J. exp. Biol. **154**, 491–507 (1990)
Printed in Great Britain © *The Company of Biologists Limited 1990*

CONTROL OF RED BLOOD CELL METABOLISM IN RAINBOW TROUT AFTER EXHAUSTIVE EXERCISE

BY CHRIS M. WOOD*, PATRICK J. WALSH†, SERGE THOMAS‡ AND STEVE F. PERRY

Department of Biology, University of Ottawa, 30 George Glinski, Ottawa, Ontario, Canada K1N 6N5

Accepted 20 June 1990

Summary

Metabolic responses (rates of CO_2 production from ^{14}C-labelled glucose or lactate, and total O_2 consumption) of red blood cells were monitored in rainbow trout (*Oncorhynchus mykiss*) at rest and during 12 h of recovery from exhaustive exercise. Extracellular acid–base status, red blood cell intracellular pH (pHi), and plasma metabolite and catecholamine levels were recorded simultaneously. Despite a post-exercise rise in plasma glucose level, glucose oxidation was depressed, at least partly because of a rise in plasma lactate level. However, lactate oxidation was stimulated markedly, especially at 0–2 h post-exercise. Subsequent multifactorial experiments *in vitro* demonstrated that augmentation of lactate oxidation was due partly to increased plasma lactate, and partly to separate stimulatory effects of elevated P_{CO_2} and catecholamine levels. Changes in pH and HCO_3^- level were not directly involved, but the stimulatory effects of catecholamines occurred only under acidotic conditions. Total red cell O_2 consumption (M_{O_2}) remained generally stable after exercise. Similar multifactorial experiments *in vitro* demonstrated that respiratory, metabolic and mixed acidoses all inhibited M_{O_2}, an effect largely attributable to the lowered pH. This inhibition was reversed by typical post-exercise levels of epinephrine and norepinephrine; again, catecholamines had no effect under control conditions. Red cell pHi regulation was achieved without an increase in M_{O_2} above resting levels. Our results indicate a complex sensitivity of red cell metabolism to acid–base status and a shift in substrate preference for oxidation after strenuous exercise. The mobilization of catecholamines plays an important coordinating role and helps sustain

* Present address: Department of Biology, McMaster University, 1200 Main St West, Hamilton, Ontario, Canada L8S 4K1.

† Present address: Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA.

‡ Present address: CNRS, Laboratoire de Physiologie Animale, Faculté des Sciences et Techniques, Université de Bretagne Occidentale, 6 Avenue Victor Le Gorgeu, F-29283 Brest, France.

Key words: *Oncorhynchus mykiss*, red blood cells, metabolism, exercise, catecholamines, acid–base status, lactate, glucose, oxygen consumption.

normal rates of oxidative metabolism by red cells in the face of post-exercise blood acidosis.

Introduction

In teleost fish, the red blood cell (RBC) is not only the vehicle that carries oxygen to the tissues but it is also a significant consumer of this oxygen (Hunter and Hunter, 1957; Eddy, 1977). Indeed, it has been shown recently that aerobic metabolism accounts for more than 90 % of resting nucleoside triphosphate (NTP) production in salmonid RBCs (Ferguson and Boutilier, 1988, 1989; Ferguson *et al.* 1989). These workers demonstrated a close coupling between aerobic metabolism, maintenance of intracellular NTP levels and regulation of intracellular pH (pHi). Thus, in response to stressful situations such as strenuous exercise, the energy demand of the RBC may increase above resting levels in association with adrenergic activation of RBC Na^+/H^+ exchange. This mechanism serves to regulate RBC intracellular pH and thereby maintain blood O_2 transport capacity in the face of extracellular acidosis (Primmett *et al.* 1986; Milligan and Wood, 1987). In addition to elevated plasma catecholamine levels, a variety of other variables can potentially affect RBC metabolism after strenuous exercise. These include altered blood pH, P_{CO_2} and HCO_3^- levels (typically a mixed respiratory/metabolic acidosis occurs), as well as altered plasma concentrations of oxidative substrates, in particular greatly elevated plasma lactate (Wood and Perry, 1985). Pervasive direct actions of acid–base variables and catecholamines on the metabolism of hepatic cells in salmonids have been demonstrated recently (Walsh *et al.* 1988; Moon *et al.* 1988; Mommsen *et al.* 1988; Wright *et al.* 1989). Therefore, it seems likely that flux through metabolic pathways of RBCs could be subject to similar perturbation during acid–base stress and adrenergic pHi regulation, though nothing is known on this point.

The preceding paper described the basic metabolic pathways present in trout RBCs, developed *in vitro* methods for assessing their oxidative metabolism under defined acid–base conditions, identified lactate and glucose as important oxidative substrates and characterized the kinetic relationships involved (Walsh *et al.* 1990). The goal of the present study was to extend this experimental framework to an analysis of RBC metabolism *in vivo* after strenuous exercise in the rainbow trout *Oncorhynchus mykiss*. We first assessed the conditions present in the bloodstream (acid–base status, catecholamines, substrate levels) and the oxidative metabolism of the resident RBCs (substrate oxidation rates, total O_2 consumption rates) using blood withdrawn from catheterized fish at rest and at selected times after exhaustion. During the measurements, the RBCs were incubated *in vitro* under conditions closely duplicating those monitored *in vivo*. Subsequently, we established the mechanisms of exercise-induced alterations in metabolism by simulating the various altered parameters both singly and in combination. When combined with the kinetic relationships determined in the preceding paper (Walsh *et al.*

1990), this multifactorial modelling approach allowed an assessment of the independent and integrated effects of catecholamines, substrate levels and the various components of acid–base status on RBC metabolism.

Materials and methods

Animals and surgical preparation

Rainbow trout (*Oncorhynchus mykiss*=*Salmo gairdneri*; 150–400 g) were obtained and held at 9–12 °C as described earlier (Walsh *et al.* 1990). All fish, whether employed experimentally in the exercise trials or used as a source of blood in the factorial modelling experiments, were fitted with indwelling arterial catheters (Soivio *et al.* 1972). Prior to sampling or exercise, the fish were allowed to recover for 24–48 h in darkened Perspex chambers served with aerated flowing tapwater at the experimental temperature (10±1 °C).

