J. exp. Biol. **104**, 247–268 (1983)
Printed in Great Britain © The Company of Biologists Limited 1983

LACTATE AND PROTON DYNAMICS IN THE RAINBOW TROUT (*SALMO GAIRDNERI*)

BY JEFFREY D. TURNER*, CHRIS M. WOOD AND DONNA CLARK†

Department of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

(*Received 25 May 1982—Accepted 12 January 1983*)

SUMMARY

Chronically cannulated rainbow trout were subjected to 6 min of severe burst exercise and monitored over a 12 h recovery period. There were short-lived increases in haematocrit, haemoglobin, plasma protein, Na^+ and Cl^- levels. Plasma [Cl^-] later declined below normal as organic anions accumulated. A much larger and more prolonged elevation in plasma [K^+] probably resulted from intracellular acidosis. An intense extracellular acidosis was initially of equal respiratory (i.e. P_{a,CO_2}) and metabolic (i.e. ΔH_m^+) origin. However P_{a,CO_2} was rapidly corrected while the metabolic component persisted. Plasma ammonia increases had negligible influence on acid-base status. Elevations in blood lactate (ΔLa^-) were equal to ΔH_m^+ immediately post-exercise but later rose to twice the latter. Simultaneous white muscle biopsies and blood samples demonstrated that muscle to blood gradients of lactate and pyruvate were maximal immediately post-exercise. As blood levels rose and muscle levels declined, an approximate equilibrium was reached after 4 h of recovery. Intra-arterial infusions of lactic acid in resting trout produced a severe but rapidly corrected metabolic acidosis. The rates of disappearance of ΔH_m^+ and ΔLa^- from the blood were equal. Infusions of similar amounts of sodium lactate produced a small, prolonged metabolic alkalosis with a much slower ΔLa^- disappearance rate. It is suggested that the excess of ΔLa^- over ΔH_m^+ in the blood after exercise is associated with differential release rates of the two species from white muscle rather than differential removal rates from the bloodstream, and that the majority of the lactic acid load in muscle is removed by metabolism *in situ*.

INTRODUCTION

In mammals, lactic acid generation and movement between body compartments has been extensively studied. During recovery from exhausting exercise in man, blood lactate reaches a peak 5–10 min into recovery, then falls rapidly, returning to resting levels in less than 60 min (Johnson & Edwards, 1937; Crescitelli & Taylor, 1944; Bergström, Guarnieri & Hultman, 1971; Karlsson & Saltin, 1971).

* Present address: Department of Physiology and Biophysics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, U.S.A.

† Present Address: Pharmaceutical Research Department, Ortho Pharmaceutical (Canada) Ltd., 19 Green Belt Drive, Don Mills, Ontario M3C 1L9, Canada.

Key words: Lactate, exercise, trout.

Lower animals in general exhibit a very different blood lactate response to severe activity. In fish, the typical pattern of lactate accumulation in the blood during recovery requires 2–4 h to reach a maximum level and a total of 12–24 h to return fully to normal (Jones & Randall, 1978). These and other observations have spawned many hypotheses for the mechanisms that might control lactate movement; these include the influence of such parameters as acid-base status (Mainwood & Worsley-Brown, 1975), muscle perfusion (Stevens & Black, 1966; Stevens, 1968), temperature (Black, Chiu, Forbes & Hanslip, 1959), or circulating catecholamines (Wardle, 1978). There is no doubt that all of these factors can affect lactate movement in specific circumstances; however, their relative importance to the overall control mechanism(s) remains to be established.

Another interesting difference between mammals and lower animals is the apparent manner in which lactic acid leaves the myotome and accumulates in the blood. Lactate and protons are formed in stoichiometrically equivalent amounts during the anaerobic component of severe exercise (Krebs, Woods & Alberti, 1975). During recovery in man, the quantity of lactate in the blood always closely approximates the base deficit or metabolic acid load, which is representative of the quantity of metabolic protons buffered by the blood (Turrel & Robinson, 1942; Bouhuys, Pool, Binkhorst & van Leeuwen, 1966; Keul, Keppler & Doll, 1967).

In lower animals, the process does not usually occur in this way. Two basic patterns appear to exist, in which blood lactate accumulates either in excess or deficit of the metabolic proton load. In general, when lactate was found in excess of metabolic protons, the explanations offered were those of preferential proton retention by the muscle, proton buffering elsewhere in the animal, or preferential proton excretion into the environment (e.g. Piiper, Meyer & Drees, 1972; Mainwood & Worsley-Brown, 1975; McDonald, McMahon & Wood, 1979; Wood & Randall, 1981). On the other hand, when metabolic protons were found to accumulate in excess of lactate, the discrepancy was attributed to a more rapid efflux of protons from the muscle or the presence of an unmeasured organic acid in the blood (e.g. Wood, McMahon & McDonald, 1977; Benadé & Heisler, 1978; Smatresk, Preslar & Cameron, 1979; Boutilier, McDonald & Toews, 1980; McDonald, Boutilier & Toews, 1980).

The present report represents part of an investigation into the whole question of lactate versus metabolic proton discrepancy after anaerobic activity in teleost fish. The active, pelagic rainbow trout (*Salmo gairdneri*) has been chosen for the present study (also Graham, Wood & Turner, 1982; Wood, Turner & Graham, 1982) as it has now become the model teleost in fish respiratory and exercise physiology (cf. Jones & Randall, 1978). An accompanying paper examines the problem in a very different species, the sluggish benthic flathead sole *Hippoglossoides elassodon* (Turner, Wood & Hōbe, 1983). Lactate metabolism in the rainbow trout was extensively studied in the pioneering investigations of Black and co-workers (Black, 1957; Black *et al.* 1959; Black, Robertson, Hanslip & Chiu, 1960; Black, Connor, Lam & Chiu, 1962; Black, Manning & Hayashi, 1966; Stevens & Black, 1966). Calculations based on these data suggest that blood lactate load may have exceeded blood metabolic proton load in some of Black's experiments. However, blood samples were drawn by cardiac puncture and muscle samples taken by excision after first netting the fish, and the acid-base technology was deficient by modern standards. In the present study,

We developed a virtually non-disturbing, instantaneous technique for terminal muscle biopsy and have employed chronic cannulation for blood sampling. The importance of the latter in lactate studies in fish has been stressed by Driedzic & Kiceniuk (1976).

Experiments have focussed on: (i) the nature of the blood acid-base and lactate disturbance after severe exercise; (ii) white muscle-to-blood gradients of lactate and pyruvate and plasma ammonia levels during recovery from exercise; and (iii) the relative rates of removal of exogenous lactate and metabolic protons from the bloodstream when these were infused in equal or unequal amounts. In each case, basic haematological parameters and plasma electrolyte levels were followed, the latter because of the well established interactions between the mechanisms of acid-base and ionic homeostasis in fish (e.g. Heisler, 1980). The results describe in detail the blood acid-base, ionic, haematological and metabolite consequences of severe burst exercise.

MATERIALS AND METHODS

Experimental animals

Rainbow trout (*Salmo gairdneri* Richardson) of both sexes weighing 278 ± 8 g ($\bar{x} \pm 1$ S.E.M., $N = 57$) were purchased from Spring Valley Trout Farm, Petersburg, Ontario and acclimated to 15 ± 1 °C in dechlorinated, aerated fresh water (hardness ~ 140 mg l^{-1} as $CaCO_3$) for 2 weeks. During holding, the fish were fed *ad libitum* on trout pellets (Martin Feed Mills, Elmira, Ontario) and were starved for at least 7 days prior to experimentation.

Dorsal aortic catheters were implanted in all fish for chronic blood sampling. Cannulae consisted of Clay-Adams PE-50 polyethylene tubing tipped with 22 gauge needles and filled with heparinized (50 i.u. ml^{-1}) Cortland saline (Wolf, 1963). Catheterization was performed while the animal was under 1: 15 000 MS-222 (Sigma) anaesthesia on an operating table (Smith & Bell, 1964; Holeton & Randall, 1967). Following surgery, the fish were allowed to recover for a minimum of 24 h in darkened acrylic boxes served with a water flow of 300–450 ml min^{-1} (Kobayashi & Wood, 1980).

Experimental series

Series I

The first experiment employed 13 fish to elucidate the acid-base, metabolite, haematological and ionic disturbances in the blood associated with recovery from severe burst exercise. To exercise an individual trout, it was quickly transferred to a circular 500 l tank (diameter = 91 cm) and chased vigorously with a blunt stick for 6 min. By the end of the period, all fish appeared incapable of further 'burst' performance, but some continued to swim very slowly around the tank. This condition probably represented an exhaustion of the largely anaerobic white muscle while aerobic red muscle continued to support low level activity (e.g. Johnston, 1977). The fish was then returned to its individual box and monitored over the following 12 h recovery period. Anaerobically drawn samples of 600 μl whole blood were taken at rest

(control), immediately following exercise (0 h), and at 0·5, 1, 2, 4, 8 and 12 h. Bl[redacted] samples were replaced with an equal volume of Cortland saline. The blood was analysed for pH_a, total CO_2 (in whole blood and true plasma), haematocrit (Ht), haemoglobin concentration (Hb), lactate concentration (La^-), and plasma levels of sodium (Na^+), potassium (K^+), chloride (Cl^-) and total protein. It is noteworthy that five fish died from the effects of exercise during the recovery period. The data from these fish were not utilized in this report but are presented in detail elsewhere (Wood, Turner & Graham, 1982).

Series II

The second study utilized the same exercise protocol to examine La^- and pyruvate gradients between muscle and blood at various recovery times. Comparisons were also made between these gradients and other blood parameters. Twenty-nine fish were cannulated and exercised as in the previous study and placed in specially designed biopsy boxes. Each animal was used only once for a terminal muscle and blood sample. Sampling times included rest, 0, 1, 4, 8 and 12 h. Anaerobically drawn samples of 1000 μl whole blood were analysed for pH_a, total CO_2 (whole blood and true plasma), Ht, Hb, La^- and pyruvate concentrations and plasma levels of Na^+, K^+, Cl^-, protein and total ammonia. Muscle biopsy was performed within 3 min of blood sampling.

