J. exp. Biol. **109**, 97–108 (1984)
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DISTRIBUTION OF FLOW AND PLASMA SKIMMING IN ISOLATED PERFUSED GILLS OF THREE TELEOSTS

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Accepted 14 October 1983

SUMMARY

Distribution of flow and red cells between the efferent (epibranchial) and venous pathways was examined with an isolated perfused gill adapted to collect the two effluents separately. Gills from two fish species (Ictalurids) with abundant prelamellar arteriovenous anastomoses (AVAs) were compared with those of the trout which contain few AVAs. The gills were perfused with Ringer or Ringer containing ^{51}Cr-tagged red cells (blood).

In Ringer-perfused gills, efferent outflow decreased as efferent pressure increased. Epinephrine prevented the decrease in efferent flow at elevated efferent pressures. In all species around one-third of the control blood perfusing the gill drained *via* the venous pathway. At constant efferent pressure epinephrine increased and acetylcholine decreased efferent outflow. These results suggest that tonic adrenergic stimulation is necessary for normal branchial perfusion.

The haematocrit of efferent effluent was greater than venous effluent in all species. No consistent effects of epinephrine or acetylcholine on plasma skimming were observed. Comparison of measured microhaematocrit and haematocrit values calculated from ^{51}Cr red cell space suggests that the red cells in the venous effluent are larger than those from the efferent pathway and supports the concept of a nutritive function for the venous pathway.

INTRODUCTION

The functional unit of the fish gill, the gill filament, contains two and perhaps three distinct vascular networks. The first, the respiratory pathway, consists of the afferent filamental artery, afferent lamellar arteriole, lamellar sinus, efferent lamellar arteriole and efferent filamental artery. In this network systemic venous blood is oxygenated at the lamellae and returned to the systemic circulation *via* the epibranchial and hypobranchial arteries. The second pathway is contained within the body of the gill filament. This pathway, variously called the central venous sinus, filamental sinus, venolymphatic sinus or interlamellar pathway (cf. Boland & Olson, 1979), shows considerable variability both within and between species. For simplicity, this second pathway will collectively be referred to as the central sinus in the present discussion. In a number of fish the central sinus appears as a simple sack-like structure (Laurent

Key words: Fish, gills, blood flow.

& Dunel, 1976), whereas in others it exists as a well-organized network traversing the filament in register with the interlamellar filamental epithelium (Boland & Olson, 1979; Olson, 1981; Olson & Kent, 1980). In many species, ranging from cyclostomes to teleosts, the central sinus is perfused with postlamellar blood *via* small diameter vessels from the efferent lamellar arterioles, efferent filamental artery or even the efferent branchial artery (Gannon, Campbell & Randall, 1973; Vogel, Vogel & Kremers, 1973; Vogel, Vogel & Schlote, 1974; Laurent & Dunel, 1976; Smith, 1976; Vogel, Vogel & Pfautsch, 1976; Dunel & Laurent, 1977; Nakao & Uchinomiya, 1978; Cooke & Campbell, 1980; Cooke, 1980; Olson & Kent, 1980; Farrell, 1980; Olson, 1981). These vessels constitute the postlamellar arterio-venous anastomoses (postlamellar AVAs). Prelamellar arterio-venous anastomoses (prelamellar AVAs) have also been described in several species (Steen & Kruysse, 1964; Dunel & Laurent, 1977; Laurent & Dunel, 1976; Vogel *et al.* 1973, 1974; Cooke, 1980; Cooke & Campbell, 1980; Olson & Kent, 1980). Fluid in the central sinus exits the filament and arch *via* the venous (or venolymphatic) vessels and returns to the sinus venosus. The third pathway, a nutritive pathway is frequently considered as part of the central sinus system. This pathway consists of the aforementioned postlamellar AVAs which, during their often tortuous course across the filament to the central sinus, are believed to deliver oxygenated blood to the inner filament. In some species, however, the nutritive system is distinct from the central sinus at the level of the filament (Boland & Olson, 1979). Frequently the orifice of pre- and postlamellar AVAs (or nutrient vessels) at their origin is modified, apparently to restrict entry of cellular elements (Vogel, 1978*a,b*; Vogel *et al.* 1973, 1974, 1976; Laurent, Delaney & Fishman, 1978). However, this is not a universal finding (Cooke & Campbell, 1980).

