THE RELATIONSHIP BETWEEN INTRACELLULAR pH AND SWIMMING PERFORMANCE OF BROWN TROUT EXPOSED TO NEUTRAL AND SUBLETHAL pH

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Summary

Adult brown trout were acclimated for 2–4 weeks to artificial soft water ([Ca^{2+}] $25 \mu mol\,l^{-1}$) at neutral pH and at summer (15°C) temperature. During this period they swam against a current of approximately $0.25\,m\,s^{-1}$. They then had their dorsal aorta cannulated and were exposed to neutral or sublethal pH (4.5) for 4 days in still water.

After 4 days of exposure to sublethal pH, critical swimming speed (U_{crit}) was 35% lower than that for fish at neutral pH. There were significant increases in arterial P_{CO_2} and in blood lactate concentrations at U_{crit} compared with the values in resting fish at neutral pH and these led to significant reductions in plasma pH. There were no such changes in fish at sublethal pH. There were no significant changes in intracellular pH (pHi) of red blood cells at U_{crit}, probably as a result of increases in the levels of plasma catecholamines. There were significant reductions in pHi of red and white muscle fibres at U_{crit}. It is argued that these values were not as low in the white fibres as those seen in previous studies after fish have been chased to exhaustion and, therefore, that the fish in the present study were not completely exhausted, although they would no longer swim at a steady speed. As pHi of the red muscle was the same at U_{crit} for fish at neutral and at sublethal pH, it is suggested that U_{crit} (fatigue) coincides with a particular pHi of the red muscles and possible mechanisms are discussed.

Introduction

There is increasing evidence that acute exposure to low environmental pH has a detrimental effect on the swimming performance of salmonid fish. Although Waiwood and Beamish (1978) found that exposure to water at pH6 had no effect on the swimming performance of fingerling rainbow trout, a more thorough study by Graham and Wood (1981) found that critical swimming speed (U_{crit}; Brett, 1964) was significantly depressed at pH values below 4.6 in soft water. The fish had not been acclimated to the test pH and no physiological measurements were made, but the authors suggested that impairment of gas exchange and/or of oxygen transport were the major factors affecting the fishes' swimming performance. More recently, Ye and Randall (1991) found that 24h of exposure to high (>pH9) or low pH (<pH6) reduced swimming performance in adult

rainbow trout and Ye *et al.* (1991) suggested, but did not demonstrate, that exposure to low pH reduced the oxygen content of arterial blood and, therefore, that oxygen transport capacity was impaired. This contention was not supported by a study on brown trout in which there was no reduction in arterial oxygen content (Ca_{O_2}) in fish exposed to sublethal pH for 4 days: in fact at 15°C, there was a significant increase in Ca_{O_2} as the result of a rise in haemoglobin concentration (Butler *et al.* 1992). There were also no signs of any impairment of gas exchange. There were, however, signs of haemoconcentration in these fish and the authors did not rule out the possibility of an increase in blood viscosity which could have had subtle, adverse effects on the local circulation and thus impaired oxygen transport to the locomotor muscles. In fact, McDonald *et al.* (1980) reported an increase in blood lactate concentration in rainbow trout after exposure to low pH, which could be indicative of impaired oxygen transport. Thus, upon exposure to low pH, any impairment of oxygen transport resulting from haemoconcentration, which is itself the result of disturbances in ionoregulation (McDonald *et al.* 1980; Milligan and Wood, 1982), is likely to lead to a reduction in swimming performance (Butler *et al.* 1992).

There is much debate as to the possible causes of fatigue during exercise in vertebrates and one possible cause is a reduction in intracellular pH (pHi) (Metzger and Fitts, 1987). Jones and Randall (1978) discuss the possible causes of fatigue in fish, where problems associated with ionoregulation may be important (see also Gonzalez and McDonald, 1992). They dismiss the role of pHi in swimming failure in fish 'in the absence of a profligate bout of burst swimming'. This is probably true if they were referring to *complete* swimming failure. However, fish swum to U_{crit} are still able to undergo burst activity: they are merely unable or unwilling to swim at a steady speed. Their inability to maintain a constant speed for a set time could well be the result of a critical pHi having been reached in the red muscle. The fact that trout have a lower U_{crit} in acid water than in neutral water could indicate that protons have an inhibitory effect on exercise (Nelson, 1989) and thus provides an ideal model for testing the above hypothesis.

The purpose of the present study was, therefore, to determine pHi of locomotor (and cardiac) muscles in resting fish and in fish at U_{crit} acclimated to water at either neutral or at sublethal pH. Also, as circulating levels of cortisol and catecholamines increase during swimming at or near U_{crit} in trout (Zelnick and Goldspink, 1981; Butler *et al.* 1986) and as catecholamines, at least, may enhance swimming performance (Butler *et al.* 1989; Johnson *et al.* 1991), the concentrations of these two hormones were also determined.

