HCO_3^- DEHYDRATION IN THE PLASMA OF RAINBOW TROUT: THE ROLE OF BUFFERING CAPACITY

K.M. Gilmour
Department of Biology, Carleton University, 1125 Colonel By Dr.,
Ottawa, ON, K1S 5B6, Canada
Phone (613) 520-2600 x1608; Fax (613) 520-2569;
E-mail kgilmour@ccs.carleton.ca

P.R. Desforges
Department of Biology, University of Ottawa

Abstract

HCO_3^- entry into the red blood cell is thought to be the rate-limiting step in CO_2 excretion in fish. By theory, the addition of bovine carbonic anhydrase (CA) to the circulation should provide an extracellular site for HCO_3^- dehydration, enhancing CO_2 excretion, and lowering the post-branchial blood CO_2 tension ($PaCO_2$). In agreement with theory, intravascular injection of bovine CA (5 mg kg^{-1}) into rainbow trout caused a small but significant reduction in $PaCO_2$. The contribution of extracellular HCO_3^- dehydration to CO_2 excretion may, however, be limited by proton availability owing to the low nonbicarbonate buffering capacity (ß) of trout plasma. To test this hypothesis, the effect on the plasma HCO_3^- dehydration rate of manipulating plasma ß using HEPES was examined both *in vivo* and *in vitro*. *In vivo*, a greater than three-fold increase in plasma ß (from -3.8 to -14 mmol L^{-1} pH $unit^{-1}$) had no significant impact on the extent of the $PaCO_2$ decrease following CA injection. However, an increase in plasma ß from -4.9 to -12 mmol L^{-1} pH $unit^{-1}$ increased the *in vitro* HCO_3^- dehydration rate of separated plasma significantly in both the absence and presence of bovine CA. These data suggest that HCO_3^- dehydration in fish plasma is limited by proton availability. Under normal conditions *in vivo*, however, treatment with HEPES to increase plasma buffering capacity has little effect on the decrease in $PaCO_2$ achieved by the addition of bovine CA to the circulation of the fish because proton availability for HCO_3^- dehydration is so much greater in the red cell owing to both the buffering power of haemoglobin, and the oxylabile release of protons from haemoglobin (Haldane effect).

Introduction

CO_2 excretion in rainbow trout (*Oncorhynchus mykiss*) follows the typical vertebrate pattern, in which the majority of CO_2 is carried as HCO_3^- ions in the blood plasma (reviewed by Perry, 1986; Tufts and Perry, 1998). At the gills, HCO_3^- ions enter the red blood cell (RBC) via a Cl^-/HCO_3^- exchanger (AE1 or band 3 protein) and are dehydrated to molecular CO_2, which then diffuses out of the blood to the ventilatory water along its partial pressure gradient. The protons required for HCO_3^- dehydration are provided primarily by haemoglobin, either through its buffering capacity or the Haldane effect, and the dehydration reaction is catalysed by RBC carbonic anhydrase (CA). The rate-limiting step in this process is thought to be HCO_3^- entry in the RBC via the anion exchanger (Perry and Gilmour, 1993, reviewed by Tufts and Perry, 1998). Because plasma CO_2 reactions in rainbow trout do not have access to extracellular CA activity at the gills (Perry et al., 1997; Gilmour et al., 1999), all catalysed HCO_3^- dehydration for CO_2 excretion must take place through the RBC. HCO_3^- dehydration in the plasma occurs at the uncatalysed rate and therefore makes a negligible contribution to CO_2 excretion owing to the brief residence time of blood in the gill [~1-3 sec (Cameron and Polhemus, 1974) versus ~25-90 sec for the half-time of the uncatalysed HCO_3^- dehydration reaction at fish body temperatures (Perry, 1986)].

Given that CO_2 excretion is limited by HCO_3^- entry into the RBC, the addition of CA to the blood plasma of rainbow trout would be predicted to enhance CO_2 excretion by providing an extracellular site for HCO_3^- dehydration. However, experimental results to date do not appear to support this prediction. Wood and Munger (1994) found that the effects of CA injection (10 mg kg^{-1}) injection into resting rainbow trout were similar to those of saline injection - very minor decreases in arterial CO_2 tension ($PaCO_2$) and HCO_3^- concentration and increases in arterial pH (pHa) were observed, changes that were close to the limit of reliable detection. Similar results (small increases in pHa, little or no change in $PaCO_2$ or blood total CO_2 content) were obtained by Lessard et al. (1995) and Gilmour et al. (1994).

