# CARDIAC OUTPUT AND REGIONAL BLOOD FLOW IN GILLS AND MUSCLES AFTER EXHAUSTIVE EXERCISE IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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## SUMMARY

Rainbow trout (*Salmo gairdneri*) were electrically stimulated to exhausting activity and the changes in cardiac output and blood flow distribution to gills and systemic tissues resulting from the developing severe lactacidosis were repeatedly measured by the microsphere method (15 $\mu$m). Determination of cardiac output by application of the Fick principle resulted in values not significantly different from cardiac output measured by the indicator dilution technique, suggesting that cutaneous respiration, oxygen consumption, and arterio-venous shunting were insignificant under these conditions.

Following muscular activity, cardiac output was elevated by up to 60%. In the gills, the blood flow distribution in the gill arches showed a consistent pattern, even during lactacidosis, with a higher perfusion in gill arches II and III, and in the middle sections of individual gills. Blood flow to white and red muscle was increased much more than cardiac output (+230 and +490%, respectively) such that blood flow to other tissues was actually reduced.

We conclude that the elimination of lactate from muscle cells during the recovery period from strenuous exercise is delayed, not as a result of an impaired post-exercise muscle blood flow, but probably as a result of a high diffusion resistance in the cell membrane.

## INTRODUCTION

Fish are generally equipped with two types of muscle, the highly vascularized red muscle utilized for aerobic low to medium level swimming and positioning activity, and the poorly vascularized white muscle. When high level activity is required, energy is to a large extent produced anaerobically in the white muscle, resulting in the accumulation of large quantities of acidic metabolic end products, mainly lactic acid, in the muscle cells (e.g. Wittenberger & Diaciuc, 1965; Stevens & Black, 1966; Burt & Stroud, 1966; Wardle, 1978). The elimination of lactate from the muscle cells of fish is a rather slow process: peak values in the blood are not attained before 2–4 h after exercise in salmonid fishes (Black, 1957a,b,c; Black, Chiu, Forbes & Hanslip, 1959;

Black, Connor, Lam & Chiu, 1962; Holeton, Neumann & Heisler, 1980; Heisler, 1982) and in tench and dogfish not before 6 h after exercise (Secondat & Diaz, 1942; Piiper, Meyer & Drees, 1972; Heisler & Holeton, 1979; Holeton & Heisler, 1983). In comparison, peak blood lactate concentrations in man are attained a few minutes after exercise (e.g. Margaria & Edwards, 1934; Margaria, Edwards & Dill, 1933; Margaria, Cerretelli & Mangili, 1964; Margaria et al. 1963; Bang, 1936; Crescitelli & Taylor, 1944; Andersen, Bolstad & Sand, 1960).

This delay of the lactate efflux in fish has been interpreted as the result of a considerably impaired muscle perfusion during and following strenuous activity (Black et al. 1962; Stevens & Black, 1966; Wood, McMahon & McDonald, 1977; Wardle, 1978). Muscle blood content has been investigated and found to be unchanged after exercise (Stevens, 1968; Wardle, 1978). Blood flow through fish muscle tissue during or after exercise, however, has not been measured.

The aim of the present study was to evaluate the contribution of a possible blood flow limitation to the slow lactate efflux from muscles tissues after exercise. Rainbow trout were stimulated to strenuous activity in an identical manner to a recent study of the acid-base regulation and the lactate efflux from muscle tissues (Holeton, Neumann & Heisler, 1983), and the distribution of cardiac output to gills and systemic tissues before and after muscle activity was determined by application of the microsphere method.

## MATERIALS AND METHODS

Hatchery raised rainbow trout (*Salmo gairdneri*), weight 1400–1700 g, both sexes) were obtained from a local fish hatchery and were acclimated for at least 3 weeks to a temperature of $15 \pm 0.5$ °C. The water was aerated vigorously so that $P_{O_2}$ was kept higher than 140 mmHg and $P_{CO_2}$ lower than 0.4 mmHg. The aquaria with volumes of more than $150 \, l \, fish^{-1}$ were flushed with tap water, previously dechlorinated in activated charcoal columns, at a rate of more than $200 \, l \, fish^{-1} \, day^{-1}$. The fish were fed on commercial trout food pellets until 3–5 days before experimentation. Experiments were conducted on 19 specimens.

### Catheterization of blood vessels

More than 36 h before the experiment the fish were anaesthetized by immersion in urethane/water solution $(20 \, g \, l^{-1})$ until reactivity ceased, and were placed on an operating rack. The anaesthesia was maintained by artificial ventilation of the gills with an aerated solution of $2.5 \, g$ urethane $l^{-1}$ water.

The dorsal aorta was punctured from the roof of the mouth with a sharpened stainless steel wire inserted into a PE 50 polyethylene catheter, which was conically pulled out at one end, such that the tip of the wire protruded just beyond the tip of the catheter. After puncture, the wire was withdrawn and the catheter pushed 1.5–2 cm forward into the dorsal aorta. This procedure has been reported in detail previously (Ultsch, Ott & Heisler, 1981; Holeton et al. 1983) and is a modification of the method of Soivio, Westman & Nyholm (1972) and Soivio, Nyholm & Westman (1975). The caudal artery and the caudal vein were catheterized in a similar way afte

exposure of the vessel by a sideline incision near the root of the tail fin and spreading of the covering muscle layers. The wound was closed again with two layers of atraumatic sutures (muscles and skin) and the catheters were secured to the skin.

