could partly account for a sustained cardiac output are: first, an increase in capillary density, even if myoglobin levels and enzyme activities are not increased (508); second, the absence of polycythemia (50), eliminating any cellular induced increase in viscous resistance and cardiac work. In addition to mechanisms that facilitate and enhance oxygenation, two local vascular adaptations compensate for shortcomings in a respiratory system designed to function best when PO_2 does not chronically deviate from sea level standard values.

The first local vascular compensation mechanism is a reduction in HPV. If PO_2 deviates chronically, as occurs at high-altitude, pulmonary vasoconstriction occurs in an attempt to locally match ventilation with perfusion in different regions of the lungs. Obviously, this effort cannot compensate for environmental hypoxia, but it occurs nonetheless, accompanied by pulmonary hypertension and eventually pulmonary edema. Blunting of the HPV is, therefore, beneficial and has been documented in human populations from Tibet (230) and such animal species as yaks (149), pikas (210), and bar-headed geese (167). In bar-headed geese, pulmonary arterial pressure (PAP) only increases at PO_2 below 3.33 kPa. For comparison, PAP is elevated below 6.00 kPa in ducks and below 10.67 kPa in chickens (167). The mechanism blunting HPV is not well understood, but the rate of NO release is higher at high altitude than at sea level in the pulmonary endothelium of Tibetans at high altitude (32, 269). NO is a vasodilator that not only maintains PAP and increases pulmonary flow but can also contribute to systemic vasodilation and systemic flow by increasing the circulating concentration of bioactive NO products more than tenfold (161).

A second local vascular mechanism seen in animals adapted at high altitudes involves the preservation of brain perfusion by preventing the vasoconstrictor effect of hypocapnia. Hypocapnia is an inevitable side effect of the hyperventilatory response to environmental hypoxia. If hypocapnic vasoconstriction outcompetes cerebral hypoxic vasodilation, blood flow to the brain is reduced, triggering confusion and ataxia, the first symptoms of high-altitude cerebral edema (625). In contrast to what happens in mammals, CBF in birds is not reduced when PCO_2 is lowered, even below 1.33 kPa [reviewed in (165)]. Because hypocapnic insensitivity has been documented in bar-headed geese (166), ducks (231), pigeons (441), and chickens (640), its relevance in altitude adaptation is dubious, and suggests birds are less sensitive to low PCO_2 even if they readily respond to increased PCO_2 with vasodilation (165). The mechanism responsible for the lower sensitivity of birds to hypocapnia has not been characterized.

The cardiorespiratory adjustments detailed earlier have been graphically depicted in a recent review and reproduced here as Figure 10 (509). By means of these physiological adjustments, bar-headed geese can carry on with their yearly migrations following a route started before the rise of the Himalayan mountain range during the Eocene epoch, lasting from 55 to 34 million years ago.

Cardiovascular adaptations to gravity: Giraffes as a case study

The effects of gravity on the circulatory system were empirically recognized during the 19th century, when supine positioning of humans, instead of the more ancient practice of humoral bloodletting, to treat syncope in hemorrhaged patients [reviewed by (261)]. Simply laying the patients horizontally led to many of them recovering consciousness quickly. The rational was in the sitting position, the heart did not have enough force to overcome gravitational pressure, but that if the gravitational effect were removed by laying down, brain perfusion would resume. The gravitational effect on blood pressure is 10.27 kPa m^{-1} of vertical height. This is undisputed, but it has been argued that gravitational gradients do not increase cardiac work. As stated, "it is no harder, in the circulation, for the blood to flow uphill than downhill," and "differences in level of different parts of the vascular bed do not in any way affect the driving forces for flow, and so do not directly affect the circulation" (85). The siphon controversy was fostered by early pump-and-hose models of the circulation and generated a lively debate between the