The apparent lack of significant effect of exogenous CA injection on CO_2 excretion in trout may be the consequence of limited proton availability in plasma. The nonbicarbonate buffering capacity (ß) of separated trout plasma is -2.6 mmol L^{-1} pH $unit^{-1}$, whereas that of whole blood (at a haematocrit of 25%) is -9.7 mmol L^{-1} pH $unit^{-1}$ (Wood et al., 1982), the higher value for whole blood reflecting the buffering properties of haemoglobin. Because the dehydration of

one mole of HCO_3^- consumes one mole of H^+, HCO_3^- dehydration in the plasma of rainbow trout may be limited by proton availability owing to the relatively low plasma buffering capacity. Thus, the objective of the present study was to test the hypothesis that an increase in plasma nonbicarbonate buffering capacity would increase the extent to which exogenous CA activity enhanced (plasma) HCO_3^- dehydration in rainbow trout both *in vivo* and *in vitro*.

Materials and Methods

Experimental animals.

Rainbow trout were obtained from Linwood Acres Trout Farm (Campbellcroft, ON) and maintained on a 12L:12D photoperiod in large fibreglass aquaria supplied with flowing, aerated and dechlorinated City of Ottawa tap water at 13°C. Trout were fed to satiation on alternate days with commercial trout pellets and food was withheld for 24 h prior to experimentation. Two groups of fish were used; one group (N = 13) acted as blood donors for *in vitro* experiments while blood respiratory variables were measured *in vivo* using an extracorporeal arterial blood loop on the second group of fish [mass = 527 ± 22 (mean ± SEM); N = 48].

Surgical procedures.

All fish were anaesthetised in an aerated solution of benzocaine (ethyl-*p*-aminobenzoate; 0.1 g L^{-1}) and then transferred to an operating table that permitted the gills to be irrigated with the same anaesthetic solution throughout the surgery. For the fish used as blood donors for *in vitro* experiments, a single indwelling polyethylene cannula (Clay-Adams PE 50) was inserted into the dorsal aorta according to the basic method of Soivio et al. (1975). For continuous measurements of blood respiratory variables *in vivo* using the extracorporeal loop, the caudal vein and caudal artery were cannulated. Cannulation of the caudal vessels involved making a lateral incision at the level of the caudal peduncle so as to separate the epaxial and hypaxial musculature and expose the haemal arch. Catheters (PE50) were then fed into the caudal artery and vein in the anterior direction and were secured with ligatures to the skin; the incision was tightly closed with silk sutures. Small (1 cm^2) brass plates were stitched to the external surface of each operculum to allow the measurement of ventilation parameters using an impedance converter. After surgery, fish were transferred to individual opaque acrylic boxes supplied with

aerated, flowing water for a 24 h recovery period. Cannulae were flushed with heparinised (100 i.u. mL^{-1} sodium heparin) Cortland saline.

Experimental protocol – In vitro *experiments*.

The objective of this series of experiments was to assess HCO_3^- dehydration rates *in vitro* in separated plasma under various conditions of ß, CA activity and plasma HCO_3^- concentrations ($[HCO_3^-]$). HCO_3^- dehydration rates were measured using the radioisotopic assay of Wood and Perry (1991). Approximately 30 mL of separated plasma was required for a typical single experimental run (*i.e.* $N = 1$). Thus, it was necessary to use pooled blood obtained by slow withdrawal from the dorsal aortic cannulae of 2-3 fish. Following blood withdrawal/pooling and separation by brief centrifugation to obtain plasma, 1.2 mL of 180 mmol L^{-1} HEPES was added to one half of the plasma pool to achieve a nominal final concentration of 10 mmol L^{-1} HEPES; ß was measured for both the control (*i.e.* naturally-occurring ß) and HEPES-treated plasma (see below). For each ß, three levels of CA activity (0, 0.001 and 1 mg mL^{-1}) and two levels of HCO_3^- (nominally 10 and 15 mmol L^{-1}) were tested. Bovine CA was added (50 µl injection volume, bovine CA dissolved in saline) immediately prior to assaying the sample. Plasma HCO_3^- levels were elevated abruptly at the start of the assay by 'spiking' the plasma with 10 or 20 µL of isotope prepared in 500 mmol L^{-1} $NaHCO_3$. The nominal HCO_3^- levels were verified by analysing the plasma total CO_2 concentration (CCO_2); actual HCO_3^- concentrations were 11.0 ± 0.3 (48) and 15.5 ± 0.3 (48) mmol L^{-1} [mean ± SEM (*N*)]. Plasma pH was measured prior to and following the assay (pH glass and reference electrodes in a thermostatted blood gas cell connected to a blood gas analyser; Cameron Instruments).

