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COMPARTMENTAL DISTRIBUTIONS OF CARBON DIOXIDE AND AMMONIA IN RAINBOW TROUT AT REST AND FOLLOWING EXERCISE, AND THE EFFECT OF BICARBONATE INFUSION

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Summary

The carbon dioxide content of the intracellular compartment of fish muscle was determined by direct measurements of CO_2 and pH in tissue homogenates of rainbow trout, *Oncorhynchus mykiss*. The results agree with the concept that compartmental distribution of CO_2 is pH-dependent and that muscle membranes are not very permeable to bicarbonate. The interaction between CO_2 and ammonia excreted from fish muscle was also investigated by altering plasma CO_2 content using bicarbonate infusion following exhaustive exercise. Removal of the acid boundary layer in white muscle by bicarbonate infusion resulted in retention of ammonia in the muscle, indicating that ammonia excretion across the muscle membrane might be enhanced by the hydration of excreted CO_2 in the extracellular fluid. Passive diffusion of NH_3, rather than NH_4^+ transfer, is probably the dominant pathway of ammonia excretion through fish muscle membranes.

Introduction

The mechanisms of CO_2 transport in the blood and its excretion across the gills have been extensively studied in various fish (see reviews by Perry, 1986; Randall, 1990), but little is known about CO_2 distribution or the factors determining that distribution in body tissues of fish. Because of a lack of adequate methods for direct measurement, tissue CO_2 content is calculated by assuming that tissue P_{CO_2} is equal to venous P_{CO_2}, based on the theory of non-ionic diffusion of weak acids (Milne *et al.* 1958). The validity of such calculations, however, has not been tested, especially in cases where large changes of CO_2 content occur in a short period, such as following exhaustive exercise. The first objective of the present study was

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therefore to evaluate intracellular CO_2 content in the white muscle of rainbow trout at rest and following exhaustive exercise, based on direct total CO_2 and pH measurements in tissue homogenates.

The distribution and excretion of ammonia have been investigated in some fish (see review by Randall, 1990). The excretion of ammonia and carbon dioxide across fish gills is linked (Wright *et al.* 1989). In brief, CO_2 excretion across the gills acidifies the water layer next to the gill surface as CO_2 is hydrated at a catalysed rate to form HCO_3^- and H^+. NH_3 excretion however, raises boundary layer pH with the formation of NH_4^+. The latter effect is usually masked by the much larger CO_2 excretion. The formation of an acidic boundary layer at the gill as a result of CO_2 excretion will, therefore, enhance ammonia excretion by trapping NH_3 as NH_4^+. Carbonic anhydrase has been observed on the sarcolemma of skeletal muscle in a variety of vertebrates (Gros and Dodgson, 1988) including fish (Sanyal *et al.* 1982). The presence of this enzyme on the sarcolemma raises the possibility that similar interactions between carbon dioxide and ammonia may also occur at the tissue level. The role of carbonic anhydrase on the sarcolemma of muscle tissue is probably to facilitate CO_2 diffusion. However, the functional influence of a catalysed CO_2 hydration reaction on ammonia excretion has not been considered. The second objective of the present study was, therefore, to investigate the possible interactions between carbon dioxide and ammonia transfer from white muscle. This was approached by altering plasma CO_2 content using bicarbonate infusion following exhaustive exercise in rainbow trout, and then examining CO_2 and ammonia contents in extra- and intracellular compartments.

Wright *et al.* (1988) concluded that fish muscle membranes, unlike those of mammals, were permeable to NH_4^+. This conclusion has been challenged on the grounds that the metabolic cost that must result from the subsequent short-circuiting of proton transport is too high (Heisler, 1990). If the muscle membrane is very permeable to ammonium ions, then the removal of the acid boundary layer by bicarbonate infusion should enhance ammonia excretion from the muscle. If the muscle membrane is permeable to ammonia rather than to ammonium ions then the removal of the acid boundary layer should retard ammonia excretion from the muscle.

Materials and methods

Animals and preparation

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)], weighing 180–250 g, were obtained from a local hatchery, and held outdoors at the University of British Columbia, Canada, in dechlorinated Vancouver tap water (11–13 °C) for at least 2 weeks before experimentation. The animals were fed with commercial trout pellets, but feeding was suspended 4 days prior to surgery. Under MS-222 anaesthesia (1:10 000 in $NaHCO_3$-buffered fresh water), fish were fitted with dorsal aortic catheters. Following surgery, fish were allowed to recover for at least

48 h in the darkened Plexiglas box of a 3-l water recirculating system at constant temperature (12°C).

Experimental protocol

Series 1. CO_2 distribution at rest and following exercise

For measurements at rest, blood samples (800 μl) were taken from the dorsal aorta and immediately analyzed for plasma pH (pHa), plasma total CO_2 (Ta_{CO_2}), plasma total ammonia (Ta_{amm}) and whole-blood lactate concentration ($[Lactate]_a$). The animals then received a bolus injection through the dorsal aortic cannula of 1 mg per 300 g body mass of *d*-tubocurarine chloride (a neuromuscular junction blocker, Sigma). When the animal was unable to move (approximately 10 s after injection), it was quickly removed from the chamber, and a sample of white muscle was immediately excised from beside the spine starting at the middle of the dorsal fin and cutting 3–4 cm caudally. Samples were immediately freeze-clamped and stored in liquid nitrogen prior to analysis. The time between injection and freezing was 20–30 s. The animals were then killed by anaesthetic overdose.

