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IN VIVO ANALYSIS OF GAS TRANSPORT IN ARTERIAL AND VENOUS BLOOD OF THE SEA LAMPREY *PETROMYZON MARINUS*

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Summary

Exercise in sea lampreys resulted in a significant decrease in the extracellular pH (pHe) in both arterial and venous blood. At rest, the erythrocyte pH (pHi) of venous blood was significantly greater than the pHi of arterial blood. Despite the considerable extracellular acidosis after exercise, both arterial and venous pHi were maintained throughout the recovery period. In the venous blood, there was a reversal of the pH gradient (ΔpH) across the erythrocyte membrane immediately after exercise. Exercise also resulted in significant reductions in the partial pressure of oxygen and hemoglobin oxygen-carriage and a significant increase in the partial pressure of CO_2 in arterial and venous blood. Although the total CO_2 concentration of the plasma decreased after exercise, erythrocyte total CO_2 concentrations ($C_{CO_2,i}$) increased. In venous blood, the $C_{CO_2,i}$ immediately after exercise was double the resting value. At rest, partitioning of the total CO_2 content between plasma and erythrocytes indicated that 16 % and 22 % of the total CO_2 could be attributed to the erythrocytes in arterial and venous whole blood, respectively. After exercise, these percentages increased to 25 % (arterial) and 38 % (venous). Changes in $C_{CO_2,i}$ accounted for 62 % of the arteriovenous difference in whole-blood total CO_2 at rest. This increased to 78 % immediately after exercise. Thus, unlike other vertebrates, CO_2 transport in the lamprey *in vivo* is largely dependent on erythrocyte CO_2-carriage.

Introduction

The characteristics of carbon dioxide transport in blood have been thoroughly described in a number of vertebrates both *in vitro* and *in vivo*. *In vitro*, CO_2 carriage has often been examined within the different compartments; whole blood, true plasma and red blood cells. Typically, this analysis indicates that the total carbon dioxide concentration of true plasma is considerably greater than that of the erythrocytes over a range of CO_2 tensions (Roughton, 1964; Boutilier *et al.* 1979; Heming, 1984). *In vivo*, changes in the total CO_2 content of the plasma

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during passage of blood through the respiratory organ also account for the majority of the CO_2 excreted in those vertebrates studied to date (Roughton, 1964; Heming, 1984; Klocke, 1987). Thus, the plasma has a prominent role in CO_2 carriage in the blood of most vertebrates.

Recent studies have demonstrated that the *in vitro* CO_2 transport properties of lamprey blood are markedly different from those of other vertebrates (Tufts and Boutilier, 1989, 1990; Nikinmaa and Matsoff, 1991). In lampreys, the total CO_2 content of the erythrocytes exceeds that of true plasma as CO_2 tensions are increased *in vitro* (Tufts and Boutilier, 1989, 1990). These results may be attributable to reduced quantities of chloride/bicarbonate exchange protein in the erythrocyte membrane of agnathans (Nikinmaa and Railo, 1977; Ellory *et al.* 1977). In most vertebrates, a large amount of the bicarbonate formed within the erythrocyte after the hydration of CO_2 is rapidly transferred to the plasma in exchange for chloride *via* the chloride/bicarbonate exchange protein, capnophorin (Band 3), within the erythrocyte membrane (Swenson, 1990). Thus, chloride/bicarbonate exchange reduces the total CO_2 content of the erythrocyte and increases that of the plasma at any given CO_2 tension. In lampreys, sodium-dependent movements of protons across the erythrocyte membrane may also contribute to the unique CO_2 transport properties of blood observed *in vitro* (Tufts, 1992). The relative importance of these two factors has not been clearly determined.

Based on the blood CO_2 transport properties observed *in vitro*, Tufts and Boutilier (1989) proposed that CO_2 transport in lampreys is largely dependent on CO_2 carriage by the erythrocyte rather than by the plasma. The reverse is true for most vertebrates. In view of the phylogenetically primitive position of these animals, such a novel strategy for CO_2 transport may provide important insights towards understanding the evolution of gas transport in vertebrates. Indeed, one can predict that transport of carbon dioxide within the erythrocyte may have important consequences for the coupling of oxygen and carbon dioxide transport. To date, however, there have been no detailed investigations of gas transport in lampreys *in vivo*. Consequently, the purpose of the present study was to examine blood gas transport in both arterial and venous blood of the sea lamprey, *Petromyzon marinus*, at rest and during recovery from exercise.

Materials and methods

Adult sea lampreys, *Petromyzon marinus* (L.) (250–400 g: $N=12$), were collected during their spawning migration in the Shelter Valley River in Eastern Ontario. The animals were transported to the Biology Department at Queen's University where they were held in freshwater tanks at 8–10 °C for at least 2 weeks before experiments. The composition of the water used to hold the animals and in the experiments was as follows (mequiv l^{-1}): $[Na^+]$ 2.1; $[K^+]$ 0.05, $[Ca^{2+}]$ 2.2, $[Cl^-]$ 1.3, $[HCO_3^-]$ 1.5, pH 7.4.