The radioisotopic HCO_3^- dehydration assay was carried out as described by Wood and Perry (1991). In brief, plasma samples (0.8 mL) were equilibrated with a humidified gas mixture of 0.5% CO_2 in air (GF-3/MP gas mixing flowmeter; Cameron Instruments) for 60 min in a shaking water bath held at 10°C. To start the assay, 74 kBq of sodium [^{14}C]bicarbonate was added to each sample and the vial containing the sample was then immediately sealed with a cap containing a CO_2 trap (a filter paper impregnated with 150 µL hyamine hydroxide). At the end of the 3 min assay period, filter paper and plasma ^{14}C activities were determined by liquid scintillation counting (Packard TR 2500) with automatic quench correction. Filter papers were counted in 10 mL of Bio-Safe NA (Research Products Int.) while 50 µL of plasma was counted in 10 mL of ACS (Amersham) scintillation cocktail. Plasma CCO_2 was measured on 20

μL duplicate samples (Capni-Con 5 total CO_2 analyser; Cameron Instruments). The HCO_3^- dehydration rate for each vial was then calculated by dividing filter paper ^{14}C activity by plasma specific activity and time.

Experimental protocol – In vivo *experiments*.

The objective of this series of experiments was to assess the effects of exogenous CA injection on CO_2 excretion *in vivo* under normal conditions and following elevation of plasma ß by treatment with HEPES. CO_2 excretion was assessed indirectly by monitoring arterial CO_2 tension ($PaCO_2$) and pH (pHa) for 90 min following the intra-arterial injection of either 5 mg kg^{-1} bovine CA (in 1 mL saline) or the saline vehicle only. The effects of these treatments in fish at their naturally-occurring plasma ß were compared with those in fish that had received an injection of HEPES (2 mL kg^{-1} of 1.5 mol L^{-1} stock solution to achieve a nominal final circulation concentration of 10 mmol L^{-1} HEPES) to elevate plasma ß 2 h prior to CA or saline injection; plasma ß was measured at the end of each experiment.

Blood respiratory variables were measured using an extracorporeal shunt in which blood was withdrawn from the caudal artery cannula and passed by means of a peristaltic pump through an external circuit containing pH, PCO_2 and PO_2 electrodes (Thomas, 1994). The flow rate through the external loop, which contained approximately 1 mL of blood, was 0.40 mL min^{-1}. To prevent clotting, the circuit was rinsed with at least 10 mL of heparinised (540 i.u. mL^{-1}) saline before initiating the blood flow. Arterial blood pH, PCO_2 and PO_2 were monitored using Radiometer or Cameron Instruments (CO_2, O_2), and Metrohm (pH) electrodes housed in thermostatted cuvettes and connected to a blood gas analyser (Cameron Instruments). Blood gas electrodes were calibrated by pumping water equilibrated with appropriate gas mixtures (supplied by a GF-3/MP gas mixing flowmeter; Cameron Instruments) through the circuit; precision buffer solutions were used to calibrate the pH electrode. In addition to the blood respiratory variables, the frequency and amplitude of opercular displacements were assessed as an index of ventilation using a custom-built impedance converter that measured the changes in impedance between the electrodes sutured to the opercula. All analog signals (blood gases and impedance) were converted to digital data by interfacing with a data acquisition system (Biopac Systems Inc.) using Acknowledge™ data acquisition software and a Pentium™ PC. Thus, continuous data recording were obtained for blood gases (PaO_2, $PaCO_2$) and pH (pHa) as well as ventilation frequency (V_f;

automatic rate calculation from the raw impedance trace) and amplitude (V_{amp}, the difference between maximum and minimum impedance values).

Measurement of plasma nonbicarbonate buffering capacity.