Interest in the relationship between the central sinus and respiratory pathway stems from the hypothesis of Steen & Kruysse (1964), who suggested that the central sinus serves as a lamellar bypass in times of low oxygen demand and thus ameliorates the untoward osmotic fluxes that would otherwise occur across the delicate lamellar epithelium. Although it is now known that the central sinus cannot serve as a lamellar bypass in the true sense (arterial-arterial), interest in this complex and ubiquitous vascular network has not waned and numerous other physiological functions have been suggested for it (see Discussion). Unfortunately the much needed experimental evidence is still lacking.

Other mechanisms of vascular mediation of lamellar gas exchange that would change functional surface area have been postulated as an alternative to the bypass hypothesis. These include intralamellar, interlamellar and interfilamental apportionment of cardiac output (Hughes, 1972; Smith & Johnson, 1977; Farrell, Sobin, Randall & Crosby, 1980; Holbert, Boland & Olson, 1979). However, Booth (1979) has argued against the logic of changing functional surface area from an osmotic standpoint. How these mechanisms would influence central sinus perfusion is unknown.

Regulation of blood flow through the gill has been of interest since Krawkow (1913) demonstrated the sensitivity of the gill vasculature to vasoactive hormones. Recently there has been considerable interest in the identification and location of some of these vascular receptors (Bergman, Olson & Fromm, 1974; Wood, 1975; Smith, 1977; Colin, Kirsch & Leray, 1979; Walqvist, 1980) but most of the attention has been

focused on the ability of the gill vasculature to regulate functional surface area. Much of the available information on the regulation of flow through the central sinus has been obtained with the isolated perfused head technique developed by Payan & Matty (1975). In this preparation the central sinus outflow (gill venous) is compared to dorsal aortic outflow. However, the 'central sinus' flow in these experiments is an admixture of gill venous outflow and the entire head systemic venous outflow plus the flow from a number of severed systemic arteries. Therefore, caution must be exercised in evaluating intrafilamental central sinus events when using the whole head preparation.

The purpose of the present experiments is to examine branchial flow distribution using an isolated perfused gill preparation from which both efferent branchial and central sinus outflows can be collected. In view of the unique structure of the AVAs supplying the central sinus in many fish and of the numerous histological reports of an unusually low haematocrit in central sinus blood, the presence of plasma skimming in both pathways was examined. Two species of fish were chosen for these experiments based on the presence or absence of prelamellar AVAs and the organization of the central sinus. The channel catfish (*Ictalurus punctatus*) contains a highly developed interfilamental (central sinus) vasculature and numerous prelamellar AVAs (Boland & Olson, 1979), whereas the central sinus of the rainbow trout (*Salmo gairdneri*) is less well developed and prelamellar AVAs are quite rare (Morgan & Tovell, 1973; Laurent & Dunel, 1976; Smith, 1976). The bullhead (*Ictalurus melas*) was also used in these studies as the catfish became unavailable. The vascular anatomy of *I. melas* has not been examined in detail but preliminary studies have shown that it is similar to that of the catfish (K. R. Olson, unpublished observation).

MATERIALS AND METHODS

Hatchery-reared rainbow trout (*Salmo gairdneri* Richardson), 150–300 g, and black bullheads (*Ictalurus melas* Rafinesque), 200–600 g were obtained from a local dealer. Wild channel catfish (*Ictalurus punctatus* Rafinesque), 400–800 g, were purchased from commercial fishermen. The fish were maintained in several 2000-l round, fibreglass tanks with continuous through-flowing aerated well water at 13–15 °C. The trout and bullheads were fed commercial fish pellets but the catfish would not eat in captivity and were not fed.

