

THE EFFECTS OF OXYGEN SUPPLY, EPINEPHRINE, AND ACETYLCHOLINE ON THE DISTRIBUTION OF BLOOD FLOW IN TROUT GILLS

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SUMMARY

Injecting vitally stained blood cells into the ventral aorta of unrestrained, cannulated fish, and rapid freezing in liquid nitrogen, permitted the examination of the effects of oxygen supply, epinephrine and acetylcholine on branchial lamellar perfusion. Compared to the conditions in resting fish in air-saturated water, hypoxia and injection of epinephrine significantly increased the proportion of secondary lamellae receiving stained cells, and acetylcholine caused a significant reduction, but hyperoxia did not significantly affect the proportion of lamellae containing stained cells. Perfusion of the filamental central compartment was not affected by the treatments. It is concluded that trout can respond to changes in oxygen supply by varying the number of secondary lamellae perfused with blood, and that the distribution of blood flow is regulated by cholinergic and adrenergic receptors. It is suggested, however, that lamellar recruitment would not be useful in minimizing the costs of osmo- and iono-regulation.

INTRODUCTION

Teleosts are apparently capable of altering the diffusion capacity of their gills to salts, water, and respiratory gases (Steen & Krusysse, 1964; Randall, Holeton & Stevens, 1967; Motais & Isaia, 1972; Randall, Baumgarten & Malyusz, 1972; Bergman, Olson & Fromm, 1974; Haywood, Isaia & Maetz, 1977). Steen & Krusysse (1964) believed that at least part of the changes could be explained by variations in the functional surface area of the gills due to non-respiratory vascular shunts, but later researchers have failed to find such a pathway (Vogel, Vogel & Kremers, 1973; Gannon, Campbell & Randall, 1973; Cameron, 1974; Vogel, Vogel & Pfautsch, 1976; Laurent & Dunel, 1976). It is now generally believed that the functional surface area of the gills is changed by varying the number of secondary lamellae receiving blood (Randall, 1970; Hughes, 1972). However, until recently, the gross change in filament blood flow observed by Davis (1972) was the only evidence from live, unanaesthetized, intact fish supporting the lamellar recruitment model.

Booth (1978) showed that in unanaesthetized, intact rainbow trout, the conditions necessary for lamellar recruitment occurred; that is, in fish exposed to air-saturated

water, only 58% of the secondary lamellae were perfused with blood. If lamella recruitment is one of the means by which the branchial diffusion capacity is altered, it should be possible to demonstrate that factors previously shown to affect branchial vascular resistance and diffusion capacity also cause changes in the number of secondary lamellae perfused with blood.

The original concept of a variable gill diffusion capacity was based on the idea that, for water breathers, there must be a balance between the necessity to minimize passive water and ion exchange with the environment, and the need to obtain oxygen from an environment in which the concentration of this gas is low and variable (Steen & Kruyse, 1964; Randall *et al.* 1967). Since the maximum diffusion capacity of the gills of a fish has presumably evolved to cope with extreme conditions, it is greater than would be required by the fish when the oxygen supply is plentiful or the demand for oxygen is low (Saunders, 1962; Holeton & Randall, 1967*b*). It has therefore generally been assumed that the reduction in the functional surface area of the gills would serve to reduce the costs of iono- and osmo-regulation (Steen & Kruyse, 1964) when the full gas exchange capacity of the gills is not required.

The first purpose of this study was to test the prediction that variations in ambient oxygen concentrations should affect the proportion of the total number of secondary lamellae receiving blood in the gills of the rainbow trout (*Salmo gairdneri*).

The vasoactive agents epinephrine and acetylcholine cause changes in the branchial gas, ion, and water diffusion rates as well as in branchial vascular resistance (Keys & Bateman, 1932; Östlund & Fänge, 1962; Bergman *et al.* 1974; Wood, 1974, 1975; Payan & Girard, 1977; Smith, 1977; Wood, McMahon & McDonald, 1978; Shuttleworth, 1978). If adrenergic and cholinergic nerves, along with circulating catecholamines, represent the dominant regulators of branchial blood flow (Bergman *et al.* 1974; Wood, 1974, 1975; Payan & Girard, 1977; Smith, 1977), then injections of epinephrine or acetylcholine into the bloodstream might also affect the recruitment of secondary lamellae.