The ventral aorta was punctured from the buccal cavity as described for the dorsal aorta, but only for the first experiments. It turned out that the vigorous movements of the fish during stimulation often pulled out the catheter, which was usually in the vessel with a length of only less than 1 cm. In order to overcome these problems, in most experiments the ventral body wall and the pericardium were cut open and a PE 50 catheter mounted with a metal tip was stabbed through the wall of the bulbus arteriosus and pushed forward into the ventral aorta. The metal tip consisted of a 5 mm length of 0·55 mm o.d. stainless steel tube with a drop of silver solder on one end (diameter of 0·95–1·0 mm) with five connections of 0·4 mm drilled to the lumen of the stainless steel tube. The pericardium and the body wall were closed with fine atraumatic sutures and the catheter secured to the body wall. This arrangement stayed patent and the rounded metal piece prevented the catheter from penetrating the vessel even during extreme muscular activity of the fish.

After preparation, the gills of the fish were irrigated with thermostatted (15 ± 0·2 °C) fresh water until the fish became active and showed apparently normal ventilation after introduction into the experimental chamber. The water in the chamber was recirculated through an oxygenator system (see Heisler, 1978) where the water was oxygenated, decarbonated and thermostatted. The apparatus was flushed with fresh water at a rate of $0·5–1 \, \mathrm{l \, min^{-1}}$ during the entire recovery period before experimentation.

### Experimental procedure

The experiments were conducted in a respirometer (Fig. 1), which was flushed with recirculated, freshly oxygenated and decarbonated water from a thermostatted (15 ± 0·2 °C) oxygenator system (Heisler, 1978). The oxygenator was by-passed and the respirometer was closed only during determination of the oxygen uptake ($\dot{V}_{O_2}$) of the fish by continuous measurement of the oxygen partial pressure in the respirometer. The water was mixed in the respirometer at a rate of about $5 \, \mathrm{l \, min^{-1}}$ by a recirculation pump. Temperature was maintained during the time when the respirometer was closed by pumping cooling fluid through a cooling coil in the bottom of the chamber which was regulated according to the temperature sensed by a thermistor in the box.

After habituation of the animals during the recovery period after anaesthesia, control measurements of $\dot{V}_{O_2}$, dorsal aortic pH, $P_{CO_2}$, $P_{O_2}$ and blood oxygen content ($C_{O_2}$) in dorsal aortic and ventral aortic blood were performed. Simultaneously, radioactively labelled microspheres were injected into the caudal vein and the dorsal aorta, respectively. In some experiments cardiac output was additionally determined by application of the indicator dilution technique by withdrawal of blood from the caudal artery with inulin and microspheres used as indicators (see Berger & Heisler, 1977).

After the control measurements, the fish were prodded and stimulated to vigorous activity by mild electric shocks (a.c., 2–4 V, 50 Hz, supplied by transformer) for a period of 5 min in an identical manner as performed in a previous study (Holeton *et al*. 1982). The end of stimulation was defined as the time zero. Five min, 30 min and
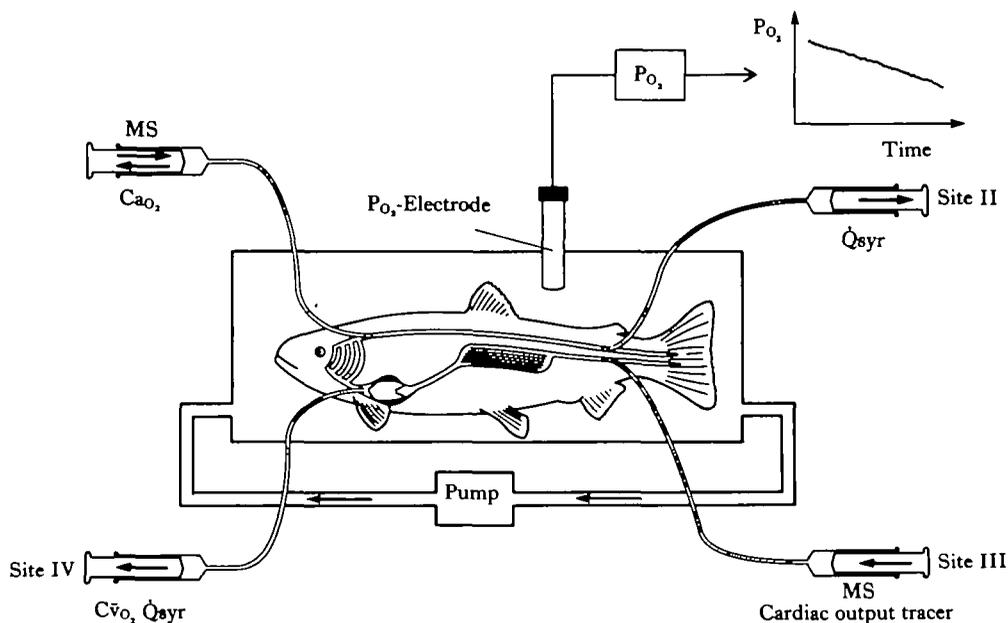
Fig. 1. Experimental apparatus. The fish is confined in a closed respirometer and $P_{O_2}$ is continuously monitored for the determination of $\dot{V}_{O_2}$. Blood samples are withdrawn from the dorsal aorta (site I), caudal artery (site II) and ventral aorta (site IV). Microspheres (MS) and cardiac output tracer are injected into the caudal vein (site III) and into the dorsal aorta (site I).

