*J. exp. Biol.* **123**, 123–144 (1986)
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# TISSUE INTRACELLULAR ACID–BASE STATUS AND THE FATE OF LACTATE AFTER EXHAUSTIVE EXERCISE IN THE RAINBOW TROUT

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*Accepted 14 January 1986*

## SUMMARY

Exhaustive 'burst-type' exercise in the rainbow trout resulted in a severe acidosis in the white muscle, with pHi dropping from 7·21 to a low of 6·62, as measured by DMO distribution. An accumulation of lactate and pyruvate, depletions of glycogen, ATP and CP stores, and a fluid shift from the extracellular fluid to the intracellular fluid of white muscle were associated with the acidosis. The proton load was in excess of the lactate load by an amount equivalent to the drop in ATP, suggesting that there was an uncoupling of ATP hydrolysis and glycolysis. Initially, lactate was cleared more quickly than protons from the muscle, a difference that was reflected in the blood. It is suggested that during the early period of recovery (0–4 h), the bulk of the lactate was oxidized *in situ*, restoring pHi to a point compatible with glyconeogenesis. At that time, lactate and $H^+$ were used as substrates for *in situ* glyconeogenesis, which was complete by 24 h. During this time, lactate and $H^+$ disappearance could account for about 75 % of the glycogen resynthesized. The liver and heart showed an accumulation of lactate, and it is postulated that this occurred as a result of uptake from the blood. Associated with the lactate load in these tissues was a metabolic alkalosis. Except for an apparent acidosis immediately after exercise, the acid–base status of the brain was not appreciably affected. Despite the extracellular acidosis, red cell pHi remained nearly constant.

## INTRODUCTION

Bouts of exhaustive exercise in teleost fish result in an accumulation of lactate and, presumably, protons within the working muscle. A number of studies have focused on the fate of lactate after exercise (Black, Robertson, Hanslip & Chiu, 1960; Black, Robertson, Lam & Chiu, 1962; Stevens & Black, 1966; Wardle, 1978; Batty & Wardle, 1979; Turner, Wood & Clark, 1983; Turner, Wood & Hōbe, 1983). The general pattern emerging from these investigations is that lactate clearance from the muscle is quite slow, requiring 8–12 h. Similarly, restoration of the depleted glycogen store is not complete until up to 24 h after exercise. Little is known, however, about the fate of the metabolically produced protons, or their consequence(s) for muscle. In the previous paper (Milligan & Wood, 1986), it was shown that exhaustive exercise in the trout does, indeed, result in a severe intracellular

Key words: lactate, pHi, muscle, trout, exercise.

acidosis in the whole body, with the bulk of this metabolic proton load remaining in the intracellular compartment.

The present study investigates further the intracellular acid–base disturbance associated with exhaustive exercise in trout. Since lactate and proton production are inextricably linked, the question of muscle acid–base regulation cannot be considered in isolation from metabolism (Hochachka & Somero, 1984). Thus, muscle metabolite, as well as acid–base status, was followed after exercise. In addition, the consequences of the exercise-induced disturbances in extracellular acid–base and metabolite status for other tissues (heart, liver, brain and red blood cells) were also examined. This investigation explores not only the question of intracellular acid–base regulation after exercise in fish, but also its relationship to metabolism.

## MATERIALS AND METHODS

### *Experimental animals*

Adult rainbow trout (*Salmo gairdneri*, 180–420 g) were purchased at various times throughout the year from Spring Valley Trout Farm, Petersburg, Ontario, and held as previously described (Milligan & Wood, 1986).

Following catheterization of the dorsal aorta, fish were placed in 5-l darkened fish boxes supplied with a continual flow of fresh dechlorinated Hamilton tap water at 15 ± 1 °C and allowed to recover for 48 h prior to experimentation.

### *Experimental protocol*

In these experiments, fish were terminally sampled rather than sequentially sampled. Approximately 12 h prior to sampling, trout were infused with a 1 ml $kg^{-1}$ dose of 5 $\mu$Ci $ml^{-1}$ [$^{14}$C]DMO (5,5-dimethyl-2,4-oxazolidinedione) (New England Nuclear; specific activity: 50 mCi $mmol^{-1}$) plus 20 $\mu$Ci $ml^{-1}$ [$^{3}$H]mannitol (New England Nuclear; specific activity: 27·4 mCi $mmol^{-1}$) in Cortland saline (Wolf, 1963) followed by an equal volume of saline. Individual fish (6–9 at each time) were terminally sampled prior to (rest) and immediately following (0 h) exercise, as well as at 0·5 h, 1, 2, 4, 8, 12 and 24 h after exercise. A blood sample (2000 $\mu$l) was drawn from the arterial catheter, and the volume replaced with saline; then the fish was grasped firmly by one investigator, immediately removed from the water, placed on a sponge and wiped dry. Ten approx. 100-mg muscle samples were taken by a second investigator by punching 10 biopsy needles (i.d. 4·78 mm) through the dorsal epaxial muscle, just anterior to the dorsal fin. Samples were immediately frozen in the needles in liquid nitrogen. The fish was quickly killed by a cephalic blow, the liver, heart and brain excised and immediately frozen in liquid nitrogen. Larger samples of muscle and liver (approx. 1–2 g) were then taken for determination of total tissue water. The carcass was dried to a constant weight at 85 °C for determination of total body water.

Data from fish which struggled unduly during the sampling procedure were discarded. This method of tissue sampling proved almost as quick as that of Turner *et al.* (1983) and resulted in less struggling. Average times from first grasping the fish

to freezing the samples were approx. 10 s for muscle biopsy and 30–60 s for the other tissues.

Blood was analysed for pH, total $CO_2$ (in both whole blood and plasma), whole blood [lactate], [glucose], [haemoglobin], [pyruvate], haematocrit and plasma levels of protein, $^3H$ and $^{14}C$ radioactivity. Heart, liver and muscle were analysed for levels of lactate, pyruvate, $^3H$ and $^{14}C$ radioactivity and total water content with the additional measurements of glycogen in liver and muscle and adenosine triphosphate (ATP) and creatine phosphate (CP) in muscle. Brain was assayed for $^3H$ and $^{14}C$ radioactivity and water content only. The red blood cells were analysed only for intracellular pH by direct measurement (described below).

*Analytical techniques and calculations*

Arterial blood pHa, total $CO_2$, [lactate], [glucose], [haemoglobin], haematocrit and plasma levels of $^3H$ and $^{14}C$ radioactivity were measured as described previously (Milligan & Wood, 1986), except that scintillation counting was done on an LKB Wallac scintillation counter. Whole blood [pyruvate] was measured enzymatically (lactate dehydrogenase method using Sigma reagents) on 400 $\mu$l of the extract prepared for lactate analysis. Plasma [protein] was measured by refractometry (American Optical). Red cell pHi was measured directly by the freeze–thaw technique of Zeidler & Kim (1977) as described by Milligan & Wood (1986).