Sixteen to 24 h prior to gill perfusion, blood was withdrawn from a donor fish and the red cells washed three times in phosphate-buffered Ringer solution (NaCl, 7·37 g l^{-1}; KCl, 0·31 g l^{-1}; $CaCl_2$, 0·10 g l^{-1}; $MgSO_4$, 0·14 g l^{-1}; KH_2PO_4, 0·46 g l^{-1}; Na_2HPO_4, 2·02 g l^{-1}; glucose, 0·90 g l^{-1}; pH adjusted to 7·8) and resuspended in four volumes of Ringer solution. The suspended cells were then incubated overnight with sodium chromate (^{51}Cr, New England Nuclear, Boston, MA) at 5 °C. Slow rotation of the incubator vials (16 rev./min) ensured gentle mixing. On the day of the perfusion the cells were washed three times in four volumes of Ringer solution and resuspended to a final haematocrit of 15 ± 2·0 in Ringer solution containing 40 g l^{-1} bovine albumin fraction V (Sigma, St. Louis, MO). Addition of albumin to the Ringer solution necessitated readjustment of the pH back to 7·8. The perfusate was divided into aliquots to be used as control and experimental perfusates. If red cells were not radiolabelled, blood was harvested on the day of the experiment.

The isolated perfused gill technique of Bergman *et al.* (1974) and Holbert *et al.* (1979) was used with slight modifications in the present experiments and will be only briefly described. Fish were anaesthetized in benzocaine (ethyl-*p*-aminobenzoate, Sigma) and the first or second gill arches removed. The afferent branchial artery was cannulated with polyethylene tubing (selected to match size of vessel) and a ligature of heavy cord secured around the entire arch and cannula. This whole-arch ligature prevented loss of perfusate from the hypobranchial and ventral venous vessels which would otherwise occur. The efferent filamental artery was then teased free and cannulated with polyethylene tubing and a ligature of fine thread secured around the vessel. The arch was suspended in a 150 ml bath of 2% Ringer solution (Ringer-distilled water) with the efferent cannula and distal epibranchial arch slightly out of the bath. A plastic collar was then cut from a flexible vinyl glove and glued with a cyanoacrylic glue around the distal arch adjacent to the last filaments. Care was taken to ensure that the collar did not constrict the arch at any point. The collar was then formed into a trough that funnelled all outflow other than efferent branchial from the arch into a collection vial.

The gills were perfused at constant flow with a peristaltic pump. A 'T' in the inlet cannula near the arch was connected to a pressure transducer and pressures continuously monitored during the experiment. Perfusion flow rate was adjusted to give a mean pressure between 20 and 30 mmHg (2·7–4·0 kPa). The height of the efferent cannula was adjusted to simulate the desired dorsal aortic pressure. Timed outflow from the efferent branchial cannula and venous collar was collected into tared vials and determined gravimetrically. A Beckman Gamma 4000 was used to count ^{51}Cr. Chromium-calculated haematocrits were determined by multiplying the haematocrit of the test perfusate (prior to perfusion) by the ratio of the activity (per unit volume) of the effluent perfusate to the activity (per unit volume) of the test perfusate. Microhaematocrit was determined in duplicate for each sample following scintillation counting.

A typical experiment consisted of 20–30 min perfusion with Ringer solution to rinse the blood from the gills followed by 20–45 min perfusion with control blood (labelled with chromium but without a vasoactive drug added). The experimental blood (containing a vasoactive drug) followed the control for an additional 20–45 min. During an experiment the cells were kept in suspension by continuous stirring. A four-way stopcock connecting any two sequential perfusion solutions with the perfusion pump prevented flow interruption while switching solutions. Gills were discarded when less than 90% of the perfusate was recovered. No differences in efferent-venous flows or haematocrits were observed between gills with 90 or 100% recovery.