To measure plasma ß in either series of experiments, a plasma sample of approximately 3 mL was gassed first with CO_2 for 5 min and then several times briefly with air. Following each equilibration, approximately 0.7 mL of plasma were withdrawn and analysed for CCO_2 (20 µL in duplicate; Capni-Con 5 total CO_2 analyser; Cameron Instruments) and pH (pH glass and reference electrodes in a thermostatted blood gas cell connected to a blood gas analyser; Cameron Instruments). Total CO_2 was plotted against pH and the nonbicarbonate buffering capacity was taken as the slope of the resultant linear regression line.

Statistical analysis.

Data are presented as means ± 1 standard error of the mean (SEM) (*N*). For *in vivo* experiments, mean blood gas and ventilatory data were compiled for 2 min periods at time = 0, immediately prior to saline or CA injection, and time = 90 min after saline or CA injection. Differences between the time = 0 and time = 90 min samples within a treatment were analysed statistically using a paired Student's *t*-test. A two-way repeated measures ANOVA followed by Tukey's post-hoc multiple comparisons test (as appropriate) was used to analyse the *in vitro* data for statistically-significant differences. In all cases, the fiducial limit of significance was 5%.

Results and Discussion

The addition of bovine CA to the circulation of rainbow trout resulted in a small, but significant, decrease in $PaCO_2$ coupled with a corresponding (non-significant) increase in pHa, whereas saline injection was without effect on either variable (Table 1). However, while HEPES treatment was successful in elevating the nonbicarbonate buffer capacity of the plasma more than three-fold (a significant increase from -3.82 ± 0.38 to -14.0 ± 2.76 mmol L^{-1} pH $unit^{-1}$; unpaired Student's *t*-test, $P < 0.05$), this increase in plasma ß did not enhance the effect of CA injection *in vivo*. As in fish of normal plasma ß, CA injection in HEPES-treated trout was associated with a small, significant decrease in $PaCO_2$ of about 0.2 Torr (Table 1). Again, saline injection was without effect on either $PaCO_2$ or pHa (Table 1), and ventilation in all groups was unaffected by either saline or CA injection (data not shown).

Table 1. The effect of saline or bovine CA (5 mg kg^{-1}) injection on arterial PCO_2 and pH in control fish and fish treated with HEPES to elevated plasma nonbicarbonate buffer capacity.

Treatment	**pHa**		**$PaCO_2$ (Torr)**	
	Time = 0 min	**Time = 90 min**	**Time = 0 min**	**Time = 90 min**
Control ß, saline (12)	7.92 ± 0.02	7.92 ± 0.02	2.01 ± 0.15	1.95 ± 0.14
Control ß, CA (22)	7.90 ± 0.02	7.93 ± 0.03	2.54 ± 0.17	2.30 ± 0.16*
High ß, saline (6)	7.80 ± 0.05	7.83 ± 0.04	2.22 ± 0.19	2.30 ± 0.17
High ß, CA (8)	7.94 ± 0.03	7.95 ± 0.04	1.73 ± 0.16	1.54 ± 0.15*

* indicates a significant difference (paired *t*-test, $P < 0.05$) within a treatment from the corresponding time = 0 value.

On the basis of these data, it is clear that the addition of exogenous CA to the circulation of rainbow trout enhances CO_2 excretion, presumably by providing an extracellular site for HCO_3^- dehydration. However, the extent to which exogenous CA enhanced CO_2 excretion was limited, nor was this limitation relieved by increasing the plasma nonbicarbonate buffering capacity. There are at least two plausible explanations for these results: (1) CO_2 excretion *in vivo* under resting conditions is not limited to a significant extent by the rate of anion exchange across the RBC, and hence bypassing this step through the addition of extracellular CA to the circulation has little or no effect on $PaCO_2$; or (2) HCO_3^- dehydration in the plasma is limited by proton availability, even under conditions of elevated plasma ß, or is limited by some factor other than proton availability.