For measurements following exhaustive exercise, animals were chased to exhaustion in a 500-l circular tank (approximately 6 min). Immediately after exercise, blood and white muscle samples were taken as described above.

Series 2. Effects of bicarbonate infusion following exercise

Animals were exercised to exhaustion as described above. Each fish was then held in the darkened chamber of the recirculating system. The animal was immediately infused with 5 ml kg^{-1} body mass of saline (control group) or 3 mol l^{-1} $NaHCO_3$ in saline (experimental group), using a peristaltic pump to deliver the solution into the dorsal aorta over a period of approximately 10 min. At 15 min or 30 min post-exercise (about 5 min or 20 min after infusion), samples of blood and white muscles were taken as described above. For measurement of ammonia excretion in the external medium over the experimental period (0–15 min or 0–30 min post-exercise), water samples from the recirculating system were taken at 0 and 15 or 30 min following exercise.

Analytical procedures and calculations

Plasma pH (pHa) was determined with a microcapillary pH electrode (Radiometer G279/G2) coupled to a PHM84 pH meter. Plasma total CO_2 (Ta_{CO_2}) was measured using a gas chromatography method (Boutilier *et al.* 1985) on samples obtained anaerobically. Plasma CO_2 tension (Pa_{CO_2}) and bicarbonate concentration ($[HCO_3^-]_a$) were calculated using a rearrangement of the Henderson–Hasselbalch equation with values of plasma pK′ and CO_2 solubility coefficients for trout blood at 12°C (Boutilier *et al.* 1984). Plasma total ammonia (Ta_{amm}) was assayed based on the L-glutamic dehydrogenase/NAD method (Sigma reagents). Plasma partial pressure of ammonia (Pa_{NH_3}) and ammonium ion concentration ($[NH_4^+]_a$) were calculated from the Henderson–Hasselbalch equation, using pK and αNH_3 values given by Cameron and Heisler (1983). Whole-blood lactate

levels ($[Lactate]_a$) were analyzed using the L-lactate dehydrogenase/NAD method (Sigma, 1982). Water ammonia concentration was measured by a modification of the salicylate–hypochlorite reaction (McDonald and Wood, 1981). The net fluxes of ammonia (in $\mu mol\,h^{-1}\,kg^{-1}$) were calculated from changes in their respective concentrations in the water of the recirculating system.

For intracellular measurements of white muscle, samples were ground to a fine powder under liquid nitrogen using a precooled mortar and pestle. The powder was always kept wet with liquid nitrogen (to avoid CO_2 loss) and was immediately subjected to the following procedures. Muscle intracellular pH (pHi) and Ti_{CO_2} were determined by direct measurement of tissue homogenates using fluoride and nitrilotriacetic acid as metabolic inhibitors, as described recently by Pörtner *et al.* (1990). In brief, about 150 mg of tissue powder (wet with liquid nitrogen) was transferred to a preweighed 0.5 ml Eppendorf tube containing 0.2 ml of ice-cold medium (150 mmol l^{-1} potassium fluoride, 6 mmol l^{-1} nitrilotriacetic acid). The tube (containing the mixture of tissue powder and medium) was quickly weighed, filled with more medium until almost full (to avoid air contamination), briefly stirred with a needle, capped and reweighed. The mixture was then stirred with a vortex mixer for 3–4 s, and centrifuged for 3–5 s at 12 000 revs min^{-1}. Samples of the supernatant were immediately taken for measurements of pH and T_{CO_2} as described above. The measured pH of the supernatant was taken as the pH of the tissue homogenate since the effect of dilution by the medium on the pH value is negligible in this case. The values of white muscle intracellular pH were calculated from the pH of the supernatant, taking the estimated influence of extracelluar compartments into consideration (see Pörtner *et al.* 1990, for details of the calculation). Values of muscle Ti_{CO_2} on the basis of intracellular fluid (mmol l^{-1} ICF) were calculated as:

$$Ti_{CO_2} = \frac{\text{whole-tissue } T_{CO_2} - (Q \times \text{extracellular } T_{CO_2})}{1 - Q}, \quad (1)$$

where whole-tissue T_{CO_2} =

$$\text{measured supernatant } T_{CO_2} \times \frac{MV + (TM \times WTFV)}{TM \times WTFV}. \quad (2)$$

MV is the medium volume (l) used in the tissue and medium mixture. WTFV is the whole-tissue fluid volume ($l\,g^{-1}$ tissue) taken from Milligan and Wood (1986). TM is the tissue mass (g) used in the homogenate preparation. Q is the fraction (%) of extracellular fluid volume in whole-tissue fluid volume, taken from Milligan and Wood (1986). Since only arterial plasma values were measured in the present study, the extracellular T_{CO_2} values in equation 1 were assumed to be the same as the venous levels, which were estimated from arterial values by assuming that venous–arterial differences in T_{CO_2} and pHa were the same as those determined by Milligan and Wood (1986), who employed the same experimental protocol as that used in this study. This adjustment was not performed when calculating Ti_{CO_2} at rest, since Milligan and Wood (1986) found no significant venous–arterial

differences in pH or T_{CO_2} in resting trout. Muscle Pi_{CO_2} and $[HCO_3^-]_i$ were then calculated using a rearrangement of the Henderson–Hasselbalch equation, based on the value of muscle intracellular pK and the CO_2 solubility coefficient calculated using the equations given by Heisler (1984). Values of Pi_{NH_3} and $[NH_4^+]_i$ were similarly calculated using appropriate constants from Cameron and Heisler (1983).