When the perfusate was changed from Ringer solution to blood, afferent pressure increased, presumably due to the higher viscosity of the latter. To offset this, the perfusion pump flow rate was decreased and physiological pressures were maintained. Once steady state conditions were attained, usually within 5 min of changeover, further adjustments of pump flow were unnecessary. The pump flow rate was not changed when blood containing the vasoactive agents was introduced.

From 4 to 10 efferent and 3 to 8 venous samples were collected for analyses of flow rate and haematocrit at each experimental period (Ringer solution, control blood and experimental blood). Unless otherwise stated, only the last sample, prior to changin

perfusate, was used for statistical analysis of flow rate and microhaematocrit. All samples from a specific perfusate except the first two (i.e. those immediately following a perfusate change) were pooled for comparison of measured haematocrit with ^{51}Cr-calculated haematocrit. A paired Students *t*-test was used for all analyses. The data are expressed plus or minus the standard deviation of the mean difference (S.D.M.D.).

RESULTS

The effects of increased efferent branchial artery pressure on outflow distribution between the efferent branchial artery and central sinus are shown in Fig. 1. Without epinephrine, the gill was unable to maintain efferent flow at even subphysiological pressures. Above 15 mmHg (2·0 kPa) efferent pressure, efferent outflow usually ceased unless epinephrine was present in the perfusate (not shown). After red cells had been added to the perfusate there was a tendency for the percent efferent flow to decrease (Table 1). However, this was only statistically significant for the catfish. Epinephrine (10^{-7} M) increased and acetylcholine (10^{-7} M) decreased the efferent arterial outflow relative to the central sinus (Table 1). In the catfish, addition of 10^{-7} M epinephrine to Ringer perfusate increased the percent efferent flow to nearly 100 % (Fig. 1) at 10 mmHg (1·33 kPa) efferent pressure, whereas only 85 % efferent flow was obtained at the same epinephrine concentration and efferent pressure in perfusate containing red cells (Table 1). In the bullhead, propranolol, a β-blocker, slightly decreased percent efferent flow in the epinephrine-stimulated gill, although this was not statistically significant.

The haematocrit of efferent branchial blood was higher than that of central venous blood in all species studied (Table 2). This relationship was maintained in the presence of epinephrine and acetylcholine.

The effects of epinephrine and acetylcholine *per se* on haematocrit from either efferent or central venous pathways were quite variable. Epinephrine had no effect on efferent haematocrit in the catfish, increased it in the bullhead ($P < 0{\cdot}05$), and

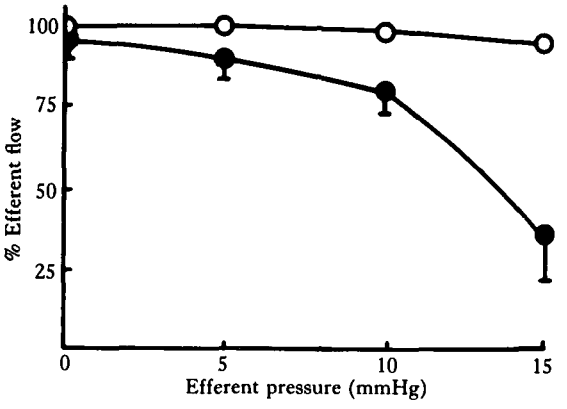

Fig. 1. Effect of efferent branchial artery pressure on percent efferent outflow (efferent artery outflow/efferent artery outflow + central sinus outflow) in the perfused catfish gill. Solid circles, phosphate-buffered Ringer perfusate; open circles, phosphate-buffered Ringer with epinephrine (10^{-7} M). Vertical lines equal one standard deviation of the mean (solid circles) and are all within radius of open circles ($N = 8$). $P < 0{\cdot}05$ at 0 mmHg pressure; $P < 0{\cdot}02$ at 5 mmHg and $P < 0{\cdot}001$ at 10 and 15 mmHg efferent artery pressure.