To determine tissue levels of lactate and pyruvate, heart (65–110 mg), muscle (80–120 mg) and liver samples (50–200 mg) were homogenized in 1 ml of 8 % ice-cold perchloric acid with a Turmax Tissuemizer for 2 min. The homogenate was then transferred to 1·5 ml centrifuge tubes and centrifuged for 3 min at 9000 ***g***. The supernatant was neutralized with Trizma buffer (Sigma) and analysed as previously described (Turner *et al.* 1983). Muscle and liver glycogen were measured on tissues of similar weight homogenized in 1·1 ml acetate buffer. The glycogen was broken down with amyloglucosidase (Boehringer-Mannheim) and analysed enzymatically for glucose using the linked hexokinase/glucose-6-phosphate dehydrogenase (Boehringer-Mannheim) assay described by Bergmeyer (1965). Muscle concentrations of ATP and CP were measured on approx. 100 mg samples homogenized in 2 ml 6 % perchloric acid. The homogenate was centrifuged for 10 min at 5000 ***g***, the supernatant withdrawn, neutralized with 10 mol $l^{-1}$ KOH, recentrifuged and the resultant supernatant analysed. The technique employed was that described by Bergmeyer (1965) in which the [NADP] is measured fluorometrically and is directly proportional to the [ATP] as linked by the glucose-6-phosphate dehydrogenase/hexokinase enzyme system. [CP] was then measured by the same assay after the addition of ADP and phosphocreatine kinase (Boehringer-Mannheim). Tissue water content was determined by drying to a constant weight at 85 °C. Tissue levels of all metabolites were eventually expressed on an intracellular basis – i.e. per litre of intracellular fluid (ICF) (see below) with appropriate correction for the metabolite levels trapped in the ECF.

Levels of $^3H$ and $^{14}C$ radioactivity in brain, liver, heart and muscle were determined by digesting 50–150 mg of tissue in a 2 ml NCS tissue solubilizer

(Amersham) for 2–4 days until a clear solution was obtained. The digests were neutralized with 60 $\mu$l glacial acetic acid, then 10 ml fluor (OCS; Amersham) was added. Samples were stored in the dark overnight, to reduce chemiluminescence, then counted on an LKB Wallac liquid scintillation counter. Dual-label quench correction was performed using quench standards prepared from the tissue of interest and the external standard ratio method (Kobayashi & Maudsley, 1974).

The non-bicarbonate buffer capacity ($\beta$) of muscle, liver, heart and brain was determined by acid titration of tissue homogenates as described by Cameron & Kormanik (1982). Tissue (1–2 g) was frozen and pulverized with a mortar and pestle under liquid nitrogen, then suspended in 3 ml 0·9 % NaCl. The tissue homogenate was titrated to pH 8·0 with 1 mol $l^{-1}$ NaOH, allowed to stabilize, then back titrated to pH 6·5 with 0·02 mol $l^{-1}$ HCl under a nitrogen atmosphere at 15 °C. A Radiometer G-202 pH electrode and associated acid–base analyser were used to measure pH. The slope of the curve relating pH to mmol HCl added over the pHi range observed *in vivo* for each tissue (Fig. 2) was taken as the buffer capacity of the tissue in mmol $pH^{-1}$ $kg^{-1}$ wet weight, and then converted to mmol $pH^{-1}$ $l^{-1}$ ICF, as for the tissue metabolites.

Tissue extracellular fluid volume (ECFV) was calculated according to the equation:

$$\text{ECFV (ml g}^{-1}) = \frac{\text{Tissue [[}^3\text{H]mannitol] (d.p.m. g}^{-1})}{\text{Plasma [[}^3\text{H]mannitol] (d.p.m. g}^{-1})/\text{plasma } H_2O}, \quad (1)$$

where plasma water content was calculated from the refractive index. Intracellular fluid volume (ICFV) was calculated as the difference between total tissue water and ECFV. [$^3$H]mannitol distributions after 12 h equilibration proved inadequate for estimating ECFV in liver, producing values often as high as or greater than the total liver water content. This obvious artifact was attributed to permeation of the ICFV by mannitol, and perhaps subsequent metabolism. Also, mannitol sometimes yielded suspiciously high estimates for the heart. Control experiments with inulin indicated that this was a problem only for these two tissues, and not for brain or white muscle. Thus in place of these erroneous values, inulin-derived ECFV values (liver: 193·6 ml $kg^{-1}$; heart: 180·3 ml $kg^{-1}$; C. M. Wood & S. Munger, unpublished results) were used.

Tissue pHi was calculated according to equation 6 of Milligan & Wood (1986), where pHe was arterial plasma pH, $pK_{DMO}$ was taken from Malan, Wilson & Reeves (1976), and [DMO]e was calculated from equation 7 of Milligan & Wood (1986) and

$$[\text{DMO]i (d.p.m. ml}^{-1}) = \frac{\text{tissue [[}^{14}\text{C]DMO] (d.p.m. g}^{-1}) - [\text{ECFV (ml g}^{-1}) \times [\text{DMO]e (d.p.m. ml}^{-1})]}{\text{ICFV (ml g}^{-1})}. \quad (2)$$

$Pa_{CO_2}$ and [$HCO_3^-$] (in plasma and whole blood) were calculated from measured pHa and total $CO_2$ contents using the Henderson–Hasselbalch equation, with values of pK′ and $\alpha CO_2$ for rainbow trout from Boutilier, Heming & Iwama (1984).

Intracellular $[HCO_3^-]$ in tissues was similarly calculated from $Pa_{CO_2}$ and pHi, assuming plasma $Pa_{CO_2}$ was in equilibrium between the ECF and ICF. While the use of venous data would yield slightly higher values of intracellular $[HCO_3^-]$ (Milligan & Wood, 1986), this would have negligible effect on the calculated changes in $\Delta H^+m$ (see below), which was the purpose of these calculations.

The 'metabolic' acid load ($\Delta H^+m$) after exercise in various tissue compartments and whole blood was calculated as outlined for blood by Milligan & Wood (1986):

$$\Delta H^+m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2), \quad (3)$$

using the appropriate values of pH, $[HCO_3^-]$ and $\beta$ for the compartment in question. As each fish was sampled only once, $\Delta H^+m$ was calculated from the mean values at each time. Similarly, Δlactate was calculated as the difference between the means of [lactate] at rest and each time after exercise.

### *Statistical analysis*

Means ± 1 S.E.M. ($N$) are reported throughout, unless stated otherwise. Differences between groups were tested for significance ($P \leq 0.05$) with Student's two-tailed $t$-test, unpaired design.

## RESULTS

### *Tissue buffer capacities*

White muscle had the greatest buffer capacity of the tissues examined, followed by, in descending order, liver, heart and brain (Table 1). It is recognized that these values represented total physicochemical buffer capacity of the tissues (i.e. non-bicarbonate + bicarbonate). However, as $P_{CO_2}$ was kept low during titration, intracellular bicarbonate levels would be quite low ($<1$ mmol $l^{-1}$) and would not contribute significantly to the measured buffer capacity. Thus, in essence, these values were representative of the non-bicarbonate buffer capacity, $\beta$, of the tissue.

### *Extracellular acid–base and metabolite status*

Changes in extracellular (i.e. plasma) acid–base status after 6 min of exercise (Fig. 1) were qualitatively similar to those described in the previous paper (Milligan & Wood, 1986). However, in the present study, the faster pHa recovery and the

Table 1. *Buffer capacities of brain, heart, liver and white muscle in the rainbow trout*

| | mmol $pH^{-1}$ $kg^{-1}$ wet weight | mmol $pH^{-1}$ $l^{-1}$ ICF |
|---|---|---|
| Brain | −17·41 ± 0·34 (5) | −24·83 ± 0·39 (5) |
| Heart | −20·92 ± 1·04 (6) | −30·39 ± 1·50 (6) |
| Liver | −25·44 ± 2·88 (6) | −42·76 ± 4·84 (6) |
| White muscle | −51·32 ± 4·56 (7) | −73·59 ± 4·87 (7) |

Means ± 1 S.E.M. ($N$).

