Circulatory Anatomy in Bimodally Breathing Fish

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SYNOPSIS. The development of air-breathing organs in bimodally breathing fish has necessitated a degree of vascular remodelling in order to enhance gas exchange and support other homeostatic activities. Macrocirculatory changes include several plumbing schemes that allow perfusion of the gills, air-breathing organ, and systemic circulations in a variety of in-parallel and in-series arrangements. The incorporation of structural adaptations designed to minimize admixture of oxygenated and deoxygenated blood in transit through the heart as well as vascular shunts further increases the efficiency of the gas exchange process. A number of anatomical modifications in capillary architecture and endothelial cell structure are found in air-breathing fish and appear to be unique to these vertebrates. The physiological significance of the microcirculatory adaptations remains, to a large extent, speculative.

INTRODUCTION
Anatomical remodelling, evident in the vasculature of bimodally breathing fish, appears to convey a number of physiological attributes designed to enhance gas exchange and, perhaps, support other homeostatic functions. Vascular modifications can be broadly classified as either macrocirculatory or microcirculatory based on the approximate size of the vessel in question, arteriolar and smaller vessels comprising the latter group. Three categories of macrocirculatory modifications are evident: 1) those that affect blood distribution, i.e., the “conduit” vessels that connect the heart, gills, accessory respiratory organs and the systemic circulation; 2) those that minimize mixing of oxygenated and deoxygenated blood (axial flow separation) during transit through the heart; and 3) spatial and temporal shunts. Microcirculatory modifications are found in the form of three-dimensional rearrangement of small-vessel architecture and in endothelial cell specializations. Although limited in number, physiological studies suggest that the macrocirculatory modifications enhance oxygen delivery to tissues. Verification of the efficacy (or even intent) of microvascular specialization is, to a large extent, lacking.

Accessory respiratory organs have evolved independently in a considerable variety of fish and are derived from a number of unrelated organs, e.g., gill, swim bladder, or gastrointestinal tract (Johansen, 1970; Satchell, 1976; Graham, 1992; see also other papers from this symposium). Consequently, there does not appear to be a phylogenetic progression in the level of sophistication of the macrocirculation, in fact, the cardiovascular adaptations of the supposedly primitive Dipnoi appear to be the most advanced. It is beyond the scope of this review to catalogue vascular specializations in all bimodally-breathing fish, instead, I will emphasize the major variations in the macrocirculation that appear to be of homeostatic benefit. Other reviews (Johansen, 1970; Munshi, 1976; Satchell, 1976; Burggren and Johansen, 1986; Graham, 1992) can be consulted for additional information. By comparison, little information is available on microcirculatory adaptations and I have chosen to emphasize this topic in order to encourage further investigation.

MACROCIRCULATION

Conduit vessels

In addition to their well-known respiratory function, the gills of water-ventilating fish are the primary sites for nitrogen excretion, ionoregulation, and acid-base balance (see reviews in Hoar and Randall, 1984a, b). Gills are also important in regulating a variety of circulating hormones, an activity enhanced by the in-series arrangement of the gill and systemic circulations (Olson, 1991). Neither kidneys nor skin appear to be sufficiently developed to fulfill all the needs of hydromineral and acid-base regulation and nitrogen excretion (Randall et al., 1981). Thus in bimodally breathing fish there is an implied need to retain gill tissue for these functions. This, in turn, requires that the vasculature accommodate both gills and accessory respiratory organs in the overall perfusion scheme.

Figure 1 shows the vascular arrangement in a typical water-ventilating fish (Fig. 1A) and in an 'ideal' air-breather, such as a mammal, in which water balance and nitrogen excretion are regulated exclusively by non-respiratory organs (Fig. 1B). In both animals, respiratory and systemic vascular beds are in series. The mammal has two pumps to enable precise regulation of intravascular pressure to respiratory and systemic tissues whereas, in the fish, blood pressure in one area can be affected by resistance changes in the other.

The imposition of an accessory air-breathing organ (ABO) requires some vascular compromises. These are dictated by the anatomical location of the ABO, by the added vascular resistance of the ABO, and by the desired gas exchange efficiency of the gills and ABO. An in-series gill-ABO-systemic vasculature, which would optimize both non-respiratory and gas exchange activities, is not found in any fish, presumably because this would greatly increase total vascular resistance.