Exercise experiments

Trout were transferred, without air exposure, to a 500 l tank, and then exercised to exhaustion by vigorous chasing for 6 min (see Turner *et al.* 1983). At the end of exercise, fish were returned to their individual chambers and sampled at various times during recovery. Arterial blood samples (approximately 2.0 ml, 50 i.u. heparin ml^{-1}) were drawn anaerobically into ice-cold gas-tight syringes (Hamilton) at rest, at 0 h (immediately post-exercise, prior to return to the holding chamber), and at 1, 2, 4 and 12 h of recovery. Fish used for the rest samples were not subsequently exercised in case the removal of blood altered swimming performance. At other times, fish were sampled no more than twice, at intervals no shorter than 2 h, and the blood removed was replaced with an equal volume of non-heparinized Cortland saline (Wolf, 1963).

Blood samples were analyzed for arterial pH (pHa), RBC intracellular pH (pHi), true plasma total CO_2 content (Ca_{CO_2}), haemoglobin (Hb), haematocrit (Hct), plasma glucose, lactate, epinephrine and norepinephrine levels, and *either* RBC CO_2 production rates individually from lactate and glucose *or* total RBC O_2 consumption rate (M_{O_2}). Note that P_{CO_2} values were calculated and, if samples were not at equilibrium, it is possible that the 0 h post-exercise P_{CO_2} values may be slight overestimates. Not all parameters were measured on all samples. For the metabolic determinations, the samples were placed in 20 ml glass vials in volumes of either 700 μl (for CO_2 production measurements) or 1400 μl (for M_{O_2} measurements) and additionally heparinized (125 i.u.). The vials were placed in a shaking water bath at 10±1 °C and equilibrated for 15 min to the P_{CO_2} appropriate to that sample time, prior to the start of the rate measurement (see below). The open gassing system described by Walsh *et al.* (1990) was employed with the following humidified CO_2 mixtures (balance air) to maintain *in vivo* acid–base status: 0.25 %

CO_2 for rest, 4 h and 12 h samples; 1.00 % for 0 h samples, 0.50 % for 1 h samples; and 0.40 % for 2 h samples.

Factorial experiments

Blood was withdrawn from the arterial catheters of resting trout (2–5 ml per fish) as described by Walsh *et al.* (1990), care being taken to avoid struggling or disorientation of the fish. The blood was initially heparinized at 150 i.u. ml^{-1} and kept on ice for up to 30 min until use. For each run in a matrix experiment, blood from several different fish was pooled. The pooled blood was very lightly centrifuged (500 *g* for 60 s), an appropriate volume of plasma removed, and the RBCs resuspended in the remaining plasma to ensure a representative Hct (25–30 %) after addition of other solutions. Each pooled preparation was considered as one sample for statistical purposes. In the CO_2 production experiments, it was possible to run all treatments in the matrix simultaneously on each pool. This was not possible in the O_2 consumption experiments because of equipment constraints. Therefore, a slightly different protocol was used in which a subset of the matrix was examined in each pool, but the appropriate control was always included.

In the matrix experiments that examined CO_2 production from [U-^{14}C]L-lactate, the pooled blood was placed in 20 ml glass vials in 700 μl volumes, each receiving an additional 125 i.u. of heparin. The appropriate volume of a neutralized sodium lactate stock solution (140 mmol l^{-1}) was added to each vial to create a final nominal concentration of 5 mmol l^{-1}. Experimental factors were added by equilibration with elevated P_{CO_2} (for respiratory acidosis), and/or appropriate volumes of 140 mmol l^{-1} HCl (for metabolic acidosis), 140 mmol l^{-1} $NaHCO_3$ (for metabolic alkalosis), and a stock solution of 3×10^{-6} mol l^{-1} L-epinephrine bitartrate (Sigma) plus 3×10^{-7} mol l^{-1} L-norepinephrine bitartrate (Sigma) in 140 mmol l^{-1} NaCl (for elevated catecholamines). The final nominal concentrations of added catecholamines were 150 nmol l^{-1} epinephrine and 15 nmol l^{-1} norepinephrine. An appropriate amount of 140 mmol l^{-1} NaCl was added to each vial so that the total sample volume was always 850 μl. In the matrix experiments that examined total O_2 consumption, the treatments were identical, but the initial samples of blood were 1400 μl, so the volumes of all additions were doubled to yield final sample volumes of 1700 μl.

Once all the additions were complete, the vials were placed in a shaking water bath at 10±1 °C, and equilibrated for 30 min to the appropriate P_{CO_2} before the start of metabolism measurements. The open gassing system described by Walsh *et al.* (1990) was employed, using humidified mixtures of 0.25 % CO_2 (balance air) for normocapnia and 1.00 % CO_2 (balance air) for respiratory acidosis. CO_2 production or O_2 consumption rates were then determined as outlined below. At the end of all runs, measurements were made of blood pH (pHe), true plasma C_{CO_2}, Hb, Hct and cell mass, either on dummy vials or on samples recovered from the M_{O_2} measurement chamber. RBC pHi was also measured in some experiments.

O_2 consumption and CO_2 production determinations

For O_2 consumption measurements in both the exercise and factorial experiments, samples were prepared as outlined above, then transferred by gas-tight Hamilton syringe to an M_{O_2} measurement chamber thermostatted to 10±1 °C. In the factorial experiments, 5 mmol l^{-1} sodium lactate was added to all vials. The rate of O_2 consumption was measured over a 15 min period exactly as described by Walsh *et al.* (1990).

In the exercise experiments, CO_2 production rates were measured separately from [U-^{14}C]L-lactate (90–105 mCi mmol^{-1}; ICN) and [U-^{14}C]D-glucose (310 mCi mmol^{-1}; ICN) using the native substrate levels present in the blood, which were measured in both initial and final samples. In the factorial experiments, CO_2 production rates from [U-^{14}C]L-lactate were measured using nominal added concentrations of 5 mmol l^{-1} unlabelled sodium lactate; exact concentrations were measured in both initial and final samples. Methodology was identical to that described by Walsh *et al.* (1990) and gassing conditions and other experimental considerations have been outlined above. Experimental runs lasted 2 h, except in one case where a 30 min run was carried out on 0 h and 2 h post-exercise samples to ensure that transient events were not being missed. The results were the same as in the 2 h runs.

Analytical and statistical procedures

All analytical methods and calculations were identical to those described by Walsh *et al.* (1990). All values are presented as means±1 S.E.M. (N). The statistical significance ($P<0.05$) of differences was assessed using Student's two-tailed t-test, paired or unpaired format as appropriate to the design of the experiments. In the factorial experiments, a model III two-way analysis of variance (ANOVA) together with Student–Newman–Keuls and Duncan's new multiple-range test was employed (Zar, 1974).