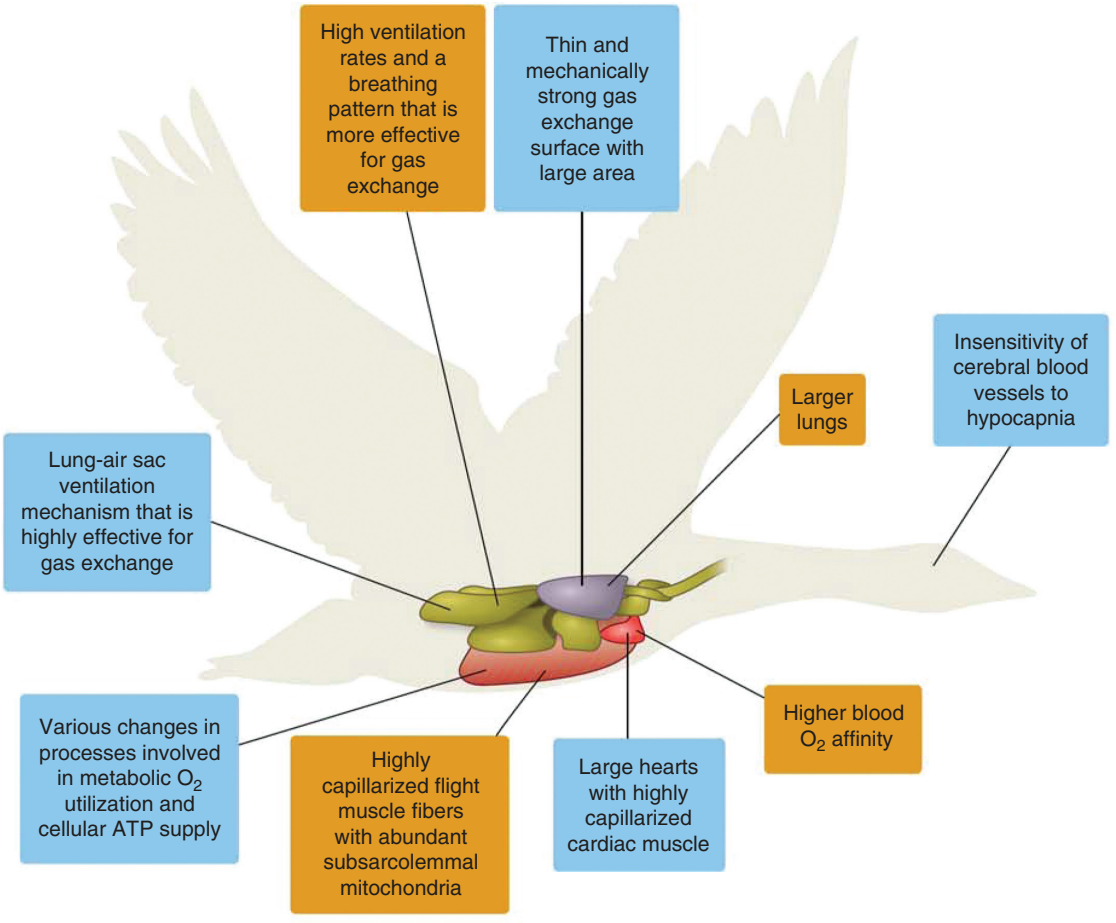

Figure 10 Summary of features aiding to high-altitude flight in bar-headed goose either as avian general traits (blue boxes) or species-specific specializations (orange boxes). Reprinted from (509) with permission.

prosiphon advocates (26,27,257,258) and the no-siphon critics (301,443,444,514,515). The debate is not fully settled, but most would agree today that the heart does work against gravity (137,215). The siphon effect has not been fully discounted, and the existence of negative pressures in the cranial venous vasculature is most easily explained by the siphon principle (259). Subatmospheric pressures occur in the cranial veins and sinuses of standing humans and can cause venous air embolism during sitting neurosurgical procedures (67, 141). Subatmospheric pressures have also been measured in the cranial jugular veins in anaesthetized giraffes (67).