Materials and methods

The animals and their holding conditions were as described by Butler *et al.* 1992), except that in the present experiments only summer (June to mid-September) fish kept at 15°C were used. Briefly, brown trout, *Salmo trutta* (L.) (total length 30–46cm, mass 320–520g), were obtained from the Leadmill trout farm, Hathersage, Derbyshire, and kept for 2–4 weeks in large (1400l) circular glass fibre tanks through which dechlorinated Birmingham tapwater flowed at a rate of $120 l h^{-1}$. The water was aerated vigorously and circulated around the tank using a pump and spray bar which produced jets of water that

were nearly horizontal when they hit the water surface. The fish were provided with plastic tubes (10cm diameter, 55cm length) suspended in mid water, in which they could position themselves. Because the water was circulating around the tank (at approximately $0.25\,\mathrm{ms}^{-1}$) and through the tubes, the fish had to swim to maintain position.

Following the initial acclimation period, the fish were transferred into a similar tank containing artificial lakewater (Dalziel *et al.* 1985), also circulating at $0.25\,\mathrm{ms}^{-1}$. The concentration of Ca^{2+} ($25\,\mu\mathrm{mol}\,l^{-1}$) is similar to that found in some areas of the UK, e.g. Galloway (Harriman *et al.* 1987), mid Wales and north Wales (Turnpenny *et al.* 1987). Titanium cooling coils and small aquarium heaters maintained the temperature of the water in the two large holding tanks at 15°C (June to mid-September, 12–16°C at the trout farm). The pH of this water was maintained at 7±0.1 (range). The fish were kept under these conditions for a further 2–4 weeks (see Booth *et al.* 1988) after the initial period in Birmingham tapwater. Throughout the acclimation period the fish were exposed to the natural photoperiod and were fed daily on floating pellets [Mainstream trout diet, B.P. Nutrition (U.K.) Ltd]. All uneaten pellets were removed from the tank an hour after feeding. No food was given the day before transfer of the fish to an experimental tank, or during the experimental period. Sublethal pH for the fish was determined as described by Butler *et al.* (1992) and was pH4.5.

After acclimation, fish were anaesthetised in buffered MS222 and the dorsal aorta was cannulated. The animals were placed into a Blazka-type water channel and left to recover for 2 days. They were then left for a further 4 days at neutral pH or at sublethal pH. Experiments were performed alternately at neutral and sublethal pH to avoid seasonal bias. On the fifth day (third experimental day) the fish were infused with $0.22\mathrm{MBqkg}^{-1}$ ($6\,\mu\mathrm{Cikg}^{-1}$) $[^{14}\mathrm{C}]\mathrm{DMO}$ and $0.74\mathrm{MBqkg}^{-1}$ ($20\,\mu\mathrm{Cikg}^{-1}$) $[^{3}\mathrm{H}]$mannitol. At the end of the exposure period (at least 18h after injection of $[^{14}\mathrm{C}]\mathrm{DMO}$ and $[^{3}\mathrm{H}]$mannitol), data were collected from resting fish or from fish that had been swum up to their U_{crit} (see Butler *et al.* 1992, for details). 2–3ml of arterial blood was removed from the dorsal aorta for the determination of arterial plasma pH using a Radiometer BMS3 blood micro system and a PHM 73 pH/blood gas monitor, plasma carbon dioxide content ($Ca_{\mathrm{CO_2}}$) using a Corning 965 CO_2 analyser and the intracellular pH (pHi) of red blood cells (RBCs) using the freeze/thaw method (Zeidler and Kim, 1977). $Pa_{\mathrm{CO_2}}$ was determined from the Henderson–Hasselbalch equation, for which αCO_2 (solubility of CO_2 in plasma) and pK^1 were obtained from the formulae presented by Boutilier *et al.* (1984). pHi of RBCs, cardiac muscle, red and white skeletal muscle were determined by the DMO method (Waddell and Butler, 1959; Heisler, 1975; Milligan and Wood, 1985, 1986*a,b*), with $[^{3}\mathrm{H}]$mannitol being used to determine extracellular fluid volume (ECFV).

Following the removal of the arterial blood sample, the fish was carefully removed from the swim tube. It was found that, by placing a large piece of filter foam over the animal before such removal, it did not struggle. The fish was then immediately stunned and decapitated and the heart removed. The ventricle was divided into three 100–150mg portions which were lightly blotted to remove any blood and placed into pre-weighed Eppendorf tubes. Similar-sized samples of red and white muscle were similarly prepared, care being taken to remove the thin layer of fat and any white muscle fibres from the red muscle and not to use the superficial layer of dorsal white muscle as it tends to be fatty.