We have chosen to report CO_2 production rates of RBCs from specific substrates in units of μmol CO_2 g^{-1} cell mass h^{-1}, the standard unit in the fish cell metabolism literature (e.g. French *et al.* 1981; Mommsen *et al.* 1988; Perry and Walsh, 1989; Walsh, 1989). In contrast, we have reported RBC O_2 consumption rates in terms of nmol O_2 g^{-1} Hb min^{-1}, the standard unit in the fish red cell respiration and acid–base literature (e.g. Ferguson and Boutilier, 1988; Ferguson *et al.* 1989). A reported advantage of the latter is that it corrects for changes in RBC mass occurring simply because of water flux, i.e. swelling and shrinking. The mean cell haemoglobin concentration (MCHC=Hb/Hct), which is often considered an index of this water flux (e.g. Milligan and Wood, 1987; Ferguson *et al.* 1989), was monitored in all experiments. While MCHC did change significantly as a result of some treatments, the absolute changes were never greater than 25 %, and would not have affected any of the basic trends in metabolism seen in either data set. At a typical MCHC of 0.25 g Hb ml^{-1} RBC, the conversion is 1 μmol g^{-1} cell h^{-1}=67 nmol g^{-1} Hb min^{-1}.

Results

Responses to exercise in vivo

Strenuous exercise in rainbow trout, using an established protocol (Turner *et al.* 1983; Milligan and Wood, 1986*a*,*b*, 1987), caused typical changes in blood acid–base status and catecholamine and metabolite levels (Figs 1,2,3). Arterial blood displayed a pronounced mixed respiratory/metabolic acidosis, as shown by an immediate elevation of Pa_{CO_2} and longer-lasting depression of plasma HCO_3^- concentration (Fig. 1A,B,C). The acidosis was greatest at 0– 1 h post-exercise,

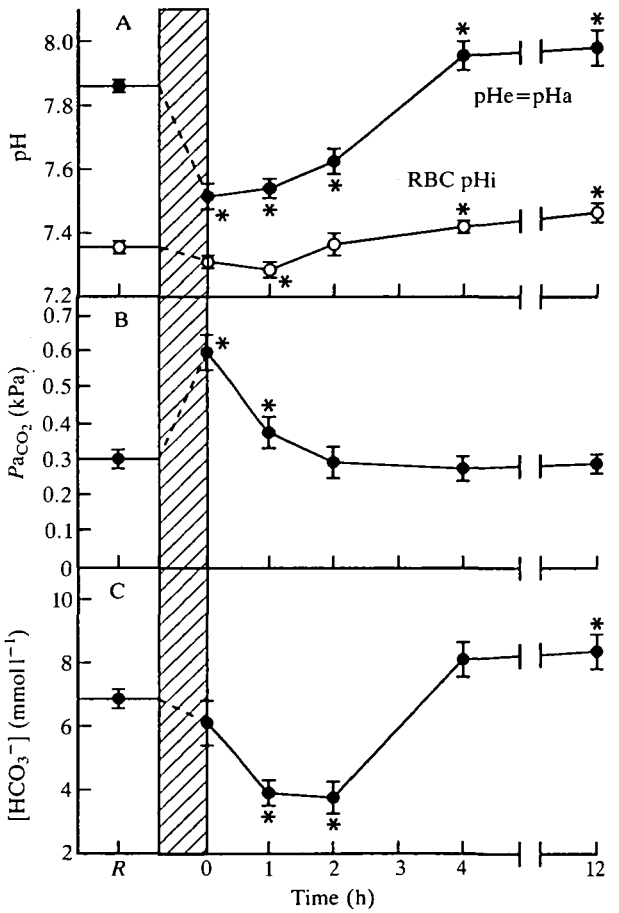

Fig. 1. The effects of exhaustive exercise and recovery on arterial blood acid–base status in rainbow trout: (A) blood plasma pHa (=extracellular pH, pHe) and red blood cell intracellular pH (RBC pHi); (B) CO_2 tension (Pa_{CO_2}); and (C) plasma bicarbonate concentration $[HCO_3^-]$. Means±1 S.E.M. *R* indicates control value at rest, shaded vertical bar indicates 6 min of exhaustive exercise, and 0 h is immediately post-exercise. *N* numbers at each sample time are: *R*=16–21, 0 h=11–20, 1 h=6–10, 2 h=6–15, 4 h=9–18, 12 h=9–19.* indicates a significant difference ($P<0.05$) from the corresponding rest value.

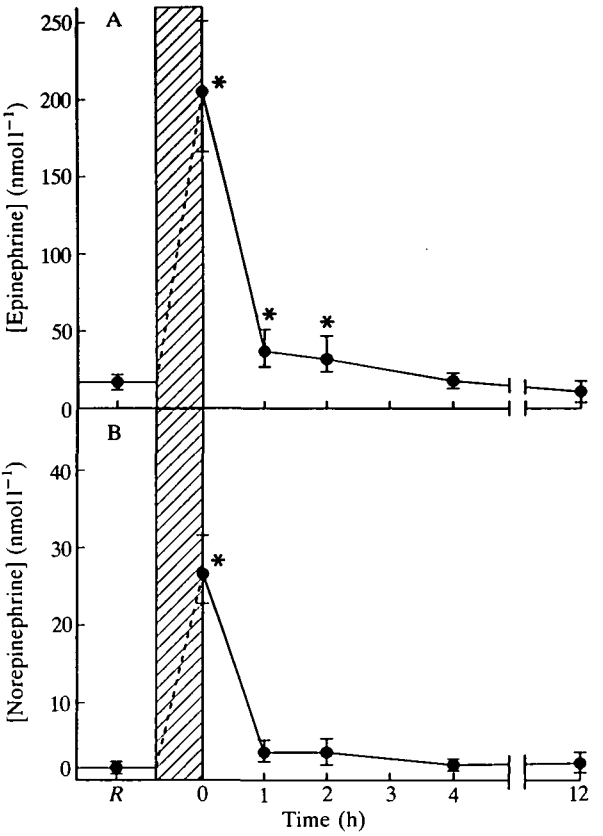

Fig. 2. The effects of exhaustive exercise and recovery on arterial plasma catecholamine levels in rainbow trout: (A) epinephrine; and (B) norepinephrine. Means ±1 S.E.M. The data were normalized by logarithmic transformation for averaging and statistical tests, resulting in asymmetrical S.E.M. bars. Other details as in legend of Fig. 1.

and had been corrected by 4 h with a slight alkalotic overshoot which persisted at 12 h. RBC pHi, in contrast, was extremely well regulated in the face of this extracellular acidosis. RBC pHi fell only marginally at 1 h (by 0.06 units relative to an extracellular drop of 0.32 units), was completely compensated at 2 h, and manifested a significant alkalosis at 4 and 12 h (Fig. 1A).