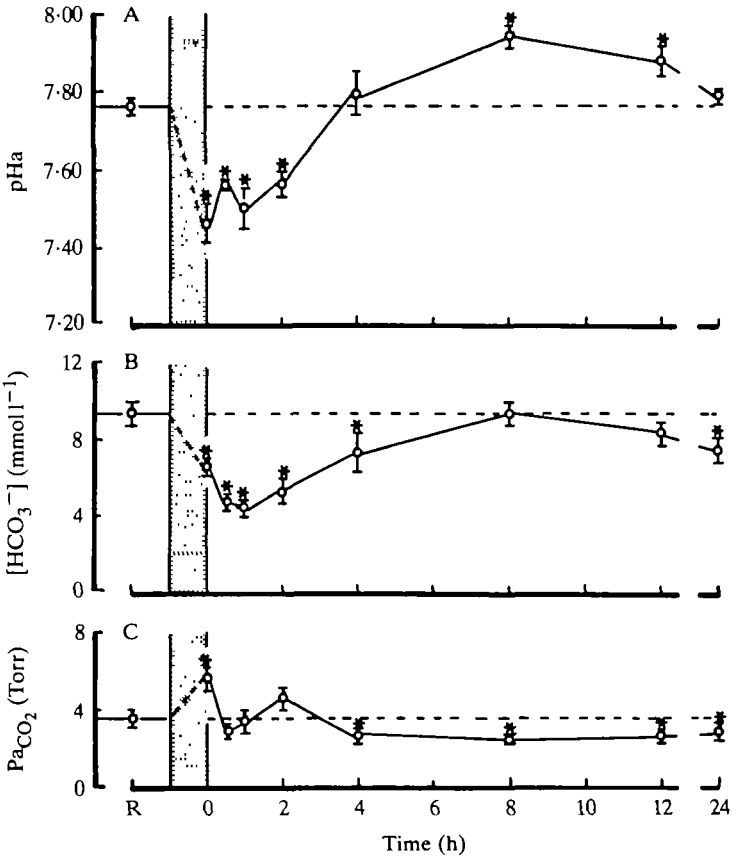


Fig. 1. Changes in arterial plasma pH (A), $[HCO_3^-]$ (B) and $P_{CO_2}$ (C) after exhaustive exercise in the rainbow trout. Means ± 1 S.E.M. Shaded bar indicates 6 min of exercise, R indicates rest, 0 immediately after exercise. Dashed line indicates the mean of the rest value. $N = 9$ at rest, 8 at 0 h, 6 at 0·5 h, 7 at 1 h, 6 at 4 h, 6 at 8 h, 7 at 12 h and 7 at 24 h. * indicates a significant ($P < 0·05$) difference from corresponding rest value.

alkalosis at 8–12 h post-exercise had a partial 'respiratory' origin in these fish due to a reduction in $Pa_{CO_2}$ below the resting level which was not observed in the previous study. The reason for this difference is unknown. The haematological changes accompanying exercise in the present study were similar to those reported in the previous paper (Milligan & Wood, 1986), and will not be reported here. The diluting effect of repetitive blood sampling was not a complication in the present study, and calculations based on the observed 30–40 % increase in both plasma protein and haemoglobin concentrations suggest that the previously measured 27 % decline in whole body ECFV (Milligan & Wood, 1986) was fully reflected in a similar decline in blood plasma volume.

As before, whole blood [lactate] reached a peak close to 20 mmol $l^{-1}$ 2 h after exercise, thereafter falling (Table 2). Whole blood [pyruvate] followed a similar pattern, rising slowly and reaching a plateau at 2–4 h into recovery, then slowly declining, although the absolute levels and elevations of [pyruvate] were far less than those of [lactate]. Whole blood [glucose] tended to increase after exercise,

Table 2. *Whole blood levels of lactate, pyruvate and glucose prior to and following exhaustive exercise in the rainbow trout*

| | Time after exercise (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rest | 0 | 0·5 | 1 | 2 | 4 | 8 | 12 | 24 |
| | | | | Lactate | | | | |
| 0·58 ± 0·08 (9) | 8·82 ± 2·15 (8)* | 10·95 ± 1·03 (6)* | 15·55 ± 1·69 (7)* | 19·73 ± 1·48 (6)* | 15·26 ± 1·54 (6)* | 3·81 ± 1·30 (6)* | 1·72 ± 1·11 (7) | 0·65 ± 0·21 (7) |
| | | | | Pyruvate | | | | |
| 0·05 ± 0·01 (9) | 0·13 ± 0·02 (8)* | 0·26 ± 0·05 (6)* | 0·42 ± 0·13 (7)* | 0·34 ± 0·16 (6)* | 0·45 ± 0·17 (6)* | 0·11 ± 0·03 (6) | 0·08 ± 0·04 (7) | 0·03 ± 0·01 (7) |
| | | | | Glucose | | | | |
| 3·79 ± 0·11 (8) | 4·35 ± 0·19 (7) | 4·79 ± 0·26 (6)* | 5·47 ± 0·79 (6) | 4·78 ± 0·56 (6) | 5·45 ± 0·93 (6) | 6·07 ± 0·40 (6)* | 4·57 ± 0·86 (7) | 2·17 ± 0·35 (7)* |

* Significantly different ($P < 0·05$) from corresponding rest value.
Mean ± 1 S.E.M. ($N$).
All values are expressed as mmol $l^{-1}$ whole blood.

although the changes were significant only at 0·5 and 8 h. At 24 h [glucose] declined significantly.

*Tissue intracellular acid–base and metabolite status*

At rest, mean white muscle [lactate] was much higher than that in blood (approx. 14 *versus* approx. 0·6 mmol $l^{-1}$, Tables 2, 3) and the average muscle pHi was 7·21 ± 0·04 ($N = 9$; Fig. 2A), about 0·56 units lower than extracellular pH. Exercise to exhaustion drove pHi to a low of 6·62 ± 0·06 ($N = 6$), increasing the pHe–pHi gradient to approx. 0·8 units. Muscle pHi did not show any signs of recovery until 2–4 h, requiring up to 12 h for full recovery. Unlike the extracellular compartment, there was no evidence of intracellular alkalosis in the later stages of recovery. A plot of the muscle data on a pH *versus* [$HCO_3^-$] diagram (Fig. 4A) indicates that the intracellular acidosis was mainly of metabolic origin, with little contribution from $P_{CO_2}$ except, perhaps, immediately after exercise. In plotting these data, it was assumed that arterial $P_{CO_2}$ was representative of intracellular $P_{CO_2}$. While this may underestimate intracellular $P_{CO_2}$, it does not appreciably alter the interpretation. Accompanying this metabolic acidosis was a 4- to 5-fold increase in muscle [lactate] to almost 50 mmol $l^{-1}$ and a slightly larger relative elevation in [pyruvate] to approx. 1·5 mmol $l^{-1}$ (Table 3) and a near depletion of glycogen, ATP and creatine phosphate stores (Fig. 3). The latter changes indicate that exercise was, indeed, exhaustive. The breakdown of muscle glycogen accounts for about 70 % of the lactate produced, the remainder presumably arising from blood-borne glucose.

Lactate was cleared relatively quickly from the muscle, so that by 8 h resting levels were obtained. However, [lactate] continued to decline, and by 24 h it was only about 30 % of that at rest (Table 3). Glycogen restoration was very slow, requiring up to 24 h for full recovery (Fig. 3C). Resynthesis of glycogen stores did not occur while pHi was maximally depressed (0–2 h), but began to show signs of recovery once pHi began to increase. In contrast, ATP resynthesis was quite rapid and complete (at 1 h) well before pHi began to recover (Fig. 3A). In fact, [ATP] continued to accumulate, reaching levels at 24 h, 3–4 times those at rest. [Creatine phosphate] restoration followed a different pattern still, with about 50 % restoration by 2 h, during the period of maximal acidosis, with full restoration complete by 4 h (Fig. 3B). There was some evidence of overshoot at 8 and 24 h.