The muscle samples were taken from the same position on the fish each time (red, anterior; white, anterior, dorsal). These muscle samples, together with 50–100 μl samples of plasma and red blood cells, were dried to constant weight over a period of 7–10 days. Samples were processed in a biological oxidiser (OX400, R. J. Harvey Instrument Corporation, USA) to release and separate the [^{14}C] and [^{3}H] isotopes. Comparison of oxidised with unoxidised known standards gave recovery efficiencies between 69 and 83%. [^{14}C] and [^{3}H] activities were determined with a Beckman LS 1700 scintillation counter using 'Optisorb S' and 'Optisorb 4' (LKB Scintillation Products, UK), respectively, as scintillation solutions.

The concentrations of plasma catecholamines, noradrenaline (Nadr) and adrenaline (Adr) were determined using reverse-phase, ion-pair HPLC with electrochemical detection (Ehrenström and Johansson, 1985, 1987; Butler *et al.* 1989). Plasma cortisol concentration was measured by radioimmunoassay (Hargreaves and Ball, 1977; Kenyon *et al.* 1985). Water in the holding tanks and water channel was monitored weekly for P_{O_2}, CO_2 content and total ammonia content (Verdouw *et al.* 1978). The first was always greater than 20kPa, the second less than $0.1 \text{mmol} \, l^{-1}$ and the third less than $20 \, \mu\text{mol} \, l^{-1}$ in the holding tanks and less than $10 \, \mu\text{mol} \, l^{-1}$ in the water channel.

All values are given as the mean ± S.E. Between-treatment comparisons were analysed by two-way analysis of variance (ANOVA). If significant ($P<0.05$), pairwise comparisons were made with the Tukey multicomparison (honestly significant different) test.

Results

U$_{crit}$

As reported earlier (Butler *et al.* 1992), exposure to sublethal pH had significant effects on U_{crit} in these fish. At neutral pH, U_{crit} was 2.21 ± 0.08 bodylengths per second, $\text{bl} \, \text{s}^{-1}$ ($0.73\pm0.02 \, \text{ms}^{-1}$), whereas at sublethal pH it was $1.37\pm0.08 \text{bl} \, \text{s}^{-1}$ ($0.48\pm0.03 \, \text{ms}^{-1}$). N=7 and 8 respectively.

Pa_{CO_2} *and blood lactate concentration*

There was a significant increase in Pa_{CO_2} in response to swimming at neutral pH, but no effect of exposure to sublethal pH nor of swimming at sublethal pH (Fig. 1A). Blood lactate concentration in resting fish was not affected by exposure to sublethal pH. At U_{crit}, there was a significant, fourfold increase in blood lactate concentration above the resting value at neutral pH but no significant change at sublethal pH (Fig. 1B). These last two values were significantly different from one another.

Plasma and intracellular pH

There was no significant difference in any of the values of pHi of RBCs obtained by the freeze/thaw and DMO methods (Fig. 2B,C). Swimming to U_{crit} at neutral pH, but not at sublethal pH, caused a significant decrease in arterial pH. It had no effect on pHi of RBCs or on that of cardiac muscle at either environmental pH although there were significant decreases in pHi of the red and of the white muscles (Figs 2, 3).
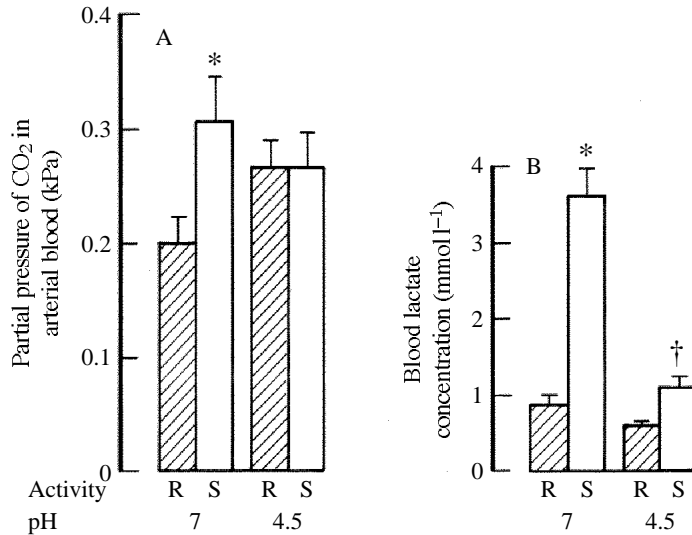
Fig. 1. Histograms showing mean values (+ S.E.) of partial pressure of CO_2 (A) and blood lactate concentration (B) in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (7) or after 4 days at sublethal pH (4.5). * indicates significant effect of swimming at U_{crit} (compared with rest) at a given pH. † indicates a significant effect of pH at a given level of activity. Seven fish were used for each treatment at pH7 and eight at pH4.5. For partial pressure of CO_2, F_{calc} pH=0.62, F_{calc} activity=4.30, F_{calc} pH×activity=3.47. For blood lactate, F_{calc} pH=53.55, F_{calc} activity=74.68, F_{calc} pH×activity=34.38. $F_{0.05(1), 1, 26}$=4.23.

