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PHYSIOLOGICAL CONSEQUENCES OF SEVERE EXERCISE IN THE INACTIVE BENTHIC FLATHEAD SOLE (*HIPPOGLOSSOIDES ELASSODON*): A COMPARISON WITH THE ACTIVE PELAGIC RAINBOW TROUT (*SALMO GAIRDNERI*)

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SUMMARY

Chronically cannulated flathead sole were subjected to 10 min of either moderate or exhausting burst exercise and monitored over a 12 h recovery period. Acid-base disturbances were more severe after exhausting exercise, but ionic and haematological changes were the same in the two treatments. Most effects were qualitatively similar to those previously described in severely exercised rainbow trout (Turner, Wood & Clark, 1983). Specific differences are discussed and related to the different external environments (sea water *vs* fresh water), exercise capabilities and ecologies of the two species. The most striking divergence occurred in lactate (La^-) and metabolic proton dynamics. Post-exercise La^- levels in white muscle in sole were less than half those in trout but declined much more slowly. In contrast to the situation in trout, muscle [La^-] remained significantly elevated and a large muscle to blood La^- gradient persisted even after 12 h recovery. Blood [La^-] underwent only minimal elevation (< 2 mequiv l^{-1}), and blood metabolic proton load (ΔH_m^+) greatly exceeded ΔLa^- throughout the recovery period, effects directly opposite those in trout. This observed excess of ΔH_m^+ over ΔLa^- in the blood of exercised sole is probably not due to a preferential removal mechanism, because ΔH_m^+ and ΔLa^- disappeared from the blood at similar rates after an intra-arterial infusion of lactic acid in resting animals. It is therefore argued that the phenomenon reflects a differential release of the two metabolites from the white muscle of the sole, La^- being strictly retained in the muscle for gluconeogenesis *in situ*.

INTRODUCTION

While the rainbow trout, *Salmo gairdneri*, continues to be the teleost which is most

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extensively studied by cardiorespiratory physiologists, there has been a recent surge of interest in other species. Members of the Order Pleuronectiformes, the flatfishes, have attracted particular attention (Edwards, 1971; Watters & Smith, 1973; Woods, Johansen & Weber, 1975; Hayden, Cech & Bridges, 1975; Cech, Bridges, Rowell & Balzer, 1976; Cech, Rowell & Glasgow, 1977; Wood, McMahon & McDonald, 1977, 1979*a*,*b*; Kerstens, Lomholt & Johansen, 1979; Steffensen, Lomholt & Johansen, 1981; Wood, McDonald & McMahon, 1982). The general picture which has emerged is that of a fish whose cardiorespiratory system is designed to operate with great efficiency at rest, to perform well under environmental hypoxia, but to offer little scope for increasing O_2 delivery during exercise. Such strategies appear to be exactly opposite to those employed by the active, pelagic rainbow trout (Wood *et al.* 1979*a*,*b*) and are probably linked to the various structural and behavioural adaptations which have allowed successful exploitation of the benthic habitat by the Pleuronectiformes.

Flatfish have an extremely high body density (Harden-Jones & Marshall, 1953) and a very low content of 'aerobic' red muscle relative to 'anaerobic' white muscle (Mannan, Fraser & Dyer, 1961; Greer-Walker & Pull, 1975). They are browsing feeders, generally do not migrate long distances, and rely heavily on tidal transport (Hart, 1973; Greer-Walker, Harden-Jones & Arnold, 1978). Their defensive strategy is to bury in the substrate and remain motionless. Not surprisingly, their locomotory capabilities are rather limited. With the recent development of a tilting tunnel respirometer (Priede & Holliday, 1980), it has been shown that flatfish are capable of only brief bouts of activity at very modest velocities (< 2 body lengths s^{-1}). On the basis of both energetic arguments and physiological measurements (Wardle, 1978; Wood *et al.* 1979*b*), it seems likely that their activity metabolism may have a large anaerobic component. Indeed in the latter study, severely anaemic flounder showed similar burst exercise capabilities and post-exercise changes in blood [La^-] to animals with normal blood O_2 transport capacity.

Two reported features of the post-exercise physiology of flatfish are particularly interesting in the light of our preceding study on the rainbow trout (Turner, Wood & Clark, 1983). Firstly, despite this supposed reliance on anaerobic metabolism during activity, blood lactate levels in exhausted flatfish are only about one tenth of those in exhausted trout (Dando, 1969; Wood *et al.* 1977, 1979*b*; Wardle, 1978). Secondly, Wood *et al.* (1977), working on the starry flounder, concluded that metabolic acid levels in the blood exceed lactate anion levels during the recovery period, though only two specimens were examined in detail. This situation is exactly opposite to that in the trout (Turner *et al.* 1983) and the dogfish (Piiper, Meyer & Drees, 1972), the only other fish species which have been investigated. The aim of the present study was to confirm and extend these observations in a different pleuronectid, the flathead sole, *Hippoglossoides elassodon*, as part of our overall studies on lactate and metabolic proton regulation in teleost fish. Within the limitations of a field situation, the experiments were designed to duplicate as closely as possible those on the trout (Turner *et al.* 1983), thereby permitting comparison of the post-exercise responses of a sluggish, benthic, flatfish in sea water with those of an active, pelagic roundfish in fresh water.

MATERIALS AND METHODS

Experimental animals

Flathead sole (*Hippoglossoides elassodon* Jordan and Gilbert) of both sexes, weighing 223 ± 10 g ($\bar{x} \pm 1$ S.E.M., $N = 48$), were collected during a series of 30 min otter trawls during August, 1980 in Barkley Sound, off Bamfield, British Columbia. At Bamfield Marine Station, fish were held in large sand-covered tanks with flow-through sea water (salinity ~31 ‰, P_{O_2} ~90 Torr) at 11·5 ± 1 °C for at least 5 days before experimentation. The animals were not fed during this period. Mortalities due to capture stress were negligible (less than 5 %).

Fish were anaesthetized on an operating table with 1: 15 000 MS-222 (Sigma) and the caudal artery or vein chronically catheterized with Clay-Adams PE50 polyethylene tubing, as described by Watters & Smith (1973) and Wood *et al.* (1977). The incision was dusted with Furanace (Nifurpironal, Dainippon Pharmaceutical), a wide-spectrum fish antibiotic, and closed with silk sutures. Fish were allowed to recover for 48–72 h, in covered, sand-filled plastic mesh enclosures (30 × 40 × 10 cm deep) placed on a wet table with flow through sea water. Long recovery periods and access to sand are required to establish resting conditions in flatfish (Wood *et al.* 1979*a*). Fish typically buried into the sand with only the eyes and mouth exposed, and remained motionless.

In vitro *experimentation*

In vitro CO_2 titration was performed to obtain the relationship between haemoglobin (Hb) concentration and β, the non-bicarbonate buffer capacity, in whole blood of the flathead sole. β, as predicted from [Hb], was required for the calculation of blood metabolic acid load (ΔH_m^+) in the *in vivo* experiments (cf. Turner *et al.* 1983). Blood was drawn from the arterial catheters of four resting sole, heparinized at 100 i.u. ml^{-1} (sodium heparin; Sigma), pooled, gently centrifuged to separate plasma from RBCs, then reconstituted at 5 haematocrits (Ht) ranging from 0 % to 30 %. Tonometry, analyses and estimation of β at the experimental temperature for each Ht and [Hb] were carried out as described by Wood *et al.* (1982).

