J. exp. Biol. **123**, 409–421 (1986)
Printed in Great Britain © *The Company of Biologists Limited 1986*

PLASMA CATECHOLAMINES IN THE LESSER SPOTTED DOGFISH AND RAINBOW TROUT AT REST AND DURING DIFFERENT LEVELS OF EXERCISE

BY P. J. BUTLER, J. D. METCALFE AND S. A. GINLEY

Department of Zoology and Comparative Physiology, University of Birmingham, Birmingham B15 2TT, UK

Accepted 2 April 1986

SUMMARY

The hypothesis that there is an increase in plasma catecholamines during exercise in fish and that they play an important role in the cardiovascular adjustments during exercise was investigated in the lesser spotted dogfish and rainbow trout. In resting fish plasma catecholamines were at a concentration of 10^{-9}–10^{-8} mol l^{-1}. During spontaneous swimming in the dogfish, adrenaline increased by 3·3 times to $1{\cdot}9\times10^{-8}$ mol l^{-1} and noradrenaline increased by 2·3 times to $3{\cdot}2\times10^{-8}$ mol l^{-1}. In rainbow trout swimming at a steady 1 body length s^{-1} ($L\,s^{-1}$) in a water channel, the levels of both amines decreased to approximately 25% of the resting values. When swimming to apparent exhaustion at approximately 2 $L\,s^{-1}$, adrenaline was 10 times the resting value at $1{\cdot}4\times10^{-8}$ mol l^{-1}, whereas noradrenaline was 2·2 times the resting value at $2{\cdot}3\times10^{-8}$ mol l^{-1}. Only after repeated burst swimming for 2–3 min did the levels of plasma catecholamines increase substantially above the resting values. In the dogfish, both amines were at 10^{-7} mol l^{-1}, whereas in the rainbow trout, noradrenaline was at $8{\cdot}5\times10^{-8}$ mol l^{-1} and adrenaline was at 2×10^{-7} mol l^{-1}.

These levels were compared with the concentrations of catecholamines used by other workers to elicit changes in the branchial vasculature, gas exchange at the gills or gas transport to the tissues. In lesser spotted dogfish, the levels of adrenaline and noradrenaline present in the plasma during spontaneous swimming have 80% and 50% of maximum effect on gill blood vessels, respectively, whereas in rainbow trout the levels present when swimming to apparent exhaustion have approximately 20% of maximum effect on the branchial vasculature. The levels present in the trout after repeated burst swimming have 40% of maximum effect on blood vessels in the gills. The difference between the dogfish and the trout may be related to the lack of innervation of the gill blood vessels in the former. Enhancement of gas exchange across the gills of rainbow trout can be demonstrated by using adrenaline at the concentration found after repeated burst swimming. It is possible, however, that the concentration of adrenaline found in the plasma of trout after swimming to apparent exhaustion may cause an increase in the *concentration* of oxygen in arterial blood, thus enhancing oxygen delivery to the tissues.

It therefore appears that, in rainbow trout, only the levels of plasma catecholamines attained during repeated burst swimming are likely to have clear effects on oxygen delivery to the tissues. Burst swimming is powered by the white anaerobic

Key words: fish, exercise, catecholamines.

muscle fibres, so oxygen delivery *during* the exercise will not be of value. An elevated supply of oxygen *after* such exercise could well be important, although it is not certain to what extent a fish would engage in repeated burst swimming for several minutes in its natural environment.

INTRODUCTION

Tight control of the circulatory and respiratory systems during an increased demand for oxygen is of paramount importance for all animals and yet the details of this control during exercise are poorly understood even for mammals (Whipp & Ward, 1982, 1985), let alone for other vertebrates. When swimming at maximum sustainable speed (critical swimming speed) at 10°C there is approximately an eight-fold increase in oxygen uptake in the rainbow trout (Kiceniuk & Jones, 1977), and in spontaneously swimming dogfish at 18°C there is almost a doubling of oxygen consumption (Piiper, Meyer, Worth & Willmer, 1977). It would seem reasonable that the nervous system is involved, at least to some extent, in initiating and/or maintaining the respiratory and cardiovascular responses that occur in fish during exercise (see Kiceniuk & Jones, 1977) in order to deliver the required oxygen to the active muscles. There is, however, little or no sympathetic innervation of the heart and systemic blood vessels in elasmobranchs (Gannon, Campbell & Satchell, 1972; Opdyke, McGreehan, Messing & Opdyke, 1972), and no innervation of the branchial blood vessels at all (Metcalfe & Butler, 1984*b*). So, in these fish at least, any *adrenergic* control of the cardiovascular system during exercise will be by way of circulating catecholamines released from the chromaffin tissue.

Ever since the work of Krawkow (1913) on pike and Keys & Bateman (1932) on eels it has been known that catecholamines cause overall vasodilatation in the gills of teleost fish, a response that would seem ideally suited to the increased oxygen demand of exercise. This effect of adrenaline and noradrenaline on the branchial blood vessels has been verified for teleosts (see, for example, Ostlund & Fänge, 1962; Rankin & Maetz, 1971; Wood, 1974; Wahlqvist, 1980) and demonstrated to occur in elasmobranchs (Davies & Rankin, 1973: Metcalfe & Butler, 1984*b*). Catecholamines also have positive inotropic and chronotropic effects on the hearts of teleosts (Holmgren, 1977; Cameron & Brown, 1981) and elasmobranchs, although in the latter noradrenaline has a negative chronotropic effect (Capra & Satchell, 1977).