Exposure to sublethal pH had no significant effects on plasma pH or on pHi of RBCs or of red, white and cardiac muscles (Figs 2B,C, 3A–C). It is interesting to note that, at U_{crit}, values of pHi in red and white muscles were similar in fish exposed to neutral pH and in those exposed to sublethal pH (Fig. 3A,B). None of the experimental variables (exercise to U_{crit}, sublethal pH) had any effect on pHi of cardiac muscle.

Intra- and extracellular fluid volumes

Values of total muscle water content, extracellular fluid volume (ECFV) and of intracellular fluid volume (ICFV) in resting fish at neutral pH are given in Table 1. Exposure to sublethal pH had no effect on ECFV or ICFV. Swimming at U_{crit} had no significant effect on ICFV or on ECFV in cardiac muscle (Fig. 4C) but in red muscle it caused a reduction in ECFV (and a rise in ICFV) at neutral pH and a rise in ECFV (together with a fall in ICFV) at sublethal pH. The values of ECFV and of ICFV at U_{crit} in red muscle from fish acclimated to neutral and to sublethal pH were significantly different from each other (Fig. 4A,D). There was a significantly higher total water content of the white muscle (795 ± 3mlkg^{-1}) in fish exposed to sublethal pH (N=8) compared with that (778 ± 5mlkg^{-1}) in fish at neutral pH (N=7). The effects of swimming at U_{crit} on ECFV and ICFV of white muscle were not so clear. Under no condition were there significant, complementary changes in both variables. At neutral pH, there was a significant increase in ICFV at U_{crit} but no change in ECFV.
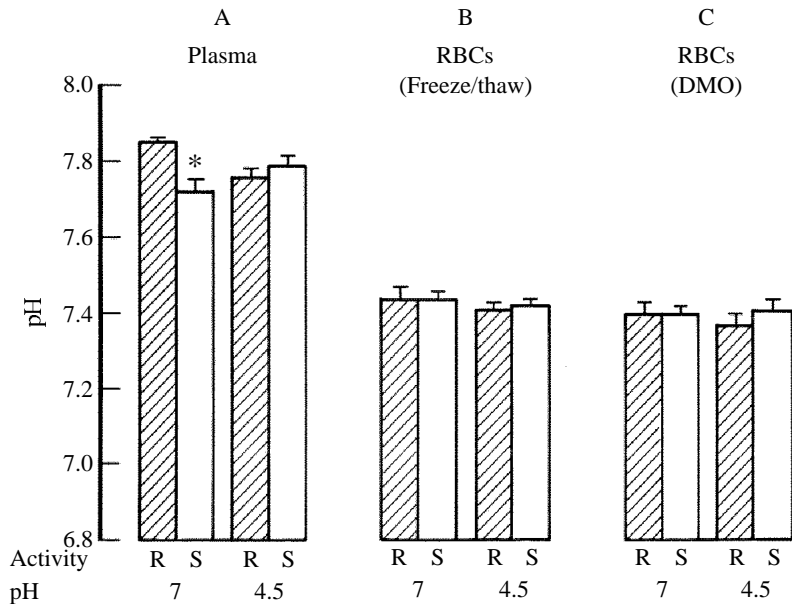
Fig. 2. Histograms showing mean values (+ S.E.) of plasma pH (A) and of intracellular pH of red blood corpuscles (RBCs) obtained by the freeze/thaw (B) and DMO (C) techniques in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (7) or after 4 days at sublethal pH (4.5). * indicates a significant effect of swimming at U_{crit} (compared with rest) at a given pH. Seven fish were used for each treatment at pH7 and eight at pH4.5. For plasma pH, F_{calc} pH=0.08, F_{calc} activity=4.69, F_{calc} pH×activity=11.98. For RBCs (freeze/thaw), F_{calc} pH=0.04, F_{calc} activity=2.40, F_{calc} pH×activity=2.81. For RBCs (DMO), F_{calc} pH=0.04, F_{calc} activity=0.59, F_{calc} pH×activity=0.84. $F_{0.05(1), 1, 26}$=4.23.