For the measurements of muscle intracellular ammonia and lactate concentrations, about 500 mg of the tissue powder (wet with liquid nitrogen) was transferred to a preweighed vial containing 1 ml of ice-cold 0.6 mol l^{-1} perchloric acid (PCA) and then reweighed. A further 2 ml of PCA was added and the mixture was immediately homogenized twice on ice for 15 s using an Ultra-Turrax homogenizer. The homogenate was then centrifuged for 10 min at 13 000 revs min^{-1} and 4 °C. A known volume of supernatant was immediately neutralized (pH 7.0) with medium containing 1.5 mol l^{-1} K_2CO_3 and 0.5 mol l^{-1} triethamolamine, and stored in liquid nitrogen prior to analysis. The concentrations of ammonia and lactate in the supernatant were analyzed in the same way as described above. The values of muscle Ti_{amm} and $[Lactate]_i$ (mmol l^{-1} ICF) were calculated in the same way as that for Ti_{CO_2} (see equations 1 and 2).

If carbon dioxide is distributed between muscle intra- and extracellular compartments according to the pH gradient, then intra- and extracellular $[CO_2]$ should be equal. To determine whether this was the case, muscle Ti_{CO_2} and pHi were predicted from the following formula:

$$\text{predicted muscle pHi} = \text{pK} + \log \frac{Ti_{CO_2} - \text{extracellular } [CO_2]}{\text{extracellular } [CO_2]}, \tag{3}$$

$$\text{predicted muscle intracellular } T_{CO_2} = \text{extracellular } [CO_2] \{\text{antilog (measured pHi} - \text{pK}) + 1\}. \tag{4}$$

Similarly, assuming that intra- and extracellular $[NH_3]$ were equal, the following predictions can be made:

$$\text{predicted muscle pHi} = \text{pK} + \log \frac{\text{extracellular } [NH_4^+]}{\text{intracellular } [NH_4^+]}, \tag{5}$$

$$\text{predicted muscle intracellular } T_{amm} = \text{extracellular } [NH_3] + \frac{\text{extracellular } [NH_3]}{\text{antilog (measured pHi} - \text{pK)}}. \tag{6}$$

The equilibrium potentials for HCO_3^- ($E_{HCO_3^-}$) and NH_4^+ ($E_{NH_4^+}$) across the muscle cell membranes were calculated from the Nernst equation:

$$E_{HCO_3^-} = \frac{RT}{ZF} \ln \frac{\text{intracellular } [HCO_3^-]}{\text{extracellular } [HCO_3^-]} \tag{7}$$

$$E_{NH_4^+} = \frac{RT}{ZF} \ln \frac{\text{extracellular } [NH_4^+]}{\text{intracellular } [NH_4^+]} \tag{8}$$

where R, T, Z and F have their usual meanings.

Statistical analysis

Mean values±S.E. are reported throughout. Differences between groups were analyzed statistically using unpaired Student's *t*-test. $P<0.05$ was taken as the fiducial limit of significance.

Results

The present studies are the first direct measurement of carbon dioxide content in the intracellular compartment of fish muscle (Fig. 1; Table 1). In resting animals Pi_{CO_2} is similar to Pa_{CO_2} and Pv_{CO_2}, whereas after exercise Pi_{CO_2} is about 0.2 kPa greater than Pa_{CO_2} but is similar to the value calculated for venous blood (Fig. 1A; Table 2). The correlation between Pi_{CO_2} and Pa_{CO_2} or Pv_{CO_2} is demonstrated in Fig. 2. T_{CO_2}, however, is much higher in plasma than in muscle during both rest

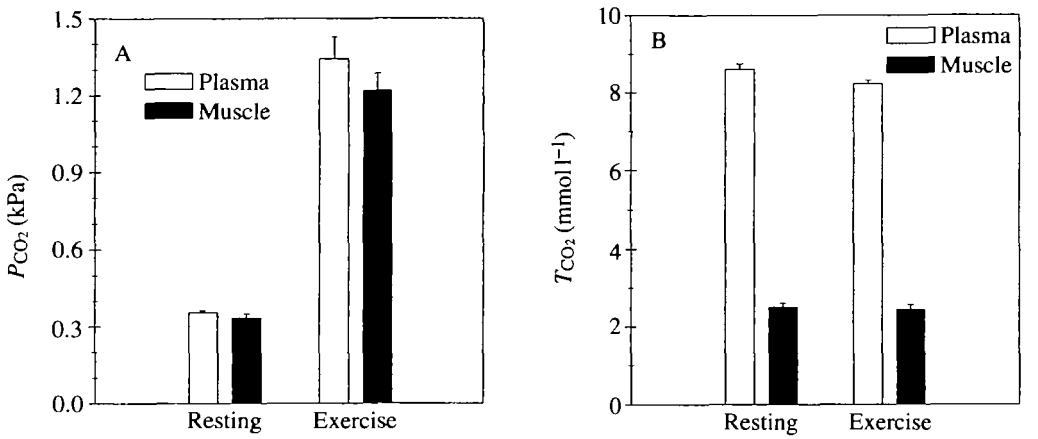

Fig. 1. (A) Partial pressure of CO_2 (P_{CO_2}) and (B) total CO_2 content (T_{CO_2}) in trout venous plasma (derived from our arterial values and the arterial–venous differences reported by Milligan and Wood, 1986) and white muscle at rest and immediately following exhaustive exercise. Values are shown as means+S.E. ($N=7$).

