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INTRACELLULAR AND EXTRACELLULAR ACID–BASE STATUS AND H^+ EXCHANGE WITH THE ENVIRONMENT AFTER EXHAUSTIVE EXERCISE IN THE RAINBOW TROUT

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SUMMARY

Exhaustive exercise induced a severe short-lived (0–1 h) respiratory, and longer-lived (0–4 h) metabolic, acidosis in the extracellular fluid of the rainbow trout. Blood 'lactate' load exceeded blood 'metabolic acid' load from 1–12 h after exercise. Overcompensation occurred, so that by 8–12 h, metabolic alkalosis prevailed, but by 24 h, resting acid–base status had been restored. Acid–base changes were similar, and lactate levels identical, in arterial and venous blood. However, at rest venous RBC pHi was significantly higher than arterial (7·42 *versus* 7·31). After exercise, arterial RBC pHi remained constant, whereas venous RBC pHi fell significantly (to 7·18) but was fully restored by 1 h. Resting mean whole-body pHi, measured by DMO distribution, averaged approx. 7·25 at a pHe of approx. 7·82 and fell after exercise to a low of 6·78 at a pHe of approx. 7·30. Whole-body pHi was slower to recover than pHe, requiring up to 12 h, with no subsequent alkalosis. Whole-body ECFV decreased by about 70 ml kg^{-1} due to a fluid shift into the ICF. Net H^+ excretion to the water increased 1 h after exercise accompanied by an elevation in ammonia efflux. At 8–12 h, H^+ excretion was reduced to resting levels and at 12–24 h, a net H^+ uptake occurred. Lactate excretion amounted to approx. 1% of the net H^+ excretion and only approx. 2% of the whole blood load. Only a small amount of the anaerobically produced H^+ in the ICF appeared in the ECF and subsequently in the water. By 24 h, all the H^+ excreted had been taken back up, thus correcting the extracellular alkalosis. The bulk of the H^+ load remained intracellular, to be cleared by aerobic metabolism.

INTRODUCTION

In fish, exhaustive 'burst-type' exercise results in the production of CO_2, lactate and H^+ within the working muscle. Efflux of anaerobically produced H^+ and aerobically produced CO_2 from the working muscle results in a pronounced and often persistent extracellular acidosis, with recovery requiring up to 12 h. This acid–base disturbance has been well characterized in terms of its nature (i.e. respiratory *versus* metabolic), severity and time course of recovery (e.g. Black, Chiu,

Key words: trout, exercise, pHi, acid excretion.

Forbes & Hanslip, 1959; Piiper, Meyer & Drees, 1972; Wood, McMahon & McDonald, 1977; Turner, Wood & Clark, 1983*a*; Turner, Wood & Hōbe, 1983*b*; Holeton & Heisler, 1983; Schwalme & Mackay, 1985). The metabolic consequences of strenuous exercise, on both the extracellular compartment (ECF) and the working muscle, have also been extensively investigated (e.g. Black, Robertson, Hanslip & Chiu, 1960; Black, Robertson, Lam & Chiu, 1962; Stevens & Black, 1966; Wardle, 1978; Batty & Wardle, 1979; Turner *et al.* 1983*a*,*b*; Milligan & Farrell, 1986). At least in the ECF, it is now clear that lactate anions and acidic equivalents of metabolic origin ('metabolic acid') appear in unequal amounts, with the exact pattern varying between species. In the rainbow trout, the lactate load in the blood greatly exceeds the 'metabolic acid' load (Turner *et al.* 1983*a*; Holeton, Neumann & Heisler, 1983). Recently, some progress has been made in elucidating the mechanisms leading to this inequality of H^+ and lactate appearance. Both the gills and muscle are potential control sites, though it is controversial whether differential release or differential excretion into the water, or a combination of these and other factors, is responsible (Holeton & Heisler, 1983; Holeton *et al.* 1983; Neumann, Holeton & Heisler, 1983; Turner *et al.* 1983*a*; Turner & Wood, 1983).

There remain two other major gaps in our knowledge. First, nothing is known about changes in acid–base status within the intracellular compartment (ICF) of muscle or other tissues after exhaustive exercise. Previous studies have relied on model calculations rather than measurement (e.g. Heisler, 1982; Holeton & Heisler, 1983). Secondly, little is known about the possible controlling relationships between acid–base status and metabolism in either the ICF or ECF *in vivo*, though *in vitro* studies suggest that important interactions should occur (Hochachka & Somero, 1984).

Recently, we have shown that the DMO technique (Waddell & Butler, 1959) for measuring mean tissue intracellular pH (pHi) can reliably detect pHi transients on a time course similar to that likely to occur after exhaustive exercise in fish (Milligan & Wood, 1985). In the present study, we have applied this methodology to these areas of interest. This first paper focuses on acid–base changes in the whole body ICF, ECF (arterial and venous blood) and environmental water after strenuous exercise in the rainbow trout, with particular emphasis on transcompartmental 'metabolic acid' and lactate exchanges. The paper also assesses the behaviour of the radiolabels used in the DMO technique ([^{3}H]mannitol, [^{14}C]DMO) during long-term experiments, the possible influence of arterial *versus* venous sites of measurement, and changes in erythrocyte pHi after exhaustive exercise. The second paper (Milligan & Wood, 1986) investigates changes in the acid–base and metabolite status of individual tissues and their contributions to post-exercise recovery in the whole animal.

MATERIALS AND METHODS

Experimental animals

Adult rainbow trout (*Salmo gairdneri*) of both sexes (280–1200 g) were purchased from Spring Valley Trout Farm, Petersburg, Ontario at various times of the year and

held indoors in 600-l fibreglass tanks supplied with a continuous flow of aerated, dechlorinated Hamilton tap water at seasonal temperatures (5–19°C). During holding, fish were fed twice weekly with commercial trout pellets. At least 1 week prior to experimentation, fish were acclimated to experimental temperature (15 ± 1°C), during which period they were starved in order to minimize possible dietary influence on acid–base status. Acclimation and experimental water had the following composition (in mequiv l^{-1}): Na^+ 0·6; Cl^- 0·8; Ca^{2+} 1·6; Mg^{2+} 0·3; K^+ 0·05 and titration alkalinity 2·0; total hardness 140 mg l^{-1} as $CaCO_3$; pH 8·0.

Trout were anaesthetized with MS 222 (1:10 000; Sigma) and surgically fitted with dorsal aorta cannulae (Soivio, Westman & Nyholm, 1972) and, in some cases, ventral aortic cannulae (Kiceniuk & Jones, 1977). Fish were allowed to recover at least 48 h prior to experimentation in 10-l darkened Lucite flux boxes of the design described by McDonald (1983). For measurements of acidic equivalent, ammonia and lactate excretion, these boxes were operated as low volume (3–6 l) recirculating systems at 15 ± 1°C. Temperature was controlled by bathing the boxes with chilled water.

Experimental protocols

Series I

The first series focused on post-exercise changes in arterial acid–base and lactate status, whole body pHi and ECFV, acidic equivalent exchanges with the environment, and excretion of [^{3}H]mannitol and [^{14}C]DMO (5,5-dimethyl-2,4-oxazolidinedione). The fish [mean weight = 346·3 ± 43·5 (S.E.M.) g; N = 18] were fitted with arterial catheters only. Parallel experiments were performed on two separate groups, only one of which was subjected to exhaustive exercise (N = 8). The other (N = 10) served as a control for handling and sampling effects; these fish were left at rest throughout but otherwise treated identically to the experimental group.

Twenty-four hours prior to exercise, the inflow to the flux box was closed, the volume standardized to 1 l per 100 g body weight, and thereafter, water recirculated within the box by means of aeration. The box was thoroughly flushed with fresh water at experimental temperature (15 ± 1°C) for approximately 20 min at 12 h prior to exercise, at the time of exercise, then again at 12 and 24 h post-exercise, at which point the experiment was terminated. Thus two 12 h control periods at rest were followed by two 12 h periods post-exercise. The water was flushed at 12 h intervals to prevent ammonia levels in the box from exceeding 500 μmol l^{-1}. At the beginning of the 12 h flux period prior to exercise, fish were infused with an exact 1 ml kg^{-1} dose of 5 μCi ml^{-1} [^{14}C]DMO (5,5-dimethyl-2,4-oxazolidinedione, New England Nuclear, specific activity: 50 mCi $mmol^{-1}$) plus 20 μCi 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. This allowed 12 h for marker distribution before the first blood sample was taken. Water samples were taken for analysis of ammonia and titratable acidity flux at the start and end of each 12 h period prior to exercise and at 0, 1, 2, 4, 8, 12 and 24 h after exercise. Thus, net fluxes of

ammonia and acidic equivalents, as well as losses of markers to the environmental water, could be followed both before and after exercise.

Fish were exercised by vigorously chasing them around a large circular tank (500 l) for 6 min (cf. Turner *et al.* 1983*a*), at which point they did not respond to further stimulation. They were then immediately returned to the experimental chambers. Fluxes of titratable acidity and ammonia and loss of markers to the water could not be measured during the brief exercise period. However, even if ammonia and titratable acidity flux had occurred at up to five times the immediately post-exercise rate during the 6-min exercise period, their influence on all calculated parameters would have been negligible.

Blood samples (500 μl) were drawn prior to ('rest'), immediately after (time 0) exercise, as well as at 0·25, 0·5, 1, 2, 4, 8, 12 and 24 h post-exercise. Samples were analysed for pH, total CO_2 (in both whole blood and plasma), haematocrit, [haemoglobin], whole blood [lactate] and [glucose], and plasma levels of ^3H and ^{14}C radioactivity. The volume of blood sampled was replaced with saline.