Vascular connections between gill, ABO, and systemic tissues of air-breathing fish can be described by one of three basic models (Fig. 1C–E). In Figure 1C, the ABO is perfused by pre-branchial blood from the ventral aorta or one of its branches. Oxygenated blood is returned via systemic veins and mixes with de-oxygenated systemic blood prior to entering the heart. This pattern, in which the ABO is in parallel with the gill-systemic circulation, is found in Electrophorus, Monopterus and Periophthalmus. In Clarias and Heteropneustes, effluent from both gills and the ABO enters the systemic arterial circulation (Fig. 1D). Thus the two respiratory organs are in parallel with each other and together they are in series with the systemic circulation. Fish that obtain oxygen from the gastrointestinal tract (e.g., Ancistrus, Hoplosternum and Plecostomus) or swim bladder (Lepisosteus) have their ABO circulation in parallel with other systemic tissues and this single ABO-systemic circulation is in-series with the gills (Fig. 1E). Figures 1F and 1G represent variations of the basic pattern illustrated in Figure 1E. In all three instances (Fig 1E–G), ventral aortic blood initially perfuses the gill arches. However, in Figures 1F and 1G, effluent from specific, and often structurally modified, gill arches may be directed to either systemic tissues or the ABO. Gas exchange efficiency may be variably enhanced in these latter two systems (Fig. 1F, G) by additional modifications, such as anatomical and temporal shunting away from potentially undesirable gas exchange surfaces, and control of axial flow separation (see below). The simplest of the two patterns (Fig. 1F) is found in Amia and Polypterus where all gill arches are well developed, and there are few anatomical modifications that suggest an attempt to prevent mixing of venous effluent from systemic tissues and ABOs. The most complex pattern is found in the lungfish, Protopterus, Lepidosiren, and Neoceratodus (Fig. 1G), where a number of shunts and anti-mixing modifications have developed. Other bimodal breathing fish, such as Anabas and Channa are found somewhere in between Amia and Protopterus in both the degree of anatomical modification and in gas exchange efficiency. Interestingly, the ABO in Amia, Polypterus, and the lungfish is derived from the swimbladder and is the basis of a true pulmonary circulation, whereas the ABO of Anabas and Channa is derived from branchial tissue (Graham, 1992).
Greenwood and Liem (1984) have described a fourth circulatory arrangement in Arapaima gigas (not shown in Fig. 1) in which the ABO is perfused from the caudal vein via the renal-portal vessels. This partial in-series gill-systemic-ABO system has not been observed in any other species of fish nor was it identified in Arapaima in previous reports (Farrell, 1978; Randall et al., 1978). Additional examination is required to resolve this discrepancy.

Three physiological problems arise from the inclusion of an ABO into the vasculature: 1) enforced partial unsaturation; 2) transbranchial oxygen loss; and 3) pressure regulation (Satchell, 1976; Graham, 1992). Enforced partial unsaturation results from the admixture of oxygenated ABO venous blood with deoxygenated systemic venous blood prior to perfusion of systemic tissues. Transbranchial oxygen loss is possible when the partial pressure of oxygen in ambient water falls below that of the blood traversing the gill lamellae and the resultant gradient favors oxygen diffusion from blood to water. Problems with pressure regulation become evident when the ABO vasculature is interposed either in series or in parallel with the branchial and/or systemic circulations. Modifications designed to affect axial flow through the heart or produce spatial and/or temporal shunting have repeatedly appeared in air-breathing fish in an attempt to minimize these problems.

*Axial flow*

Axial mixing can be minimized by decreasing the linear distance in which two fluids share the same conduit. This can be accomplished by formation of separate, parallel vessels to conduct oxygenated and deoxygenated blood to and from the heart and by intravascular valves and septa that reduce mixing of blood in a common vessel or chamber. To be fully effective, axial flow separation must extend from the confluence of ABO and systemic venous effluents and continue through the heart and into the arterial origin of the ABO and systemic circulations.

Venous vascular separation appears to be present in many air-breathing fish because the ABO and systemic veins either empty directly into, or anastomose near, the atrium. This is especially evident in Protopterus, Lepidosiren (Burggren and Johansen, 1986) and Gymnarchus (Graham, 1992) where ABO and systemic veins have separate connections with the atrium.

Multiple vessel separation of ventral aortic streams at, or immediately after, the origin of the conus or bulb is a feature also found in many air-breathers. In Protopterus and Lepidosiren, the conus has a central septum that emerges into individual ventral aortae. The latter proceed directly to the branchial arches (Burggren and Johansen, 1986). Multiple ventral aortas are also found in Gymnarchus (Graham, 1992), Heteropneustes (Olson et al., 1990), Anabas (Olson et al., 1986) and Channa (Ishimatsu et al., 1979).