2 h after exercise the same parameters were remeasured and microspheres with a different label were injected.

After the final microsphere injection the fish were killed by injection of an overdose of anaesthetic, and tissue samples of white muscle and red muscle and the gill arches were excised. The gill filaments of each gill arch were divided into eight samples (see Fig. 3) and counted separately in order to obtain a rough pattern of blood flow distribution along the gill arches. The remainder of the fish was also cut into small pieces (up to 3 g) and all samples were analysed for the activity of the different microsphere labels by $\gamma$-scintillation counting.

### Determination of tissue blood flow

About $3–5 \times 10^5$ microspheres (MS) with a nominal diameter of 15 $\mu$m, radio-actively labelled with either $^{141}Ce$, $^{54}Cr$, $^{85}Sr$ or $^{46}Sc$, were suspended in 0·5 ml 10 % dextran solution by means of an ultrasonic homogenizer (see also Berger & Heisler, 1977). Samples were checked for aggregation of MS by light microscopy prior to injection. The samples of 0·5 ml were drawn into 2 ml syringes, 1·5 ml blood was aspirated from the vessel (either dorsal aorta or caudal vein), and the MS suspension was thoroughly mixed with the blood by means of a steel sphere in the syringe and a magnet immediately before the mixture was injected. The catheter was then flushed with 1 ml dextran. The relative amount of activity in tissue samples was taken as the relative blood perfusion. Absolute blood flow was determined from measurement of the cardiac output by application of the Fick principle, using the indicator dilution

echnique with the MS label just injected, or by using tritium-labelled inulin as an indicator. Blood was sampled for the indicator dilution technique from the caudal artery at rates between 0·6 and 2·0 ml min$^{-1}$ into pre-weighed vials by means of a roller pump. The MS activity in the sampled blood was determined together with the tissue samples by $\gamma$-scintillation counting with a multichannel analyser (Model 5986, Packard Instruments, Inc.). The activity of the four individual labels was recalculated by a computer, programmed with an efficiency matrix obtained from standard countings, immediately before counting of the samples. The activity of $^3$H-inulin injected and recovered in the plasma of the withdrawn blood was analysed by liquid-scintillation counting after appropriate oxidation of the samples in a sample oxidizer (Model 306, Packard Instr., Inc.; see Heisler, Weitz & Weitz, 1976). For calculations see Berger & Heisler (1977).

Homogeneous mixing of the injected microspheres with the blood flowing in the dorsal aorta was checked in two specimens by injection less than 2 min apart of two batches of microspheres with different labels. This procedure was repeated with another pair of labels about half an hour later.

The ratio of the relative blood flows indicated by the fraction of the injected activity of each of the two labels found in the tissues averaged $101·8 \pm 3·1\%$ ($\bar{x} \pm$ s.D., $N = 224$).

The actual diameter of the microspheres was checked by light microscopy to be $15·1 \pm 1·5\,\mu$m for $^{141}$Ce, $14·8 \pm 1·4\,\mu$m for $^{51}$Cr, $15·4 \pm 1·8\,\mu$m for $^{85}$Sr, and $14·9 \pm 1·7\,\mu$m for $^{46}$Sc ($\bar{x} \pm$ s.D., $N = 100$ for each label).

### Determination of microsphere recirculation

In order to determine the relative amount of microspheres not trapped during the first pass in the gills or the systemic tissues, microspheres with different labels were injected into the caudal vein (site III) and into the dorsal aorta (site I). Blood was withdrawn at a constant rate of about 0·6 ml min$^{-1}$ from the caudal artery (site II) and from the ventral aorta (site IV). The activity of the MS label injected into the caudal vein (dorsal aorta) was related to the activity of the same label trapped in the systemic tissues (gills) and in the blood withdrawn from the caudal artery (ventral aorta). For evaluation of the blood samples, the ratio of rate of withdrawal to cardiac output was taken into account.

About 30–70% of the microspheres injected into the caudal vein were trapped in the portal vessel system of the kidneys, but the relative amount of MS not trapped during the first passage in gills and systemic tissues never exceeded 0·5% of the total amount, irrespective of the method of determination (MS trapped in the 'wrong' vascular bed, or withdrawn from the 'wrong' site).

### Determination of respiratory parameters

Blood from the dorsal and ventral aorta was withdrawn anaerobically into heparinized syringes. Subsamples of $20\,\mu$l were analysed for arterial and mixed venous oxygen content using a Lex-O$_2$-Con apparatus (Lexington Instr. Inc., Waltham, Mass.) calibrated with atmospheric air. Blood was also analysed for plasma pH, $P_{CO_2}$ and $P_{O_2}$ using appropriate microelectrodes (Radiometer, Copenhagen) thermostatted to $15 \pm 0·1\,°$C and calibrated with precision phosphate buffers (S 1500, S 1510,

Radiometer) and appropriate humidified gas mixtures provided by gas mixing pump (Wösthoff, Bochum, FRG).