Series II

The second series employed much larger trout (mean weight = 806·4 ± 172 g; $N = 5$) fitted with both arterial and venous catheters. The goals of this series were to compare post-exercise changes in venous *versus* arterial acid–base and lactate status, to assess possible differences in [^{14}C]DMO and [^{3}H]mannitol distribution between arterial and venous blood and their impact on pHi calculations, and to measure directly changes in red cell (RBC) pHi after exercise. With the following exceptions, the experimental protocol was identical to that of the exercised group in the first series. The larger size of the fish necessitated running the flux boxes on a continual flow-through basis, thereby precluding measurement of acidic equivalent exchange with the water or radiolabel excretion, and therefore calculation of ECFV or pHi. Simultaneous arterial and venous blood samples were drawn at each time, and these were of larger volume (900 μl) to facilitate the RBC pHi determinations. Samples were analysed for pH, total CO_2 (in both whole blood and plasma), haematocrit, whole blood [lactate], RBC pHi, and plasma levels of ^3H and ^{14}C radioactivity.

Series III

The third series employed non-cannulated fish (mean weight = 216·4 ± 8·8 g; $N = 4$) to assess lactate excretion into the water after exercise in comparison to net acidic equivalent exchange during the period of maximum blood lactacidosis. The 'flux' boxes were operated as closed low-volume systems, and water samples taken for analysis of lactate, ammonia and titratable acidity flux at 0, 2 and 4 h post-exercise.

Analytical techniques and calculations

Blood pH was determined on 40 μl samples injected into a Radiometer pH microelectrode (type E5021) maintained at 15 ± 1 °C and linked to a Radiometer PHM 71 or 72 acid–base analyser. Total CO_2 in both whole blood and plasma was measured on 50 μl samples by the method of Cameron (1971) using Radiometer CO_2

electrodes. P_{CO_2} and $[HCO_3^-]$ in blood and plasma were calculated using the Henderson–Hasselbalch equation, inserting αCO_2 and pK′ values determined for rainbow trout plasma at 15 °C by Boutilier, Heming & Iwama (1984).

Whole blood metabolic acid load ($\Delta H^+ m_{WB}$) was calculated in the cumulative fashion described by Turner *et al.* (1983*a*) using the following equation for each interval:

$$\Delta H^+ m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pHa_1 - pHa_2) \quad (1)$$

and summing (taking account of the sign) for each period from the rest sample onwards. In this equation, $[HCO_3^-]$ was the value for whole blood because $\Delta H^+ m_{WB}$ was compared to whole blood lactate, and β, the non-bicarbonate buffer capacity of whole blood, was estimated from the blood haemoglobin concentration at each time using the regression relationship derived by Wood, McMahon & McDonald (1982) for rainbow trout blood at 15 °C:

$$\beta = -1{\cdot}073 \times [\text{haemoglobin}] - 2{\cdot}48. \quad (2)$$

Haematocrit (by centrifugation), [haemoglobin] (as cyanmethaemoglobin) and their ratio (mean cell haemoglobin concentration) were measured according to Milligan & Wood (1982). Whole blood [lactate] was determined enzymatically (L-lactate dehydrogenase/NADH) as described by Turner *et al.* (1983*a*). Whole blood [glucose] was analysed on 100 μl samples deproteinized in 900 μl ice-cold trichloroacetic acid, using the *o*-toluidine method of Hyvarinon & Nikkita (1962) and Sigma reagents.

For determination of [^{3}H]mannitol and [^{14}C]DMO activity in the extracellular fluid, 50 μl of plasma was added to 10 ml scintillation fluid (ACS; Amersham) and counted on a Beckman LS-250 scintillation counter. The injected stock was similarly assayed. Radiotracer loss to the water was determined by counting 5 ml water in 10 ml ACS fluor. Dual-label quench correction was performed using the external standard ratio method as described by Kobayashi & Maudsley (1974).

Red cell pHi was measured on a pellet of cells obtained by centrifuging 400 μl of whole blood for 2 min at 9000 ***g***. The plasma was removed, the pellet sealed and the red blood cells were lysed by repeated freezing and thawing in either dry ice or liquid nitrogen and warm water, respectively (Zeidler & Kim, 1977). The pH was then measured directly on the homogenate (approx. 40 μl) using a Radiometer pH microelectrode and associated acid–base analyser, maintained at 15 °C.

Whole body extracellular fluid volume (ECFV) at each time was calculated as:

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

where $\sum$sampled refers to loss of marker *via* blood sampling and was calculated as

$$\frac{(1-\text{Ht}) \times \text{vol (ml)} \times \text{plasma}[[^3\text{H}]\text{mannitol}]\ (\text{d.p.m. ml}^{-1})}{\text{plasma } H_2O\ (\text{ml ml}^{-1})}, \quad (4)$$

where Ht was haematocrit as a decimal and vol was the volume of each blood sample. $\sum$excreted at each time was calculated as:

$$\text{water } [[^3\text{H}]\text{mannitol}] \text{ (d.p.m. ml}^{-1}) \times \text{water volume (ml)}, \quad (5)$$

taking into account the exchange of water during flushes. Intracellular fluid volume (ICFV) was calculated as the difference between total body water and ECFV. Plasma water was determined to be 0·956 ml ml^{-1} (Milligan & Wood, 1986).

Mean whole body pHi at each time was calculated according to the equation

$$\text{pHi} = \text{pK}_{\text{DMO}} + \log\left\{\frac{[\text{DMO}]\text{i}}{[\text{DMO}]\text{e}} \times (10^{(\text{pHe}-\text{pK}_{\text{DMO}})} + 1) - 1\right\}, \quad (6)$$

where pK_{DMO} was taken from Malan, Wilson & Reeves (1976), pHe was plasma pH, and [DMO]e and [DMO]i represent extracellular and intracellular [DMO], respectively. These were calculated as:

$$[\text{DMO}]\text{e (d.p.m. ml}^{-1}) = \frac{\text{plasma } [[^{14}\text{C}]\text{DMO}] \text{ (d.p.m. ml}^{-1})}{\text{plasma } \text{H}_2\text{O (ml ml}^{-1})} \quad (7)$$

and

$$[\text{DMO}]\text{i (d.p.m. ml}^{-1}) = \{[^{14}\text{C}]\text{DMO injected (d.p.m.)} - \sum\text{excreted (d.p.m.)} - \sum\text{sampled (d.p.m.)} - \{\text{ECFV(ml)} \times [\text{DMO}]\text{e (d.m.p. ml}^{-1})\}\}/\text{ICFV (ml)}, \quad (8)$$

where $\sum$sampled and $\sum$excreted were calculated as in equations 4 and 5, respectively. The importance of accounting for marker excretion and loss due to sampling in whole body pHi calculations has been demonstrated by Hōbe, Wood & Wheatly (1984) and Wood & Cameron (1985).

Total metabolic acid load to the whole body at each time was calculated as:

$$\Delta\text{H}^+\text{m (mequiv kg}^{-1}) = \Delta\text{H}^+\text{m}_{\text{ECFV}} \text{ (mequiv kg}^{-1}) + \Delta\text{H}^+\text{m}_{\text{ICFV}} \text{ (mequiv kg}^{-1}), \quad (9)$$

where

$$\Delta\text{H}^+\text{m}_{\text{ECFV}} \text{ (mequiv kg}^{-1}) = \text{BV (l kg}^{-1}) \times \Delta\text{H}^+\text{m}_{\text{WB}} \text{(mequiv l}^{-1}) + \{(\text{ECFV}-\text{PV})(\text{l kg}^{-1}) \times \Delta\text{H}^+\text{m}_{\text{ISF}} \text{ (mequiv l}^{-1})\}. \quad (10)$$

BV and PV were blood and plasma volumes, respectively, using values measured in rainbow trout at 15°C by Milligan & Wood (1982) and $\Delta\text{H}^+\text{m}_{\text{WB}}$ was calculated according to equation 1. To estimate $\Delta\text{H}^+\text{m}_{\text{ISF}}$, the 'metabolic acid' load to the interstitial fluid (ISF), the β value of the ISF was taken to be the same as that for separated plasma, $-2{\cdot}59$ mequiv pH^{-1} l^{-1} (Wood *et al.* 1982), and interstitial [HCO_3^-] was assumed to be the same as that of true plasma. For details concerning calculation of $\Delta\text{H}^+\text{m}_{\text{ICFV}}$, 'metabolic acid' load to the intracellular compartment, see Discussion.

The exchange of acidic equivalents (H^+) with the environmental water was calculated as the sum of titratable acidity flux and ammonia flux, signs considered. Titratable acidity flux was calculated from titratable alkalinity measurements on

10-ml water samples as outlined in McDonald & Wood (1981) and water [ammonia] was determined using a micro-modification of the phenol–hypochlorite method of Solorzano (1969).

Since lactate levels in the water were quite low (0·5–2·2 μmol l^{-1}), 500-ml water samples were freeze-concentrated 100-fold (Birchard, 1977), then assayed for lactate as described. In addition–recovery tests, this method was found to yield 60 ± 1·3 % ($N = 8$) recovery and experimental values were corrected accordingly.

Statistical analysis

Means ± 1 S.E.M. are reported throughout. Student's two-tailed t-test (paired design) was used to assess significant differences ($P \leq 0{\cdot}05$) within groups (e.g. arterial *versus* venous, post-exercise *versus* rest) using each fish as its own control. A non-paired t-test was used for between group comparisons.