Plasma cortisol and catecholamines

Neither exposure to sublethal pH nor swimming to U_{crit} had any effect on plasma cortisol concentration (Fig. 5A). Exposure to sublethal pH had no significant effects on the concentrations of plasma Nadr and Adr levels (Fig. 5B,C), whereas swimming at U_{crit} did cause significant increases in both Nadr and Adr at both neutral and sublethal pH. Nadr level increased to approximately three times the resting values and Adr concentration increased to 10–14 times the resting values.

Discussion

Critique of the DMO method for determining pHi

The chosen method for determining pHi of the various muscles of brown trout depends on an estimate of ECFV of each muscle and, in a recent paper, Munger *et al.* (1991) have compared values of ECFV using different types of radiolabelled markers after different equilibration times. They used immature (180–320g) rainbow trout acclimated to 15°C. They concluded that [^{3}H]polyethylene glycol (PEG) is the best of the range of markers they used for [^{14}C]DMO determinations of pHi in fish. They point out, however, that the
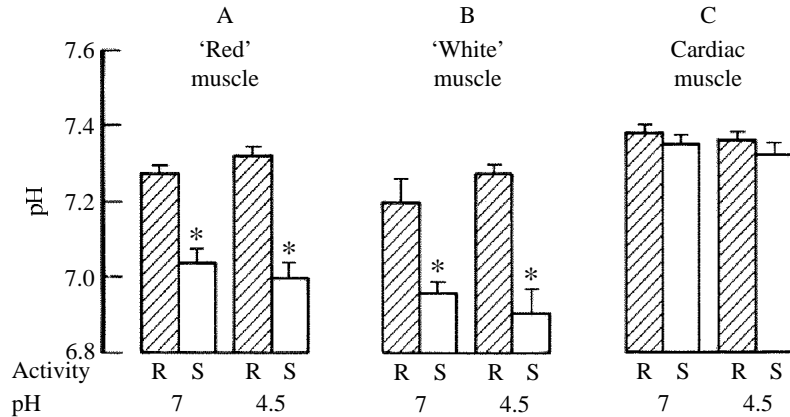
Fig. 3. Histograms showing mean values (+ S.E.) of intracellular pH of red (A), white (B) and cardiac (C) muscles in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (7) or after 4 days at sublethal pH (4.5). * indicates a significant effect of swimming at U_{crit} (compared with rest) at a given pH. Seven fish were used for each treatment at pH7 and eight at pH4.5. For 'red' muscle, F_{calc} pH=0.52, F_{calc} activity=98.49, F_{calc} pH×activity=4.62. For 'white' muscle, F_{calc} pH=0.18, F_{calc} activity=51.64, F_{calc} pH×activity=2.10. For cardiac muscle, F_{calc} pH=1.72, F_{calc} activity=2.15, F_{calc} pH×activity=0.15. $F_{0.05(1), 1, 26}$=4.23.

Table 1. *Total muscle water content, extracellular fluid volume (ECFV) and intracellular fluid volume (ICFV) of cardiac, red and white muscles from seven resting brown trout at 15°C and in water at neutral pH*

	Cardiac muscle	Red muscle	White muscle
Total muscle water	811±14	796±4	778±5
ECFV	237±14	135±6	86±7
ICFV	574±15	661±7	692±8

Values are mean ± S.E. ($mlkg^{-1}$).

