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HCO_3^- dehydration by the blood of rainbow trout following exhaustive exercise

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Abstract

Exhaustive exercise causes Pa_{CO_2} elevation, intense acidosis, and a mobilization of catecholamines into the blood of trout. A radioisotopic assay (Wood and Perry, *J. Exp. Biol. 157: 349–366, 1991*) was used to examine changes in HCO_3^- dehydration rate in blood sampled by dorsal aortic catheter from exercised trout. The rate increased about 50% during and throughout the 30 min period following exercise when the blood was assayed immediately at the acid-base status prevailing in vivo. However when the blood from exercised fish was rapidly equilibrated to resting levels of P_{CO_2} prior to assay, the rate was decreased by 15–35% relative to resting values. This effect was seen only in whole blood, not in plasma, indicating an inhibition at the level of the red cells. Conversely, when blood from resting trout was equilibrated to the elevated Pa_{CO_2} seen in vivo after exercise, the measured dehydration rate was greater than twice that in samples from exercised fish. Thus a *relative* inhibition of HCO_3^- dehydration through the red cells occurs after exercise, an effect likely associated with adrenergic activation of Na^+/H^+ exchange.

Keywords: Bicarbonate, dehydration rate; Carbon dioxide, excretion; Exercise, HCO_3^- dehydration rate; Fish, trout (*Oncorhynchus mykiss*); Mediators, catecholamines

1. Introduction

CO_2 excretion in fish follows the standard vertebrate scheme whereby plasma HCO_3^- enters the red blood cell (RBC) in electroneutral exchange for Cl^- via the band 3 protein, is dehydrated to CO_2 by erythrocytic carbonic anhydrase, and diffuses through the plasma and branchial epithelium to the external environment. The flux is driven by the P_{CO_2} gradient from RBC to water, and HCO_3^- entry appears to be the rate-limiting step (see Perry, 1986; Perry and Laurent, 1990, for reviews). In this paper,

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the term "HCO_3^- dehydration rate" will be used to describe the *net* rate of conversion of plasma HCO_3^- to excreted CO_2 by whole blood, thereby encompassing the processes of HCO_3^-/Cl^- exchange entry on band 3, carbonic anhydrase catalyzed conversion of HCO_3^- to CO_2 in the RBC, as well as uncatalyzed conversion in the plasma, and CO_2 diffusion to the excretion sink.

Wood and Perry (1985) proposed that catecholamine mobilization during severe exercise would inhibit the relative rate of HCO_3^- dehydration and therefore CO_2 excretion by RBCs at a time of increased CO_2 production by the systemic tissues. As a result, blood P_{CO_2} levels would increase throughout the fish, thereby contributing to the post-exercise increases in Pa_{CO_2} and Pv_{CO_2} which have been observed in many studies. These ideas have remained controversial.

Tufts et al. (1988), who employed a modified "boat" assay (Booth, 1938) to measure HCO_3^- dehydration rates, reported a marked *increase* in HCO_3^- dehydration rates when arterial blood sampled from exhaustively exercised trout was assayed. This increase appeared to be of non-adrenergic origin. Unfortunately the modified boat assay has been plagued by artifacts when used for whole blood (Booth, 1938; Haswell and Randall, 1976; Heming and Randall, 1982). In addition, the acid-base status of the sample is dramatically altered during the assay, and the resulting blood CO_2 excretion rates which are measured are several hundred-fold greater than estimated in vivo rates. The results are therefore difficult to interpret.

Wood and Perry (1991) developed a new in vitro assay to directly measure the net rate of HCO_3^- dehydration by blood under acid-base conditions which more closely duplicate those occurring in vivo. The assay employs ^{14}C-labelled plasma HCO_3^- and direct collection of evolved CO_2, and yields rates much closer to estimated in vivo values. Using this assay, Wood and Perry (1991) and Perry et al. (1991) confirmed that catecholamines do inhibit the dehydration of plasma HCO_3^- by RBCs when added to blood in vitro. Furthermore they demonstrated that the effect only occurred when the blood was acidified to duplicate post-exercise conditions, that it was short-lasting (~ 30 min), and that it was linked to the β_1-activation of the well-known Na^+/H^+ exchange mechanism in the RBC membrane (see Nikinmaa and Tufts, 1989; Motais et al., 1989 for reviews). Band 3 is thought to be the overall rate-limiting step in HCO_3^- dehydration; as discussed by Perry et al. (1991), the mechanism of inhibition probably reflects an alteration in the electrochemical gradient for HCO_3^- entry on band 3 and/or CO_2 recycling from plasma to RBCs (Motais et al., 1989). Adrenaline appears to have no direct effect on carbonic anhydrase activity itself (Wood and Perry, 1991).