Table 1. *Effects of red cells and vasoactive drugs on percent efferent outflow (efferent artery outflow/efferent artery outflow + central sinus outflow) at constant efferent artery pressure (10 mmHg)*

Species (*N*)	Ringer perfusate	Control blood perfusate	Blood plus acetylcholine 10^{-7} M	Blood plus epinephrine 10^{-7} M	Blood plus epinephrine 10^{-7} M propranolol 10^{-6} M	Standard deviation of mean difference
Ictalurus punctatus						
(21)	81·8	71·9				2·9†
(6)		69·9	41·3			6·9†
(12)		69·9		85·5		3·2‡
Ictalurus melas						
(11)	79·2	68·8				68·4
(5)		51·3	31·4			6·4*
(7)		50·6		75·3		6·3†
(4)				71·9	64·6	9·1
Salmo gairdneri						
(6)	72·2	55·0				16·5
(6)		55·0		76·3		14·8

Paired analysis, N = number of pairs; * = $P \leq 0{\cdot}05$; † = $P \leq 0{\cdot}01$; ‡ = $P \leq 0{\cdot}001$.

Table 2. *Haematocrit of efferent and venous perfusate*

Species	Control Efferent	Control Venous	10^{-7} M epinephrine Efferent	10^{-7} M epinephrine Venous	10^{-7} M acetylcholine Efferent	10^{-7} M acetylcholine Venous
Ictalurus punctatus	15·4	11·8	15·7	12·8	18·4	14·8
	(1·11)†		(1·35)*		(3·03)	
	$N = 21$		$N = 12$		$N = 6$	
Ictalurus melas	14·8	8·5	18·6	7·9	19·3	9·8
	(1·95)†		(1·78)‡		(2·47)*	
	$N = 11$		$N = 7$		$N = 4$	
Salmo gairdneri	18·4	12·0	15·7	8·4		
	(0·96)†		(2·03)*			
	$N = 5$		$N = 5$			

Paired analysis, N = number of pairs, standard deviation of mean difference in parenthesis, * = $P < 0{\cdot}05$; † = $P < 0{\cdot}01$; ‡ = $P < 0{\cdot}001$.

decreased it in the trout ($P < 0{\cdot}01$). A slight but not significant increase in venous haematocrit in catfish and a decrease in venous haematocrit in bullheads and trout was also observed. Acetylcholine increased haematocrit in both efferent and venous outflow in catfish and bullheads, but the increase was significant only in the catfish venous flow ($P < 0{\cdot}01$). In all species, no significant changes were observed in either efferent or venous microhaematocrit in the three consecutive sample periods following changeover from control to perfusate containing either epinephrine or acetylcholine.

The mean difference between the observed microhaematocrit and haematocrit

Table 3. *Mean difference (paired analysis) between observed microhaematocrit (Obs) and haematocrit calculated from chromium-labelled red blood cell space (Cr)*

Species	Control		Epinephrine		Epinephrine + propranolol		Acetylcholine	
	Efferent	Venous	Efferent	Venous	Efferent	Venous	Efferent	Venous
Ictalurus punctatus								
Cr *vs* Obs	2·47	−0·63	3·00	2·56				
	(12)	(12)	(8)†	(8)				
Ictalurus melas								
Cr *vs* Obs	1·71	−0·71	0·64	−1·60	0·79	−2·04	2·13	−0·02
	(42)*	(42)*	(25)	(25)†	(15)	(11)‡	(20)	(20)
Salmo gairdneri								
Cr *vs* Obs	1·25	−0·80	0·57	−0·37				
	(20)*	(20)*	(20)	(20)				

A positive mean difference indicates that the calculated haematocrit is larger than the observed haematocrit. E = efferent; V = venous; N = number of samples; * = $P < 0{\cdot}05$; † = $P < 0{\cdot}01$; ‡ = $P < 0{\cdot}001$.

calculated from chromium-labelled red cell space is shown in Table 3. A positive value indicates that the haematocrit calculated from the red cell space is larger than the observed microhaematocrit. In all species, and in all experimental groups except one, haematocrit calculated from red cell space was larger than the observed microhaematocrit in the efferent perfusate and smaller than that in the venous perfusate. These differences were statistically significant in 7 out of 16 experimental groups.