The biopsy chambers, while of the same size as the standard acrylic boxes, were modified by the addition of a moveable plastic liner and large (5 cm i.d.) port in the bottom. For biopsy sampling, the box was rapidly drained (< 2 s) by opening the port while the plastic liner was pulled rapidly upwards. This trapped the fish on its side, pressed firmly against the acrylic lid and covered with only a thin film of water. Biopsy needles were then punched through a slit in the lid and through the underlying epaxial muscle mass just posterior to the dorsal fin. This technique allowed lateral biopsy of the epaxial myotome without contamination from environmental water, major blood vessels or viscera. The biopsy needles were stainless steel trocars of i.d. = 4·78 mm (Arnold-Nasco Ltd., Guelph, Ontario) mounted in duplicate, 1 cm apart in an acrylic holder. Each needle obtained a sample of 93·2 ± 4·2 mg ($N = 41$), which was immediately frozen in liquid nitrogen. The total elapsed time from initial positioning of the fish to freezing the sample was under 5 s.

Series III

The third study examined the removal rates of La^- and metabolic protons from the blood space following either a lactic acid or sodium lactate infusion. In the lactic acid experiments, eight animals were infused by means of the dorsal aortic catheter with 5 ml kg^{-1} body weight of an L(+)-lactic acid (Sigma) solution [248 ± 18(8) μmol ml^{-1}] in 120 mM-NaCl. The mean infused load was 1242 ± 93(8) μmol kg^{-1} body weight. The lactic acid solution was prepared from crystalline lactic acid prior to each experiment. Due to the labile and hygroscopic nature of this compound, the mean infused load varied between experiments.

In a parallel study, seven animals were infused via the dorsal aortic catheter with 5 ml kg^{-1} body weight of a sodium L(+)-lactate (Sigma) solution [185 ± 9(7) μmol ml^{-1}] in 120 mM-NaCl. The mean infused load was 920 ± 12(7) μmol kg^{-1} bo[redacted]

ght. The sodium lactate solution was prepared through equivolumetric reaction of 400 mM-L(+)-lactic acid and 400 mM-NaOH, both in 120 mM-NaCl. The pH of the resultant 200 mM-sodium L(+)-lactate solution was adjusted to pH 7·1 by the appropriate minute addition of L(+)-lactic acid and NaOH. The infusate La^- concentrations in both the lactic acid and sodium lactate trials were measured directly by enzymatic analysis prior to each experiment.

The infusions required 10 min, then the catheter was flushed by the injection of 2 ml kg^{-1} body weight of 120 mM-NaCl and a further 5 min were allowed for mixing before post-infusion sampling commenced (0 h). This infusion duration was chosen as it was the minimum time in which ~1 mmol kg^{-1} body weight lactic acid could be infused into the trout without initiating vigorous locomotion. Such activity produces endogenous lactic acid which would confound data analysis. The lactic acid loads infused (~1 mmol kg^{-1} body weight) approximated the maximum tolerable dose (Kobayashi & Wood, 1980). No fish died as a result of the infusion. The blood sampling technique and times were the same as in the first study, except that the 12 h sample was omitted. Blood samples were analysed for pH_a, total CO_2 (whole blood and true plasma), Ht, Hb and La^- concentrations and plasma levels of Na^+, K^+, Cl^- and total protein.

Analytical techniques

Blood pH_a was determined by injecting a 40 μl aliquot of whole blood into a Radiometer microelectrode (type E5021) thermostatted to the experimental temperature and linked to a Radiometer PHM 71 or PHM 72 acid-base analyser. Total CO_2 was measured on 50 μl samples of whole blood and true plasma using the method of Cameron (1971) and a Radiometer CO_2 electrode (type E 5036/0). Percent haematocrit (Ht) was measured by centrifuging 80 μl of whole blood in sealed heparinized capillary tubes (Radiometer type D) at 5000 ***g*** for 5 min. This also allowed the separation of true plasma for total CO_2 determinations (see above); plasma was transferred anaerobically to the Cameron chamber. Haemoglobin concentration (Hb) was determined colorimetrically on 20 μl of blood using the cyanmethaemoglobin method (Blaxhall & Daisley, 1973) and Sigma reagents.

The remainder of the blood sample was centrifuged at 9000 ***g*** for 2 min to separate plasma. Total plasma protein was determined with an American Optical Goldberg refractometer (Alexander & Ingram, 1980). Plasma concentrations of Na^+ and K^+ were appropriately diluted and measured against known standards using flame photometry (EEL Mark II). Swamping was used to eliminate the effect of Na^+ on K^+ emissions. $[Cl^-]$ was determined via coulometric titration (Radiometer CMT10).

Blood $[La^-]$ was measured on 100 μl of whole blood, deproteinized with 200 μl of chilled 8 % $HClO_4$ and centrifuged at 9000 ***g*** for 3 min. The supernatant was analysed enzymatically (L-lactic dehydrogenase/NADH) for lactate using Sigma reagents (Sigma, 1977*a*). Similarly, blood pyruvate determinations used 400 μl of whole blood deproteinized with 800 μl of 6 % $HClO_4$, centrifuged as above and the supernatant analysed enzymatically (L-lactic dehydrogenase/NAD) for L(+) pyruvate (Sigma, 1977*a*). No measurements were performed to partition blood lactate or pyruvate concentrations into plasma and RBC components. According to McDonald, Boutilier oews (1980), whole blood levels are generally representative of plasma levels, but

may underestimate the latter after exercise because of slow lactate permeation of erythrocyte. Plasma ammonia was measured on 67 μl of plasma via enzymatic (L-glutamate dehydrogenase/NAD) analysis (Sigma, 1977*b*).

To determine muscle concentrations of La^- and pyruvate, individual muscle samples (~90 mg) were trimmed of skin and bloodstained portions while frozen, weighed, then extracted in 1000 μl of chilled 6% $HClO_4$ with a glass homogenizer for 5 min. The homogenate was spun at 9000 ***g*** for 3 min and the supernatant neutralized with Trizma buffer (Sigma) and analysed for L-lactate and L-pyruvate as above. The values obtained were then corrected for La^- and pyruvate trapped in extracellular fluid (assumed to be the same concentration as whole blood) using trout white muscle extracellular fluid volume estimates of 73·2 ml kg^{-1} muscle tissue (Milligan & Wood, 1982). Final concentrations were expressed as mequiv kg^{-1} of muscle cells, as it could not necessarily be assumed that all intracellular lactate and pyruvate was in solution in cell water. Cell water averages 698 ml kg^{-1} muscle tissue in trout white muscle (Milligan & Wood, 1982).

Calculations

From pH_a and total CO_2 meausurements, (true plasma), P_{a,CO_2} levels were calculated by rearrangement of the Henderson-Hasselbalch equation for the bicarbonate/carbonic acid system (Albers, 1970):

$$P_{a,CO_2} = \frac{\text{total } CO_2}{\alpha CO_2\,(1 + \text{antilog } (pH_a - pK'))} \tag{1}$$

where αCO_2 and pK′ are given by Severinghaus (1965) and Albers (1970) respectively at the appropriate pH and temperature. Then plasma bicarbonate was estimated:

$$[HCO_3^-] = \text{total } CO_2 - \alpha CO_2 \cdot P_{a,CO_2} \tag{2}$$

Whole blood bicarbonate levels were also calculated by substituting the whole blood measurement of total CO_2 in equation (2). While values for αCO_2 in whole blood are not available, any errors from this source are likely to be very small.

The relative contribution of respiratory acidosis (i.e. P_{a,CO_2} elevation) and metabolic acidosis (i.e. non-volatile acid) to the overall pH_a depression at any time were estimated by the graphical techniques described by Wood *et al.* (1977).

The concentration of metabolic protons (ΔH_m^+) added to the whole blood over any time period (1 to 2) was calculated by the equation of McDonald, Hōbe & Wood (1980):

$$\Delta H_m^+ = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_{a,1} - pH_{a,2}) \tag{3}$$

which derives from principles described in Davenport (1974), Woodbury (1974), Wood *et al.* (1977), and McDonald, Boutilier & Toews (1980). In this equation $[HCO_3^-]$ is the value for whole blood because ΔH_m^+ is compared to whole blood $[La^-]$, and β, the non-bicarbonate buffer capacity of whole blood, is estimated from the blood haemoglobin concentration (Hb; g 100 ml^{-1}) at time 2 using the relationship determined *in vitro* by Wood, McDonald & McMahon (1982).

$$\beta = -1{\cdot}073\,[Hb] - 2{\cdot}48$$

The total blood metabolic acid concentration at any time, relative to the resting level as zero, was calculated by summing the ΔH_m^+s (taking account of the sign) for each period from the rest sample onwards. Mean cell haemoglobin concentration (MCHC, in g Hb ml^{-1} of erythrocytes) was also estimated from the Hb measurement:

$$MCHC = \frac{[Hb]}{Ht} \quad (5)$$

In the lactic acid and sodium lactate infusion experiments, the lactate space (theoretical equilibrium volume of La^- distribution in the fish, in ml kg^{-1} body weight) was calculated by the formula of Kobayashi & Wood (1980):

$$\text{Lactate space} = \frac{\text{injected } La^- \text{ load}}{[La^-]_0 - [La^-]_R} \quad (6)$$

$[La^-]_R$ represents the resting blood La^- level and $[La^-]_0$ is the theoretical concentration of La^- in whole blood at time 0 h if the injected load were fully mixed throughout the lactate space. After an initial brief mixing phase, blood $[La^-]$ followed an exponential decline in all fish. $[La^-]_0$ was estimated by extrapolation of $\log[La^-]$ versus time curves back to time 0 h, ignoring the 0 h sample itself as it was within the mixing phase (see Fig. 4 of Kobayashi & Wood, 1980 for a representative plot).

Statistical analyses

Data are reported as means ±1 S.E.M. (N). The significance of changes from the rest sample within individual data sets was determined using the paired Student's two-tailed t-test ($P \leq 0.05$). Each fish was used as its own control. Differences between data sets were tested using the unpaired Student's two-tailed t-test ($P \leq 0.05$).

RESULTS

Severe exercise in rainbow trout caused major acid-base, metabolite and ionic disturbances, most of which were corrected during 12 h of recovery.