Plasma catecholamine levels were elevated dramatically at 0 h post-exercise, but declined rapidly thereafter (Fig. 2). Epinephrine was the dominant catecholamine both at rest and after exhaustive exercise, approximately 10-fold higher than norepinephrine, with a persistent slight elevation at 1 and 2 h. MCHC (not shown) fell significantly from 0.251±0.011 (16) g ml^{-1} at rest to 0.224±0.007 (15) g ml^{-1} at 0 h, indicating a slight swelling of the RBCs, but there were no other significant changes. Swelling may have been slightly lower than prior studies because the changes in P_{CO_2} and pHe were not as pronounced in these fish. Plasma lactate

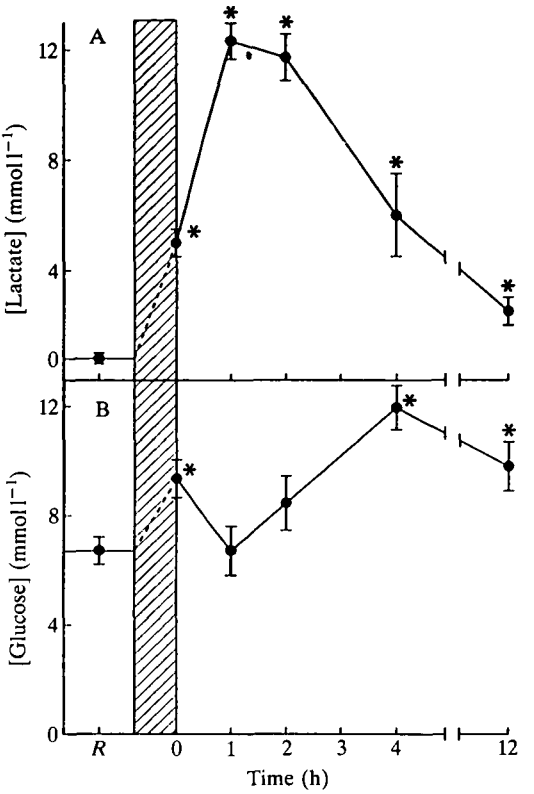

Fig. 3. The effects of exhaustive exercise and recovery on arterial plasma (A) lactate and (B) glucose concentrations in rainbow trout. Means±1 S.E.M. Other details as in legend of Fig. 1.

levels increased immediately post-exercise, reaching a peak of about 12 mmol l^{-1} at 1–2 h and declining thereafter; levels remained significantly elevated at 12 h (Fig. 3A). Plasma glucose levels exhibited a biphasic response (Fig. 3B) with a significant immediate rise at 0 h, a return to resting levels at 1 and 2 h, and a second elevation at 4 and 12 h. On both a relative and an absolute basis, these changes in glucose were much smaller than those in lactate.

Metabolic rates of the RBCs were measured directly on samples of this same blood under acid–base conditions closely approximating those *in vivo*. At rest, CO_2 production from glucose was approximately three times higher than from lactate, as reported by Walsh *et al.* (1990). However, this situation changed markedly after exercise (Fig. 4). Glucose oxidation was depressed by 30–50 %, despite the fact that plasma glucose levels stayed the same or increased (Fig. 3B). The depression became significant at 1 h and roughly coincided with the peak of plasma lactate (Fig. 3A). Glucose oxidation thereafter recovered to control levels at 4 and 12 h. CO_2 production from lactate, in contrast, increased by about 250 % immediately after exercise, and reached a peak 450 % increase at 2 h. By 12 h, lactate oxidation had returned to resting levels. Thus, at 1–2 h post-exercise,

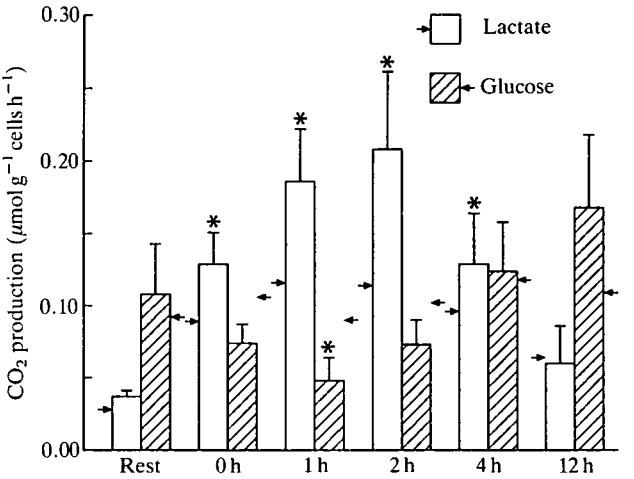

Fig. 4. The effects of exhaustive exercise and recovery on rates of CO_2 production from lactate and glucose by red blood cells of rainbow trout. Substrate concentrations were those native to the blood when sampled (measured in an initial and a final sample), and acid–base status was maintained close to levels measured *in vivo*. Arrows indicate the rates predicted from the effects of the measured changes in substrate concentrations only, based on the kinetic relationships established in Fig. 1 of Walsh *et al.* (1990). See text for additional details. Means±1 S.E.M. *N* numbers at each sample time are: rest=13, 0 h=10, 1 h=6, 2 h=6, 4 h=10, 12 h=6. * indicates a significant difference ($P<0.05$) from the corresponding rest value.

lactate oxidation was 3–4 times faster than glucose oxidation. These changes in lactate oxidation closely followed the changes in plasma lactate concentrations (cf. Fig. 3A). However, it is apparent that rates at 0, 1 and 2 h post-exercise were elevated substantially beyond the levels predicted by the measured plasma lactate concentrations (Fig. 3A) and the kinetic relationship established for resting blood (cf. Fig. 1 of Walsh *et al.* 1990, and present Fig. 4). Conversely, at these same times, rates of CO_2 production from glucose were depressed below the levels predicted by the measured plasma glucose concentrations (Fig. 3B) and the comparable kinetic relationship for glucose oxidation by resting blood (cf. Fig. 1 of Walsh *et al.* 1990, and present Fig. 4).