The second purpose of this study was to determine if stimulation of branchial receptors, through injection of epinephrine or acetylcholine, would cause changes in the number of secondary lamellae perfused in the gills of rainbow trout.

MATERIALS AND METHODS

I. General procedures

Rainbow trout (190–480 g) obtained from a commercial fish hatchery, were kept indoors in 850 l tanks of aerated dechlorinated water at 10 °C. For each experiment a fish was cannulated in the ventral aorta (Holeton & Randall, 1967*a*) and a suture wire loop was attached to each operculum. The fish was then allowed to recover for 24 h in the experimental chamber, supplied with aerated recirculated water at 5 °C.

Following the experimental treatments, the gills were examined using a technique described by Booth (1978) and briefly described again here.

Blood, 0.5 ml, stained with acridine orange was injected into the fish through the ventral aorta cannula. While the blood was still entering the fish, a spring-stopper retractor was slipped into the wire loops and the fish was removed from the chamber

and plunged into a container of liquid nitrogen. As the fish fell, the retractor pulled on the loops, exposing the gills to the liquid nitrogen. Measurements indicated that the temperature among the gill filaments reached -10°C less than 2 s after the procedure was initiated. The gills were removed from the fish and sectioned, while still frozen, in the plane of the gill arch, using a microtome mounted in a cryostat. The frozen sections were then placed on a warm slide where they thawed and dried. The sections were then examined with a fluorescence microscope in order to determine the location of the stained blood cells.

II. *Experimental treatments*

All experiments were carried out using recirculated dechlorinated water at 5°C .

(A) *Controls*

Control values were obtained from undisturbed fish exposed to air-saturated water ($157\text{ mmHg } P_{\text{O}_2}$) (Booth, 1978).

(B) *Hypoxia*

The air supply was replaced by nitrogen and the O_2 concentration of the water was reduced to the desired level between 1.8 and 2.8 mg/l (23 and $35\text{ mmHg } P_{\text{O}_2}$, respectively) and held there for 30 min. The fish was then frozen and the gills examined using the techniques described above.

(C) *Hyperoxia*

The air supply was replaced by pure oxygen. The oxygen concentration of the water was increased to 35 mg/l ($440\text{ mmHg } P_{\text{O}_2}$), and held at this level for 30 min before the fish was frozen and the gills examined.

(D) *Epinephrine*

A dose of approximately $2.5 \times 10^{-4}\text{ g}$ epinephrine (BDH) per kg fish was administered in 0.5 ml of Cortland saline (Wolf, 1963) via the ventral aorta cannula. The fish were frozen 1.5 or 5 min after the injection of epinephrine and prepared as described above.

(E) *Acetylcholine*

Cortland saline, 0.25 ml , containing $1.3 \times 10^{-5}\text{ g}$ acetylcholine (BDH) per kg body weight was injected into the fish through the cannula. The fish were frozen 2 min after injection and the gills sectioned for examination.

III. *Treatment of the data*

For each fish, every fifth filament of each hemibranch from one side of the fish was examined. A count was obtained of the number of secondary lamellae which were 'perfused' (more than 75% of visible blood cells stained), 'half-perfused' (26% to 75% of visible blood cells stained), and 'unperfused' (fewer than 25% of the visible blood cells stained) in each filament. To estimate the proportion of secondary lamellae perfused within each hemibranch, the three values were summed separately for all of the filaments sampled and applied to the equation:

$$\text{proportion of lamellae perfused} = \frac{x + 0.5y}{z},$$

x = total number of perfused lamellae, y = total number of half-perfused lamellae
 z = total number of lamellae sampled. To estimate the proportion of secondary lamellae perfused in a fish the three values were summed for all the filaments sampled, and the totals were applied to the above equation.

The results obtained for each experimental treatment were compared to the control values using a one-way analysis of variance (Sokal & Rolff, 1969).