In 1967 it was demonstrated by Nakano & Tomlinson that plasma adrenaline and noradrenaline increased 26-fold and eightfold, respectively, in rainbow trout in response to 'physical disturbance'. This disturbance consisted of forcing the fish to swim vigorously 'by repeatedly grasping them by the tail' and the fish were close to exhaustion after 15 min of such activity. Largely as a result of their paper, however, it has been claimed that increased levels of circulating catecholamines occur during exercise in fish (elasmobranchs as well as teleosts) and that they may play an important role in the cardiovascular adjustments to exercise (Randall, 1970, 1982). Indeed, some authors have used a similar technique to that of Nakano & Tomlinson (1967) to induce activity yet have still referred to the effect of *exercise* on release of catecholamines (Opdyke, Carroll & Keller, 1982). In a recent review, though, Wood

& Perry (1985), while attributing many of the compensatory responses that occur in fishes during exercise to circulating catecholamines, point out that their evidence is 'circumstantial rather than direct' as the levels of plasma catecholamines have rarely been measured during the 'experimental treatments'.

In the present study the levels of plasma adrenaline and noradrenaline in an elasmobranch and a teleost were measured during different degrees of activity: at rest; while swimming either spontaneously or at different speeds in a water channel; and during repeated burst swimming. These levels of catecholamines were then compared with the concentrations used in other studies to produce changes in branchial blood flow, or to produce effects on gas transfer in isolated head preparations, and with the doses used to produce effects on gas transport in the whole animal.

MATERIALS AND METHODS

Data were obtained from eight lesser spotted dogfish, *Scyliorhinus canicula*, of either sex and of mean mass (±S.E. of mean) 0·79 ± 0·024 kg, and from 25 female rainbow trout, *Salmo gairdneri*, of mean mass 0·41 ± 0·015 kg and of mean length 33 ± 0·7 cm. The dogfish were obtained either from the Plymouth laboratories of the Marine Biological Association of the UK or from the Marine Biology Laboratory, UCNW, Menai Bridge. They were transported in oxygenated sea water to aquaria in Birmingham where they were held in aerated, recirculated sea water, maintained at 15°C, for at least 2 weeks prior to experimentation. They were fed periodically on sprats obtained from a local fishmonger. The rainbow trout were obtained from Bibury Trout Farm, Gloucestershire, and transported in oxygenated water to aquaria in Birmingham where they were held in aerated, dechlorinated Birmingham tap water maintained at 12°C for at least 2 weeks prior to experimentation. They were fed once a day on trout pellets.

Attempts to train the dogfish to swim in a water channel, similar in design to that described by Johnston & Moon (1980), were unsuccessful. They merely angled their pectoral fins so that the water flowing over them generated a downward force, thus enabling the fish to remain motionless on the bottom. Even placement beneath the fish of a mesh inclined at an angle to the water flow was not effective. It was decided, therefore, to record data from dogfish during bouts of spontaneous swimming activity which occur predominantly at night (Metcalfe & Butler, 1984*a*). The ventral aorta was cannulated under MS 222 anaesthesia using a modification of the method described by Smith & Bell (1964). A length of polyethylene tubing (i.d. 0·76 mm, o.d. 1·22 mm) was positioned in the blood vessel with the aid of a stainless steel wire sharpened to a point at the end (Soivio, Nyholm & Westman, 1975). The cannula was led out through the spiracle and the fish left at least 24 h in an actograph (Metcalfe & Butler, 1984*a*) before measurements were taken. It was possible to take a mixed venous blood sample *via* this cannula in resting fish and immediately after periods of spontaneous swimming activity, as indicated by the actograph. Unfortunately, there was so much movement artefact on the pressure trace during swimming that it was not possible to record heart rate or blood pressure.

The rainbow trout would swim in the water channel. Water velocity could be varied between 0 and 0·9 m s^{-1} and was measured by a Braystoke BFM 002 current flowmeter. The dorsal aorta of the fish was cannulated under MS 222 anaesthesia, as described above for the dogfish. Each fish was left in the water channel overnight to recover from the anaesthesia and handling and swam for 30 min at approximately 1 body length per second (1 L s^{-1}) on 3–4 occasions during the next 2 days. If the fish rested against the back grid during the swimming bouts it was encouraged to swim again by gently tapping the grid. Covering the anterior portion of the swimming chamber while brightly illuminating the rear also encouraged the fish to continue swimming. After each swimming period the fish was left for at least 6 h at rest in the water channel. The dechlorinated water in the channel was kept well-aerated and at 12 °C. On the fourth day, resting values of the measured variables were taken before the water flow in the channel was increased, either to a velocity approximately equivalent to 1 L s^{-1} and left there for 30 min or progressively at increments of 10 cm s^{-1} every 10 min until the fish ceased to swim and rested against the posterior grid. The only attempt that was made to encourage the fish to swim during the latter experiment was to reduce the water velocity slightly after the fish had stopped swimming. Invariably it began to swim again and the water velocity was then returned to its former value. This usually gave sufficient time for a blood sample (approximately 1·2 ml) to be taken before the fish stopped swimming again. The fish performed little or no burst swimming during this procedure and apparent exhaustion occurred at approximately 2 L s^{-1}. This compares with a critical swimming speed of 0·5–1·5 L s^{-1} for the (somewhat larger) instrumented rainbow trout used by Kiceniuk & Jones (1977). Blood pressure was measured continuously.

