Gill Circulation: Regulation of Perfusion Distribution and Metabolism of Regulatory Molecules

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ABSTRACT   The fish gill is the primary regulatory interface between internal and external milieu and a variety of neurocrine, endocrine, paracrine, and autocrine signals coordinate and control gill functions. Many of these messengers also affect gill vascular resistance, and they, in turn, may be inactivated (or activated) by branchial vessels. Few studies have critically addressed how flow is distributed within the gill filament, the physiological consequences thereof, or the impact of gill hormone metabolism on gill and systemic homeostasis. In most fish, the entire cardiac output perfuses the arterioarterial pathway, and this network probably accounts for the majority of passive- and stimulus-induced changes in vascular resistance. The in-series arrangement of the extensive gill microcirculation with systemic vessels is also indicative of a high capacity for metabolism of plasma-borne messengers as well as xenobiotics. Adenosine, arginine vasotocin (AVT), and endothelin (ET) are the most potent gill constrictors identified to date, and all decrease lamellar perfusion. Perhaps not surprising, they are also inactivated by gill vessels. Acetylcholine favors perfusion of the alamellar filamental vasculature, although the physiological relevance of acetylcholine-mediated responses remains unclear. Angiotensin, bradykinin, urotensin, natriuretic peptides, prostaglandins, and nitric oxide are vasoactive to varying degrees, but their effects on intrafilamental blood flow are unknown. If form befits function, then the complex vascular anatomy of the gill suggests a level of regulatory sophistication unparalleled in other vertebrate organs. Resolution of these issues will be technically challenging but unquestionably rewarding. *J. Exp. Zool. 293:320–335, 2002.* © 2002 Wiley-Liss, Inc.

GILL PERFUSION

Knowledge of the factors that contribute to gill resistance, and their control, is of paramount importance in understanding both the specific function of different gill circuits and the impact of vasoactive agents on gill physiology. To date, this information is quite limited and it may be further confounded by the fact that many vasoactive substances may also directly affect gill exchange processes. Krakow ('13) was the first to demonstrate that resistance of isolated gills could be altered pharmacologically. Since then innumerable reports have cataloged the vasoactive effects of practically every molecule with known vasoactivity in mammalian vessels. This has provided valuable information on branchial receptors and gill vascular resistance; however, relatively few experiments have critically addressed the effects these vasoactive molecules have on flow distribution within the gill and their ultimate impact on gill physiology. The first part of this review examines the components of gill vascular resistance and focuses on studies that have directly addressed regulation of intrafilamental blood flow. A summary of the potential metabolic impact of the gill circulation on these vasoactive molecules is considered in the second part. The enormity of the task ahead will become obvious.

FLOW DISTRIBUTION

Vascular pathways in the gill filament are detailed in this issue (Olson, 2002) and presented schematically in Figures 1 and 2. The effects of
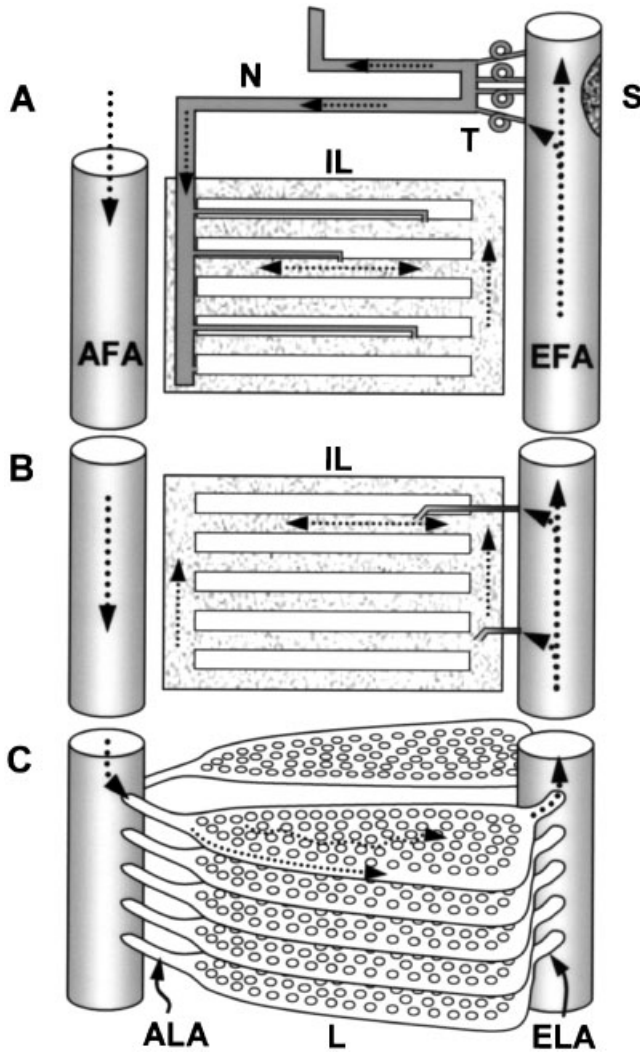
Fig. 1. Vascular pathways in the gill filament include two arteriovenous (panels **A** and **B**) and one arterioarterial (panel **C**) pathway. (**A**) Nutrient circulation (N) is formed from numerous tortuous vessels (T) that arise from the efferent filamental artery (EFA) near the muscular sphincter (S). Nutrient vessels drain into the interlamellar system (IL), or in some species, into filamental veins. (**B**) Interlamellar system is also supplied by narrow-bore feeder (F) vessels from the medial wall of the EFA. (**C**) Respiratory lamellae (L) are supplied by afferent and efferent lamellar arterioles (ALA and ELA, respectively). Dotted arrows indicate direction of blood flow.

filament are perfused (Booth, '78; Farrell et al., '79), thus recruitment of peripheral lamellae could enhance gill functional surface area (Hughes, '72). Redistribution of flow within individual lamellae could also affect functional surface area. The innermost channels of individual lamellae are sufficiently imbedded in filamental tissue that they probably do not participate in gas exchange (Fig. 2; Farrell et al., '79; Pärt et al., '84; Tuurala et al., '84). Redistribution of flow between the inner and outer margins could affect transbranchial exchange (Farrell et al., '79; Pärt et al., '84; Tuurala et al., '84), or the ability of pillar cells to metabolize blood borne molecules (Olson, '98; see also below). The physiology of the alamellar circulation in the filament core could be affected by the rate of blood flow, or its direction. Flow into the interlamellar system is probably regulated by narrow-bore feeder vessels emanating from the efferent filamental artery and by the nutrient
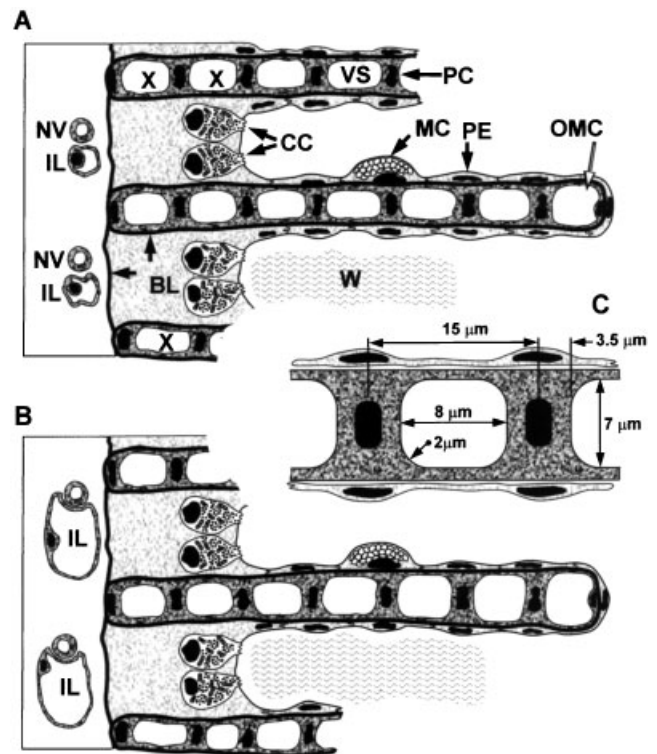

Fig. 2. Schematic of cross-section through filament perpendicular to lamellae. (**A**) Low-pressure perfusion; (**B**) with elevated intravascular pressure channels in the lateral lamella distend as does the interlamellar (IL) system. Abbreviations: BL, basal lamina; CC, chloride cell; OM, outer marginal channel; PC, pillar cell; VS, vascular space; W, respiratory water; X, vascular channel with extended blood–water diffusion distance. Based on studies of Farrell et al. ('80) and Olson ('83). (**C**) Dimensions used for calculating vascular surface area in a "typical" trout lamella; adapted from Olson ('98).

several vasoactive agents on resin distribution in vascular corrosion replicas are shown in Figures 3–8.