RESULTS

The average proportions of secondary lamellae perfused with stained blood cells in the fish exposed to each experimental treatment are presented in Table 1. There were significant differences between the control group and the epinephrine-treated group ($P < 0.01$), between the controls and the acetylcholine-treated group ($P < 0.01$) and between the controls and the fish exposed to hypoxia ($P < 0.05$). Fish exposed to hyperoxia did not differ significantly from the controls. There was no significant difference (student's t test) between fish frozen 1.5 min ($n = 5$) and 5 min ($n = 6$) after epinephrine injections.

The distribution of perfused lamellae between hemibranchs for the hypoxia, epinephrine-treated, and acetylcholine-treated and control groups is shown in Fig. 1. It is interesting to note that while the overall proportion of secondary lamellae perfused is increased in the hypoxic and epinephrine-treated fish, the average proportion of secondary lamellae perfused in the hemibranchs of the first arch in both groups is less than in the same hemibranchs of the control group.

Although three of the experimental treatments significantly affected the proportion of secondary lamellae perfused, compared to the controls (Table 1), the basic distribution pattern of stained cells within the hemibranchs was not changed by any of the treatments. In every group there was a preference for perfusion of lamellae near the base of the filaments, and of filaments near the dorsal end of the arch. Although there was never any exclusive perfusion of either the marginal or basal channel around the outer edges of the secondary lamellae, stained cells in the 'half-perfused' lamellae were more often found near the base or outer margin than the centre of the lamellae.

Comparisons of the size of the central compartment and the number of stained cells which it contained, indicated no effect by any of the treatments. In each treatment group the size of the central compartment and the number of stained blood cells in it varied considerably between filaments, even within the same hemibranch. There appeared to be a direct relationship between the presence of stained cells in the central compartment and the number of secondary lamellae on the same filament which were perfused with stained blood cells. In other words, filaments in which a high proportion of the lamellae received stained blood tended to have more stained cells in the central compartment.

Distribution of blood flow in trout gills

Table 1. *The proportion of secondary lamellae perfused in the gills of rainbow trout exposed to different experimental treatments at 5 °C*

Treatment	Sample size	Proportion of lamellae perfused (mean \pm s.e.)
Epinephrine	11	0.750 ± 0.046 *
Acetylcholine	6	0.428 ± 0.038 *
Hypoxia	9	0.710 ± 0.036 †
Controls	10	0.582 ± 0.035
Hyperoxia	6	0.559 ± 0.093 N.S.

* Significantly different from controls ($P < 0.01$).
 † Significantly different from controls ($P < 0.05$).
 N.S. Not significantly different from controls.