For determination of cardiac output by application of the Fick principle the oxygen consumption of the fish was measured by monitoring the fall in $P_{O_2}$ during the time the respirometer was closed (Fig. 1). The time period of closing the respirometer was kept small enough to lower $P_{O_2}$ in the box from more than 140 mmHg to not below 110 mmHg. $\dot{V}_{O_2}$ was calculated using a value of $0.00208 \, mmol \, l^{-1} \, mmHg^{-1}$ for the solubility of oxygen in water.

### RESULTS

Dorsal aortic plasma pH fell immediately from the control value of about 7·8 to values below 7·4 and only slowly started to recover towards control values during the 2 h of the post-exercise observation period (Fig. 2, upper panel). This response was very similar to that observed recently by Holeton et al. (1982) in slightly smaller rainbow trout in a study of exercise-induced acid-base disturbances. Arterial $O_2$ content was slightly reduced initially (probably due to Root and/or Bohr effects resulting from the considerably reduced plasma pH), but recovered after 2 h (Table 1). In addition, mixed venous $O_2$ content dropped during the recovery period to rather low values, resulting in $O_2$ extraction ratios of up to 86 % (5 min). Accordingly, the difference between arterial and venous $O_2$ content (a−v difference) remained relatively constant in spite of the considerably increased $O_2$ consumption.

Cardiac output during control conditions averaged $45.9 \pm 5.5 \, ml \, min^{-1} \, kg^{-1}$ ($\bar{x} \pm$ s.D.) and was increased after exercise to $68.8 \pm 14.4$ after 5 min, $71.9 \pm 9.4$ after 30 min, and almost recovered to control values ($47.1 \pm 6.1 \, ml \, min^{-1} \, kg^{-1}$) 120 min after activity (Fig. 2, middle panel). No significant differences between values determined by application of the Fick principle or by the indicator dilution method using MS or inulin could be found when these methods were applied simultaneously in four animals. The microsphere dilution method yielded $105 \pm 6.2 \%$, and the inulin dilution method $96 \pm 5.3 \%$ of the respective values determined by the Fick principle method. In about half of the specimens the highest values for cardiac output were observed 5 min, in the other half 30 min, after the end of exercise. This distribution suggests that peak values were attained between 5 and 30 min after exercise and were missed by the experimental procedure.

Table 1. *Arterial and mixed venous blood oxygen content ($C_{O_2}$), blood oxygen extraction (E), and oxygen consumption ($\dot{V}_{O_2}$), in* Salmo gairdneri *during control conditions and following strenuous muscular activity*

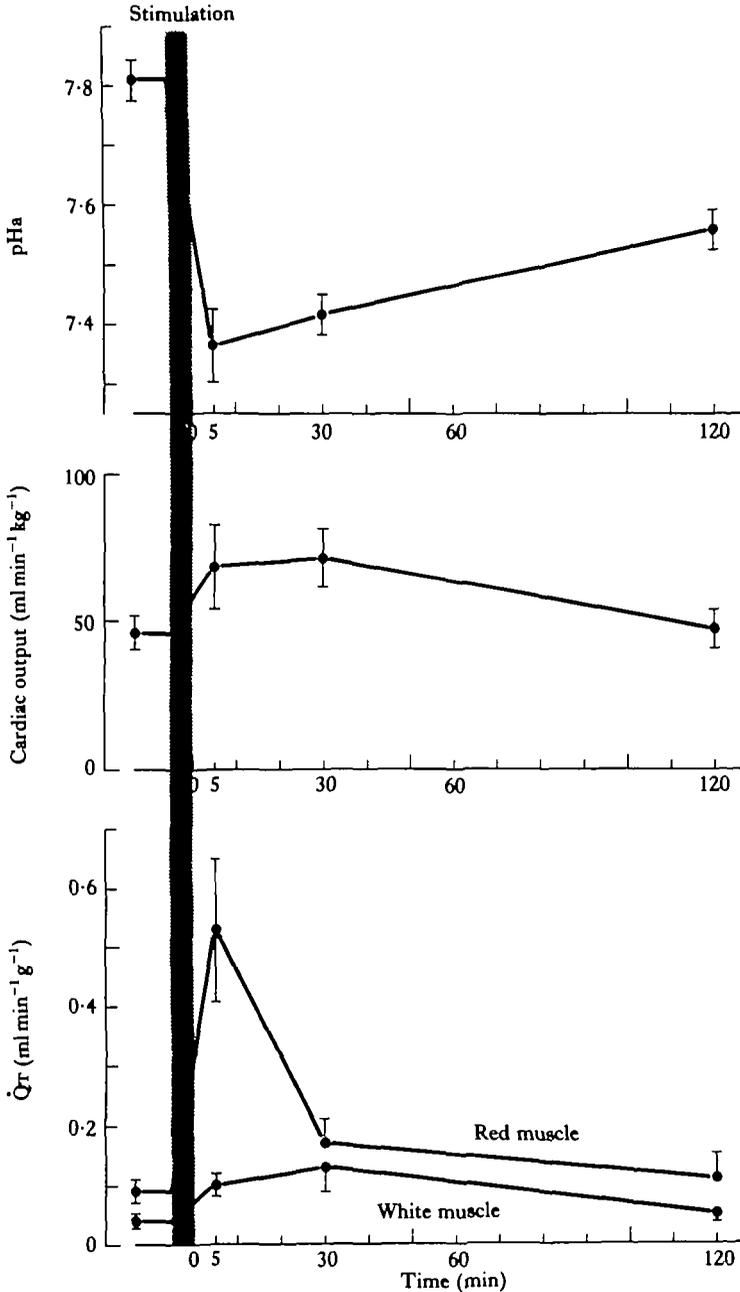| | $C_{a_{O_2}}$ (Vol %) | $C_{\bar{v}_{O_2}}$ (Vol %) | $C_{a_{O_2}} - C_{\bar{v}_{O_2}}$ (Vol %) | E (%) | $\dot{V}_{O_2}$ (ml min$^{-1}$ kg$^{-1}$) |
|---|---|---|---|---|---|
| Control | 7·6 ± 1·2 | 2·8 ± 0·8 | 4·8 ± 0·9 | 64 | 2·2 ± 0·3 |
| 5 min | 5·7 ± 0·8 | 0·8 ± 0·3 | 4·9 ± 0·8 | 84 | 3·1 ± 0·6 |
| 30 min | 5·5 ± 0·9 | 1·0 ± 0·4 | 4·5 ± 0·5 | 82 | 3·1 ± 0·5 |
| 120 min | 6·7 ± 1·6 | 2·3 ± 0·9 | 4·4 ± 0·9 | 66 | 2·1 ± 0·4 |