Table 1. *Measured and predicted muscle pHi and T_{CO_2} in rainbow trout at rest and immediately following exercise*

	Measured		Predicted		Calculated
	Muscle pHi	Muscle T_{CO_2} (mmol l^{-1})	Muscle pHi	Muscle T_{CO_2} (mmol l^{-1})	$E_{HCO_3^-}$ (mV)
Rest	7.40±0.01	2.51±0.10	7.39±0.02	2.58±0.05	−31±1
Exercise	6.74±0.02*	2.45±0.12	6.70±0.03*	2.61±0.13	−35±1

* Significantly different from rest value ($P<0.05$).
The predicted values are based on the assumption that CO_2 is distributed according to pH.
Values are mean±S.E. ($N=7$).

Table 2. *Acid–base status in the plasma of rainbow trout at rest and immediately following exhaustive exercise*

	pHa	Ta_{CO_2} (mmol l^{-1})	Pa_{CO_2} (kPa)	$[HCO_3^-]$ (mmol l^{-1})	$[Lactate]_a$ (mmol l^{-1})	Ta_{amm} (mmol l^{-1})
Rest	7.89±0.01	8.62±0.14	0.35±0.01	8.45±0.13	0.15±0.04	0.024±0.008
Exercise	7.32±0.03*	6.89±0.10*	1.02±0.06*	6.60±0.21*	5.32±0.47*	0.296±0.024*

*Significantly different from rest value ($P<0.05$).
Values are mean±S.E. ($N=7$).

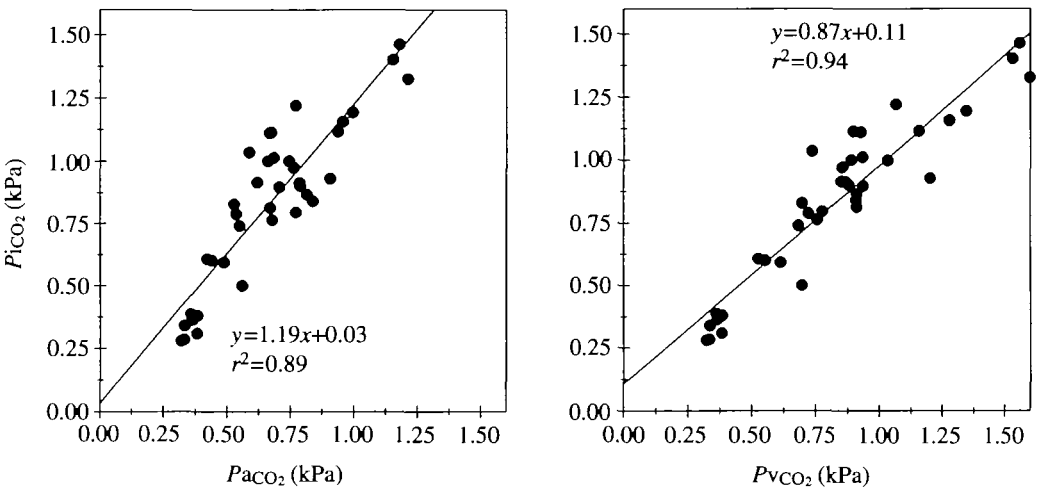

Fig. 2. Correlations between Pi_{CO_2} and Pa_{CO_2} and between Pi_{CO_2} and values of Pv_{CO_2} derived as described in the caption of Fig. 1. Data were pooled from both resting/exercise and saline/bicarbonate infusion experiments.

and exercise (Fig. 1B). This reflects the fact that carbon dioxide is distributed according to pH between muscle and blood under all conditions tested (Table 1). Calculated values of muscle pH and total carbon dioxide levels, based on the assumption that carbon dioxide was distributed according to pH, were similar to measured values. Membrane potential was not measured but it is unlikely that it was similar to the calculated equilibrium potential for bicarbonate (Table 1). Plasma and muscle P_{CO_2} increased markedly following exercise but T_{CO_2} did not change (Fig. 1). There was a negative linear relationship between Pa_{CO_2} and pHa (Fig. 3).

Changes in blood and muscle pH and ammonia concentration were similar to those previously reported for exhaustive activity in trout (Tang and Boutilier, 1991; Wright and Wood, 1988) except that resting ammonia levels were significantly lower than those reported by Wright and Wood (1988). Muscle Pi_{NH_3} and Ti_{amm} levels were higher than those in the plasma, and all increased following

