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Circulatory changes in secondary lamellae of Salmo gairdneri gills in hypoxia and anaesthesia

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The secondary lamellae of rainbow trout gills from hypoxic and anaesthetized fish were analysed stereologically for the blood volume and haematocrit value. In hypoxia the gills undergo vasodilatation, which is accompanied by slight haemodilution. When oxygen is available the situation changes; there is a tendency to vasoconstriction, but a continuing decrease in the haematocrit value. In anaesthesia the vasodilatation seen in the secondary lamellae is combined with haemoconcentration. During recovery vasodilatation continues, while the haematocrit value falls to below that of controls. Explanations for these observations are offered in terms of the circulation through the gills.

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1. Introduction

With the increasing use of the electron microscope for morphometry, detailed knowledge has become available regarding the structure of trout gills (TOVELL *et al.* 1970, HUGHES 1972, MORGAN & TOVELL 1973, VOGEL *et al.* 1976). These studies, although mainly morphological, have shown how greatly knowledge of ultrastructure can help in solving functional problems. Recently HUGHES & PERRY (1976) described a light microscope method suitable for morphometric analysis of gills. But, in spite of the morphological information obtained, the physiological situation was still not clear, especially with regard to the circulation.

WESTFALL (1943) reported that erythrocytes accumulate in the gills of goldfish (*Carassius auratus*) during anoxia.

The circulatory resistance in the gills has been shown to increase when rainhow trout are exercised (STEVENS & RANDALL 1967) or when there is a fall in the oxygen tension of the water (HOLETON & RANDALL 1967). Simultaneously the haematocrit value in the dorsal aorta increases, mainly because of swelling of the erythrocytes (Soivio, unpublished data).

FROMM *et al.* (1971) have shown that MS-222 significantly increases the resistance to fluid flow through isolated-perfused gills of *Salmo gairdneri*. A marked haemoconcentration in the blood of the central circulation of anaesthetized rainbow trout has been shown (SOIVIO *et al.* 1977) to be due mainly to swelling of the erythrocytes.

In the light of this physiological knowledge an attempt was made to analyse the circulatory status and haematocrit value in the secondary lamellae of trout in different physiological conditions.

2. Material and methods

Gills were obtained from 2-year-old rainbow trout (*Salmo gairdneri* Richardson), stock of Laukaa Fish Culture Research Station. The fish were transferred from outdoor ponds (temperature 2° C at the end of January) to 4 m^3 fibreglass tanks holding 2 m^3 of water and kept in a covered rearing hall. The water was warmed gradually (less than 0.5° C/h) and kept at the experimental temperature (10° C) for 2 weeks before the experiment. The oxygen concentration of the water was 104 % of saturation (Winkler) and the water

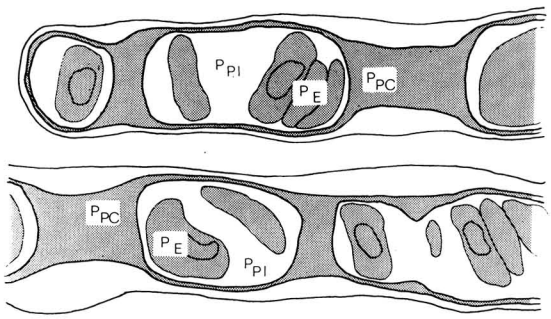

Fig. 1. Tracing of a typical field showing two secondary lamellae and the structures counted. For abbreviations see text.

quality as follows: pH 6.9, specific conductivity 48 μS/cm, $KMnO_4$ 21.1 mg/l, total hardness 0.86 °dH, Ca hardness 0.67 °dH.

Each fish was enclosed in a separate chamber for 1 day before the experiment (Soivio *et al.* 1975). The water flow through the chamber was about 2 l/min. After the appropriate treatment the fish were taken from the chamber in a net and immobilized with a sharp blow on the head; the ventral aorta was clamped with a tourniquet which also opened the mouth and helped the fixative to pass into the gills, and the fish was suspended by the tail with the gills in the fresh fixative (2.5 % glutaraldehyde (Taab) in 0.1 M phosphate buffer (pH 7.2) at 10° C) for 30 min. To help the fixative to circulate, the fish were carefully moved 3 or 4 times during this period. The third right gill arch was then dissected out, fixed *in vitro* for a further 24 h at 4 °C and washed in phosphate buffer. Five filaments from the lateral third of the gill arch were postfixed with 1 % OsO_4 in phosphate buffer for 30 min and embedded individually in Epon.