The giraffe is the tallest animal on Earth, and therefore is exposed to the greatest gravitational loads. Pioneering studies carried in the 1950s and 1960s unveiled, but could not fully explain, how the giraffe cardiovascular system copes with gravity. Giraffes have a high blood pressure (220) and defy the medical dogma that systemic hypertension leads to morbidity. In restrained but conscious animals, mean arterial pressure at heart ranges between 10.27 and 38.93 kPa (222), with similar values in free-ranging giraffes (608). Such high blood pressures maintain cerebral perfusion by compensating for the negative effects of gravity (67, 242, 609). As a result, blood pressures of >10.27 kPa at brain level in giraffes (222, 607) are relatively similar to the cerebral perfusion pressures in other mammals, including humans (610). This would allow for cerebral autoregulation, which is stable within pressure ranges of 8.00 to 18.67 kPa in many species (343) and 9.33 to 28.00 kPa in giraffes (370).

The gravitational loads on the circulatory system of the giraffe are extreme. When giraffes raise their heads after drinking, the brain circulation would experience a gravitational pressure change of at least 30.67 kPa, corresponding to a 3-m change in elevation. Prior investigators estimated a drop of 36.67 kPa in pressure for a 14-ft-tall giraffe (394) based on the early measurements in conscious giraffes (607). A much smaller pressure change would render a human unconscious. Orthostatic hypotension, one of the common causes of syncope and transient loss of consciousness, is defined by the American Autonomic Society and the American Academy of Neurology as a drop in systolic blood pressure of 2.67 kPa within 3 min of standing. The opposite pressure changes occur when giraffes lower their heads to drink, and distal carotid pressures increase in conscious (607) and in anesthetized animals (67) as expected for the gravitational loads.

In terrestrial vertebrates, buffering the substantial effects of postural changes on blood pressure is crucial to brain perfusion and for preventing brain damage due to excess pressure. Several mechanisms operating at different time scales are responsible for such homeostatic response. First is the baroreflex, the fastest mechanism for blood pressure compensation on a beat-to-beat basis (235). Giraffes lack internal carotid arteries and carotid sinuses (221), which led early studies to disregard the importance of baroreflex responses (222). Careful histological studies later confirmed that the junction between the carotid and the occipital arteries closer to the base of the brain display features which are typical for baroreceptive areas, namely an elastic tunica media and a rich innervation through a branch of the glossopharyngeal nerve (312). In fact, circumstantial evidence of a postural baroreflex tachycardia upon standing up from a horizontal position, and bradycardia while lowering the head for drinking has been shown (607). From the original paper (607), the estimated baroreflex gain of the cardiac limb is 97 ms kPa^{-1} when standing up; this is almost double the gain estimated for rabbits, which is 39 to 59 ms kPa^{-1} (324, 479) as previously calculated (10).

When a giraffe raises its head, the baroreflex is assisted by an increased venous return caused by sudden emptying of the jugular veins (67) that, in turn, raises cardiac output and increases carotid blood pressure through the Frank-Starling mechanism (395). Immediately after head raising and concurrent with the baroreflex response, CBF could be maintained by blood flow redistribution between intracranial and extracranial arteries (395), and by preferential draining through the vertebral venous plexus instead of the jugular veins (394). This would result in an increase in intracranial perfusion despite the initial drop in carotid pressure. Although speculative, this scenario is in line with the observed counter-intuitive increase in cerebral vascular resistance upon head-lifting contributed mainly by extracranial arteries (395), and also with the histological findings of rich sympathetic innervation of the carotid arteries (314, 415), but poor innervation of intracranial arteries (314). Confirmation of these mechanisms would require that cranial blood flow can shift quickly between the collapsible jugular veins and the noncollapsible vertebral venous plexus, as previously suggested (394).