There are a number of perfusion options that could potentially affect function. Gas exchange could be altered by regulating the number of perfused lamellae, i.e., lamellar recruitment. Under resting conditions only lamellae in the proximal two-thirds of the trout or lingcod
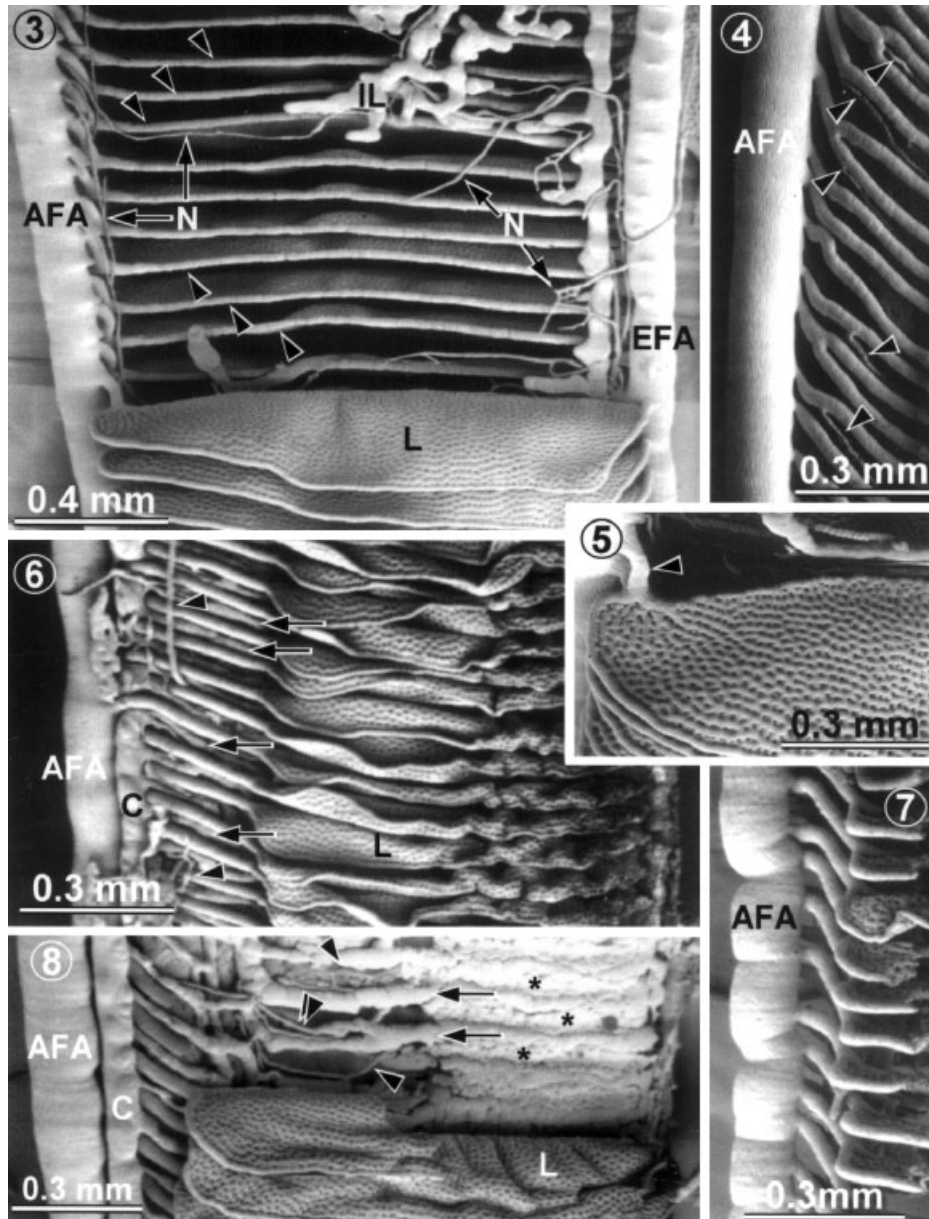
Fig. 3. (Figures 3–8 are scanning electron micrographs of methyl methacrylate corrosion replicas of channel catfish, *Ictalurus punctatus*, gills.) Gill filament pre-perfused with 10^{-5} M epinephrine. Afferent (AFA) and efferent (EFA) filamental arteries and lamellae (L) in arterioarterial respiratory pathway are well filled, whereas there is little resin in either interlamellar (IL) or nutrient (N) vessels thereby exposing the inner margins of lamellae on the opposite side of the filament (arrowheads); adapted from Olson ('80).

Fig. 4. Afferent filamental artery (AFA), afferent lamellar arterioles and even a few pre-lamellar arteriovenous anastomoses of filament pre-perfused with 10^{-5} M epinephrine are well filled. Compare to Fig. 7 (Olson, unpublished observation).

Fig. 5. Lamella from gill pre-perfused with 10^{-5} M epinephrine is well filled with resin. A pronounced outer marginal channel and vascular channels between pillar cells are evident. Arrowhead, afferent lamellar arteriole (Olson, unpublished observation).

Fig. 6. Gill filament pre-perfused with 10^{-6} M acetylcholine. Lamellae (L) appear well filled on afferent end but less resin is present toward the efferent margin. The interlamellar system (arrows), afferent collateral vessel (C), and nutrient vessels are also well filled. AFA, afferent filamental artery (Olson, unpublished observation).

Fig. 7. Afferent filamental artery (AFA) from gill pre-perfused with 10^{-6} M acetylcholine appears partially constricted. Note incomplete filling of lamellae on right. Compare with Fig. 4 (Olson, unpublished observation).

Fig. 8. Gill filament pre-perfused with 10^{-5} M sodium nitroprusside. The filling pattern of the afferent filamental artery (AFA), lamella (L), and collateral vessel (C) appears similar to that produced by acetylcholine (Fig. 6). The interlamellar vessels (arrows) are well filled; however, there appears to be considerable extravasation from the interlamellar system (*). Arrowheads indicate nutrient vessels and the double arrowhead shows a prelamellar arteriovenous anastomosis (Olson, unpublished observation).

vessels originating from the basal segment of the efferent filamental artery. Within each filament there are multiple possibilities for countercurrent flow; water versus lamellar blood flow, lamellar versus interlamellar, and interlamellar versus nutrient. The presence of prelamellar arteriovenous anastomoses (AVAs; Figs. 4 and 8) in a few fish adds to the perfusion possibilities. In most fish there is a heavily muscularized and well innervated sphincter in the basal efferent filamental artery (Bailly and Dunel-Erb, '86; Dunel-Erb and Bailly, '86), which could (and undoubtedly does) also affect perfusion at the filamental, lamellar and intralamellar levels (Dunel and Laurent, '77).

Most fish, with the exception of eels, catfish (and perhaps a few others) lack prelamellar AVAs and it is assumed that the entire cardiac output perfuses the lamellae. Perfusion of the alamellar filamental vasculature has been estimated in vivo, in whole-head preparations, and in perfused gills by measuring inflow into the head or arch relative to dorsal aortic or efferent branchial outflow while venous flow is either derived from the difference (Girard and Payan, '76; Colin et al., '79) or, less commonly, measured directly (Nilsson and Pettersson, '81; Ishimatsu et al., '88; Sundin and Nilsson, '92). In whole-head or multiple-holobranch preparations the percent venous flow has been reported to be 8–18% in the cod, *Gadus morhua* (Pettersson and Johansen, '82; Sundin and Nilsson, '92), and between 10% and 44% in the trout, *Oncorhynchus mykiss* (Girard and Payan, '76; Payan and Girard, '77; Colin et al., '79; Perry et al., '85; Gardaire et al., '91). Percent venous flow in perfused gills ranges from 10% to 30% in trout (Olson, '84; Nekvasil and Olson, '86; Olson et al., '86) to 18% in channel catfish, *Ictalurus punctatus*, and 21% in black bullhead, *Ictalurus melas* (Olson, '84), to 28% in the flounder, *Platichthys flesus* (Stagg and Shuttleworth, '84). Ishimatsu et al. ('88) compared hematocrits of branchial venous, sinus venosus, and dorsal aortic blood in trout in vivo and estimated that branchial venous flow was 7% of the total. Perhaps the main drawback of these preparations is the difficulty in collecting venous flow exclusively from filamental arteriovenous (A-V) vessels because many extra-filamental nutrient vessels also drain from the gill arches and because whole-head preparations usually include hypobranchial and cephalic circulations. These extra-filamental circuits likely contribute to an over-estimation of intra-filamental A-V flow, a point that seems to be supported by morphometric measurements of gill resistance (see below). These problems may be further compounded by unphysiological perfusion conditions relative to back pressure, pulse pressure, viscosity, anesthetics, or lack of other endogenous stimuli.

VASCULAR RESISTANCE

Gill vascular resistance has been measured in vivo, in perfused heads and isolated gill arches, and in rare instances it has been estimated morphometrically. In most teleosts, gill resistance is between 25% and 35% of systemic resistance (Perry et al., '84; Olson, '97). Thus for a generic teleost with ventral and dorsal aortic pressures of 35 and 25 mmHg, respectively and cardiac output of 15 mL·kg^{-1}·min^{-1}, total branchial resistance is around 0.7 mmHg·mL^{-1}·kg·min and resistance of a single gill arch is 0.09 mmHg·mL^{-1}·kg·min (Perry et al., '84; Olson, '97). Obviously, these estimates could be further refined by correcting for hemodynamic variables of specific fish and variability in the relative size of the four pairs of arches. These estimates also generally ignore the resistance contribution of non-respiratory pathways. Because the filament is generally considered the functional unit of the gill, emphasis will be placed on perfusion of the filamental vasculature.

Farrell ('80) measured vessel dimensions in fixed gills and corrosion replicas of lingcod, *Ophiodon elongatus*, and calculated the resistance of individual vessel segments (Table 1; Farrell, '80; Farrell and Sobin, '85; additional details provided in Randall and Daxboeck, '84). As shown in Table 1, afferent lamellar arterioles have the highest resistance of any arterioarteriolar vessel in the filament (over 6 times that of lamellar resistance and 30 times that of the efferent lamellar arteriole).