The possibility that CO_2 excretion *in vivo* under resting conditions is not limited by access of plasma HCO_3^- to red cell CA via Cl^-/HCO_3^- exchange was also raised by Wood and Munger (1994) to explain the negligible effect of CA injection on resting $PaCO_2$ in their experiments. By contrast, the large, significant elevation of $PaCO_2$ that normally follows exhaustive exercise was attenuated by 50% in rainbow trout treated with CA prior to exercise, suggesting that the rate of anion exchange is limiting under conditions of elevated CO_2 production (Wood and Munger, 1994). Similarly, Lessard et al. (1995) found little effect of CA injection in rainbow trout under normoxic conditions but calculated that $PaCO_2$ would be decreased significantly (by ~1 Torr) by CA infusion during hypoxia, suggesting that the rate of anion exchange would be limiting under conditions of elevated CO_2 excretion. The hypothesis that CO_2

excretion *in vivo* is limited by entry of plasma HCO_3^- into the red cell only under conditions of elevated CO_2 production or excretion is attractive in terms of explaining the small effect of CA injection on $PaCO_2$ under resting, normoxic conditions observed in the present study and the lack of effect observed previously (Wood and Munger, 1994; Lessard et al., 1995). However, this hypothesis also necessitates that some other factor in the CO_2 excretion pathway acts to limit CO_2 excretion under normoxic conditions at rest, and it is difficult to conceive of what this factor would be. CO_2 diffusion in biological media is rapid and should never be rate-limiting during the course of CO_2 transfer (Swenson, 1990), yet CO_2 excretion *in vivo* is thought to behave as a diffusion-limited system (Malte and Weber, 1985; Julio et al., 2000). Recently, the apparent diffusion limitations on CO_2 excretion in fish were demonstrated experimentally to be the consequence of chemical reaction limitations (Julio et al., 2000). Julio et al. (2000) found that the increase in $PaCO_2$ elicited by reducing the surface area for gas exchange using gill ligation in rainbow trout could be reversed by treatment of the fish with bovine CA. The demonstration that CO_2 excretion *in vivo* is limited by chemical reaction kinetics, coupled with the fact that CA injection into normoxic, resting rainbow trout does a produce significant decrease in $PaCO_2$ (Table 1), confirm that HCO_3^- entry to the RBC via the anion exchanger is indeed the rate limiting step in CO_2 excretion *in vivo*.

Given Cl^-/HCO_3^- exchange as the rate-limiting step in CO_2 excretion *in vivo*, it might be expected that bypassing this step by providing a site for extracellular HCO_3^- dehydration at the catalysed rate would result in larger decreases in $PaCO_2$ than have been measured experimentally (Table 1; Wood and Munger, 1994; Lessard et al., 1995). The small effect of CA injection on $PaCO_2$ *in vivo* suggests that HCO_3^- dehydration in the plasma is limited by some factor, and an obvious candidate for such a factor is the plasma nonbicarbonate buffering capacity, since each mole of HCO_3^- ions dehydrated requires a mole of protons. Typically, the arterial-venous difference in blood total CO_2 concentration in rainbow trout is about 1.5 mmol L^{-1} (Brauner, 1995). This clearance of CO_2 as the blood passes through the gills is accompanied by a decrease in PCO_2 from about 3.7 Torr in the pre-branchial blood to about 2.3 Torr in the post-branchial blood (Tufts and Perry, 1998). The arterial-venous pH difference is dependent upon a variety of environmental factors (Brauner and Randall, 1998), but tends to be small under resting, normoxic conditions (arterial pH > venous pH) (Brauner, 1995). Thus, under normal conditions, ~1.5 mmol L^{-1} HCO_3^- is lost from the plasma with little change in pH (Fig. 1); the 1.5 mmol L^{-1} H^+ required for HCO_3^- dehydration is provided by the buffering capacity of haemoglobin and by Bohr protons released from haemoglobin during oxygenation (Tufts and

Perry, 1998; Brauner and Randall, 1998). However, were HCO_3^- dehydration to take place only in the plasma, where ß is -2.6 mmol L^{-1} pH $unit^{-1}$ (rather than the whole blood value of -9.7 mmol L^{-1} pH $unit^{-1}$), a decrease in PCO_2 from 3.7 to 2.3 Torr would elicit only an approximately 0.5 mmol L^{-1} decrease in plasma HCO_3^-; a 1.5 mmol L^{-1} change in the plasma HCO_3^- concentration would require an approximately 6 Torr decrease in PCO_2 together with a 0.5 unit pH change (Fig. 1). Clearly, by theory the low buffering capacity of the plasma will limit proton availability and hence constrain the extent of HCO_3^- dehydration that can take place in this compartment.