It remains to be seen whether such an inhibition actually occurs in vivo. On the one hand, exogenous or endogenous additions of catecholamines to the bloodstream generally raise Pa_{CO_2} in vivo (e.g. Perry and Vermette, 1987; Perry and Thomas, 1991). On the other, attempts to detect decreases in CO_2 excretion on a whole animal basis in salmonids which have been exhaustively exercised or infused with catecholamines have been unsuccessful (Steffensen et al., 1987; Playle et al., 1990). This is not surprising, because whole animal CO_2 production increases greatly in exercised fish (Steffensen et al., 1987; Milligan and McDonald, 1988; Scarabello et al., 1992) so CO_2 excretion must increase almost in parallel if lethal "CO_2 retention" is to be avoided. Any inhibition which occurs must be a *relative* one (with respect to the flux which would have

occurred in the absence of adrenergic stimulation) so as to "reset" blood P_{CO_2} to a higher level. The resulting increased P_{CO_2} gradient from blood to water must be sufficient to drive the necessary CO_2 excretion despite the *relative* inhibition of flux through the RBC.

The present study employed the assay of Wood and Perry (1991) to measure the actual rates of HCO_3^- dehydration in arterial blood sampled from trout in vivo during the period of Pa_{CO_2} elevation after exhaustive exercise. To test whether there was a *relative* inhibition of HCO_3^- flux through the RBCs, assays were also run after the P_{CO_2} of the post-exercise samples had been quickly restored to resting levels. As an additional test, assays were also performed on resting blood samples in which the acid-base status was adjusted to simulate post-exercise levels. The results confirm that a *relative* inhibition of HCO_3^- dehydration rate by the RBCs occurs after exercise despite an elevation of absolute rates.

2. Materials and methods

Experimental animals, exercise, and blood sampling Experiments were performed on 92 adult rainbow trout (*Oncorhynchus mykiss* Walbaum; 150–350 g) obtained from Spring Valley Trout Farm, Petersburg, Ontario and held at seasonal temperatures (9–16 °C) in flowing dechlorinated Hamilton tapwater (moderately hard water; composition as in Scarabello et al., 1992). The fish were acclimated to the experimental temperature (15 ± 1 °C) for at least 7 days prior to experiment. Each fish was fitted with an indwelling dorsal aortic catheter for blood sampling (Soivio et al., 1972) and allowed to recover for 24–48 h in a darkened individual fish box.

Blood samples were taken from fish at rest or at various times up to 30 min after exhaustive exercise. Exercise was induced by transferring the fish, without air exposure, to a 500 l tank and then vigorously chasing for 6 min, following which the fish was returned to its chamber. Only one blood sample was drawn from each fish. Blood samples (1.0 to 3.0 ml, depending on the experiment; 175 i.u. heparin·ml^{-1}) were drawn anaerobically into gas-tight syringes (Hamilton) and then aliquotted for the HCO_3^- assay or other analyses as appropriate. The assay is sensitive to RBC concentration, but only below a resting hematocrit of about 15% (Wood and Perry, 1991; Perry and Gilmour, 1993). Therefore small samples were drawn from all trout prior to test, for measurement of resting hematocrit (5000 × *g* for 5 min); anemic fish (hematocrit < 15%) were rejected.