Vascular resistance of the two A-V networks in the gill filament (see Fig. 1 for pathways) has not been quantified. However, there is now enough anatomical information (Olson, 2002) to add to the study of Farrell ('80) and provide at least a qualitative estimate of relative resistances and thereby permit further evaluation of physiological studies. With the Poiseuille relationship for a Newtonian fluid, vessel resistance R is equal to $8·\eta·l/\pi·r^4$; where η=viscosity; l=vessel length, and r=vessel radius. The dimensions and resistance of feeder vessels (those that arise from the medial wall of the efferent filamental artery along

TABLE 1. *Vascular resistance, pressure drop and blood transit time for individual vessels in the lingod, Ophiodon elongatus, filament*[*]

	Diameter (μm)	Length (cm)	R (mmHg·mL^{-1}·min)[a]	ΔP (mmHg)[a]	Transit time (sec)
AFA[b]	200	1.44	1.36×10^2	1.6	2.1
ALA-prox[b]	20	0.06	0.94×10^5	3.5	
ALA-cent[b]	17	0.03	0.89×10^5	3.4	0.34[b]
ALA-dist[b]	14	0.015	0.98×10^5	3.7	
Lamell[b]	10	0.8	1.45×10^4	0.6	2.53
ELA[b]	28	0.009	2.92×10^3	0.11	0.01
Feeder AVA[c]	7	0.015	1.57×10^6		
Tortuous	15	0.015	7.43×10^4		

[*]Abbreviations: AFA, afferent filamental artery; ALA and ELA, afferent and efferent filamental arterioles, respectively; proximal, central, and distal denote positions of ALA along filament.
[a]Converted from cm H_2O to mmHg; similar values for all ALA.
[b]From Farrell ('80).
[c]Measurements of several teleosts from Olson ('02).

its length) and tortuous vessels (those that arise from the base of the efferent filamental and condense to form the nutrient system) are shown in Table 1. The resistance of a single feeder is 16 times the total resistance of the in-series afferent lamellar arteriole, lamella, and efferent lamellar arteriole (ALA-L-ELA) pathway. As there are anywhere from 3–10 pairs of in-parallel ALA-L-ELA per single feeder vessel (Olson, 2002), the resistance of a single feeder is 85 times that of 3 pairs of ALA-L-ELA and 280 times greater than 10 pairs of ALA-L-ELA. Resistance of the capacious interlamellar vessels is difficult to estimate because their diameter is highly susceptible to transmural pressure (Olson, '83) and the direction of flow across the filament body (hence vessel length) is uncertain. However, since the interlamellar vessels are in-series with the feeder vessels, they further increase the total resistance of the feeder-interlamellar network. The high-resistance feeder vessels probably serve at least two important physiological functions: (i) they insure that only a small fraction of the cardiac output is diverted away from the arterioarterial (A-A) circulation, and (ii) they step down efferent filamental arterial pressure to prevent over-distension of the highly compliant interlamellar vessels.

The comparatively high resistance of the feeder–interlamellar system has several implications for physiological studies. First, because resistance of the feeder–intralamellar system is nearly 100 times greater than that of the ALA-L-ELA vessels, it seems unlikely that a physiological change in feeder resistance will produce a perceptible change in input pressure either in a perfused preparation or in vivo. Second, it also seems unlikely that a change in resistance of the feeder vessels alone

will greatly affect effluent flow from the A-A circulation.

The contribution of the tortuous vessels to intrafilamental vascular resistance may also be minor. The resistance of a single tortuous vessel is $7.4 \cdot 10^4$ mmHg·mL^{-1}·min (Table 1). A single efferent filamental artery of a 300-g rainbow trout may have 100–200 of these vessels (Olson unpublished observation), which reduces their collective resistance to $(7.4 \cdot 10^2)$–$(3.7 \cdot 10^2)$ mmHg·mL^{-1}·min. As these vessels arise from near the base of the filament, their total resistance is in-parallel with the terminal segment of the efferent branchial artery which, being very short, is only around 7 mmHg·mL^{-1}·min. Thus the origin of the nutrient system is also a relatively high resistance pathway relative to the in-parallel segment of the respiratory circuit and a change in nutrient resistance either in vivo or in a perfused preparation may be difficult to detect. Furthermore, as only a fraction of the nutrient circulation goes to the filament core (much goes to nutrient supply for the arch tissue and the adductor musculature of the filament) resistance measurements will over-estimate the flow into the filament core.

A significant number of prelamellar AVAs are present in eels (Laurent and Dunel, '76; Donald and Ellis, '83) and catfish (Boland and Olson, '79). In catfish, prelamellar AVAs are 2–3 times longer and 80% narrower than afferent lamellar arterioles (cf. Figs. 4 and 8), which means that their resistance is well over 1,000 times greater. Furthermore, because they are less frequent than lamellar vessels their overall contribution to filamental vascular resistance is probably minimal. Hughes et al. ('82) used the Fick method and

estimated that 30% of the cardiac output is diverted into prelamellar AVAs in the eel, *Anguilla anguilla*. However, in corrosion replicas of gills from the Australian eel, *Anguilla australis* (Donald and Ellis, '83), the relative resistance of prelamellar AVAs appears considerably higher than that suggested by physiological estimates. Thus the actual contribution of these vessels to filamental blood flow remains unclear. Nevertheless, because prelamellar AVAs are relatively uncommon in most fish (Olson, 2002), they probably do not make a significant contribution to the vascular resistance of the alamellar filamental circulation.

A number of assumptions were made in the above calculations, most notably that blood is a Newtonian fluid with a viscosity of 5 cP ($= 0.615 \cdot 10^{-6}$ mmHg·min; Farrell, '80). How much the Farhaeus Lindqvist effect (blood viscosity is less dependent on hematocrit in capillary-size vessels) is offset by the low shear rates in the gill microcirculation, is unknown. Nevertheless, it seems reasonable to assume that it is difficult, if not impossible, to evaluate resistance changes in the non-respiratory portion of the filamental circulation based solely on changes in input pressure or flow in an intact arch.

PHYSICAL FACTORS AFFECTING GILL BLOOD FLOW

As transmural pressure in the gill increases, gill vascular resistance usually decreases (Wood, '74; Farrell et al., '79), although resistance was linearly related to inlet pressure in the perfused flounder gill (Stagg and Shuttleworth, '84). Calculations based on vessel geometries suggested that the decrease in gill resistance could be due to lamellar recruitment (Muir and Brown, '71; Wood, '74), however, this has been questioned (Farrell et al., '79). Intralamellar blood volume also increases when transmural pressure increases (Olson, '83). Pulsatile input pressure reduces vascular resistance (Farrell et al., '79; Daxboeck and Davie, '82) and increases perfusion of peripheral lamellae even at the same mean pressure (Farrell et al., '79). As would be expected, an increase in efferent pressure in perfused gills increases flow through the venous system and decreases flow from the efferent branchial artery (Olson, '84; Stagg and Shuttleworth, '84). Thus gill hemodynamics may be affected by changes in cardiac output and stroke volume (pre-gill pressures), systemic vascular resistance, and central venous pressure.

PHYSIOLOGICAL FACTORS AFFECTING GILL BLOOD FLOW

Endocrine stimuli

Angiotensin

Angiotensin II (ANG II) constricts perfused gills of trout (Olson et al., '94) and eels (Fenwick and So, '81). The trout response appears confined to the gill microcirculation because ANG II either has no effect on gill conductance arteries (efferent branchials), or it slightly dilates them via release of an endothelial derived prostaglandin (Conklin and Olson, '94). Gills do not appear to be under any tonic ANG control; in vivo inhibition of endogenous ANG II production with the angiotensin converting enzyme inhibitor, lisinopril, does not affect gill resistance, whereas systemic resistance is nearly halved (Olson et al., '97b). All teleost vascular ANG II receptors appear to be similar to the AT_1 type except that they are relatively insensitive to sartan-type inhibitors (Russell et al., 2001). Eel gill vessels are refractory to ANG II (Oudit and Butler, '95a). There is no information regarding the effects of angiotensins on intrabranchial flow.

Arginine vasotocin

Arginine vasotocin (AVT) is a potent vasoactive hormone in most fish. In intact eels it increases A-V blood flow (Chan and Chester Jones, '69; Oudit and Butler, '95b), whereas it decreases A-A flow without affecting A-V flow in perfused gills (Bennett and Rankin, '86). In trout, where essentially all A-V flow has a post lamellar origin, AVT is one of the most potent gill constrictors. A 100 pM·kg^{-1} AVT bolus injected into unanesthetized fish doubles branchial resistance while systemic resistance only increases by 15% (Conklin et al., '97). AVT has two effects in perfused trout gills (Conklin et al., '97). At low doses (10^{-12} M) it increases flow through the A-A pathway and decreases A-V flow without affecting total flow or input pressure; at high doses (10^{-8} M) it increases input pressure and increases A-V flow at the expense of both A-A and total flow. The former response is generated at physiological AVT levels (Warne and Balment, '97), whereas the high dose apparently produces tissue damage. The trout vascular receptor is pharmacologically similar to the mammalian AVP V_{1a} and OXY receptors and it

is distinct from the previously reported gill epithelial receptor (Conklin et al., '99).

Bradykinin

Homologous bradykinins (BK) slightly increase gill resistance in unanesthetized trout via secondary release of prostaglandins, but by themselves they do not have measurable vasoactivity in vivo (Olson et al., '97c) or on efferent branchial arteries in vitro (Conlon et al., '96).

Cholecystokinin and caerulein

Cholecystokinin-8 and caerulein constrict both A-A and AV pathways in Atlantic cod (Sundin and Nilsson, '92).

Epinephrine/norepinephrine

The effects of biogenic amines and autonomic nerves on gill perfusion are described elsewhere in this issue (Sundin and Nilsson, 2002).