Surgery

Lampreys were anesthetized in an aerated and pH-balanced solution of tricaine methane sulfonate (66.7 mg l^{-1} MS-222 and 1333 mg l^{-1} $NaHCO_3$). The animals were then transferred to a surgical table and a mid-ventral incision (3–4 cm) was made approximately half-way down the body. Cannulae of polyethylene tubing (PE 50) were implanted in the dorsal aorta and the posterior cardinal vein. It was not feasible to sample prebranchial blood from the ventral aorta because of the extensive network of cartilage and blood vessels associated with the gill pouches, but blood from the posterior cardinal vein empties into the sinus venosus of the heart and these samples should therefore be similar to prebranchial blood. The cannulae extended through the incision, which was then closed with sutures. During the surgical procedure, the lamprey's body was wrapped in a damp cloth and the head and gills were kept moist by intermittent immersion in the anesthetic solution. Following the 5–10 min surgery, the lampreys were allowed to recover in a lightproof Perspex box containing aerated flowing fresh water at 10 °C for at least 24 h before experiments.

Protocol

After recovery, 600 μl blood samples were taken into Hamilton gas-tight syringes from both the arterial and venous cannulae. Whole-blood total carbon dioxide concentration was measured immediately on 100 μl of the sample. Triplicate hematocrit measurements were made using 200 μl of blood and the remainder was dispensed into 0.5 ml Eppendorf tubes and centrifuged at 10 000 *g* for 4 min at 10 °C. The C_{CO_2} of true plasma was then determined on a 100 μl sample of plasma taken from the hematocrit tubes using a 100 μl gas-tight Hamilton syringe. Plasma pH (pHe) was measured immediately from the supernatant in the Eppendorf tube. The remaining plasma was removed from the tube and the pellet was frozen in liquid nitrogen before determination of erythrocyte pH (pHi).

After the control sample had been taken, the lamprey was moved to a cylindrical tank containing aerated water at 10 °C, where it was manually chased to exhaustion in 5 min. The exhausted lamprey was then returned to the Perspex container and a second 600 μl sample was removed from each cannula. Identical analyses to those described for the control sample were performed on this blood sample. Additional samples were also taken at 0.5, 1 and 4 h of recovery from exercise. Throughout the experiment, blood samples were replaced with a similar volume of heparinized (20 i.u. ml^{-1}) saline after both the arterial and venous samples had been taken.

In a second series of experiments, lampreys were also cannulated in the dorsal aorta and the posterior cardinal vein. After recovery, 1 ml samples of blood were taken into Hamilton gas-tight syringes. The whole-blood oxygen content (C_{O_2}) and oxygen tension (P_{O_2}) were measured immediately using about 300 μl of these samples. Another 50 μl of whole blood was used to determine hemoglobin concentration. The remaining blood from each syringe was divided equally between two 0.5 ml Eppendorf tubes and centrifuged. After centrifugation, 200 μl

of plasma supernatant was removed from the tubes and frozen for later analysis of plasma Cl^- concentrations. Any remaining plasma was discarded and the red blood cell pellets were saved for the determination of erythrocyte water content and Cl^- concentrations. As in the first series of experiments, lampreys were exercised to exhaustion after the control sample. A 1 ml sample was removed from each cannula as soon as the lamprey was returned to the Perspex box. These post-exercise samples were treated in an identical manner to the control samples. Samples were also taken after 0.5, 1 and 4 h of recovery. However, at these times, only 350 μl was removed from each cannula and only C_{O_2}, P_{O_2} and hemoglobin were analyzed. Thus, in both series of experiments about 5 ml of blood was removed from each animal. In a previous study, Tufts (1991) demonstrated that this sampling protocol would not significantly affect the variables measured in the present experiments.

Analyses

Plasma pH (pHe) and erythrocyte pH (pHi) were determined with a PHM73 pH meter and associated micro-pH unit (Radiometer, Copenhagen, Denmark) thermostatted at 10°C. Erythrocyte pellets were frozen and thawed twice in liquid nitrogen prior to the determination of pHi according to the method of Zeidler and Kim (1977). The whole-blood P_{O_2} was measured with an E5046 oxygen electrode (Radiometer, Copenhagen, Denmark), also thermostatted at 10°C, and an associated oxygen meter (Cameron Instrument Co., Texas, USA). Another E5046 oxygen electrode was used to determine the total oxygen content (C_{O_2}) of whole-blood samples using the method of Tucker (1967). Total CO_2 concentrations (C_{CO_2}) of whole blood and plasma were measured with a Corning model 965 CO_2 analyzer (Ciba Corning Canada Inc.). Analysis of hemoglobin concentration was performed by Drabkin's method (Drabkin and Austin, 1935) using Sigma reagents. For the determination of erythrocyte chloride concentration, the erythrocyte pellet was first dissolved in 8% perchloric acid. The chloride concentration of the extract was then measured with a CMT10 chloride titrator (Radiometer, Copenhagen).