The HCO_3^- dehydration assay The assay was performed as described by Wood and Perry (1991; see their Fig. 1) with minor modification. The assay measures the *net* rate of plasma HCO_3^- dehydration and resulting evolution of CO_2 by the whole blood sample during brief exposure to an external P_{CO_2} of 0 Torr, rather comparable to the situation of blood passing through the gills. The measurement therefore includes both HCO_3^- dehydration at the (presumably) uncatalyzed rate in the blood plasma, and the much larger dehydration flux occurring through the RBCs by band 3 and catalyzed by erythrocytic carbonic anhydrase. It does not include the dehydration of HCO_3^- already

inside the RBC's at the time of assay. The coefficient of variation (CV = SD/mean) for multiple assays run on a common pool is $<5\%$.

In brief, 1.0 ml of whole blood was added to a 20 ml glass scintillation vial which was placed in a shaking water bath at 15 ± 1 °C. After appropriate pre-treatment as required (see below), the assay was started by the addition of 2 μCi (40 $\mu Ci \cdot ml^{-1}$) of sodium [^{14}C]bicarbonate (ICN Biomedicals Inc.) in 5 $mmol \cdot L^{-1}$ HCO_3^- Cortland salmonid saline (volume added = 50 μl). The vial was immediately sealed with a rubber stopper containing a $^{14}CO_2$ trap, and shaking was continued for exactly 3 min. The CO_2 trap was a fluted filter paper (Whatman GF/A 2.4 cm glass microfibre filter) impregnated with 150 μl of 1 $mol \cdot L^{-1}$ hyamine hydroxide in methanol. After 3 min, the filter was immediately removed and assayed for ^{14}C-radioactivity, and the blood was drawn into a gas-tight syringe for immediate measurement of pHe, and in some experiments hematocrit, hemoglobin concentration, and RBC pHi. The remaining blood was then centrifuged ($12000 \times g$ for 2 min) in a sealed tube for determination of true plasma CO_2 content (C_{CO_2}) (2×50 μl) and ^{14}C-radioactivity (2×50 μl), and thereby plasma specific activity (dpm.μmol^{-1}). The CO_2 excretion rate for each 1 ml sample was calculated by dividing filter paper ^{14}C-radioactivity (dpm, corrected to 100% based on the measured $^{14}CO_2$ trapping efficiency of 76%) by plasma specific activity (dpm.μmol^{-1}) and time (0.05 h). The red cell pellet was frozen in liquid N_2 if required for measurement of RBC pHi.

Experimental series

Series 1 This series was designed to measure the rates of HCO_3^- dehydration in blood sampled from trout at various times after exhaustive exercise. Samples were drawn at rest (control), after 2 min of exercise (i.e. during the exercise bout), after 6 min of exercise (the end of the exercise bout, time 0), and at 6, 15, and 30 min post-exercise. The HCO_3^- dehydration assay was run immediately without prior treatment of the blood. Thus each assay was started at the acid-base status prevailing in vivo, and blood acid-base status was measured at the end of each 3 min assay. Other parameters were not measured in this series, except in the case of the time 0 sample, where the same measurements as in Series 3 were performed (see below).

Series 2 This series was designed to detect whether there was a *relative* inhibition of HCO_3^- dehydration after exercise by assaying all the samples at a P_{CO_2} approximating the normal resting value in vivo. The goal was to eliminate any differences in P_{CO_2} as a complicating factor. Samples were drawn at rest (control), after 2 min of exercise (i.e. during the exercise bout), after 6 min of exercise (the end of the exercise bout, time 0), and at 7 and 22 min post exercise. Part of the sample was used for immediate measurement of acid-base status at all times, and for preservation of plasma for catecholamine analysis at rest, time 0, and at 22 min (storage at −80 °C in the presence of 25 $mmol \cdot L^{-1}$ each of reduced glutathione and EGTA). The remainder (1.0 ml) was aliquotted into a 20 ml glass scintillation vial in the shaking water bath. The vial was immediately sealed with a rubber stopper containing a hyamine hydroxide filter paper trap (exactly as above) to rapidly lower the P_{CO_2} of the sample. This was then followed

by equilibration on open-circuit with a humidified precision gas mixture of $P_{CO_2} = 2$ Torr, $P_{O_2} = 155$ Torr, balance N_2. The relative times of the "scrubbing" phase with the CO_2 trap and the "gassing" phase were varied as necessary to achieve the final desired P_{CO_2}, but in all cases the total equilibration time after sampling was 8 min. At this time, the HCO_3^- dehydration assay was started by adding [^{14}C] bicarbonate and sealing the vial with a fresh $^{14}CO_2$ trap. At the end of the assay, the sample was processed in the regular fashion, with measurements of blood acid-base status, RBC pHi, hemoglobin, and hematocrit.