Experimental series

Series I

The first study employed 10 fish fitted with arterial cannulae to examine the acid-base, metabolite, haematological and ionic disturbances in the blood associated with two exercise intensities, which we have termed moderate and exhausting. Moderate exercise ($N = 5$) was achieved by manually chasing the fish in a shallow tank (100 × 100 × 20 cm deep) for 10 min, the procedure used by Wood *et al.* (1977, 1979*b*) to exhaust the starry flounder. By the end of the exercise period, flathead sole failed to respond to further tactile stimulation but were not exhausted as they still showed righting responses, and the use of electric shocks could elicit further swimming activity. Exhausting exercise ($N = 5$) was produced by chasing the fish in a deeper tank (150 × 100 × 90 cm deep) with an electric wand. The wand consisted of 16 gauge copper wire unravelled into two fan-shaped electrodes, 12 cm apart, and connected to

a 60 V a.c. power supply. When the electric field approached the fish, a startle response lasting 5–20 s was elicited. Direct contact stimulation was strictly avoided. After 10 min of such exercise, the fish were completely refractory to further stimulation and exhibited no righting responses, indicating that they were exhausted. Upon completion of either exercise protocol, the fish were immediately returned to their enclosures, where they soon buried into the sand again.

Samples (600 μl) of whole blood were drawn anaerobically at rest (control), immediately following exercise (0 h), and at 0·5, 1, 2, 4, 8 and 12 h after exercise. The blood removed was replaced with an equal volume of Cortland saline (Wolf, 1963) adjusted to 160 mequiv l^{-1} sodium concentration with NaCl (Wood *et al.* 1979*a*). The blood was analysed for pH_a, total CO_2 (in whole blood and true plasma), Ht, [Hb], lactate (La^-) concentration, and plasma levels of sodium (Na^+), chloride (Cl^-), potassium (K^+) and osmolality. No fish died during either exercise regime or recovery period. These fish were subjected to terminal muscle and blood sampling at 12 h as part of the second experimental series (see below).

Series II

The second study used 34 animals and examined white muscle-to-blood La^- gradients and blood pyruvate and plasma ammonia levels at various recovery times after both moderate and exhausting exercise. Each fish yielded one pair of terminal, almost simultaneous, muscle and blood samples. Sampling times included rest, 0 and 12 h after moderate exercise, and 0, 2 and 12 h after exhausting exercise. The rest and 0 h samples were taken from sole fitted with venous catheters in order to compare venous blood data for the control and immediately post-exercise conditions with the corresponding arterial data collected in the first series. The remaining samples were drawn from fish bearing arterial catheters. Animals of the first experimental series provided the 12 h samples.

Whole blood samples (1000 μl) were drawn anaerobically and analysed for pH, total CO_2 (in whole blood and true plasma), and plasma levels of Na^+, K^+, Cl^-, osmolality and total ammonia. Muscle biopsy was completed within 60 s of blood sampling. Immediately after the blood was drawn, the fish was quickly removed from the water, placed on a sponge, and wiped dry. All animals maintained their motionless defence posture while on the sponge. Two simultaneous muscle samples were taken by manually punching two biopsy needles (i.d. = 4·78 mm, Arnold Nasco Ltd., Guelph, Ontario) through the dorsal apaxial muscle, 5–7 cm from the head. The samples, averaging 115·0 ± 6·0 mg ($N = 34$), were immediately frozen in a dry ice/ethanol mixture. The total elapsed time from removal of the fish from the water to freezing the sample was less than 5 s.

Series III

The third study examined the removal rates of La^- and metabolic protons from the blood when equal amounts of the two substances were loaded into the fish by means of lactic acid infusion. Five sole were infused with 5 ml kg^{-1} body weight of an L(+)-lactic acid (Sigma) solution [248 ± 8(5) μmol ml^{-1}] in 160 mM-NaCl by means of a caudal arterial catheter. The total load delivered was 1241 ± 42(5) μmol kg^{-1} body weight. The infusion required 10 min, then the catheter was flushed by the injecti

[redacted] ml kg^{-1} body weight of 160 mM-NaCl. A further 5 min was allowed for mixing before post-infusion sampling commenced (0 h). (For full details, see Turner *et al.* 1983.) The blood sampling times and techniques were the same as in the first series except that the 12 h sample was omitted. Blood samples were analysed for pH_a, total CO_2 (in whole blood and true plasma), Ht, [Hb], and [La^-].

Analytical techniques, calculations, and statistical analyses

Methods were all identical to those described in detail by Turner *et al.* (1983), with the following minor exceptions. Plasma protein and muscle pyruvate levels were not measured, but plasma osmolalities were determined by means of a Wescor Vapour Pressure Osmometer (Model 5100 B). Muscle La^- concentrations (expressed as mequiv kg^{-1} of muscle cells) were corrected for La^- trapped in extracellular fluid (assumed to be the same concentration as whole blood) using a white muscle extracellular fluid volume estimate of 91·6 ml kg^{-1} muscle tissue, the figure given by Batty & Wardle (1979) for plaice, *Pleuronectes platessa*. Values of β needed for the calculation of the whole blood metabolic acid load (ΔH_m^+) were predicted from [Hb] using the regression relationship established in the *in vitro* experiments (see above) of the present study. Regression lines were fitted by the method of least squares and the significance of the correlation coefficient (r) determined.

RESULTS

Blood non-bicarbonate buffer capacity

In vitro tonometry demonstrated linear relationships between β, the non-bicarbonate buffer capacity of whole blood, and either [Hb] or Ht. The two regression relationships were:

$$\beta = -1{\cdot}575[\text{Hb}] - 1{\cdot}88 \quad (N = 5,\ r = 0{\cdot}99,\ P \leqslant 0{\cdot}01) \tag{1}$$

$$\beta = -32{\cdot}55\text{Ht} - 2{\cdot}06 \quad (N = 5,\ r = 0{\cdot}99,\ P \leqslant 0{\cdot}01) \tag{2}$$

where β is in slykes, [Hb] is in g 100 ml^{-1} blood and Ht is expressed as a decimal.

Series I. Blood parameters after exercise

Exhausting exercise caused a larger and more prolonged disturbance in acid-base balance than did moderate exercise (Fig. 1). In the exhausting regime, arterial pH fell from 7·803 ± 0·016(5) at rest to 7·352 ± 0·024(5) at 0 h, and required 8 h to recover (Fig. 1A). The acidosis after moderate exercise was only about half this size and was corrected by 2 h. Plasma [HCO_3^-] declined more gradually from 4·94 ± 0·34(5) mequiv l^{-1} at rest to a minimum of 3·22 ± 0·32(5) mequiv l^{-1} at 2 h after exhausting exercise, before recovering by 8 h (Fig. 1B); this parameter did not change significantly after moderate exercise. Exhausting exercise increased P_{a,CO_2} from 2·02 ± 0·17(5) Torr at rest to 5·31 ± 0·29(5) Torr at 0 h, but the deviation was rapidly corrected by 1 h (Fig. 1C). As with pH_a, the change in P_{a,CO_2} in the moderate regime was only about half of that in the exhausting regime. An analysis based on principles [redacted]ined in Wood *et al.* (1977) showed that the acidosis caused by exhausting activity