Series I. Blood parameters after exercise

Acid-base disturbances were severe (Fig. 1). Arterial pH fell from 7·880 ± 0·014(8) at rest to 7·370 ± 0·031(8) at 0 h then rose slowly to control levels, overshot significantly at 8 h before returning to control values at 12 h (Fig. 1A). Plasma $[HCO_3^-]$ dropped more slowly, from 8·34 ± 0·68(8) mequiv l^{-1} at rest to a low of 3·58 ± 0·29(8) mequiv l^{-1} at 1 h before returning to resting levels at 8 h (Fig. 1B). P_{a,CO_2} more than doubled from 3·03 ± 0·22(8) Torr at rest to 7·79 ± 0·64(8) Torr at 0 h, but was rapidly corrected by some 76 % in 30 min and completely recovered by 2 h (Fig. 1C). These data are indicative of a mixed respiratory and metabolic acidosis (cf. Davenport, 1974). An analysis based on the principles outlined in Wood *et al.* (1977) showed that the acidosis was 48 % respiratory and 52 % metabolic at 0 h, but greater than 75 % metabolic after 0·5 h.

By definition, ΔH_m^+ was equal to zero at rest (equation 3). Blood La^- levels averaged 0·83 ± 0·09(8) mequiv l^{-1} at rest. In Fig. 2, only the changes (Δ) in H_m^+ and La^- with

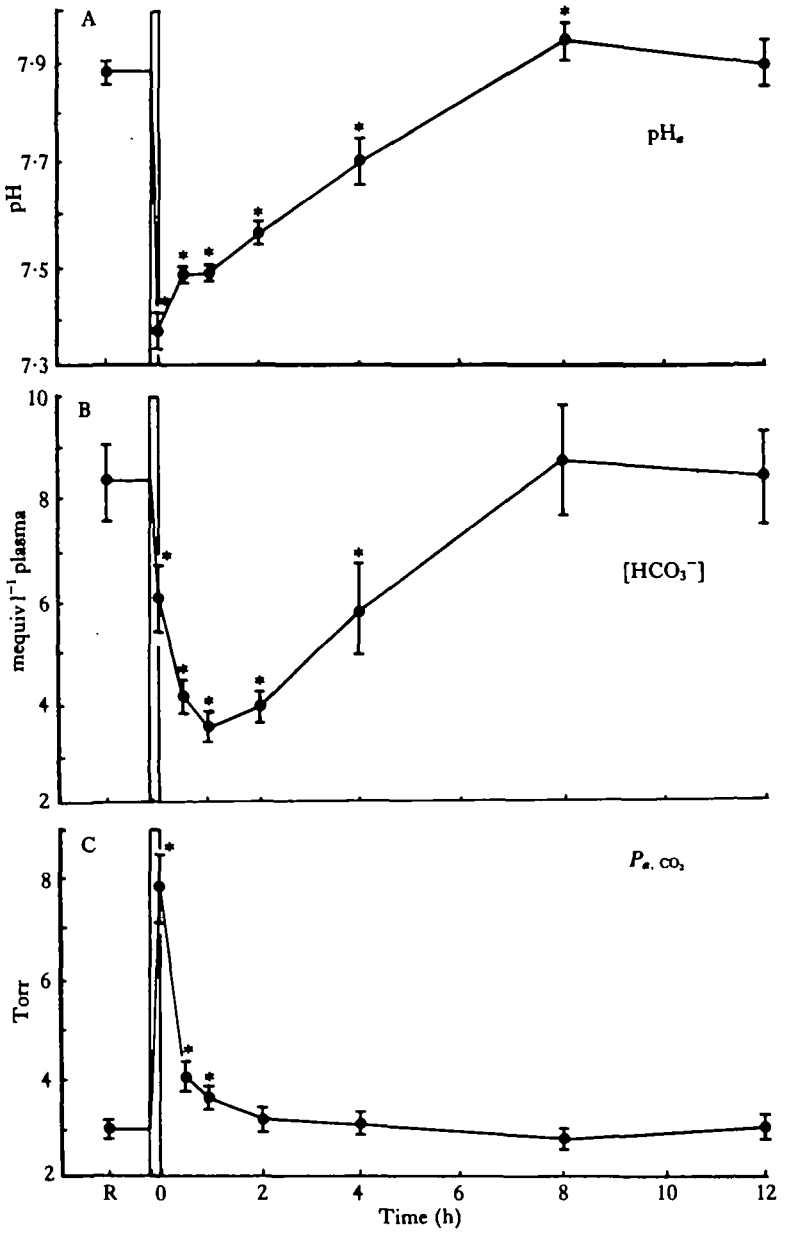

Fig. 1. Blood acid-base status [(A) arterial pH, (B) arterial true plasma bicarbonate concentration, (C) arterial CO_2 tension] in the rainbow trout prior to and following severe exercise. Means ± 1 S.E.M. ($N = 8$). R = rest; bar indicates 6 min of severe activity; 0 h = immediately post-exercise; *indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

respect to resting levels are illustrated. At 0 h, ΔH_m^+ and ΔLa^- were present in the blood in approximately equivalent amounts, ΔLa^- at 5·17 ± 0·27(8) mequiv l^{-1} and ΔH_m^+ at 6·65 ± 0·65(8) mequiv l^{-1}. As recovery progressed, ΔLa^- accumulated in excess of ΔH_m^+; ΔLa^- was significantly greater than ΔH_m^+ at all times from 0·5 h to 12 h. Indeed, by 2 h, ΔLa^- had doubled to a maximum value of 13·15 ± 0·39(8) mequiv

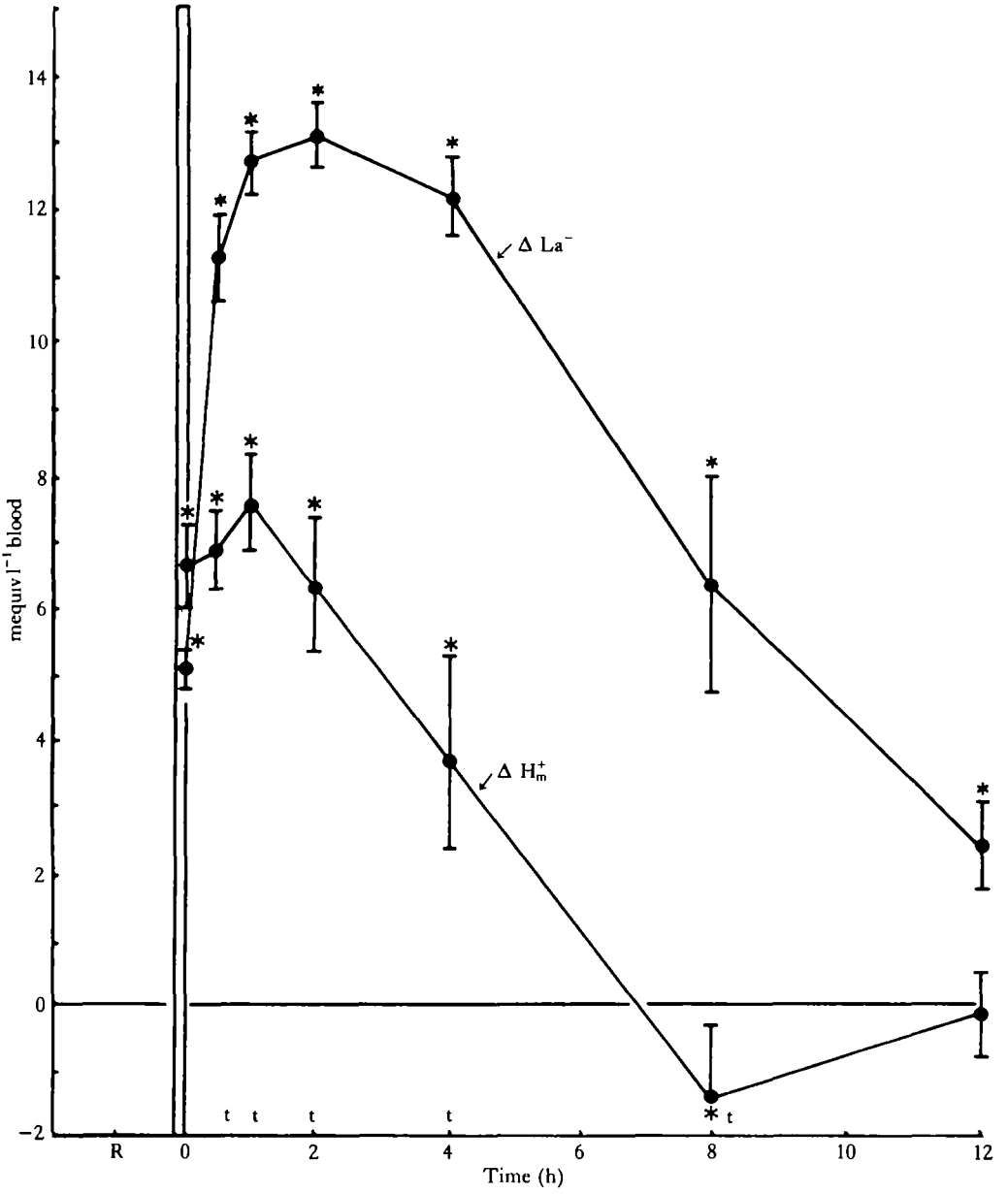

Fig. 2. Changes in blood lactate (ΔLa^-) and blood metabolic acid load (ΔH_m^+) during recovery from severe exercise in the rainbow trout. Means ± 1 S.E.M. ($N = 8$). R = rest; bar indicates 6 min of severe activity; 0 h = immediately post-exercises; *indicates a significant difference ($P \leq 0.05$) from rest; t indicates a significant difference ($P \leq 0.05$) between ΔLa^- and ΔH_m^+ at the same sample time.

while ΔH_m^+ had actually declined slightly to $6.30 \pm 0.83(8)$ mequiv l^{-1}. Between 2 h and 8 h, both species disappeared from the bloodstream at similar rates. By 8 h, ΔH_m^+ had been overcorrected while ΔLa^- was still only reduced to one half its maximum. Lactate remained significantly elevated at the 12 h sample. This discrepancy between

ΔLa^- and ΔH_m^+ could be caused by a preferential removal of protons from the blood space and/or by a slower proton release (with respect to La^-) from the muscle.