In order to confirm that the *relative* inhibition of CO_2 excretion observed in this series was a function of the RBCs rather than the plasma, the rest and immediately post-exercise points (time 0) were repeated, with duplicate 1.5 ml samples for true plasma and whole blood. After the 8 min period of equilibration, one of the samples was centrifuged (12000 × *g* for 2 min) in a sealed tube for separation of true plasma. Plasma (1.0 ml) and whole blood (1.0) samples were then added to fresh vials and the HCO_3^- dehydration assay was performed in the standard fashion.

Series 3 In order to assess the extent of inhibition of HCO_3^- dehydration actually occurring at the P_{CO_2} levels observed in vivo after exhaustive exercise, blood was drawn from *resting* fish and equilibrated to elevated P_{CO_2} in vitro. The latter was representative of P_{CO_2}'s observed in vivo after exercise. Duplicate 1.0 ml aliquots were added to glass scintillation vials and equilibrated on open-circuit with a humidified gas mixture of $P_{CO_2} = 7.5$ Torr, $P_{O_2} = 155$ Torr, balance N_2 for 30–60 min. One sample was employed for determination of pre-assay acid-base status; the other was used in the HCO_3^- dehydration assay in the standard fashion. Measured dehydration rates in these samples were compared with those determined immediately post-exercise in Series 1. Post-assay blood acid-base status and RBC pHi were also measured for these comparisons.

Analytical techniques Catecholamines (adrenaline and noradrenaline) were determined on alumina-extracted plasma samples by HPLC (Waters 510 pump, reverse phase C-18 column) with electrochemical detection (Waters M460). Hematocrit was determined by centrifuging 60 μl of blood in a heparinized capillary tube for 5 min at 5000 × *g*. Hemoglobin was measured by the cyanmethemoglobin procedure, using Sigma reagents. Mean cell hemoglobin concentration (MCHC), an index of RBC swelling, was calculated as [hemoglobin] ($g \cdot ml^{-1}$) divided by hematocrit ($ml \cdot ml^{-1}$). RBC pHi was determined by the freeze-thaw lysate method of Zeidler and Kim (1977).

Intracellular (RBC pHi) and whole blood extracellular pH (pHe) were measured with a micro-capillary electrode (G297/G2) thermostatted to the experimental temperature and connected to a PHM-71 Mk2 acid-base analyzer (Radiometer-Copenhagen). Plasma C_{CO_2} was measured with either a Corning 965 or a Capni-con Model II Total CO_2 Analyzer (Cameron Instruments). P_{CO_2} and plasma [HCO_3^-] were estimated using the Henderson-Hasselbalch equation with appropriate values of αCO_2 and pK′ for rainbow trout plasma from Boutilier et al. (1984).

Scintillation counting with quench correction on an LKB Rackbeta 1217 Counter was employed to measure ^{14}C radioactivity in plasma (50 μl in 10 ml Amersham

ACS II) and filter papers (in 10 ml of customized cocktail containing 2.0 g PPO + 0.1 g POPOP in 0.8 L of toluene + 0.2 L of 95% ethanol).

All data are expressed as means ± 1 SEM (N) in Figures and Tables. Statistically significant differences ($P \leq 0.05$) were assessed using Student's two-tailed t-test, paired or unpaired format as appropriate to the design of the experiments. Whenever multiple comparisons were involved, the t-value was adjusted by the Bonferroni procedure.