($\bar{x} \pm$ s.D., $N = 9$).

Fig. 2. Dorsal aortic plasma pH (pHa), cardiac output (C.O.) and blood flow to red and white musculature ($\dot{Q}_T$) before and after strenuous exercise. ($\bar{x} \pm$ s.d., $N = 9$).

The perfusion of white muscle was increased after exercise from about 0·040 ml min$^{-1}$ (g muscle weight)$^{-1}$ during control conditions to 0·102 ml min$^{-1}$ (g muscle weight)$^{-1}$ after 5 min, and to 0·133 ml min$^{-1}$ (g muscle weight)$^{-1}$ after 30 min, and returned to control values after 120 min (Fig. 2, lower panel). The increase in red
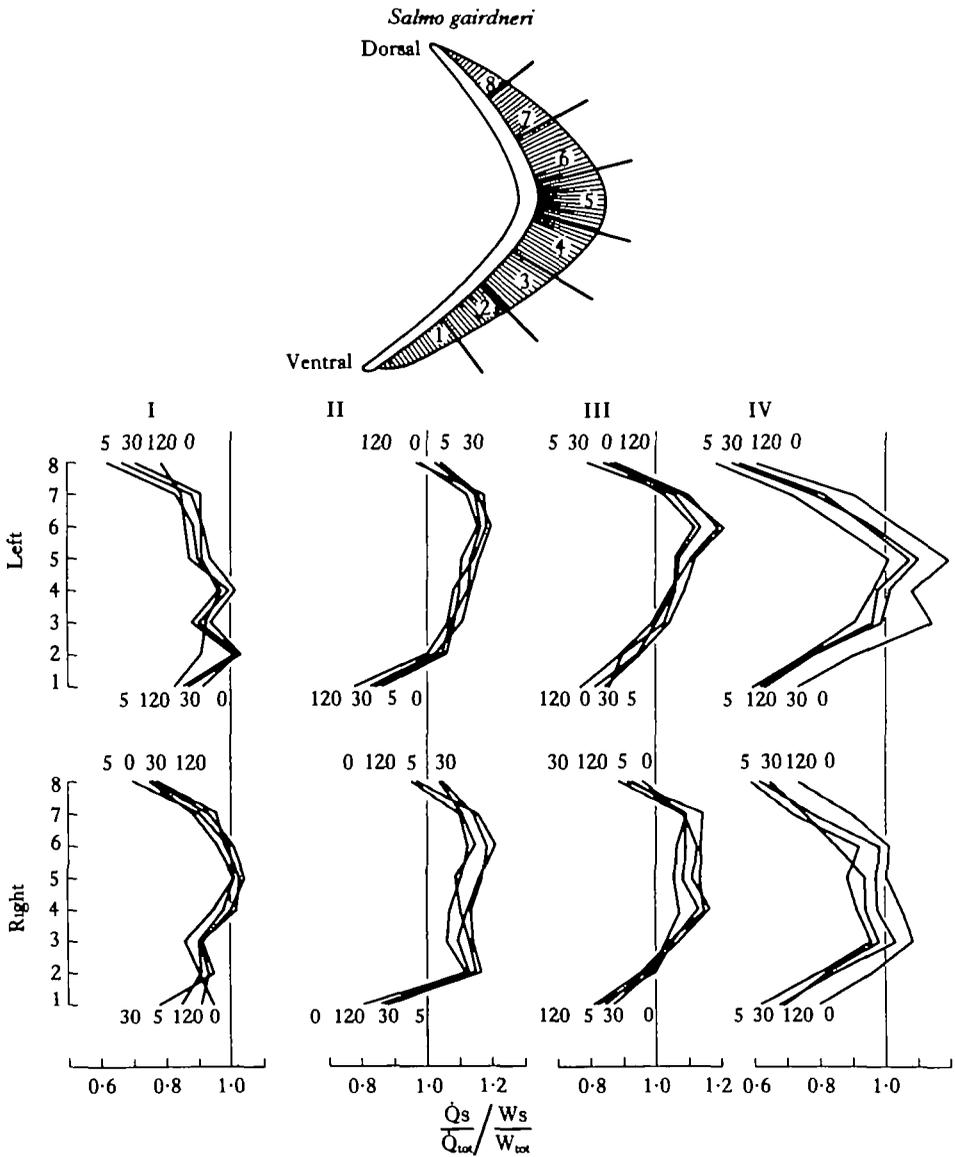
Fig. 3. Relative perfusion of eight filament subsamples of each gill arch. Lines drawn through the average values from nine specimens are not significantly different 5, 30 and 120 min after exercise from control values (0) and from each other. Values higher than '1' indicate higher than average perfusion, lower than '1', lower than average perfusion. Standard deviations of average values were usually below ±0·050, in some cases up to ±0·095.