As far as possible, the specimens were sectioned perpendicularly to the surface of the secondary lamellae and stained with toluidine blue. Slides were viewed under a Wild automatic sampling microscope (1410 ×) fitted with a projection head containing a Weibel 168-point grid. These points were used to estimate the relative volumes of the components in a single section from each block (Fig. 1).

Counts were made of the number of points falling upon the following structures within the pillar cell system (PCS):

Pillar cells + basement membrane (P_{PC}), red blood cells (P_E), blood plasma (P_{Pl}). The epithelium was excluded, because the aim was to analyse the circulatory changes in the secondary lamellae. Initially counts (about 1000/section) were made separately for these points in different parts of the secondary lamella, but for the final analysis points for similar components were summed. Ratios were calculated between the numbers of points as follows:

P_{Pl}/P_{PCS} to indicate plasma volume
P_E/P_{PCS} to indicate erythrocyte volume
$P_{Pl} + P_E/P_{PCS}$ to indicate blood volume
$P_E/P_E + P_{Pl}$ to indicate haematocrit value (Hct).

From well-established stereological principles (Underwood 1970) it is known that the counting of points falling upon given components withint ransverse sections of structures such as secondary lamellae gives a measure of the relative volumes of those structures (Hughes 1972). The use of ratios gives no information regarding absolute volumes, but from the ratio $P_E/P_E + P_{Pl}$ values are obtained for the secondary lamellar Hct.

The effects of hypoxia and anaesthesia were tested on five groups:

Hypoxic (H): The P_{O_2} of the water circulating in the fish chambers was reduced to 50 mmHg for 3 hours before the gills were fixed. The hypoxic water was produced using the partial vacuum principle described by Mount (1961).

Recovery from hypoxia (RH): After exposure to hypoxic water for 3 h these fish were allowed to recover in normoxic water for 3 h before the gills were fixed.

Anaesthetized (A): The fish were anaesthetized for 15 min at 10° C in a trough containing MS-222 (0.1 g/l) to induce deep anaesthesia (Soivio *et al.* 1977) before the gills were fixed.

Recovery from anaesthesia (RA): After 15-min exposure to MS-222 (as above) the fish were allowed to recover for 15 min before the gills were fixed.

Controls (C): Gills fixed from previously untreated fish.

3. Results

In hypoxia, as seen from Table 1, the gills (H) underwent vasodilation combined with a slight decrease in Hct. However, there was a simultaneous increase in the total volume of erythrocytes in the secondary lamellae. During recovery from hypoxia (RH) the vasodilatation tended to subside but there was a continuing decrease in Hct. This was presumably due to a small increase in plasma volume and a decrease in erythrocyte volume within the secondary lamellae.

During anaesthesia (A) vasodilatation occurs in the secondary lamellae and continues even through the recovery stage (RA). This vasodilatation in anaesthetized fish is combined with an increase in Hct, although the plasma volume is slightly increased. During recovery (RA) the Hct drops to below the control value, mainly because of an increase in plasma volume. However, there are indications of decreasing total erythrocyte volume within the secondary lamellae.

Table 1. The calculated ratios in all groups studied. Means ± SEM and numbers of sections counted (n) are given.

Ratio and parameter indicated	RH	Hypoxic	Control	Anaesth.	RA
P_{Pl}/P_{PCS} (plasma volume)	.266± .036	.255± .018	.226± .017	.231± .030	.260± .012
P_E/P_{PCS} (erythrocyte volume)	.454± .017	.492± .019	.482± .029	.528± .037	.514± .038
$P_{Pl} + P_E/P_{PCS}$ (blood volume)	.720± .043	.747± .013	.708± .030	.759± .026	.774± .037
$P_E/P_E + P_{Pl}$ (haematocrit value)	.637± .030	.658± .024	.675± .026	.695± .040	.651± .021
n	6	13	14	5	5