RESULTS

Behaviour of [^{14}C]DMO and [^{3}H]mannitol

The concentrations of both [^{14}C]DMO and [^{3}H]mannitol in arterial and venous blood plasma were identical at rest (Table 1). Immediately after exercise (0 h), arterial concentrations of both radiolabels were significantly elevated over venous values by about 5 %. There were no significant differences at any other time. The impact of arterial *versus* venous measurement sites on whole body ECFV and pHi calculations (see below) turned out to be negligible.

Significant excretion of both radiolabels occurred into the environmental water (Table 2). Excretion rates were highly variable between individual fish, but were more or less linear over time and of about the same absolute size for [^{14}C]DMO and [^{3}H]mannitol. There were no significant differences at any time between the control and experimental groups, indicating that exercise did not influence the rate of marker loss. Cumulative losses due to repetitive blood sampling amounted to only about 10 % of losses due to excretion. By time 0 (i.e. 12 h post-infusion), less than 90 % of the injected [^{14}C]DMO and [^{3}H]mannitol doses remained in the animals, and by 24 h (i.e. 36 h post-infusion) this was reduced to about 70 % (Table 2). Failure to take these losses into account would have led to overestimates of mean whole body pHi by 0·13 (time 0) to 0·37 units (24 h) and overestimates of ECFV by 28 (time 0) to 107 ml kg^{-1} (24 h).

ECFV estimates were also complicated by the lengthy marker equilibration time. At the rest sample, [^{3}H]mannitol had been in the animal for about 12 h, and by the end of the experiment, 36 h had elapsed since marker infusion. At rest (12 h post-infusion), the calculated ECFV in both groups was about 250 ml kg^{-1} (Table 3), which is in close agreement with two previous determinations of ECFV in freshwater rainbow trout obtained by entirely different methods (Milligan & Wood, 1982; M. V. E. Attygalle, G. Shelton & P. C. Croghan, in preparation). However, during the experimental period (12–36 h post-infusion), the control group exhibited a gradual rise in ECFV, so that by 2 h, the rise was significant, and by 24 h, ECFV had

Table 1. *Ratio of arterial to venous plasma concentrations of [^{14}C]DMO and [^{3}H]mannitol*

	Time after exercise (h)								
Rest	0	0·25	0·5	1	2	4	8	12	24
[^{14}C]DMO arterial/venous									
1·007 ± 0·016 (5)	1·032 ± 0·008* (5)	1·061 ± 0·033 (5)	1·022 ± 0·021 (5)	1·015 ± 0·011 (5)	1·016 ± 0·010 (5)	1·019 ± 0·011 (5)	0·994 ± 0·016 (5)	1·021 ± 0·021 (4)	1·012 ± 0·008 (3)
[^{3}H]mannitol arterial/venous									
1·009 ± 0·011 (5)	1·052 ± 0·011* (5)	1·024 ± 0·019 (5)	1·017 ± 0·023 (5)	1·049 ± 0·029 (5)	1·038 ± 0·023 (5)	1·034 ± 0·020 (5)	1·005 ± 0·023 (5)	1·023 ± 0·013 (5)	1·012 ± 0·016 (5)

* Value is significantly different ($P \leq 0·05$) from 1·000.
Mean ± 1 S.E.M. (N).

Table 2. *Percentage of injected marker doses excreted to the environment in exercised and non-exercised rainbow trout*

Time after injection (h)	12	14	16	20	24	36
Experimental time (h)	0	2	4	8	12	24
Control group						
[^{14}C]DMO	11·61 ± 4·36% (10)	14·03 ± 4·29% (10)	16·44 ± 4·48% (10)	18·99 ± 4·59% (10)	24·16 ± 5·21% (10)	39·17 ± 9·68% (4)
[^{3}H]mannitol	14·00 ± 3·23% (10)	17·00 ± 4·02% (10)	18·78 ± 4·46% (10)	21·48 ± 4·51% (10)	22·99 ± 4·47% (10)	40·44 ± 11·91% (4)
Exercise group						
[^{14}C]DMO	10·45 ± 2·03% (8)	13·45 ± 2·19% (8)	15·13 ± 2·28% (8)	17·51 ± 2·28% (8)	22·06 ± 1·33% (8)	29·20 ± 0·83% (4)
[^{3}H]mannitol	13·27 ± 3·16% (8)	16·47 ± 2·95% (8)	17·42 ± 2·92% (8)	21·77 ± 4·19% (8)	23·54 ± 4·61% (8)	39·00 ± 6·86% (8)

There were no significant differences at any time between the control and experimental group.
Mean ± 1 S.E.M. (*N*).

Table 3. *Fluid volume distributions (uncorrected) (ml kg^{-1} body weight) in exercised and non-exercised rainbow trout*

	Time after exercise (h)								
Rest	0	0·25	0·5	1	2	4	8	12	24
Control									
235·2 ± 10·8	246·5 ± 12·1 (10)	254·2 ± 10·2 (10)	246·0 ± 6·8 (10)	257·2 ± 10·9 (10)	258·9 ± 8·3* (10)	277·8 ± 13·9* (10)	285·1 ± 10·5* (10)	299·9 ± 16·5* (10)	303·0 ± 28·4* (4)
Exercise									
253·5 ± 11·6 (8)	210·4 ± 14·1* (8)	201·9 ± 12·2* (8)	209·1 ± 9·6* (8)	231·8 ± 15·2* (8)	230·7 ± 7·2 (8)	249·9 ± 13·2 (8)	303·2 ± 19·3* (6)	315·2 ± 32·9* (6)	355·3 ± 18·6* (4)

* Significantly different ($P < 0·05$) from corresponding rest value.
Mean ± 1 S.E.M. (N).

increased by nearly 30% (Table 3). This apparent expansion of the extracellular space was due to gradual [^{3}H]mannitol penetration into the intracellular space of the liver, and occasionally heart, after lengthy equilibration; other tissues (brain, white muscle, red muscle) were unaffected (Milligan & Wood, 1986; C. M. Wood & S. Munger, unpublished results). In the exercised fish, calculated ECFV showed a significant decline for the first 2 h after exercise, but thereafter exhibited a gradual rise similar to that in the control group (Table 3). Clearly, the true post-exercise changes were masked by this artifact. In order to 'unmask' these changes, rest values (at 12 h post-infusion), which agreed with previous ECFV determinations (see above), were assumed to be correct. The mean increase over rest values in the control group (Table 3) was then subtracted from the exercise value at each respective time to yield the corrected ECFV values of Fig. 4A. These corrected values were then used in the calculation of mean whole body pHi (Fig. 5).

Blood acid–base and metabolite status

In the first series, exhaustive exercise resulted in a pronounced arterial blood acidosis, with pHa maximally depressed immediately after exercise (Fig. 1A). Blood acid–base status recovered relatively quickly, showing an alkalosis at 8 and 12 h post-exercise, and by 24 h recovery was complete. The initial acidosis was of mixed respiratory and metabolic origin, as indicated by the rapid elevation of Pa_{CO_2} (Fig. 1C) and slower depression of [HCO_3^-] (Fig. 1B). Analysis based upon the principles of Wood *et al.* (1977) revealed that immediately after exercise, the respiratory and metabolic components contributed equally to the acidosis. The respiratory component dissipated quickly, as P_{CO_2} returned to rest levels (Fig. 1C), so that by 2 h the metabolic component prevailed, as reflected in the continuing depression of plasma [HCO_3^-] (Fig. 1B). However, at 8 and 12 h, [HCO_3^-] was elevated above the resting level, causing the observed alkalosis at these times. Blood acid–base status in the control group was unaffected by sampling (Fig. 1A–C).

Blood lactate followed an entirely different pattern, rising slowly after exercise and peaking at about 17·5 mmol l^{-1} at 2–4 h, a time when blood pH was beginning to recover. Lactate levels then fell slowly, returning to rest values by 24 h (Fig. 1D). In the control group, [lactate] did not deviate significantly from resting levels.

Whole blood [glucose] rose by about 50% during the first hour after exercise and remained significantly elevated for 12 h (Fig. 2A). There were very much smaller elevations in the control group. [Haemoglobin] was elevated slightly up to 2 h after exercise, despite the dilution due to sampling which, in the control group, caused [haemoglobin] to decline significantly over this period (Fig. 2B). The sampling effect in the exercise group became evident at 4 h, when [haemoglobin] began to decline. Haematocrit initially rose by more than 50% after exercise, again, despite the diluting effect evident in the control group (Fig. 2C). By 8 h haematocrit fell as a consequence of continual sampling. Mean cell [haemoglobin] (MCHC), calculated as the ratio of [haemoglobin]/haematocrit, fell significantly after exercise, remaining depressed up to 2 h (Fig. 2D), which suggests swelling of the red cell and/or