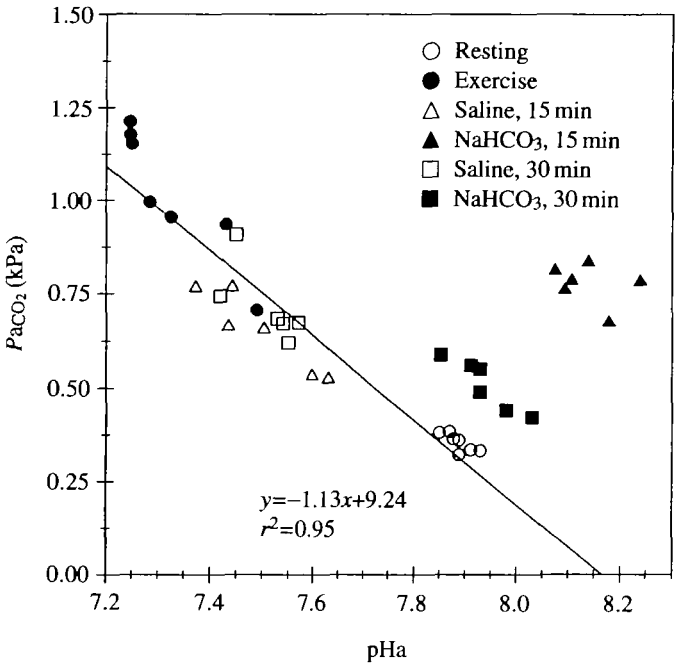

Fig. 3. Correlation between partial pressure of CO_2 (Pa_{CO_2}) and pH (pHa) in plasma. Data were pooled from both resting/exercise and saline/bicarbonate infusion experiments. The bicarbonate infusion data are not included in the linear regression.

exercise (Fig. 4). NH_4^+ was not distributed according to pH but according to membrane potential (Table 3). Calculated values based on the assumption that ammonia is distributed according to pH were different from measured values, whereas the calculated equilibrium potential was similar to that expected for muscle (Hodgkin and Horowicz, 1959), indicating that NH_4^+ is distributed according to membrane potential.

The data from the saline and bicarbonate infusion experiments cannot be compared directly with the initial data set because the initial set was collected immediately following exercise, whereas data following infusion were collected 15 or 30 min after exercise. Bicarbonate infusion resulted in a rise in plasma pHa, Ta_{CO_2} and bicarbonate levels, which were partially corrected after 30 min (Fig. 5). Pa_{CO_2} and Pi_{CO_2}, however, were lower 30 min after exercise in bicarbonate-infused animals and muscle Ti_{CO_2} and bicarbonate and lactate concentrations were unaffected (Fig. 5; Table 4). Muscle pHi increased significantly 30 min after exercise in bicarbonate-infused animals (Table 4). The CO_2 partial pressure differences between muscle and plasma ($Pi_{CO_2}-Pa_{CO_2}$) decreased 15 min after exercise in bicarbonate-infused animals but returned to normal 30 min after infusion (Table 4).

Bicarbonate infusion caused a significant reduction in plasma total ammonia and

Table 3. *Measured and predicted muscle pHi and T_{amm} in rainbow trout at rest and immediately following exercise*

	Measured		Predicted		Calculated
	Muscle pHi	Muscle T_{amm} (mmol l^{-1})	Muscle pHi	Muscle T_{amm} (mmol l^{-1})	$E_{NH_4^+}$ (mV)
Rest	7.40±0.01	0.661±0.048	6.33±0.12†	0.069±0.021†	−84±7
Exercise	6.74±0.02*	6.748±0.229*	5.82±0.06*†	0.857±0.115*†	−81±2

* Significantly different from rest value ($P<0.05$).

† Significantly different from measured value ($P<0.05$).

The predicted values are based on the assumption that ammonia is distributed according to pH.

Values are mean±S.E. ($N=7$).

NH_4^+ levels at 15 min, while plasma NH_3 partial pressure and lactate levels were elevated at 30 min (Fig. 6). Bicarbonate infusion also raised the muscle total ammonia, NH_3 and NH_4^+ levels and the NH_3 partial pressure differences between muscle and plasma (Table 4). The ratio of total ammonia in plasma to that in muscle was lowered after bicarbonate infusion (Table 4). Ammonia excretion by the fish was unaffected by bicarbonate infusion. Ammonia excretion was 536±37 μmol h^{-1} kg^{-1} (mean±S.E., $N=6$) following saline infusion compared with 543±33 μmol h^{-1} kg^{-1} in bicarbonate-infused fish during the first 30 min of the post-exercise period.

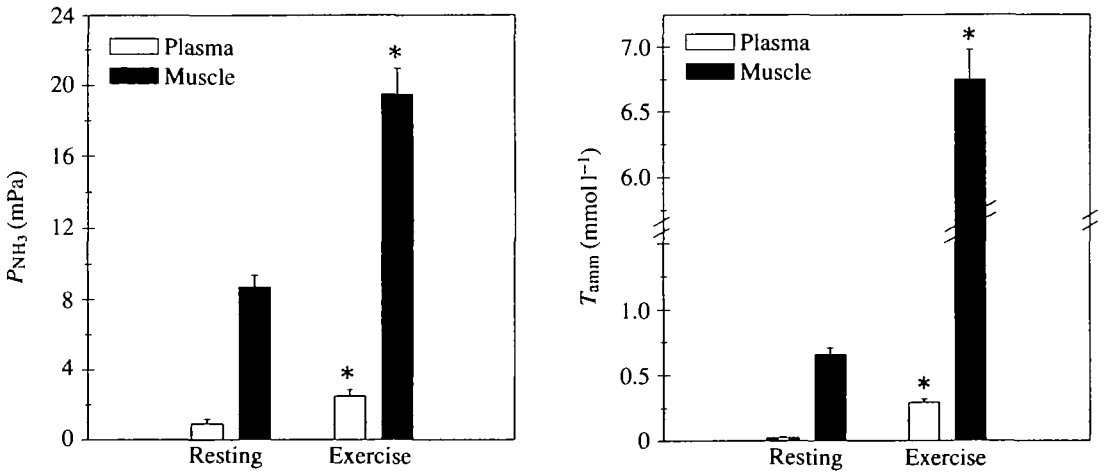

Fig. 4. Partial pressure of ammonia (P_{NH_3}) and total ammonia content (T_{amm}) in trout plasma and white muscle at rest and following exhaustive exercise. Values are shown as means+S.E. ($N=7$). * indicates a significant difference ($P<0.05$) from resting values.