4. Discussion

The vasodilatation revealed by our results on hypoxic gills and the evident increase in the amounts of blood in the secondary lamellae agree well with the observed accumulation of erythrocytes in the gills of asphyctic goldfish (Westfall 1943). These observations are confirmed by a preliminary study which shows that the gill filaments of rainbow trout show a significant accumulation of haemoglobin during hypoxia (Soivio, unpublished data). The slight decrease in the Hct contrasts with the oedema-like leakage of plasma observed in the kidneys of asphyctic or anaesthetized rainbow trout (Soivio *et al.* 1974a), which is evidently due to the increased blood pressure (Holeton & Randall 1967; Stevens & Randall 1967) and the high capillary permeability in fish (Hargens *et al.* 1974). The reason for this discrepancy is probably that hypoxia is not entirely comparable with asphyxia.

The vasodilatation and simultaneous increase in the Hct of the secondary lamellae during anaesthesia, an asphyctic condition as pointed out by Soivio *et al.* (1977), agree well with the pressure changes referred to previously and even with the oedema-like situation observed in the kidney of anaesthetized rainbow trout (Soivio *et al.* 1974), because most of the increase in blood volume is due to the erythrocytes present in the secondary lamellae. This may partly be due to the swelling capacity of the trout erythrocytes in lowered P_{O_2} (Soivio *et al.* 1973; 1974) and to the small increase in haemoglobin concentration during anaesthesia (Soivio *et al.* 1977). This "mechanical" explanation by no means excludes the complicated regulatory processes that are possible in the gill circulation (see Bolis & Rankin 1975, Girard & Payan 1976, Ristori & Laurent 1977, Smith 1977). The increased amount of catecholamines in the blood of stressed fish (Mazeaud *et al.* 1977) complicates the situation, because hypoxia (evidently a stress) decreases the total flow through the isolated-perfused gills (Ristori & Laurent 1977) and perfusion with adrenaline increases the total flow through gill preparations (Smith 1977), and through the secondary lamellae of living fish (Davis, 1972).

Several investigators have studied the effects of adrenaline and hypoxia on the gill network, using isolated-perfused preparations; their results, however, seem to be inconsistent. The situation is even more complicated because some degree of autoregulation has been suspected for the gills (Wood 1974, Kent & Pierce 1975). Noradrenaline and adrenaline increase both pre- and postbranchial blood pressure in free-swimming *Gadus morhua* (Helgason & Nilsson 1973), a situation resembling that reported by Holeton & Randall (1967) for hypoxic rainbow trout. Our results seem to agree with the physiological situation reported from intact fish but we have several difficulties when trying to reconcile them with the results reported from perfusion experiments. However, the circulatory and haematological differences between our experimental groups and especially the differences in plasma volume could be interpreted in terms of the circulatory model of Vogel *et al.* (1976) based on EM studies. The afferent arterio-venous anastomoses (AVA), although rarely seen in their deeply anaesthetized trout, will allow plasma to bypass the respiratory circulation, so leading to a relative increase of erythrocytes in the secondary lamellae. It is much more difficult to explain the haemodilution seen in the hypoxic group and the two recovery groups, because the resistance in all the prevailing shunts will always allow the plasma to pass through before the erythrocytes. One, but by no means the only, explanation is that, even in the normal situation, the pre-respiratory AVAs function as the main regulators of plasma volume, while most of the regulation of the

erythrocyte circulation falls on the post-respiratory shunts. In such a scheme haemodilution would result from a reduction in the volume of plasma shunted off via the AVA system.

The lack of AVAs in rainbow trout gills analysed by conventional histological methods and casting techniques (LAURENT & DUNEL 1976) opposes the explanation suggested above. However, the oedema-like situation in kidneys of similarly treated fish (SOIVIO *et al.* 1974a) shows that plasma may be able to escape from the circulation even through the endothelium.

In work of this kind, however, the autonomic innervation of the gills combined with the vasoconstricting effect of electrical stimulation (NILSSON 1973) cannot be overlooked as a source of artifacts. A mechanical stimulus, possibly originating from the stunning of the fish, might lead to a "constriction artifact", counteracted in the "stressed" groups by the raised levels of catecholamines in the circulation.

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