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THE EFFECT OF EPINEPHRINE AND ACETYLCHOLINE ON THE DISTRIBUTION OF RED CELLS WITHIN THE GILLS OF THE CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

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SUMMARY

The second pair of gill arches were perfused with ^{51}Cr tagged red cells at constant pressure or constant flow and in the presence of 10^{-5} M epinephrine or 10^{-6} M acetylcholine. The tissues were quick frozen, lyophilized and the lamellae and alamellar filaments from the distal, mid and basal thirds of the filaments counted for radioactivity. At constant flow, epinephrine increased red cell space (RCS) in lamellae from basal and mid regions and decreased RCS in peripheral lamellae. The RCS in all alamellar filaments decreased. With constant pressure perfusion, epinephrine increased RCS in all lamellae while alamellar filamental RCS remained unchanged. Acetylcholine, at constant flow perfusion, decreased distal lamellar RCS and increased basal lamellar RCS. RCS decreased in distal and mid alamellar filaments during the same period. These data support the theory of lamellar recruitment as a means of increasing respiratory exchange and stress the importance of concomitant central cardiovascular changes in eliciting maximal response.

INTRODUCTION

The physiology and morphology of the teleost gill with respect to respiration and ion regulation has received considerable attention in the last 50 years. In addition, there has been a revival of interest in the vasculature of this tissue and there is some debate as to the importance (or existence) of physiological and/or anatomical shunt pathways and the efficacy of these shunts in regulating the physiological function of the gill.

Systemic metabolic requirements presumably regulate respiratory exchange across the gill and provide the minimum set point for branchial ventilation and perfusion. The maximum set point is thought to be the osmotic imbalance incurred when ventilation and perfusion become excessive. The latter situation will not provide any respiratory advantages but could increase the diffusive exchange of salts and water and place an additional burden on the osmoregulatory system. Increased metabolic demands such as exercise would require increased capabilities for oxygen extraction

from the surrounding medium. Part of these demands is met by increased ventilation of gill tissue (Holeton & Randall, 1967; Stevens & Randall, 1967; Dejours, 1972; Hughes, 1972; Jones & Schwarzfeld, 1974; Kiceniuk & Jones, 1977) and by increased cardiac output (Stevens & Randall, 1967; Kiceniuk & Jones, 1977). There is however, relatively little direct evidence on the ability of gill vasculature to affect respiratory exchange by modulating lamellar perfusion.

Several theories have been proposed by which gaseous exchange across the secondary lamellae could be regulated through vasoactivity. Steen & Kruysse (1964) drawing on their own experiments and those of Riess (1881) described a complete bypass of the lamellar circulation in 12 species of teleosts. This was later verified in the trout, *Salmo gairdneri*, by Richards & Fromm (1969). In this model, afferent branchial blood need not traverse the secondary lamellae but can be shunted from the afferent filament artery to the efferent filament artery through the filament sinus or around the tip of the filament. These pathways, having relatively large volume flow capabilities and thick blood–water diffusion distances, would help ameliorate osmotic imbalances. Subsequent investigations have failed to find anatomical evidence for lamellar bypass shunts in several species of teleosts (Munshi, Byczkowska-Smyk & Morgan; in Hughes, 1972; Gannon, Campbell & Randall, 1973; Morgan & Tovell, 1973; Vogel, Vogel & Kremers, 1973; Cameron, 1974; Vogel, Vogel & Schlote, 1974; Dunel, 1975; Smith, 1976; Vogel, Vogel & Pfautsch, 1976) and the model of Steen and Kruysse has been questioned. This is not a consensus for all teleosts, however, as connections between the prelamellar vasculature and the central venous sinus have been described recently in several species (Dunel & Laurent, 1977; Olson, Holbert & Boland, 1978).

A second mechanism for regulation of branchial gas exchange is intralamellar shunting. Bettex-Galland & Hughes (1972) have reported contractile elements in the pillar cells of secondary lamellae. Motility of these cells could either increase lamellar blood volume or redistribute blood flow between the inner preferential channel (reclused within the body of the filament proper and therefore of minimal importance in gaseous exchange) and the outer preferential channel, an enlarged vascular channel circumnavigating the distal border of the lamellae. The efficiency of this mechanism has recently been examined with a computer model (Smith & Johnson, 1977).