60% increase in white muscle ECFV between [3H]PEG and [3H]mannitol reduced pHi by only 0.06 unit. As the estimate of ECFV in white muscle of resting trout in water at neutral pH in the present study is similar to that obtained by Munger *et al.* (1991) using [3H]mannitol, the small error indicated above may apply to the present values of pHi for white muscle. The value of ECFV obtained for red muscle of trout at neutral pH in the present study is very similar to that obtained by Munger *et al.* (1991) using [3H]PEG (and [3H]mannitol, as it happens), so the present values of pHi in red muscle should be acceptable. Milligan and Wood (1986*b*) found that the ECFV values for cardiac muscle when obtained using [3H]mannitol were unacceptably high and used inulin-derived estimates for their calculations. Although the estimate of ECFV of cardiac muscle from trout at neutral pH in the present study is some 40% greater than that obtained by Munger *et al.* (1991) using [3H]PEG, the value of pHi (7.38±0.02) is similar to that obtained by Milligan and Wood (1986*b*) from resting adult rainbow trout acclimated to 15°C (approximately 7.35).
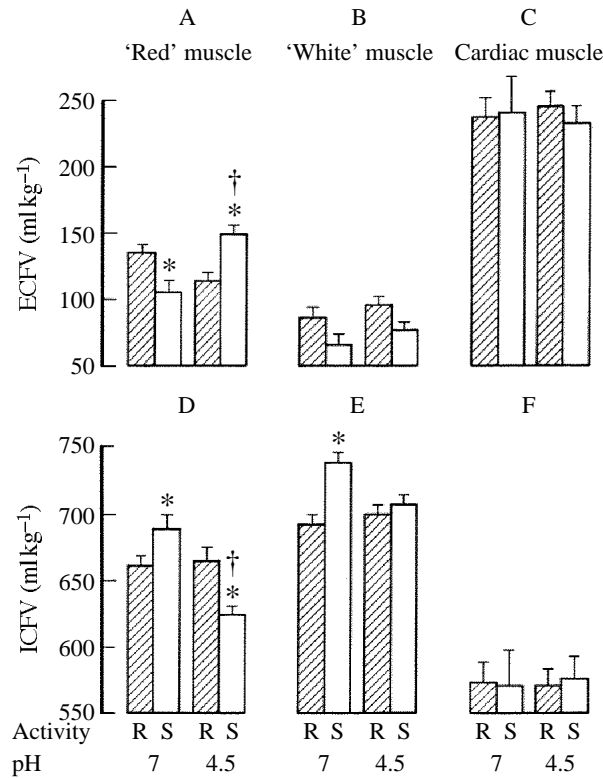
Fig. 4. Histograms showing mean values (+ S.E.) of extracellular fluid volume (ECFV) of red (A), white (B) and cardiac (C) muscles and of intracellular fluid volume (ICFV) of red (D), white (E) and cardiac (F) muscles in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (7) or after 4 days at sublethal pH (4.5). * indicates a significant effect of swimming at U_{crit} (compared with rest) at a given pH. † indicates a significant effect of pH at a given level of activity. Seven fish were used for each treatment at pH7 and eight at pH4.5. For 'red' muscle ECFV, F_{calc} pH=2.98, F_{calc} activity=0.18, F_{calc} pH×activity=26.26. For 'white' muscle ECFV, F_{calc} pH=2.43, F_{calc} activity=9.40, F_{calc} pH×activity=0.08. For 'cardiac' muscle ECFV, F_{calc} pH=0.00, F_{calc} activity=0.11, F_{calc} pH×activity=2.44. For 'red' muscle ICFV, F_{calc} pH=7.11, F_{calc} activity=61.44, F_{calc} pH×activity=4.77. For 'white' muscle ICFV, F_{calc} pH=2.80, F_{calc} activity=15.50, F_{calc} pH×activity=8.64. For cardiac muscle ICFV, F_{calc} pH=0.00, F_{calc} activity=0.00, F_{calc} pH×activity=0.03. $F_{0.05(1), 1, 26}$=4.23.

Another possible source of error of the DMO method for determining pHi in the present set of experiments is the time taken for full equilibration of DMO between the intra- and extracellular compartments. Milligan and Wood (1985) demonstrated that, provided the markers had already fully equilibrated throughout the fish, the DMO method could reliably detect changes in pHi of white muscle 15min after the onset of the transient. Milligan and Wood (1986b) then demonstrated that after 6min of exhaustive exercise of rainbow trout, pHi of white muscle may be overestimated by approximately 0.1 unit. It seems inevitable, therefore, that, for the relatively poorly perfused white muscle, pHi at U_{crit} may only be accurate in those fish that swam at the final speed for 10–15min. The shorter the time below this value, the greater could be the error. The error
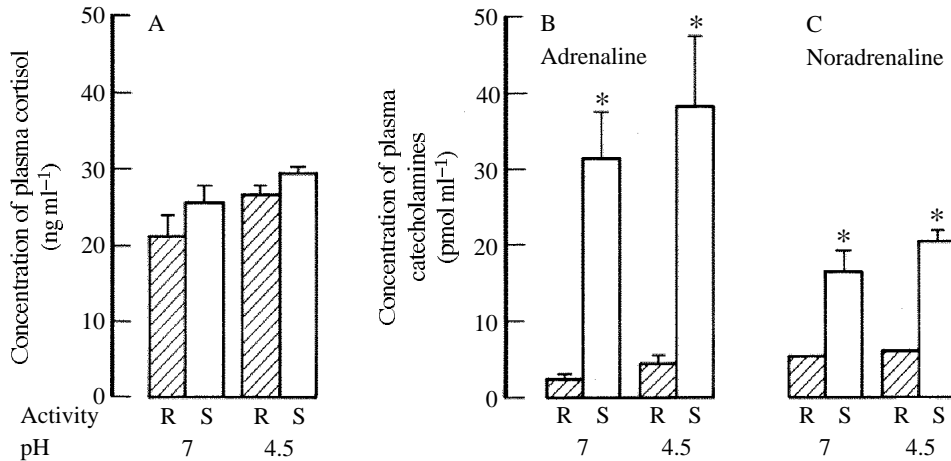
Fig. 5. Histograms showing mean values (+ S.E.) of plasma concentrations of cortisol (A), noradrenaline (B) and adrenaline (C) in adult brown trout at rest (R) and while swimming at U_{crit} (S) in soft water at neutral pH (7) or after 4 days at sublethal pH (4.5). * indicates a significant effect of swimming at U_{crit} (compared with rest) at a given pH. Seven fish were used for each treatment at pH7 and eight at pH4.5. For plasma cortisol, F_{calc} pH=6.99, F_{calc} activity=4.86, F_{calc} pH×activity=0.29. For plasma adrenaline, F_{calc} pH=0.60, F_{calc} activity=31.75, F_{calc} pH×activity=0.22. For plasma noradrenaline, F_{calc} pH=2.29, F_{calc} activity=67.81, F_{calc} pH×activity=0.96. $F_{0.05(1), 1, 26}$=4.23.