Natriuretic peptides

Natriuretic peptides (NPs) are consistent branchial dilators in vivo (Olson et al., '97b) and in vitro (Evans et al., '89; Olson and Meisheri, '89). Homologous NPs, (tANP, tVNP, and tCNP) increase cGMP production in isolated trout efferent branchial arteries and their ability to partially or completely relax pre-contracted vessels appears to be independent of the nature of the contractile stimulus (Olson, Smith, Russell, and Takei, unpublished observation). In perfused trout gills, ANP has little effect on otherwise un-stimulated gills whereas it decreases A-A resistance in epinephrine-constricted gills from winter trout (Olson and Meisheri, '89).

Prolactin, cortisol, growth hormone, insulin-like growth factor, and thyroid hormones

To my knowledge, the effects of these molecules on gill perfusion have not been examined.

Urotensin

Urotensin II slightly increases gill vascular resistance in unanesthetized trout and contracts efferent branchial arteries in vitro (LeMevel et al., '96).

Paracrine stimuli

Adenosine

Adenosine increases gill resistance in vivo and in vitro in trout (Ristori and Laurent, '77; Colin and Leray, '79; Colin et al., '79; Sundin and Nilsson, '96), borchs, *Pagothenia borchgrevinki* (Sundin et al., '99), and cichlids, *Oreochromis niloticus* (Okafor and Oduleye, '86). In perfused trout heads it decreases A-A outflow and increases flow through the A-V pathway; efferent branchial arteries appear more responsive than afferent branchials (Colin et al., '79). In vivo video microscopy of trout filaments suggest that adenosine decreases perfusion of peripheral lamellae, thereby ''de-recruiting'' lamellae and also diverting flow to the A-V pathway (Sundin and Nilsson, '96), and this is supported by a decrease in oxygen uptake by adenosine-treated borchs (Sundin et al., '99). These responses appear to be mediated by A_1 receptors (Sundin and Nilsson, '96; Sundin et al., '99).

Prostaglandins

Prostacyclin (PGI_2) produces a biphasic transient constriction and long-lasting vasodilation in perfused elasmobranch, *Scyliorhinus stellaris* and *Torpedo marmorata*, heads and an unanticipated constriction in perfused heads of four teleosts, *A. anguilla, Conger conger, Scorpaena porcus*, and *Solea solea* (Piomelli et al., '85). Similar results have been observed in perfused arches of *A. anguilla, C. conger*, and *S. stellaris*. The constriction in teleosts was not mediated by secondary production of other prostanoids or by α-adrenoceptors or central nerves. Elasmobranch relaxations were independent of secondary prostaglandins, serotonergic, or β-adrenoceptors. Prostaglandin E_2 similarly vasoconstricts eel gills, in vivo (Janvier, '97). In perfused trout gills, with A-A and A-V effluent collected simultaneously, both indomethacin and ibuprofen decrease A-A outflow and increase A-V outflow, suggesting a contribution of endogenous prostaglandin production in gill hemodynamics (Hemker, '87).

Nitric oxide

Nitroso donors, such as sodium nitroprusside, vasodilate isolated branchial vessels (Smith et al., 2000), but in intact fish, most of their effects are on systemic vascular resistance and gill responses are minimal (Olson et al., '97b). This also reinforces the concept that branchial conductance

and resistance vessels are pharmacologically distinct. Nitric oxide synthase (NOS) activity has been detected in cod branchial nerves (Gibbins et al., '95) and the inducible form (iNOS) can be produced in trout gills within 3–6 hr after bacterial challenge (Campos-Perez et al., 2000). However the constitutive endothelial form (eNOS) has not been found in any fish vessel (Olson and Villa, '91).

Endothelin

Endothelin (ET) is one of the most potent and gill-specific vasoconstrictors identified thus far. In unanesthetized trout, homologous ET increases gill resistance 10-fold, while systemic resistance only doubles (Hoagland et al., 2000); in anesthetized cod the increase in branchial resistance is also increased 3-fold over that of systemic resistance (Stensløkken et al., '99). Elegant epi-illumination microscopic studies of trout and cod gills by Sundin and Nilsson ('98) and Stensløkken et al. ('99) have provided strong evidence that ET directly constricts the pillar cells, thereby decreasing gill exchange area and diverting the remaining blood around the outer margin of the secondary lamellae. This is consistent with the ET-mediated decrease in A-A flow seen in perfused trout gills (Olson et al., '91). It is also the first demonstration of an active pillar cell response to any vasoactive substance. ET_B receptors appear to mediate the response in the elasmobranch, *Squalus acanthias* (Evans and Gunderson, '99). Teleost ET receptors are especially abundant in the lamella but do not seem to fit the mammalian classification (Lodhi et al., '95). Immuno-reactive ET has been localized in gill neuroendocrine and endothelial cells but not in vascular smooth muscle (Zaccone et al., '96; see also Evans, this issue). It is not known if the teleostean receptors initiate physiological responses, or if they are involved in ET extraction from the circulation (see below). Similarly, it is unclear if the immunoreactive ET in endothelium is awaiting secretion, or if it has just been removed from the circulation. It is also not known if ET acts via endocrine or paracrine pathways.

Acetylcholine

Acetylcholine does not circulate in the plasma but may be released neuronally. Acetylcholine is a branchial vasoconstrictor acting through muscarinic receptors (Wood, '75; Oduleye et al., '82). It decreases the number of lamellae perfused in trout (Booth, '79) and channel catfish (Holbert et al., '79; Olson, '80). Vascular replicas of eels (Dunel and Laurent, '77), smooth toadfish (Cooke and Campbell, '80), and catfish (Olson, '80) prepared after acetylcholine infusion show increased filling of the alamellar vasculature and decreased lamellar filling (Figs. 6 and 7). The efferent filamental artery sphincter appears to be one of the predominant sites of acetylcholine constriction (Dunel and Laurent, '77; Smith, '77; Farrell and Smith, '81). Constriction of this sphincter would elevate pressure in the efferent filamental artery and increase A-V perfusion.

METABOLISM OF PLASMA SUBSTRATES

It is becoming more apparent from mammalian studies that the vasculature makes a significant contribution toward regulating titers of a variety of plasma-borne molecules, especially hormones. Endothelial cells may activate or inactivate circulating molecules directly via extracellular enzymes, or metabolism may follow internalization. Metabolic specificity is conferred by the nature of these enzymes and carriers. Metabolic capacity depends on biochemical activity, the degree of perfusion, and the extent of the vascular surface in contact with plasma. Recent studies have shown that the gill has the capability for regulating plasma-borne substrates and that its capacity may exceed that of any other organ. The following paragraphs will consider the anatomical and theoretical basis of gill metabolism and examine a few of the hormones upon which gill activation/inactivation has been demonstrated. Additional details can be found in the review by Olson ('97).

Two anatomical attributes, an extensive microvascular surface area and an in-series relationship with the systemic circulation, optimize the metabolic potential of the gill. Gill vascular surface area is considerably larger than the respiratory surface (Olson, '97). Hughes and Morgan ('73) estimated that the exposed lamellar surface area of a 300-g rainbow trout was 600 cm^2. However, as gas exchange only occurs in the spaces between pillar cells (Fig. 2c), the effective respiratory surface is probably closer to 500 cm^2. The vascular surface area of the exposed lamella, based on idealized dimensions in Fig. 2c, is around 750 cm^2. Assuming that an additional 20% of the lamellar circulation is imbedded in the filament and does not participate in gas exchange (Fig. 2a; also Olson, 2002) the vascular surface area becomes

900 cm^2, nearly twice that of the respiratory area. In fact, vascular area may be even higher because pillar cell density is greatest in the portion of the lamella imbedded in the filament.

The metabolic potential of the branchial vasculature is further enhanced because the gills are the only organ perfused by the entire cardiac output. Thus the gill can be modeled as an in-line filter in a closed, recirculated system (Olson, '98). The rate constant for branchial inactivation of plasma-borne molecules (k_G) can then be estimated from the equation; $k_G = -\ln(1-f_G) \cdot (CO/V_b)$, where f_G is the fraction of substrate (e.g., hormone) inactivated by the gill in a single pass through the branchial vasculature, CO is cardiac output, and V_b is blood volume. In most fish, CO/V_b is close to unity. This rate constant can then be used to predict the fall in plasma concentration of the active molecule from the equation; $[M_t] = [M_0]e^{-k_G t}$ where $[M_t]$ and $[M_0]$ are plasma concentrations of molecule M at time t and 0, respectively (Fig. 9a). Rate constants for nonbranchial tissues (k_{TISS}) can be determined if both the fractional inactivation (f_I) and the fraction of total cardiac output perfusing that tissue (f_P) are known, i.e., $k_{TISS} = -\ln(1-f_I \cdot f_P) \cdot (CO/V_b)$. As shown in Fig. 9b, the gill has a tremendous advantage over other tissues because it receives the entire cardiac output ($f_P = 1.0$), whereas systemic tissues receive 20% ($f_P = 0.2$), or less. Thus 20% inactivation by the gill would be as efficient as 100% inactivation by any other tissue receiving 20% of the cardiac output. If multiple tissues inactivate a molecule with the same efficiency, their contributions would be additive, based on flow.

Physiological recovery of the cardiovascular system following hormone stimulation (Fig. 9c) commonly follows a mono-exponential time course (Olson et al., '97a) and the rate constant and half-time for recovery (half-time is related to k by: $t_{1/2} = 0.693/k$) can be determined experimentally. As shown in Table 2, recovery half-time for peptides and amines (with the exception of arginine vasotocin) are 3–5 min. As these values are also similar to the rate constant for mixing of inert volume markers in the cardiovascular system (Olson et al., '97a), it can be concluded that convective distribution, and not inactivation, is the rate-limiting process in removal of many vasoactive molecules from the circulation.