Exercise caused significant increases in plasma concentrations of Na^+, K^+ and Cl^- (Fig. 3). $[Na^+]$ increased from $146 \pm 4(8)$ mequiv l^{-1} at rest to $166 \pm 5(8)$ mequiv l^{-1} at 0 h, remained approximately stable until 1 h, and then returned to control levels by

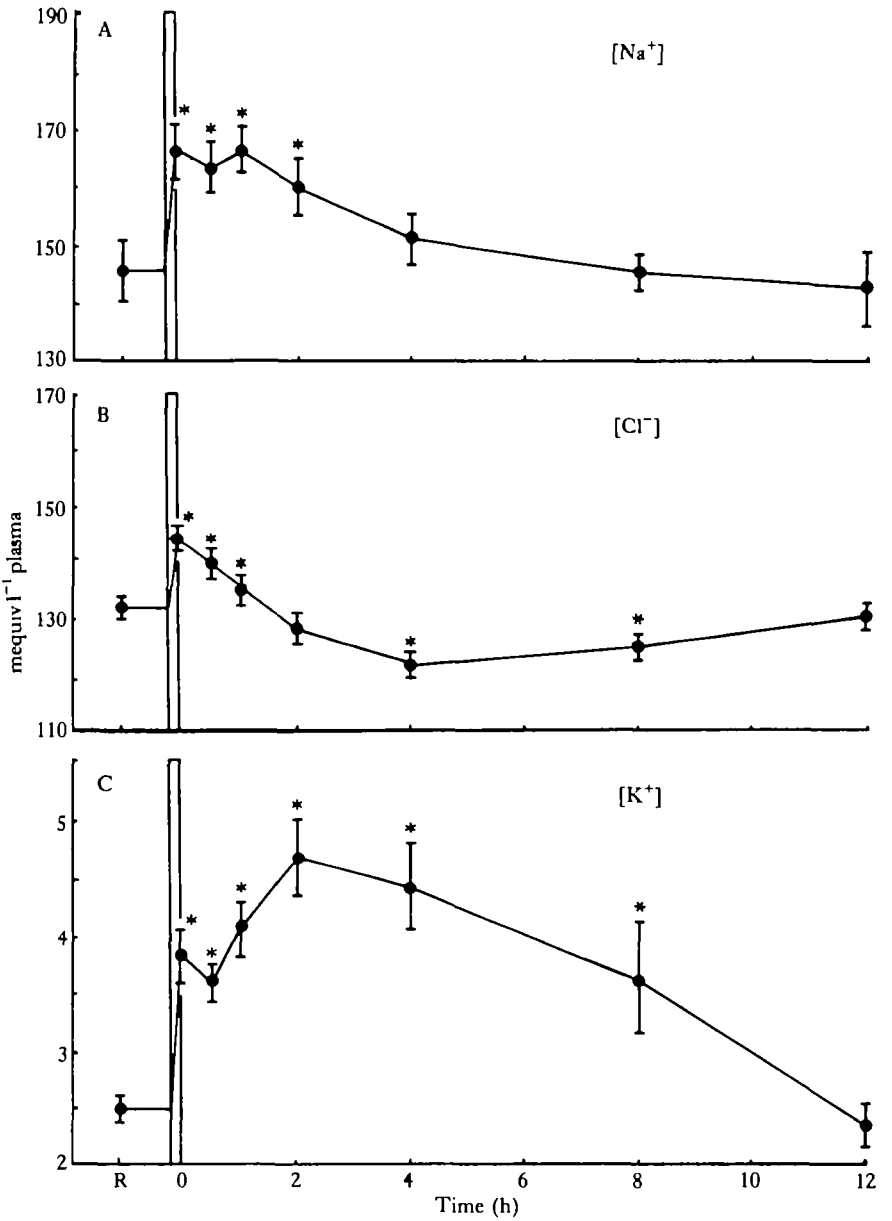

Fig. 3. Plasma concentrations of (A) sodium, (B) chloride and (C) potassium in the rainbow trout prior to and following severe exercise. Means ± 1 S.E.M. ($N = 8$). R = rest; bar indicates 6 min of severe activity; 0 h = immediately post-exercise; *indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

(Fig. 3A). $[Cl^-]$ increased briefly after exercise from $132 \pm 1(8)$ mequiv l^{-1} to $144 \pm 2(8)$ mequiv l^{-1} at 0 h but then fell well below resting levels to a low of $122 \pm 2(8)$ mequiv l^{-1} at 4 h before recovering by 12 h (Fig. 3B). $[K^+]$ was increased much more markedly by exercise and remained elevated until 12 h (Fig. 3C). Resting levels were $2{\cdot}50 \pm 0{\cdot}13(8)$ mequiv l^{-1} while a maximum of $4{\cdot}73 \pm 0{\cdot}34(8)$ mequiv l^{-1} was reached at 2 h. No haemolysis was observed, so it is unlikely that RBC destruction contributed to the K^+ elevation.

Ht, Hb and plasma protein concentrations increased during early recovery, in a pattern similar to that of plasma $[Na^+]$, but slowly decreased thereafter due to sampling losses (Table 1). MCHC fluctuated insignificantly around 0·25 g ml^{-1} RBCs throughout the experiment, indicating the lack of involvement of RBC swelling or mobilization of haemoglobin – poor RBCs in the Ht changes.

Series II. Muscle-blood relationships after exercise

In the second study, in which only a single terminal blood sample was drawn from each fish immediately prior to muscle biopsy, all blood acid-base and ionic alterations were very similar to those of the first study and therefore have not been shown. The only exception was a smaller respiratory component immediately post-exercise [$P_{a,CO_2} = 4{\cdot}80 \pm 1{\cdot}09(6)$ Torr *vs* $7{\cdot}79 \pm 0{\cdot}64(8)$ Torr] which reduced the severity of the initial acidosis [$pH_a = 7{\cdot}535 \pm 0{\cdot}066(6)$ *vs* $7{\cdot}370 \pm 0{\cdot}031(8)$]. The reasons for these differences are unknown; they may be random effects of a small sample size.

Resting epaxial muscle maintained a lactate level of $10{\cdot}06 \pm 1{\cdot}04(5)$ mequiv kg^{-1} muscle cells which increased to a maximum immediately after exercise of $43{\cdot}60 \pm 6{\cdot}44(6)$ mequiv kg^{-1} (Fig. 4A). During recovery, muscle $[La^-]$ decreased steadily to a 12 h low of $4{\cdot}04 \pm 0{\cdot}33(4)$ mequiv kg^{-1}. Blood $[La^-]$, initially $1{\cdot}01 \pm 0{\cdot}44(5)$ mequiv l^{-1} whole blood, increased much more slowly until an apparent equilibrium was reached with the muscle $[La^-]$ at 4 h. At this time, blood $[La^-]$ was $19{\cdot}15 \pm 1{\cdot}95(5)$ mequiv l^{-1} whole blood and muscle $[La^-]$ was not significantly different at $22{\cdot}59 \pm 3{\cdot}94(5)$ mequiv kg^{-1} muscle cells. During the subsequent recovery period, the decreases in muscle and blood $[La^-]$ followed a similar pattern. These results show that muscle-to-blood La^- gradients are very high at rest and immediately after

Table 1. *Haemoglobin concentration, percent haematocrit, mean corpuscular haemoglobin concentration (MCHC) and total plasma protein*

	Rest	0 h	0·5 h	1 h	2 h	4 h	8 h	12 h
Haemoglobin (g 100 ml^{-1} blood)	5·48 ± 0·47	7·08* ± 0·51	7·26* ± 0·64	6·35 ± 0·34	5·75 ± 0·28	4·84* ± 0·37	3·32* ± 0·50	2·63* ± 0·17
Haematocrit (%)	21·7 ± 1·1	28·4* ± 0·9	29·3* ± 1·2	27·8* ± 1·2	24·1 ± 0·7	22·6* ± 2·5	12·7* ± 1·2	12·1* ± 1·5
MCHC (g ml^{-1} RBCs)	0·25 ± 0·02	0·25 ± 0·02	0·25 ± 0·01	0·23 ± 0·01	0·24 ± 0·01	0·22 ± 0·02	0·28 ± 0·04	0·23 ± 0·01
Plasma protein (g 100 ml^{-1} plasma)	2·6 ± 0·2	2·8 ± 0·2	2·9* ± 0·2	2·8* ± 0·3	2·6 ± 0·3	2·4* ± 0·2	2·2 ± 0·2	2·0* ± 0·2

($\bar{x} \pm 1$ S.E.M; $N = 8$), prior to and following 6 min of severe exercise in rainbow trout.
* Indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

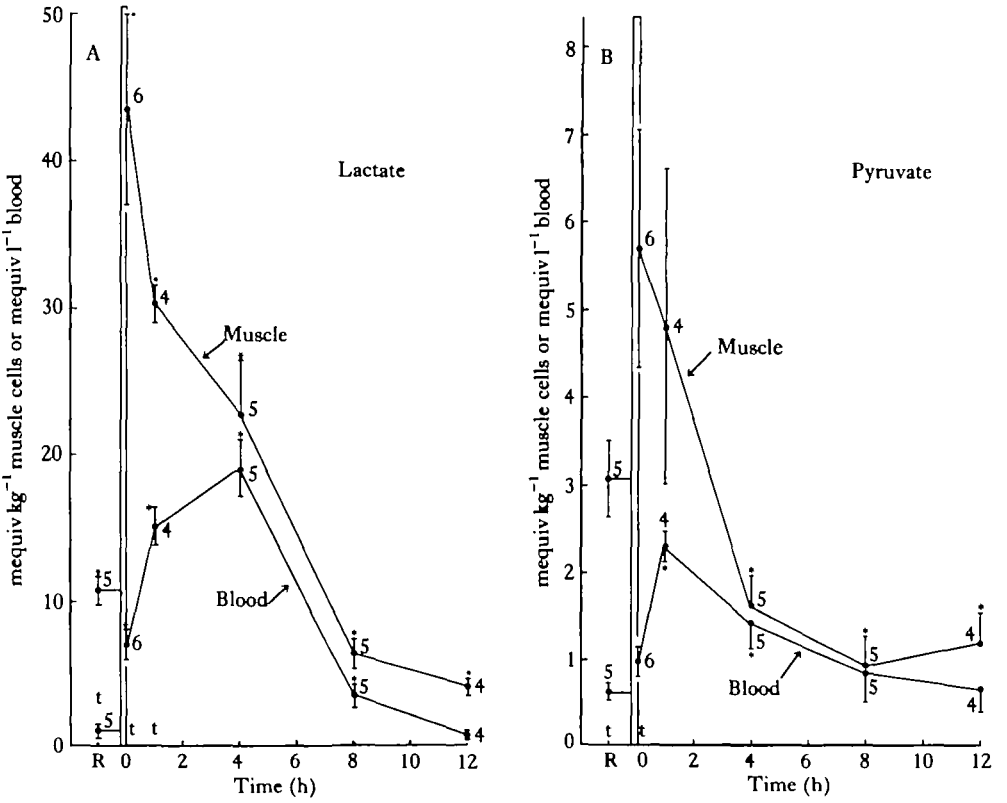

Fig. 4. The relationships between simultaneous muscle and blood concentrations of (A) lactate and (B) pyruvate in terminally sampled rainbow trout prior to and following severe exercise. Means ± 1 S.E.M. (N). R = rest; bar indicates 6 min of severe activity; 0 h = immediately post-exercise, *indicates a significant difference ($P \leq 0{\cdot}05$) from rest; t = indicates a significant difference ($P \leq 0{\cdot}05$) between muscle and blood levels at the same sample time.

exercise, but decline to very small values as blood [La^-] continues to rise. After 4 h, only a small gradient persists.