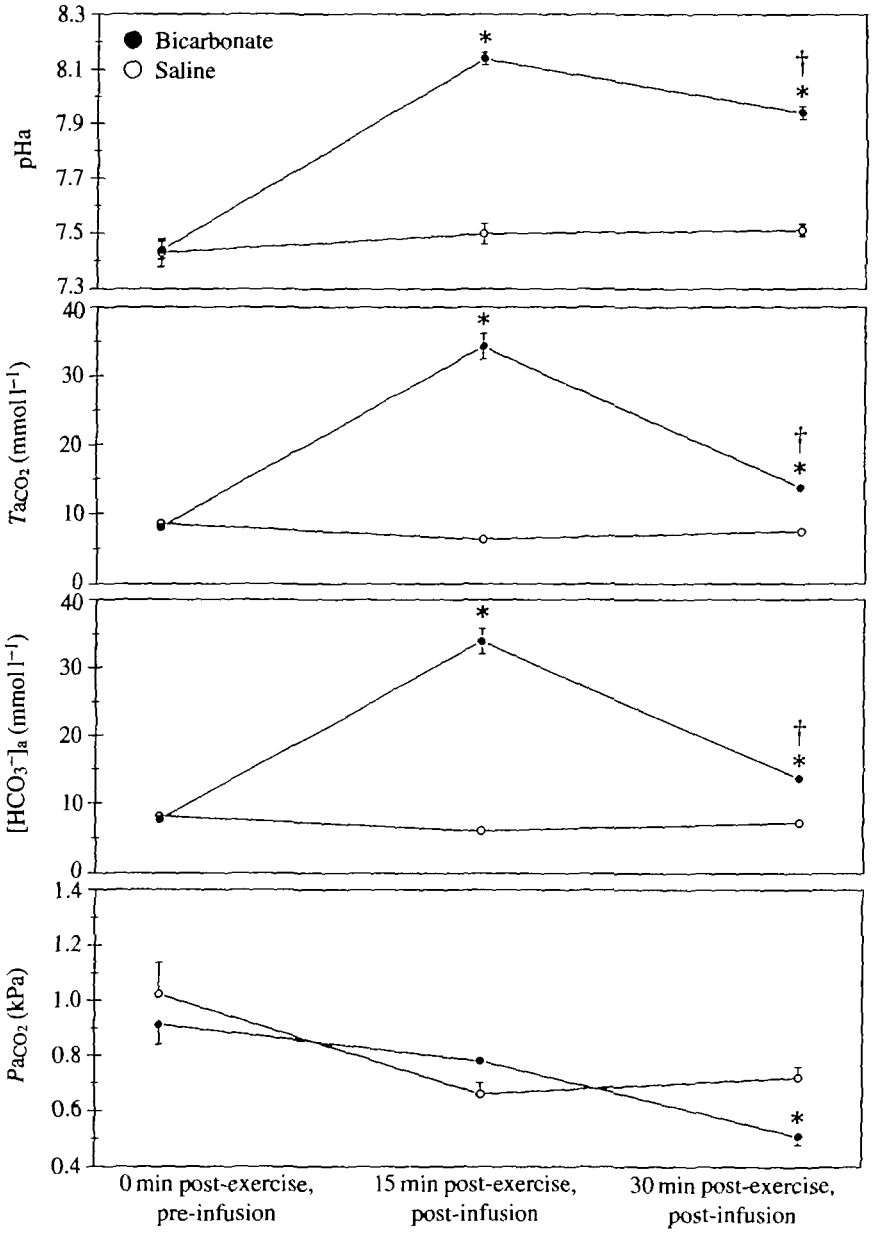

Fig. 5. pH and carbon dioxide in the plasma of saline- or bicarbonate-infused rainbow trout immediately, 15 and 30 min after exercise. Values are shown as means±S.E. (N=6). * indicates a significant difference from saline infusion values (P<0.05). † indicates a significant difference from 15 min bicarbonate infusion values (P<0.05).

Table 4. *Acid–base status in the muscle of saline- and bicarbonate-infused rainbow trout at 15 and 30 min after exercise*

	Saline 15 min	$NaHCO_3$ 15 min	Saline 30 min	$NaHCO_3$ 30 min
pHi	6.74±0.02	6.80±0.02	6.74±0.02	6.85±0.02*
Ti_{CO_2} (mmol l^{-1})	1.91±0.09	1.98±0.08	1.93±0.08	1.73±0.21
$[HCO_3^-]_i$ (mmol l^{-1})	1.45±0.06	1.55±0.08	1.46±0.07	1.39±0.18
Pi_{CO_2} (kPa)	0.96±0.07	0.88±0.03	0.96±0.04	0.68±0.07*†
$Pi_{CO_2}-Pa_{CO_2}$ (kPa)	0.30±0.06	0.10±0.03*	0.25±0.05	0.17±0.06
Ti_{amm} (mmol l^{-1})	6.00±0.32	7.27±0.33*	4.96±0.64	7.47±0.41*
$[NH_4^+]$ (mmol l^{-1})	5.99±0.32	7.26±0.33*	4.96±0.64	7.46±0.41*
Pi_{NH_3} (mPa)	13.24±0.77	18.85±1.46*	12.45±1.75	24.64±2.25*
$Pi_{NH_3}-Pa_{NH_3}$ (mPa)	10.14±0.72	14.69±1.33*	10.76±2.03	18.87±2.12*
Ta_{amm}/Ti_{amm} (%)	5.15±0.36	1.23±0.17*	3.62±0.11	1.85±0.29*
$[Lactate]_i$ (mmol l^{-1})	40.22±2.99	38.48±3.20	35.09±1.53	34.14±1.75

* Indicates a significant difference between saline and $NaHCO_3$ infusion values ($P<0.05$).