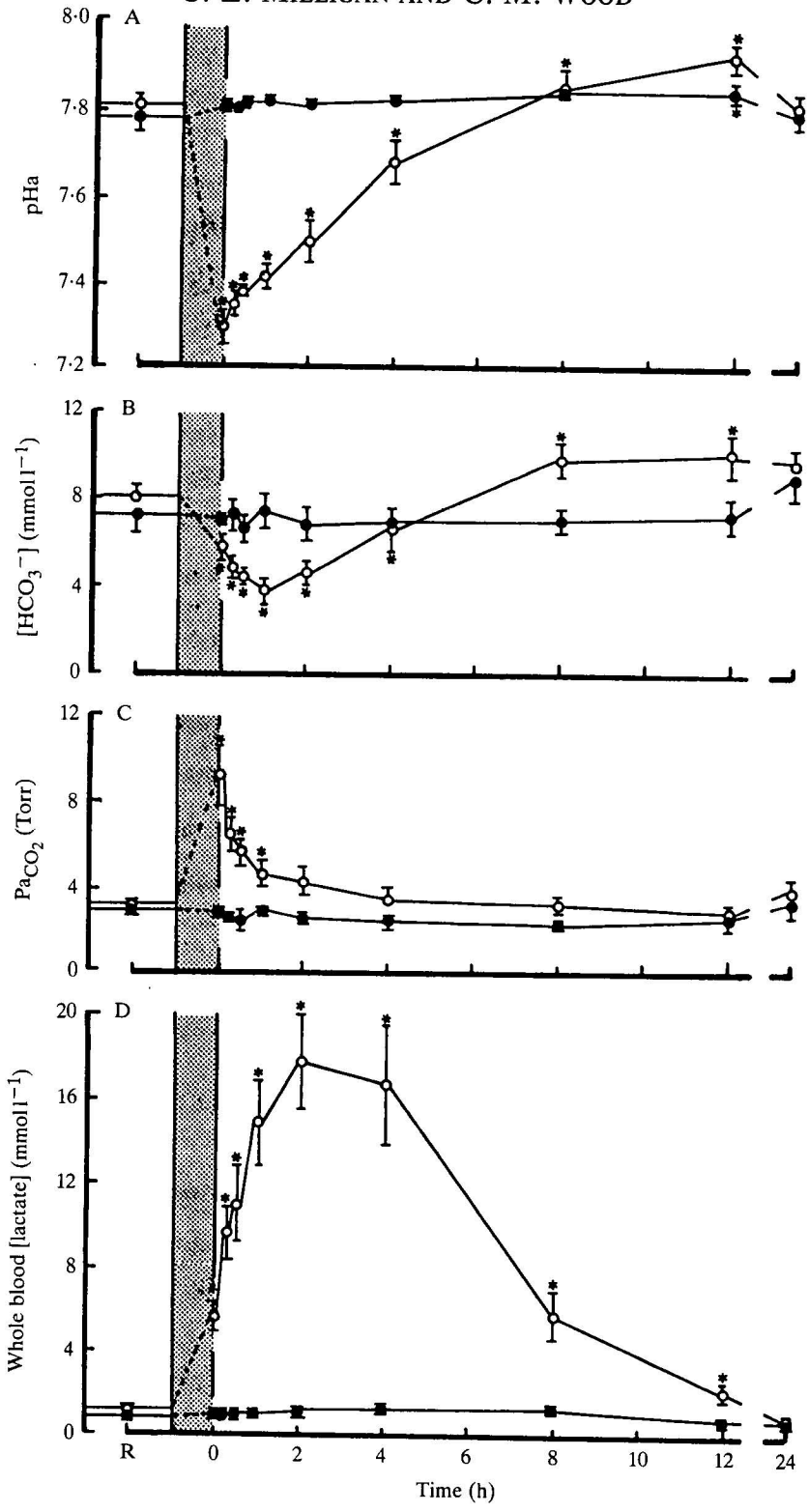
A
pHa
8·0
7·8
7·6
7·4
7·2
B
$[HCO_3^-]$ (mmol l^{-1})
12
8
4
0
C
Pa_{CO_2} (Torr)
12
8
4
0
D
Whole blood [lactate] (mmol l^{-1})
20
16
12
8
4
0
R
0
2
4
6
8
10
12
24
Time (h)

Fig. 1

recruitment of haemoglobin-poor reticulocytes. MCHC was unaffected by sampling, as it did not change in the control group (Fig. 2D).

In the second series, which compared arterial and venous responses (Table 4; Fig. 3), post-exercise changes in all arterial parameters followed the same trends as in the first series (Fig. 1). Minor quantitative differences (e.g. slightly smaller pHa depression and Pa_{CO_2} elevation, larger haematocrit declines) could have resulted from the larger fish size, greater blood sample volume, and the use of flow-through *versus* closed systems. At rest, arterial and venous pH, P_{CO_2}, and lactate were virtually identical, and the a–v difference in total CO_2 (reflected in HCO_3^-; Fig. 3B) was less than $1\,mmol\,l^{-1}$. After exercise, pHv closely followed pHa and was only significantly lower at 0 h (Fig. 3A). The a–v total CO_2 and HCO_3^- differences increased to approx. $2\,mmol\,l^{-1}$ for several hours after exercise (Fig. 3C). There were no significant differences at any time between arterial and venous lactate levels after exercise (Table 4).

At rest, RBC pHi values were 0·5 units below plasma pH (Fig. 3D). Despite the similarity of plasma pHa and pHv at rest (Fig. 3A), RBC pHi was significantly higher on the venous side. After exercise, which caused large drops in plasma pH, arterial RBC pHi did not fall and indeed tended to rise slightly, though non-significantly, later in recovery (Fig. 3D). In contrast, venous RBC pHi fell precipitously by about 0·25 units at 0 h followed by recovery to resting levels over a time course rather similar to that of the Pv_{CO_2} changes (Fig. 3C).

Whole body fluid volumes and intracellular pH

Whole-body ECFV (corrected as described above) fell by approximately 28 %, or about $70\,ml\,kg^{-1}$, after exhaustive exercise, reaching a minimum at 0·25 h and remaining significantly depressed until 8 h (Fig. 4A). As total body water did not vary significantly at any sample time (Milligan & Wood, 1986), this reflected a direct shift of fluid out of the extracellular into the intracellular compartment. The latter was therefore reciprocally expanded until 8 h (Fig. 4B).

Whole-body intracellular pH at each time in the exercise group was calculated using the corrected fluid volumes. In the control group, pHi at each time was calculated with the rest fluid volumes for each animal.

At rest, whole-body pHi in the exercise group of the first series did not differ from that of the control group, averaging about 7·25. In the control group, pHi remained stable throughout the experimental period (Fig. 5B). However, exercise resulted in a rapid, severe decline in pHi, to a low of $6{\cdot}78 \pm 0{\cdot}05$ at 0·5 h (Fig. 5A). Recovery of pHi was somewhat slower than that of pHe, for pHi had just recovered at 8–12 h, a time when pHe was overcompensated. Such an alkalosis was not observed in pHi.

Fig. 1. Blood acid–base status in exercised (○) and non-exercised (●) rainbow trout: (A) pHa; (B) arterial plasma bicarbonate concentration; (C) arterial CO_2 tension; (D) whole blood lactate concentration. Means ± 1 S.E.M. Control group: $N = 10$, except at 24 h, $N = 4$. Exercise group: $N = 8$, except at 8 and 12 h, $N = 6$ and at 24 h, $N = 4$. R = rest, bar indicates 6 min of exhaustive exercise, 0 = immediately after exercise; * indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

Table 4. *Simultaneously measured arterial and venous whole blood lactate (mmol l^{-1}) prior to and following exhaustive exercise*

	Time after exercise (h)								
Rest	0	0·25	0·5	1	2	4	8	12	24
Arterial									
2·29 ± 1·65 (5)	6·76 ± 1·44 (5)	10·00 ± 2·26* (5)	12·50 ± 2·14* (5)	13·85 ± 2·00* (5)	17·68 ± 2·01* (5)	17·11 ± 2·58* (5)	8·30 ± 1·66* (5)	3·96 ± 1·22* (4)	0·90 ± 0·40 (3)
Venous									
1·98 ± 1·32 (5)	6·32 ± 1·59* (5)	9·47 ± 1·67* (5)	12·14 ± 1·96* (5)	15·46 ± 1·90 (5)	17·66 ± 2·31* (5)	17·42 ± 2·83* (5)	7·67 ± 1·39* (5)	3·98 ± 1·05* (4)	0·92 ± 0·44 (3)

* Significantly different ($P \leq 0{\cdot}05$) from corresponding rest value. There were no significant differences at any time between arterial and venous levels.

Mean ± 1 S.E.M. (N).