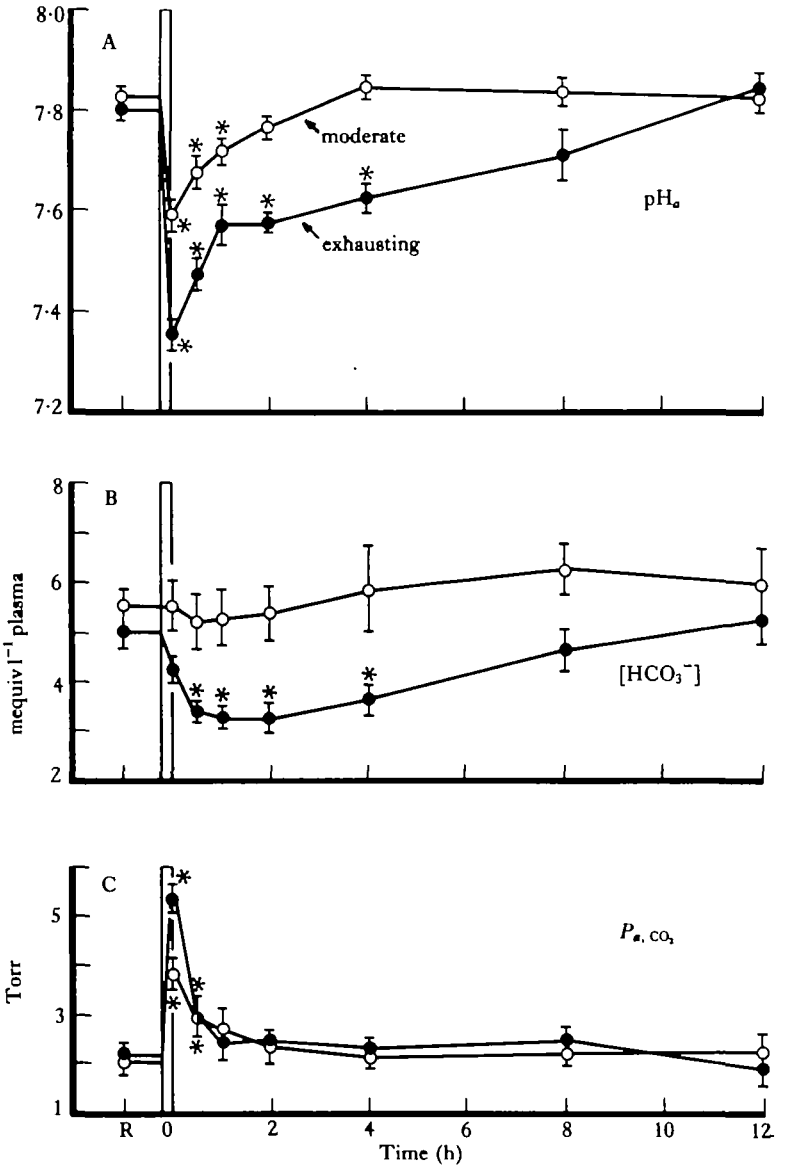

Fig. 1. Blood acid-base status [(A) arterial pH, (B) arterial true plasma bicarbonate concentration, (C) arterial CO_2 tension] in the flathead sole prior to and following either moderate (○) or exhausting (●) exercise. Means ± 1 S.E.M. ($N = 5$ for both treatments). R = rest; bar indicates 10 min of activity; 0 h = immediately post-exercise; * indicates a significant difference ($P \leq 0{\cdot}05$) from the corresponding rest value.

was initially 50 % respiratory and 50 % metabolic in origin, but greater than 75 % metabolic after 0·5 h. The smaller acidosis after moderate exercise was 67 % respiratory and 33 % metabolic at 0 h, and about 50 % attributable to each cause at 0·5 h and 1 h.

Resting arterial blood La^- levels were extremely low [0·18 ± 0·02(10) mequiv

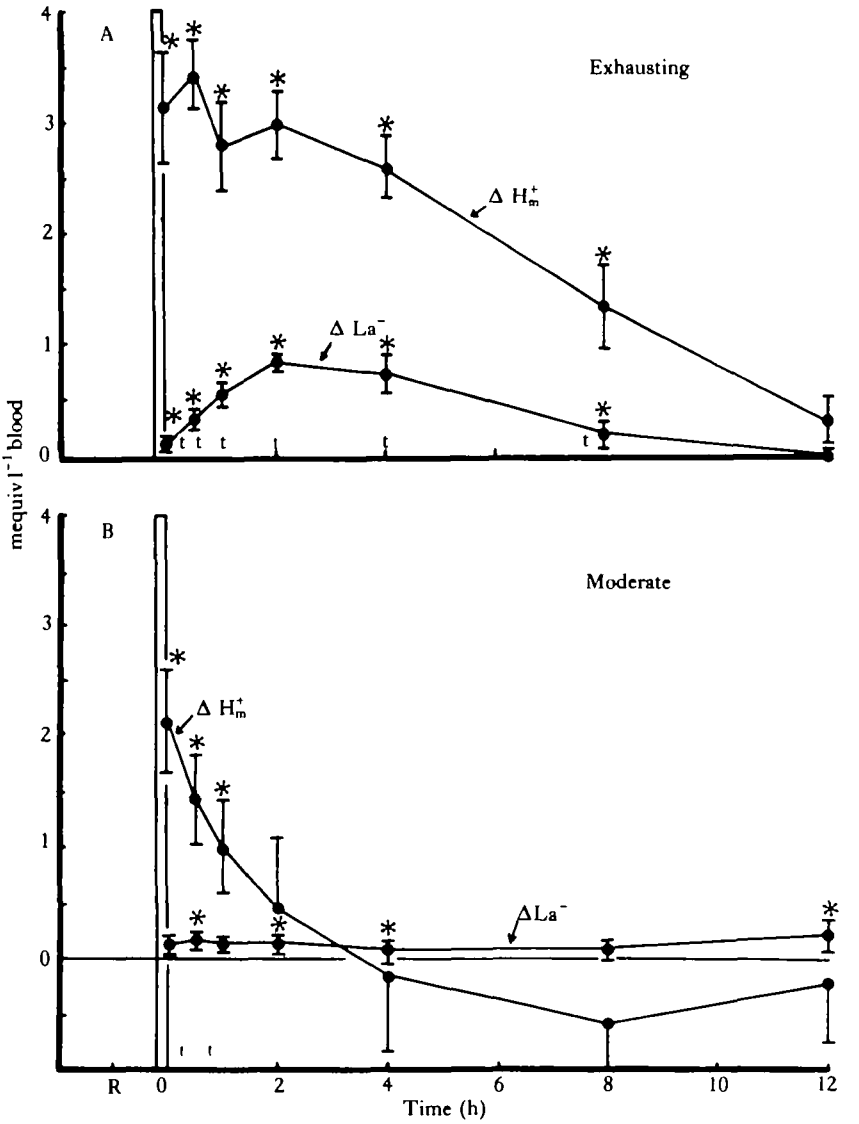

Fig. 2. Changes in blood lactate load (ΔLa^-) and blood metabolic acid load (ΔH_m^+) during recovery from either (A) exhausting or (B) moderate exercise in the flathead sole. Means ± 1 S.E.M. ($N = 5$ for both treatments). R = rest; bar indicates 10 min of activity; 0 h = immediately post-exercise; * indicates a significant difference ($P \leq 0{\cdot}05$) from the corresponding rest value; t indicates a significant difference ($P \leq 0{\cdot}05$) between ΔLa^- and ΔH_m^+ at the same sample time.

and after moderate exercise showed only a very slight rise, which was significant at some sample times (Fig. 2B). Exhausting exercise caused a more pronounced La^- increase, but the peak at 2 h was still less than 1 mequiv l^{-1} (Fig. 2A). The blood metabolic acid load (H_m^+) was always much higher than the blood lactate load (La^-) throughout recovery from exhausting exercise (Fig. 2A). The H_m^+ value was greatest [3·42 ± 0·33(4) mequiv l^{-1}] in the first 0·5 h after exercise, then slowly declined to a [illegible]l not significantly different from 0 by 12 h. A similar, though less pronounced and

prolonged, difference between H_m^+ and La^- was seen after moderate exercise (Fig. 2). These discrepancies between La^- and H_m^+ could be explained by either a preferential removal of La^- from the blood and/or by a slower release of La^- (relative to metabolic protons) from the myotome.