† Indicates a significant difference between 15 min and 30 min $NaHCO_3$ infusion values ($P<0.05$).

Values are mean±S.E. ($N=6$).

Discussion

The data reported in this study are the only direct measurements of muscle carbon dioxide content in fish. Analysis of these results shows that carbon dioxide is distributed between muscle and plasma according to pH during both rest and exercise in rainbow trout. This pattern is similar to that seen in other vertebrates. The equilibrium potential for bicarbonate is different from the probable membrane potential, indicating that the muscle membranes are not very permeable to bicarbonate. Carbon dioxide must therefore leave the muscle as molecular CO_2 rather than as bicarbonate. As expected, Pi_{CO_2} is similar to estimates of Pv_{CO_2} and is about 0.2 kPa greater than Pa_{CO_2} (Fig. 2).

Resting ammonia levels are lower than those reported by Wright and Wood (1988), presumably because of differences in sampling methods. Ammonia levels are elevated by activity and it is difficult to collect a muscle sample without disturbing the fish. Our method minimized this problem and, as a result, we observed much lower muscle ammonia levels and a significantly lower P_{NH_3} gradient from muscle to blood in resting fish than those reported by Wright and Wood (1988).

Infusion of bicarbonate after exhaustive exercise resulted in an increase in pH and T_{CO_2} and HCO_3^- and lactate levels in the blood, but little change in the muscle, except for a small increase in pHi (Figs 5, 6; Table 4). Elevated whole-blood lactate levels could be caused by reduced recycling by the liver or increased flux from the muscle, possibly as a result of the increased blood pH following bicarbonate infusion. The observation that there was a significant increase in blood lactate concentration but no change in muscle lactate concentration