Thirdly, lamellar perfusion could be increased by a synchronous increase in flow through all lamellae or as proposed by Hughes (1972) and Hughes & Morgan (1973) may be achieved by selective recruitment of previously underperfused lamellae on the more distal portions of the filament. Thus, according to Hughes (1972) and Hughes & Morgan (1973), lamellae near the basal portions of the filament are continually perfused, whereas additional peripheral lamellae are recruited for gas exchange as required by metabolic demands.

Relatively few attempts have been made to delineate intrabranchial blood flow partioning in spite of the *a priori* assumptions made of its existence. Several excellent studies have utilized visual descriptions of vascular disposition of dye (Smith, 1977), ink (Richards & Fromm, 1969), silicone resin (Dunel & Laurent, 1977), or red cells (Davis, 1972; Booth 1978). Histological localization of 15 μm or larger radiolabelled microspheres (Cameron, 1974) or the distribution of iodinated albumin in whole

trout heads (Girard & Payan, 1976) has also been reported. However, quantification of intrabranchial blood volumes and the effects of vasoactive molecules on vascular spaces has been limited.

The purpose of the present study was to measure the red cell space in the isolated perfused gill following administration of epinephrine and acetylcholine. Of interest was the distribution of ^{51}Cr tagged red cells in lamellae from proximal, mid and distal portions of the filament and in similar areas within the filament body. Shifts in red cell distribution are assessed in view of current theories of branchial perfusion.

MATERIALS AND METHODS

Experimental animals

Channel catfish (*Ictalurus punctatus*), 400–600 g, were purchased locally and maintained in 2000 l round fibre-glass tanks with continuously through-flowing aerated well water at 15 °C. The fish were fed a maintenance diet of commerical catfish pellets, but were starved 1 week prior to experimentation.

Experimental procedure

On the day prior to perfusion, blood was withdrawn from a donor fish and the red cells washed 3 times in phosphate-buffered saline (PBS) (NaCl, 7·37 g/l; KCl, 0·31 g/l; $CaCl_2$, 0·10 g/l; $MgSO_4$, 0·14 g/l; KH_2PO_4, 0·46 g/l; Na_2HPO_4, 2·02 g/l; glucose, 0·90 g/l; pH adjusted to 7·8), and resuspended in 4 volumes of PBS. ^{51}Cr in the form of sodium chromate (200–1000 Ci/g, ICN Pharmaceuticals Inc., Irvine, Calif.) was added to the blood to give approximately $2{\cdot}5 \times 10^5$ disintegrations min^{-1} ml^{-1} packed red cells. The red cells and ^{51}Cr were then slowly agitated for 15 h at 15 °C. Prior to perfusion the cells were washed 3 times in 4 vol. of PBS and resuspended to 30% haematocrit in albuminated PBS (40 g/l bovine albumin fraction V Sigma Chemical Corp., St Louis, MO.).

The isolated perfused gill technique of Bergman, Olson & Fromm (1974) was used in these experiments, with several modifications. The afferent branchial artery was grasped with fine forceps and freed of any connective tissue proximal to its entrance into the arch proper. An 18- or 20-gauge cannula was introduced into the afferent branchial artery and secured in place by passing two ligatures of 4–0 silk thread around the afferent end of the arch. This technique of including the entire arch in the ligature which secured the cannula was used for the initial three experiments with epinephrine and constant-flow perfusion. In all remaining experiments 6–0 silk sutures were placed around the cannulated afferent branchial artery, not around the entire arch. This ensured patency of the veno-lymphatic and efferent branchial vessels in the area. In the latter procedure two small holes (2 mm diameter) were placed adjacent to one another in a thin piece of styrofoam sheeting (1 cm × 2 cm × 1·5 mm), and by placing the cannula through these holes the piece of styrofoam was 'threaded' on to the cannula. The cannula was now introduced into the afferent branchial artery, and secured in place with two 6–0 silk ligatures. Residual arch musculature was used to anchor a suture connecting the arch and the styrofoam. This suture was adjusted to provide primary support for the suspended gill and to ensure that undue tension was not placed upon the cannulated vessel during the experiment.

After securing the cannula the gills were suspended in 300 ml of 10% PBS and perfused with albuminated PBS at constant flow (around 0·46 ml/min) until pressure measurements reached a steady state (15–20 min).