At least 1 h after swimming, either spontaneously (dogfish) or in the water channel (trout), the fish was induced into burst swimming by repeatedly touching its tail for a period of 2–3 min. This was sometimes sufficient to exhaust the fish, inasmuch as touching the tail no longer caused it to swim. However, the fish were not routinely completely exhausted by this procedure. A final blood sample was then taken.

Blood pressure was measured in the trout by a Druck pressure transducer and recorded on an Ormed pen recorder writing on rectilinear coordinates. Heart rate could be determined from the traces. Partial pressures of oxygen in the blood and in water and pH in trout blood were measured by an appropriate Radiometer electrode and a PHM 71 blood gas analyser. Haematocrit was determined with a Hawksley microhaematocrit centrifuge. Plasma lactate was assayed enzymatically using a Sigma kit (no. 826-UV) and a Beckman 25 spectrophotometer, measuring at 340 nm.

The levels of adrenaline and noradrenaline in plasma were measured using reverse phase, ion-pair, high-performance liquid chromatography with electrochemical detection from 500 μl plasma samples following extraction of the catecholamines with alumina (Hallman, Farnebo, Hamberger & Jonsson, 1978). Plasma samples were obtained from approximately 800 μl of whole fish blood following 1–2 min centrifugation (Beckman microfuge B) to precipitate the red blood cells. Plasma samples were quickly frozen and kept at below −20 °C for no longer than 7 weeks before analysis (Butler, Taylor & Davison, 1979).

Subsequently 250 μl of Tris/EDTA buffer (1·5 mol l^{-1} Tris, 0·48 mol l^{-1} EDTA, pH 8·6) and 26 mg of acid-washed alumina (AAO, Bioanalytical Systems) were added to each 500 μl plasma sample. The mix was briefly vortex-mixed for 30–40 s and then gently shaken for 5 min. The mix was centrifuged for 1 min to precipitate the AAO and the supernatant aspirated off. The AAO was washed twice, each time with 500 μl of 1/100 Tris buffer, gently shaken, centrifuged and the supernatant aspirated off. Finally the catecholamines were eluted in 300 μl of perchloric acid/EDTA (0·1 mol l^{-1} $HClO_4$, 1·0 mmol l^{-1} EDTA). This mix was briefly vortex-mixed, shaken for 5 min and centrifuged. 100 μl of the eluent was injected onto the analytical column. Recovery was calculated using paired plasma samples. One sample of each pair received 50 pmol of catecholamine ('spiked'); the other did not ('unspiked'). Percentage recovery was calculated as: ('spiked'–'unspiked'/50)×100. Recovery was always better than 75%.

Separation of the catecholamines in the perchloric acid eluent was performed on a reverse phase, ion-pair liquid chromatography column (Ultrasphere IP, 250 mm × 4·66 mm) using an aqueous mobile phase (90% 0·1 mol l^{-1} KH_2PO_4, pH 3·0, containing 37 mg EDTA and 47 mg sodium octyl sulphonic acid per litre, 10% methanol) pumped (Altex 110A) at a flow rate of 1·2 ml min^{-1}. Catecholamines were measured using electrochemical detection (Bioanalytical Systems LC-4A) at +0·72 V using a carbon paste working electrode. The output from the detector was displayed on a chart recorder (Gilson). This system was sensitive enough to detect catecholamine levels as low as $1–2\times10^{-9}$ mol l^{-1}.

Mean values are expressed ±S.E. of mean and compared using a paired *t*-test (Bailey, 1981). The term 'significant' refers to the 95% confidence level.

RESULTS

Mean values of all the measured variables are given in Table 1 and changes in the levels of plasma catecholamines are illustrated in Fig. 1.

Rest

The resting values from the two groups of trout (i.e. those swimming at 1 L s^{-1} and those swimming to apparent exhaustion) were not significantly different from each other, so they have been combined. Noradrenaline was the predominant catecholamine in the blood of both species of fish at a concentration of approximately 10^{-8} mol l^{-1}. In resting dogfish the concentration of adrenaline was 42% that of noradrenaline, whereas in trout it was 14% of the level for noradrenaline. In both animals, plasma lactate was at a concentration of approximately 10^{-3} mol l^{-1}.

Exercise

During spontaneous exercise in the dogfish, which lasted for a mean duration of 7·5 ± 2·5 min, noradrenaline increased to 2·3 times the resting value while adrenaline rose to 3·3 times resting. In trout swimming at 1 L s^{-1} for 30 min there were reductions in plasma adrenaline and noradrenaline to approximately 25% of their

resting values. In neither species was there a significant change in the level of plasma lactate under these swimming conditions. In trout there were no significant changes in arterial pH and dorsal aortic blood pressure but there were significant increases in arterial P_{O_2} and in the heart rate. When trout swam to apparent exhaustion, at approximately $2\,L\,s^{-1}$, there was a 2·2-fold increase in plasma noradrenaline and a tenfold increase in plasma adrenaline above the resting values. The levels reached when trout swam to apparent exhaustion were similar to those recorded in spontaneously swimming dogfish. Plasma lactate, however, was 2·6 times the resting value in trout swimming to apparent exhaustion, although arterial pH was similar to the resting value. This could be, at least in part, the result of a respiratory alkalosis counteracting the potential metabolic acidosis. Arterial P_{O_2} was similar to that recorded in fish swimming steadily at $1\,L\,s^{-1}$, whereas heart rate was slightly (but not