muscle perfusion was even larger: from 0·09 ml min$^{-1}$ (g muscle)$^{-1}$ to 0·53 ml min$^{-1}$ (g muscle)$^{-1}$ after 5 min. In red muscle, however, perfusion tended to normalize after only 30 min (Fig. 2), whereas white muscle at this time received maximal blood flow.

The weight-specific relative perfusion of the gills, $(\dot{Q}s/\dot{Q}_{tot})/(Ws/W_{tot})$*, was not

* $\dot{Q}s$ = sample perfusion rate, $\dot{Q}_{tot}$ = cardiac output, Ws = sample weight, $W_{tot}$ = total weight of gill filament

gnificantly different between the control period and the post-exercise period, showing the highest perfusion in the middle of the gill arch (Fig. 3). The perfusion of gill arches II and III was higher than that of arches I and IV.

The haematocrit of the fish was $25 \cdot 7 \pm 3 \cdot 5 \%$ and not significantly different from that found in previous experiments (Holeton *et al.* 1982).

Dissection of two fish specimens yielded relative tissue volumes of 46 % of the body weight for white muscle and 4 % for red muscle.

## DISCUSSION

The determination of cardiac output is influenced by a number of factors. The inulin dilution method as applied in the present study (injection at site III, sampling at site II, Fig. 1) is probably least influenced. The only source of error would be loss of indicator substance by diffusion into the extracellular space resulting in an overestimate of cardiac output. Since the transit time from site III to site II was less than 30 s, however, this factor will be insignificant. The microsphere dilution technique (injection at site I, sampling at site II) determines the flow rate in the dorsal aorta, which is the cardiac output minus the blood supply to the head and minus the blood volume which is shunted away from the dorsal aorta pre- or postbranchially towards the venous system. The Fick principle method is probably influenced to the largest extent. Prebranchial shunting would reduce cardiac output; postbranchial shunting, the oxygen consumption of the gills, and the oxygen uptake *via* the skin surface would increase the determined value. Gill consumption was estimated in cod to be about only 6 % of total $\dot{V}_{O_2}$ (Johansen & Pettersson, 1981). In the more active salmonids, with their higher routine metabolic rate, the fraction of gill $\dot{V}_{O_2}$ can be expected to be even smaller. Cutaneous respiration in 290 g trout was 13 % of total $\dot{V}_{O_2}$ (Kirsch & Nonotte, 1977). Since much larger fish ($\sim$5 times) were used in this study this factor can be estimated as negligible, especially since $\dot{V}_{O_2}$ in the present experiments was elevated by a factor of at least two compared to the standard metabolic rate. Prebranchial shunting was estimated to be about 28 % of cardiac output in the eel (*Anguilla anguilla*), which was reduced to 6 % after injection of adrenaline (Hughes, Peyraud, Peyraud-Waitzenegger & Soulier, 1980). Since no significant differences were found in our experiment between the values determined by application of the inulin and MS dilution techniques, and the Fick principle, it appears that the mentioned factors have not influenced our results to a large extent. This may be related to the elevated level of cardiac output, which was probably accompanied also by an elevated catecholamine concentration in the blood.

The average cardiac output of $45 \cdot 9$ ml min$^{-1}$ (kg body weight)$^{-1}$ determined for 'resting' trout is higher than has been measured in most previous studies (e.g. Holeton & Randall, 1967; Stevens & Randall, 1967; Cameron & Davis, 1970; Davis & Cameron, 1971; Kiceniuk & Jones, 1977). Our study, however, has been conducted at higher temperature (15 °C versus 4–10 °C), so that temperature-dependent differences in metabolic rate may account for at least part of the discrepancy (Table 2). The only study at about the same temperature (Holeton & Randall, 1967) resulted in even higher values. But even if our value is reduced according to the $Q_{10}$ of the standard metabolic rate of trout ($2 \cdot 1$) for the temperature range of 10–15 °C (Ott,

Table 2. *Cardiac output in rainbow trout* (Salmo gairdneri)

| | Weight (g) | Temperature (°C) | Cardiac output (ml min$^{-1}$ kg$^{-1}$) | |
| --- | --- | --- | --- | --- |
| | | | Rest | Activity |
| Holeton & Randall, 1967 | | 17–18 | 65–100 | |
| Stevens & Randall, 1967 | 200–400 | 4–8 | 15–30* | 67·5–135* |
| Cameron & Davis, 1970 | 175–400 | 8·7 ± 0·2 | 18·3 ± 1·9 | |
| Davis & Cameron, 1971 | 210 | 8·6 | 18·5* | |
| Kiceniuk & Jones, 1977 | 900–1500 | 9–10·5 | 17·6 ± 1·1 | 52·6 ± 2·2 |
| Present study | 1400–1700 | 15 ± 0·1 | 45·9 ± 5·5 | 71·9 ± 9·3 |

* Recalculated per kg body weight.