The results of the *in vitro* experiment support the hypothesis that proton

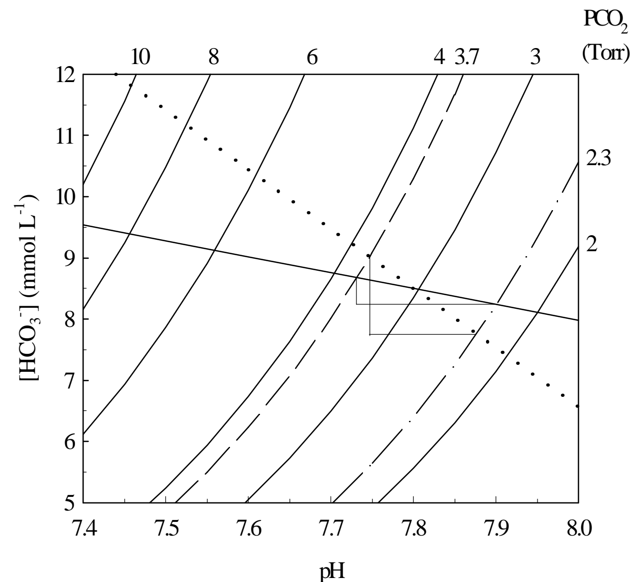

Figure 1. A pH-HCO_3^- diagram for rainbow trout at 13°C. The PCO_2 for a given combination of pH and [HCO_3^-] was calculated using the Henderson-Hasselbalch equation and the appropriate values for pK' and αCO_2 (Boutilier et al., 1984). Venous (dashed line) and arterial (dot-dash line) PCO_2 values for rainbow trout were drawn from Tufts and Perry (1998). Buffer lines for rainbow trout whole blood (dotted line) and separated plasma (solid line) were constructed using buffer values, ß, derived by Wood et al. (1982). Δ[HCO_3^-] and ΔpH for the arterial-venous PCO_2 decrease at each ß are drawn.

availability limits HCO_3^- dehydration in rainbow trout plasma. HCO_3^- dehydration rates of separated plasma at the natural buffering capacity of the fish or at elevated buffering capacity (HEPES-treated plasma) were measured at three levels of CA activity and two HCO_3^- concentrations using the radioisotopic assay of Wood and Perry (1991). As expected on the basis of previous studies (e.g. Wood and Perry, 1991; Perry and Gilmour, 1993), the HCO_3^- dehydration rate for separated plasma was low in the absence of added CA activity and increased in a dose-dependent fashion with added bovine CA (Fig. 2). Manipulation of the plasma HCO_3^- concentration had little effect on plasma HCO_3^- dehydration rates at any CA level (Fig. 2), a finding similar to that of Perry and Gilmour (1993). However, addition of 10 mmol L^{-1} HEPES to separated plasma to elevate the nonbicarbonate buffer capacity from -4.9 ± 0.7 to -12.1 ± 1.4 mmol L^{-1} pH $unit^{-1}$, a significant (paired Student's *t*-test, $P < 0.05$) approximately 2.5-fold rise, increased the HCO_3^- dehydration rate significantly at all CA levels (Fig. 2). Moreover, the higher HCO_3^- dehydration rates in HEPES-treated plasma samples were achieved with plasma pH increases that were generally smaller, although not significantly so, than those in plasma samples at the naturally-occurring buffer capacity (Table 2), despite the presumably greater consumption of protons to support the higher HCO_3^- dehydration rates.

Table 2**.** Mean changes in plasma pH (ΔpH = final pH – initial pH) as a result of the *in vitro* HCO_3^- dehydration assay.

[CA] (mg mL^{-1})	**Low ß, low [HCO_3^-]**	**Low ß, high [HCO_3^-]**	**High ß, low [HCO_3^-]**	**High ß, high [HCO_3^-]**
0 ($N = 6$)	0.05 ± 0.19	0.23 ± 0.24	0.17 ± 0.14	0.20 ± 0.13
0.001 ($N = 6$)	0.45 ± 0.19	0.41 ± 0.23	0.34 ± 0.13	0.34 ± 0.13
1 ($N = 6$)	0.53 ± 0.19	0.39 ± 0.24	0.34 ± 0.15	0.40 ± 0.17

No significant differences in ΔpH were observed within a treatment.