Table 1. *Mean values of measured variables (±S.E. of mean) in dogfish and trout at rest, during various forms of continuous swimming activity and immediately after repeated burst swimming for a period of 2–3 min*

	DOGFISH		
	Rest	Spontaneous swimming for 7·5 ± 2·5 min	Repeated burst swimming for 2–3 min
Plasma noradrenaline (10^{-9} mol l^{-1})	14·0 ± 4·6 (8)	32·5 ± 11·9 (8)	96·5 ± 38 (6)
Plasma adrenaline (10^{-9} mol l^{-1})	5·9 ± 1·2 (8)	19·3 ± 6·8 (8)	96·3 ± 28 (6)
Plasma lactate (10^{-3} mol l^{-1})	1·3 ± 0·7 (8)	1·4 ± 0·3 (8)	—
Haematocrit (%)	19·7 ± 1·2 (8)	19·9 ± 1·1 (8)	—

	TROUT			
		In a water channel		
	Rest	Steady swimming at approximately $1\,L\,s^{-1}$	Swimming to apparent exhaustion at approximately $2\,L\,s^{-1}$	Repeated burst swimming for 2–3 min
Plasma noradrenaline (10^{-9} mol l^{-1})	10·2 ± 2·4 (20)	2·5 ± 2 (13)	22·8 ± 6·1 (7)	85 ± 46 (17)
Plasma adrenaline (10^{-9} mol l^{-1})	1·4 ± 0·5 (20)	0·3 ± 0·7 (13)	14·4 ± 3·3 (7)	212 ± 89 (17)
Plasma lactate (10^{-3} mol l^{-1})	1·0 ± 0·2 (24)	1·6 ± 0·6 (13)	2·6 ± 0·7 (7)	4·4 ± 0·7 (16)
Arterial pH	7·89 ± 0·02 (12)	7·92 ± 0·03 (6)	7·84 ± 0·02 (6)	7·63 ± 0·04 (10)
Arterial P_{O_2} (kPa)	12·9 ± 0·5 (25)	15·3 ± 1·1 (13)	15·1 ± 1·1 (12)	15·5 ± 0·8 (15)
Heart rate (beats min^{-1})	56 ± 2·8 (24)	65 ± 5·0 (12)	70 ± 5·0 (12)	—
Dorsal aortic blood pressure (kPa)	3·5 ± 0·2 (25)	3·6 ± 0·2 (13)	2·8 ± 0·2 (12)	—
Haematocrit (%)	19·1 ± 1·2 (25)	16·2 ± 1·8 (13)	16·8 ± 1·6 (12)	—

Figures in parentheses give numbers of animals used.

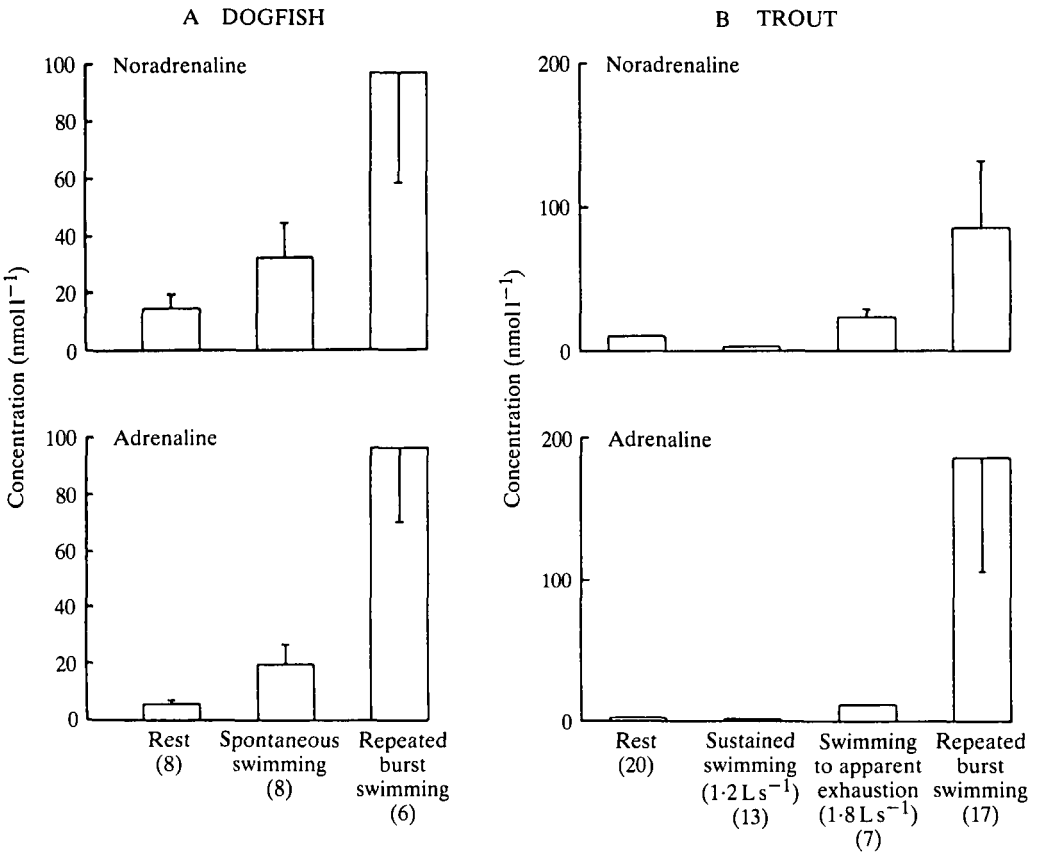

Fig. 1. Mean values (+ or − S.E. of mean) of plasma noradrenaline and adrenaline in the lesser spotted dogfish and rainbow trout at rest, while swimming spontaneously (dogfish) or at different velocities in a water channel (trout), and as a result of repeated burst swimming for 2–3 min. Number of animals used is given in parentheses beneath each histogram.