Variability between animals was relatively high for haematological and plasma

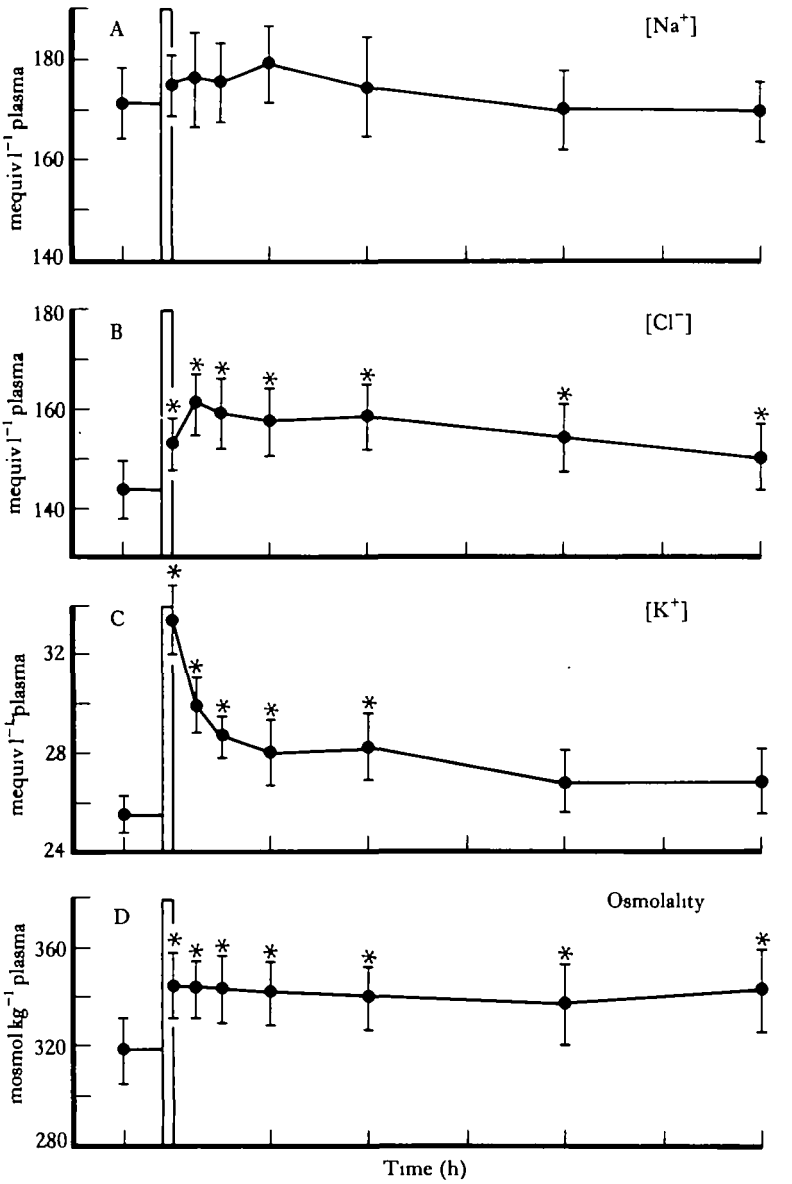

Fig. 3. Plasma concentrations of (A) sodium, (B) chloride, (C) potassium and (D) osmolality in the flathead sole prior to and following burst exercise. Means ± 1 S.E.M. ($N = 10$); data have been combined from the moderate and exhausting exercise treatments. R = rest; bar indicates 10 min of activity; 0 h = immediately post-exercise; * indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

trolyte data. No significant difference could be demonstrated between the two treatments in terms of $[Na^+]$, $[Cl^-]$, $[K^+]$, osmolality, [Hb], Ht and mean corpuscular haemoglobin (MCHC) responses after exercise. Consequently data from the two treatment groups have been combined for these parameters in Figs 3 and 4 and Table 1.

Plasma $[Na^+]$ averaged $171 \pm 9(10)$ mequiv l^{-1} at rest and underwent only a slight, non-significant increase after exercise (Fig. 3A). Plasma $[Cl^-]$, on the other hand, rose markedly from a resting value of $154 \pm 6(10)$ mequiv l^{-1} to a peak of $171 \pm 7(10)$ mequiv l^{-1} at 0·5 h, thereafter slowly declining but remaining significantly elevated for up to 12 h (Fig. 3B). Plasma $[K^+]$ increased to a much greater extent (+31 %) than $[Cl^-]$ (+11 %), rising from $2{\cdot}55 \pm 0{\cdot}09(10)$ mequiv l^{-1} at rest to a maximum of $3{\cdot}33 \pm 0{\cdot}18(10)$ mequiv l^{-1} immediately after exercise (Fig. 3C). Resting levels were re-established by 8 h. Plasma osmolality rose to about the same extent (+9 %) as plasma $[Cl^-]$, from $317 \pm 14(10)$ mosmol kg^{-1} at rest to $344 \pm 14(10)$ mosmol kg^{-1} at 0 h (Fig. 3D); however, unlike plasma $[Cl^-]$, osmolality remained stable at the elevated level for the remainder of the experiment. [Hb] and Ht rose 10–15 % immediately after exercise, despite the initial removal of RBCs in the resting sample (Table 1). Thereafter, due to sampling losses, [Hb] and Ht slowly declined to approximately 45 % of the initial values at 12 h. MCHC fluctuated insignificantly around 0·22 g ml^{-1} RBCs throughout the experiment, indicating a lack of involvement of RBC swelling or mobilization of haemoglobin-poor erythrocytes in the Ht changes (Table 1).

Series II. Muscle and blood metabolites after exercise

Biopsies demonstrated that resting epaxial muscle maintained a relatively low $[La^-]$, $2{\cdot}89 \pm 0{\cdot}51(6)$ mequiv kg^{-1} muscle cells, though this value was significantly greater than the simultaneously measured level in venous blood, $0{\cdot}37 \pm 0{\cdot}06(6)$ mequiv l^{-1} whole blood (Fig. 4). Immediately after exhausting exercise, the white muscle La^- load increased about five-fold. After 2 h recovery, the muscle level had fallen only slightly, and at 12 h was still significantly elevated above the resting concentration (Fig. 4). High muscle-to-blood La^- gradients were maintained throughout the experiment; at no time were the loads anywhere near equilibrium.

Table 1. *Haemoglobin concentration, percent haematocrit and mean corpuscular haemoglobin concentration (MCHC) ($\bar{x} \pm 1$ S.E.M; N = 10), prior to and following 10 min of exercise (data from moderate and exhausting experiments combined), in the flathead sole*

	Rest	0 h	0·5 h	1 h	2 h	4 h	8 h	12 h
Haemoglobin (g 100 ml^{-1} blood)	3·05 ± 0·31	3·35* ± 0·30	2·90 ± 0·28	2·46* ± 0·30	1·88* ± 0·23	1·81* ± 0·26	1·67* ± 0·28	1·49* ± 0·29
Haematocrit (%)	13·7 ± 1·2	15·7* ± 1·4	13·5 ± 1·3	11·5* ± 1·2	9·6* ± 1·0	7·9* ± 0·8	7·4* ± 0·9	6·1* ± 0·8
MCHC (g ml^{-1} RBCs)	0·22 ± 0·01	0·22 ± 0·01	0·22 ± 0·01	0·22 ± 0·02	0·20 ± 0·01	0·24 ± 0·02	0·22 ± 0·02	0·22 ± 0·02

*Indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

The same was true after moderate exercise. Rather surprisingly, muscle levels w[illegible] not significantly lower after moderate activity than after exhausting activity at either 0 h [12·15 ± 2·30(5) *vs* 14·91 ± 1·86(7) mequiv kg^{-1} muscle cells] or 12 h [3·74 ± 0·70(5) *vs* 6·42 ± 1·62(5) mequiv kg^{-1} muscle cells].