Erythrocyte C_{CO_2} concentration ($C_{CO_2,i}$) was determined from the C_{CO_2} of whole blood (w.bl.) and true plasma (t.pl.) and the hematocrit (Hct) according to the following equation:

$$C_{CO_2,i} = [C_{CO_2,w.bl} - C_{CO_2,t.pl.} \times (1 - Hct)]/Hct\,.$$

Measured values of true-plasma total CO_2 and pHe were used to determine the partial pressure of CO_2 (P_{CO_2}) *via* a rearrangement of the Henderson–Hasselbalch equation with the values for pK′ determined according to Boutilier *et al.* (1984) and a CO_2 solubility coefficient (αCO_2) of 8.29×10^{-3} mmol kPa^{-1} (Tufts and Boutilier, 1990).

Statistics

Means and standard errors of all values are presented. A repeated-measures

analysis of variance (ANOVA) was used to assess the significance of observed differences between sample times. If the ANOVA indicated significance ($P<0.05$), a Dunnett's multiple comparisons test was then used to determine significant differences ($P<0.05$) between resting values and post-exercise values. A paired Student's *t*-test was also used to assess the significance ($P<0.05$) of observed differences in plasma and erythrocyte chloride concentrations and erythrocyte water content between arterial and venous blood.

Results

Under resting conditions, arterial extracellular pH (pHe) in the sea lamprey was 8.040±0.027 while the venous pHe was significantly lower at 7.965±0.025 (Fig. 1A). Exhaustive exercise caused significant decreases of 0.363 and 0.455 pH units in arterial and venous pHe, respectively. Arterial pHe recovered rapidly during the post-exercise period and by 1 h had returned to 7.961±0.052, which was no longer significantly different from the resting value. Recovery of venous pHe was somewhat slower, however, and it remained significantly below the resting value until the 4 h sample.

At rest, the erythrocyte pH (pHi) of venous blood was 7.647±0.021 whereas that of arterial blood was significantly less at 7.509±0.030 (Fig. 1B). Thus, the pHi of venous blood was higher than that of arterial blood even though the venous pHe was more acidic than arterial pHe. Despite the considerable extracellular acidosis, there were no significant differences in either the arterial or venous pHi following exhaustive exercise. Maintenance of pHi resulted in very large changes in the pH gradient across the red blood cell membrane after exercise (Fig. 1C). Indeed, there was a reversal of the pH gradient in venous blood, which was −0.165±0.061 pH units immediately after exercise.

Venous P_{O_2} at rest was about 35 % of the arterial P_{O_2} (Fig. 2A). Passage of blood through the tissues under resting conditions was also associated with a 23 % reduction in haemoglobin oxygen-carriage by venous blood (Fig. 2B). Arterial and venous P_{O_2} fell after exercise by 48 % and 76 %, respectively. In arterial blood, this resulted in a 22 % decrease in haemoglobin oxygen-carriage whereas the post-exercise decline in venous haemoglobin oxygen-carriage was 76 % at 0 h. Recovery of these variables was very rapid, however, and by 30 min of recovery, there were no longer significant differences from resting values.

Exhaustive exercise also resulted in significant increases in the P_{CO_2} of both arterial and venous blood of 0.17 and 0.39 kPa, respectively (Fig. 3). Similar to the observed changes in pHe, the P_{CO_2} difference between arterial and venous blood was considerably greater after exercise. By 1 h of recovery, the P_{CO_2} in both compartments had returned to values not significantly different from rest.

The total CO_2 concentration of plasma ($C_{CO_2,t.pl.}$) of both arterial and venous blood decreased significantly after exhaustive exercise (Fig. 4A). In each case, the magnitude of the decrease was 1.4 mmol l^{-1} at 0 h. Arterial $C_{CO_2,t.pl.}$ did not recover until the 1 h sample, but venous $C_{CO_2,t.pl.}$ was no longer significantly