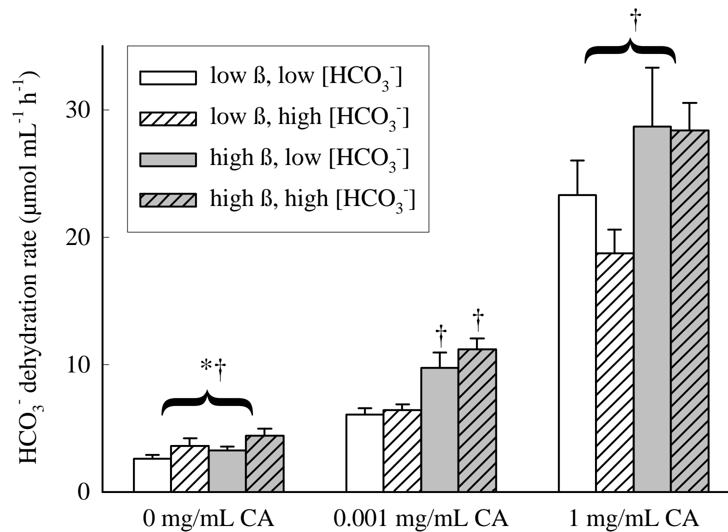

Figure 2**.** Mean HCO_3^- dehydration rates (*N* = 6 for all groups) for separated plasma samples from rainbow trout under various conditions of nonbicarbonate buffer capacity (ß), CA activity and plasma HCO_3^- concentration ([HCO_3^-]). * indicates a significant effect of [HCO_3^-] while † indicates a significant effect of ß on the HCO_3^- dehydration rate (two-way repeated measures ANOVA followed by Tukey's all-pairwise post-hoc multiple comparisons test at each CA level, $P <$ 0.05). Use of the bracket denotes that no significant interaction occurred between the two factors, [HCO_3^-] and ß, such that multiple comparisons were carried out only within a factor and not among all four groups in a treatment.

Thus, at least *in vitro*, proton availability appears to be a limiting factor on HCO_3^- dehydration in rainbow trout plasma. Interestingly, limitations on proton availability owing to the relatively low buffering capacity of plasma are thought to restrict the contribution of pulmonary capillary endothelial CA (CA IV) in mammals to less than 10% of CO_2 excretion (Bidani and Heming, 1991; Bidani, 1991; Heming and Bidani, 1992). By contrast, the high buffering capacity of

dogfish plasma relative to the whole blood value may allow gill membrane-bound CA IV in this species to make a significant (30% or more) contribution to CO_2 excretion (Gilmour et al., 1999). In rainbow trout, however, even when the plasma nonbicarbonate buffer capacity was increased more than three-fold, the impact of exogenous CA activity on $PaCO_2$ was small (Table 1). This result suggests that, under normal conditions, the high availability of protons for HCO_3^- dehydration in the red cell is of critical importance to CO_2 excretion. That is, in the presence of extracellular CA and elevated plasma buffering capacity, CO_2 excretion *in vivo* still occurs primarily via the red cell, despite the rate-limiting constraint of anion exchange, because of the high intracellular proton availability. While whole blood buffering capacity in rainbow trout is about -9.7 mmol L^{-1} pH $unit^{-1}$ (at 25% haematocrit) (Wood et al., 1982), this value takes into account the low plasma ß (-2.6 mmol L^{-1} pH $unit^{-1}$) and red cell buffering capacity is therefore presumably much higher (Tufts and Perry, 1998). Moreover, CO_2 excretion in rainbow trout is tightly linked to O_2 uptake through the release of Bohr protons from haemoglobin during oxygenation (Perry and Gilmour, 1993; Brauner et al., 1996, see reviews by Brauner and Randall, 1996, 1998). It appears that these proton sources are of overwhelming importance to CO_2 excretion *in vivo*, in that although plasma HCO_3^- dehydration rates *in vitro* were elevated by as much as 20-75% by increasing buffering capacity, the addition of bovine CA to provide an extracellular site for HCO_3^- dehydration *in vivo* in trout with elevated plasma buffering capacity had only a small impact on CO_2 excretion.

Acknowledgements

This study was supported by NSERC of Canada research and equipment grants to KMG. Dr. Steve Perry is thanked for his helpful comments on the manuscript.

References

Bidani, A. 1991. Analysis of abnormalities of capillary CO_2 exchange in vivo. J. Appl. Physiol. 70:1686-1699.

Bidani, A. & Heming, T.A. 1991. Effects of perfusate buffer capacity on capillary CO_2-HCO_3^--H^+ reactions: theory. J. Appl. Physiol. 71:1460-1468.