Liver and heart pHi at rest were 0·15–0·2 pH units higher than that of muscle (Fig. 2B,C). The response of both tissues to exercise was nearly identical. Although pHi tended to fall slightly, neither tissue showed a significant acidosis during the period of the maximal extracellular acid–base disturbance. Similarly, both tissues showed a significant alkalosis during the latter (8–24 h) period of recovery. The origin of this alkalosis was metabolic, as shown in Fig. 4B,C. Resting [lactate] in both tissues was similar (3–5 mmol $l^{-1}$) and increased 5- to 6-fold after exercise (Tables 4, 5). Liver and heart [lactate] appeared to be more or less in equilibrium with whole blood [lactate] after exercise; from 0·5 to 8 h there was no significant difference between the three compartments (Tables 2, 4, 5). Peak intracellular lactate levels were attained 2 h into recovery, although this was not associated with

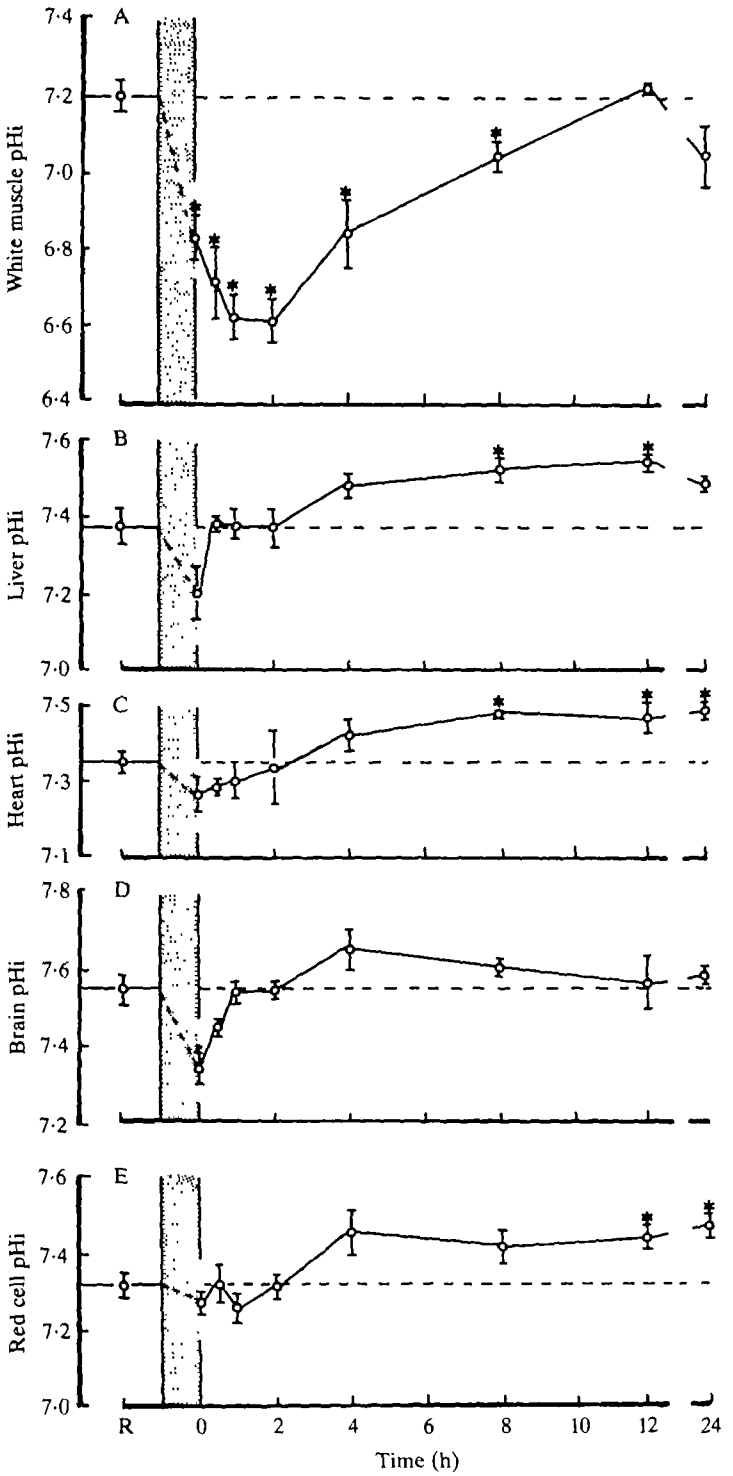


Fig. 2. Muscle (A), liver (B), heart (C), brain (D) and arterial red cell (E) pHi prior to and after exhaustive exercise. Other details as in the legend of Fig. 1.

Table 3. *White muscle levels of lactate and pyruvate prior to and following exhaustive exercise in the rainbow trout*

| | Time after exercise (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rest | 0 | 0·5 | 1 | 2 | 4 | 8 | 12 | 24 |
| | | | | Lactate | | | | |
| 13·85 ± 1·70 (9) | 48·29 ± 5·03 (8)* | 40·22 ± 3·84 (6)* | 38·69 ± 3·82 (7)* | 33·40 ± 2·18 (6)* | 23·90 ± 3·49 (6)* | 6·84 ± 0·93 (8)* | 9·84 ± 1·94 (7) | 3·94 ± 0·76 (7)* |
| | | | | Pyruvate | | | | |
| 0·18 ± 0·02 (9) | 0·93 ± 0·18 (7)* | 1·54 ± 0·38 (5)* | 1·14 ± 0·28 (7)* | 1·25 ± 0·28 (6)* | 0·39 ± 0·09 (6)* | 0·32 ± 0·09 (8) | 0·21 ± 0·06 (7) | 0·09 ± 0·02 (7) |

* Significantly different ($P < 0·05$) from corresponding rest value.
Mean ± 1 S.E.M. ($N$).
All values are expressed as mmol $l^{-1}$ intracellular water.

an accumulation of 'metabolic' acid (Fig. 4B,C). Liver pyruvate concentrations increased and then recovered over a faster time course than lactate and did not equilibrate with blood levels. Heart pyruvate levels did not change appreciably. Liver glycogen content was quite variable in each group, and no significant changes were observed after exercise (Table 4). Glycogen was not measured in the heart.

Of the tissues examined, the trout brain had the highest resting pHi, averaging $7{\cdot}55 \pm 0{\cdot}04$ ($N = 9$, Fig. 2D) at rest. Unlike heart and liver, brain pH fell