The approximate time for the complete dissection process after removing the head from the beaker was 15 min. Initial pressure during cannulation occasionally climbed to 70 mmHg but within a few minutes fell to and was maintained between 30 and 40 mmHg. If the pressure remained above 50 mmHg the gills were discarded. The gills were also discarded if the flow could be seen coming from damaged filaments or if there was no flow from the efferent branchial artery. After steady-state conditions were achieved and residual blood had been flushed from the gill, the gills were perfused with the tagged red blood cells. Both gills from the second pair of arches were used per experiment. One served as the control and was perfused with ^{51}Cr-labelled blood only. The other, the experimental, was perfused with an aliquot of blood with the same specific activity and haematocrit and to which was added either epinephrine (10^{-5} M) or acetylcholine (10^{-6} M). The gills were perfused with these solutions until a steady-state pressure was maintained for around 15 min. Usually 30 min elapsed between onset of experimental drug perfusion and termination of the experiment.

During the perfusion experiment blood would often flow down across the filaments after leaving the efferent branchial artery. Therefore, at the end of the experiment, the gills were irrigated with 10% PBS to remove any red blood cells adhering to the outside of the filaments.

The gills were then quick-frozen in 2-methylbutane quenched liquid nitrogen and lyophilized for 48 h. After lyophilization approximately 90% of the filaments were dissected from the arch and each filament was sectioned transversely into three sections of equal length: base, middle and tip. (Filaments near the ends of the arch were not used as their small dimensions made it more difficult to remove the secondary lamellae and complete perfusion of these filaments was sometimes questionable.) The filaments were placed under a stereomicroscope and the lamellae were dissected from the filament down to a level near the inner marginal channel. In this procedure, interlamellar filamental epithelium was also removed but the central filamental vasculature remained intact. The free lamellae and filaments were separated and placed on the adhesive side of tared pieces of plastic tape. The tape was re-weighed and placed in scintillation vials and radioactivity measured with a gamma counter (Packard, model 3002). Approximately 120 filaments were removed from each arch and about 260 lamellae were removed from each filament. Therefore approximately 31 200 lamellae were removed from each arch and 62 400 lamellae were removed per experiment.

Vasoactivity of a tissue irrigated by constant-flow-type perfusion will generate changes in the arteriovenous (A–V) pressure profile. This can conceivably effect vascular volume by altering intraluminal pressures. In addition, sympathetic stimulation which increases during activity in trout (Nakano & Tomlinson, 1967; Mazeaud, Mazeaud & Donaldson, 1977), decreases afferent pressure in the isolated perfused gill (Bergman *et al.* 1974) but does not lower ventral aortic pressure in the intact fish as branchial dilation is offset by an increase in cardiac output (Stevens & Randall, 1967; Kiceniuk & Jones, 1977). Thus, a constant-pressure perfusion, whereby

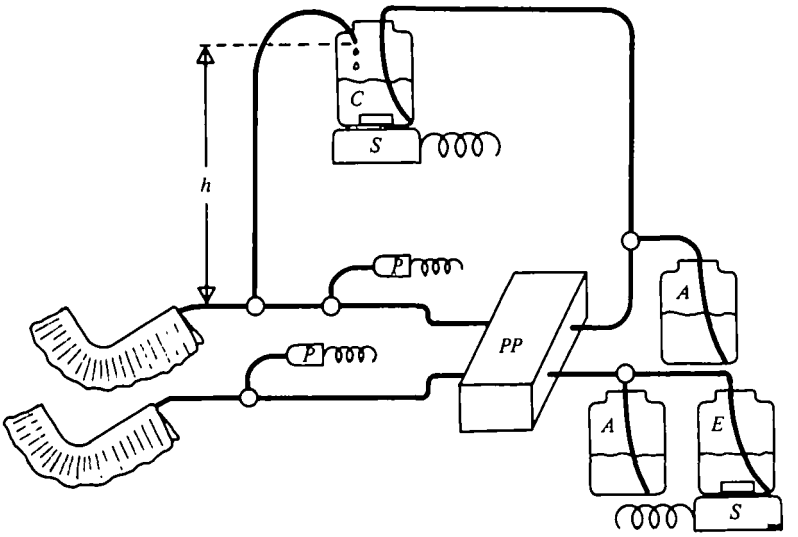

Fig. 1. Constant-pressure perfusion apparatus. *A*, Reservoir containing albuminated Ringer solution; *C*, reservoir containing ^{51}Cr-RBC (control); *E*, reservoir containing ^{51}Cr-RBC and experimental drug (micro stirring bar is seen in bottom of reservoir); *h*, height of tubing used as pressure buffer; *P*, pressure transducer; *PP*, perfusion pump; *S*, stirring apparatus.

afferent pressure to both arches can at least be kept equal, should more closely approximate *in vivo* sympathetic stimulation than constant flow-variable pressure.