When giraffes lower the head for drinking, CBF is not compromised, because the increase in carotid pressure is compensated for by similar increases in cerebral venous pressure, so that the driving cerebral perfusion pressure is maintained (394). Instead, the main problem for the giraffe would be excessive cranial pressure that could result in cerebral hemorrhage or edema. Precapillary vasoconstriction could potentially counteract this effect and keep cerebral perfusion pressure within normal boundaries, but there is no experimental evidence of this. Instead, there is a decrease in cerebral vascular resistance in anesthetized giraffes (67) that could be the result of a redistribution of blood flow toward extracranial arteries draining into the jugular veins (394). In this situation, it has been speculated that giraffes may only keep their heads down for a short period of time due to decreased CBF (394). This is unlikely in conscious giraffes, in which functional reflexes can broaden the physiological homeostatic repertoire that may be blunted in the anesthetized condition of the animals in most of the experiments to date.

Blood pressure profiles in the limb arteries and veins in the giraffe reflect the gravity load (430), as in any other terrestrial vertebrate. The main difference is the absolute pressure, which can reach 46.66 kPa at the hoof in giraffes. To support such pressures, the conduit arteries of the legs have a thick tunica media and a small lumen (221). Similar structural differences are observed in smaller limb arteries (<400 μm), but not in similarly sized arteries from the neck (448) or in skin arteries from the legs (396), indicating a structural adaptation to high pressure.

A richly innervated sphincter-like structure was reported in the tibial artery at the level of the knee (313) in giraffes, and it has also been found in the median artery below the elbow (448). This vascular narrowing constricts in response to noradrenaline and can completely eliminate flow in isolated arteries (448). Its constriction reduces distal pressure (242), which, combined with a thin, but inflexible skin rich in collagen (505), prevents interstitial edema. The tight skin of giraffe legs is an analogous structure to the anti-g suit worn by fighter pilots and astronauts subjected to high acceleration forces to prevent blackout and g-force-induced loss of consciousness.

Giraffe hypertension develops as the animals grow and the neck lengthens to support CBF. Blood pressure increases due to structural adaptations in resistance arteries that raise peripheral resistance (397). These changes are concurrent with the hypertrophic growth of the left ventricular wall that allows the heart to pump against the rising afterload. Despite the elevated blood pressure, the heart of the giraffe is not larger than that of a mammals of similar mass, constituting roughly 0.5% to 0.6% of body mass (120, 397). To generate high pressures, the heart takes advantage of its architecture, which includes increased left ventricular thickness and decreased overall radius to normalize wall stress in accordance with Laplace's law (540). This is shown by the remarkable postnatal increase in cardiomyocyte polynucleation: The number of nuclei per cell increases from 1.3 in young giraffes to 4.2 in adult giraffes, higher than in any other mammalian heart (430). This is indicative of postnatal myocyte growth by proliferation without cytokinesis occurring when blood pressure starts rising in correlation to neck extension (397). It has been argued that the pyramidal architecture of the heart imposes restrictions on stroke volume while keeping ejection fraction at a normal value (540). The only published measurement of cardiac output in giraffes records an average of 33.8 mL min^{-1} kg^{-1} in male giraffes anesthetized with alphachloralose (125). This is about 35% lower than an allometry estimate of 51.6 mL min^{-1} kg^{-1} for a mammal of similar mass, 700 kg (271, 393, 513, 549). Therefore, a reduction in stroke volume, and therefore, cardiac output, is a likely trade-off in giraffes that need to pump against a higher afterload pressure.