The relative contributions of branchial versus systemic tissues to inactivation is unknown
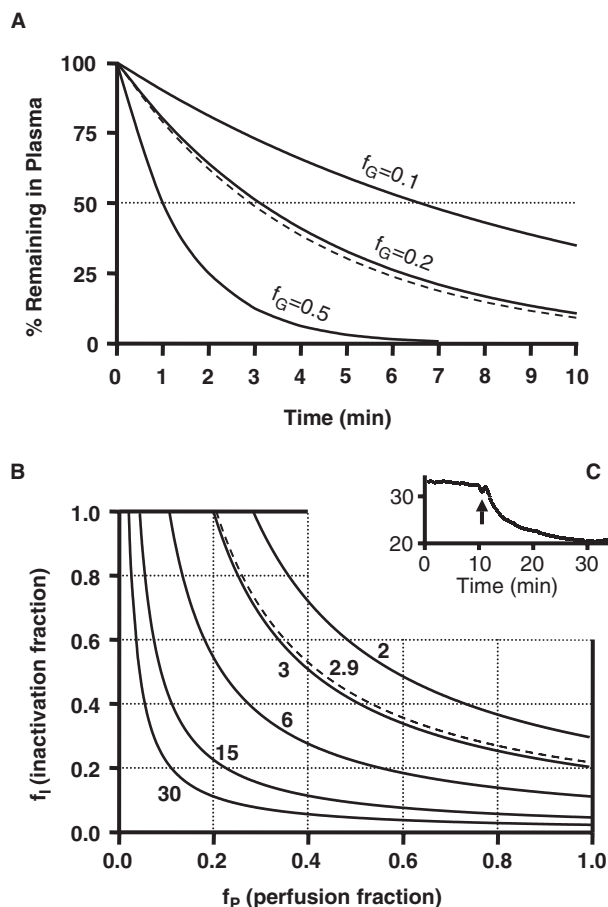

Fig. 9. Models of gill capacity for inactivating plasma-borne substrate. (**A**) Time course for inactivation of plasma substrate when fractional gill inactivation (f_G) is 0.1, 0.2, and 0.5, i.e., 10%, 20%, and 50% of the molecules are inactivated in a single transit through the branchial vasculature. A molecule with a $f_G = 0.2$ would have a 3-min half-time in the circulation. (**B**) Relationship between fractional inactivation (f_i) of a tissue and the fraction of total cardiac output that perfuses the tissue (f_p) for plasma half-times of 2, 3, 6, 15, and 30 min. Example: for a molecule to have a 6-min half-life in the circulation, gill f_i (f_G) would have to be 0.11 (gill f_p is always 1.0). Another tissue that only receives 20% of the cardiac output would need to inactivate 45% ($f_i = 0.45$) for the same 6-min half-life. (**C**) Decrease in dorsal aortic pressure upon cessation of norepinephrine infusion (at arrow). This is also shown as the dashed line in A and B [after Olson et al. ('97a) and Olson ('98)].

and needs to be examined. Isolated perfused gills have provided the most detailed information on both extraction (uptake) and metabolism, whereas most in vivo studies have focused only on branchial extraction. Although neither of these methods provide a complete picture, it is clear that the gill plays a significant, and in

TABLE 2. *Fractional extraction of biomolecules by the perfused trout gill (p) or by gills in vivo (i) and half-time ($t_{1/2}$) for recovery of cardiovascular variables following hormone infusion or blockade of angiotensin converting enzyme in trout in vivo*[a]

		Fractional extraction (or activation*)	Recovery $t_{1/2}$ (min)
Peptides	ANG II (p)	0.65*	5
	ACE inhibition		7
	BK (p, i)	0.25, 0.20	3
	ANP (p, i)	0.60, 0.60	
	AVT (i)	0.20, 0.35	18
	ET-1 (i)	0.55	
Amines[b]	Epi (p)	0.05	3
	Nepi (p)	0.16	4
	5-HT (p)	0.80	
Arachidonic acid	Ar-A (p)	0.99	
COX products	PGE$_2$ (p)	0.40	
	PGI$_2$ (p)	0.13	
Purines	Ado (p)	0.40	

[a]Abbreviations: ACE, angiotensin converting enzyme; Ado, adenosine; ANG, angiotensin; ANP atrial natriuretic peptide; Ar-A, arachidonic acid; AVT arginine vasotocin; BK, bradykinin; COX, cyclooxygenase; Epi, epinephrine; ET, endothelin; Nepi, norepinephrine; PG, prostaglandin; 5-HT, serotonin [see Olson et al. ('97a) and Olson ('98) for original references].
[b]Combined uptake and metabolism

some instances dominant, role in endocrine regulation. The activity of branchial tissue on a variety of physiological substrates is briefly summarized in the following paragraphs and itemized in Table 2. Unless otherwise indicated, the original references can be found in Olson et al. ('97a) and Olson ('98).

Angiotensin

Angiotensin converting enzyme (ACE) is found in gill vascular endothelium on the afferent side and it appears to be especially prevalent in pillar cells in the inner margin. In the perfused gill, the A-A pathway converts 65% of angiotensin I (ANG I) to angiotensin II (ANG II) in a single pass and ANG II activation appears to be only limited by convective mixing. Conversely, the A-V pathway does not form ANG II, but it metabolizes around 10% ANG II to inactive metabolites. Thus the gill is undoubtedly a significant site for ANG II formation in vivo, whereas A-V metabolism of ANG II is insufficient to account for the rapid recovery of physiological variables following ANG II infusion (Table 2).

Bradykinin

Bradykinin (BK) extraction by the perfused trout gill ranges from 40% to 20%, depending on whether BK is administered as a single bolus or continuously infused. There is relatively little BK metabolism by either pathway, which is surprising given the abundance of kininase II (ACE) in the A-A pathway and other peptidases in the A-V pathway. Extraction of ^{125}I-BK in vivo is also relatively low in trout (Fig. 10), which contrasts with the rapid recovery of blood pressure following bolus injection of BK (Table 2). Thus the trout gill does not appear to be important in BK inactivation. Recently, Takei's group (Takei and Tsuchida, 2000; Takei et al., 2001) found that ACE inhibition in the eel depresses drinking and that this effect results from increased BK levels rather than suppression of ANG II formation. Tonic inactivation of BK may be physiologically relevant in the eel, it remains to be determined if this process is mediated by branchial tissues.