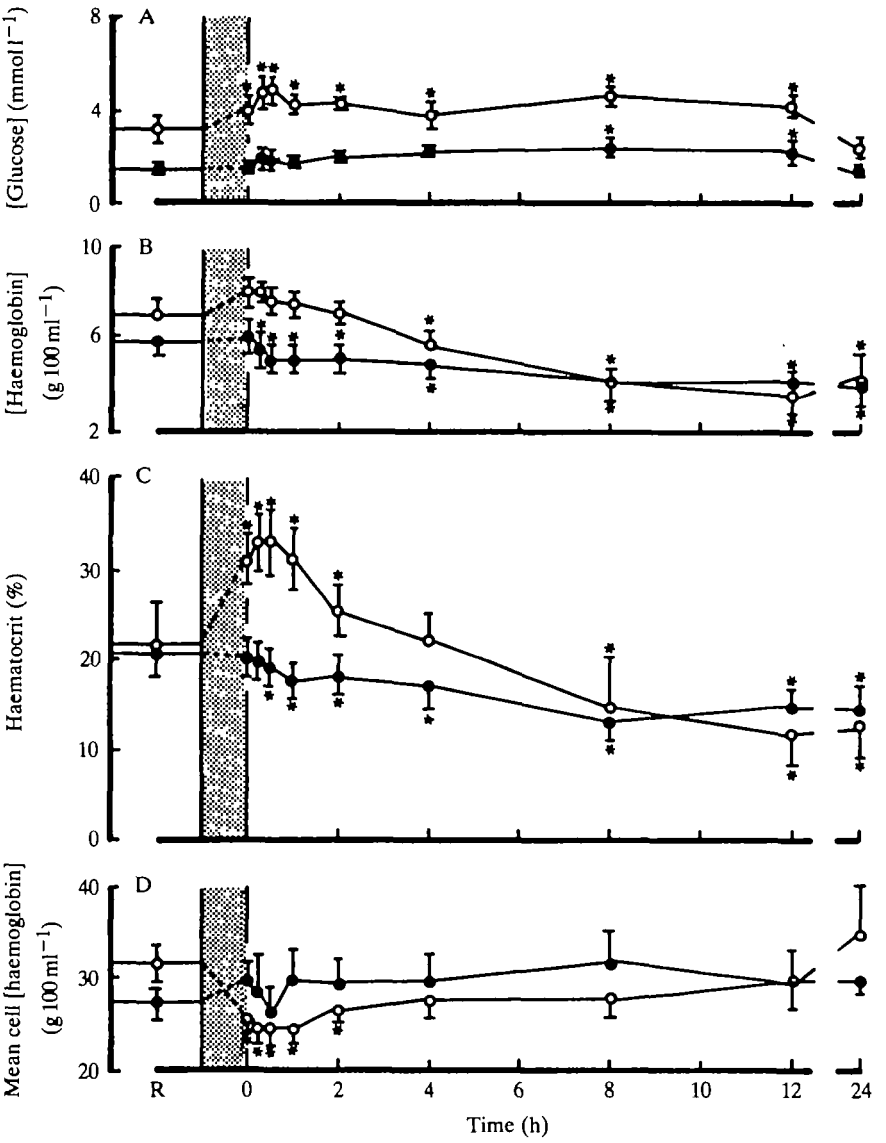

Fig. 2. (A) Blood [glucose], (B) [haemoglobin], (C) haematocrit and (D) mean cell [haemoglobin] in exercised (○) and unexercised (●) fish. Other details as in the legend of Fig. 1.

In theory, calculations of pHi should be based on interstitial fluid (ISF) as the representative ECF (Roos & Boron, 1981), though in practice this is impossible. The composition of ISF is probably between that of arterial and venous blood plasma. An index of the possible impact of this on the pHi measurements was obtained by recalculating the whole body pHi data of the first series using estimated venous values for ECF based on the mean a–v pH (Fig. 3A) and radiolabel differences (Table 2) of the second series. These differences tended to be self-compensating,

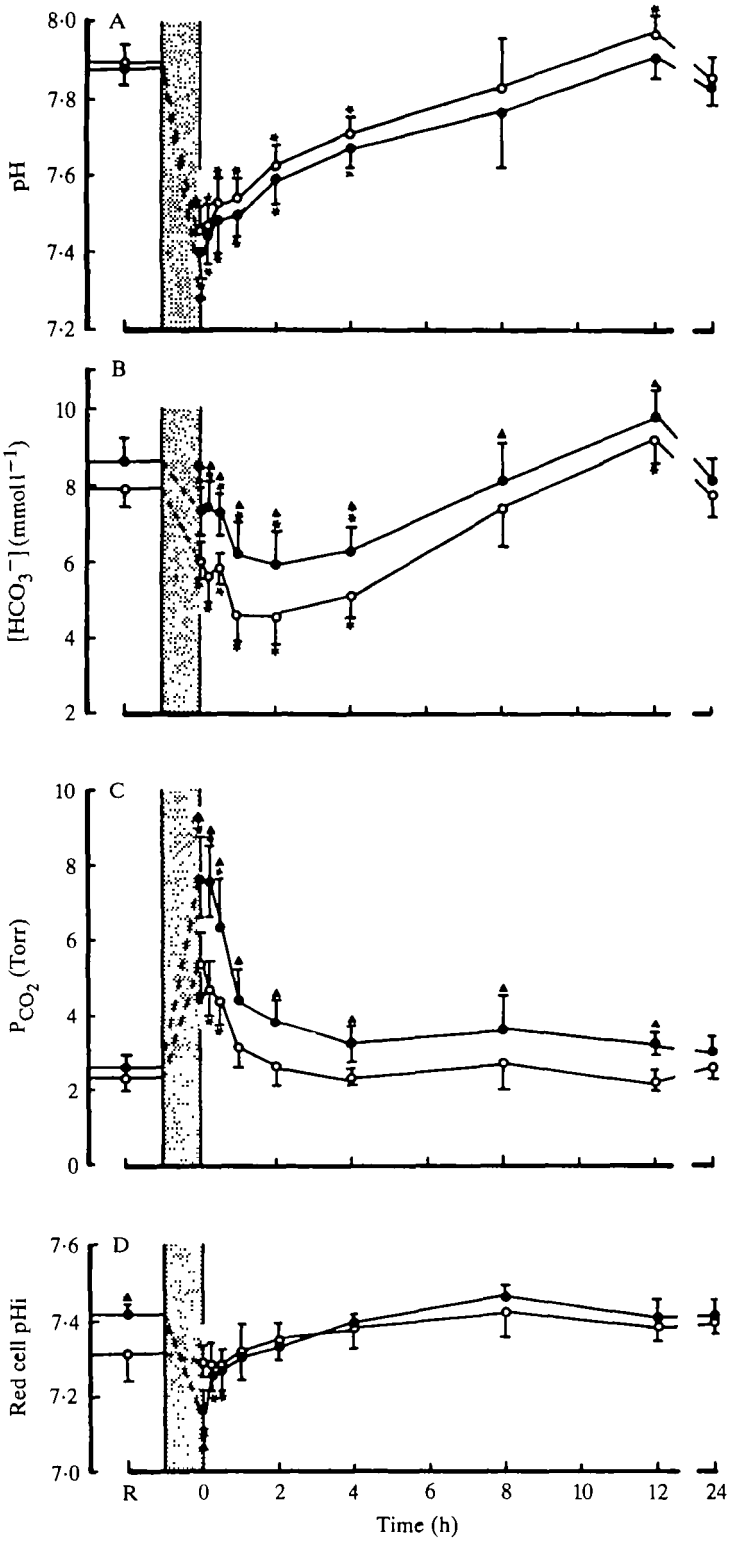
A
pH
8·0
7·8
7·6
7·4
7·2
B
$[HCO_3^-]$ (mmol l^{-1})
10
8
6
4
2
C
P_{CO_2} (Torr)
10
8
6
4
2
0
D
Red cell pHi
7·6
7·4
7·2
7·0
R
0
2
4
6
8
10
12
24
Time (h)

Fig. 3

with the result that mean whole pHi estimated from venous data was identical to that from arterial data at all times (Fig. 5A).

H^+ exchange with the environment

The methods used do not distinguish between acidic equivalent (H^+) excretion and basic equivalent uptake, or *vice versa*. Fortunately, this does not matter with respect to the acid–base status of the animal. When H^+ excretion or uptake is referred to, it could also be interpreted as basic equivalent uptake or excretion, respectively.

During the two 12-h periods prior to the experimental period, net H^+ exchange with the environment was similar in both the control and exercise group (Fig. 6A,D). Resting ammonia excretion just exceeded the titratable acidity uptake, resulting in a net H^+ flux of approx. $-30\,\mu equiv\,kg^{-1}\,h^{-1}$. This situation did not change appreciably during the experimental period in the control group (Fig. 6A–C). However, in the first hour following exercise, both titratable acidity uptake and ammonia excretion increased about fourfold; consequently, there was no change in net H^+ excretion (Fig. 6D–F). However, over the next 3 h, the situation changed; ammonia excretion remained significantly elevated, while titratable acidity uptake fell to control levels, resulting in a significant increase in net H^+ efflux. During the remainder of the experimental period, ammonia excretion returned to resting values. Titratable acidity uptake tended to increase leading to a trend towards net H^+ uptake from the environmental water, though the changes were not significant.

In the third series, excretion of lactate to the environment over the first 4 h after exercise was less than 1 % of the simultaneously measured net H^+ efflux (Table 5), despite the fact that these four fish showed rather higher net H^+ excretion rates than the general average (cf. Fig. 6D). Therefore, excretion did not contribute significantly to the clearance of lactate from the blood.

DISCUSSION

Blood acid–base changes

Exhaustive exercise led to a pronounced acidosis in arterial blood of mixed respiratory and metabolic origin (Fig. 1). These effects were very similar to those previously described by Turner *et al.* (1983*a*) and the same explanations probably apply, though it is now possible to account more fully for the Pa_{CO_2} elevation. A short-lived (approx. 1 h) respiratory acidosis following strenuous exercise is a phenomenon observed in all fish species examined to date (see Wood & Perry, 1985). This rise in P_{CO_2} has been considered a paradox, given that fish gills are thought to be

Fig. 3. Arterial (○) and venous (●) blood acid–base status prior to and after exhaustive exercise in rainbow trout. (A) pH; (B) plasma bicarbonate concentration; (C) CO_2 tension; (D) red blood cell pHi. Mean ± 1 S.E.M., $N = 5$, except at 12 h, $N = 4$ and at 24 h, $N = 3$. ▲ indicates venous values significantly different ($P \leq 0{\cdot}05$) from simultaneous arterial values. * Indicates a significant difference ($P \leq 0{\cdot}05$) from corresponding rest value.