Blood pyruvate concentrations increased immediately after exercise and remained

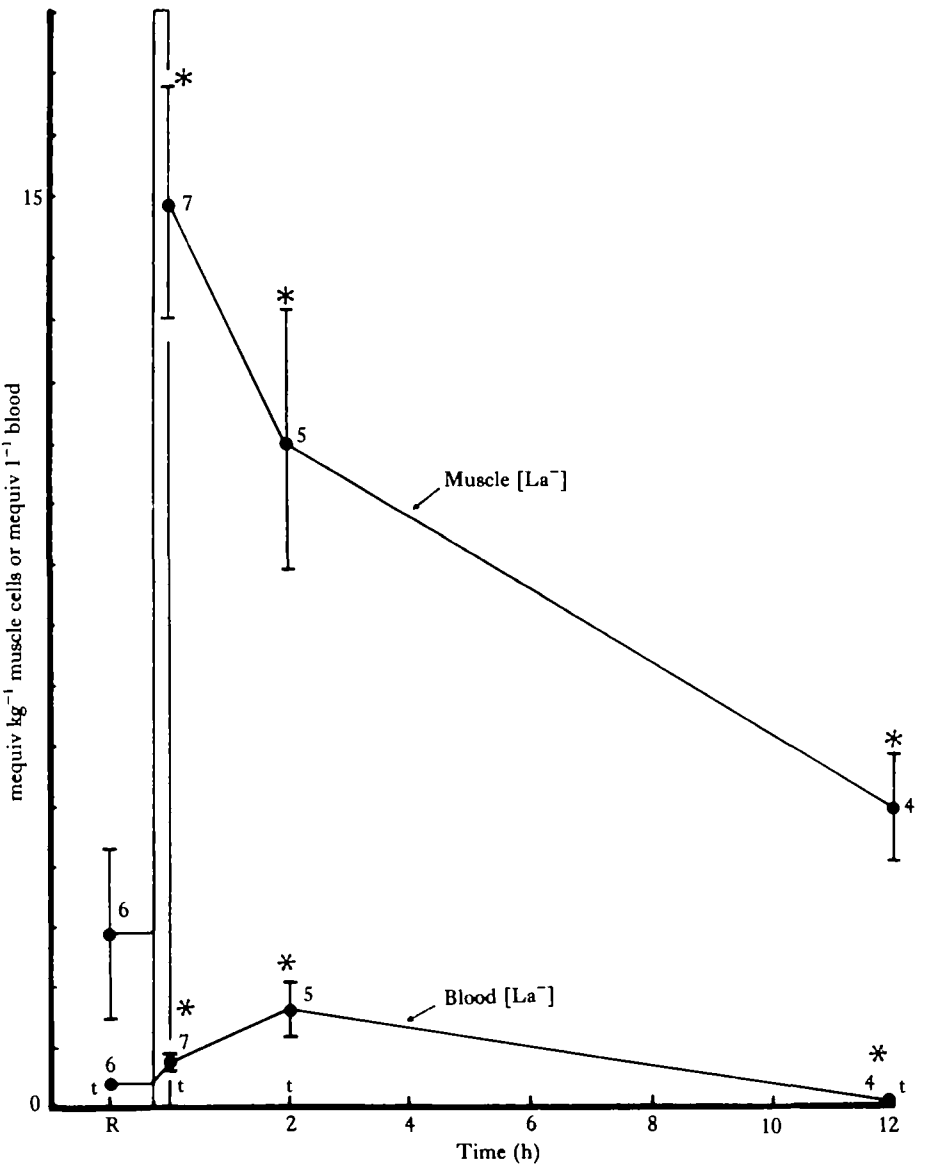

Fig. 4. The relationship between simultaneous muscle and blood concentrations of lactate in terminally sampled flathead sole prior to and following exhausting exercise. Means ± 1 S.E.M. (*N*). R = rest; bar indicates 10 min of exhausting activity; 0 h = immediately post-exercise; * indicates a significant difference ($P \leq 0{\cdot}05$) from rest; t = significant difference ($P \leq 0{\cdot}05$) between muscle and blood levels at the same sample time.

vated above resting levels for the duration of the experiment (Table 2). The magnitude of the rise was dependent upon exercise severity; moderate activity increased pyruvate levels three-fold while exhausting exercise elevated them five-fold. The lactate: pyruvate ratio declined significantly after both exercise regimes and remained depressed at 12 h, though there was an intermediate return to the resting ratio at 2 h in the exhaustively exercised group. Total plasma ammonia levels were relatively high in resting sole and did not change after moderate exercise (Table 2). After exhausting exercise, they were significantly elevated at both 0 h and 2 h, falling below resting levels by 12 h. The overall influence of these variations in pyruvate and ammonia levels on blood acid-base status would have been minimal relative to the actual changes observed (Figs 1, 2).

The venous blood samples taken at rest and 0 h after both moderate and exhausting exercise showed all the same significant changes as seen immediately post-exercise in arterial blood in series I (i.e. pH, P_{CO_2}, La^-, Cl^-, K^+, osmolality, Hb and Ht; cf. Figs 1, 2, 3; Table 1). There were only a few differences in the absolute magnitudes of arterial and venous parameters at the same sample times. Firstly, P_{CO_2} was significantly higher [5·69 ± 0·69(6) *vs* 3·84 ± 0·19(5) Torr] and pH lower [7·456 ± 0·033(6) *vs* 7·587 ± 0·036(5)] in venous than in arterial blood immediately after moderate exercise, as would be expected from elevated CO_2 production in the tissues and excretion at the gills. A similar pattern after exhausting exercise was not statistically significant. Secondly, venous La^- levels were significantly higher than arterial values both at rest [0·37 ± 0·06(6) *vs* 0·18 ± 0·02(10) mequiv l^{-1} blood] and after exhausting exercise [0·79 ± 0·10(7) *vs* 0·29 ± 0·04(5) mequiv l^{-1} blood]. These data suggest some removal of La^- from the blood between the venous and arterial sampling sites, and/or some addition of La^- between the arterial and venous sites.

Series III. Responses to lactic acid infusion

L(+)-lactic acid infusion initiated a moderate, short-lived acidosis. Arterial pH fell

Table 2. *Blood pyruvate concentrations, the lactate: pyruvate ratio in the blood and total plasma ammonia concentrations [$\bar{x}$ ± S.E.M; (N)] prior to and following 10 min of either moderate or exhausting exercise in the flathead sole*

	Rest		0 h	2 h	12 h
Blood pyruvate (mequiv l^{-1})	0·049 ± 0·005 (6)	Moderate	0·156* ± 0·016 (5)	–	0·080* ± 0·011 (5)
		Exhausting	0·270* ± 0·023 (7)	0·158* ± 0·035 (5)	0·109* ± 0·024 (4)
Blood lactate: pyruvate ratio	8·05 ± 2·04 (6)	Moderate	2·90* ± 0·58 (5)	–	4·62 ± 0·76 (5)
		Exhausting	2·99* ± 0·39 (7)	9·90 ± 1·38 (5)	0·98* ± 0·32 (4)
Plasma ammonia (mmol l^{-1})	0·71 ± 0·06 (6)	Moderate	0·87 ± 0·12 (5)	– (5)	0·72 ± 0·05
		Exhausting	1·17* ± 0·04 (7)	1·01* ± 0·07 (5)	0·53* ± 0·04 (5)

* Indicates a significant difference ($P \leq 0·05$) from rest.