significantly) higher and dorsal aortic blood pressure was significantly lower. Haematocrit was some 12–15% below the resting value in both groups of swimming trout whereas in spontaneously swimming dogfish haematocrit was the same as in resting fish.

It was only when the fish were close to physical exhaustion as a result of repeated burst swimming that plasma catecholamines increased by any substantial amount. In dogfish, this increase was to approximately seven and 16 times the resting value for noradrenaline and adrenaline, respectively. In trout, noradrenaline increased to eight times the resting value whereas adrenaline increased to an impressive 150 times resting. The highest levels of plasma catecholamines were recorded in one particular trout. Plasma adrenaline reached $1{\cdot}2\times10^{-6}$ mol l^{-1} and noradrenaline was $0{\cdot}8\times10^{-6}$ mol l^{-1} after repeated burst swimming. In trout close to physical exhaustion, plasma lactate was 4·4 times the resting value and arterial pH was significantly below the resting value, indicating an uncompensated metabolic acidosis. Arterial P_{O_2}, however, was significantly above the resting value and similar to

the values recorded during steady swimming at $1\,L\,s^{-1}$ and when swimming to apparent exhaustion at approximately $2\,L\,s^{-1}$.

DISCUSSION

When trying to measure the levels of hormones that may be released in response to exercise, it is important that the resting animal is as unstressed as possible. The resting levels of plasma adrenaline and noradrenaline found here for the dogfish are similar to those reported by Opdyke *et al.* (1982) but are somewhat lower than those reported previously for fish left 72 h after implantation of the ventral aortic catheter (Butler *et al.* 1979). This may relate to the less traumatic method used for inserting the catheter in the present experiments. In resting trout, the observed level of plasma adrenaline is similar to that reported by Primmett, Randall, Mazeaud & Boutilier (1986) but somewhat lower than that reported by Woodward (1982). On the other hand, noradrenaline is several times higher than that measured by Woodward and by Primmett *et al.*

It is interesting to note that both adrenaline and noradrenaline decreased from the resting value in trout swimming at $1\,L\,s^{-1}$ for 30 min. This could be the result of reduced production of these hormones during sustained swimming (i.e. the fish were less stressed) and/or of increased removal of the hormones from the blood, particularly by the gills. The catecholamines were measured in blood from the dorsal aorta of trout in the present study, and it has recently been demonstrated for rainbow trout (Nekvasil & Olson, 1985) and for Atlantic cod (Ungell, 1985) that the gills are able to remove noradrenaline from the blood (to a greater extent than adrenaline in cod and apparently selectively in trout). As both adrenaline and noradrenaline decreased by similar proportions from the resting values in trout during sustained swimming, increased removal from the blood by the gills would not seem to be an adequate explanation. Unfortunately, we were not able successfully to cannulate the ventral aorta in the trout or the dorsal aorta in the dogfish using techniques involving little or no surgery, so it was not possible to determine whether differences in concentrations of catecholamines do exist in venous and arterial blood and whether these concentrations vary independently during exercise. This is clearly a point worthy of further study.

In both species of fish, two blood samples were taken before the final procedure, amounting to less than 2 ml for the dogfish and approximately 2·5 ml for the trout. If blood volume/body weight ratio for dogfish is 7% (Satchell, 1971) then no more than 4% of total blood volume was removed before the final procedure and this would not be expected to have any significant effect on haematocrit and hence on oxygen-carrying capacity of the blood. In trout, blood volume is approximately 5·6% of body weight (Milligan & Wood, 1982) so that about 12% of total blood volume was removed before the final procedure. This could well, therefore, have had a significant effect on haematocrit and on oxygen-carrying capacity of the blood in these fish (cf. Soivio *et al.* 1975). The relatively large amount of blood removed from the trout could, therefore, at least partly explain the reduced haematocrit during

swimming in both groups of these fish, a trend which was not seen in the larger fish used by Kiceniuk & Jones (1977).

Resting plasma lactate values were similar in both species to those measured by other authors (Heisler, 1984). Arterial P_{O_2} and haematocrit of resting fish were somewhat less than those measured by Kiceniuk & Jones (1977) for larger rainbow trout, but were almost identical to those measured by Primmett *et al.* (1986) for fish of similar size to those used in the present study. The fish used were, therefore, in similar physiological condition to those used by other groups of workers.