The constant-pressure perfusion apparatus is shown in Fig. 1. In this system a section of tubing is inserted between the control gill and the infusion pump. The outlet of the tubing opens into the blood reservoir for the control gill. This reservoir is attached to a ring stand which allows the height of the reservoir and therefore the height, '*h*', of the open end of the connecting tubing to be regulated. Variations in *h* allow control of the input pressure to the gill. After addition of epinephrine to the experimental gill, the infusion rate was increased to maintain a constant pressure in this arch. The height of the control reservoir was then lowered to offset increased flow and maintain identical input pressure to the two arches. All perfusates containing red cells were continuously stirred with a magnetic stirring apparatus to suspend the cells and prevent haematocrit changes during perfusion.

The activity (cpm/g tissue) of each pair of tissue fractions was normalized to unity for the control tissue. Control and corresponding experimental tissue from the contralateral arch were compared by the paired *t* test. The data in Table 1 are expressed as the mean difference between the experimental and control tissues ± standard error of the mean difference.

RESULTS

Blood volumes of lamellae and alamellar filaments following epinephrine (10^{-5} M) and acetylcholine (10^{-6} M) perfusion are shown in Table 1. Positive values indicate an increase in red cell space (RCS) of the experimental gill (perfused with epinephrine or acetylcholine) when compared to the contralateral control arch. Negative values indicate a decrease in RCS in the experimental arch.

Table 1. *Effect of epinephrine and acetylcholine on the red cell space within lamellae and alamellar filaments in the isolated, perfused gill*

Tissues were taken from distal, mid and proximal thirds of filament. Whole-arch ligation; the afferent branchial artery was cannulated and secured by a ligature encircling the entire cartilaginous arch support and associated tissue. Branchial artery isolation; the afferent branchial artery was isolated, cannulated and secured by a ligature around the vessel only. The data are expressed as the mean difference between treated (Epi or Ach) and untreated controls ± standard error of the mean difference. Positive mean values indicate greater red cell space in tissues from treated arch, negative values indicate reduced red cell space in treated tissues. (*N*) = number of pairs of arches. 1 treatment and 1 contol.

	Constant-flow perfusion						Constant-pressure perfusion	
	Epinephrine 10^{-6} M				Acetylcholine 10^{-6} M		Epinephrine 10^{-6} M	
Area of filament from which tissue was taken	Whole arch ligation		Branchial artery isolation		Branchial artery isolation		Branchial artery isolation	
	Lamellae	Filament	Lamellae	Filament	Lamellae	Filament	Lamellae	Filament
Distal	−0·202 ±0·164	−0·485† ±0·199	−0·380** ±0·118	−0·653**** ±0·075	−0·168* ±0·191	−0·304** ±0·047	+2·270*** ±0·573	+0·477 ±0·340
Mid	+0·111 ±0·445	−0·593* ±0·152	+0·406 ±0·345	−0·480*** ±0·113	−0·086† ±0·041	−0·274** ±0·063	+1·308*** ±0·279	+0·351 ±0·276
Basal	+0·041 ±0·271	−0·609* ±0·156	+0·691 ±0·580	−0·537** ±0·170	+0·135* ±0·045	−0·237† ±0·118	+0·784* ±0·347	+0·176 ±0·117
	(3)		(5)		(3)		(5)	

† $P < 0·10$; * $P < 0·05$; ** $P < 0·025$; *** $P < 0·01$; **** $P < 0·001$

At constant flow the effect of epinephrine perfusion is qualitatively similar between whole-arch ligation and branchial artery isolation. Red cell space increases following catecholamine administration in basal and mid portions of the filament and decreases in lamellae from the distal third of the filament. Alamellar filament sections from all areas of the hemibranch showed significant decreases in RCS. During epinephrine perfusion, gill vascular resistance, as estimated from afferent pressure records, decreased by around 25% and remained at this level for the duration of the experiment. Before the vasodilation, however, there was a transient, sometimes barely perceptible, increase in resistance similar to that reported in trout (Bergman *et al.* 1974; Payan & Girard, 1977; Wood, 1975).