DISCUSSION

The isolated perfused head preparation developed by Payan & Matty (1975) permits qualitative separation of dorsal aortic and venous effluent and has been used by numerous investigators in studies of gill function and haemodynamics. The isolated, perfused arch used in the present study also permits separation of these two perfusion pathways and has several advantages over the whole head technique.

First, the venous effluent in the whole head preparation contains an admixture of (1) true branchial venous blood, (2) branchial efferent arterial blood from numerous severed systemic arteries that originate from the epibranchial arteries anterior to the dorsal aorta and from the hypobranchial arteries and (3) systemic venous blood from the efferent branchial arteries that perfuse the head systemic vasculature. This admixture can make it difficult to measure transbranchial molecular fluxes into or out of the two branchial vascular compartments and difficult also to identify the haemodynamic factors regulating flow distribution between them. Such an admixture probably explains the low percent dorsal aortic flow relative to total flow observed in some preparations, i.e. 27 % in the sculpin *Myoxocephalus octodecimspinosus* (Claiborne & Evans, 1980) and 63 % in the trout (Payan & Girard, 1977). In both preparations (Payan & Girard, 1977; Claiborne & Evans, 1980) dorsal aortic pressure was 0 mmHg or slightly negative which, in an otherwise unstimulated gill, should greatly favour dorsal aortic flow. In the present study with dorsal aortic pressure at 0 mmHg and

without vasostimulation, efferent arterial flow was around 97 % of the total and did not fall below 80 % until dorsal pressure exceeded 10 mmHg. As pointed out by Claiborne & Evans (1980), care must be exercised in evaluating transbranchial ionic fluxes in the whole head preparation until more is known about the haemodynamic relationships between the perfusion pathways.

Second, the isolated head technique contains an in-series vascular arrangement in the venous component, the true branchial venous pathway plus the cephalic systemic pathway. Humoral vasostimulation could result in variable resistance changes in each of these pathways and lead to errors in quantification of true branchial venous outflow.

Third, in the isolated head preparation much of the afferent and efferent nervous supply of the gill is presumably intact (cf. Pettersson & Nilsson, 1979). Because of these nerves, there is considerable potential for errors in separating primary and secondary branchial responses to infused drugs. The fish 'blood brain' barrier, unlike that of mammals, is quite permeable to small vasoactive molecules such as catecholamines (Peyraud-Waitzenegger, Savina, Laparra & Morfin, 1979) and serotonin (Genot, Morfin & Peyraud, 1981). In the whole head preparation, vasoactive compounds administered *via* the ventral aorta will not only affect the branchial receptors but they will be delivered to CNS receptors as well and could simultaneously elicit CNS-mediated branchial reflexes. Even in unstimulated preparations, innervated gill vessels may be under tonic CNS influence. Nociceptor reflex arcs, similar to those reported in an elasmobranch, *Squalus acanthias* (Poole & Satchell, 1979), are presumably intact in the isolated head and could produce additional CNS-mediated influences on the branchial vasculature. Pithing the fish prior to perfusion would remove the potential problem of CNS-mediated vasoactivity. However, there does not appear to be any report of this practice in the literature with the exception of a recent paper by Oduleye, Claiborne & Evans (1982).

The isolated perfused gill as employed in the present experiments does have several potential drawbacks. Ligation of the ventral arch around the afferent cannula not only prevents leakage from the hypobranchial vessels but also interrupts the venous drainage. This removes a portion of the efferent and venous outflow tracts from the branchial circulation and presumably increases the resistance of each pathway. There is probably only minimal perturbation of the efferent outflow because most of this is normally carried *via* the epibranchial vasculature. The extent of disruption of the venous circulation is unknown, although observation of the gills suggests this to be minimal as well. If venous outflow to many of the filaments in the anterioventral arch was drained solely *via* the ventral end of the arch then venous outflow in these filaments would be nil after the ligature is placed around the afferent cannula and arch. If this arch is then perfused with red cells but without epinephrine in the perfusate and efferent pressure is at or over 15 mmHg, the anterioventral filaments should not be perfused. This was not observed in any experiments nor could separate drainage pathways be detected in methacrylate corrosion replicas of the catfish (Boland & Olson, 1979).