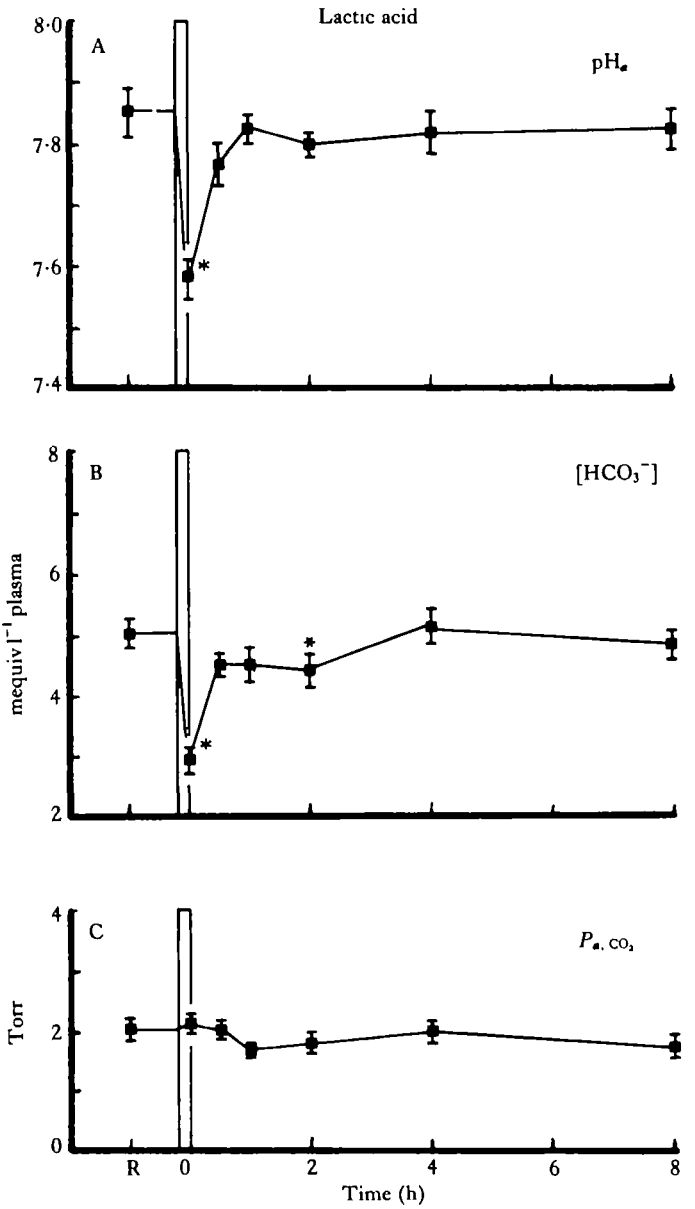

Fig. 5. Blood acid-base status [(A) arterial pH, (B) arterial true plasma bicarbonate concentration, (C) arterial CO_2 tension] in the flathead sole prior to and following an L(+)-lactic acid infusion. Means ± 1 S.E.M. ($N = 5$). R = rest; bar indicates a 15 min infusion and mixing period; 0 h = immediately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

sharply from a resting value of 7·852 ± 0·040(5) to 7·579 ± 0·032(5) at 0 h but returned to a value not significantly different from rest by 0·5 h (Fig. 5A). Plasma $[HCO_3^-]$ decreased from 5·03 ± 0·29(5) mequiv l^{-1} at rest to 2·99 ± 0·20(5) mequiv l^{-1} at 0 h, with a complete return to normal at 2 h (Fig. 5B). P_{a,CO_2} was not significantly affected (Fig. 5C). Therefore the acidosis at 0 h was 89 % metabolic in origin, as might be expected.

Table 3. *Haemoglobin concentration, percent haematocrit and mean corpuscular haemoglobin concentration (MCHC) ($\bar{x} \pm$ S.E.M; N = 5), prior to and following an* L(+)*-lactic acid infusion in flathead sole*

	Rest	0 h	0·5 h	1 h	2 h	4 h	8 h
Haemoglobin ($g\,100\,ml^{-1}$ blood)	2·69 ± 0·30	3·32* ± 0·21	2·54 ± 0·49	2·11 ± 0·43	1·80* ± 0·33	1·61* ± 0·40	1·21* ± 0·32
Haematocrit (%)	13·1 ± 1·7	15·1* ± 1·5	12·1 ± 1·7	10·4* ± 1·4	8·5* ± 1·4	6·9* ± 1·1	5·9* ± 1·2
MCHC ($g\,ml^{-1}$ RBCs)	0·21 ± 0·01	0·22 ± 0·01	0·21 ± 0·01	0·20 ± 0·02	0·21 ± 0·01	0·23 ± 0·03	0·19 ± 0·03

* Indicates a significant difference ($P \leq 0{\cdot}05$) from rest.

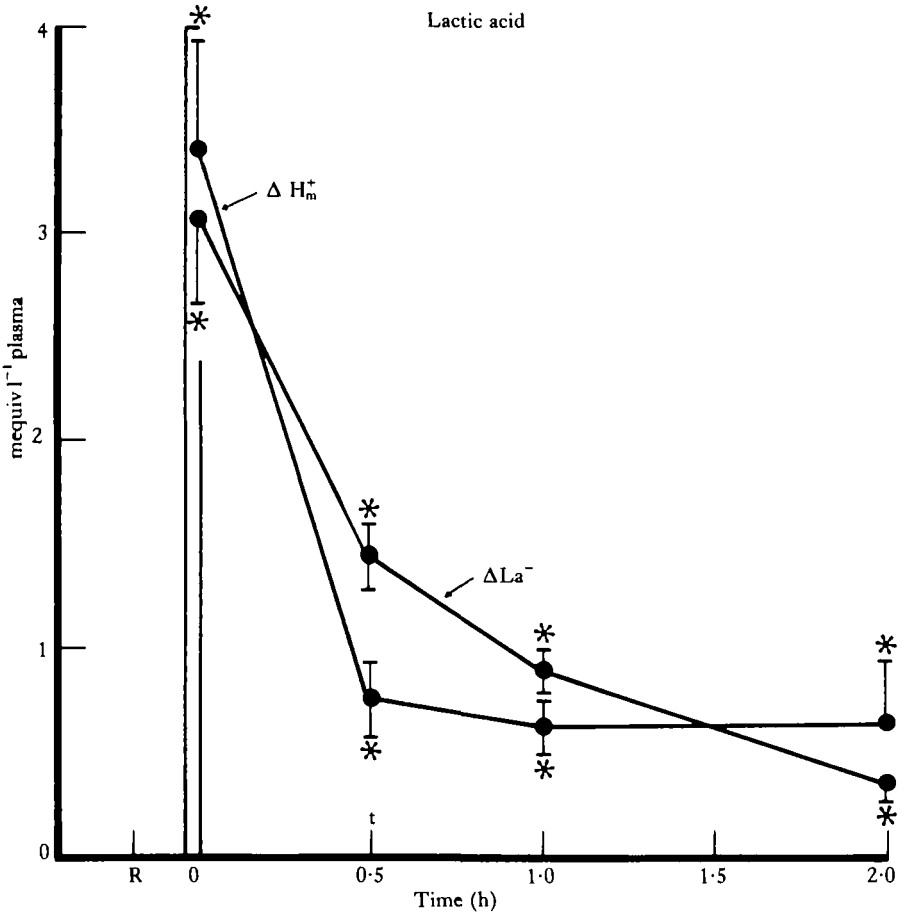

Fig. 6. Changes in blood lactate load (ΔLa^-) and blood metabolic acid load (ΔH_m^+) after infusion of L(+)-lactic acid. Means ± 1 S.E.M. ($N = 5$). R = rest; bar indicates a 15 min infusion and mixing period; 0 h = immediately after this period; * indicates a significant difference ($P \leq 0{\cdot}05$) from rest; t indicates a significant difference ($P \leq 0{\cdot}05$) between ΔLa^- and ΔH_m^+ at the same sample time.

Table 4. *A comparison of the rates of removal ($\bar{x} \pm 1$ S.E.M.; N = 5) of lactate anions and metabolic protons from the blood of the flathead sole during the first 2 h after an* L(+)*-lactic acid infusion*

	Lactate (μequiv l^{-1} blood min^{-1})	Protons (μequiv l^{-1} blood min^{-1})
0–0·5 h	54 ± 10	88 ± 18
0·5–1·0 h	18 ± 4	5 ± 7
1·0–2·0 h	9 ± 1	−2 ± 3*

* Indicates a significant difference ($P \leq 0{\cdot}05$) between the corresponding rates.

Haematological parameters changed after lactic acid infusion (Table 3) in much the same manner as after exercise (Table 1). Both [Hb] and Ht increased significantly at 0 h despite the initial removal of RBCs in the resting sample; thereafter they slowly declined in parallel due to sampling losses. MCHC again fluctuated insignificantly around 0·21 g ml^{-1} RBCs throughout the experiment.