Acetylcholine (10^{-6} M) at constant flow perfusion significantly decreased the RCS in lamellae from the tip of the filament, slightly decreased mid-lamellar RCS and significantly increased RCS in the basal lamellae. RCS decreased in all filament sections. Resistance increased by approximately 75% in arches perfused with acetylcholine and maintained steady-state conditions until tissues were frozen.

At constant-pressure perfusion, epinephrine (10^{-6} M) significantly increased RCS in lamellae from all areas of the filament. The increase in RCS observed in distal lamellae was approximately twice that of lamellae from mid filament and 3 times that of basal lamellae. No significant changes were observed in filamental RCS.

A qualitative difference was also observed between constant-flow and constant-pressure perfused gills after epinephrine administration. In the latter, but not the former, the gills appeared 'engorged' with blood, indicating the pressure dependency of this phenomenon.

DISCUSSION

The ability of the gill vasculature to regulate the exchange of materials across the gill epithelium, i.e. the concept of functional surface area (FSA), has been demonstrated in teleosts (Bergman *et al.* 1974; Haywood, Isaia & Maetz, 1977; Sorensen & Fromm, 1976) and in an elasmobranch (Kent, Eid & Peirce, 1978). However, the mechanisms by which changes in FSA might be affected are unclear. The present experiments provide quantitative evidence that regulation of FSA could be achieved by lamellar recruitment, as proposed by Hughes (1972) and Hughes & Morgan (1973). The importance of concomitant cardiogenic responses during epinephrine-induced changes in branchial resistance is also evident (cf. constant pressure and constant flow perfusion, Table 1).

Fig. 2 is a schematic diagram of the vascular anatomy of the catfish gill filament. Details of the complex intrafilamental vasculature have been omitted. The general filamental circulation is similar in many respects to that reported for other teleosts. The afferent branchial artery (AB) supplies blood to the afferent filament artery (AF) which in turn delivers it to the lamellae (L) through the afferent lamellar arterioles (AL). Blood then enters a short efferent lamellar arteriole (EL) and passes into the efferent filament artery (EF). Blood in the efferent filament artery can leave the filament via the efferent branchial artery (EB) or enter the central venous sinus (S) by way of post lamellar arteriovenous anastomoses (A) and then leave the filament and arch through the venous circuit (V). In the catfish gill, blood from the afferent lamellar arteriole can also enter the central sinus through small prelamellar arterio-