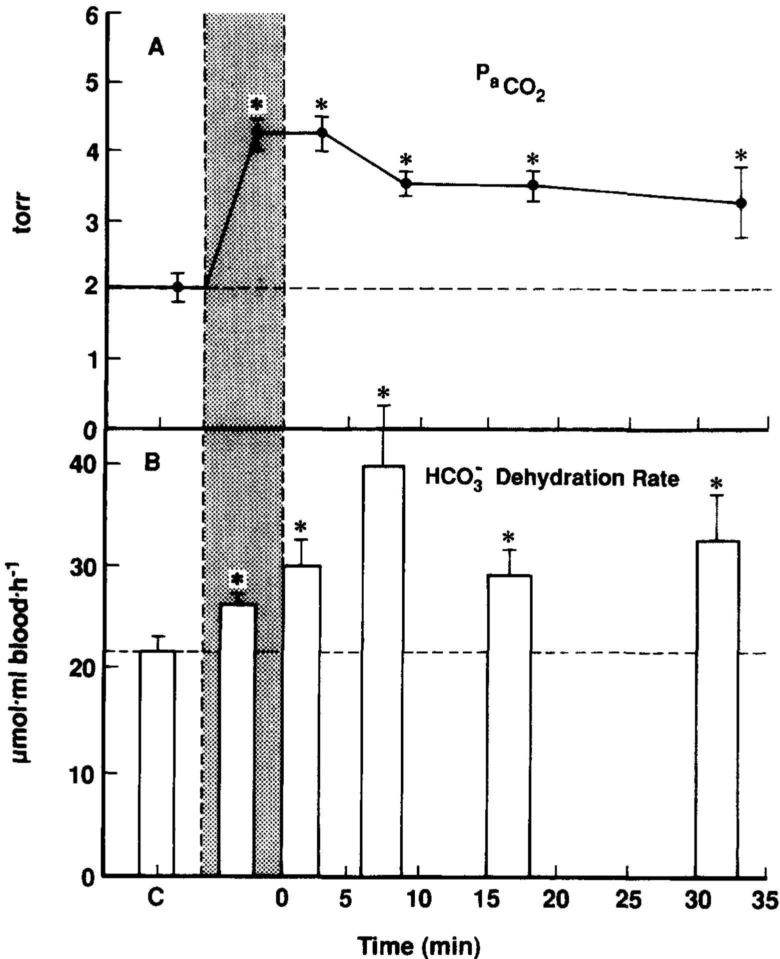

Fig. 1. The effects of 6 min of exhaustive exercise (bar) on (A) the arterial carbon dioxide tension (Pa_{CO_2}) and (B) the rate of HCO_3^- dehydration by the blood of rainbow trout. The HCO_3^- dehydration assays were started immediately after blood sampling, thereby using the acid-base conditions pertaining in vivo at the time. The Pa_{CO_2} values were determined at the end of the 3 min assays, and are plotted at the time of determination. Therefore they were undoubtedly lower than the starting in vivo values. Means ± 1 SEM (n = 6–8 at each point). Asterisks indicate significant differences ($P \leq 0.05$) from the pre-exercise control value (C).

3. Results

Series 1 In this series, the blood was assayed immediately for HCO_3^- dehydration rate under the acid-base conditions prevailing in vivo at the time, but acid-base status was routinely measured only at the end of the assay. At this time, the blood had been exposed to an external P_{CO_2} close to 0 Torr caused by the presence of the hyamine hydroxide trap. Nevertheless, Pa_{CO_2} more than doubled from about 2.0 to 4.2 Torr by 2 min of exercise, and remained significantly elevated at 6 min and throughout the