is likely to be less in the better perfused red and cardiac muscles, and for RBCs the DMO method gave accurate values of pHi (or at least, the same as those obtained by the freeze/thaw method) under all conditions.

The values of pHi in white and cardiac muscle in resting fish at 15°C are virtually identical to those obtained by Milligan and Wood (1986*b*) using the DMO technique, from resting rainbow trout at 15°C. Tang and Boutilier (1991), using the tissue homogenate technique of Pörtner *et al.* (1990), obtained a pHi value for white muscle in resting rainbow trout which is some 0.1 unit higher than that obtained in the present study. As their animals were at 10°C, a somewhat higher value might be expected (Cameron, 1984).

Exposure to sublethal pH

Exposure to sublethal pH had no effect on plasma pH, nor on pHi of RBCs and of the muscle tissues and these findings are consistent with those of Playle *et al.* (1989) and Wood (1989) for fish in very soft water ([Ca^{2+}], 0.05mequivl^{-1}). In hard water ([Ca^{2+}], 2.0mequivl^{-1}), however, there was a substantial (0.2 unit) reduction in plasma pH, significant falls in pHi of cardiac muscle and RBCs, but no change in pHi of red and white muscles (Wood, 1989). Thus, in the present experiments, the maintenance of pHi of the RBCs meant that oxygen transport was not compromised as a result of the Bohr and Root effects. It has already been demonstrated that both haemoglobin concentration, [Hb], and oxygen content of arterial blood, Ca_{O_2}, increase substantially at 15°C in trout exposed to sublethal pH (Butler *et al.* 1992). Consistent with this, and contrary to the finding of

McDonald *et al.* (1980), is the lack of increase in blood lactate concentration in response to sublethal pH. As in the present experiments, exposure to low pH in soft water has been found to have no effect on plasma cortisol after 2–3 days (Goss and Wood, 1988; Brown *et al.* 1989), whereas the latter authors did find a significant increase after 7 days' exposure. Previous studies have also shown that exposure of trout to low pH (4) does not cause a significant increase in catecholamine levels, except just before death (Ye *et al.* 1991; Brown, 1992).

Unlike the situation with rainbow trout at 14°C exposed to pH4–4.5 for 3 days (Milligan and Wood, 1982), there was no apparent movement of fluid from extracellular to intracellular space of white muscle in the present experiments, although there was a significant increase in total muscle water content of a similar magnitude to that reported by Milligan and Wood (1982). The reason(s) for this discrepancy is unknown, although the severity of acid exposure in Milligan and Wood's experiments was probably greater than that in the present experiments, where the haemodynamic responses were less extreme (see Butler *et al.* 1992).

Swimming at U$_{crit}$

Swimming at U_{crit} at neutral pH did cause a significant shift in water from the extracellular to the intracellular compartment of red and (possibly) of white muscle, which is similar to the situation found in rainbow trout chased to exhaustion (Milligan and Wood, 1986*a*). These authors attributed this, at least in part, to the production of the osmotically active lactate within the muscle. In fish exposed to sublethal pH, the movement of water in red muscle at U_{crit} was in the opposite direction. The reason for this is unclear, but there was a much lower blood lactate concentration in fish at U_{crit} at sublethal pH than at neutral pH.