Despite these large variations in blood chemistry and in rates of glucose and lactate oxidation after exhaustive exercise, rates of total O_2 consumption by RBCs stayed remarkably stable (Fig. 5). The only significant change was a 20 % decline in M_{O_2} at 2 h, a time when the blood was still acidotic (Fig. 1A), but circulating catecholamines had returned close to resting levels (Fig. 2). On an absolute basis, combined CO_2 production from both glucose and lactate remained a small fraction of total O_2 consumption, though this fraction increased from about 10 % at rest to 20–25 % post-exercise. Clearly, other substrates were of greater quantitative importance.

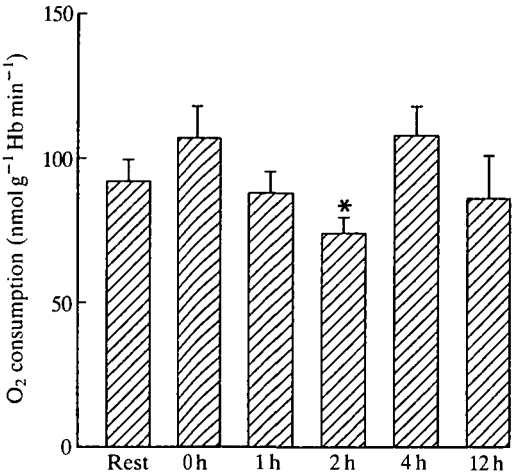

Fig. 5. The effects of exhaustive exercise and recovery on rates of total O_2 consumption by red blood cells of rainbow trout. Acid–base status was maintained close to levels measured *in vivo*. Means±1 S.E.M. N=5–6 at each sample time. * indicates a significant difference (P<0.05) from the corresponding rest value.

Factorial analyses in vitro

Since the exercise regimen produced a large number of changes in acid–base variables and catecholamine and substrate concentrations, it was difficult to determine which blood variables, if any, were responsible for the observed metabolic changes. The influence of substrate concentration has been examined by Walsh *et al.* (1990). Therefore, here we assessed the independent and combined effects of the various acid–base components and catecholamines on RBC lactate oxidation and M_{O_2} *in vitro* under simulated 0 h post-exercise conditions. Lactate (as sodium lactate) was added to all treatments at 5 mmol l^{-1}, the measured 0 h level (Fig. 3A). Metabolic acidosis was simulated by the addition of 5 mmol l^{-1} HCl, because at 0 h, the lactate and 'metabolic acid' loads to the blood were the same, as calculated from the data of Fig. 1 (see also Turner *et al.* 1983; Milligan and Wood, 1986*a*). Respiratory acidosis was simulated by 1% CO_2 (1 kPa), somewhat higher than the mean measured value at 0 h in the present experiments (Fig. 1B), but representative of that recorded in several previous studies (Turner *et al.* 1983; Milligan and Wood, 1986*a*). Catecholamine additions (150 nmol l^{-1} epinephrine plus 15 nmol l^{-1} norepinephrine) were close to measured elevations *in vivo* at 0 h (Fig. 2).

Table 1 summarizes the measured acid–base parameters and MCHC from each treatment in these factorial experiments. Respiratory and metabolic acidoses caused the same depressions in pHe and pHe–pHi, whereas the combined respiratory and metabolic treatment ('mixed acidosis') caused larger depressions in both parameters. Respiratory acidosis raised plasma HCO_3^- concentration, metabolic acidosis lowered it, while the mixed acidosis caused no significant

Table 1. *Measured acid–base parameters and cell haemoglobin concentrations (MCHC) in the factorial experiments*

Treatment	P_{CO_2} (kPa)	pHe	pHe–pHi	$[HCO_3^-]$ ($mmol\,l^{-1}$)	MCHC ($g\,ml^{-1}$)
A No added catecholamines					
Control (N=48)	0.28	7.844±0.011[a]	0.492±0.022[a]	6.18±0.14[a]	0.276±0.006[a]
Respiratory acidosis (N=24)	1.0	7.603±0.021[b]	0.315±0.028[b]	10.11±0.31[b]	0.265±0.010[a,b]
Metabolic acidosis (N=27)	0.28	7.613±0.021[b]	0.311±0.007[b]	3.15±0.20[c]	0.254±0.007[b]
Mixed acidosis (N=14)	1.0	7.364±0.021[c]	0.227±0.013[c]	5.84±0.23[a]	0.232±0.012[c]
Metabolic alkalosis (N=9)	0.28	8.086±0.056[d]	0.483±0.021[a]	11.08±0.77[b]	0.292±0.004[d]
B Catecholamines added					
Control (N=11)	0.28	7.807±0.041	0.414±0.036*	6.14±0.36	0.249±0.005*
Respiratory acidosis (N=10)	1.0	7.561±0.015	0.247±0.041	9.79±0.46	0.237±0.008*
Metabolic acidosis (N=10)	0.28	7.597±0.020	0.281±0.015	3.29±0.17	0.243±0.015
Mixed acidosis (N=11)	1.0	7.359±0.022	0.171±0.013*	5.74±0.21	0.212±0.019*

Values are means±1 S.E.M.

Respiratory acidosis was produced by elevating the P_{CO_2} to 1 kPa. Metabolic acidosis and alkalosis were produced by adding small volumes of HCl and $NaHCO_3$. Catecholamines were elevated by the addition of 150 $nmol\,l^{-1}$ epinephrine and 15 $nmol\,l^{-1}$ norepinephrine. See text for additional details.

In A, means that are *not* significantly different (P>0.05) from one another are denoted by the same letter. In B, * denotes means that are significantly different (P<0.05, paired t-test) from the respective mean in A in the absence of added catecholamines.

N represents the number of different pools measured (each pool from 3–7 fish) except for pHe–pHi, where N=4–6 in all treatments because RBC pHi was measured in only a subset of experiments.

change. Metabolic alkalosis raised pHe and plasma HCO_3^-, the latter to the same level as induced by respiratory acidosis. All acidotic treatments induced a swelling of the RBCs, as shown by a decline in MCHC, which was greatest for the mixed acidosis. Metabolic alkalosis caused a slight rise in MCHC. The addition of catecholamines caused a decrease in pHe–pHi and a decrease in MCHC, indicative of active RBC pHi regulation and swelling, effects that were significant in all treatments except metabolic acidosis. Catecholamines did not significantly affect pHe or plasma HCO_3^- in any treatment.