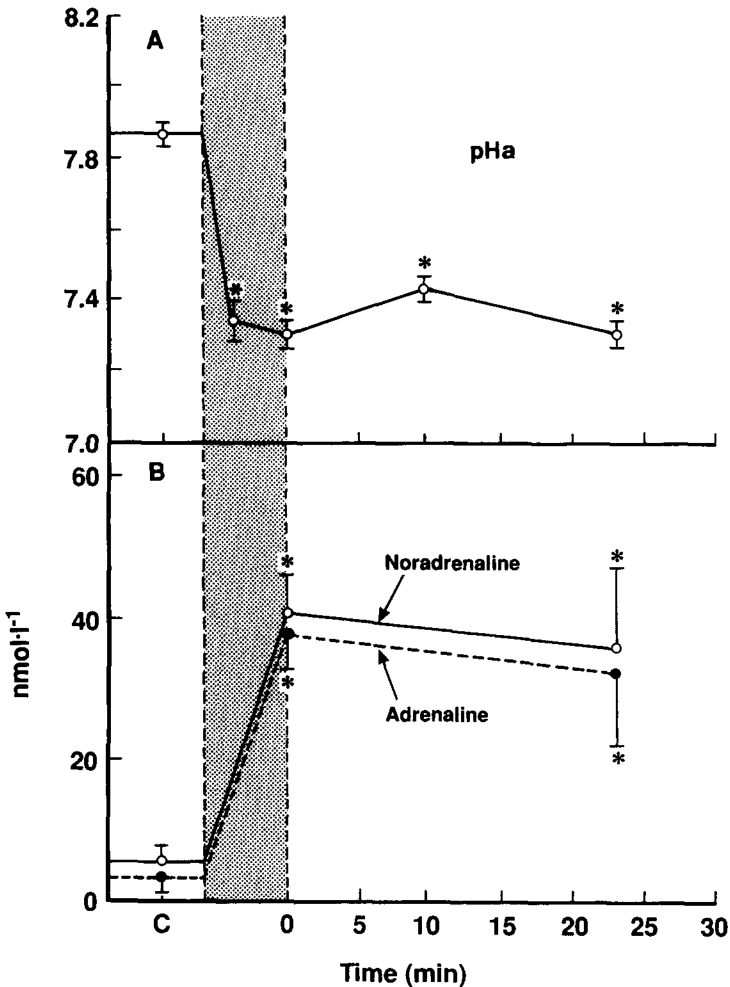

Fig. 2. The effects of 6 min of exhaustive exercise (bar) on (A) the arterial pH (pHa) and (B) the levels of catecholamines in the blood plasma of rainbow trout. The data are plotted at time of blood sampling. Means ± 1 SEM ($n = 6–9$ at each point). Asterisks indicate significant differences ($P \leq 0.05$) from the pre-exercise control value (C).

post-exercise period (Fig. 1A). Extracellular pH (i.e. pHa, not shown) fluctuated between 7.4 and 7.55 during this period, all values being significantly lower than the resting pHa of 7.86. The extent of the true respiratory acidosis in vivo at the time of sampling was clearly greater than indicated by these measurements at the end of the assay. This was shown by the data of both Series 2 and 3.