Natriuretic peptides

Atrial natriuretic peptide (ANP) has received the most attention of the three fish natriuretic peptides (NPs). Isolated trout gills continuously extract around 60% of ^{125}I-ANP during three hours of perfusion. A similar percent extraction is observed following a bolus pulse in vivo (Fig. 10) and gills accumulate 10 times more radiolabel than other tissues, irrespective of whether the peptide is injected into the ventral or dorsal aorta. C-type clearance receptors (NPR-C) are abundant in the gills and their inhibition effectively eliminates gill accumulation (Fig. 10) and produces systemic hypotension. Autoradiography of ^{125}I-ANP binding to frozen sections of eel filaments shows considerable radiolabel in the chondrocytes, although this is probably of little significance in NP clearance from the plasma. NPR-C receptors have been found in afferent and efferent arteries and arterioles and lamellae of toadfish gills (Donald et al., '94) where they likely are involved in NP uptake. A novel receptor, NPR-D has also been described in the eel [reviewed in Loretz and Pollina (2000) and Takei (2000)] and this along with the NPR-C receptor may be important in NP removal from the circulation. Interestingly, while there is a pre- to post-gill decrease in plasma ANP and VNP concentrations in freshwater eels, there is no change in NP concentrations in ventral and dorsal aortic blood of saltwater-adapted eels (Kaiya and Takei, '96). This suggests that gill
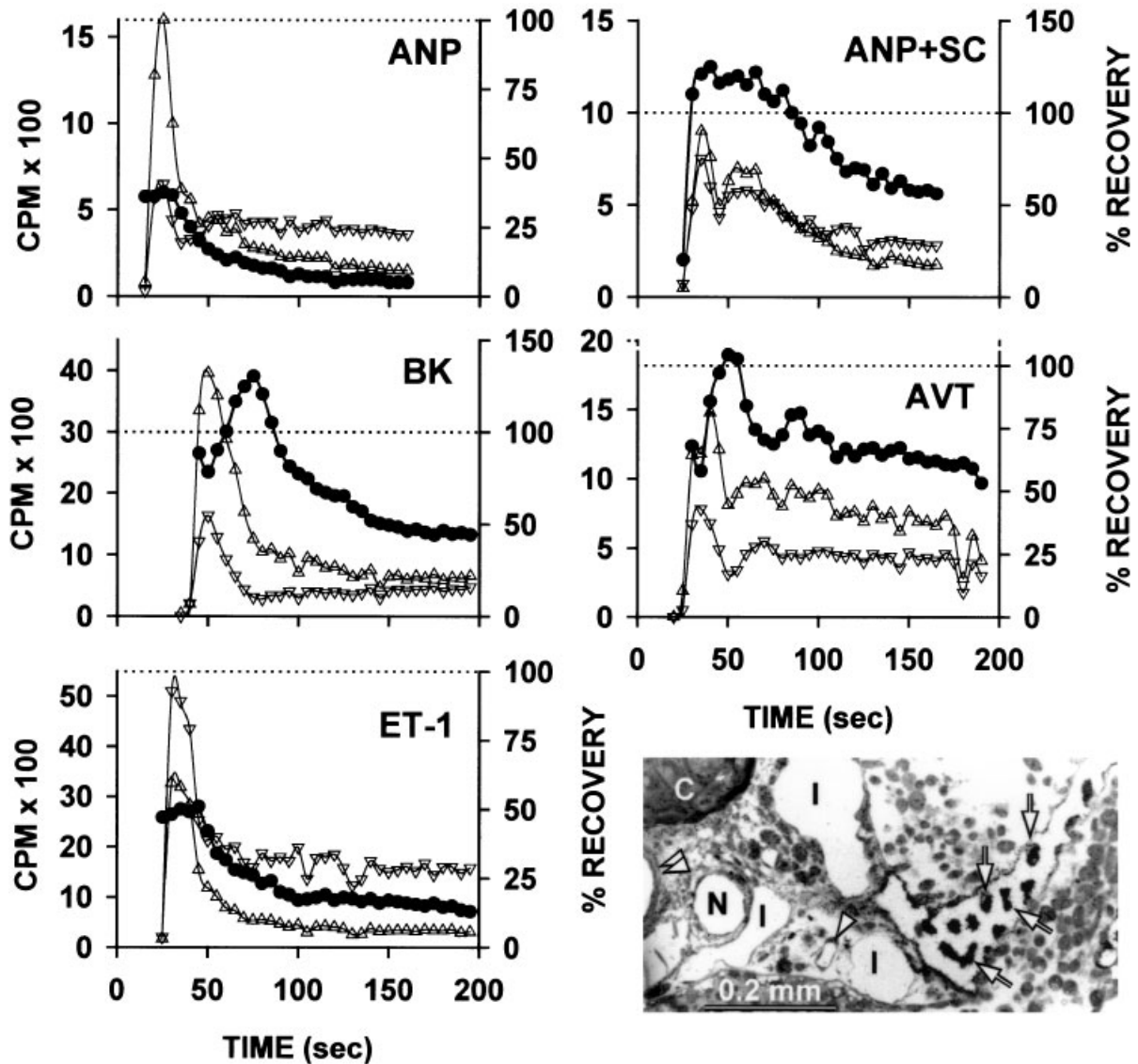
Fig. 10. Line drawings: percent recovery of radiolabeled peptides in the dorsal aorta (●) following transit through rainbow trout gills in vivo. ^{125}I-peptides (△) and an inert volume marker [58]Co-EGTA or ^{51}Cr-red blood cells (▽) were simultaneously injected into ventral aorta and blood collected at 5-sec intervals from dorsal aorta. Fractional extraction by the gill equals $1 - (\%\text{ recovery}/100\%)$. Atrial natriuretic peptide (ANP) and endothelin-1 (ET-1) are efficiently extracted by the gill, whereas bradykinin (BK) and arginine vasotocin (AVT) are not. Inhibition of the natriuretic peptide clearance receptor with SC 46542 (SC) completely inhibits ANP uptake by the gill [from Olson ('98) with permission]. Micrograph: autoradiography of ^{3}H-norepinephrine binding to pillar cells (arrows) in perfused trout gill. Section is perpendicular to filament and tangential to lamella thereby bisecting pillar cells. Label is nearly completely absent from interlamellar (I), nutrient (N and double arrowheads), or capillary-size (arrowhead) vessels; C, filament cartilage [adapted from Nekvasil and Olson ('85)].

inactivation of NPs in eels is under physiological control, and it adds another level of sophistication to branchial regulatory capacity. Gill inactivation may allow elevated intrabranchial NPs concentrations resulting from cardiac secretion while minimizing delivery of these vasodilators to the systemic circulation (Farrell and Olson, 2000). The presence of NPR-C-like receptors in hagfish gills (Toop et al., '95) probably illustrates the evolutionary value of branchial inactivation.

Arginine vasotocin

In vivo extraction of arginine vasotocin (AVT) is 20–35%. This is on the low end for peptides but is

higher than what would be predicted from the long half-time for blood pressure recovery following bolus injection (Fig. 10, Table 2). The reason for this is unknown, although it may be an artifact due to the slightly different transit times for ^{125}I-AVT and the volume marker, because recovery of ^{125}I exceeds 100% near the end of the initial pass (Fig. 10). Nevertheless, it is evident that the gill does not rapidly extract AVT from the circulation.

Endothelin

Gill extraction of endothelin-1 (ET-1) is 55%, and it is the second most aggressively extracted peptide described to date. Perhaps this helps mitigate the potent branchioconstrictor effects of this peptide. It is not known if endothelin extraction is mediated by specific receptors, or if ET is also metabolized in-transit through the gill.

Catecholamines

Gill disposition of plasma catecholamines is a complex process involving uptake, metabolism, and storage, and it is still not well understood. Epinephrine (Epi) and norepinephrine (Nepi) are extracted from the branchial circulation via an imipramine-inhibitable process that appears to be a blend of mammalian neuronal uptake (uptake$_1$) and pulmonary uptake mechanisms. Extraction efficiency depends on exposure history and it is somewhat catecholamine specific. Considerably more catecholamines are taken up following bolus injection (45% Epi, 60% Nepi extraction, all in A-A pathway) than after 20 min of continuous perfusion (2% and $\sim$0% Epi extraction in A-A and A-V and 11% and 22% Nepi in A-A and A-V pathways, respectively). There are also slightly more Nepi than Epi metabolites in the gill effluent during continuous perfusion (Nepi metabolites, 4% and 14% of total label exiting the gill via A-A and A-V pathways; Epi, metabolites, 4% and 9% in A-A and A-V pathways). Both monoamine oxidase and catechol-O-methyl transferase are present in gills, and metabolites reflecting combined deamination and O-methylation are usually found in effluent from perfused gills. Some Nepi may also be stored intact, possibly for future release. Autoradiography of gills perfused with ^{3}H-Nepi show that much of the accumulated label is in the pillar cells (Fig. 10). Thus the gill, like the mammalian lung acts as Nepi-selective filter, albeit with modest efficiency.

Serotonin

Although serotonin is an indolamine, its three-dimensional structure is similar to that of Nepi, and if present, serotonin will be taken up by mammalian adrenergic neurons and released during sympathetic stimulation. To minimize the chance of sympathetic-induced serotonergic stimulation, plasma serotonin titers are kept low by an efficient pulmonary uptake mechanism. The same appears to be the case in fish where branchial extraction and metabolism combine to remove 80% of the perfusate serotonin in a single pass. This may also provide a safety factor for other serotonergic cells (e.g., neuroepithelial cells) in the filament (see chapters by Wilson and Laurent and by Sundin and Nilsson, this issue).

Arachidonic acid and its metabolites

Arachidonic acid (Ar-A) is a membrane lipid and probably does not circulate in fish; even if it was released into the circulation, gill extraction is essentially 100%. Biologically active metabolites of Ar-A are determined by specific enzymatic pathways. Cyclooxygenase products include prostaglandins (PGs), prostacyclin and thromboxanes. Lipoxygenase produces leukotrienes and hydroxyeicosatetraeonic acids (HETEs) and cytochrome P450 epioxygenases generate epoxyeicosatrieonic acids (EETs plus 20-HETE). It is beyond the scope of this article to consider local synthesis and paracrine actions of these compounds, which undoubtedly are extensive.

Prostaglandins are the only Ar-A metabolites whose extraction from the circulation has been measured. Gill extraction of PGE$_2$ (5% from A-A and 35% from A-V pathway) and PGI$_2$ (15% from A-A and 40% from A-V pathway) is relatively low, consistent with a circulating function. Because these studies measured ^{3}H-PG extraction by the gill and not concomitant release of metabolites, it is conceivable that net inactivation may be greater. Arachidonic acid metabolism by P450 in gill microsomes is some 10–30% less than liver metabolism (Schlezinger et al., '98). It would be interesting to compare metabolism of the intact organs during physiological perfusion.

Purines

Adenosine is efficiently inactivated by the gill. The A-A pathway inactivates 40% and the A-V pathway 100%.

Xenobiotics

The gill clearly is in an opportune position to affect the flux of xenobiotic molecules both across the epithelium and in transit through its vasculature. However, compared to transepithelial processing, there is scant information on the fate of xenobiotics as they traverse the branchial vasculature. Several examples and possibilities are described below.

Cytochrome P450 1A1 is found in gills of trout and scup, *Stenotomus chrysops,* and it is especially prevalent in pillar cells. Gill P450 1A1 activity is also significantly increased (10-fold) by organics such as β-naphthoflavone. Thus this system responds to xenobiotic challenge and is ideally situated for an efficient response. However, the actual metabolic of branchial enzymes on circulating substrate is not known. By comparison, Ar-A metabolism by P450 in scup gill microsomes is some 10- to 30-fold less than liver metabolism (Schlezinger et al., '98). From the relationship between fractional inactivation and fractional perfusion discussed above, if gill Ar-A metabolism was one-tenth that of the liver, it could metabolize half as much Ar-A. Clearly, it will be quite interesting to compare metabolism of the intact organs.

Metallothioneins (MT) are heavy-metal-binding proteins that were originally shown to be induced in trout gill microsomes by environmental mercury (Olson et al., '78). Unlike cytochrome P450 enzymes, however, MTs are concentrated in chloride cells (Dang et al., 2001). The proximity of chloride cells with both the inner margin of the lamella and the interlamellar vasculature (see Olson, 2002) suggests some interesting possibilities in the role of the gill vasculature in heavy metal metabolism.