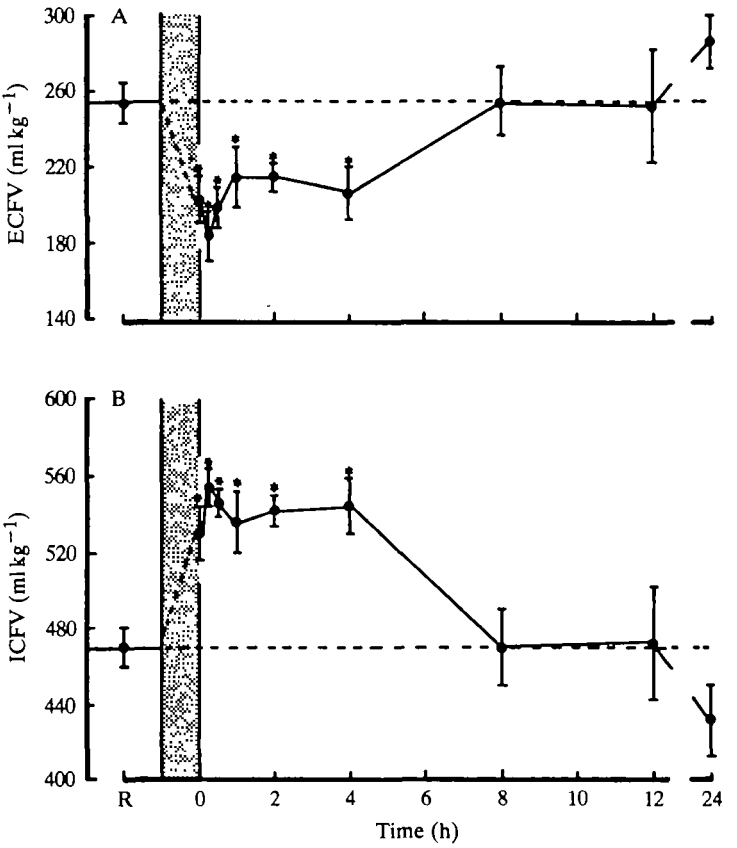

Fig. 4. Whole-body extracellular (A, ECFV) and intracellular (B, ICFV) fluid volumes prior to and following exhaustive exercise in the rainbow trout. Dashed line indicates the mean of the rest value. Other details as in the legend of Fig. 1.

hyperventilated with respect to CO_2 excretion. The rate-limiting step in CO_2 excretion in fish is thought to be HCO_3^- flux through the red cell (Perry, Davie, Daxboeck & Randall, 1982). Recently, it has been demonstrated *in vitro* that β-adrenergic stimulation inhibits HCO_3^- flux through trout red cells, thus leading to a reduction or inhibition of CO_2 excretion *in vivo* (Heming, 1984). Certainly, following exercise, the levels of circulating catecholamines increase (Mazeaud & Mazeaud, 1982). But how does the fish benefit from CO_2 retention? Since CO_2 is carried in the plasma mainly as HCO_3^-, which is the major non-protein buffer, Heming (1984) has proposed that CO_2 retention allows the benefits of HCO_3^- buffering to be shared by other tissues (e.g. heart and brain). It has also been suggested that the rise in P_{CO_2} and/or the associated fall in pHa may stimulate the post-exercise hyperventilation at a time when stimuli from other sources (e.g. arterial O_2 sensors, proprioceptors) would be minimal, thus allowing correction of the O_2 debt associated with anaerobic exercise (Wood & Perry, 1985).

While some information is available on venous acid–base changes associated with exhaustive exercise (Wood *et al.* 1977; Turner *et al.* 1983*b*; Schwalme & Mackay,

1985), the present study is the first to compare simultaneous arterial and venous measurements in detail. In general, the data (Fig. 3) indicate rather close similarity between arterial and venous changes, though with the expected larger P_{CO_2} elevations and pH depressions, and smaller HCO_3^- depressions, on the venous side. These differences undoubtedly reflected a greater CO_2 addition to the blood by the systemic tissues due to both aerobic metabolism and the titration of HCO_3^- reserves *via* 'metabolic acid' production, and the subsequent removal of this CO_2 at the gills. The slightly higher [^{14}C]DMO concentration in the arterial than venous plasma immediately after exercise (Table 2) was in accord with the slightly higher arterial pH, though it is not clear why [^{3}H]mannitol levels were similarly elevated. In any event, the data indicate that arterial *versus* venous measurement sites have negligible impact on the mean whole body pHi estimated by the DMO technique (Fig. 5A).

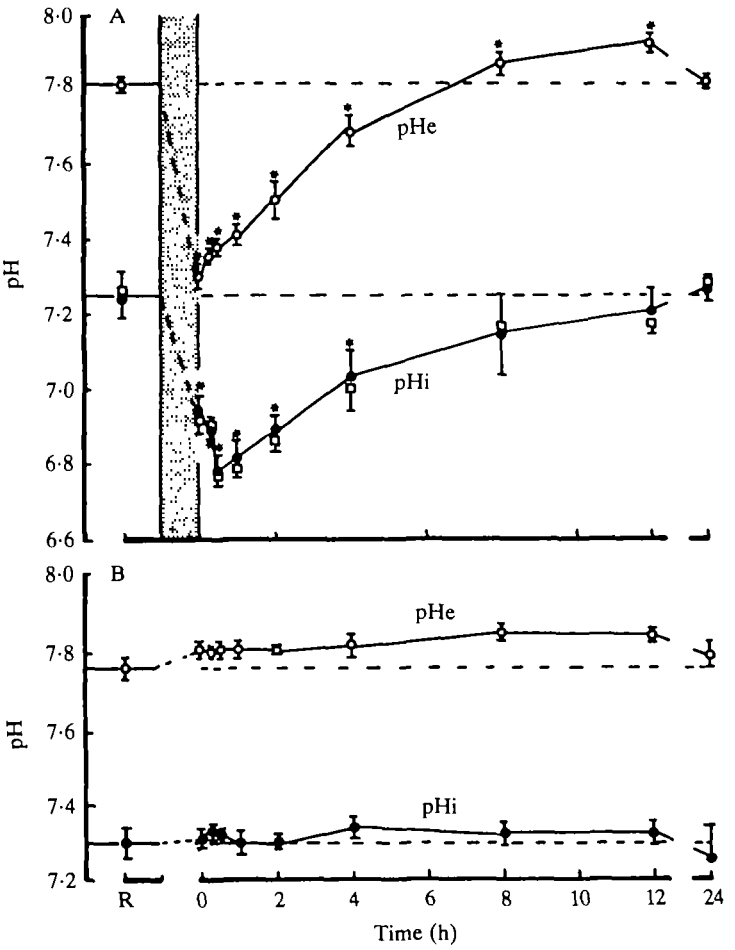

Fig. 5. Whole-body intracellular (pHi, ●) and extracellular (pHe, ○) pH in exercised (A) and control (B) fish. □ indicates pHi values calculated from venous data, see text for details. Other details as in the legend of Fig. 1.

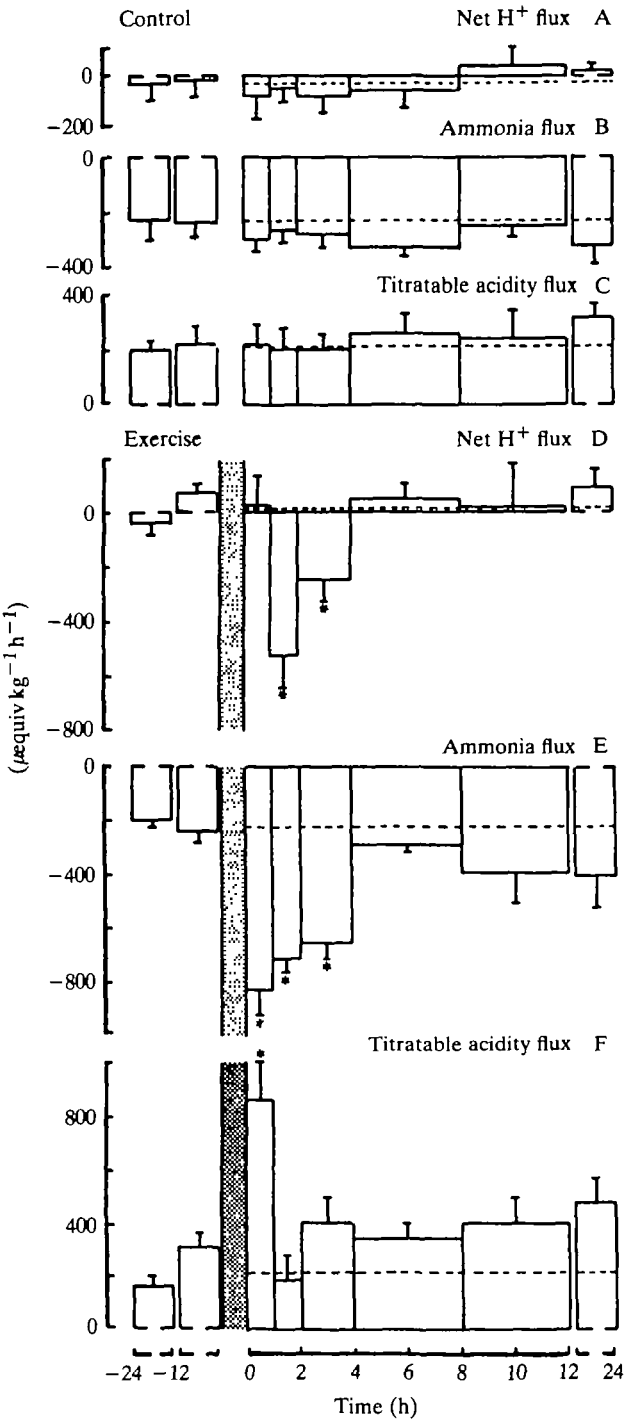

Fig. 6. Net H^+, ammonia and titratable acidity flux in control (A,B,C) and exercised (D,E,F) fish, respectively. −24, −12 h refer to two 12-h periods prior to the experimental period. Dashed line indicates mean of the two pre-experimental flux periods. Other details as in the legend of Fig. 1.