Because of variability in the data, the elevation in muscle [pyruvate] after exercise was not significant, but this parameter did fall significantly below resting levels as recovery progressed past 4 h (Fig. 4B). Blood pyruvate levels increased about four-fold to a maximum of $0{\cdot}23 \pm 0{\cdot}02(4)$ mequiv l^{-1} at 1 h before gradually returning to resting levels by 8 h. As with La^-, an approximate equilibrium was established between muscle and blood by 4 h and remained for the duration of the experiment. The lactate/pyruvate ratio calculated from mean muscle and blood levels was twice as high in muscle as in blood at rest (Table 2). After exercise it increased markedly in both compartments to very similar figures with maxima occurring at 4 h. Resting ratios were re-established by 12 h.

Total plasma ammonia concentration also increased about four-fold immediately after exercise (Table 2). A steady decline ensued, reaching a value significantly below resting level by 8 h, though normal values were re-established by 12 h. Despite these fluctuations, overall concentrations of both pyruvate and ammonia were relatively low throughout the experiment and would have had minimal effects on acid-base sta

Table 2. *The lactate:pyruvate ratio, calculated from mean muscle and blood levels, and total plasma ammonia concentrations*

	Rest	0 h	1 h	4 h	8 h	12 h
Muscle lactate:pyruvate ratio	35·2	76·5	62·5	142·1	67·1	35·0
Blood lactate:pyruvate ratio	17·4	78·5	66·0	133·9	39·4	13·3
Plasma ammonia (mmol l^{-1})	0·103 ± 0·017 (5)	0·385* ± 0·065 (6)	0·206* ± 0·027 (4)	0·187 ± 0·024 (5)	0·053* ± 0·005 (5)	0·068 ± 0·007 (7)

($\bar{x} \pm 1$ S.E.M.; N) prior to and following severe exercise in the rainbow trout.
* Indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

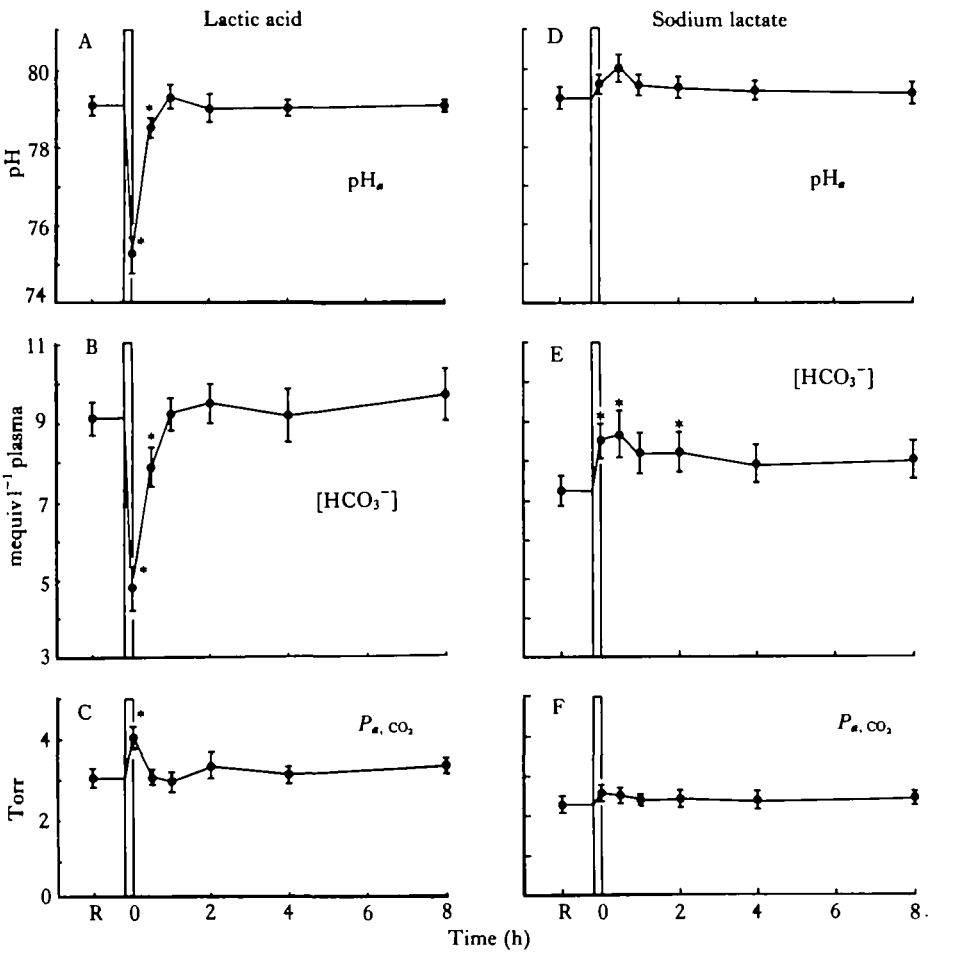

Fig. 5. Blood acid-base status [(A) and (D), arterial pH; (B) and (E), arterial true plasma bicarbonate concentration; (C) and (F), arterial CO_2 tension] in the rainbow trout prior to and following an L(+)-lactic acid infusion (A, B, C, $N = 8$) or a sodium L(+)-lactate infusion (D, E, F, $N = 7$). Means ± 1 S.E.M. R = rest; bar indicates a 15 min infusion and mixing period; 0 h = immediately after this period; *indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

Series III. Responses to infusion

Figs 5 and 6 compare the very different responses to infusions of lactic acid and sodium lactate solutions. Acid-base disturbances resulting from the infused lactic acid were severe and short-lived. Arterial pH was depressed from a resting value of 7·912 ± 0·020(8) to 7·529 ± 0·046(8) at 0 h, but then increased sharply, returning to resting levels within 1 h (Fig. 5A). Similarly, plasma [HCO_3^-] dropped promptly from 9·20 ± 0·41(8) mequiv l^{-1} at rest to 4·85 ± 0·58(8) mequiv l^{-1} at 0 h and was completely recovered by 1 h (Fig. 5B). P_{a,CO_2} increased very briefly from 3·10 ± 0·17(8) Torr at rest to 4·13 ± 0·19(8) Torr at 0 h, but returned to control by 0·5 h (Fig. 5C). As would be expected, this acidosis was determined to be greater than 80 % metabolic in origin. In contrast, sodium lactate infusion caused a small, immediate and prolonged alkalosis. The increase in pH_a was not significant ($0·5 < P < 0·1$) (Fig. 5D), but the rise in plasma [HCO_3^-] of about 1·5 mequiv l^{-1} was significant at 0, 0·5 and 2 h (Fig. 5B) and seemed to persist throughout the experiment. As P_{a,CO_2} remained unaffected (Fig. 5E), the alkalosis was totally metabolic in origin.

The primary purpose of the lactic acid infusions was to place equivalent quantities of exogenous metabolic protons and La^- in the blood in order to test whether the results of the first study could be explained by differential removal rates of the two species. As intended, blood concentrations were approximately the same at 0 h ($\Delta La^- = 10·10 \pm 0·83(8)$ mequiv l^{-1}; $\Delta H_m^+ = 8·67 \pm 1·10(8)$ mequiv l^{-1}; Fig. 6A). Both then rapidly fell; an exponential rate of decline was attained by 30 min. The disappearance rates of these species from the blood were not significantly different

Table 3. *A comparison of the rates of removal ($\bar{x} \pm 1$ S.E.M.; N) of lactate anions and metabolic protons from the blood of rainbow trout during the first 2 h after an* L(+)*-lactic acid or a sodium* L(+)*-lactate infusion*

	Lactate (μequiv l^{-1} blood min^{-1})	Protons (μequiv l^{-1} blood min^{-1})	P
Lactic acid			
0–0·5 h	234 ± 32 (7)	224 ± 24 (8)	N.S.
0·5–1·0 h	33 ± 12 (7)	57 ± 9 (8)	N.S.
1·02–2·0 h	10 ± 3 (8)	4 ± 8 (8)	N.S.
Sodium lactate			
0–0·5 h	64 ± 10* (6)	11 ± 22* (6)	N.S.
0·5–1·0 h	23 ± 3 (6)	2 ± 12 (6)	N.S.
1·0–2·0 h	5 ± 2 (6)	−8 ± 5 (6)	N.S.

P = Significance of difference in removal rates between lactate and protons.
* Indicates a significant difference ($P \leq 0·05$) from corresponding removal rate after lactic acid infusion.
N.S. Not significant.

able 3). This result suggests that the discrepancy found between ΔLa^- and ΔH_m^+ during recovery from exercise (Fig. 2) was not due to a preferential removal of protons from the blood space.

The sodium lactate infusions were performed to see if removal rates were dependent upon acid-base state. The results indicate that both the rate and pattern of the removal were affected (Fig. 6B, Table 3). While the original intention was to infuse an identical La^- load as in the lactic acid injections, the actual mean load was 74% of the latter. Nevertheless, the measured blood ΔLa^- at 0 h was only $3{\cdot}61 \pm 0{\cdot}37(7)$ mequiv l^{-1}, or 36% of the value in the lactic acid infusions. This difference was associated with a significantly greater lactate space [$392 \pm 34(7)$ ml kg^{-1} body weight] in the fish injected with sodium lactate than in those given lactic acid [$214 \pm 17(8)$ ml kg^{-1} body weight]. Conversely the disappearance rate of La^- from the blood after sodium lactate infusion was much slower than that after lactic acid infusion (Fig. 6B, Table 3). Metabolic acid levels became significantly negative immediately after infusion ($-1{\cdot}42 \pm 0{\cdot}24$ mequiv l^{-1}) and fluctuated about this level for the remainder of the experiment. Again disappearance rates of ΔLa^- and ΔH_m^+ were not significantly different, though variability in the latter was high (Table 3).