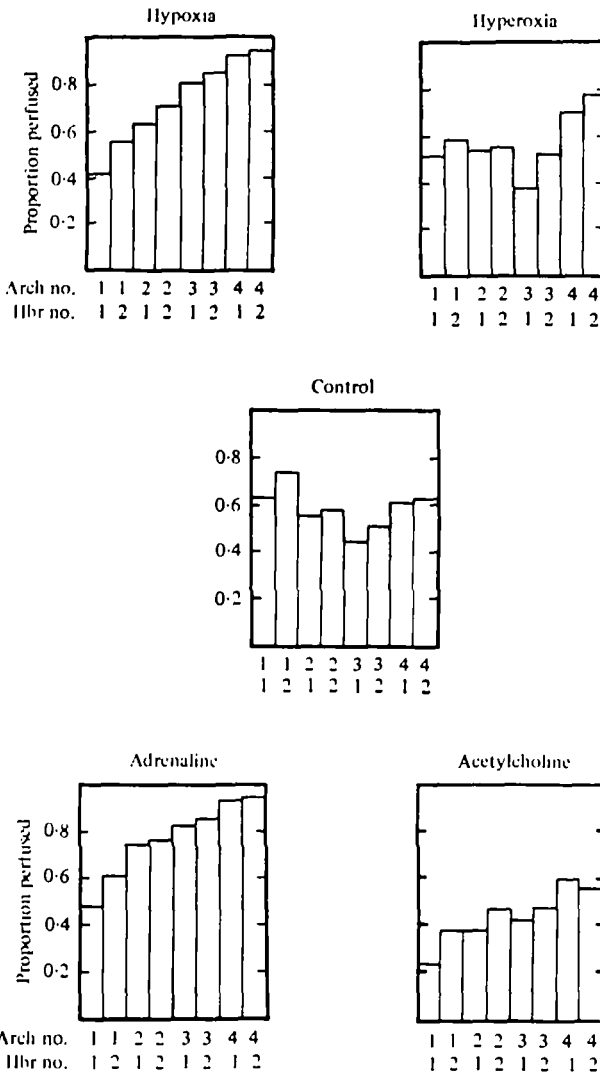


Fig. 1. *The effects of the experimental treatments on the distribution of perfused lamellae among the hemibranchs (Hbr).*

DISCUSSION

The presence of unperfused secondary lamellae in normoxic unrestrained trout has been demonstrated by Booth (1978). Using the same technique, the present study has shown that hypoxia, and high concentrations of epinephrine or acetylcholine, will cause changes in the proportion of secondary lamellae receiving stained blood cells. Since the 'central compartment' is part of the filament venous circulation (Gannon *et al.* 1973; Vogel *et al.* 1973; Vogel *et al.* 1976; Laurent & Dunel, 1976; Smith, 1977) and no other significant shunt pathway has been demonstrated, the observed variations were presumably due to lamellar recruitment.

The 'unperfused' lamellae in the present study probably represent the experimental detection of intermittent perfusion of many of the lamellae, particularly towards the distal ends of the filament. It seems most likely that, during periods of low requirements for gas exchange, none of the lamellae is shut down for long periods of time, but that perfusion of some of the lamellae occurs at intervals just long enough to supply nutrients to, and remove wastes from the lamellar cells.

There was no visible relationship between the size of pillar cells and the presence of stained cells in the secondary lamellae. Furthermore, no noticeable change in the shape of the pillar cells occurred between treatments. None of the treatments caused any restriction of stained cells to the marginal channel or basal channel of partially perfused lamellae (cf. Smith, 1977). These observations conflict with previous evidence implicating pillar cell contraction as an important mechanism in the overall control of lamellar blood flow (Hughes & Grimstone, 1965; Hughes & Byczkowska-Smyk, 1974; Smith, 1977).

The injection of large doses of epinephrine caused a significant increase in the proportion of secondary lamellae perfused with stained blood cells. The experimental dosage was chosen to approximate that which had the greatest effect on branchial vascular resistance in previous studies (Keys & Bateman, 1932; Ostlund & Fänge, 1962; Richards & Fromm, 1969; Bergman *et al.* 1974; Wood, 1974; Payan & Girard, 1977). Blood concentrations resulting from such a dose may be almost an order of magnitude greater than the concentration of epinephrine found in exercised trout (Nakano & Tomlinson, 1967). However, since the epinephrine was administered as a single pulse rather than continuously, there was some possibility that the blood epinephrine concentrations might decline rapidly, due to losses from the vascular compartment and metabolic breakdown.

In the hypoxia experiments sufficiently low oxygen concentrations were used to ensure that the fish were severely stressed. In each case, the experimental oxygen level was that which just approached the concentration at which the fish began to lose equilibrium. It was presumed that this treatment would cause the maximum possible change in lamellar perfusion.

In both the hypoxia and epinephrine experiments, only a few fish approached complete perfusion of the secondary lamellae with stained blood cells. This may, in part, be due to variations in individual sensitivity to the treatments, but the greatest single factor reducing the predicted increase in the number of secondary lamellae receiving stained cells, in both treatments, was the effect on circulation through the first arch. While the perfusion of the second, third, and fourth arches increased as

pected, the average perfusion of the first arch decreased compared to the controls. The significance of this response is not apparent. It could be an artifact arising from the methodology, or it may be related to some of the other ways in which the first arch differs from the other three.