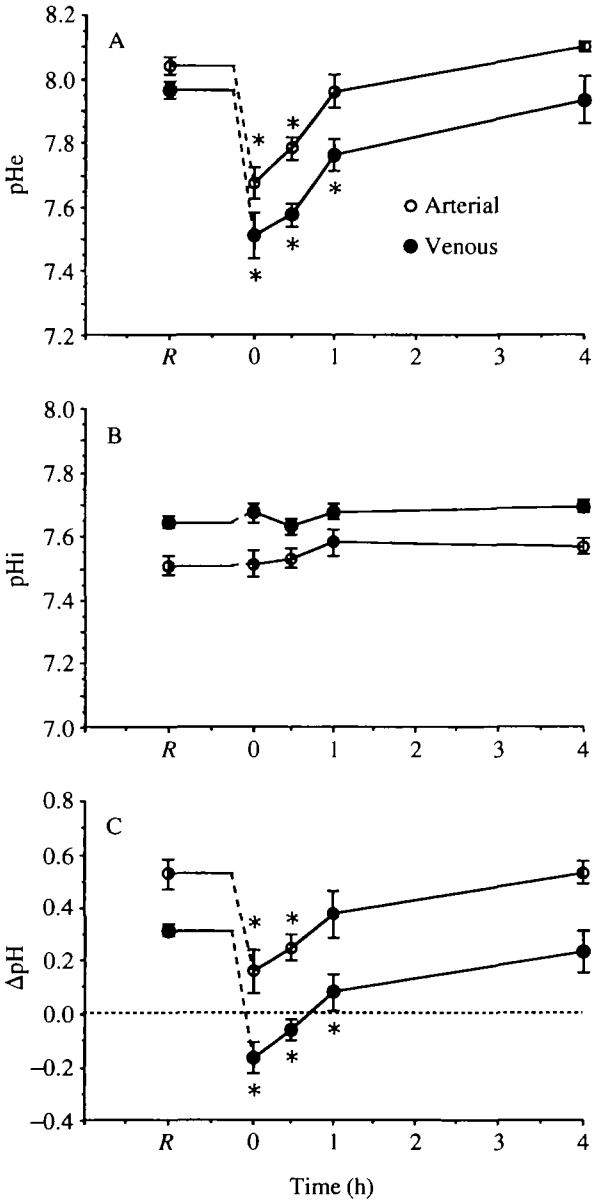

Fig. 1. (A) Extracellular pH (pHe), (B) erythrocyte pH (pHi) and (C) the pH gradient (ΔpH=pHe−pHi) across the erythrocyte membrane of arterial (○) and venous (●) blood at rest (*R*) and 0, 0.5, 1 and 4 h following exercise in *Petromyzon marinus*. The dashed lines represent the 5 min period of exercise. Values are means±standard error (N=6). Asterisks denote a significant difference from the resting value (P<0.05).

different from rest after 0.5 h. In contrast, arterial $C_{CO_2,i}$ increased significantly by 3.0 mmol l^{-1} and venous $C_{CO_2,i}$ increased significantly by 9.4 mmol l^{-1} following exercise (Fig. 4B). These changes represent increases of 60% and 104% in

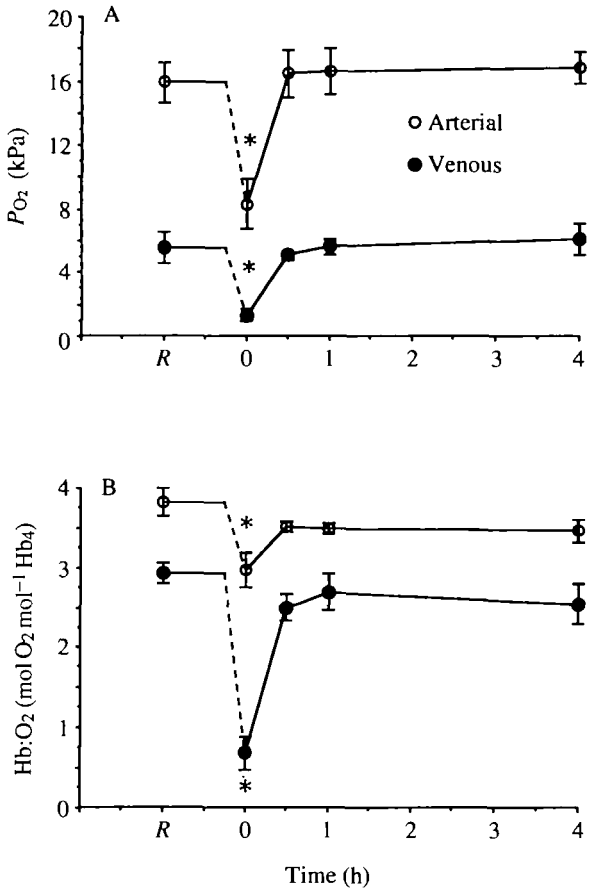

Fig. 2. (A) Oxygen tension (P_{O_2}) and (B) hemoglobin oxygen-carriage (Hb:O_2) in arterial (○) and venous (●) blood at rest (*R*) and 0, 0.5, 1 and 4 h following exercise in *Petromyzon marinus*. Values are means±standard error (N=6). Asterisks denote a significant difference from the resting value (P<0.05).

arterial and venous $C_{CO_2,i}$, respectively. The $C_{CO_2,i}$ in both compartments had returned to values which were no longer significantly different from rest by 0.5 h. No significant differences were observed in plasma or erythrocyte chloride concentrations or erythrocyte water contents between arterial and venous blood either at rest or immediately after exercise (Table 1).