Plasma electrolytes remained for the most part unaltered following both infusions (Table 4). After lactic acid administration, plasma $[Na^+]$ and $[Cl^-]$ were unchanged while $[K^+]$ increased significantly at 0·5 h and remained elevated until the end of the experiment. Since haemolysis was noted in all cases following the lactic acid infusions, elevated plasma $[K^+]$ is not surprising. Red cell swelling was noted at 0 h as a brief drop in MCHC (Table 4). Following sodium lactate infusion, all plasma ions

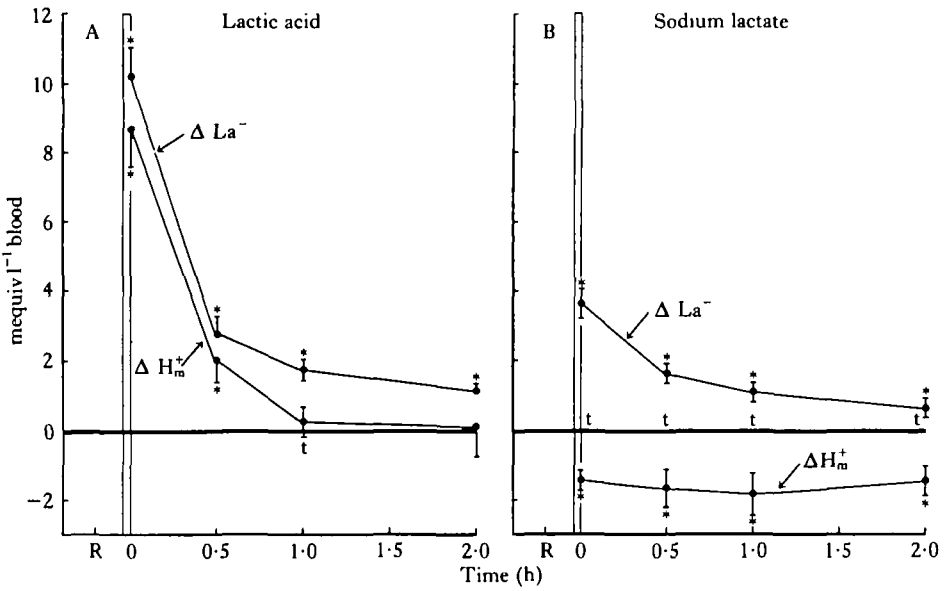

Fig. 6. Changes in blood lactate load (ΔLa^-) and blood metabolic acid load (ΔH_m^+) after infusion of (A) L(+)-lactic acid ($N = 8$) or (B) sodium L(+)-lactate ($N = 7$). Means ± 1 S.E.M. R = rest; bar indicates a 15 min infusion and mixing period; 0 h = immediately after this period; *indicates a significant difference ($P \leq 0{\cdot}05$) from rest; t indicates a significant difference ($P \leq 0{\cdot}05$) between ΔLa^- and ΔH_m^+ at the same sample time.

Table 4. *Alterations in plasma sodium, potassium and chloride levels, and mean corpuscular haemoglobin concentration ($\bar{x} \pm 1$ S.E.M.) following an L(+)-lactic acid or sodium L(+)-lactate infusion in rainbow trout*

	Rest	0 h	0·5 h	1 h	2 h	4 h	8 h
Lactic acid infusion ($N = 8$)							
Sodium (mequiv l^{-1})	145 ± 2·5	148.8 ± 2·0	150·8 ± 1·4	148·1 ± 1·9	149·9 ± 1·5	151·4 ± 2·5	148·0 ± 2·7
Chloride (mequiv l^{-1})	129·9 ± 0·9	128·5 ± 1·3	129·4 ± 1·2	129·1 ± 0·8	130·4 ± 0·7	131·8 ± 0·9	132·0 ± 0·8
Potassium (mequiv l^{-1})	2·14 ± 0·13	2·40 ± 0·19	2·98* ± 0·15	2·68* ± 0·13	2·57* ± 0·11	2·50* ± 0·09	2·53* ± 0·08
MCHC (g ml^{-1} RBCs)	0·31 ± 0·01	0·26* ± 0·01	0·30 ± 0·02	0·29 ± 0·02	0·32 ± 0·02	0·32 ± 0·02	0·32 ± 0·04
Sodium lactate infusion ($N = 7$)							
Sodium (mequiv l^{-1})	141·8 ± 2·8	139·8 ± 5·4	144·1 ± 3·5	143·3 ± 3·5	143·3 ± 3·8	142·4 ± 3·0	142·1 ± 3·7
Chloride (mequiv l^{-1})	127·9 ± 2·8	126·4* ± 3·4	127·7 ± 3·0	128·2 ± 3·2	128·2 ± 3·1	128·6 ± 2·8	127·9 ± 3·3
Potassium (mequiv l^{-1})	2·50 ± 0·14	2·36 ± 0·14	2·52 ± 0·09	2·71 ± 0·25	2·53 ± 0·10	2·51 ± 0·10	2·54 ± 0·11
MCHC (g ml^{-1} RBCs)	0·29 0·01	0·32 0·02	0·30 0·01	0·31 0·01	0·34 0·03	0·32 0·02	0·34 0·02

* Indicates a significant difference ($P \leq 0.05$) from resting levels.

remained unaltered with the exception of a small depression of [Cl^-] at 0 h. As the sodium lactate solution was near neutral pH, no cell swelling or haemolysis would be expected and none was observed. In both treatments, plasma protein, Hb and Ht levels (not shown) all slowly declined in similar fashion in response to repetitive blood sampling.

DISCUSSION

Comparison with other studies on Salmo gairdneri

Despite the improved sampling methodology used in the present study, there was generally excellent agreement with the blood and muscle [La^-] data of Black and co-workers (see Introduction for references). This suggests that [La^-] itself may not be particularly sensitive to sampling disturbance. However as anticipated, the present blood acid-base results were very different from and much more consistent than those of Black. In a recent review, Heisler (1980) summarizes the acid-base results of an unpublished experiment by G. F. Holeton, P. Neumann & N. Heisler on *S. gairdneri* which is very similar in design to the first experiment of the current study. Although there are some minor differences in absolute magnitudes, the overall agreement between the acid-base data of Figs 1 and 2 and those of Holeton *et al.* is remarkable. Plasma electrolyte data (N. Heisler, personal communication) are also generally similar in the two studies. Finally, the lactate space estimated from the lactic ac

[redacted]usion experiments in the present study (214 ± 17 ml kg^{-1} body weight) was consistent with the value (198 ± 13 ml kg^{-1} body weight) reported by Kobayashi & Wood (1980) for the same species.

Acid-base changes

Strenuous exercise caused a severe acidosis (Fig. 1) in arterial blood of mixed respiratory and metabolic origin (cf. Davenport, 1974). The respiratory component was at its maximum at 0 h, contributing almost 50 % of the total acidosis; thereafter it was rapidly corrected. P_{a,CO_2} elevation occurs during exercise when more CO_2 fluxes into the blood as a result of elevated aerobic metabolism (Jones & Randall, 1978). In addition, metabolic protons from anaerobic metabolism combine with blood HCO_3^- to form still more CO_2. However, the teleost gill is traditionally thought to be tremendously over-ventilated with respect to CO_2 excretion, so other factors must also be involved. Quite possibly, the efficiency of CO_2 excretion may be reduced by the increased cardiac output during exercise (Jones & Randall, 1978), which abbreviates the gill transit time and may prevent adequate dehydration of plasma HCO_3^- (Cameron & Polhemus, 1974). Elevated P_{a,CO_2} after exercise has been observed in several other studies (Stevens & Randall, 1967; Piiper *et al.* 1972; Wood *et al.* 1977; Turner *et al.* 1983) so it would seem to be of general occurrence. However, it must be remembered that the CO_2 equilibrium attained in measurement electrodes may never be achieved in the dynamic situation *in vivo*.

The metabolic component reached its maximum 1 h into recovery (Fig. 2), by which time the respiratory component had disappeared. The major origin of blood metabolic acidosis is probably H^+ efflux from white muscle. Since H_m^+ and La^- are produced in stoichiometrically equal amounts during exercise, the proton load in the muscle would be in excess of 40 mequiv kg^{-1} immediately post-exercise (Fig. 4). Despite the high buffer capacity of trout axial muscle (60 slykes; Castellini & Somero, 1981), a substantial intracellular acidosis could be predicted. Unfortunately no direct intracellular pH determinations have been reported after exercise in fish or any other muscle. Evidence that intracellular acidosis occurs during exercise in trout, and persists for a long time after exercise, is provided by the marked and persistent elevation of plasma $[K^+]$ (Fig. 3). Intracellular acidosis has been shown to correlate well with K^+ extrusion in mammalian skeletal muscle (Brown & Goott, 1963; Ladé & Brown, 1963).

Electrolyte and haematological changes

A generalized haemoconcentration occurred immediately after exercise, reflected in increases of plasma electrolytes (Fig. 3) and haematological parameters (Table 1) of 10–25 %. The 90 % elevation of $[K^+]$ obviously had a very different explanation (see above). While RBC mobilization was undoubtedly involved to some extent in the red cell response, the principal cause of haemoconcentration was probably water loss from the blood by diuresis and an osmotic shift into the intracellular compartment in response to the high intramuscular lactate load (Fig. 4) (Stevens, 1968; Wood & Randall, 1973). However, after the initial elevation, plasma $(Cl^-]$ fell steadily below resting levels at 4 h and 8 h while plasma $[Na^+]$ simply declined to normal values (Fig. [redacted]). The *absolute change* in $[Na^+ - Cl^-]$ (cf. Fig. 3) corresponded almost exactly to

[ΔLa^-] (Fig. 2), a phenomenon also observed by G. F. Holeton, P. Neumann N. Heisler (N. Heisler, personal communication). While it is tempting to attribute this directly to a Cl^-/La^- exchange at the cellular interface, the overall situation is probably more complicated since [Na^+] and [Cl^-] were measured in plasma and [ΔLa^-] in whole blood. Furthermore perturbations of Na^+ *vs* H^+ (NH_4^+) and Cl^- *vs* HCO_3^- (OH^-) exchanges at the gills or kidney may also occur (Heisler, 1980), as well as the movement of other charged substances into the blood. The only definite conclusion is that the overall constraint of electroneutrality must be obeyed. Indeed calculation of a more detailed charge balance ($[Na^+ + K^+] - [Cl^- + HCO_3^- + La^-]$) reveals that the difference (i.e. unmeasured negative charge) increased from ~6 to ~14 mequiv l^{-1} and then returned to normal over a similar time course to [La^-] (cf. Fig. 5 of Wood, McDonald & McMahon, 1982). This suggests that an unknown anion, perhaps that of a keto acid or a novel end product of anaerobic metabolism (e.g. succinate; Johnston, 1975; Smith & Heath, 1980) entered the bloodstream.