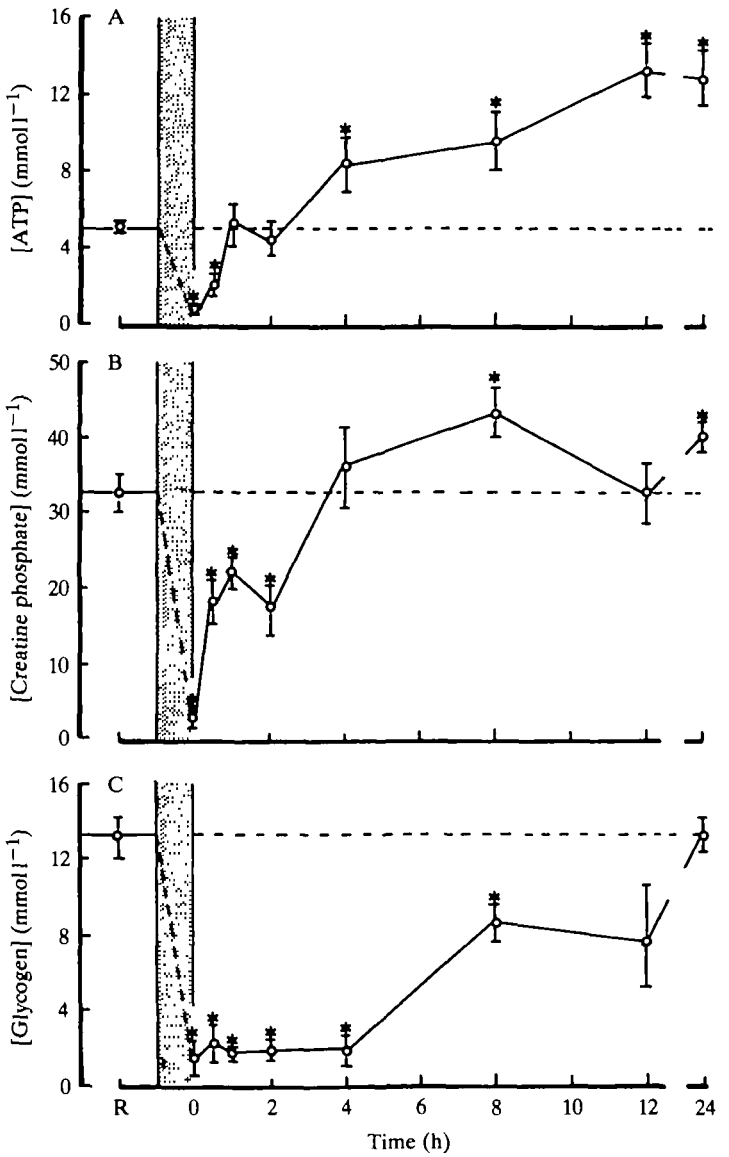


Fig. 3. Muscle concentrations of ATP (A), creatine phosphate (B), and glycogen (C) prior to and following exhaustive exercise. Other details as in the legend of Fig. 1.

immediately after exercise (Fig. 2D). While this acidosis appeared to be of mixed respiratory and metabolic origin (Fig. 4D), its significance is difficult to assess given the limitations of the method (see Discussion). No further perturbations in brain acid–base status were evident.

Arterial red cell pHi was remarkably stable after exercise (Fig. 2E), despite the increase in $P_{CO_2}$ and pronounced decline in plasma pH (Fig. 1A,C). Only at 12 and 24 h after exercise, when pHi increased above the control value, was there any significant variation from the rest value of $7{\cdot}31 \pm 0{\cdot}03$ ($N = 8$, Fig. 2E). This confirms the results of Milligan & Wood (1986).

### *Fluid volume distribution*

White muscle experienced a significant swelling after exercise, with ICFV increasing by approx. 40 ml $kg^{-1}$ from 0–2 h (Table 6). Since total tissue water did not

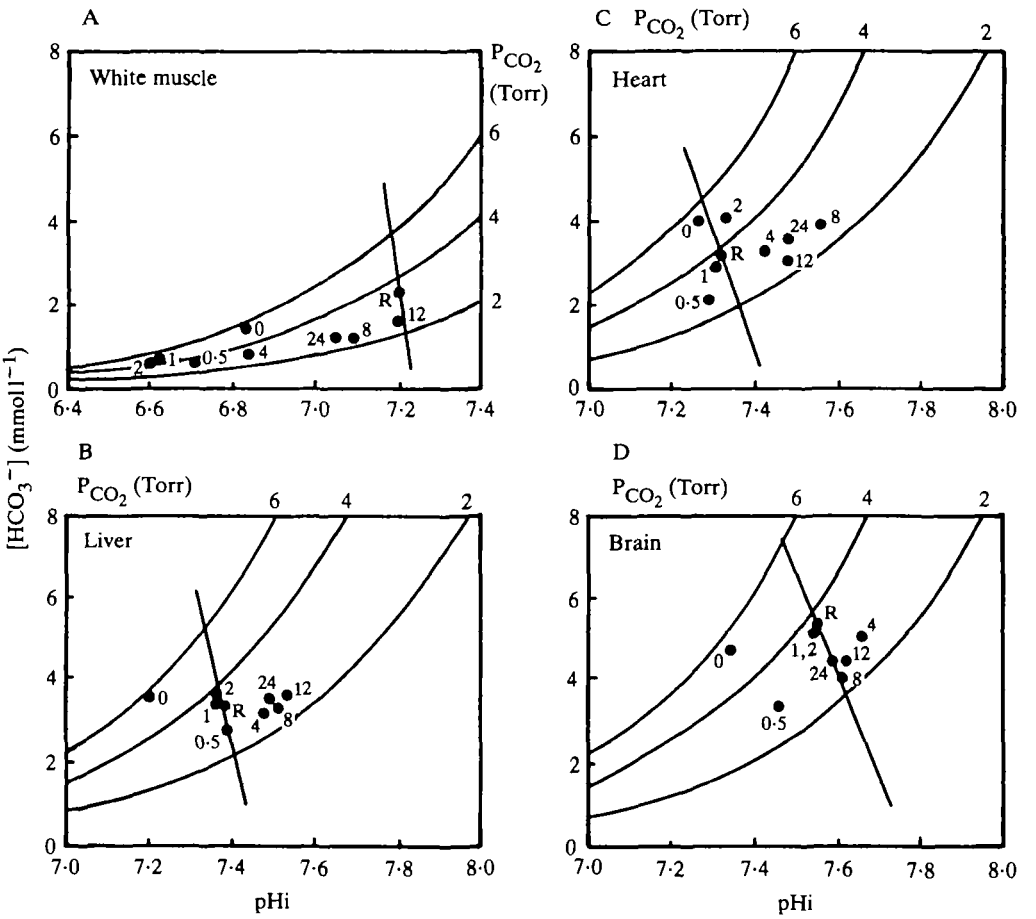


Fig. 4. pHi–$[HCO_3^-]$ diagrams of white muscle (A), liver (B), heart (C) and brain (D). Straight lines plotted are non-bicarbonate buffer values of the tissues, in mmol $pH^{-1}$ $l^{-1}$ intracellular fluid. Isopleths are $P_{CO_2}$. Means are plotted, $N$ as indicated in the legend of Fig. 1. R indicates resting value, 0 is immediately after exhaustive exercise, 0·5, 1, 2, 4, 8, 12 and 24 indicate time (in hours) after exercise.

change, the expansion of the intracellular space was at the expense of the extracellular space. The fluid distribution of the brain was not affected by exercise, as neither ECFV ($145{\cdot}8 \pm 15{\cdot}5\,ml\,kg^{-1}$, $N = 9$), ICFV ($701{\cdot}2 \pm 16{\cdot}3\,ml\,kg^{-1}$, $N = 9$) nor total water content ($845{\cdot}2 \pm 5{\cdot}6\,ml\,kg^{-1}$, $N = 9$) changed appreciably. Whilst the mannitol estimates of ECFV for liver and heart proved unreliable, they exhibited no significant change after exercise. Thus, constant inulin-based ECFV values were used in the pHi calculations for these tissues as outlined in Material and Methods. Neither liver ($788{\cdot}9 \pm 1{\cdot}4\,ml\,kg^{-1}$, $N = 9$), heart ($850{\cdot}1 \pm 5{\cdot}1\,ml\,kg^{-1}$, $N = 9$) nor whole body total water content ($724{\cdot}5 \pm 51{\cdot}3\,ml\,kg^{-1}$, $N = 9$) changed appreciably at any time as a result of exercise.