The partitioning between plasma and erythrocytes of the total CO_2 in 1 l of arterial or venous whole blood can be calculated from the C_{CO_2} of plasma and erythrocytes and the hematocrit values (Fig. 5). In arterial blood at rest, the plasma accounted for 84 % of the total CO_2. After exercise, however, there was a significant decrease in the amount of CO_2 carried by the plasma (Fig. 5A). Thus, at 0 h, the importance of the erythrocytes in CO_2 carriage has increased and the plasma component of the total CO_2 in arterial blood fell to 75 %. For the remainder of the recovery period, the amount of CO_2 carried by the plasma was

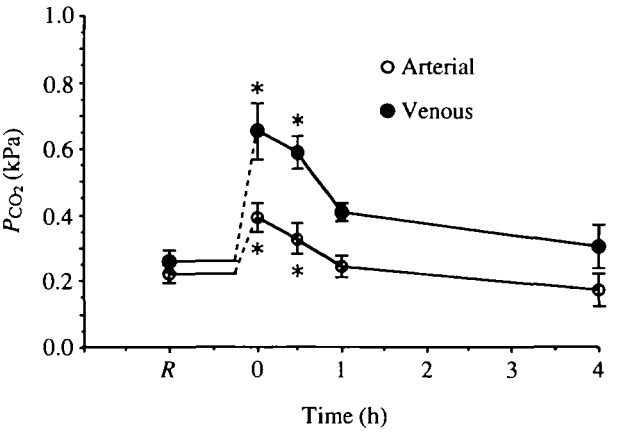

Fig. 3. CO_2 tension (P_{CO_2}) in arterial (O) and venous (●) blood at rest (R) and 0, 0.5, 1 and 4 h following exercise in *Petromyzon marinus*. The dashed lines represent the 5 min period of exercise. Values are means±standard error (N=6). Asterisks denote significant differences from resting values (P<0.05).

not significantly different from that at rest. The percentage of CO_2 in the plasma increased somewhat throughout the recovery period, but this was due to a reduction in the hematocrit caused by sampling. Hematocrits of resting lampreys were 27.4±2.3%, but they consistently declined after each sample. Immediately after exercise, mean hematocrit had fallen to 24.5±2.6% and by the end of the experiment, it was 19.2±2.8%. Thus, in the present analysis, it should be considered that, after the resting sample, the total CO_2 attributable to the erythrocytes will be somewhat underestimated. In venous blood, 78% of the CO_2 is carried within the plasma at rest (Fig. 5B). Similar to the trend in arterial blood, exercise was associated with a significant increase in the amount of CO_2 within the erythrocytes in venous blood. In this case, the CO_2 partitioned within the erythrocytes increased to 38% of the total and the plasma component, therefore, fell to 62%. During the remainder of the recovery period, the plasma CO_2

Table 1. *Chloride concentration in erythrocytes ($[Cl^-]_i$) and plasma ($[Cl^-]_{pl}$) and erythrocyte water content (% H_2O) in arterial and venous blood at rest and following exercise in* Petromyzon marinus

	Rest		Exercise	
	Arterial	Venous	Arterial	Venous
$[Cl^-]_i$ (mequiv l^{-1})	52.6±2.0 (6)	55.0±0.8 (6)	56.3±1.3 (6)	58.0±1.0 (6)
$[Cl^-]_{pl}$ (mequiv l^{-1})	118.4±1.7 (6)	120.7±4.4 (6)	117.7±2.6 (6)	118.0±2.2 (6)
H_2O (%)	67.2±0.5 (5)	67.7±0.4 (5)	66.8±0.3 (6)	66.5±0.3 (6)

All values are means±standard error (N).

There are no significant (P<0.05) differences between arterial and venous values at rest or after exercise.

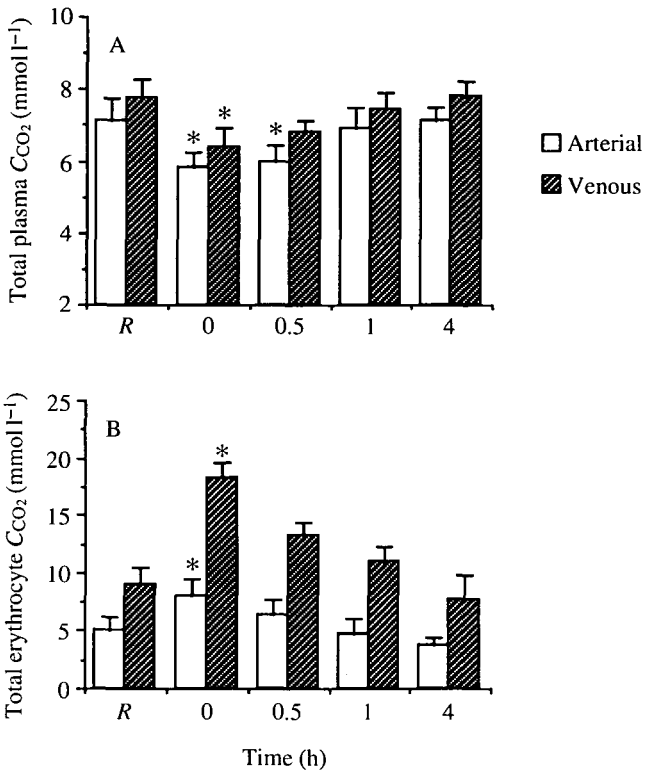

Fig. 4. Total CO_2 concentration of (A) true plasma and (B) erythrocytes in arterial (open bars) and venous (hatched bars) blood at rest (*R*) and 0, 0.5, 1 and 4 h following exercise in *Petromyzon marinus*. Values are means+standard error (N=6). Asterisks denote significant differences from resting values (P<0.05).

component returned to resting values and then slightly increased, again reflecting the reduction in hematocrit caused by sampling.