Pillar cell phagocytosis of particulates such as colloidal carbon or latex spheres has been observed in trout (Chilmonczyk and Monge, '80) but not in plaice (Ellis et al., '76). Only a small fraction (4%) of the trout pillar cells were phagocytic and these were located in the inner margin near the afferent end. Although the gill appears to be secondary to the kidney in its phagocytic ability, it will be interesting to examine the mechanism(s) of pillar cell uptake and to determine if different

materials are specifically accumulated by these cells.

LITERATURE CITED

Bailly Y, Dunel-Erb S. 1986. The sphincter of the efferent filament artery in teleost gills. I. Structure and parasympathetic innervation. J Morphol 187:219–237.

Bennett MB, Rankin JC. 1986. The effects of neurohypophyseal hormones on the vascular resistance of the isolated perfused gill of the European eel, *Anguilla anguilla* L. Gen Comp Endocrinol 64:60–66.

Boland EJ, Olson KR. 1979. Vascular organization of the catfish gill filament. Cell Tissue Res 198:487–500.

Booth JH. 1978. The distribution of blood flow in the gills of fish: application of a new technique to rainbow trout (*Salmo gairdneri*). J Exp Biol 73:119–129.

Booth JH. 1979. The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. J Exp Biol 83:31–39.

Campos-Perez JJ, Ward M, Grabowski PS, Ellis AE, Secombes CJ. 2000. Gills are an important site of iNOS expression in rainbow trout *Oncorhynchus mykiss* after challenge with the Gram-positive pathogen *Renibacterium salmoninarum.* Immunology 99:153–161.

Chan DKO. 1969. Hormonal regulation of cardiac output and regional blood flow in the eel, *Anguilla japonica.* Gen Comp Endocrinol 13:498.

Colin DA, Kirsch R, Leray C. 1979. Haemodynamic effects of adenosine on gills of the trout (*Salmo gairdneri*). J Comp Physiol 130:325–330.

Colin DA, Leray C. 1979. Interaction of adenosine and its phosphorylated derivatives with putative purinergic receptors in the gill vascular bed of rainbow trout. Pflugers Arch 383:35–40.

Conklin DJ, Olson KR. 1994. Angiotensin II relaxation of rainbow trout vessels in vitro. Am J Physiol Regul Integr Comp Physiol 266:R1856–R1860.

Conklin DJ, Chavez A, Duff DW, Weaver L Jr, Zhang Y, Olson KR. 1997. Cardiovascular effects of arginine vasotocin in the rainbow trout, *Oncorhynchus mykiss.* J Exp Biol 200:2821–2832.

Conklin DJ, Smith MP, Olson KR. 1999. Pharmacological characterization of arginine vasotocin vascular smooth muscle receptors in the trout (*Oncorhynchus mykiss*) in vitro. Gen Comp Endocrinol 114:36–46.

Conlon JM, Le Mevel J, Conklin D, Weaver L, Duff DW, Olson KR. 1996. Isolation and cardiovascular activity of a second bradykinin-related peptide ([Arg^0, Trp^5, Leu^8]bradykinin) from trout. Peptides 17:531–537.

Cooke IRC, Campbell G. 1980. The vascular anatomy of the gills of the smooth toadfish *Torquiginer glaber* (Teleostei: Tetraodontidae). Zoomorphologie 94:151–166.

Dang ZC, Berntssen MHG, Lundebye AK, Filk G, Wendelaar Bonga SE, Lock RAC. 2001.

Metallothionein and cortisom receptor expression in gills of Atlantic salmon, *Salmo salar*, exposed to dietary cadmium. Aquat Toxicol 53:91–101.

Daxboeck C, Davie PS. 1982. Effects of pulsatile perfusion on flow distribution within an isolated saline-perfused trout head preparation. Can J Zool 60:994–999.

Donald JA, Ellis AG. 1983. Arteriovenous anastomoses in the gills of Australian short-finned eel, *Anguilla australis*. J Morphol 178:89–93.

Donald JA, Toop T, Evans DH. 1994. localization and analysis of natriuretic peptide receptors in the gills of the toadfish, *Opsanus beta* (Teleostei). Am J Physiol 267: R1437–R1444.

Dunel S, Laurent P. 1977. La vascularisation branchiale chez l'anguille: action de l'acétylcholine et de l'adrénaline sur la répartition d'une résine polymérisable dans les différents compartiments vasculaires. C R Acad Sci Paris 284: 2011–2014.

Dunel-Erb S, Bailly Y. 1986. The sphincter of the efferent filament artery in teleost gills. II. Sympathetic innervation. J Morphol 187:239–246.

Evans DH, Chipouras E, Payne JA. 1989. Immunoreactive atriopeptin in plasma of fishes: its potential role in gill hemodynamics. Am J Physiol 257:R939–R945.

Evans DH, Gunderson MP. 1999. Characterization of an endothelin ET_B receptor in the gill of the dogfish shark *Squalus acanthias*. J Exp Biol 202:3605–3610.

Farrell AP. 1980. Gill morphometrics, vessel dimensions, and vascular resistance in ling cod, *Ophiodon elongatus*. Can J Zool 58:807–818.

Farrell AP, Smith DG. 1981. Microvascular pressures in gill filaments of lingcod (*Ophiodon elongatus*). J Exp Zool 216:341–344.

Farrell AP, Sobin SS. 1985. Sheet blood flow: its application in predicting shunts in respiratory systems. In: Johansen K, Burggren WW, editors. Cardiovascular shunts. Copenhagen: Munksgaard. p 269–281.

Farrell AP, Olson KR. 2000. Cardiac natriuretic peptides: a physiological lineage of cardioprotective hormones? Physiol Biochem Zool 73:1–11.

Farrell AP, Daxboeck C, Randall DJ. 1979. The effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. J Comp Physiol 133:233–240.

Fenwick JC, So YP. 1981. Effect of an angiotensin on the net influx of calcium across an isolated perfused eel gill. Can J Zool 59:119–121.

Gardaire E, Avella M, Isaia J, Bornancin M. 1991. Measurement of branchial vascular space of trout, *Oncorhynchus mykiss*: effects of adrenaline. J Comp Physiol 161:265–269.

Gibbins IL, Olsson C, Holmgren S. 1995. Distribution of neurons reactive for NADPD-diaphorase in the branchial nerves of a teleost fish, *Gadus morhua*. Neurosci Lett 193:113–116.

Girard J-P, Payan P. 1976. Effect of epinephrine on vascular space of gills and head of rainbow trout. Am J Physiol 230:1555–1560.

Hayashida N, Okui K, Fukuda Y. 1987. Modulation of vascular dynamics by spontaneous contraction of smooth muscle in the isolated carotid artery of the rat. Jpn J Physiol 37: 183–196.

Hemker DP. 1987. Metabolism and uptake of arachidonic acid in the microcirculation of the fish gill: effects of arachidonic acid metabolites and cyclooxygenase inhibitors on hemody-namics, perfusion distribution and water influx in the isolated gill arch of rainbow trout, *Salmo gairdneri*. Notre Dame, IN: University of Notre Dame.

Hoagland TM, Weaver L Jr, Conlon JM, Wang Y, Olson KR. 2000. Effects of endothelin-1 and homologous trout endothelin on cardiovascular function in rainbow trout. Am J Physiol 278:R460–R468.

Holbert PW, Boland EJ, Olson KR. 1979. The effect of epinephrine and acetylcholine on the distribution of red cells within the gills of the channel catfish (*Ictalurus punctatus*). J Exp Biol 79:135–146.

Hughes GM. 1972. Morphometrics of fish gills. Resp Physiol 14:1–25.

Hughes GM, Morgan M. 1973. The structure of fish gills in relation to their respiratory function. Biol Rev 48: 419–475.

Hughes GM, Peyraud C, Peyraud-Waitzenegger M, Soulier P. 1982. Physiological evidence for the occurrence of pathways shunting blood away from the secondary lamellae of eel gills. J Exp Biol 98:277–288.

Ishimatsu A, Iwama GK, Heisler N. 1988. In vivo analysis of partitioning of cardiac output between systemic and central venous sinus circuits in rainbow trout: a new approach using chronic cannulation of the branchial vein. J Exp Biol 137:75–88.

Janvier J-J. 1997. Cardiovascular and ventilatory effects of prostaglandin E_2 in the European eel *Anguilla anguilla*. J Comp Physiol 167:517–526.

Kaiya H, Takei Y. 1996. Atrial and ventricular natriuretic peptide concentrations in plasma of freshwater- and seawater-adapted eels. Gen Comp Endocrinol 102:183.

Krakow NP. 1913. Über die Wirkung von Giften auf die Gefässe isolierter Fishkiemen. Pfluegers Arch Gesamte Physiol Menschen Tierre 151:583–603.

Laurent P, Dunel S. 1976. Functional organization of the teleost gill. I. Blood pathways. Acta Zool (Stockholm) 57:189–209.

Le Mevel J-C, Olson KR, Conklin D, Waugh D, Smith DD, Vaudry H, Conlon JM. 1996. Cardiovascular actions of trout urotensin II in the conscious trout, *Oncorhynchus mykiss*. Am J Physiol Regul Integr Comp Physiol 271:R1335–R1343.

Lodhi KM, Sakaguchi H, Hirose S, Hagiwara H. 1995. Localization and characterization of a novel receptor for endothelin in the gills of rainbow trout. J Biochem (Tokyo) 118:376–379.

Loretz CA, Pollina C. 2000. Natriuretic peptides in fish physiology. Comp Biochem Physiol 125:169–187.