The innervation of the first arch differs in that it receives branches of the IXth cranial nerve, whereas the other three arches are innervated by the Xth cranial nerve (Bernstein, 1970). Daxboeck & Høletoen (1978) and Smith & Jones (1978) have shown that receptors associated with the bradycardia response to hypoxia exist in the region of the pseudobranch and the dorsal portion of the first arch, but not on the other arches. The first arch provides the arterial blood supply to the pseudobranch and the choroid rete of the eye (Goodrich, 1958). Davis (1971) observed that the circulation to as much as 40% of the gill surface area could be blocked without affecting dorsal aortic P_{O_2} in rainbow trout, as long as circulation to the first arch remained intact. In brown trout (*Salmo trutta*), the first and fourth arches receive a smaller proportion of the total ventilatory water than either the second or third arches (Paling, 1968). These observations, combined with the present study, suggest that the perfusion of the first arch may somehow be associated with the regulation of oxygen uptake, but the nature of the association remains a subject for speculation.

The recent observations that epinephrine alters the permeability of the branchial epithelium to specific ions (Shuttleworth, 1978) cast some doubt on the way in which catecholamines influence blood oxygenation and passive ion exchange. It is generally assumed that, in both cases, the effects of catecholamines were consequences of a single effect, namely increased perfusion of secondary lamellae (Randall *et al.* 1972). It now seems likely that the two responses may in fact be due to different effects: lamellar recruitment in the former case, and changes in epithelial permeability in the latter.

The proportion of lamellae receiving marked cells increased by only 29% in the epinephrine-treated fish, and by 22% during hypoxia, compared to the controls. Yet moderately exercised trout can increase their oxygen uptake by as much as 500% (Stevens & Randall, 1967). It seems that variations in the functional surface area of the gills, although they do occur, actually make a relatively small contribution to the overall changes in the gas exchange capacity of the gills.

In the present study, acetylcholine in large doses caused a significant reduction in the number of secondary lamellae perfused with stained blood cells. In the intact animal circulating concentrations of acetylcholine are normally insignificant. The cholinergic receptors within the gills are probably activated by cholinergic nerve fibres of vagal origin (Wood, 1965). Since the injected acetylcholine was intended to simulate the effects of neural stimulation of the cholinergic receptors, pharmacological doses were necessary to ensure that sufficient quantities reached the end organs despite the non-physiological pathways, and the rapid breakdown of acetylcholine by cholinesterases.

Acetylcholine has repeatedly been shown to cause vasoconstriction in teleost gills *in vitro* (Ostlund & Fänge, 1962; Bergman *et al.* 1974; Wood, 1975; Smith, 1977), probably via muscarinic receptors (Wood, 1975). The evidence obtained by Smith (1977) suggested two possible sites of action. Vasoconstriction might occur at the

level of the individual lamellae or at a site on the efferent filamental artery near its junction with the branchial artery. Both locations could influence the degree of lamellar recruitment, affecting the perfusion of individual lamellae in the former case, or all of the lamellae on the filament in the latter. The results of the present study are consistent with the reduction of flow at the level of individual lamellae alone, or possibly combined with reduction of flow across the whole filament. Constriction of the efferent filamental artery alone seems less likely. Unless the circulation to the filamental venous vessels from the efferent artery was also cut off, constriction of the filamental artery alone would appear more likely to result in all of the secondary lamellae of the filament becoming perfused, albeit at a lower rate, due to increased pressure in the efferent artery.

It is possible that the absence of any response to hyperoxia was due to the absence of the appropriate receptors, although this seems less likely in light of the changes in ventilation during hyperoxia observed by Randall & Jones (1973). The results for this series of experiments were extremely variable, presumably representing different sensitivities to a relatively weak stimulus. It is possible that more extensive experimentation would allow greater certainty concerning the influence of hyperoxia on gill perfusion.

The results of this study, in providing support in favour of the lamellar recruitment model, reveal that the original concept of the advantage of variations in functional surface area was based on faulty logic. The blood in the lamellae will still be exposed to the water no matter whether the circulation is proceeding normally, or is slowed down or stopped. Unless the fish permits the blood-water ionic and osmotic gradients to run down in lamellae which are 'unperfused', passive exchange of water and ions will still occur, and the fish will still have to pay the costs of active branchial exchange. If the gradient is maintained, as seems likely, then an ionic/osmotic explanation is not an acceptable rationale for varying the perfused surface area.

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