The factorial analysis demonstrated that the rate of lactate oxidation by RBCs was sensitive to blood acid–base status (Fig. 6). Respiratory acidosis (which lowered pHe and raised plasma $[HCO_3^-]$) and mixed acidosis (which lowered pHe even more without changing plasma $[HCO_3^-]$) both caused a significant 40% increase in the rate. However, metabolic acidosis alone (which lowered pHe by the

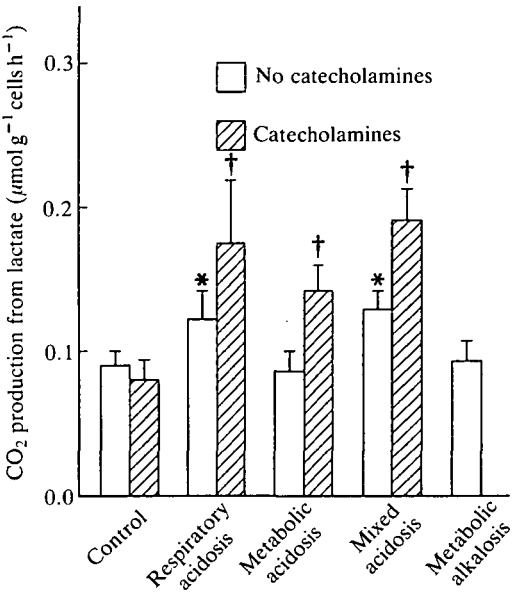

Fig. 6. The effects of acid–base variables (cf. Table 1) and added catecholamines (150 nmol l^{-1} epinephrine plus 15 nmol l^{-1} norepinephrine) on rates of CO_2 production from lactate (nominal concentration 5 mmol l^{-1}) by red blood cells of rainbow trout. Means±1 S.E.M. N=4–6 pools for each treatment. * indicates a significant difference (P<0.05) from the rest value, † indicates a significant difference from the respective value in the absence of catecholamines.

same amount as respiratory acidosis, but also lowered plasma [HCO_3^-]) had no effect. These results suggest that lactate oxidation is enhanced by increased P_{CO_2} and/or [HCO_3^-], while pHe is without influence. However, when the cells were subjected to metabolic alkalosis (which raised pHe and elevated plasma [HCO_3^-] to the same level as in respiratory acidosis), there was no effect on lactate oxidation. This indicates that the rate enhancement during respiratory and mixed acidoses was due solely to increased P_{CO_2} and not to changes in either [HCO_3^-] or pHe.

Elevated plasma catecholamine levels also significantly altered the rate of lactate oxidation, but in an interactive manner with acid–base status (Fig. 6). Under control conditions, catecholamines were without effect. However, under all three types of acidosis (respiratory, metabolic and mixed), the rate was significantly enhanced by typical post-exercise levels of epinephrine and norepinephrine. In each case, the elevation was approximately 60 % of the control rate. Thus, the combination of respiratory (or mixed) acidosis plus catecholamines accounted for about a 100 % increase.

The rate of total O_2 consumption by RBCs was also sensitive to acid–base status and catecholamines, but in a very different pattern from that of lactate oxidation (Fig. 7). Both respiratory and metabolic acidoses caused a 35 % decrease in M_{O_2}; however, these effects were not additive, because mixed acidosis (where the pHe

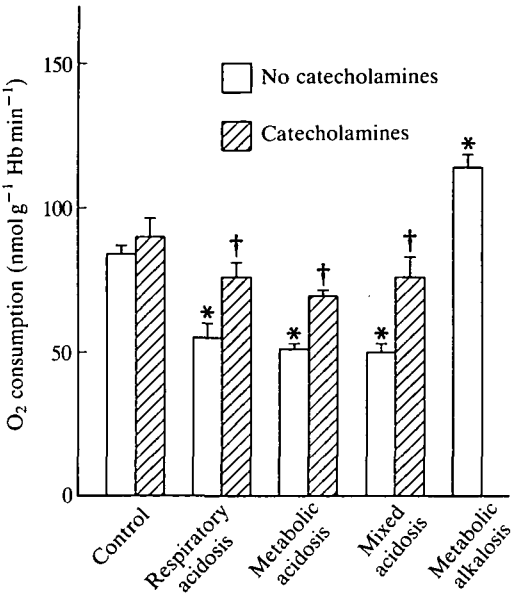

Fig. 7. The effects of acid–base variables (cf. Table 1) and added catecholamines (150 nmol l^{-1} epinephrine plus 15 nmol l^{-1} norepinephrine) on total rates of O_2 consumption by red blood cells of rainbow trout. Means±1 S.E.M. $N>6$ pools for each treatment. * indicates a significant difference ($P<0.05$) from the rest value, † indicates a significant difference from the the respective value in the absence of catecholamines.

decrease was greater) also caused a 35 % decrease. In contrast, metabolic alkalosis induced a significant 30 % increase above control M_{O_2}. In general, these results indicate that pHe, but not Pa_{CO_2} or plasma [HCO_3^-], was the important acid–base variable affecting RBC M_{O_2}.

The role of catecholamines was again interactive with acid–base status (Fig. 7). Elevated epinephrine and norepinephrine levels did not affect the control M_{O_2}, but significantly enhanced the lower rates seen under all three acidotic conditions, returning them close to the control rate. Thus, catecholamines served to reverse the depression in total aerobic metabolism of RBCs caused by acidosis.

Discussion

During strenuous exercise and recovery in rainbow trout, there were distinct variations in blood acid–base status, lactate and glucose concentrations and levels of circulating catecholamines (Figs 1,2,3; see also Turner *et al.* 1983; Primmett *et al.* 1986; Milligan and Wood, 1986*a*,*b*, 1987). The relative oxidation rates of lactate and glucose by RBCs *in vivo* would be expected to vary under these circumstances, based solely on the observed changes in substrate concentrations (Fig. 3) and the pathway K_m values for lactate and glucose determined by Walsh *et al.* (1990). However, observed changes in CO_2 production rates from lactate or glucose following strenuous exercise did not entirely follow these simple kinetic

patterns (Fig. 4). Lactate oxidation was elevated above the predicted level, while glucose oxidation was depressed below the predicted level during the 0–2 h post-exercise period. Subsequent experiments designed to factor out the potential effects of the many altered blood variables (Fig. 6) demonstrated that: (i) CO_2 production from lactate was stimulated by the respiratory component of the post-exercise acidosis (i.e. elevated Pa_{CO_2}); and (ii) elevated plasma catecholamine levels were effective in stimulating lactate oxidation under the acidotic conditions prevailing post-exercise (but not under control conditions). The combination of these two factors with the increased blood lactate levels was sufficient to explain the observed lactate oxidation rates, at least at 0 and 1 h post-exercise. The mechanisms of the P_{CO_2} and catecholamine effects are not known, though direct actions on lactate entry appear unlikely, for this is a passive process, at least in tuna, *Katsuwonus pelamis*, RBCs (Moon *et al.* 1987). It is of interest to ask whether these elevated rates of lactate oxidation could serve as a significant route for lactate clearance from the bloodstream after exhaustive exercise. While exact calculations are problematical (see also Milligan and McDonald, 1988), the answer is clearly negative – the observed rates would remove less than 0.5 % h^{-1} of the peak blood lactate load.