It is also possible to determine the relative contribution of changes in $C_{CO_2,i}$ and $C_{CO_2,t.pl.}$ to the difference in CO_2 content between arterial and venous whole blood. In contrast to the previous analysis, this will indicate the relative importance of the erythrocytes and the plasma in the transport of CO_2 from the metabolizing tissues to the gills and will also indicate the source (plasma *versus* erythrocytes) of the CO_2 excreted by the gills. This analysis showed that the majority (62 %) of the CO_2 added to the blood by the tissues at rest is transported by the erythrocytes (Fig. 6). Moreover, changes in $C_{CO_2,i}$ account for 78 % of the CO_2 difference between arterial and venous whole blood after exercise. During the remainder of the recovery period, the involvement of the erythrocytes falls and it then declines further because of the hematocrit changes mentioned earlier.

Discussion

Exhaustive exercise, in the sea lamprey, causes a considerable extracellular

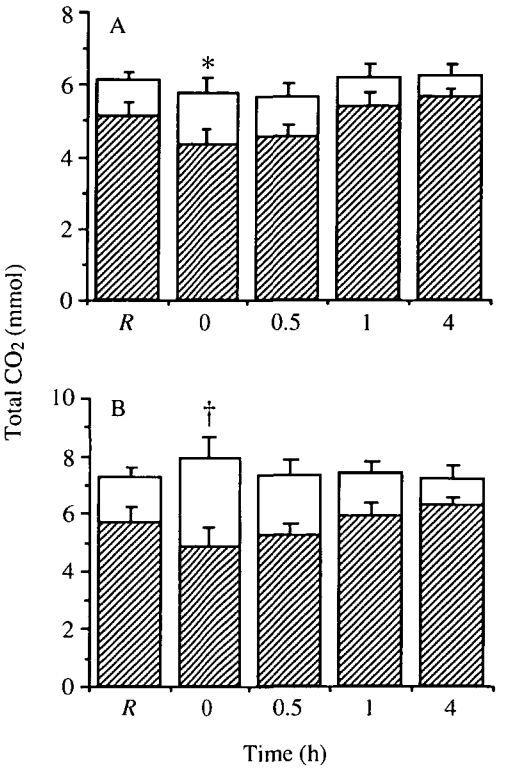

Fig. 5. Total CO_2 partitioned between plasma (hatched bars) and erythrocytes (open bars) in 1l of (A) arterial and (B) venous blood at rest (*R*) and 0, 0.5, 1 and 4h following exercise in *Petromyzon marinus*. Values are means+standard error (N=6). An asterisk denotes a significant difference from the resting value for plasma. A dagger denotes a significant difference from the resting value for erythrocytes (P<0.05).

acidosis (Tufts, 1991; Fig. 1A). Previously, Tufts (1991) demonstrated that arterial pHi was maintained during the extracellular acidosis following exercise in the sea lamprey. The present results indicate that this is also the case in venous blood (Fig. 1B). In fact, venous pHi is consistently higher than arterial pHi. The pH gradient across the erythrocyte membrane is therefore smaller in venous blood and becomes reversed after exercise (Fig. 1C). One can speculate that the maintenance of venous pHi may be beneficial for oxygen uptake following exercise. Conversely, the absence of significant reductions in venous pHi could also have a deleterious effect on oxygen delivery to the tissues by minimizing the impact of the Bohr effect on haemoglobin oxygen-carriage.

In the river lamprey, *Lampetra fluviatilis*, Na^+/H^+ exchange is involved in the regulation of erythrocyte pH *in vitro* (Nikinmaa, 1986; Nikinmaa *et al.* 1986). Recently, Tufts (1992) demonstrated that erythrocyte pH in the sea lamprey, *Petromyzon marinus*, may also be determined by a sodium-dependent mechanism *in vitro*. It is possible, therefore, that the observed regulation of pHi after exercise

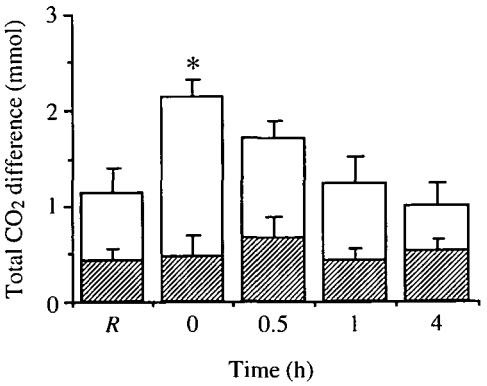

Fig. 6. Total CO_2 difference between 1 l of arterial and venous whole blood partitioned between plasma (hatched bars) and erythrocytes (open bars) at rest (*R*) and 0, 0.5, 1 and 4 h following exercise in *Petromyzon marinus*. Values are means+standard error (N=6). An asterisk denotes a significant difference from the resting value for erythrocytes.