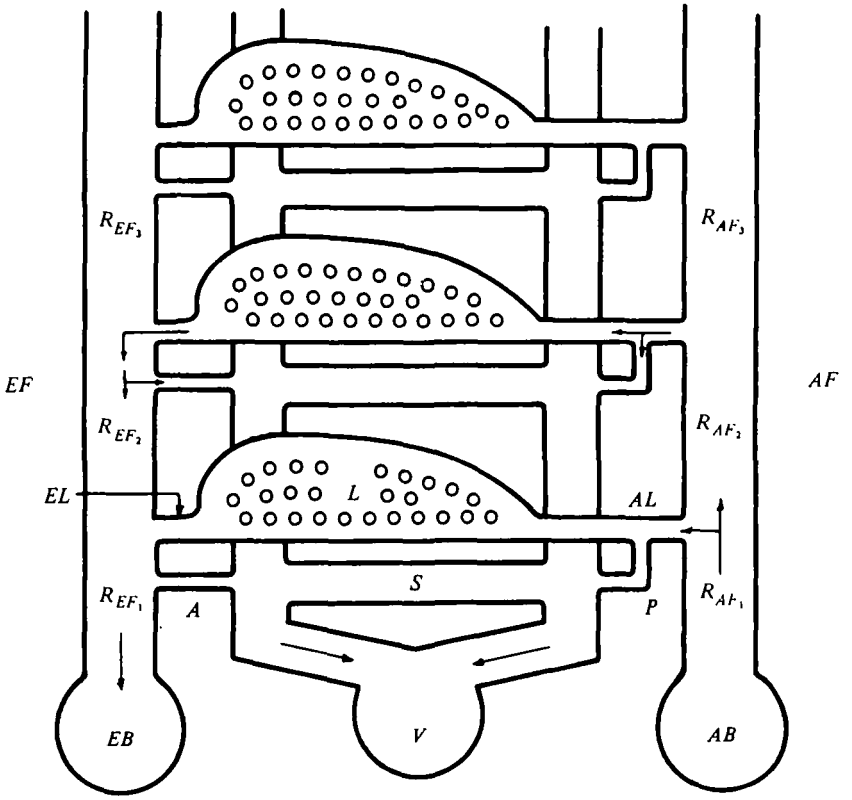

Fig. 2. Schematic diagram of the circulatory pathways in a gill filament from the channel catfish. *AB*, Afferent branchial artery; *AF*, afferent filament artery, *AL*, afferent lamellar arteriole, *L*, lamellae; *EL*, efferent lamellar arteriole, *EF*, efferent filament artery; *P*, prelamellar AVA; *A*, postlamellar AVA; *EB*, efferent branchial artery, *R*, resistance segments for the afferent and efferent filament vessels.

venous anastomoses (P) and thus bypass the lamellar sinus (Olson *et al.* 1978). Similar shunt pathways have recently been found in *Anguilla* (Dunel & Laurent, 1977) and *Tilapia* (Vogel *et al.* 1974) although they are very small in diameter and infrequent in the latter. (The central filament vessels are by convention collectively called the central venous sinus. However, in the catfish these vessels remain as distinct parallel vessels and do not form a cavernous sinus.) Filamental vascular resistances include the in-series resistances of afferent and efferent filament arterial segments $R_{AF_{1,2,3}}$ and $R_{EF_{1,2,3}}$ respectively and the in-parallel lamellar circuits and central sinus vessels.

The effects of epinephrine perfusion can best be explained by vasoactivity at several sites within the filamental vasculature. At constant flow perfusion, 10^{-5} M epinephrine decreased the RCS in distal lamellae and slightly increased RCS in lamellae from basal and mid filament (Table 1). The decrease in RCS within the distal lamellae is expected even if prelamellar vessels to all lamellae are dilated uniformly. This is because the vascular arrangement of a single afferent filament artery and the in-parallel lamellae exhibit an exponential flow decrement distally which is analogous to the linear cable theory for current conduction down a nerve axon (Olson, in preparation). Decreases in resistance of the lamellar circuit (afferent lamellar arteriole

– lamellar sinus – efferent lamellar arteriole) are predicted to favour basal lamellar flow at the expense of delivery to distal areas (Olson, in preparation) which is verified experimentally in Table 1. With constant-pressure perfusion, epinephrine greatly increases RCS in peripheral lamellae while RCS in basal lamellae only moderately increases. If the resistance in all lamellar circuits decreased uniformly, the basal lamellae would be predicted by linear cable theory to show the greatest increase in RCS. Since this is clearly not the case, selective dilation of peripheral lamellar circuits must be assumed.

Although the afferent lamellar arteriole is a logical site for regulating lamellar blood flow, the role of the afferent and efferent filament arteries, the efferent lamellar arteriole and the lamellar sinus, as additional regulators of FSA, cannot be discounted. Dilations in the efferent lamellar arteriole similar to those in the trout (Smith, 1976) were observed under phase contrast microscopy in catfish gills perfused with washed red cells at a 5% haematocrit (Holbert, unpublished observations). Red cells leaving the lamellae would accumulate in the dilated area and produce a log jam effect similar to that seen in sickle cell anaemia. When epinephrine was added to the perfusate the accumulated red cells were dispersed and the disappearance of the red-cell clusters proceeded sequentially toward the distal end of the filament. In this preparation only the distal half of the filaments could be observed and it is not known if basal areas behave in a similar manner.

If lamellar recruitment is operative then the basal lamellae must be moderately well perfused during resting conditions. Additional increases in blood flow to these lamellae which would accompany elevated ventral aortic pressures during exercise would then be superfluous. An increase in basal lamellar resistance during exercise could prevent inordinate flow to these areas. The magnitude of the resistance increase need not reduce flow but merely insure that basal lamellar flow does not become excessive. Epinephrine has been shown to produce a transient branchial α vasoconstriction followed by a prolonged β vasodilation (Bergman *et al.* 1974; Wood, 1975; Payan & Girard, 1977). Payan & Girard (1977) have demonstrated that the constriction is maintained but masked by the more pronounced dilation. The authors attribute this to constriction of the central sinus and expulsion of blood stored within. However, as the constrictor response precedes dilation, it is probable that some of the α receptors are proximal to the β, i.e. in the basal lamellar circuit.