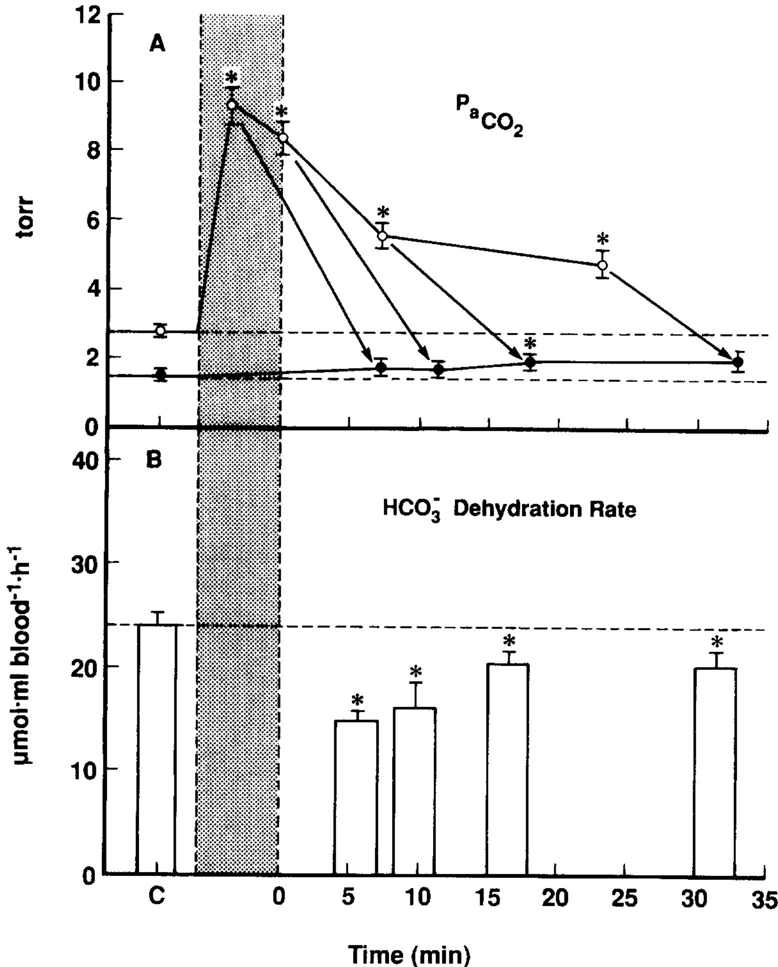

Fig. 3. The effects of 6 min of exhaustive exercise (bar) on (A) the arterial carbon dioxide tension (Pa_{CO_2}) and (B) the rate of HCO_3^- dehydration by the blood of rainbow trout. In this experiment, the in vivo Pa_{CO_2} values were determined at the time of blood sampling (open circles, plotted at the time of sampling), and then the blood was rapidly equilibrated over an 8 min period to a constant P_{CO_2} (2 Torr) representative of resting levels. The HCO_3^- dehydration assay (3 min) was then performed, and Pa_{CO_2} determined at the end of the assay (closed circles, plotted 11 min after sampling). The arrows indicate the change in P_{CO_2} for each sample. The HCO_3^- dehydration data are plotted at the time when the assays were performed (8–11 min post-sampling). Means ± 1 SEM (n = 6–9 at each point). Asterisks indicate significant differences ($P \leq 0.05$) from the relevant pre-exercise control value (C).

The HCO_3^- dehydration rate of blood sampled from trout under resting conditions was about 22 $\mu mol \cdot ml^{-1} . h^{-1}$ (Fig. 1B). Exhaustive exercise caused a substantial increase in this rate, measured under in vivo acid-base conditions. The increase, while somewhat variable, averaged about 50% overall and was significant throughout the period from 2 min of exercise to 30 min of post-exercise recovery (Fig. 1B).

Series 2 In this series, measurements of blood acid-base status at the time of sampling indicated a decrease in pHa from about 7.88 at rest to 7.35 after only 2 min of severe exercise (Fig. 2A), and an accompanying large increase in Pa_{CO_2} from 2.8 to 9.4 Torr (Fig. 3A). This marked depression in pHa persisted through 6 min of exercise and over the subsequent recovery period. On the other hand, Pa_{CO_2} tended to fall with time after exercise (Fig. 3A) despite the constant pHa, and metabolic acidosis made a progressively larger contribution to the acid-base disturbance. Nevertheless, Pa_{CO_2} was still significantly elevated to about 4.8 Torr at 22 min of post-exercise recovery. Throughout this period, there was a significant mobilization of both adrenaline and noradrenaline into the bloodstream to about 35–40 $nmol \cdot L^{-1}$ for each catecholamine, approximately 10-fold resting levels (Fig. 2B).

The equilibration procedure was largely successful in standardizing all of the blood samples to the same P_{CO_2} (Fig. 3A). The mean P_{CO_2} determined at the end of the assays was 1.8 Torr, close to the resting level of Series 1, and the resting rate of HCO_3^- dehydration was about 24 $\mu mol \cdot ml^{-1} . h^{-1}$. Assays at this uniform P_{CO_2} revealed a substantial inhibition of HCO_3^- dehydration (Fig. 3B) throughout the post-exercise period of catecholamine mobilization and respiratory acidosis. The inhibition was greatest (about 35%) in samples assayed at 4–7 and 8–11 min post-exercise (samples drawn at 2 and 6 min of exercise), but the effect persisted (15–20%) through 30–33 min (samples drawn at 22 min). These results indicate that despite the *absolute* increase in HCO_3^- dehydration rates seen at in vivo P_{CO_2}'s (Fig. 1B), there was a *relative* inhibition once the stimulatory effect of elevated P_{CO_2} was removed. This inhibition occurred as a result of RBC processes, rather than events in the plasma (Table 1). There was no change in the HCO_3^- dehydration rate measured with plasma alone after exercise, despite a marked decrease in the rate for whole blood (Table 1).