The levels of catecholamines measured during the different forms of exercise can be related to the concentrations that are required to cause physiological effects on the gills and gas transporting system of the particular species of fish. Studies on elasmobranchs are sparse. Davies & Rankin (1973) looked at the effects of adrenaline and noradrenaline on the flow of filtered saline perfusate through the isolated fifth gill of the dogfish, *Scyliorhinus canicula*. Unfortunately, the exact concentrations of the catecholamines in the perfusates are not known, but the amounts of the drugs given were in approximately 1 ml of perfusate (J. C. Rankin, personal communication). Thus, the molar concentrations were approximately 1000 times greater than the values given in the paper. On this basis, it would appear from the dose/response curves in fig. 3 of Davies & Rankin (1973) that the concentration of adrenaline present in the blood of the dogfish during spontaneous swimming in the present study has near maximum effect (80%) on the gill blood vessels. The level of noradrenaline present during spontaneous swimming in the dogfish has approximately 50% of maximum effect. Perfusion of the whole head of the rainbow trout with saline containing a colloid substitute for plasma proteins (polyvinyl pyrrolidone) enabled Wood (1974) to construct concentration/response curves for the effects of adrenaline and noradrenaline on the gill vasculature. The levels of plasma catecholamines present in rainbow trout swimming to apparent exhaustion in the water channel would have approximately 20% of maximum effect on the gill vasculature, while the levels present after repeated burst swimming would have 40% of maximum effect on the blood vessels in the gills. The apparent difference in the responses of the branchial blood vessels of the dogfish and trout to the levels of plasma catecholamines present during spontaneous or sustained swimming could well be related to the absence of adrenergic innervation in the former (Metcalfe & Butler, 1984*b*).

It is not really possible to predict the physiological significance of these levels of catecholamines from their *in vitro* effects on overall resistance of the gill vasculature. This is partly because other hormones such as cortisol are also released during sustained swimming activity in the trout (Zelnick & Goldspink, 1981), and these may potentiate the β-adrenergic effects of the catecholamines (Newsholme & Leech, 1983), and partly because we do not know the relationship between overall branchial vascular resistance and the effectiveness of gas exchange across the gills.

More detailed studies of the role of circulating catecholamines on gas transport have been made almost exclusively on teleosts and particularly on the rainbow trout. In resting trout in well-aerated water some 40% of the secondary lamellae are not

perfused with blood (Booth, 1978) and even in those lamellae that are perfused, up to 20% of the blood may be in basal channels which are probably non-respiratory because of their greater distance from the water than the other lamellar blood spaces (Tuurala, Pärt, Nikinmaa & Soivio, 1984). Adrenaline causes interlamellar recruitment *in vivo* and *in vitro* (Booth, 1979; Holbert, Boland & Olson, 1979) and an increase in perfusion pressure (which could be mediated by catecholamines) causes inter- and intralamellar recruitment *in vitro* (Farrell, Daxboeck & Randall, 1979; Farrell, Sobin, Randall & Crosby, 1980). During intralamellar recruitment a greater proportion of blood perfuses the central lamellar spaces, i.e. there is a move away from the basal channels. There may also, therefore, be a thinning of the epithelium of the lamellae, thus reducing the diffusion distance between blood and water. It has been demonstrated *in vitro* that adrenaline increases the permeability of the gills of freshwater-adapted rainbow trout to butanol and water, and it has been stated that it has the same effect on oxygen (Isaia, 1984) although direct experimental evidence is lacking.

Thus, there are a number of ways in which plasma catecholamines could affect oxygen transfer across the gills. Indeed, addition of adrenaline to the perfusate does increase $Pa_{O_2}-Pv_{O_2}$ (Wood, McMahon & McDonald, 1978) and oxygen uptake (Perry, Daxboeck & Dobson, 1985) in isolated saline-perfused head preparations of rainbow trout. The concentrations used were 10^{-5} and 10^{-7} mol l^{-1}, respectively, the latter level being almost an order of magnitude greater than that recorded in the plasma of trout swimming to apparent exhaustion, but similar to the level reached following repeated burst swimming. Similarly, the initial concentration of adrenaline required to cause an increase in the *concentration* of oxygen in the arterial blood of intact trout is approximately 10^{-7} mol l^{-1} (calculated from Nikinmaa, 1982). Even though adrenaline from a single injection disappears from the plasma very quickly (Ungell & Nilsson, 1979) there is still an effect, albeit reduced, on haematocrit 10 min after the injection (Nikinmaa, 1982).

With the possible exception, therefore, of increasing the concentration of the oxygen in arterial blood, the level of adrenaline in the plasma of rainbow trout swimming to apparent exhaustion is not likely to cause an increase in oxygen delivery to the active muscles by any of the mechanisms discussed above. However, if repeated burst swimming occurs, as a result of some sort of physical disturbance (perhaps being chased by a predator), then plasma catecholamines could well reach an effective level. This would be the classical 'fight or flight' response. During burst swimming, however, it is the white, anaerobic muscle fibres that generate the high level of power necessary for the quick darting movements. So, the functional significance of the increased levels of circulating catecholamines would not be to enhance the supply of oxygen to the locomotor muscles, at least not during the period of the exercise itself. Enhanced oxygen supply would, presumably, be of some importance after the period of burst swimming and, in addition to the mechanisms mentioned previously, hyperventilation may be sustained as a result of CO_2 retention mediated by the elevated plasma catecholamines (Wood & Perry, 1985). These authors point to other areas in which circulating catecholamines may be

important after exhaustive exercise, e.g. in controlling the release of lactate from the white muscle into the blood and in maintaining intracellular pH of the red blood cells, thus preventing a decrease in oxygen affinity of the haemoglobin.