## DISCUSSION

### *Methodology*

The DMO method has been successfully employed for measuring pHi in a variety of tissues from a range of animals (see Roos & Boron, 1981). The estimate of pHi is dependent upon full equilibration of DMO between the intra- and extracellular compartments. As discussed earlier (Milligan & Wood, 1986) the ability of the DMO method reliably to detect pHi transients in trout tissues has only been validated to 15 min after initiation of the transient (Milligan & Wood, 1985), so the reliability of the pHi estimates immediately following 6 min exercise (0 h) is in doubt. If DMO were not redistributing, then tissue pHi would track the changes in pHe (see equation 6 in Milligan & Wood, 1986). The observation that this did not occur and that the [DMO]i/[DMO]e distribution ratio did, in fact, change in all tissues (e.g. in liver: [DMO]i/[DMO]e = $0{\cdot}45 \pm 0{\cdot}01$, $N = 9$, at rest and $0{\cdot}59 \pm 0{\cdot}02$, $N = 6$, at time 0) shows that DMO was redistributing in response to the pH change. However, if DMO redistribution were not complete at 0 h, then the calculated pHi would be low, thereby overestimating the actual change in tissue pHi. The error this introduces would be dependent upon the extent of the DMO disequilibrium. If, for example, DMO redistribution was only 50 % complete at time 0 then the calculated pHi would underestimate the 'actual' pHi by 0·2–0·3 pH units. In the present study, the error was probably not so severe. For instance, if it is assumed that $H^+$ and lactate were produced in stoichiometric quantities, then based on a lactate load of $34{\cdot}4\,mmol\,l^{-1}$ (Table 3) and a buffer value of $-69{\cdot}77\,mmol\,pH^{-1}\,l^{-1}$ (Table 1, using the time 0 h intracellular fluid volume of Table 6), then the predicted pHi in white muscle at time 0 would be 6·71, compared to the calculated value of $6{\cdot}82 \pm 0{\cdot}04$ ($N = 8$). Certainly by 0·5–1 h the estimates are considered reliable, since the time for marker equilibration was more than adequate, though some 'blurring' of fine changes might have occurred (Milligan & Wood, 1986).

Another potential source of error in the pHi estimate is the value chosen for pHe. In this study, arterial plasma pH (pHa) was assumed to be representative of the extracellular fluid. Muscle and brain are perfused by arterial and venous blood, with the true interstitial pHe probably lying between pHa and pHv. However, this is not the case for the liver, as it is supplied mainly by venous blood (Smith & Bell, 1976). The heart receives both venous and arterial blood, but the coronary arteries serve

Table 4. *Liver levels of lactate, pyruvate and glycogen prior to and following exhaustive exercise in the rainbow trout*

| Time after exercise (h) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rest | 0 | 0·5 | 1 | 2 | 4 | 8 | 12 | 24 |
| | | | | Lactate | | | | |
| 4·33 ± 1·07 (9) | 8·98 ± 1·48 (8)* | 15·53 ± 2·21 (6)* | 19·24 ± 2·03 (7)* | 21·94 ± 3·06 (5)* | 15·21 ± 1·36 (6)* | 6·00 ± 0·99 (7) | 5·61 ± 0·88 (7) | 3·89 ± 0·35 (7) |
| | | | | Pyruvate | | | | |
| 0·07 ± 0·01 (8) | 0·18 ± 0·04 (6)* | 0·42 ± 0·12 (6)* | 0·19 ± 0·06 (6) | 0·10 ± 0·06 (5) | 0·14 ± 0·8 (4) | 0·06 ± 0·01 (7) | 0·22 ± 0·02 (6) | 0·03 ± 0·01 (6) |
| | | | | Glycogen† | | | | |
| 126·68 ± 22·61 (7) | 166·94 ± 105·50 (6) | 240·98 ± 139·54 (4) | 247·63 ± 65·83 (7) | 87·21 ± 40·34 (6) | 126·26 ± 52·66 (6) | 109·49 ± 14·74 (8) | 109·71 ± 33·12 (7) | 127·45 ± 24·79 (7) |

* Significantly different ($P < 0·05$) from corresponding rest value.
† Glycogen expressed as mmol $l^{-1}$ glucose units.
Mean ± 1 S.E.M. ($N$).
All values are expressed as mmol $l^{-1}$ intracellular fluid.

Table 5. *Heart levels of lactate and pyruvate prior to and following exhaustive exercise in the rainbow trout*

| Time after exercise (h) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rest | 0 | 0·5 | 1 | 2 | 4 | 8 | 12 | 24 |
| | | | | Lactate | | | | |
| 3·72 ± 0·34 (6) | 13·00 ± 2·16 (4)* | 12·76 ± 1·44 (4)* | 15·75 ± 2·43 (5)* | 22·69 ± 2·25 (4)* | 14·61 ± 1·33 (4)* | 3·90 ± 1·22 (5) | 4·58 ± 1·38 (4) | 3·56 ± 0·50 (6) |
| | | | | Pyruvate | | | | |
| 0·14 ± 0·04 (6) | 0·23 ± 0·08 (4) | 0·22 ± 0·09 (4) | 0·20 ± 0·04 (5) | 0·11 ± 0·06 (4) | 0·14 ± 0·03 (4) | 0·09 ± 0·05 (5) | 0·13 ± 0·03 (4) | 0·08 ± 0·04 (6) |

* Significantly different ($P < 0·05$) from corresponding rest value.
Mean ± 1 S.E.M. ($N$).
All values are expressed as mmol $l^{-1}$ intracellular fluid.

Table 6. *Muscle fluid distribution prior to and following exhaustive exercise in the rainbow trout*

| Time after exercise (h) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rest | 0 | 0·5 | 1 | 2 | 4 | 8 | 12 | 24 |
| ECFV | | | | | | | | |
| 86·0 ± 8·4 (9) | 49·9 ± 5·3 (8)* | 48·5 ± 5·6 (6)* | 50·2 ± 3·0 (6)* | 49·4 ± 2·7 (6)* | 96·2 ± 2·6 (6) | 86·2 ± 8·3 (6) | 90·9 ± 8·8 (7) | 91·7 ± 5·7 (7) |
| ICFV | | | | | | | | |
| 697·4 ± 8·2 (9) | 735·2 ± 5·5 (8)* | 731·2 ± 9·6 (6)* | 739·5 ± 3·8 (6)* | 743·9 ± 4·1 (6)* | 699·5 ± 9·0 (6) | 693·1 ± 5·3 (6) | 693·4 ± 8·3 (7) | 693·9 ± 12·1 (7) |
| Total $H_2O$ | | | | | | | | |
| 783·4 ± 2·2 (9) | 786·2 ± 3·1 (8) | 779·6 ± 9·6 (6) | 790·6 ± 3·5 (6) | 793·2 ± 2·7 (6) | 795·7 ± 3·7 (6) | 799·3 ± 5·0 (6) | 784·3 ± 1·8 (7) | 785·5 ± 10·2 (7) |

* Significantly different ($P \leq 0{\cdot}05$) from corresponding rest value.
Mean ± 1 S.E.M. ($N$).
All values are expressed as ml $kg^{-1}$ wet weight.
ECFV, extracellular fluid volume; ICFV, intracellular fluid volume.

only the outer compressed layer of the ventricle, which represents about 20 % of total ventricle weight (Poupa, Gesser, Jonsson & Sullivan, 1974). The remaining 80 % is served by the venous supply. In any event, we have shown in the previous paper that arterial–venous pH, [DMO] and [mannitol] differences were insignificant except immediately after exercise (0 h) and even at this time, they tended to be self-compensating so as to have negligible effects on calculated mean whole-body pHi (Milligan & Wood, 1986). Comparable calculations for the individual tissues of the present study similarly indicated a negligible influence of arterial *versus* venous measurement site, and did not alter the significance of any of the differences demonstrated.