The depression in glucose oxidation 0–2 h after exercise (Fig. 4) in the face of unchanged or increased plasma glucose concentrations (Fig. 3B) was at least partially explained by the increase in plasma lactate levels at these times. Walsh *et al.* (1990) demonstrated that elevated lactate levels had a small inhibitory effect on glucose oxidation at high concentrations of glucose and lactate. Additional factors that were not assessed in the present study could include the post-exercise changes in acid–base variables and catecholamines, though Tse and Young (1990) have reported that catecholamines did not affect glucose uptake by trout RBCs. Furthermore, it should be remembered that our methods utilized uniformly labelled [^{14}C]glucose and thus measured CO_2 production from both the tricarboxylic acid cycle and the pentose-phosphate shunt (Wood and Katz, 1958; Walsh *et al.* 1990). It is possible that the marked depression in CO_2 production from glucose included a decrease in pentose-shunt activity, a pathway that produces NADPH and five-carbon sugars for biosynthesis. Clearly the control of glucose metabolism by RBCs *in vivo* deserves further study.

Total O_2 consumption rates of RBCs *in vivo* were remarkably stable after exhaustive exercise (Fig. 5), despite the clear evidence of RBC pHi regulation (Fig. 1A) and elevation in plasma catecholamine levels (Fig. 2). *In vitro*, Ferguson and Boutilier (1988) and Ferguson *et al.* (1989) have reported that adrenergically induced pHi regulation is accompanied by a marked increase in RBC M_{O_2}, the presumed metabolic cost of activation of the secondarily active Na^+/H^+ exchange. However, these results were obtained with 5×10^{-4} mol l^{-1} epinephrine, more than three orders of magnitude greater than the physiological levels of catecholamines measured *in vivo* after exhaustive exercise in the present study. Interestingly, Ferguson *et al.* (1989) also reported the results of an *in vivo* exercise experiment rather similar to that of the present study, though single M_{O_2}

measurements were made over a 2 h period starting at 0 h post-exercise. They found no consistent response – in one experimental group, RBC M_{O_2} increased significantly after exercise, while in another it exhibited a non-significant decrease. It is notable that, in the present study, catecholamines only stimulated O_2 consumption under conditions of low pHe, where it is well known that adrenergically stimulated Na^+/H^+ exchange is potentiated.

The present *in vivo* M_{O_2} results (Fig. 5) were entirely consistent with the results of the *in vitro* factorial experiments (Fig. 7), which showed that: (i) M_{O_2} was inhibited by all types of acidosis, an effect attributable mainly to the fall in pHe; and (ii) this inhibition was reversed by catecholamines, though these had no effect under control conditions. Thus, the stability of RBC M_{O_2} in the face of post-exercise acidosis was explained by the protective effect of elevated levels of circulating catecholamines. This inhibitory effect of acidosis became evident *in vivo* at 2 h (Fig. 5), when the elevation in plasma catecholamine levels had largely disappeared (Fig. 2) but acidosis persisted (Fig. 1A). *In vitro*, physiological levels of epinephrine and norepinephrine never elevated RBC M_{O_2} above control levels, yet Na^+/H^+ exchange was clearly activated, as shown by decreases in the pHe–pHi gradient and MCHC (Table 1). These results suggest that the aerobic cost of RBC pHi regulation may be lower than estimated by Ferguson and Boutilier (1988) and Ferguson *et al.* (1989) when physiological levels of catecholamines are involved. In support of this argument, decreases in RBC NTP stores after exhaustive exercise *in vivo* (Milligan and Wood, 1987) were also much smaller than those seen with high levels of epinephrine *in vitro* (Ferguson and Boutilier, 1988; Ferguson *et al.* 1989). Indeed, the constancy of RBC M_{O_2} *in vivo* after exercise means that the erythrocytes consume an even smaller percentage of whole-animal O_2 uptake than the 2–3 % at rest (Walsh *et al.* 1990). One note of caution should be added regarding the correlation of our *in vivo* and *in vitro* results: we cannot eliminate the possibility that additional red blood cells (with different metabolic capabilities) were recruited during exercise by release from the spleen.

It is now becoming evident that acid–base effects on metabolism are widespread in fish, but their nature appears specific to the individual tissue, pathway and species, and no general conclusions can yet be drawn. Indeed, even within the relatively narrow area of aerobic respiration by trout RBCs, there is a marked dichotomy in the present results. Thus, elevated P_{CO_2} stimulated lactate oxidation while pHe and HCO_3^- had little effect (Fig. 6). This is the first demonstration to our knowledge of a stimulatory effect of P_{CO_2} on CO_2 production from a single substrate in fish tissue. However, total M_{O_2} was depressed by respiratory acidosis, an effect largely correlated with the drop in pHe, with little influence of P_{CO_2} or $[HCO_3^-]$ (Fig. 7). By way of contrast, elevated P_{CO_2} and lowered pHe independently inhibited lactate oxidation and gluconeogenesis in trout hepatocytes, while elevated HCO_3^- stimulated these processes (Walsh *et al.* 1988; Mommsen *et al.* 1988). However, in toadfish (*Opsanus beta*) hepatocytes, lowered pHe stimulated gluconeogenesis from lactate (Walsh, 1989), while elevated $[HCO_3^-]$ stimulated

urea synthesis (Walsh *et al.* 1989); in both cases, other acid–base variables had no effect. Clearly a variety of patterns exists, but almost nothing is known regarding the specific mechanisms involved.