Muir BS, Brown CE. 1971. Effects of blood pathway on the pressure drop in fish gills, with special reference to tunas. J Fish Res Bd Can 28:947–955.

Nekvasil NP, Olson KR. 1985. Localization of ^{3}H-norepinephrine binding sites in the trout gill. J Exp Zool 235: 309–313.

Nekvasil NP, Olson KR. 1986. Extraction and metabolism of circulating catecholamines by the trout gill. Am J Physiol Regul Integr Comp Physiol 250:R526–R531.

Nilsson S, Pettersson K. 1981. Sympathetic nervous control of blood flow in the gills of the Atlantic cod, *Gadus morhua*. J Comp Physiol 144:157–163.

Oduleye SO, Claiborne JB, Evans DH. 1982. The isolated, perfused head of the toadfish, *Opsanus beta*. I. Vasoactive responses to cholinergic and adrenergic stimulation. J Comp Physiol 149:107–113.

Okafor MC, Oduleye SO. 1986. Haemodynamic effects of purinergic receptor stimulation in isolated fish gills. Acta Physiol Hung 67:45–51.

Olson KR. 1980. Application of corrosion casting procedures in identification of perfusion distribution in a complex microvasculature. In: Johari O, editor. Scanning electron microscopy, Vol 3. Chicago: SEM, Inc. p 357–364.

Olson KR. 1983. Effects of perfusion pressure on the morphology of the central sinus in the trout gill filament. Cell Tissue Res 232:319–325.

Olson KR. 1984. Distribution of flow and plasma skimming in isolated perfused gills of three teleosts. J Exp Biol 109:97–108.

Olson KR. 1997 The cardiovascular system. In: Evans DH, editor. The physiology of fishes. Boca Raton: CRC Press. p 129–154.

Olson KR. 1998. Hormone metabolism by the fish gill. Comp Biochem Physiol 119A:55–65.

Olson KR. 2002. Vascular anatomy of the fish gill. J Exp Zool 293:214–231.

Olson KR, Meisheri KD. 1989. Effects of atrial natriuretic factor on isolated arteries and perfused organs of trout. Am J Physiol Regul Integr Comp Physiol 256:R10–R18.

Olson KR, Villa J. 1991. Evidence against non-prostanoid endothelial-derived relaxing factor(s) in trout vessels. Am J Physiol Regul Integr Comp Physiol 260:R925–R933.

Olson KR, Chavez A, Conklin DJ, Cousins KL, Farrell AP, Ferlic R, Keen JE, Kne T, Kowalski KA, Veldman T. 1994. Localization of angiotensin II responses in the trout cardiovascular system. J Exp Biol 194:117–138.

Olson KR, Conklin DJ, Conlon JM, Kellogg MD, Smith MP, Weaver L Jr, Bushnell PG, Duff DW. 1997a. Physiological inactivation of vasoactive hormones in rainbow trout. J Exp Zool 279:254–264.

Olson KR, Conklin DJ, Farrell AP, Keen JE, Takei Y, Weaver L, Smith MP, Zhang Y. 1997b. Effects of natriuretic peptides and nitroprusside on venous function in trout. Am J Physiol 273:R527–R539.

Olson KR, Conklin DJ, Weaver L Jr, Herman CA, Wang X, Conlon JM. 1997c. Cardiovascular effects of homologous bradykinin in rainbow trout. Am J Physiol 272:R1112–R1120.

Olson KR, Duff DW, Farrell AP, Keen J, Kellogg MD, Kullman D, Villa J. 1991. Cardiovascular effects of endothelin in trout. Am J Physiol Heart Circ Physiol 260:H1214–H1223.

Olson KR, Kullman D, Narkates AJ, Oparil S. 1986. Angiotensin extraction by trout tissues in vivo and metabolism by the perfused gill. Am J Physiol Regul Integr Comp Physiol 250:R532–R538.

Olson KR, Squibb KS, Cousins RJ. 1978. Tissue uptake subcellular distribution, and metabolism of $^{14}CH_3HgCl$ and $CH_3^{203}HgCl$ by rainbow trout (Salmo gairdneri). J Fish Res Board Can 35:381–390.

Oudit GY, Butler DG. 1995a. Angiotensin II and cardiovascular regulation in a freshwater teleost, Anguilla rostrata LeSueur. Am J Physiol 269:R726–R735.

Oudit GY, Butler DG. 1995b. Cardiovascular effects of arginine vasotocin, atrial natriuretic peptide and epinephrine in freshwater eels. Am J Physiol 268:R1273–R1280.

Pärt P, Tuurala H, Nikinmaa M, Kiessling A. 1984. Evidence for a non-respiratory intralamellar shunt in perfused rainbow trout gills. Comp Biochem Physiol 79(A):29–34.

Payan P, Girard J-P. 1977. Adrenergic receptors regulating patterns of blood flow through the gills of trout. Am J Physiol 232:H18–H23.

Perry SF, Davie PS, Daxboeck C, Ellis AG, Smith DG. 1984. Perfusion methods for the study of gill physiology. In: Hoar WS, Randall DJ, editors. Fish physiology, Vol X Part B. New York: Academic Press, Inc. p 325–388.

Perry SF, Daxboeck C, Dobson GP. 1985. The effect of perfusion flow rate and adrenergic stimulation on oxygen transfer in the isolated, saline-perfused head of rainbow trout (Salmo gairdneri). J Exp Biol 116:251–269.

Pettersson K, Johansen K. 1982. Hypoxic vasoconstriction and the effects of adrenaline on gas exchange efficiency in fish gills. J Exp Biol 97:263–272.

Piomelli D, Pinto A, Tota B. 1985. Divergence in vascular actions of prostacyclin during vertebrate evolution. J Exp Zool 233:127–131.

Randall D, Daxboeck C. 1984 Oxygen and carbon dioxide transfer across fish gills. In: Hoar WS, Randall DJ, editors. Fish physiology, Vol. XA: Gills—Anatomy, gas transfer, and acid–base regulation. New York: Academic Press, Inc. p 263–314.

Ristori MT, Laurent P. 1977. Action de l'hypoxie sur le système vasculaire branchial de la tâte perfusée de truite. C R Seances Soc Biol 171:809–813.

Russell MJ, Klemmer AM, Olson KR. 2001. Angiotensin signaling and receptor types in teleost fish. Comp Biochem Physiol 128:41–51.

Schlezinger JJ, Parker C, Zeldin DC, Stegeman JJ. 1998. Arachidonic acid metabolism in the marine fish Stenotomus chrysops (Scup) and the effects of cytochrome P450 1A inducers. Arch Biochem Biophys 353:265–275.

Smith DG. 1977. Sites of cholinergic vasoconstriction in trout gills. Am J Physiol 233:R222–R229.

Smith MP, Takei Y, Olson KR. 2000. Similarity of vasorelaxant effects of natriuretic peptides in isolated blood vessels of salmonids. Physiol Biochem Zool 73:494–500.

Stagg RM, Shuttleworth TJ. 1984. Hemodynamics and potentials in isolated flounder gills: effects of catecholamines. Am J Physiol 246:R211–R220.

Stensløkken K-O, Sundin L, Nilsson GE. 1999. Cardiovascular and gill microcirculatory effects of endothelin-1 in Atlantic cod: evidence for pillar cell contraction. J Exp Biol 202:1151–1157.

Sundin L, Nilsson GE. 1996. Branchial and systemic roles of adenosine receptors in rainbow trout: an in vivo microscopy study. Am J Physiol 40:R661–R669.

Sundin L, Nilsson GE. 1998. Endothelin redistributes blood flow through the lamellae of trout gills. J Comp Physiol 168:619–623.

Sundin L, Nilsson S. 1992. Arteriovenous branchial blood flow in the Atlantic cod Gadus morhua. J Exp Biol 165:73–84.

Sundin L, Nilsson S. 2002. Branchial innervation. J Exp Zool 293:232–248.

Sundin L, Axelsson M, Davison W, Forster ME. 1999. Cardiovascular responses to adenosine in the Antarctic fish, Pagothenia borchgrevinki. J Exp Biol 202:2259–2267.

Takei Y. 2000. Structural and functional evolution of the natriuretic peptide system in vertebrates. Int Rev Cytol 194:1–66.

Takei Y, Tsuchida T. 2000. Role of the renin–angiotensin system in drinking of seawater-adapted eels Anguilla japonica: a re-evaluation. Am J Physiol 279:R1105–R1111.

Takei Y, Tsuchida T, Li Z, Conlon JM. 2001. Antidipsogenic effects of eel bradykinins in the eel *Anguilla japonica*. Am J Physiol 281:R1090–R1096.

Toop T, Donald JA, Evans DH. 1995. Localisation and characteristics of natriuretic peptide receptors in the gills of the Atlantic hagfish *Myxine glutinosa* (Agnatha). J Exp Biol 198:117–126.

Tuurala H, Part P, Nikinmaa M, Soivio A. 1984. The basal channels of secondary lamellae in *Salmo gairdneri* gills: a non-respiratory shunt. Comp Biochem Physiol 79(A):35–39.

Warne JM, Balment RJ. 1995. Effect of acute manipulation of blood volume and osmolality on plasma [AVT] in seawater flounder. Am J Physiol 269:R1107–R1112.

Wood CM. 1974. A critical examination of the physical and adrenergic factors affecting blood flow through the gills of rainbow trout. J Exp Biol 60:241–265.

Wood CM. 1975. A pharmacological analysis of the adrenergic and cholinergic mechanisms regulating branchial vascular resistance in the rainbow trout (*Salmo gairdneri*). Can J Zool 53:1569–1577.