Under experimental conditions it is necessary to use some form of physical contact with the fish to induce repeated burst swimming, so it is not possible to dissociate the effect of the physical contact from the effect of the burst activity itself on the release of the catecholamines. It is clear that the levels of plasma catecholamines are quite different between fish that have refused to swim any longer in a water channel and those that have performed repeated bouts of burst swimming, and the inference is that the difference is attributable to the different type of swimming activity. There is a high level of mortality in rainbow trout within 4–8 h of experimentally induced burst swimming for a period of 6 min (Wood, Turner & Graham, 1983). This raises the questions: do such levels of mortality occur following more naturally induced burst swimming of similar duration, or indeed do fish only rarely perform such repeated bouts of burst swimming under natural conditions, for example when they are negotiating currents during spawning migration? Even then they are able to rest at will.

This study was supported by the SERC. The authors wish to thank an anonymous referee for some particularly useful comments.

REFERENCES

BAILEY, N. H. T. (1981). *Statistical Methods in Biology*, 2nd edn. London: Hodder & Stoughton.

BOOTH, J. H. (1978). The distribution of blood flow in the gills of fish: application of a new technique to rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **73**, 119–129.

BOOTH, J. H. (1979). The effects of oxygen supply, epinephrine, and acetylcholine on the distribution of blood flow in trout gills. *J. exp. Biol.* **83**, 31–39.

BUTLER, P. J., TAYLOR, E. W. & DAVISON, W. (1979). The effect of long term, moderate hypoxia on acid–base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula*. *J. comp. Physiol.* **132**, 297–303.

CAMERON, J. S. & BROWN, S. E. (1981). Adrenergic and cholinergic responses of the isolated heart of the goldfish, *Carassius auratus*. *Comp. Biochem. Physiol.* **70**, 109–115.

CAPRA, M. F. & SATCHELL, G. H. (1977). Adrenergic and cholinergic responses of the isolated saline-perfused heart of the elasmobranch fish *Squalus acanthias*. *Gen. Pharmac.* **8**, 59–65.

DAVIES, D. T. & RANKIN, J. C. (1973). Adrenergic receptors and vascular responses to catecholamines of perfused dogfish gills. *Comp. gen. Pharmac.* **4**, 139–147.

FARRELL, A. P., DAXBOECK, C. & RANDALL, D. J. (1979). The effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. *J. comp. Physiol.* **133**, 233–240.

FARRELL, A. P., SOBIN, S. S., RANDALL, D. J. & CROSBY, S. (1980). Intralamellar blood flow patterns in fish gills. *Am. J. Physiol.* **239** (*Reg. int. comp. Physiol.* **8**), R428–R436.

GANNON, B. J., CAMPBELL, G. D. & SATCHELL, G. H. (1972). Monoamine storage in relation to cardiac regulation in the Port Jackson shark *Heterodontus portusjacksoni*. *Z. Zellforsch. mikrosk. Anat.* **131**, 437–450.

HALLMAN, H., FARNEBO, L., HAMBERGER, B. & JONSSON, G. (1978). A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. *Life Sci.* **23**, 1049–1052.

HEISLER, N. (1984). Acid–base regulation in fishes. In *Fish Physiology*, vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 315–401. New York: Academic Press.

HOLBERT, P. W., BOLAND, E. J. & OLSON, K. R. (1979). The effect of epinephrine and acetylcholine on the distribution of red cells within the gills of the Channel catfish (*Ictalurus punctatus*). *J. exp. Biol.* **79**, 135–146.

HOLMGREN, S. (1977). Regulation of the heart of a teleost, *Gadus morhua*, by autonomic nerves and circulating catecholamines. *Acta physiol. scand.* **99**, 62–74.

ISAIA, J. (1984). Water and nonelectrolyte permeation. In *Fish Physiology*, vol. XB (ed. W. S. Hoar & D. J. Randall), pp. 1–38. New York: Academic Press.

JOHNSTON, I. A. & MOON, T. W. (1980). Endurance exercise training in the fast and slow muscles of a teleost fish (*Pollachius virens*). *J. comp. Physiol.* **135**, 147–156.

KEYS, A. & BATEMAN, J. B. (1932). Branchial responses to adrenaline and pitressin in the eel. *Biol. Bull. mar. biol. Lab., Woods Hole* **63**, 327–336.

KICENIUK, J. W. & JONES, D. R. (1977). The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. *J. exp. Biol.* **69**, 247–260.

KRAWKOW, N. P. (1913). Über die Wirkung von Giften auf die Gefässe isolierter Fischkiemen. *Arch. ges. Physiol.* **151**, 583–603.

METCALFE, J. D. & BUTLER, P. J. (1984*a*). Changes in activity and ventilation in response to hypoxia in unrestrained, unoperated dogfish (*Scyliorhinus canicula* L.). *J. exp. Biol.* **108**, 411–418.

METCALFE, J. D. & BUTLER, P. J. (1984*b*). On the nervous regulation of gill blood flow in the dogfish (*Scyliorhinus canicula*). *J. exp. Biol.* **113**, 253–267.

MILLIGAN, C. L. & WOOD, C. M. (1982). Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **99**, 397–415.

NAKANO, T. & TOMLINSON, N. (1967). Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance. *J. Fish. Res. Bd Can.* **24**, 1701–1715.