Lactate vs *metabolic proton levels in the blood*

While blood levels of ΔLa^+ and ΔH_m^+ were similar immediately after exercise (0 h), proton accumulation was substantially less than that of La^- for the remainder of the recovery period (0·5–12 h). Similar discrepancies have been reported in the dogfish (Piiper *et al.* 1972) and two species of crabs (McDonald *et al.* 1979; Wood & Randall, 1981) after exhaustive exercise. One possible explanation for the discrepancy would be metabolic base addition to the blood. The only likely candidate here is ammonia produced as NH_3. While total ammonia levels did increase after exercise (Table 2), probably as a result of elevated amino acid or adenylate deamination (Driedzic & Hochachka, 1976), the absolute levels remained low and would have had minimal influence on ΔH_m^+. In any event, comparable increases in pyruvic acid (Fig. 5B) would have opposed any alkalinizing influence of NH_3. Therefore the ΔLa^- *vs* ΔH_m^+ discrepancy must result from either a preferential removal mechanism which extracts protons from the blood faster than La^- and/or a differential release effect in which proton efflux from muscle occurs at a slower rate than La^-.

Protons may be removed from the blood by a number of processes which include excretion *via* branchial or renal exchanges, intracellular buffering in muscle, and sequestering by tissues other than muscle (Heisler, 1980; Kobayashi & Wood, 1980; Cameron & Kormanik, 1982). To examine the net effect of these processes on the relative removal rates of protons and lactate from the blood, lactic acid was infused. This process placed equivalent quantities of protons and lactate in the blood space. The results (Table 3) clearly illustrate that lactate and protons disappeared at the same rate. The net effect was the removal of 'lactic acid' from the blood, most likely by metabolism. Additional support for this concept of equal removal rates can be drawn from the rate of decrease of blood ΔH_m^+ and ΔLa^- following exercise (Fig. 2). During recovery from 2–8 h, the rates of disappearance of ΔLa^- and ΔH_m^+ from the blood were not significantly different at $18{\cdot}7 \pm 4{\cdot}3(8)$ μequiv l^{-1} min^{-1} and $21{\cdot}1 \pm 4{\cdot}0(8)$ μequiv l^{-1} min^{-1} respectively (cf. Fig. 2). Indeed the removal of 'lactic acid' resulted in a negative ΔH_m^+ value at 8 h (Fig. 2) and drove the acid-base status into metabolic alkalosis (Fig. 1). Similarly, when La^- was placed in the blood in the absence of associated protons by way of a sodium lactate infusion, metabolic alkalos

sulted (Figs 5, 6), and the disappearance rate of ΔLa^- was greatly retarded in the absence of protons (Fig. 6, Table 3). As a result, the La^- was able to distribute through a much larger 'lactate space' than in the lactic acid infusions. All these data suggest that 'lactic acid' is removed from the blood by metabolic processes, and argue against a preferential removal of protons as an explanation for the observed discrepancy.

A differential release of lactate and protons from the muscle to the blood is the other possible explanation. Recent work from this laboratory (J. D. Turner and C. M. Wood, unpublished) using a modification of the isolated, perfused trout trunk (Wood & Shelton, 1975; Moen & Klungsoyr, 1981) has demonstrated the presence of such a differential release phenomenon. When pre-exercised preparations were perfused with a haemoglobin containing saline approximating to resting blood acid-base status, the net rates of La^- and metabolic proton efflux from the myotome were equal. However, when the perfusate simulated the acid-base status of post-exercise acidosis, (similar to 0 h, Fig. 1), the efflux of protons was significantly smaller than the efflux of La^-. La^- effluxes were the same in the two treatments. *In vivo*, the P_{a,CO_2} rise and pH_a drop seen immediately after exercise (Fig. 1) probably develop gradually rather than instantaneously during 6 min of 'burst' activity. Therefore, the initial effluxes of protons and La^-, from muscle to blood, would be comparable (i.e. 0 h, Fig. 2) but the discrepancy would later develop as the sustained blood acidosis inhibited the proton efflux. Indeed, when the measured La^- and proton effluxes from the pre-exercised trunk musculature into the perfusate over the first 30 min of perfusion were distributed in the known blood volume of the trout ($55{\cdot}8\ ml\ kg^{-1}$ body weight; Milligan & Wood, 1982), the calculated ΔH_m^+ and ΔLa^- values were almost identical to those observed at 0·5 h in the present study (Fig. 2). This evidence, though preliminary, indicates that release of protons from muscle is modulated by extracellular acid-base status and that differential proton and La^- release alone can explain the observed discrepency *in vivo*.

However Heisler (1980) presents evidence from the unpublished work of G. F. Holeton, P. Neumann & N. Heisler on trout that a large, rapid efflux of metabolic protons occurs across the gills after exercise and explains the discrepancy. This efflux is said to appear mainly in the form of NH_4^+ and to represent a temporary 'storage' of protons in the external environment. Clearly further work is needed to solve this apparent conflict.

One further complication is that any movement of La^- and protons from the muscle cells into the blood must occur through the interstitial fluid compartment of the ECF. While La^- and HCO_3^- should be distributed between plasma and interstitial fluid according to a simple Donnan ratio with a value close to unity, it is probable that the absolute buffer capacity of the interstitial fluid is much lower than that of blood. Clearly events in the interstitial fluid compartment are of key importance and deserve attention in future work.

Ultimate fate of lactic acid

The branchial and/or renal excretion of lactic acid has been examined and seems to be of little importance. Kobayashi & Wood (1980) found that rainbow trout excreted through the kidney only a small percentage (2–25 %) of the protons and lactate

anions associated with an infused (1 mequiv kg^{-1} body weight) or endogenously produced (hypoxia) lactic acid load over a period of 3 days. In the short term (12 h), the low urinary proton and La^- efflux rates were virtually identical. Similarly, Cameron & Kormanik (1982) found that combined branchial and renal H^+ excretion over 24 h following a 1 mequiv kg^{-1} body weight lactic acid infusion in the catfish was less than 2 % of the infused load; urinary La^- excretion was negligible. Attempts to detect branchial La^- excretion have been unsuccessful (Piiper *et al.* 1972; Heisler, 1980). Therefore it would seem that metabolism, which consumes equivalent amounts of H^+ and La^-, is ultimately responsible for the removal of this valuable energetic substrate from the blood. However the exact site and nature of this metabolism remains unclear.

Since post-exercise La^- levels in excess of 40 mequiv kg^{-1} muscle cells (Fig. 4) occur in the white muscle – a tissue which makes up the greatest percentage of the body mass – the major portion of the whole body lactic acid burden must initially lie within this compartment. It may subsequently either be exported to the blood for metabolism by other tissues, some of which have high La^- oxidation capabilities (Bilinki & Jonas, 1972), or remain in the myotome for biochemical utilization *in situ*. Fig. 4 might suggest that much of the muscle La^- moves into the blood and forms an equilibrium. However, it must be remembered that the sizes of the two compartments are vastly different (blood volume = 55·8 ± 1·6 ml kg^{-1} body weight, total ICFV = 459·1 ± 9·4 ml kg^{-1}; Milligan & Wood, 1982) and only small amounts of La^- need efflux from muscle to form the equilibrium observed. Furthermore, the simple measurement of concentration gives no reliable estimate of lactate flux through the blood space. However, the results of the experiments with the pre-exercised perfused trunk (J. D. Turner & C. M. Wood, unpublished) show that only ≃10 % of the measured La^- disappearance from muscle *in vivo* can be accounted for by La^- efflux into the blood; the same is true of protons. We suggest that lactic acid is largely retained within the muscle and that removal by metabolic process *in situ* (i.e. oxidation and/or glycogen resynthesis) is the principal fate of endogenously produced lactic acid. This conclusion is in accord with the interpretation of Black *et al.* (1966), Wardle (1978) and Batty & Wardle (1979). While this opposes the traditional view that La^- is exported to the liver to be reprocessed into glucose by the Cori cycle, it should be noted that La^- retention and metabolism *in situ* may not be a uniquely piscine phenomenon. Exercised human leg muscles have been shown to release only 10 % of their total La^- load into the bloodstream; up to 75 % may be resynthesized to glycogen *in situ* (Hermansen & Vaage, 1977).

We thank Mark Graham and Mary Rogano for excellent technical assistance. Dr N. Heisler and the late Dr G. Holeton provided very helpful communication during the course of the study. Supported by an operating grant to CMW from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

ALBERS, C. (1970). Acid-base balance. In *Fish Physiology*, Vol. IV, (eds W. S. Hoar & D. J. Randall). New York: Academic Press Inc.

ALEXANDER, J. B. & INGRAM, G. A. (1980). A comparison of five of the methods commonly used to measure protein concentrations in fish sera. *J. Fish Biol.* **16**, 115–122.

BATTY, R. S. & WARDLE, C. S. (1979). Restoration of glycogen from lactic acid in the anaerobic swimming muscle of plaice, *Pleuronectes platessa* L. *J. Fish. Biol.* **15**, 509–519.

BENADÉ, A. J. S. & HEISLER, N. (1978). Comparison of efflux rates of hydrogen and lactate ions from isolated muscle *in vitro*. *Resp. Physiol.* **32**, 369–380.

BERGSTRÖM, J., GUARNIERI, G. & HULTMAN, E. (1971). Carbohydrate metabolism and electrolyte changes in human muscle tissue during heavy work. *J. appl. Physiol.* **30**, 122–125.