Non-physiological irrigation at the arch is a second problem with the isolated arch preparation. In the present experiments the gills were irrigated with a magnetic stirring bar. This can leave unstirred layers in the medium around the gill (Payan & Matty, 1975), and although this might affect transbranchial fluxes it would seem to

be less critical for gill perfusion pathways. Jackson & Fromm (1981) have shown that even the unstirred layer effect can be minimized by high stirring rates.

The sensitivity of efferent and venous flow to changes in efferent pressures in the unstimulated gill is striking (Fig. 1). This suggests that either there is a large potential for shunting postlamellar blood back to the heart or that some degree of adrenergic tone is required to maintain a relatively high proportion of efferent outflow into the systemic circulation. There does not seem to be any advantage to a large volume shunt and it must be assumed that *in vivo* this is not the case. Evidence for tonic adrenergic control has been presented in elasmobranchs (Holcombe, Wilde & Opdyke, 1980) and teleosts (Wood, 1974; Mazeaud, Mazeaud & Donaldson, 1977; Petterson & Nilsson, 1979; Wahlqvist & Nilsson, 1980). The present experiments demonstrate the physiological necessity of tonic regulation of branchial resistance.

It has been suggested that the central filamental vasculature serves as a blood reservoir (Vogel *et al.* 1973). Girard & Payan (1976), using a trout whole head preparation, observed that as much as 5 % of the total blood volume may be returned to the circulation by an adrenergic stimulus that empties the venous sinus. The results of the present experiments do not agree with this hypothesis. In all three species examined, no changes were observed in microhaematocrit and venous flow rate in sequential samples collected following adrenergic stimulation. Holbert *et al.* (1979) also failed to observe changes in alamellar filamental red cell space following adrenergic stimulation, and Booth (1979) did not find any change in filamental sinus volume or red cell concentration after epinephrine treatment. In histological studies the red cell density is also reported to be lower in the filamental sinus (Hughes, 1972; Skidmore & Tovell, 1972; Cooke & Campbell, 1980).

Plasma skimming by the central sinus has been predicted by numerous anatomical studies of gill vascular space and vessel structure. Reduced red cell densities have been observed in the central sinus (Hughes, 1972; Skidmore & Tovell, 1972; Cooke & Campbell, 1980) and very high intralamellar vascular haematocrits have also been reported (Hughes, Tuurala & Soivio, 1978; Soivio & Hughes, 1978; Soivio & Tuurala, 1981). The latter, calculated from morphometric analysis, were in excess of 64 %. Microvillous extensions of cells at the origin of pre- and postlamellar AVAs have been observed to project into the lumen of the parent vessel (Nakao & Uchinomiya, 1978; Vogel *et al.* 1974, 1976; Vogel, 1978*a,b*) and in general the small diameter of most AVAs would seem to impede red cell transit (Vogel *et al.* 1973, 1974, 1976; Vogel, 1978*a,b*; Boland & Olson, 1979; Cooke & Campbell, 1980). Cooke & Campbell (1980), however, did not observe microvillous extensions at the AVA entrance in gills from *Torquiginer glaber* nor have they been found in *Ictalurus punctatus* (E. J. Boland and K. R. Olson, unpublished observations). This feature may be species-dependent.