Boutilier, R.G., Heming, T.A. & Iwama, G.K. 1984. Appendix: Physicochemical parameters for use in fish respiratory physiology. In: W.S. Hoar & D.J. Randall (Eds.). Fish Physiology. Academic Press, London, pp. 403-430.

Brauner, C.J. 1995. An analysis of the transport and interaction of oxygen and carbon dioxide in fish. Ph.D. thesis, University of British Columbia. 148pp.

Brauner, C.J., Gilmour, K.M. & Perry, S.F. 1996. Effect of haemoglobin oxygenation on Bohr proton release and CO_2 excretion in the rainbow trout. Respir. Physiol. 106:65-70.

Brauner, C.J. & Randall, D.J. 1996. The interaction between oxygen and carbon dioxide movements in fishes. Comp. Biochem. Physiol. 113A:83-90.

Brauner, C.J. & Randall,D.J. 1998. The linkage between oxygen and carbon dioxide transport. In: S.F. Perry & B.L. Tufts (Eds.). Fish Respiration. Academic Press, San Diego, pp. 283-319.

Cameron, J.N. & Polhemus, J.A. 1974. Theory of CO_2 exchange in trout gills. J. exp. Biol. 60:183-194.

Gilmour, K.M., Perry, S.F., Bernier, N.J., Henry, R.P. & Wood, C.M. Extracellular carbonic anhydrase in dogfish, *Squalus acanthias*: A role in CO_2 excretion. Respir. Physiol., submitted.

Gilmour, K.M., Randall, D.J. & Perry, S.F. 1994. Acid-base disequilibrium in the arterial blood of rainbow trout. Respir. Physiol. 96:259-272.

Heming, T.A. & Bidani, A. 1992. Influence of proton availability on intracapillary CO_2-HCO_3^--H^+ reactions in isolated rat lungs. J. Appl. Physiol. 72:2140-2148.

Julio, A.E., Desforges, P.R. & Perry, S.F. 2000. Apparent diffusion limitations for CO_2 excretion in rainbow trout are relieved by injections of carbonic anhydrase. Respir. Physiol. 121:53-64.

Lessard, J., Val, A.L., Aota, S. & Randall, D.J. 1995. Why is there no carbonic anhydrase activity available to fish plasma? J. exp. Biol. 198:31-38.

Malte, H. & Weber, R.E. 1985. A mathematical model for gas exchange in the fish gill based on non-linear blood gas equilibrium curves. Respir. Physiol. 62:359-374.

Perry, S.F. 1986. Carbon dioxide excretion in fishes. Can. J. Zool. 64:565-572.

Perry, S.F., Brauner, C.J., Tufts, B.L. & Gilmour, K.M. 1997. Acid-base disequilibrium in the venous blood of rainbow trout (*Oncorhynchus mykiss*). Experimental Biology Online 2, 1.

Perry, S.F. & Gilmour, K.M. 1993. An evaluation of factors limiting carbon dioxide excretion by trout red blood cells *in vitro* . J. exp. Biol. 180:39-54.

Soivio, A., Nynolm,K. & Westman,K. 1975. A technique for repeated sampling of the blood of individual resting fish. J. exp. Biol. 62:207-217.

Swenson, E.R., 1990. Kinetics of oxygen and carbon dioxide exchange. In: R.G. Boutilier (Ed.). Advances in Comparative and Environmental Physiology. Springer-Verlag, Berlin, pp. 163-210.

Thomas, S. 1994. Extracorporeal circulation. In: P.W. Hochachka & T.P. Mommsen (Eds.). Biochemistry and molecular biology of fishes: Analytical Techniques. Elsevier, Amsterdam, pp. 161-167.

Tufts, B.L. & Perry, S.F. 1998. Carbon dioxide transport and excretion. In: S.F. Perry & B.L. Tufts (Eds.). Fish Respiration. Academic Press, San Diego, pp. 229-281.

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

Wood, C.M. & Munger, R.S. 1994. Carbonic anhydrase injection provides evidence for the role of blood acid-base status in stimulating ventilation after exhaustive exercise in rainbow trout. J. exp. Biol. 194:225-253.

Wood, C.M. & Perry, S.F. 1991. A new *in vitro* assay for carbon dioxide excretion by trout red blood cells: effects of catecholamines. J. exp. Biol. 157:349-366.