BILINSKI, E. & JONAS, R. E. E. (1972). Oxidation of lactate to carbon dioxide by rainbow trout (*Salmo gairdneri*) tissues. *J. Fish. Res. Bd Can.* **29**, 1467–1471.

BLACK, E. C. (1957). Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. I. Kamloops trout, *Salmo gairdneri*. *J. Fish. Res. Bd Can.* **14**, 117–134.

BLACK, E. C., CHIU, W., FORBES, F. D. & HANSLIP, A. (1959). Changes in pH, carbonate and lactate of the blood of yearling Kamloops trout, *Salmo gairdneri*, during and following severe muscular activity. *J. Fish. Res. Bd Can.* **16**, 391–402.

BLACK, E. C., CONNOR, A. R., LAM, K. C. & CHIU, W. (1962). Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. *J. Fish. Res. Bd Can.* **19**, 409–435.

BLACK, E. C., MANNING, G. T. & HAYASHI, K. (1966). Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate and lactate in venous blood of rainbow trout (*Salmo gairdneri*) during and following severe muscle activity. *J. Fish. Res. Bd Can.* **23**, 783–795.

BLACK, E. C., ROBERTSON, A. C., HANSLIP, A. R. & CHIU, W. (1960). Alterations in glycogen, glucose, and lactate in rainbow trout and Kamloops trout, *Salmo gairdneri*, following muscular activity. *J. Fish. Res. Bd Can.* **17**, 487–500.

BLAXHALL, P. C. & DAISLEY, K. W. (1973). Routine haematological methods for use with fish blood. *J. Fish. Biol.* **5**, 771–781.

BOUHUYS, A., POOL, J., BINKHORST, R. A. & VAN LEEUWEN, P. (1966). Metabolic acidosis of exercise in healthy males. *J. appl. Physiol.* **21**, 1040–1046.

BOUTILIER, R. G., MCDONALD, D. G. & TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid-base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis*; a comparison with the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 289–302.

BROWN, E. B., JR. & GOOTT, B. (1963). Intracellular hydrogen ion changes and potassium movement. *Am. J. Physiol.* **204**, 765–770.

CAMERON, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**, 632–634.

CAMERON, J. N. & KORMANIK, G. A. (1982). The acid-base responses of gills and kidneys to infused acid and base loads in the channel catfish, *Ictalurus punctatus*. *J. exp. Biol.* **99**, 143–160.

CAMERON, J. N. & POLHEMUS, J. A. (1974). Theory of CO_2 exchange in trout gills. *J. exp. Biol.* **60**, 183–194.

CASTELLINI, M. A. & SOMERO, G. N. (1981). Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. *J. comp. Physiol.* **143**, 191–198.

CRESCITELLI, F. & TAYLOR, C. (1944). The lactate response to exercise and its relationship to physical fitness. *Am. J. Physiol.* **141**, 630–640.

DAVENPORT, H. W. (1974). *The ABC of Acid-Base Chemistry*, 6th ed. Chicago: The University of Chicago Press.

DRIEDZIC, W. R. & HOCHACHKA, P. W. (1976). Control of energy metabolism in fish white muscle. *Am. J. Physiol.* **230**, 579–582.

DRIEDZIC, W. R. & KICENIUK, J. W. (1976). Blood lactate levels in free-swimming rainbow trout (*Salmo gairdneri*) before and after strenuous exercise resulting in fatigue. *J. Fish. Res. Bd Can.* **33**, 173–176.

GRAHAM, M. S., WOOD, C. M. & TURNER, J. D. (1982). The physiological responses of the rainbow trout to strenuous exercise: interactions of water hardness and environmental acidity. *Can. J. Zool.* **60**(12), 3153–3164.

HEISLER, N. (1980). Regulation of the acid-base status in fishes. In *Environmental Physiology of Fishes*, Series A, Volume 35, (ed. M. A. Ali), NATO Advanced Study Institutes Series. New York: Plenum Press.

HERMANSEN, L. & VAAGE, C. (1977). Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *Am. J. Physiol.* **233**, E422–E429.

HOLETON, G. F. & RANDALL, D. J. (1967). Changes in blood pressure in the rainbow trout during hypoxia. *J. exp. Biol.* **46**, 297–305.

JOHNSON, R. E. & EDWARDS, H. T. (1937). Lactate and pyruvate in blood and urine after exercise. *J. biol. Chem.* **118**, 427–432.

JOHNSTON, I. A. (1975). Studies on the swimming musculature of the rainbow trout. II. Muscle metabolism during severe hypoxia. *J. Fish. Biol.* **7**, 459–467.

JOHNSTON, I. A. (1977). A comparative study of glycolysis in red and white muscles of the trout (*Salmo gairdneri*) and mirror carp (*Cyprinus carpio*). *J. Fish. Biol.* **11**, 575–588.

JONES, D. R. & RANDALL, D. J. (1978). The respiratory and circulatory systems during exercise. In *Fish Physiology*, Vol. VII, (eds W. S. Hoar & D. J. Randall). New York: Academic Press Inc.

KARLSSON, J. & SALTIN, B. (1971). Oxygen deficit and muscle metabolites in intermittent exercise. *Acta. physiol. scand.* **82**, 115–122.

KEUL, I., KEPPLER, D. & DOLL, E. (1967). Standard bicarbonate, pH, lactate and pyruvate concentrations during and after muscular exercise. *German Medical Monthly* **12**, 156–158.

KOBAYASHI, K. A. & WOOD, C. M. (1980). The response of the kidney of the freshwater rainbow trout to true metabolic acidosis. *J. exp. Biol.* **84**, 227–244.

KREBS, H. A., WOODS, H. F. & ALBERTI, K. G. M. M. (1975). Hyperlactataemia and lactic acidosis. *Essays Med. Biochem.* **1**, 81–103.

LADÉ, R. I. & BROWN, E. B. (1963). Movement of potassium between muscle and blood in response to respiratory acidosis. *Am. J. Physiol.* **204**, 761–764.

MAINWOOD, G. W. & WORSLEY-BROWN, P. (1975). The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J. Physiol., Lond.* **250**, 1–22.

MCDONALD, D. G., BOUTILIER, R. G. & TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid-base balance in the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 273–287.

MCDONALD, D. G., HÖBE, H. & WOOD, C. M. (1980). The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *J. exp. Biol.* **88**, 109–131.

MCDONALD, D. G., MCMAHON, B. R. & WOOD, C. M. (1979). An analysis of acid-base disturbances in the haemolymph following strenuous activity in the Dungeness crab (*Cancer magister*). *J. exp. Biol.* **79**, 47–58.

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

MOEN, K. & KLUNGSOYR, L. (1981). Metabolism of exogenous substrates in perfused hind parts of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **68**B, 461–466.

PIIPER, J., MEYER, M. & DREES, F. (1972). Hydrogen ion balance in the elasmobranch, *Scyliorhinus stellaris*, after exhausting activity. *Resp. Physiol.* **16**, 290–303.

SEVERINGHAUS, J. W. (1965). Blood gas concentrations. In *Handbook of Physiology, Section 3, Volume 2.*, (eds W. O. Fenn & H. Rahn), pp. 1475–1487. American Physiological Society, Washington, D.C.

SIGMA (1977*a*). The quantitative determination of pyruvic acid and lactic acid in whole blood at 340 nm. Sigma Technical Bulletin 726-UV and 826-UV. Sigma Chemical Co., St. Louis.

SIGMA, (1977*b*). The quantitative determination of ammonia in serum at 340 nm. Sigma Technical Bulletin 170-UV. Sigma Chemical Co., St. Louis.

SMATRESK, N. J., PRESLAR, A. J. & CAMERON, J. N. (1979). Post-exercise acid-base disturbance in *Gecarcinus lateralis*, a terrestrial crab. *J. exp. Zool.* **210**, 205–210.

SMITH, L. S. & BELL, G. R. (1964). A technique for prolonged blood sampling in free-swimming salmon. *J. Fish. Res. Bd Can.* **21**, 1775–1790.

SMITH, M. J. & HEATH, A. G. (1980). Responses to acute anoxia and prolonged hypoxia by rainbow trout (*Salmo gairdneri*) and mirror carp (*Cyprinus carpio*) red and white muscle: use of conventional and modified metabolic pathways. *Comp. Biochem. Physiol.* **66**B, 267–272.

STEVENS, E. D. (1968). The effect of exercise on the distribution of blood to various organs in the rainbow trout. *Comp. Biochem. Physiol.* **25**, 615–625.

STEVENS, E. D. & BLACK, E. C. (1966). The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, *Salmo gairdneri*. *J. Fish. Res. Bd Can.* **23**, 471–485.

STEVENS, E. D. & RANDALL, D. J. (1967). Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. exp. Biol.* **46**, 329–337.

TURNER, J. D., WOOD, C. M. & HÖBE, H. (1983). Physiological consequences of severe exercise in the inactive benthic flathead sole (*Hippoglossoides elassodon*); a comparison with the active, pelagic rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **104**, 269–288.

TURREL, E. S. & ROBINSON, S. (1942). The acid-base equilibrium of the blood in exercise. *Am. J. Physiol.* **137**, 742–745.

WARDLE, C. S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice, *Pleuronectes platessa* L.: a stress reaction. *J. exp. Biol.* **77**, 141–155.

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

WOOD, C. M., MCDONALD, D. G. & MCMAHON, R. B. (1982). The influence of experimental anaemia on blood acid-base regulation *in vivo* and *in vitro* in the starry flounder (*Platichthys stellatus*) and the rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **96**, 221–237.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1977). An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. *J. exp. Biol.* **69**, 173–185.

WOOD, C. M. & RANDALL, D. J. (1973). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **82**, 257–276.

WOOD, C. M. & RANDALL, D. J. (1981). Haemolymph gas transport, acid-base regulation, and anaerobic metabolism during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**, 23–35.

WOOD, C. M. & SHELTON, G. (1975). Physical and adrenergic factors affecting systemic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. exp. Biol.* **63**, 505–523.

WOOD, C. M., TURNER, J. D. & GRAHAM, M. S. (1983). Why do fish die after severe exercise? *J. Fish. Biol.* **22**(2), 189–201.

WOODBURY, J. W. (1974). Body acid-base state and its regulation. In *Physiology and Biophysics II: Circulation, Respiration, and Fluid Balance, 20th Edition*, (eds T. C. Ruch, H. D. Patton & A. M. Scher). Toronto: W. B. Saunders Co.