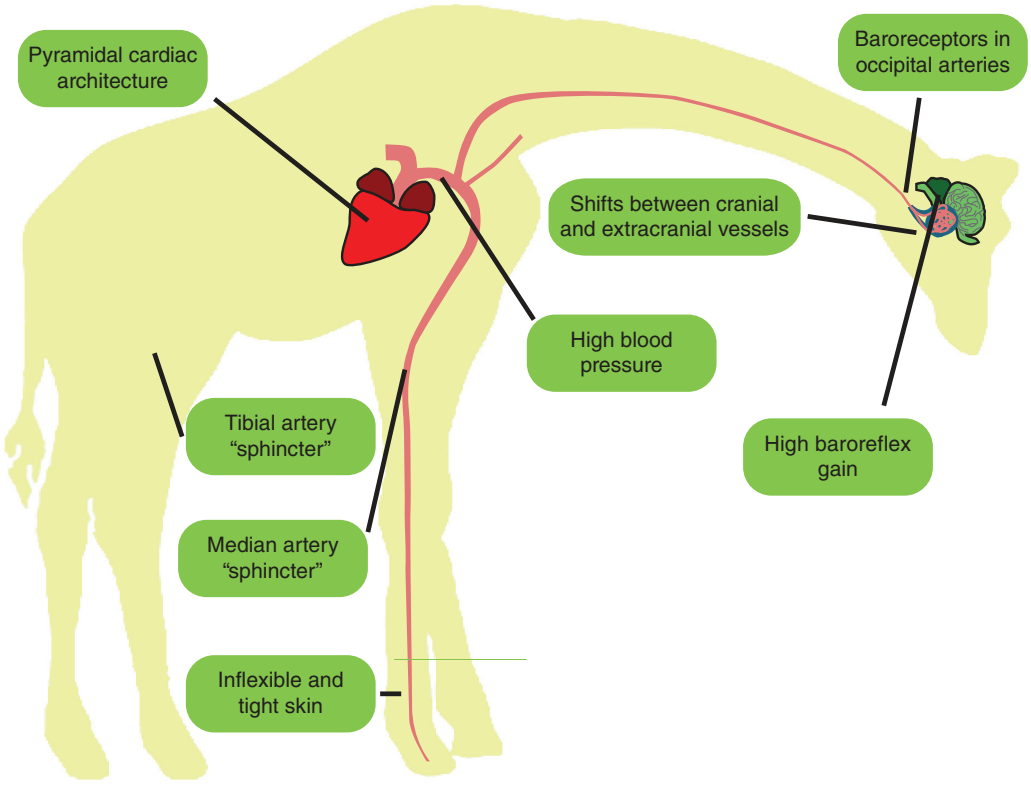

Figure 11 Summary of specializations to account for gravitational effects in the cardiovascular system of the giraffe.

Five decades of intermittent research on giraffe cardiovascular physiology show that relatively small adjustments in the cardiovascular system provide suitable regulation of blood pressure. From tip to toe, intricate and still poorly characterized blood shifts between intracranial and extracranial vessels, a powerful baroreflex, sphincter-like narrowing in limb conduit arteries, resistance arteries with a thickened media layer, and tight inflexible skin on the legs are all instrumental in explaining the giraffe's success facing large gravitational loads. These mechanisms are graphically summarized in Figure 11.

Conclusions and future directions

Novel adaptive mechanisms explain the ability of selected species to thrive in harsh and challenging environments where other species cannot survive. Studies of diving seals, high-flying geese, and giraffes outline a myriad of small changes in several organ systems that support their fitness in these environments, not just in the heart and the vasculature. Although our understanding of these systems is still fragmented, it is a combination of small changes that allows long dives in seals, but not in manatees, high flights in bar-headed geese, but not in Pekin ducks, and high blood pressure in giraffes, but not humans.

Conclusion

The challenges for future studies of the cardiovascular system are multifaceted, but, in terms of the relevance of the cardiovascular system to animal adaptation, they could be divided into three areas: first, future studies can help to achieve a similar level of understanding in other physiologically challenging scenarios such as postprandial responses, hibernation, and estivation. Second, future studies could take advantage of novel technical developments for the remote and less invasive monitoring of animals in their home ranges. This could lead to breakthroughs in the understanding of deep sea diving by whales, for instance. Third, including phylogenetic comparisons in the analysis of animal adaptations could provide a historical context for the appearance of novel physiological mechanisms.

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