The goal of the infusion was to place equivalent concentrations of lactate and metabolic protons in the blood compartment at time 0 h; this was achieved [La^- = 3·06 ± 0·41(5) mequiv l^{-1} blood; $H_m^+ = 3{\cdot}39 \pm 0{\cdot}51$ mequiv l^{-1} blood; Fig. 6]. The rather low La^- relative to the infused load reflected an extremely large lactate space in the flathead sole, 624 ± 64(5) ml kg^{-1} body weight. La^- and H_m^+ declined in an exponential fashion from 30 min onwards (Fig. 6), resting levels being regained by 4 h. Between 0 h and 1 h post-infusion, the rates of H_m^+ and La^- removal were not significantly different (Table 4). If anything, H_m^+ fell more quickly, a lower absolute level being attained by 0·5 h (Fig. 6). These results suggest that the discrepancy found between H_m^+ and La^- levels during recovery from exercise (Fig. 2) cannot be explained by a preferential removal of La^- from the blood space. It must be noted that between 1 h and 2 h, the apparent disappearance of La^- was faster than that of protons, but this is of little consequence as the rates were extremely low by this time, less than 10 μequiv l^{-1} min^{-1} (Table 4).

DISCUSSION

Comparison with other studies on exercised flatfish

The present data on *Hippoglossoides elassodon*, a species whose acid-base and exercise physiology has not previously been studied, confirms the two unusual features of the post-exercise physiology of Pleuronectiformes which were raised in the Introduction. Firstly, in agreement with the studies of Dando (1969) and Wardle (1978) on the plaice, *Pleuronectes platessa*, and of Wood *et al.* (1977, 1979*b*) on the starry flounder, *Platichthys stellatus*, blood La^- levels after exhausting exercise remained extremely low (< 2 mequiv l^{-1}; Figs 2, 4). Secondly, metabolic acid levels (ΔH_m^+) exceeded lactate anion levels in the blood after exercise (Fig. 2) to an even greater extent than that reported by Wood *et al.* (1977) for the starry flounder. The overall pattern of post-exercise changes in acid-base status of the blood (Fig. 1) w

qualitatively similar but quantitatively different from that seen in the starry flounder (see below).

Despite the similarity in the blood La^- concentrations, post-exercise levels of La^- in the white muscle of the flathead sole (Fig. 4) were less than half those in similarly exercised plaice (Wardle, 1978). This appears to be a real species difference as Dando (1969) also reported higher muscle levels in plaice recovering from trawling stress. It is interesting that in the present study, muscle [La^-] was almost as high after moderate as after exhausting exercise. There is certainly no doubt that the latter treatment produced total exhaustion, so the capacity for intracellular La^- generation appears to be rather limited in *Hippoglossoides elassodon*.

Acid-base changes

The acidoses occurring after both moderate and exhausting exercise in the flathead sole were of mixed respiratory (i.e., P_{CO_2} elevation) and metabolic (i.e., ΔH_m^+ elevation) origin (cf. Davenport, 1974) (Figs 1, 2). The smaller acid-base disturbance after moderate exercise reflected smaller contributions from both factors. In each case, the respiratory component was maximal immediately post-exercise, and was then rapidly corrected, while the metabolic component was more prolonged. In arterial blood, the respiratory contribution to the total pH depression at 0 h was 67 % after moderate exercise and 50 % after exhaustive exercise. The greater pH depression in venous blood in both treatments mainly reflected larger P_{CO_2} elevations; ΔH_m^+ could not be precisely calculated for the venous samples as they came from different animals at different times, but estimates based on mean values suggested that this parameter was of about equal magnitude in venous and arterial blood. Based on their measurements in venous blood of starry flounder, Wood *et al.* (1977) postulated that a temporal separation occurs between respiratory and metabolic acidoses to prevent overloading of the very low buffer capacity of fish blood. The present measurements on arterial blood of both sole and trout (Turner *et al.* 1983) partially support this hypothesis. The metabolic component certainly persisted long after the P_{a,CO_2} elevation had been corrected (Figs 1, 2; Figs 1, 2 of Turner *et al.* 1983), but values of ΔH_m^+ were already close to or at maximum by 0 h, the time when values of P_{a,CO_2} were at their peaks.

The size of the acidosis in arterial blood of the flathead sole after exhausting exercise (−0·45 pH units) was very similar to that seen in the rainbow trout after strenuous exercise (−0·51 pH units) and the relative causations (50 % respiratory, 50 % metabolic) were identical. Qualitatively, the physiological explanations for the effects were probably the same in the two species, and have been outlined in Turner *et al.* (1983). However, despite the similar pH_a depressions, the P_{a,CO_2} elevation (+3·29 *vs* +4·76 Torr) and the ΔH_m^+ increase (+3·4 *vs* +7·7 mequiv l^{-1} blood) were both considerably smaller in the sole than in the trout. The explanation of the apparent contradiction is complex. Firstly, the average whole blood non-bicarbonate buffer capacity (β) in sole (6·7 slykes) was somewhat lower than in trout (8·5 slykes), a difference resulting from a much lower [Hb] partially compensated by a higher β per unit [Hb] [compare Equation (1) of the present study with Equation (4) of Turner *et al.* 1983]. Secondly, the blood bicarbonate buffer capacity (i.e., whole blood [HCO_3^-]) was also lower in the sole than in the trout (4·7 *vs* 7·1 mequiv l^{-1} blood). Thirdly, because of the linear/log relationship between pH and P_{CO_2} (Wood *et al.*

1977, 1982), the smaller P_{a,CO_2} elevation in the sole, which started from a lo[illegible] absolute value than in the trout (2·02 *vs* 3·03 Torr), would have had a greater relative effect on pH_a.

Flathead sole obviously performed far less exercise prior to exhaustion than did trout, and this probably accounted for their lower post-exercise levels of both P_{a,CO_2} and ΔH_m^+. The major source of the latter was probably H^+ efflux from white muscle. The post-exercise rise in muscle intracellular [La^-] in the sole was less than half that in the trout (±12·0 *vs* +33·5 mequiv kg^{-1} muscle cells), so initial increases in intracellular proton loads probably differed to a comparable extent. We have previously argued that the post-exercise elevation in plasma [K^+] reflects intracellular acidosis (Turner *et al.* 1983). The much smaller elevations in this parameter in exhausted sole than in severely exercised trout (+1·01 *vs* +2·23 mequiv l^{-1} plasma) is in accord with this interpretation.

Electrolyte and haematological changes

Like the rainbow trout in fresh water (Turner *et al.* 1983), the flathead sole in sea water exhibited a general haemoconcentration after exercise, plasma ions, osmolality, Ht and [Hb] all increasing by 5–15 % (Fig. 3, Table 1). The mechanism is probably similar, i.e. a shift of water out of the vascular compartment in response to the increased intracellular La^- load and/or diuresis. However, it is also possible that elevated salt influx from and/or water loss to the hypertonic external environment occurred at the gills. Indeed, the greater rise in plasma [Cl^-] than in plasma [Na^+] (Fig. 3) supports this interpretation as Cl^- is more concentrated than Na^+ in sea water. This decrease in plasma [$Na^+ - Cl^-$] after exercise was opposite to the situation in trout, a difference presumably correlated with the fact that sole demonstrated very small increases in blood [La^-] (Figs 2, 4), while trout exhibited large increases both in this parameter and in another supposed organic anion in the blood after exercise (Turner *et al.* 1983). The observed changes in [Na^+], [Cl^-] and [K^+] were large enough to account for the immediate rise in osmolality (Fig. 3), but late in recovery, osmolality remained high while these ions had largely returned to normal. Elevated plasma glucose levels at this time may have contributed to the effect (cf. Wardle, 1972).