Swimming to U_{crit} caused a significant reduction in plasma pH in fish at neutral pH, and this reduction was accompanied by an increase in blood lactate to a level similar to that recorded by Driedzic and Kiceniuk (1976) and in Pa_{CO_2} (see Thomas *et al.* 1987). Plasma pH at U_{crit} was the same for animals at neutral pH as it was for those at sublethal pH. Swimming at U_{crit} had no effect on pHi of the RBCs under any condition; this was probably related to the significant increases in catecholamine levels (Primmett *et al.* 1986). Neither did swimming to U_{crit} have a significant effect on pHi of cardiac muscle, which is similar to the situation in rainbow trout after being chased to exhaustion (Milligan and Wood, 1986*b*). The mechanism(s) responsible for this is unknown, although it has been suggested that it may involve the uptake of lactate (Farrell and Milligan, 1986).

In both red and white muscles, pHi values are similar at U_{crit} when the fish are exposed to neutral or to sublethal pH. Studies on rainbow trout by Milligan and Wood (1986*b*) at 15°C and Tang and Boutilier (1991) at 10°C indicate that pHi of white muscle after the fish had been chased to exhaustion can be as low as 6.8–6.6, which is lower than that in fish at U_{crit} and neutral pH in the present study. Also, in other studies (Milligan and Wood, 1986*a*; Wood *et al.* 1990), it was found that rainbow trout chased to exhaustion have substantially higher concentrations of blood lactate (5–6mmol l^{-1}) at the end of the period of activity than those found in the present study. Thus, in terms of *burst* exercise, it

is assumed that the fish in the present study had not reached complete exhaustion and could, if prompted, have performed further burst swimming. They were, nonetheless, unable (or unwilling) to swim at a *constant* speed any longer. It would appear, therefore, that sustained swimming is terminated when pHi of the red muscle reaches a particular value of approximately 7.0.

In salmonids, white muscle fibres are recruited during sustained swimming (Johnston and Moon, 1980) and lactate accumulates in both red and white muscles, at least during the early stages of sustained swimming in rainbow trout (Wokoma and Johnston, 1981). These phenomena can explain the reduction in pHi at U_{crit} in these two types of muscle in brown trout, at least for the fish in water at neutral pH. However, there is no increase in blood lactate level in fish at U_{crit} in water at sublethal pH, although the reductions in pHi of the red and white muscles are similar to those at U_{crit} at neutral pH. Nelson (1989) and Nelson and Mitchell (1992) have described a similar phenomenon in yellow perch, *Perca flavescens,* where fish from lakes at neutral pH have a reduced U_{crit} when exposed to acid water yet have a much lower blood lactate concentration than when swimming at U_{crit} in neutral water. Lactate concentration in the white muscle is also lower at U_{crit} in fish in acid water (Nelson, 1990), which indicates a reduced capacity for anaerobiosis under these conditions.

If this also occurred in the fish exposed to sublethal pH in the present experiments, then when sufficient power could not be provided by aerobic metabolism, at a relatively low swimming speed, ATP consumption would exceed its production, thus producing sufficient protons to cause a reduction in pHi of the red and white muscles (see Milligan and Wood, 1986*b*). Two factors may have exacerbated this situation: aerobic metabolism itself may have been compromised as a result of impaired oxygen transport (see Introduction) and a low environmental pH may reduce the ability of the fish to excrete at the gills the metabolically produced H^+. It should be noted that work on humans has indicated that the decrease in strong ion difference (SID) in exercising muscle, which accounts for most of the increase in $[H^+]$, is as much the result of a fall in $[K^+]$ as of a rise in [lactate] (Kowalchuk *et al.* 1988).

The causal relationship between acidosis and muscle fatigue is not clear. Work on mammals indicates that an increase in $[H^+]$ may have a direct effect on excitation–contraction coupling (Metzger and Fitts, 1987) or may reduce the rate and extent of the cross-bridge transition from the low to the high force state (Thompson *et al.* 1992). There is also a close relationship between $[H^+]$ and Ca^{2+}-ATPase activity, indicating that acidity may impair Ca^{2+} transport in the sarcoplasmic reticulum (O'Brien *et al.* 1991). Other evidence suggests that H^+ may impair muscle function by causing a decrease in phosphocreatine (PCr) concentration (Sahlin, 1986; Katz *et al.* 1986), either directly, by displacing the creatine kinase reaction ($H^+ + ADP + PCr \rightleftharpoons ATP + Cr$) towards breakdown of PCr (but see Meyer *et al.* 1991), or indirectly, by way of increased [ADP]. It has also been suggested for mammals, that the loss of K^+ from exercising muscle may itself impair contractility (Sjøgaard, 1986), as well as contributing to the increase in $[H^+]$ (see above). Further studies on fish, particularly using isolated muscle fibres (Johnson *et al.* 1991), may help elucidate more clearly the relationship between $[H^+]$ and fatigue in vertebrate muscle.

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