NEKVASIL, N. P. & OLSON, K. R. (1985). Localization of ^{3}H-norepinephrine binding sites in the trout gill. *J. exp. Zool.* **235**, 309–313.

NEWSHOLME, E. A. & LEECH, A. R. (1983). *Biochemistry for the Medical Sciences*. Wiley: Chichester. 737 pp.

NIKINMAA, M. (1982). The effects of adrenaline on the oxygen transport properties of *Salmo gairdneri* blood. *Comp. Biochem. Physiol.* **71**, 353–356.

OPDYKE, D. F., CARROLL, R. G. & KELLER, N. E. (1982). Catecholamine release and blood pressure changes induced by exercise in dogfish. *Am. J. Physiol.* **242** (*Reg. int. comp. Physiol.* **11**), R306–R310.

OPDYKE, D. F., MCGREEHAN, J. R., MESSING, S. & OPDYKE, N. E. (1972). Cardiovascular responses to spinal cord stimulation and autonomically active drugs in *Squalus acanthias*. *Comp. Biochem. Physiol.* **42**, 611–620.

OSTLUND, E. & FÄNGE, R. (1962). Vasodilation by adrenaline and noradrenaline, and the effects of some other substances on perfused fish gills. *Comp. Biochem. Physiol.* **5**, 307–309.

PERRY, S. F., DAXBOECK, C. & DOBSON, G. P. (1985). The effect of perfusion flow rate and adrenergic stimulation on oxygen transfer in the isolated, saline-perfused head of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **116**, 251–269.

PIIPER, J., MEYER, M., WORTH, H. & WILLMER, H. (1977). Respiration and circulation during swimming activity in the dogfish *Scyliorhinus stellaris*. *Respir. Physiol.* **30**, 211–239.

PRIMMETT, D. R. N., RANDALL, D. J., MAZEAUD, M. & BOUTILIER, R. G. (1986). The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. *J. exp. Biol.* **122**, 139–148.

RANDALL, D. J. (1970). The circulatory system. In *Fish Physiology,* vol. IV (ed. W. S. Hoar & D. J. Randall), pp. 133–172. New York: Academic Press.

RANDALL, D. J. (1982). The control of respiration and circulation in fish during exercise and hypoxia. *J. exp. Biol.* **100**, 275–288.

RANKIN, J. C. & MAETZ, J. (1971). A perfused teleostean gill preparation: vascular actions of neurohypophysial hormones and catecholamines. *J. Endocr.* **51**, 621–635.

SATCHELL, G. H. (1971). *Circulation in Fishes*. Cambridge: Cambridge University Press.

SMITH, L. S. & BELL, G. R. (1964). A technique for prolonged blood sampling in free-swimming salmon. *J. Fish. Res. Bd Can.* **21**, 711–717.

SOIVIO, A., NYHOLM, K. & WESTMAN, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *J. exp. Biol.* **62**, 207–217.

TUURALA, H., PÄRT, P., NIKINMAA, M. & SOIVIO, A. (1984). The basal channels of secondary lamellae in *Salmo gairdneri* gills – a non-respiratory shunt. *Comp. Biochem. Physiol.* **79**, 35–39.

UNGELL, A.-L. (1985). Accumulation of intra-arterially administered [^{3}H]adrenaline and [^{3}H]noradrenaline in various tissues of the Atlantic cod, *Gadus morhua*. *Comp. Biochem. Physiol.* **81**, 25–28.

UNGELL, A.-L. & NILSSON, S. (1979). Metabolic degradation of [^{3}H]-adrenaline in the Atlantic cod, *Gadus morhua*. *Comp. Biochem. Physiol.* **64**, 137–141.

WAHLQVIST, I. (1980). Effects of catecholamines on isolated systemic and branchial vascular beds of the cod, *Gadus morhua*. *J. comp. Physiol.* **137**, 139–143.

WHIPP, B. J. & WARD, S. A. (1982). Cardiopulmonary coupling during exercise. *J. exp. Biol.* **100**, 175–193.

WHIPP, B. J. & WARD, S. A. (1985). Cardiopulmonary system responses to muscular exercise in man. In *Circulation, Respiration and Metabolism: Current Comparative Approaches* (ed. R. Gilles), pp. 64–80. Heidelberg: Springer-Verlag.

WOOD, C. M. (1974). A critical examination of the physical and adrenergic factors affecting blood flow through the gills of the rainbow trout. *J. exp. Biol.* **60**, 241–265.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1978). Oxygen exchange and vascular resistance in the totally perfused rainbow trout . *Am. J. Physiol.* **234** (*Reg. int. comp. Physiol.* **3**), R201–208.

WOOD, C. M. & PERRY, S. F. (1985). Respiratory, circulatory, and metabolic adjustments to exercise in fish. In *Circulation, Respiration and Metabolism: Current Comparative Approaches* (ed. R. Gilles), pp. 2–22. Heidelberg: Springer-Verlag.

WOOD, C. M., TURNER, J. D. & GRAHAM, M. S. (1983). Why do fish die after severe exercise? *J. Fish. Biol.* **22**, 189–201.

WOODWARD, J. J. (1982). Plasma catecholamines in resting rainbow trout, *Salmo gairdneri* Richardson, by high pressure liquid chromatography. *J. Fish Biol.* **21**, 429–432.

ZELNICK, P. R. & GOLDSPINK, G. (1981). The effect of exercise on plasma cortisol and blood sugar levels in the rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **19**, 37–43.