Separation flow while in transit through the heart further enhances the efficiency of air-breathing mechanisms and approaches
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Fig. 2. Vascular corrosion replica of shunts between afferent (A) and efferent (E) filamental arteries in the third gill arch of Channa punctata. Arrows indicate rudimentary lamellar sinusoids. Bar = 50 μm. From Olson et al., 1994, with permission.

Spatial (anatomical) and temporal shunts are employed by many air-breathing fish to increase the efficiency of gas transfer. Arterio-arterial spatial shunts are found at many levels in the circulation and may provide a low-resistance pathway to enhance flow between vascular beds or, they may be a high-resistance pathway to step down arterial pressure. True arteriovenous (arterial to central venous) shunts do not appear to convey any particular hemodynamic advantage to bimodal-breathing fish and do not seem to be associated with the evolution of ABO. Venovenous shunts may minimize venous admixture or circumvent portal circuits. Temporal shunts permit regulation of perfusion distribution to different tissues when dictated by environmental conditions or tissue needs.

Arterio-arterial shunts may be transbranchial, intrabranchial, or post-branchial. Direct continuation of the afferent branchial artery as the efferent branchial artery provides a ventral to dorsal aortic transbranchial shunt, thereby preventing oxygen loss in an hypoxic environment and simultaneously lowering vascular resistance. Gill shunting may be complete, i.e., all four gill arches as in Electrophorus, or only one (fourth arch in Monopterus) or two (first and second arches in Protopterus and Lepidosiren) arches may be bypassed. Intrabranchial shunts, as afferent-efferent branchial, or afferent-efferent filamental artery anastomoses (Fig. 2), are quite common and serve a similar purpose. Even in unmodified gill lamellae, there is a possibility for a functional shunt if the blood preferentially flows through the portion of the lamellar sinus that is embedded in the filamental tissue (Fig. 3; Farrell et al., 1980). This type of shunt would not only reduce oxygen loss to the environment but would also maintain pillar cell contact with plasma, a necessary requisite for hormone metabolism (Olson, 1991).

Shunts between the dorsal aorta and the ABO artery, functionally analogous to the ductus arteriosus of mammals (Fishman et
al., 1985), are found in fish such as *Amia*, *Hoplerythrinus*, *Gymnarchus*, *Polypterus* and *Protopterus* where the ABO is perfused by epibranchial arteries or their extensions. In these fish, dorsal aortic and ABO artery pressures are similar. *Heteropneustes* has multiple connections between the dorsal aorta and efferent ABO arteries that could shunt oxygenated blood directly to the posterior of the fish (Olson et al., 1990). Many species also exhibit temporal shunting between the systemic circuit and ABO that is coordinated with ventilation of the ABO (Johansen et al., 1968; Farrell, 1978; Smith and Gannon, 1978).

Venovenous shunts appear in the form of an interrenal vein (Satchell, 1976) in a number of intestinal air-breathing fish (*Ancistrus*, *Callichthys*, and *Hoplosternum*) and in some fish that breathe with a modified swim bladder (*Erythrinus*). This vein bypasses the hepatic portal system thereby preventing oxygen extraction by the metabolically active liver. It also exposes the capillaries of the air-breathing organ to the *vis a fronte* suction of the heart and eliminates the potential problem of hepatic resistance affecting ABO flow (Satchell, 1976).

**MICROCIRCULATION**

**Microvascular architecture**

The three-dimensional organization of arteriole and capillary-size vessels in both gills and ABO of some bimodally breathing fish suggests other circulatory adaptations. Additional work is needed to determine if these adaptations directly enhance gas exchange or if they are secondary ionoregulatory or metabolic adaptations necessitated by bimodal breathing. Nevertheless, they clearly illustrate the complexity and plasticity of the cardiovascular system.

Lamellae of the rudimentary second and third gill arches in *Monopterus* do not have pillar cells but instead consist of a single vessel, about the size of an outer marginal channel (see Fig. 3), that may serve as an afferent to efferent filamental artery shunt. However, unlike the short, large-bore lamellar shunts found in *Channa* (Fig. 2), the shunts in *Monopterus* are long tortuous vessels that follow a zig-zag course, and in

Figs. 4 and 5. Corrosion replica of ABO capillaries in *Monopterus cuchia* (Fig. 4) and *Channa punctata* (Fig. 5). Position of respiratory epithelium is indicated by dashed line. Long arrow indicates direction of blood flow, note dilation in capillary segment nearest respiratory epithelium and constrictions on both sides of dilated segment (short arrows). Bar = 10 μm both figures. From Munshi et al., 1990 (Fig. 4) and Olson et al., 1994 (Fig. 5), with permission.
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Fig. 6. SEM of the respiratory vasculature from Channa punctata ABO. The respiratory epithelium (X) has been removed over the surface of the capillaries to reveal the underlying endothelial cell (E) and associated microvilli. Two red blood cells (R) can be seen squeezing through the constricted segment (see Fig. 5). Bar = 5 µm. From Olson et al., 1994, with permission.