Heisler & Ultsch, 1980) some literature data are still considerably lower (Table 2). Also the oxygen consumption of 2·2 ml min$^{-1}$ (kg body weight)$^{-1}$ (Table 1), which is about twice the value determined as the standard metabolic rate of trout at 15 °C (Ott *et al.* 1980), suggests that our fish were more active than in some of the other studies. Activity periods of several hours with a three-fold increase of oxygen consumption are frequently (up to 30 % of total time) observed, even in absolutely undisturbed trout (Ultsch, Ott & Heisler, 1980). Thus it appears likely that in the present experiments in spite of all precautions, such as shielding of the respirometer etc., the fish were more active and more disturbed by the handling of the catheters and the microsphere injections that in other studies at lower temperatures. Augmentation of cardiac output by a decrease in blood oxygen capacity (Cameron & Davis, 1970) is ruled out by the normal haematocrit in our experiments. Also, an effect of the opening of the pericardium on intrapericardial pressure and cardiac output (Randall, 1970) is unlikely in trout with their highly compliant pericardium.

After strenuous exercise cardiac output is increased by about 60 %. This increase is small compared to the 2·4-fold increase found after swimming at maximal speed (Kiceniuk & Jones, 1977), and to the 4·5-fold increase during moderate swimming activity (Stevens & Randall, 1967) in rainbow trout. Part of the difference may be related to the evidently higher baseline levels in our study, part to the fact that peak values in our study were probably missed by the gap between measurements 5 min and 30 min after exercise. Also, the oxygen consumption during aerobic swimming activity (Stevens & Randall, 1967), and thus the cardiac output, would be expected to be larger than during recovery from anaerobic exercise (Table 1), where oxygen consumption is limited by the metabolic processing of lactic acid.

Cardiac output was distributed to the gills in an apparently very consistent pattern (Fig. 3). The weight-specific relative perfusion of each of the eight filament regions of each gill arch was extremely consistent in individual animals with time and even during the considerable enhancement of cardiac output during exercise-induced lactacidosis. Also, the differences between individuals were very small compared to the regional perfusion in all other tissues. Thus, if during conditions of increased cardiac output additional lamellae are recruited (Booth, 1978), these must be distributed along the gill arch in a very uniform manner. In contrast to the extremely constant perfusion observed in our experiments, considerable blood flow redistribution among various gill

rches was observed during hypoxia and hyperoxia and after adrenaline and acetyl-choline administration in a study using fluorescently labelled erythrocytes (Booth, 1979). In addition, the blood flow distribution along the individual gill arches and among the four gill arches is different. In the present experiments, gill arches II and III, and the middle segments of individual arches, received higher perfusion per g weight, whereas gill arches I and IV and the ventral and dorsal segments of the arches received reduced perfusion (Fig. 3). In the experiments of Booth (1978, 1979), perfusion tended to be lower in arches II and III and was higher in the dorsal part of individual arches. Some of these discrepancies may be related to differences in the weight ratio between secondary lamellae and supporting tissue at various sites of the gill apparatus.

The higher relative perfusion of gill arches II and III may very well be related to a larger ventilatory water flow to these arches, as was found in brown trout (*Salmo trutta*) (Paling, 1968). Such adjustment would then serve to establish a homogeneous $\dot{V}g/\dot{Q}$ ratio and thus optimize gas and ion exchanges in the gills.

The distribution of blood flow to various tissues of fish has only rarely been investigated. White muscle of freely swimming *Squalus acanthias* received $0.024 \, \text{ml g}^{-1} \, \text{min}^{-1}$, about one quarter of the amount distributed to red muscle (microsphere technique, Kent, Pierce & Bever, 1973), whereas in the arctic grayling (*Thymallus arcticus*) the relative perfusion of white muscle was only one tenth of that in red muscle (Cameron, 1975). In the present study the ratio of red muscle/white muscle perfusion was 2.25 and thus very well correlated with the capillary density ratio of red and white muscle in fish (Cameron & Cech, 1970). After strenuous exercise, the perfusion of white muscle was enhanced by a factor of more than 3.3 after 30 min and perfusion of red muscle by a factor of 5.9 after 5 min. These increases in perfusion are much larger than can be accounted for by the increased cardiac output, indicating a considerable redistribution of blood flow to various tissues. The percentage of cardiac output received by white and red musculature can be roughly calculated based on the average perfusion of the analysed tissue samples and the relative quantity of the respective tissue type in the organism (Table 3). As most of the muscle samples were taken from the fillets of the caudal half of the fish, presumably the more active part of the fish, this may have resulted in an overestimate. Nevertheless, this estimate shows that after exercise the fraction of cardiac output supplied to the musculature is increased by a factor of more than two, at the expense of the other tissues. What mechanism is responsible for this blood flow redistribution cannot be judged from the present data. We can only speculate that the severe reduction of pH in the blood passing through the musculature induces the observed vasodilation. When pH

Table 3. *Estimate of the percentage of cardiac output distributed to white muscle and red muscle tissues before and after strenuous exercise*

|  | White muscle (%) | Red muscle (%) |
|---|---|---|
| Control | 40.0 | 7.8 |
| 5 min | 68.2 | 30 |
| 30 min | 83.2 | 9.4 |
| 120 min | 48.8 | 9.3 |

Calculations based on the average perfusion of tissue samples and relative quantity of the respective tissue.

recovers towards control values (Fig. 2) perfusion of the musculature is reduced. The extreme temporary increase in red muscle perfusion 5 min after exercise may then be explained on the basis of the same mechanism, but primarily induced by accumulation of $CO_2$ in the more aerobic red muscle; the $CO_2$ would be washed out of the tissue in a few minutes.