In this study, it was found that mannitol was not a suitable ECFV marker for trout liver or heart, so inulin-derived ECFV values were used in the pHi calculations for these tissues. These inulin-derived ECFV estimates from resting fish (C. M. Wood & S. Munger, unpublished results) were similar to those obtained from PEG-4000 distribution and $Cl^{-}$–$K^{+}$ space estimates in trout (Houston & Mearow, 1979). Inherent in this correction is the assumption that exercise did not cause a fluid shift in either the liver or heart. In the calculation of pHi, a $\pm 50\,ml\,kg^{-1}$ change in ECFV will alter pHi by $\pm 0{\cdot}02$–$0{\cdot}03$ pH units, which is outside the limits of detection of the DMO method.

*Resting tissue acid–base status*

White muscle had the greatest buffer value of the tissues examined, followed by liver, heart and brain, the latter with a buffer value only one-third that of muscle (Table 1). The high $\beta$ value of white muscle is to be expected, for it has a high anaerobic potential (Castellini & Somero, 1981). While these $\beta$ values were similar to those reported for heart and white muscle in dogfish (Heisler & Neumann, 1980) and channel catfish (Cameron & Kormanik, 1982), they were very different from those determined by the same methods in the sea raven (white muscle: approx. 39, heart: approx. $85\,mmol\,pH^{-1}\,l^{-1}$ ICF; Milligan & Farrell, 1986). The lower $\beta$ value in the sea raven muscle probably reflected the lower anaerobic potential of this fish (Castellini & Somero, 1981).

The resting pHi values followed almost the reverse pattern to that of $\beta$ values, with brain having the highest pHi, followed by liver, heart, red cell and white muscle (Fig. 2). Similar trends have been observed in the American eel (Walsh & Moon, 1982) and the channel catfish (Cameron & Kormanik, 1982), as well as in the rat (Roos & Boron, 1981). Thus, it is evident that there is a great deal of intertissue heterogeneity with respect to pHi. These differences may reflect differences in resting membrane potential and/or metabolic profiles of the tissues (Roos & Boron, 1981). Note, for example, the highest resting lactate level in the tissue with the lowest resting pHi – white muscle (Table 3; Fig. 2).

*Post-exercise changes*

After exercise, white muscle experienced a severe drop in pHi, which was due almost entirely to an accumulation of metabolic protons. The brain exhibited an

apparent mixed 'respiratory' and 'metabolic' acidosis immediately after exercise (though see cautionary note above) but had recovered by 0·5 h. The acid–base status of the heart and liver did not change appreciably (Figs 2, 4). Similarly, red cell pHi in arterial blood, the measurement of which was independent of DMO, did not fall, despite the erythrocytes' intimate contact with the acidotic extracellular fluid. Passive non-bicarbonate buffering, which is much higher than in the blood, obviously plays an important role in minimizing the pHi depressions (Fig. 4). A post-exercise catecholamine surge is also thought to play an important role in pHi regulation in red blood cells, as discussed in Milligan & Wood (1986). However, the role of circulating catecholamines in pHi regulation in other tissues (e.g. liver, brain, heart) is less clear. In mammalian cardiac muscle *in vitro*, it has been suggested that circulating catecholamines may stimulate $Cl^-/HCO_3^-$ exchange in the myocardium, thus aiding pHi regulation (Fenton, Gonzalez & Clancy, 1978; Gonzalez & Clancy, 1984). However, preliminary studies indicate that adrenaline does not appear to be important in rainbow trout cardiac muscle pHi regulation (A. P. Farrell & C. L. Milligan, unpublished results).

The source of metabolic proton production during anaerobiosis is a complex issue (Hultman & Sahlin, 1980; Hochachka & Mommsen, 1983). In brief, protons are generated by ATP hydrolysis, which, when tightly coupled to glycolysis, will result in a 1:1 stoichiometry between lactate and proton accumulation in vertebrate muscle. If, however, these processes should become uncoupled (i.e. ATP consumption exceeds production), then this 1:1 ratio is lost, with protons accumulating in excess of lactate. This appears to have occurred in trout white muscle after exercise (Fig. 5A). The proton excess ($\Delta H^+m$) of approx. 7 mequiv $l^{-1}$ over $\Delta$lactate was equivalent to the decline in ATP levels (see Fig. 3A). Since terminal rather than serial samples were taken, with $\Delta H^+m$ and $\Delta$lactate calculated from the mean at each time, the significance of the discrepancy between $H^+$ and lactate cannot be assessed statistically. However, a similar pattern ($\Delta H^+m > \Delta$lactate) has been observed after exercise in the white muscle of the sea raven (*Hemitripterus americanus*; Milligan & Farrell, 1986) and the starry flounder (*Platichthys stellatus*; C. L. Milligan & C. M. Wood, unpublished results).

The pattern of lactate accumulation in the liver (Fig. 5C) and heart (Fig. 5D) was similar to that of the blood (Fig. 5B), with peak levels attained 2 h after exercise. However, in contrast to the blood, liver and heart did not show a $\Delta H^+m$ accumulation but a $\Delta H^+m$ deficit. It is not possible to determine if the appearance of lactate in these tissues was from glycolysis *in situ* or uptake from the blood, though a number of factors suggest the latter. First, both these tissues are capable of taking up and oxidizing exogenous lactate (Bilinski & Jonas, 1972). In fact, lactate is a preferred substrate for the trout heart (Lanctin, McMorran & Driedzic, 1980). Secondly, the pattern of lactate appearance paralleled that of the blood, and absolute levels of lactate were equal in the three compartments from 0·5 to 8 h. Had lactate appeared in these tissues as a result of exercise-induced glycolysis *in situ*, peaks at 0 h, as in white muscle, rather that at 2 h would have been anticipated. Finally, there

was no accumulation of metabolic protons, which would be expected if lactate had been produced *in situ*.

The mechanism(s) of lactate uptake is (are) poorly understood. In some tissues, the bulk of lactate is transported in conjunction with $H^+$ or in exchange for $OH^-$ (e.g. human red cells, Dubinsky & Racker, 1978; Ehrlich-Ascites tumour cells, Spencer & Lehninger, 1976), whereas in others cotransport with $Na^+$ predominates (e.g. rat kidney, Ullrich, Rumrich & Kloss, 1982). As lactate metabolism is a net $H^+$-consuming process (Hochachka & Mommsen, 1983), a net proton deficit (i.e. metabolic alkalosis) would result if lactate were transported in conjunction with $Na^+$ or in exchange for $Cl^-$. In perfused rat liver, a direct relationship between intracellular alkalinization and lactate uptake was observed (Cohen *et al.* 1971). The alkalosis associated with lactate accumulation in the trout heart and liver (Fig. 5C,D) suggests that at least some of the lactate is transported without an accompanying proton and subsequently metabolized (see below).