Fig. 7. SEM of the respiratory vasculature from Anabas testudineus ABO. The respiratory epithelium (X) has been removed over the surface of the capillaries to reveal the tongue-like projections (T) and valve-like flaps (arrows) of the endothelium. Arrows also indicate direction of blood flow. Bar = 5 µm. From Munshi et al., 1986, with permission.

As shown in Figures 4 and 5, the lumen of the coiled ABO capillaries in Monopterus and Channa is noticeably constricted immediately prior to, and after, the vessel passes closest to the respiratory epithelium, whereas it is dilated when juxtaposed near the epithelium (Munshi et al., 1990; Olson et al., 1994). The significance of this in mixing blood, promoting a rapid arteriovenous pressure drop, removing old red cells (or other functions) is unknown.

Endothelial specializations

Although reports are limited, both transmission (TEM) and scanning (SEM) electron microscopy of gill and ABO vessels have shown curious structural modifications in the endothelium that appear to be unique to air-breathing fish. In Channa punctata ABO, numerous short microvilli cover the surface of the endothelial cell (Fig. 6; Olson et al., 1994) at the point of transition from a constricted to a dilated cap-

Coiled and uniquely shaped capillaries are found in ABO of the distantly related Monopterus (Fig. 4; Munshi et al., 1990) and Channa (Fig. 5; Olson et al., 1994) indicating that this type of vasculature has evolved independently. These vessels may also step down arterial pressure thereby enabling direct anastomosis of ABO and systemic venous effluents. (However, this could also be achieved with a precapillary arteriolar constriction which would produce a more uniform intravascular pressure along the length of the capillary.) The increased length of the capillaries may be important in intra-vascular mixing or in increasing the capacity of the endothelium to metabolize circulating hormones. The latter would compensate for the loss of gill metabolic capability associated with the reduction in branchial mass in these fish.
illary (Fig. 5). In contrast, few (2–4) microvilli are found on endothelia from *Channa gachua* (Olson et al., 1994) and they are absent altogether from *Monopterus ABO* (Munshi et al., 1990). The function of the microvilli is unknown.

Anabas ABO capillaries are long, straight vessels with two distinct endothelial cell modifications (Hughes and Munshi, 1973; Munshi et al., 1986; Munshi and Hughes, 1991). The most obvious is the tongue-like projection of the endothelium from the tissue side of the capillary (Figs. 7, 8). This may occlude a considerable portion of the lumen and thereby force the red cell to come in close contact with the gas exchange epithelium, it may also create turbulence and thereby promote mixing. This projection will also increase the endothelial surface area, if needed, for hormone metabolism. The endothelial cells also have valve-like cytoplasmic flaps on their upstream margins (Fig. 7) that may lift the tongue-like process closer to the respiratory surface when capillary flow increases (Munshi et al., 1990).

There are also complex modifications in the gill endothelium from the hyoidean hemibranch and gill arches 3 and 4 of *Protoperus* (Fig. 9; Laurent et al., 1978). Here, bulbous endothelial cell valves appear to promote flow of interstitial fluid, first into specialized cisternae, and then into the venous circulation. This cisternal-venous drainage is found in all species of *Protoperus* examined (Laurent et al., 1978) and in *Lepidosiren* (Morgan and Wright, 1989) but is absent from *Neoceratodus* (Gannon et al., 1983) and has not been reported in any other fish. Presumably this system is involved in hydromineral regulation.

**CONCLUSIONS**

The plasticity of the vertebrate cardiovascular system is exemplified in the macro- and
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microcirculatory modifications expressed in bimodally breathing fish. Nowhere is this more evident than in the primitive Dipnoi, especially *Protopterus* and *Lepidosiren*, where virtually all types of macrocirculatory adaptations are found. Collectively, a separate pulmonary circulation, intracardiac septation and central vessel separation, transbranchial, intrabranchial and postbranchial shunts, and the vasomotor mechanisms for temporal shunting, create in these fish nearly complete separation of pulmonary and systemic circulations that is more efficient than that found in many amphibians (Burggren and Johnson, 1986). Clearly these variations in large vessel architecture impart a hemodynamic advantage and increase gas exchange efficiency. Structural alterations in the microcirculation in many instances are unique to bimodal-breathing fish. The adaptive significance of these microvascular changes can only be surmised and this remains an important field for further investigation.

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