The role of catecholamines in metabolic regulation is becoming more obvious, from both the present study and other recent investigations (Walsh *et al.* 1988; Mommsen *et al.* 1988; Moon *et al.* 1988; Perry *et al.* 1988; Wright *et al.* 1989). In general, these substances appear to have little influence under control circumstances, when they would not normally be mobilized anyway. However, at times of acid–base disturbance, they become extremely important in stimulating, protecting or modifying metabolism, thereby fulfilling their basic role in adaptation to stress. This is particularly clear in the trout RBC, where catecholamines have small or negligible effects on pHi, haemoglobin O_2-affinity (Nikinmaa and Tufts, 1989) and oxidative metabolism under normal conditions, but extremely important actions during acidotic or hypoxic stresses. Trout RBCs represent an appealing experimental system for further studies of the interrelationships between acid–base status, catecholamines and metabolic control at the molecular level.

This research was supported by grants from NSERC of Canada (CMW and SFP), NSF and NIH (PJW) and CNRS (ST). We thank Richard Kinkead and Tina Goodison for valuable assistance, and Dr Tom Mommsen for commenting on an earlier draft of this paper.

References

EDDY, F. B. (1977). Oxygen uptake by rainbow trout blood, *Salmo gairdneri*. *J. Fish Biol.* **10**, 87–90.

FERGUSON, R. A. AND BOUTILIER, R. G. (1988). Metabolic energy production during adrenergic pH regulation in red cells of the Atlantic salmon, *Salmo salar*. *Respir. Physiol.* **74**, 65–76.

FERGUSON, R. A. AND BOUTILIER, R. G. (1989). Metabolic–membrane coupling in red blood cells of trout: the effects of anoxia and adrenergic stimulation. *J. exp. Biol.* **143**, 149–164.

FERGUSON, R. A., TUFTS, B. L. AND BOUTILIER, R. G. (1989). Energy metabolism in trout red cells: consequences of adrenergic stimulation *in vivo* and *in vitro*. *J. exp. Biol.* **143**, 133–147.

FRENCH, C. J., MOMMSEN, T. P. AND HOCHACHKA, P. W. (1981). Amino acid utilization in isolated hepatocytes from rainbow trout. *Eur. J. Biochem.* **113**, 311–317.

HUNTER, A. S. AND HUNTER, F. R. (1957). A comparative study of erythrocyte metabolism. *J. cell. comp. Physiol.* **49**, 479–502.

MILLIGAN, C. L. AND MCDONALD, D. G. (1988). *In vivo* lactate kinetics at rest and during recovery from exhaustive exercise in coho salmon (*Oncorhynchus kisutch*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **135**, 119–131.

MILLIGAN, C. L. AND WOOD, C. M. (1986*a*). Intracellular and extracellular acid–base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 93–121.

MILLIGAN, C. L. AND WOOD, C. M. (1986*b*). Tissue intracellular acid–base status and the fate of lactate after exhaustive exercise in the rainbow trout. *J. exp. Biol.* **123**, 123–144.

MILLIGAN, C. L. AND WOOD, C. M. (1987). Regulation of blood oxygen transport and red cell pHi after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *J. exp. Biol.* **133**, 263–282.

MOMMSEN, T. P., WALSH, P. J., PERRY, S. F. AND MOON, T. W. (1988). Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. *Gen. comp. Endocr.* **70**, 63–73.

MOON, T. W., BRILL, R. W., HOCHACHKA, P. W. AND WEBER, J.-M. (1987). L-(+)-Lactate translocation into the red blood cells of the skipjack tuna (*Katsuwonus pelamis*). *Can. J. Zool.* **65**, 2570–2573.

MOON, T. W., WALSH, P. J., PERRY, S. F. AND MOMMSEN, T. P. (1988). Effects of *in vivo* beta-adrenoreceptor blockade on hepatic carbohydrate metabolism in rainbow trout. *J. exp. Zool.* **248**, 88–93.

NIKINMAA, M. AND TUFTS, B. (1989). Regulation of acid and ion transfer across the membrane of nucleated erythrocytes. *Can. J. Zool.* **67**, 3039–3045.

PERRY, S. F. AND WALSH, P. J. (1989). Metabolism of isolated fish gill cells. *J. exp. Biol.* **144**, 507–520.

PERRY, S. F., WALSH, P. J., MOMMSEN, T. P. AND MOON, T. W. (1988). Metabolic consequences of hypercapnia in the rainbow trout, *Salmo gairdneri*; beta-adrenergic effects. *Gen. comp. Endocr.* **69**, 439–447.

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

SOIVIO, A., WESTMAN, K. AND NYHOLM, K. (1972). Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout. *Finnish Fish. Res.* **1**, 11–21.

TSE, C.-M. AND YOUNG, J. D. (1990). Glucose transport in fish erythrocytes: variable cytochalasin-B-sensitive hexose transport activity in the common eel (*Anguilla japonica*) and transport deficiency in the paddyfield eel (*Monopterus albus*) and rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **148**, 367–383.

TURNER, J. D., WOOD, C. M. AND CLARK, D. (1983). Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 247–268.

WALSH, P. J. (1989). An *in vitro* model of post-exercise hepatic gluconeogenesis in the gulf toadfish *Opsanus beta*. *J. exp. Biol.* **147**, 393–406.

WALSH, P. J., MOMMSEN, T. P., MOON, T. W. AND PERRY, S. F. (1988). Effects of acid–base variables on *in vitro* hepatic metabolism in rainbow trout. *J. exp. Biol.* **135**, 231–241.

WALSH, P. J., PARENT, J. J. AND HENRY, R. P. (1989). Carbonic anhydrase supplies bicarbonate for urea synthesis in toadfish (*Opsanus beta*) hepatocytes. *Physiol. Zool.* **62**, 1257–1272.

WALSH, P. J., WOOD, C. M., THOMAS, S. AND PERRY, S. F. (1990). Characterization of metabolism in red blood cells of rainbow trout. *J. exp. Biol.* **154**, 475–489.

WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progve Fish Cult.* **25**, 135–140.

WOOD, C. M. AND PERRY, S. F. (1985). Respiratory, circulatory, and metabolic adjustments to exercise in fish. In *Circulation, Respiration and Metabolism* (ed. R. Gilles), pp. 1–22. Berlin: Springer-Verlag.

WOOD, H. G. AND KATZ, J. (1958). The distribution of C14 in the hexose phosphates and the effect of recycling in the pentose cycle. *J. biol. Chem.* **233**, 1279–1282.

WRIGHT, P. A., PERRY, S. F. AND MOON, T. W. (1989). Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. *J. exp. Biol.* **147**, 169–188.

ZAR, J. H. (1974). *Biostatistical Analysis*. Englewood Cliffs: Prentice-Hall.