### *The fate of lactate*

During the first 4 h of recovery, lactate was cleared from the muscle mass much more quickly than was the proton load, a difference which was reflected in the whole blood, where $\Delta$lactate was greater than $\Delta H^+m$ (Fig. 5A,B). What is the fate of this lactate and what is the overall contribution of the heart, liver and other lactate-utilizing 'aerobic tissues' (e.g. red muscle, kidney, gills; Bilinski & Jonas, 1972) to the clearance of the muscle lactate load? In a 1-kg trout, the total lactate load immediately after exercise averaged 17·3 mmol, assuming that the muscle mass constituted 60 % of the body weight (Stevens, 1968), that whole blood lactate levels were representative of the average extracellular level, and that the whole body ECFV estimates of Milligan & Wood (1986) were applicable. Of this total, 15·2 mmol was in the white muscle (Table 3). By 4 h, 11·0 mmol had disappeared from the muscle, of which only 1·6 mmol could be accounted for by additional accumulation in the extracellular fluid (Tables 2, 3). Excretion to the water was insignificant (Milligan & Wood, 1986). The observations that neither liver nor muscle glycogen were elevated over this 0–4 h period (Tables 3, 4) and that the rise in blood [glucose] was quite small (Table 2) suggest that the bulk of this 9·4 mmol lactate which disappeared was oxidized. For this to occur, a whole animal $O_2$ consumption rate of at least 225 mg $O_2\,kg^{-1}\,h^{-1}$ would be required. This value is not unreasonable, for $O_2$ consumption can reach levels as high as 800–1000 mg $O_2\,kg^{-1}\,h^{-1}$ following exercise in salmonids (Brett, 1972). If the burden of oxidation were placed solely on the 'aerobic' tissues (approx. 7 % of body weight; Stevens, 1968), then minimal $O_2$ consumptions of approx. 3200 mg $O_2\,kg^{-1}\,h^{-1}$ would be required of this tissue mass. An oxygen consumption of about 2500 mg $O_2\,kg^{-1}\,h^{-1}$ has been observed for the *in situ* perfused trout heart (A. P. Farrell & C. L. Milligan, unpublished results) and *in vitro* red muscle preparations from a variety of fish (Gordon, 1972*a*), so the requirement of 3200 mg $O_2\,kg^{-1}\,h^{-1}$ does not seem unreasonable. However, these reported tissue $O_2$ consumption rates represent total aerobic metabolism, of which lactate oxidation is

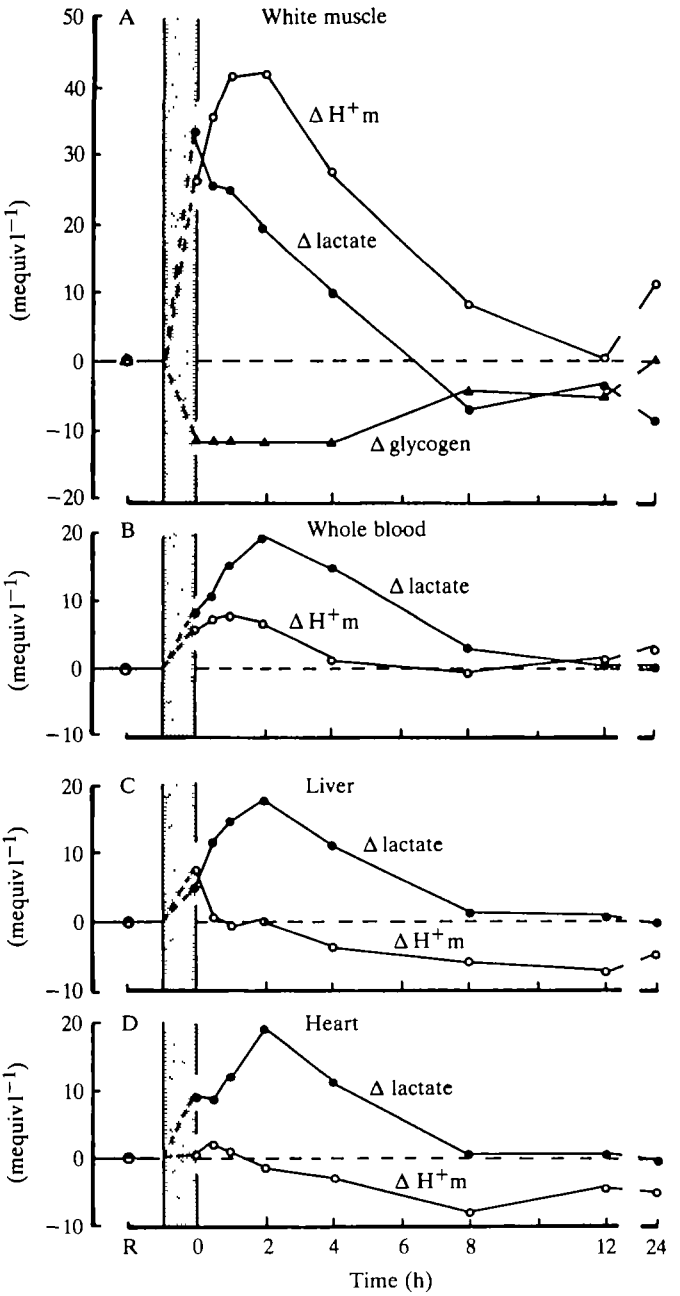


Fig. 5. White muscle (A) lactate (Δlactate, ●), metabolic acid ($\Delta H^+m$, ○) and glycogen (Δglycogen, ▲) loads and whole blood (B), liver (C) and heart (D) lactate (Δlactate, ●) and metabolic acid ($\Delta H^+m$, ○) loads after exercise. By definition, Δlactate and $\Delta H^+m$ and Δglycogen are zero at rest (R). Bar indicates period of exercise; 0 indicates immediately after exercise. Values plotted are calculated from means at each time. See text for details.

only a part; thus a minimal requirement of $3200\,mg\,O_2\,kg^{-1}\,h^{-1}$ is probably an underestimate.

The white muscle is also capable of lactate oxidation, albeit at rates lower than the 'aerobic' tissues (Bilinski & Jonas, 1972). As white muscle constitutes the bulk of the body mass, it does not seem unreasonable to suggest that lactate may be oxidized *in situ*. Indeed, this may have supplied the energy for the restoration (and overshoot) of ATP and CP stores which occurred at this time (Fig. 3). The observation of Turner & Wood (1983) that only about 10 % of the lactate produced after exercise leaves the muscle mass supports this contention. Given this scenario, then, 'aerobic' tissue $O_2$ consumption requirement would only be about $320\,mg\,O_2\,kg^{-1}\,h^{-1}$ and that of the white muscle about $340\,mg\,O_2\,kg^{-1}\,h^{-1}$, well within the reported *in vitro* values (Gordon, 1972*a*,*b*). Thus, in trout it would appear that, in the early period of recovery, lactate clearance can be attributed to a combination of export to the 'aerobic' tissues and *in situ* oxidation.

During the remainder of the recovery period, from 4 to 24 h, lactate and proton clearance from the muscle were about equal and correlated with a restoration of glycogen stores (Fig. 5A) as pHi returned to resting levels (Fig. 2A). In fact, by 24 h about 75 % of the glycogen resynthesized could be accounted for by the disappearance of lactate and protons. It appears that the muscle even dipped into its lactate 'reserve' to replenish its glycogen store, for by 24 h lactate had fallen to about 28 % of the resting level (Table 3; Fig. 5A).

The classical picture of the restoration of glycogen stores after exercise is the Cori cycle: lactate leaves the muscle, is taken up by the liver, and synthesized into glucose which then enters the bloodstream to be taken up by the muscle and made into glycogen. However, the results of this study and others (Hermansen & Vaage, 1977; Connett, 1979; McLane & Holloszy, 1979; Constable, Young, Higuchi & Holloszy, 1984) indicate that vertebrate white muscle is capable of *in situ* glyconeogenesis. These physiological studies, in conjunction with biochemical investigations (Dyson, Cardenas & Barsotti, 1975; Hochachka & Somero, 1984) argue against a prominent role for the Cori cycle in restoring muscle glycogen stores in vertebrate muscle after exercise. Instead, it is postulated that lactate and proton clearance from the muscle initially occurs *via* export to 'aerobic' tissues and *in situ* oxidation, which aids in restoring muscle pHi to a level compatible with glycogen resynthesis. When this point is reached, lactate and protons are used as substrates for *in situ* glyconeogenesis.

We wish to thank S. Munger for excellent technical assistance, S. Brown for advice on ATP, CP and glycogen assays and Dr C. E. Booth for helpful comments on the manuscript. Financial support was provided by grants to CMW from the Natural Sciences and Engineering Research Council of Canada and the Canadian National Sportsmen Fund. CLM was supported by a Natural Sciences and Engineering Research Council Postgraduate scholarship.

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