in vivo involves the activation of ion transport processes across the red cell membrane. In addition, a considerable Haldane effect is present in the blood of both of these lamprey species (Nikinmaa and Matsoff, 1991; R. A. Ferguson, N. Sehdev, B. Bagatto and B. L. Tufts, unpublished data). Thus, deoxygenation of hemoglobin and associated buffering of H^+ probably contributes both to the differences in pHi between arterial and venous blood and to the maintenance of pHi immediately following exercise in *P. marinus*. In this regard, Fig. 2 illustrates the measured differences in P_{O_2} and haemoglobin oxygen-carriage between arterial and venous blood and highlights the significant decreases in these variables immediately after exercise.

Titration of plasma bicarbonate by protons would account for the observed increase in arterial and venous P_{CO_2} after exercise (Tufts, 1991; Fig. 3). A post-exercise increase in CO_2 production by the tissues undoubtedly contributes further to the greater elevation in venous P_{CO_2} (Fig. 3). As noted previously by Tufts (1991), it should be remembered that these P_{CO_2} values have been calculated. Thus, while relative differences will be reflected in these values, the absolute values may be somewhat different if, for any reason, the CO_2 reactions in the plasma have not reached equilibrium. Titration of plasma bicarbonate by protons could also explain the reductions in $C_{CO_2,t.pl.}$ of both arterial and venous blood after exercise (Fig. 4A). The changes in $C_{CO_2,i}$, however, are markedly different from those in the plasma. In arterial and venous blood, exercise causes a significant increase in $C_{CO_2,i}$ (Fig. 4B). In venous blood, $C_{CO_2,i}$ at 0 h was more than double the resting value. An increase in $C_{CO_2,i}$ after exercise would be expected because pHi is maintained at a time when P_{CO_2} is increased. However, the increase in $C_{CO_2,i}$ in venous blood is well above that predicted from the CO_2 dissociation curves for sea lamprey erythrocytes (Tufts and Boutilier, 1989, 1990).

This discrepancy can probably be attributed to the Haldane effect in sea lamprey blood (Nikinmaa and Matsoff, 1991; R. A. Ferguson, N. Sehdev, B. Bagatto and B. L. Tufts, unpublished data). Venous haemoglobin oxygen-carriage falls by 76% immediately after exercise (Fig. 2B). In venous blood, the amount of bicarbonate formed within the erythrocyte at any given P_{CO_2} will therefore be greater than that in arterial blood as a result of the increased number of protons which can be buffered by deoxygenated hemoglobin.

In most vertebrates, a large portion of the bicarbonate formed after hydration of CO_2 within the erythrocyte is transferred to the plasma in exchange for chloride (Roughton, 1964; Cameron, 1979; Perry, 1986; Swenson, 1990). In the rainbow trout, this process results in arteriovenous differences in erythrocyte chloride concentration of 15 mmol l^{-1} at rest and 22 mmol l^{-1} after exercise (Nikinmaa and Jensen, 1986). *In vitro* studies have indicated that chloride/bicarbonate exchange may be absent or very limited in agnathan erythrocytes (Ellory *et al.* 1987; Nikinmaa and Railo, 1987). However, there has been no *in vivo* evidence to support this view. Furthermore, the characteristics of CO_2 transport and acid–base regulation in arterial blood of the sea lamprey after exercise are not markedly different from those of other lower vertebrates (Tufts, 1991). In the present study, there are no significant differences in erythrocyte chloride concentration or erythrocyte water content between arterial and venous blood at rest or after exercise (Table 1). Thus, unless erythrocyte chloride is rapidly redistributed across the erythrocyte membrane by some other mechanism, these results indicate that the importance of erythrocyte chloride/bicarbonate exchange for CO_2 transport *in vivo* in the sea lamprey must be minimal. It should also be noted that the present values for erythrocyte chloride concentration are considerably higher than those reported by Tufts and Boutilier (1989). Extraction of dried samples with nitric acid, as in Boutilier and Tufts (1989), was later found to be an ineffective method for the determination of chloride concentrations in lamprey erythrocytes (B. L. Tufts and R. G. Boutilier, unpublished data). The large increase in $C_{CO_2,i}$ in venous blood after exercise may be further evidence that there is minimal exchange of chloride and bicarbonate across the erythrocyte membrane *in vivo* (Fig. 4B). In most vertebrates, much of the bicarbonate formed within the erythrocyte under these conditions would be redistributed to the plasma in exchange for chloride. Since erythrocyte chloride concentrations do not change significantly after exercise, one can predict that the majority of the bicarbonate formed within the erythrocyte would remain within the cytosol and, as observed, result in a very large increase in venous $C_{CO_2,i}$ after exercise.