The first direct evidence for plasma skimming was obtained by Soivio, Nikinmaa, Nyholm & Westman (1981). They compared haematocrits of dorsal and ventral aortic blood taken from catheterized trout at 9 °C and found that dorsal haematocrits and haemoglobin concentrations were significantly higher. The authors also reported an unpublished study in which blood drawn from anaesthetized or stunned trout *via* cardiac puncture had a higher haematocrit than blood taken from the ductus Cuvieri (Soivio *et al.* 1981). Nikinmaa (1981) did not observe any difference between dorsal

and ventral aortic haemoglobin concentrations in trout adapted to 15 °C but at 8 °C there was again a significantly higher haematocrit in dorsal aortic blood. The present study confirms the existence of plasma skimming in the trout even at 15 °C and also demonstrates plasma skimming in gills of two Ictalurids.

The mechanisms by which plasma is skimmed into the central sinus are unclear. Although the microvillous nature of the cells at the origin of the AVAs would certainly restrict red cell entry into the AVA (Vogel, 1978*a*) the failure to observe this cell type in the catfish, *I. punctatus*, suggests that its presence is not a requirement for skimming. Presumably the geometric relationships and relative flow velocities between the parent (afferent lamellar arteriole or efferent filamental artery) and daughter (AVA) vessel determine the degree of skimming, a condition similar to that found in mammalian systems (Johnson, 1971; Yen & Fung, 1978). That is, plasma skimming by the daughter vessel increases as its diameter decreases relative to that of the parent and/or as flow velocity through the daughter relative to that of the parent decreases. Soivio & Hughes (1978) attribute the high lamellar haematocrit observed in their morphometric analysis of trout gills to plasma skimming by the prelamellar AVAs. The authors acknowledge the paucity of prelamellar AVAs in trout gills and suggest that prelamellar AVAs are involved in plasma volume regulation whereas postlamellar AVAs regulate red cell circulation. Based on this hypothesis one would expect to observe considerably greater skimming in species with numerous prelamellar AVAs, such as the catfish (Boland & Olson, 1979), eel (Dunel & Laurent, 1977) or lamprey (Nakao & Uchinomiya, 1978). This does not appear to be true for the catfish as the extent of skimming is about the same as for the trout (Table 2). It will be interesting to evaluate plasma skimming efficiency in the eel and lamprey in this light.

The present experiments show that two or perhaps more parameters are involved in changing the haematocrit of blood perfusing the two pathways through the gill. The first and predominant effect is that of plasma skimming. This significantly lowers observed and ^{51}Cr-calculated haematocrit of venous relative to efferent perfusate (only observed haematocrits are shown in Table 2). Another less obvious effect is suggested in Table 3. Chromium-calculated haematocrit consistently increased relative to observed haematocrit in efferent perfusate and decreased in venous perfusate. It seems unlikely that this could be due to instability of the ^{51}Cr–red cell bond because this would require that chromium be removed from the venous circuit and be added to the efferent. Two alternative explanations would be that there is either isosmotic water movement into or out of the blood or redistribution of water between the cell and plasma fractions. In order to achieve an increased mean difference in calculated *vs* observed haematocrits in the efferent perfusate by isosmotic water movement, water would have to leave the perfusate. This is highly unlikely because the perfusate is hyperosmotic relative to the bath. Thus it appears that the resulting changes in calculated *vs* observed haematocrits are due to redistribution of water between the red cell and plasma. In the efferent pathway, the difference between calculated and observed haematocrit can be explained by water loss from the red cell, whereas the red cells gain water in the venous pathway. The difference in red cell volume can be explained by the specific physiological function of each pathway, red cell oxygenation in the lamella and deoxygenation of venous blood perfusing metabolically active filamental tissues. Red cell volume is then affected by changes in oxygen

tension (Soivio, Westman & Nyholm, 1974*a,b*) or a concomitant chloride shift (Comroe, 1975; Cameron, 1978).

Supported in part by NSF Grant No. PCM 79-23073. The author wishes to thank Drs W. Gingerich and R. Kingsley for numerous helpful comments and suggestions and Ms D. Kullman, K. Drajus and Mr D. Cecconi for their valuable technical assistance. Appreciation is also expressed to Ms D. Hopper and J. Smith for typing the manuscript.

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