Finally, the data (Table 4) did not substantiate the claim of Driedzic & Kiceniuk (1976) that lactate is removed from the blood by the gills, though these workers were similarly unable to demonstrate any significant a–v lactate difference. Certainly, there was very little excretion of lactate into the water (Table 5).

Blood lactate and 'metabolic acid' loads

After exercise, there was a severe acidosis in the whole body intracellular compartment (Fig. 5), which reflected, to a large extent, changes occurring in the white muscle mass (Milligan & Wood, 1986). Both H^+ and lactate, the major end products of anaerobic metabolism, are released from the muscle after exercise, the former causing the metabolic acidosis. Lactate and H^+ efflux appear to be governed by different factors, for the 'metabolic acid' load in the blood peaked immediately post-exercise, whereas the lactate load continued to rise, not peaking until about 2 h into recovery (Fig. 7). In addition, the lactate load appeared in excess of the H^+ load from about 1 h onwards, reflecting differential rates of release and/or removal. However, up until this time (1 h post-exercise), net H^+ excretion into the environmental water remained at resting levels (essentially zero; Fig. 6D), so the results tend to support the hypothesis of differential release of H^+ and lactate anion from the muscle, as earlier proposed (Turner *et al.* 1983*a*; Turner & Wood, 1983). Turner *et al.* (1983*a*) have discounted differential uptake of H^+ over lactate by tissues as a contributing factor to the H^+ *versus* lactate differential in blood, since after lactic acid infusion the rates of lactate and H^+ removal were equal. Furthermore, evidence is presented in the accompanying paper (Milligan & Wood, 1986) which suggests that lactate is taken up by tissues (heart and liver) without any accompanying H^+ which, if anything, would tend to cause an excess of H^+ *over* lactate in the blood, the reverse of the observed pattern. The efflux of H^+ is thought to be a passive process, subject to 'equilibrium limitations', which include such factors as pH gradient between the intra- and extracellular compartment and extracellular buffer capacity (Holeton & Heisler, 1983; Turner & Wood, 1983). The transfer of lactate is somewhat more complex, for the relative movements of the dissociated and undissociated forms are unknown. If lactate moved as the free acid, the efflux would be subject to the same limitations as H^+ (e.g. pH, Mainwood & Worsley-Brown, 1975). If, as appears to be the case, lactate moves as the free anion, perhaps with the involvement of a carrier (Hochachka & Mommsen, 1983; Turner & Wood, 1983*)*, then its movement may be independent of the movement of H^+. This is an intriguing problem that deserves further inquiry.

Table 5. *Lactate and net H^+ excretion in the 4 h following exhaustive exercise in the rainbow trout*

	0–2 h	2–4 h
Lactate (μequiv kg^{-1} h^{-1})	6·08 ± 2·99	4·42 ± 1·08
Net H^+ (μequiv kg^{-1} h^{-1})	865·8 ± 223·8	693·2 ± 216·6

Mean ± 1 S.E.M. ($N = 4$).

Red blood cell pHi

The RBC pHi results cast some new light on the functional role of the red cell (Fig. 3D). At rest, the pHi of venous erythrocytes was considerably higher than that of arterial cells, despite similar plasma pHe values. This was probably due to the Haldane effect, whereby relative deoxygenation of venous blood raised the H^+-binding capacity of the haemoglobin. The phenomenon has not previously been observed *in vivo* in fish, but has been demonstrated *in vitro* in sheatfish blood (Albers, Götz & Welbers, 1981). After exhaustive exercise, arterial pHi remained stable in the face of a large plasma acidosis. Similar results have recently been reported in both striped bass (Nikinmaa, Cech & McEnroe, 1984) and rainbow trout (Primmet, Randall, Mazeaud & Boutilier, 1986) and have been attributed to a β-adrenergic influence of catecholamines mobilized into the blood by exercise stress. Catecholamines potentiate red cell swelling and increase RBC pHi, especially at low pHe, and thereby help maintain the O_2 affinity and capacity of the haemoglobin in the face of extracellular acidosis (Nikinmaa, 1982, 1983; Nikinmaa *et al.* 1984; Heming, 1984). While this effect clearly helps sustain O_2 loading at the gills, the question has been raised whether in fact it would impede O_2 unloading at the

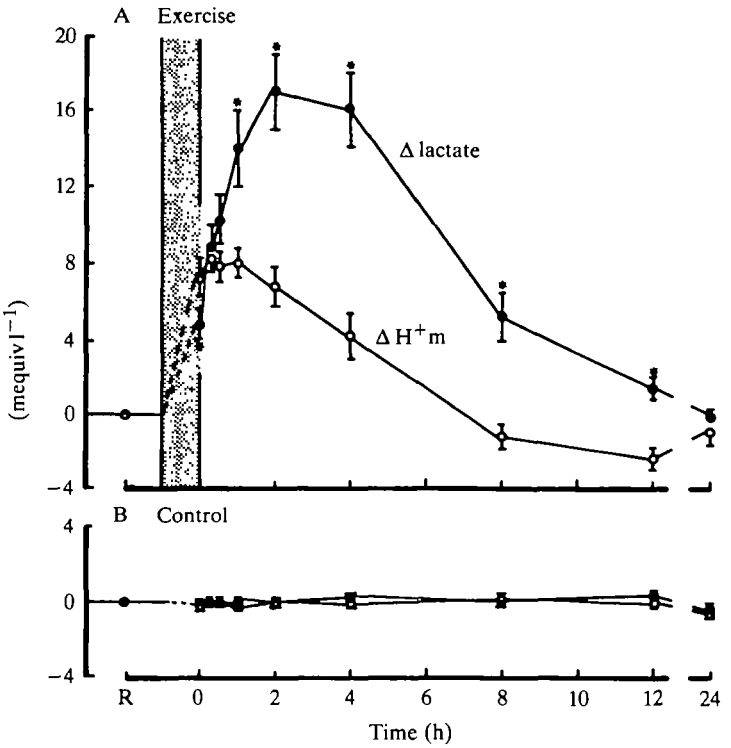

Fig. 7. Changes in blood lactate (Δlactate, ●) and blood metabolic acid load (ΔH^+m, ○) during recovery from exercise, see text for details of calculation. (A) Exercise group, (B) control group; * indicates a significant difference ($P < 0{\cdot}05$) from ΔH^+m. Other details as in the legend of Fig. 1.

systemic tissues (Nikinmaa, 1982). The present data suggest that the Root and Bohr effects still operate effectively at the tissues, because venous RBC pHi dropped precipitously after exercise. It is not clear why venous pHi was not regulated to the same extent as arterial, but it may be related to the much larger elevations in P_{CO_2} on the venous side (Fig. 3C) which surpassed some regulatory threshold. This is not to say that there was no pHi regulation in the venous RBCs. Based on a recently established *in vitro* relationship for trout blood equilibrated to varying P_{CO_2} at 15 °C (pHi = 0·73pHe+1·74; Milligan & Wood, 1985), venous pHi, which fell by 0·25 units *in vivo* at 0 h, would have declined by 0·36 units had there been only passive physicochemical buffering. Arterial pHi, which did not change *in vivo*, would have fallen by 0·32 units.

Whole-body pHi

In fish, whole-body pHi reflects to a large extent that of the white muscle, though is biased upwards by tissues with higher pHi, such as liver, brain and heart (Cameron & Kormanik, 1982). The resting whole-body pHi estimates in the present study (Fig. 5), were similar to those measured in rainbow trout by Hōbe *et al.* (1984). After exercise, the drop in pHi was of similar magnitude to that observed in white muscle and followed a similar time course of recovery (Milligan & Wood, 1986), though again, the absolute values were biased upwards.

The accuracy of the estimate of pHi in a dynamic situation is dependent upon full equilibration of DMO between the intracellular and extracellular compartments. In trout white muscle perfused *in vitro*, we have shown that full equilibration is attained within 15 min of a step change in pH (Milligan & Wood, 1985); shorter periods were not tested. Thus, the reliability of the present 0 h pHi estimate, obtained immediately after the 6-min exercise period, cannot be assessed, though some qualitative conclusions can be drawn. The [DMO]i/[DMO]e distribution ratio (of equation 6) significantly increased after exercise. Thus there was a net movement of DMO from extracellular to intracellular compartments. If, at time 0, this re-equilibration was *not* complete, then the pHi estimate at this time would have been too low, and the magnitude of the true pHi change would have been *overestimated*. The disappearance of this disequilibrium during longer recovery periods (1–2 h) would be expected to increase calculated pHi substantially. The fact that this did not occur (Fig. 5) suggests that disequilibrium, if it occurred at all, was not a serious problem, a conclusion substantiated by studies on white muscle (Milligan & Wood, 1986). It seems likely that true pHi undergoes continuous rather than step changes *in vivo*, so some blurring of the transients is likely with the DMO method.