In the trout and the eel a sphincter-like segment has been described in the efferent filament artery immediately prior to the anastomosis of this vessel with the efferent branchial artery (Gannon, personal communication; Dunel & Laurent, 1977; Smith, 1977). Adrenergic dilation of the sphincter will presumably allow more oxygenated blood to enter the efferent branchial artery and reduce flow into the central sinus unless cardiac output increases or, in the eel, the prelamellar shunt vessels dilate. Cholinergic constriction of the sphincter would favour sinus flow. Although sphincter dilation favours delivery of oxygenated blood to the dorsal aorta it cannot account for lamellar recruitment. In fact, dilation would favour basal lamellar flow because the pressure differential across the basal circuits would increase more than the pressure differential across the peripheral lamellae. Thus, a greater proportion of the cardiac output would perfuse the basal areas during adrenergic stimulation. This is not supported by the present findings (Table 1). However, if in addition to lamellae,

whole filaments can be recruited the efferent filament artery sphincter is in an ideal location to affect filamental perfusion.

Acetylcholine (10^{-6} M) increased branchial resistance and decreased RCS in all gill areas except the basal lamellae in which RCS increased. This suggests 'derecruitment' whereby constrictor activity afferent to the lamellae and prelamellar arteriovenous anastomoses (or including these vessels) prevents distal blood flow. The basal lamellae must transport the extra blood since they have less constrictor activity and total flow through the gills is maintained constant by pump perfusion.

The presence of pre-lamellar arteriovenous anastomoses adds a further degree of complexity to blood flow regulation. Because they carry deoxygenated venous blood to the central filament their significance as nutrient vessels seems questionable unless they transport non-respiratory nutrients to central tissues that would otherwise be lost during transit through the lamellae. It also does not seem probable that they deliver red cells to the central sinus for storage as the post lamellar arteriovenous anastomoses could accomplish this with oxygenated blood. Since, in the catfish, the central filamental vasculature appears to perfuse the interlamellar filamental epithelium, the prelamellar arteriovenous anastomoses might be involved in ion transport. How they affect filamental blood flow is unknown.

The present technique offers the distinct advantage of quantifying the red blood cell distribution within the gill vasculature. There are, however, several procedural limitations which must be realized. First, two variables determine FSA: the volume-area of lamellar blood perfusion and the linear flow velocity. Since the present experiments measure only the volume-area of lamellar perfusion, the ability of the fish to regulate gas exchange by changing flow velocity is unknown. However, Smith & Johnson (1977) predicted, in a computer simulated trout gill, that increases in lamellar flow rate would decrease oxygen exchange. If this is also true for catfish, then regulating linear flow velocity is not a significant physiological method of increasing FSA and the RCS determined in the present study is a good index of FSA. Secondly, increased or decreased red cell space within a particular vascular segment cannot necessarily be interpreted as an indication of vasoactivity of that specific segment. Changes in resistance proximal or distal to the segment in question can alter transluminal pressures and produce volume changes within the segment. In addition, failure to maintain physiological venous pressures, as pointed out by Smith (1977), could alter blood pathways as well as have transluminal effects. Thirdly, since only red-cell volumes were measured, it is not possible to correlate the data with blood volumes. If plasma skimming were to occur, the RCS would not be an index of total blood volume. However, red-cell distribution is a better estimate of respiratory exchange potential (Booth, 1978), and RCS values are more applicable to FSA measurements than plasma markers. Girard & Payan (1976) perfused trout heads with radioidionated serum albumin at constant pressure and found large decreases in filamental vascular volume after 10^{-5} M epinephrine administration. These results conflict with the present study where, at constant pressure perfusion, 10^{-5} M epinephrine had no significant effect on filamental RCS. The difference between these two experiments could be due to variation in the vascular organization of the gills of the two species or more probably reflect the difference between red cell and plasma distribution within the gill vasculature. It would be interesting to perfuse gills with ^{51}Cr

and ^{125}I albumin and assess plasma skimming in the lamellar and filamental compartments. Clearly, additional interspecies comparisons with various blood volume–flow indices are certainly required before generalizations on gill blood flow can be attempted.

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