Wood *et al.* (1977) reported a rise in Ht after exhausting exercise in the starry flounder similar to that in the flathead sole, and attributed it to RBC swelling as a result of high venous P_{CO_2}. The explanation does not apply to the present study, because MCHC remained unchanged in both venous and arterial samples. Simple haemoconcentration by plasma fluid loss and/or RBC mobilization appears more likely. Almost identical changes in haematological parameters occurred after lactic acid infusion (Table 3). Perhaps blood acidosis in some way elicits these effects, for example by causing diuresis, splenic contraction or increased diffusive permeability of the gills.

Lactate vs *metabolic proton levels in the blood*

The most striking difference between the responses of the sole and the trout was in the relationship between blood La^- and metabolic proton levels. In the trout, ΔLa^- exceeded ΔH_m^+ at all times after 0 h (cf. Fig. 2 of Turner *et al.* 1983), but exactly t[illegible]

posite was true in the sole, even at 0 h (Fig. 2). Wood *et al.* (1977) hypothesized that a somewhat smaller excess of ΔH_m^+ over ΔLa^- in the exercised starry flounder reflected the presence of an 'unknown acid' in the blood. NH_4^+, while not measured, was suggested as a candidate, based on the report that NH_4^+ ions were released from white muscle after strenuous exercise in the carp (Driedzic & Hochachka, 1976). Total plasma ammonia levels did increase after exhaustive exercise in the flathead sole (Table 2), as in the rainbow trout (Turner *et al.* 1983), but the magnitude of the effect was far too small to have significant influence on ΔH_m^+ (Fig. 2). In any event, it now seems more likely that this ammonia would be produced metabolically as NH_3, rather than as NH_4^+, thereby having a slight alkalizing influence (Kormanik & Cameron, 1980). Pyruvate measurements (Table 2) indicated that increases in pyruvic acid levels would also be far too small to alter ΔH_m^+ significantly. Nevertheless, the possibility of elevation of ΔH_m^+ by the presence of other organic acids produced in exercise metabolism cannot be eliminated without detailed biochemical analysis of the blood plasma.

If, on the other hand, metabolic protons and lactate anions originate mainly from lactic acid production in white muscle, then the excess of ΔH_m^+ over ΔLa^- can only be explained by differential removal or differential release phenomena. Differential removal seems unlikely, since as in the trout study, ΔH_m^+ and ΔLa^- disappeared from the blood at similar rates during lactic acid infusion experiments (Fig. 6, Table 4). We therefore favour the differential release explanation, which has also been proposed to account for the opposite ΔH_m^+ *vs* ΔLa^- discrepancy in the trout (Turner *et al.* 1983). In *Salmo gairdneri*, the hypothesis has been strongly supported by direct measurements of a differential release of lactate anions in excess of metabolic protons in an isolated, perfused trunk preparation (J. D. Turner and C. M. Wood, unpublished). Similar experiments with the perfused trunk of *Hippoglossoides elassodon* should therefore clarify the situation.

Similar patterns of ΔH_m^+ excess over ΔLa^- in the blood have been observed in two amphibians (McDonald, Boutilier & Toews, 1980; Boutilier, McDonald & Toews, 1980), the related starry flounder (Wood *et al.* 1977), and apparently also in the muskellunge, *Esox masquinongy* (Beggs, Holeton & Crossman, 1980), though a complete blood acid-base analysis was not performed in the latter study. Rather sedentary life styles and the capability for only short bursts of vigorous exercise, supported largely by anaerobic metabolism, are features common to all these animals. The trout (Turner *et al.* 1983) and the dogfish (Piiper *et al.* 1972), which show the opposite discrepancy, ΔLa^- exceeding ΔH_m^+ in the blood after exercise, are active fish capable of extended cruising or endurance exercise supported largely by aerobic metabolism. They rely on glycolysis mainly to support short sprinting performance. Nevertheless, their absolute scope for energy production by anaerobic metabolism, and therefore lactic acid production, may well be greater than that of the more sedentary animals.

In an ecological context, the strategy of the active animals after burst performance may be to minimize proton loading into the blood from the high intramuscular lactic acid load in order to minimize interference with blood O_2 transport by the Bohr and Root effects. Maintenance of adequate O_2 transport is necessary to support continued ivity by aerobic red muscle, an essential requirement for survival in an animal

adopting a cruising pelagic life style. The majority of La^- is probably metaboli within the muscle (cf. Turner *et al.* 1983), but some may be released as a fuel for essential organs such as the heart, gills and red muscle (Bilinski & Jonas, 1972; Lanctin, McMorran & Driedzic, 1980).

The strategy of the normally sluggish, sedentary animals, as typified by the flathead sole, may be to minimize La^- release from white muscle, so that the majority of the La^- can be utilized for glycogen resynthesis *in situ* (see below). Proton release into the blood may be tolerated so that white muscle intracellular pH returns rapidly to a level compatible with the gluconeogenic processes. Any detrimental effect of ΔH_m^+ on blood O_2 transport would be relatively unimportant as burrowing, immobility and camouflage are the principal defence strategies. It is also possible that some of the protons may be excreted; McDonald, Walker, Wilkes & Wood (1982) have recently demonstrated a high branchial capacity for H^+ excretion in a related flatfish, *Parophrys vetulus*, the lemon sole.

This restriction on La^- release from muscle to blood probably accounted for the extremely unusual lactate: pyruvate ratios observed in the blood of sole after exercise (Table 2). Rather than increasing in the standard fashion, as in the trout (Turner *et al.* 1983), the ratios decreased significantly after both moderate and exhausting exercise. The transitory return of the ratio to resting level at 2 h resulted from the modest blood lactate peak at this time (Figs 2, 4). Therefore, pyruvate movement from the myotome into the blood does not appear to be restricted in the same fashion as La^- movement. Unfortunately, pyruvate levels and lactate: pyruvate ratios were not measured in the white muscle in the flathead sole. However, in the white muscle of plaice recovering from trawling stress, Dando (1969) found that these parameters changed in the standard pattern. The lactate: pyruvate ratio in blood must therefore be interpreted with caution in lower vertebrates where La^- release can be differentially controlled.

Ultimate fate of lactic acid

The present results are entirely in accord with the view that La^- is actively retained within the myotome so that the major portion may be converted back to glycogen by gluconeogenis *in situ* (Wardle, 1978; Batty & Wardle, 1979). A similar argument for lactic acid retention and metabolism *in situ* has been presented for the trout (Turner *et al.* 1982), but the evidence for flatfish appears even more clear cut. Blood La^- levels stayed extremely low after exercise (Fig. 2) and white muscle levels remained significantly elevated even after 12 h (Fig. 4). La^- retention in white muscle appears to be under catecholaminergic control (Wardle, 1978), and there is some evidence that La^- may actually be taken up against a gradient and incorporated into glycogen by the muscle cells (Batty & Wardle, 1979). The extremely high La^- space in the resting flathead sole, 624 ml kg^{-1} body weight, also suggests that La^- is taken up into the muscle cells after lactic acid infusion. There is also evidence against the operation of the Cori cycle in flatfish. Dando (1969) found minimal levels of LDH activity in plaice liver and little evidence for La^- uptake or metabolism by this site in animals recovering from trawling stress. Furthermore, Batty & Wardle (1979) demonstrated that blood glucose turnover rates were far too low to account for measured glycogen resynthesis rates in the white muscle of plaice recovering from exercise stress. Howev

serious argument against this scheme of glycogen resynthesis from lactate within the white muscle of flatfish remains. Phosphoenolpyruvate carboxykinase, thought to be an essential enzyme in gluconeogenesis, has been found in a variety of white muscles, including those of fish (Opie & Newsholme, 1967; Crabtree, Higgins & Newsholme, 1972), but is absent from the white muscle of plaice according to Johnston & Moon (1979) and Moon & Johnston (1980). Perhaps further investigation will yield an alternative pathway or find activity of this enzyme with some alteration in the assay.

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