In vitro analyses of CO_2 transport in sea lamprey blood suggest that the erythrocytes carry a much greater proportion of CO_2 than in other vertebrates (Tufts and Boutilier, 1989, 1990). Based on these results, Tufts and Boutilier (1989) built a predictive model for CO_2 in sea lampreys which primarily involves carriage by erythrocytes. Simply, bicarbonate formed within the erythrocyte would remain there until the blood reaches the gills. At this point, the intracellular bicarbonate would combine with a proton to be excreted as CO_2. The present

results can now be used to compare the relative importance of the erythrocyte and the plasma for CO_2 transport *in vivo*.

In resting sea lampreys, only 16 % of the total CO_2 present in arterial blood can be attributed to the erythrocytes. In venous blood, this percentage is somewhat greater (22 %), but the majority of the CO_2 is found in the plasma. By comparison, calculations based on data from Nikinmaa (1990) indicate that the erythrocytes can account for approximately 20 % of the total CO_2 in both arterial and venous blood in humans. In rainbow trout, which have a hematocrit closer to that of sea lampreys, the erythrocytes carry about 8 and 10 % of the total CO_2 in arterial and venous blood, respectively (Heming, 1984). Similarly, in the amphibian *Bufo marinus*, the erythrocytes account for about 10 % of the total CO_2 present in arterial blood at rest (Boutilier *et al.* 1979). Thus, under resting conditions, the distribution of total CO_2 between plasma and erythrocytes in arterial and venous blood of the sea lamprey is not markedly different from that in mammals, but the proportion carried in the erythrocytes appears to be somewhat greater than that in other lower vertebrates. Immediately after exercise, there are significant increases in $C_{CO_2,i}$ in arterial and venous blood and significant decreases in $C_{CO_2,t.pl.}$. This causes a change in the partitioning of CO_2 between plasma and erythrocytes in the sea lamprey (Figs 4, 5). The percentage of total CO_2 partitioned within the erythrocytes increases to 25 % in arterial blood and 38 % in venous blood, despite the fact that repetitive blood sampling resulted in a small decrease in hematocrit. The reported contribution of the erythrocytes will, therefore, be marginally underestimated. Thus, a considerable amount of the total CO_2 in sea lamprey blood is partitioned within the erythrocytes after exercise, particularly in the venous system.

When the total CO_2 differences between arterial and venous blood are analyzed, the actual contribution of the erythrocytes to 'CO_2 transport' in the sea lamprey becomes more apparent. At rest, 62 % of the difference between arterial and venous whole-blood C_{CO_2} can be attributed to changes in C_{CO_2} between arterial and venous erythrocytes. Even though the erythrocytes represent a much smaller fraction of the whole blood than the plasma, the difference in $C_{CO_2,i}$ between arterial and venous blood is much greater (4.0 mmol) than it is in the plasma (0.6 mmol; Fig. 4). In comparison, Heming (1984) reported that 82 % of the difference in whole-blood C_{CO_2} across the gills of rainbow trout was caused by changes in plasma bicarbonate concentration, whereas changes in red cell bicarbonate concentration accounted for only 8 % of the arteriovenous C_{CO_2} difference. Table 2 summarizes the arteriovenous differences for variables relevant to CO_2 transport in the sea lamprey and compares them to values for the rainbow trout. When the total CO_2 differences between arterial and venous blood are compared, the present *in vivo* experiments support the view that the strategy for CO_2 transport in sea lampreys may be markedly different from that in other vertebrates. After exercise, the difference in $C_{CO_2,i}$ between arterial and venous blood is even greater (Fig. 4B). At 0 h post-exercise, these differences account for 78.3 % of the total CO_2 difference between arterial and venous whole blood,

Table 2. *Comparison of resting arteriovenous differences in the sea lamprey and the rainbow trout*

	Sea lamprey	Rainbow trout
Total arteriovenous difference in CO_2 (mmol l^{-1} blood)	1.13	0.99[1]
Percentage of total arteriovenous difference in CO_2 from plasma	38	85[1]
Percentage of total arteriovenous difference in CO_2 from red blood cells	62	15[1]
Δrbc arteriovenous [Cl^-] (mequiv l^{-1})	2.4	15.0[2]
Δplasma arteriovenous [Cl^-] (mequiv l^{-1})	2.3	2.0[2]
Hematocrit (%)	27.4	23.2[2]

[1] Data from Heming (1984) and [2] data from Nikinmaa and Jensen (1986).

indicating that the role of the erythrocyte in CO_2 carriage is even more important after exercise in sea lampreys.

In conclusion, the present results indicate that the pattern of CO_2 transport in sea lampreys *in vivo* is markedly different from the standard model of CO_2 transport in vertebrates. As proposed by Tufts and Boutilier (1989), CO_2 transport in the sea lamprey *in vivo* is largely dependent on erythrocyte CO_2 carriage. In view of the phylogenetically primitive position of lampreys, one can speculate that this may represent an early stage in the evolution of gas transport. Transport of bicarbonate within the erythrocyte, however, appears to minimize the changes in pHi that are normally present in other vertebrates. Thus, a possible selective pressure for the evolution of the strategy of CO_2 transport present in most vertebrates may have been to maximize the impact of the Bohr effect in O_2 delivery.

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