Fluid volume distribution

Resting ECFV in both the control and exercise groups (Fig. 4) was similar to values previously reported for the rainbow trout (Milligan & Wood, 1982; M. V. E. Attygalle, G. Shelton & P. C. Croghan, in preparation). Exercise resulted in a relatively long-lived (approx. 4 h) contraction of the extracellular space, and as there was no change in total body water (Milligan & Wood, 1986), the intracellular space

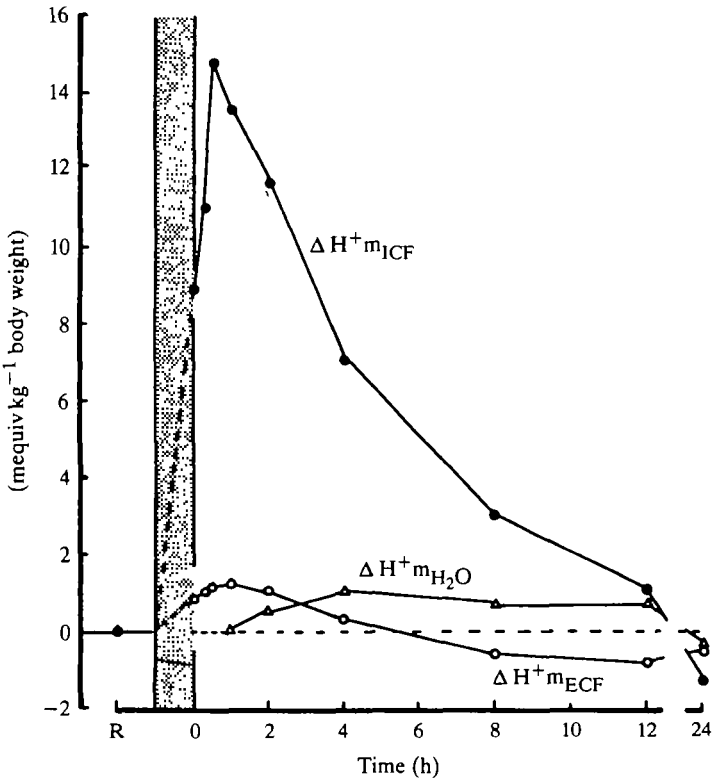

Fig. 8. Distribution of 'metabolic acid' load between the intracellular (ΔH^+m_{ICF}, ●), extracellular compartment (ΔH^+m_{ECF}, ○) and environmental water ($\Delta H^+m_{H_2O}$, △) following exhaustive exercise in trout, see text for details of calculation. R = rest, bar indicates 6 min of exhaustive exercise, 0 = immediately after exercise.

exhibited a reciprocal expansion. The breakdown of glycogen to the osmotically more active lactate within the muscle was undoubtedly an important contributor to the water shift. A significant reduction in white muscle ECFV and expansion of white muscle ICFV has been observed after exercise in rainbow trout (Milligan & Wood, 1986). M. V. E. Attygalle, G. Shelton & P. C. Croghan (in preparation) report a similar, though much shorter-lived, reduction in ECFV in trout after exercise to fatigue in a water tunnel. However, this was followed by a significant expansion of the ECFV at 2–8 h into recovery, a phenomenon which did not occur in the present study. These authors attribute this expansion to a net influx of water across the gills combined with a reduction in urine flow rate. Under the conditions of our experiments, urine flow remained elevated until 12 h after exercise (C. M. Wood & M. S. Rogano, unpublished results). A possible reason for the difference between the present study and that of M. V. E. Attygalle, G. Shelton & P. C. Croghan (in preparation) is that, in the latter study, fish were swum during recovery while, in the present study, fish were sedentary.

The reduction in ECFV resulted in a pronounced haemoconcentration, as indicated by the increases in [haemoglobin] and haematocrit (Fig. 2). Other factors

which probably also contributed to the rise in haematocrit include red cell swelling in response to P_{CO_2} and catecholamine elevations, and mobilization of red cells from the spleen in response to adrenergic stimulation (Yamamoto, Itzawa & Kobayashi, 1980). The latter would also contribute to the rise in [haemoglobin].

Whole blood [glucose] followed a different pattern from [haemoglobin] or haematocrit after exercise, suggesting that factors other than haemoconcentration were involved, such as mobilization of glucose from glycogen reserves in the liver (Driedzic & Hochachka, 1975).

Mechanism of H^+ exchange with the environment

After exercise, trout exhibited large increases in both ammonia and acidic equivalent excretion to the environmental water (Fig. 6). By analogy with the results of Holeton *et al.* (1983), the major portion of both fluxes would have occurred at the gills rather than at the kidney. However, it must be noted that there were important differences between the experimental conditions of the two studies (see below). The increased ammonia excretion post-exercise probably resulted from increased production within the muscle, as fish white muscle is capable of anaerobic ammonia production (Driedzic & Hochachka, 1976).

Whether the animal exploited this increased ammonia production for acid–base regulation is unclear, as relative excretions of NH_3 and NH_4^+ could not be determined with the present methods. Recently, Wright & Wood (1985) have examined ammonia excretion in resting trout under conditions very similar to those of the present study (i.e. same water quality, pH approx. 8·0, same flux conditions) and have concluded that both NH_3 diffusion and NH_4^+/Na^+ exchange occur, with the latter clearly dominant. They have furthermore suggested that the relative contribution of NH_3 and NH_4^+ to ammonia excretion in rainbow trout is flexible, dependent upon such factors as water pH, [ammonia] and, perhaps, acid–base status of the fish. The present data suggest that ammonia was excreted in both forms (Fig. 6). In the first hour after exercise, the elevation in titratable acidity uptake nearly equalled that in ammonia flux, suggesting that NH_3 excretion predominated, while over the next 3 h, titratable acidity uptake declined with ammonia excretion still elevated, suggesting that NH_4^+ excretion predominated and served as a route of acidic equivalent excretion. This conclusion differs from that of Holeton *et al.* (1983), who argued that NH_3 excretion predominated throughout after exhaustive exercise in trout, and that acidic equivalent excretion was achieved largely by H^+/Na^+ exchange. However, it must be noted that the lower water pH (approx. 7·4) in their study would have favoured NH_3 diffusion, and that again the method used was incapable of absolutely separating NH_3 and NH_4^+ movements.

It has been claimed previously that lactate transfer to the environmental water does not occur after exhaustive exercise in fish (Holeton & Heisler, 1983; Holeton *et al.* 1983). However, by increasing the sensitivity of the assay 100-fold by freeze-concentration of water samples, we have been able to demonstrate a very small lactate transfer (Table 5). This amounted to less than 1 % of the simultaneous H^+ transfer

and only about 2% of the total blood lactate load. Thus it is clear that the excretion of H^+ as free lactic acid was negligible, and that lactate excretion made almost no contribution to the final disposition of the lactate burden. It is likely that at least some of this small loss occurred at the kidney, as the elevated blood lactate levels after exercise would have surpassed the renal lactate threshold (Kobayashi & Wood, 1980).

H^+ distribution between ECF, ICF and the environment

The data presented allow construction of a 'metabolic acid load' (ΔH^+m) budget to the three compartments: intracellular, extracellular and environment (Fig. 8). Analysis of ΔH^+m in the ECF was calculated as described in Materials and Methods. Calculation of ΔH^+m in the entire ICF was complicated by the fact that the β value for this compartment is unknown. However, ΔH^+m in the ICF was calculated by using the β value of white muscle, $-51{\cdot}3\,mmol\,pH^{-1}\,kg^{-1} = -73{\cdot}6\,mmol\,pH^{-1}\,l^{-1}$ ICF (Milligan & Wood, 1986) and the calculated mean whole-body pHi changes of Fig. 5 and assuming that the bulk of intracellular buffer capacity is in the white muscle, contributions from other tissues being negligible. Muscle was assumed to occupy 60% of the body mass (Stevens, 1968). Therefore this estimate of ΔH^+m in the ICF will tend to err on the conservative side. On the other hand, if the estimates of whole-body pHi changes are in error due to DMO disequilibrium, then the absolute value of ΔH^+m in the ICF will also be in error. However, given that the error in pHi is probably small, as previously discussed, this would translate into a small overestimate of ΔH^+m in the ICF. These errors will not greatly affect the interpretation of the ΔH^+m budget. H^+ loading to the environment was calculated assuming that pre-exercise excretion rates represented basal production. The mean of the two pre-exercise periods was then subtracted from the means of subsequent post-exercise periods to estimate exercise-induced H^+ excretion.

Analysis of ΔH^+m distribution (Fig. 8) revealed that the bulk of the H^+ load remained in the intracellular compartment at all times during recovery with only small amounts transferred to the ECFV or transiently 'stored' in the water. Thus, the results do not support the contention that the major portion of the H^+ load is stored in the water during the later stages of recovery, thereby allowing full correction of intracellular acidosis prior to final lactate metabolism (cf. Heisler, 1982; Holeton & Heisler, 1983; Holeton *et al.* 1983). However, in the studies of Heisler and co-workers, there occurred a fourfold greater H^+ load to the environment, despite a post-exercise blood acid–base status similar to that of the present study. The reason for this difference may be related to the type of exercise, electrical stimulation (Holeton *et al.* 1983) *versus* manual chasing (present study), or water quality. Indeed, less than 10% of the 'metabolic acid' initially produced by exhaustive exercise in the present study was transferred to the ECFV and then subsequently stored in the water. This H^+ excretion to the environment appeared to expedite the recovery of the extracellular compartment and was associated with the metabolic H^+ deficit (or metabolic alkalosis) in the ECFV at 8–12 h. After 24 h of recovery, all H^+ excreted to the water had been taken back up by the fish, thus correcting the

extracellular alkalosis. In contrast, clearance of H^+ from the intracellular compartment was not by export, but rather dependent upon aerobic metabolism, a point which is dealt with in detail by Milligan & Wood (1986).

In summary, the present data suggest that in rainbow trout, correction of the post-exercise extracellular acidosis takes priority over the intracellular compartment, perhaps as a means of maintaining adequate blood O_2 transport once the protective effect of the catecholamine surge on RBC pHi has declined.

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