Speculations about an impairment of muscle blood flow as an explanation for the slow lactate efflux after strenuous exercise can be ruled out on the basis of the tissue flow rates determined in the course of this study. If the lactate transfer is considered
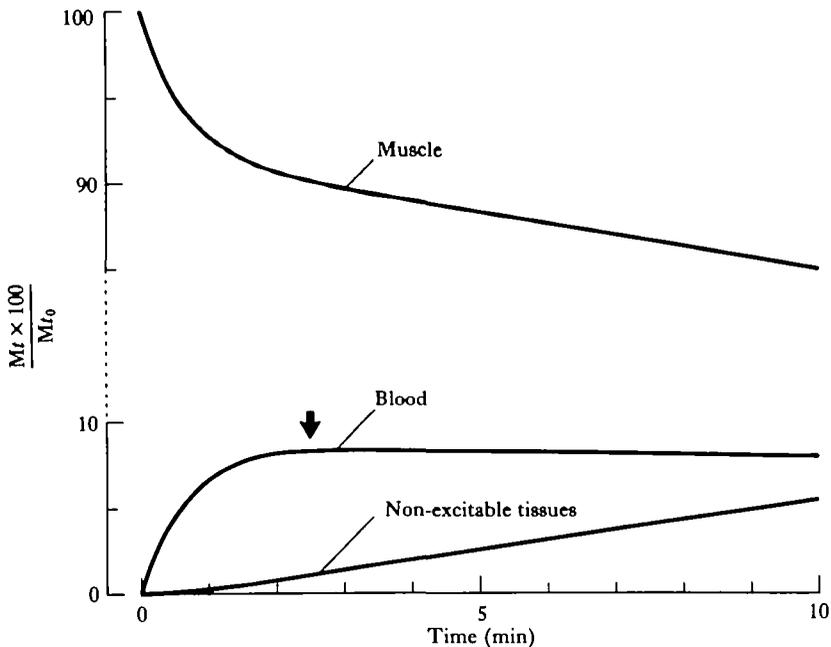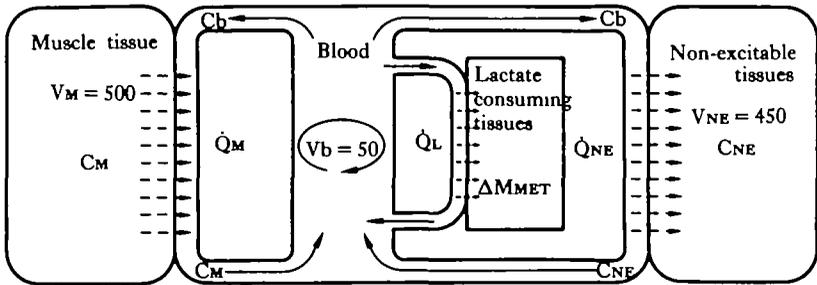


Fig. 4. The efflux of lactate from the muscle tissue into blood and other tissues modelled as being perfusion limited. The estimate is based on complete equilibrium between tissue and blood concentrations after passage, complete mixing of venous blood return with the blood pool, the relative volume of the respective tissue type $V_M$, $V_{NE}$, ml (kg body weight)$^{-1}$, the perfusion rates ($\dot{Q}_M$, $\dot{Q}_{NE}$) determined in this study (see Results), and a constant rate of further aerobic processing of lactic acid in the metabolism of 10 % of the originally produced amount per hour. Presented is the percentage in the respective tissue ($M_t$) of the originally produced lactate ($M_{t_0}$). The amount in the blood has already reached a maximum by 2·5 min (arrow), whereas *in vivo* peak blood lactate concentration is attained only after more than 2 h (Holeton, Neumann & Heisler, 1982). NE denotes non-excitable tissue; M denotes muscle; b denotes blood.

be exclusively perfusion-limited, a simple three-compartment model for the transfer between muscle tissue, blood and non-excitable tissues can be established, based on the perfusion rates determined, the compartment volumes, and the rate of lactate removal from the organism by further aerobic processing (Holeton *et al.* 1982). The efflux of lactate from the white muscle can then be simulated by computer-aided Bohr integration (Fig. 4). The calculations indicate that less than 2·5 min are required to attain peak blood lactate concentration. This time is more than 50 times shorter than the 2 h required *in vivo* (Holeton *et al.* 1982). Thus the efflux of lactate from muscle tissue is evidently not perfusion limited, but the result of slow transmembrane diffusion.

After exhaustive exercise the blood flow to the white and red musculature is increased by 230 and 490%, respectively. This is much more than can be attributed to the increase in cardiac output (+60%) and indicates a considerable redistribution of cardiac output during muscular lactacidosis. In contrast to the redistribution of blood flow to various systemic tissues, the distribution of blood flow in the gills remained unaffected by the general acidosis. We conclude that the slow elimination of lactate from the muscle tissues is not the result of a perfusion limitation induced by a blood flow impairment after exercise, but probably due to the large diffusion resistance of the muscle cell membrane.

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