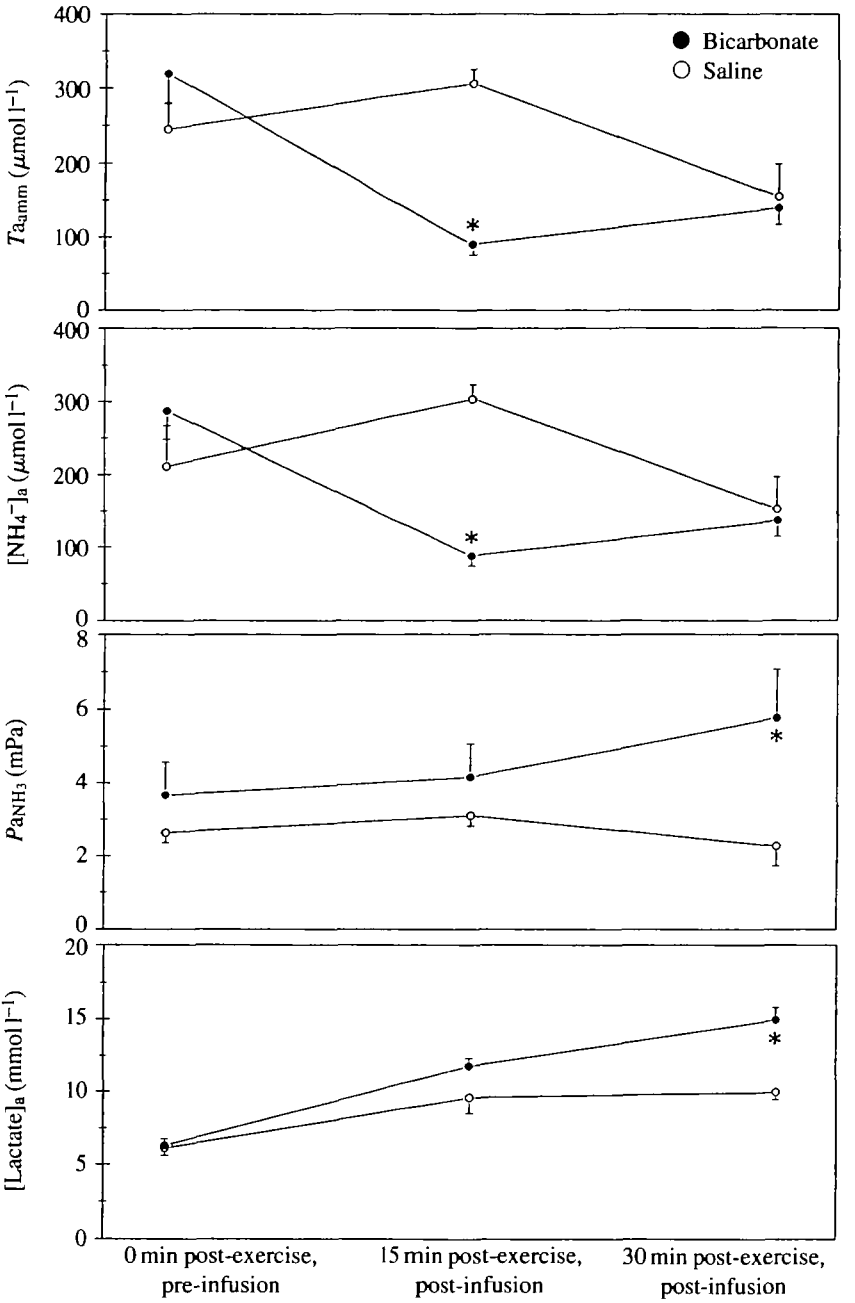

Fig. 6. Ammonia and lactate contents in the plasma of saline- or bicarbonate-infused rainbow trout immediately, 15 and 30 min after exercise. Values are shown as means±S.E. (N=6). * indicates a significant difference from saline infusion values (P<0.05).

following bicarbonate infusion can be explained by the large difference in the pool size of the two compartments. The blood pool of lactate represents only about 3 % of the total pool; hence, changes in blood levels would represent only a 1 % reduction in the muscle pool and would not be detectable by our methods.

Bicarbonate infusion initially lowered blood T_{amm} and NH_4^+ levels but subsequently raised blood NH_3 levels. It also caused an increase in muscle NH_3 and NH_4^+ concentrations. This increase in T_{amm} could be due to an increase in ammonia production or increased ammonia retention in the muscle. The pattern seen during exercise, however, was identical and so it seems unlikely that there were differences in ammonia production. Thus, it seems probable that the increase in muscle T_{amm} following bicarbonate infusion was due to ammonia retention. Muscle NH_3 concentration increased more than total ammonia concentration, reflecting the small increase in muscle pH following bicarbonate infusion, but this cannot explain ammonia retention in the muscle. We did not measure the pH of extracelluar fluid surrounding the muscle, but we assume that bicarbonate infusion resulted in an elevation in pH of fluid around the muscle as well as in blood. The more alkaline extracellular environment will raise NH_3 levels and reduce NH_3 flux from the muscle; the reverse, however, will be true for NH_4^+. If NH_4^+ transfer is the dominant form of ammonia excretion from the muscle, then raising extracellular pH should augment ammonia excretion by reducing extracellular ammonium ion concentrations. Our observations, however, indicate that alkaline conditions cause ammonia retention in the muscle. This indicates that the muscle membrane in fish, like that of mammals, is permeable to NH_3 but not to NH_4^+. This is at odds with our calculations indicating that fish muscle is permeable to NH_4^+ (Table 3). Clearly this requires further investigation. It appears, however, that an acid extracellular environment enhances ammonia removal from the muscle. The location of carbonic anhydrase on the muscle surface (Sanyal *et al.* 1982) ensures that any CO_2 excreted by the muscle will be rapidly hydrated and will acidify the fluid surrounding the muscle. Thus, it seems likely that CO_2 excretion by the muscle will also augment ammonia excretion from that tissue, in a manner similar to that seen in the gills (Wright *et al.* 1989). Extracellular carbonic anhydrase activity plays a role, therefore, not only in facilitating CO_2 transfer but also in augmenting ammonia transfer.

Muscle ammonia concentrations increased but ammonia excretion remained the same in fish infused with bicarbonate compared with those infused with saline, indicating increased ammonia production or a decreased rate of muscle ammonia utilization during recovery. The breakdown of adenylate appears to be the major source of muscle ammonia (Mommsen and Hochachka, 1988). This occurs during exhaustive exercise and was the same in both groups because bicarbonate or saline infusion was given after exercise. During recovery, ammonia and IMP are used as substrates to restore the adenylate pool and this process is well under way after 30 min of recovery (Mommsen and Hochachka, 1988), when we measured muscle ammonia concentrations. The only other measured change in the muscle following bicarbonate infusion was a small rise in pH. This may have been sufficient to delay

restoration of the adenylate pool. Alternatively, adenylate breakdown may have continued after exhaustive exercise and resulted in a greater ammonium production in the less acidotic, bicarbonate-infused fish. The pH sensitivity of the various components of the purine nucleotide cycle in fish is not known.

P_{CO_2} was elevated in both blood and muscle following exercise, as reported by others (see Perry and Wood, 1989), and this was inversely related to pH (Figs 2, 3). A reduction in blood pH causes a Root shift and decreases the oxygen content of the blood, but this does not occur during the acidosis following exercise because of β-adrenergic activation of Na^+/H^+ exchange by elevated levels of circulating catecholamines, resulting in a rise in erythrocytic pH (Primmett *et al.* 1986). Thus, as blood flows through the gills, the protons produced by haemoglobin oxygenation fuel both bicarbonate dehydration and Na^+/H^+ transfer in the red blood cell. In fact, these two mechanisms compete for the available protons as blood passes through the gills. The dumping of protons into the plasma from red blood cells will create a CO_2/HCO_3^- disequilibrium, resulting in a rise in Pa_{CO_2} as blood flows away from the gills. Na^+/H^+ exchange is enhanced at low pH (Nikinmaa *et al.* 1987), so the extent of proton dumping will be increased by low pH and will result in a larger rise in Pa_{CO_2}. This explains the observed inverse relationship between Pa_{CO_2} and pHa (Fig. 3) in trout following exhaustive exercise, when levels of circulating catecholamines are elevated. Pa_{CO_2} is further elevated following bicarbonate infusion because of an exacerbated CO_2/HCO_3^- disequilibrium.

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