Table 1

A comparison of HCO_3^- dehydration rates measured in whole blood and plasma samples drawn from resting trout and from exercised fish at time 0; in both cases the samples were equilibrated to the same P_{CO_2} = 2 Torr

		Rest (n = 7)	Exercised[a] (n = 6)
P_{CO_2} (Torr)		1.97 ± 0.13	2.03 ± 0.20
HCO_3^- Dehydration Rate	Whole Blood	24.1 ± 1.1	$17.8^* \pm 1.2$
($\mu mol \cdot ml \cdot blood^{-1} \cdot h^{-1}$)	Plasma	3.1 ± 0.3	3.6 ± 0.2

[a]Assayed at 8–11 min post-exercise.
Significantly different ($P \leq 0.05$) from rest value.

Table 2
Extracellular pHe, red cell intracellular pHi, the transmembrane pH gradient, and mean red cell haemoglobin concentration (MCHC) in the blood of trout at various times after severe exercise; values were measured after all samples had been equilibrated at the same $P_{CO_2} = 2$ Torr and processed through the HCO_3^- dehydration assay

Time post exercise[a]	pHe	RBC pHi	pHe–pHi	MCHC ($g \cdot ml^{-1}$)
Rest ($n = 7$)	8.028 ± 0.028	7.464 ± 0.015	0.564 ± 0.025	0.4479 ± 0.0320
7 min ($n = 7$)	7.726* ± 0.036	7.459 ± 0.024	0.267* ± 0.014	0.3820 ± 0.0100
11 min ($n = 8$)	7.601* ± 0.039	7.416 ± 0.034	0.185* ± 0.008	0.3696* ± 0.0174
18 min ($n = 7$)	7.670* ± 0.032	7.347* ± 0.015	0.323* ± 0.023	0.2955* ± 0.0061
33 min ($n = 7$)	7.447* ± 0.043	7.247* ± 0.018	0.200* ± 0.023	0.2512* ± 0.0073

[a] See Fig. 3A for actual times of sampling.
* significantly different from rest value ($P \leq 0.05$).

Data summarized in Table 2 suggest that the period of inhibited HCO_3^- dehydration coincided with a period of adrenergically mediated Na^+/H^+ exchange in the RBCs. In particular there was a substantial decrease in the transmembrane pH gradient (pHe – pHi) and a progressive fall in MCHC, indicative of RBC swelling. Note that these data were taken at end of the equilibration and assay periods at constant $P_{CO_2} \simeq 1.8$ Torr, so the effects seen cannot be attributed to the presence of elevated P_{CO_2}.

Table 3
A comparison of HCO_3^- dehydration rates and blood acid-base status between samples drawn from exercised fish at time 0 and assayed immediately and samples drawn from resting fish and equilibrated in vitro to simulate the acid-base status of exercised fish

	Exercised ($n = 8$)	Simulated ($n = 8$)
Pre-assay		
pHe	7.303 ± 0.027	7.382 ± 0.034
P_{CO_2} (Torr)	6.40 ± 0.53	6.74 ± 0.27
Post-assay		
pHe	7.445 ± 0.026	7.488 ± 0.024
P_{CO_2} (Torr)	4.20 ± 0.26	4.00 ± 0.16
$[HCO_3^-]$ ($mmol \cdot L^{-1}$)	4.74 ± 0.26	5.08 ± 0.17
pHi	7.287 ± 0.014	7.206* ± 0.018
pHe–pHi	0.158 ± 0.014	0.243* ± 0.012
HCO_3^- Dehydration rate ($\mu mol \cdot ml\ blood^{-1} \cdot h^{-1}$)	30.2 ± 2.2	72.4* ± 2.7

* Significantly different ($P \leq 0.05$) from exercised value.

Series 3 In order to confirm that this *relative* inhibition of HCO_3^- dehydration occurred at the P_{CO_2} levels observed in vivo after exhaustive exercise, and to assess its magnitude, blood drawn from resting trout was equilibrated in vitro to the P_{CO_2} seen at time 0 post-exercise. There were no significant differences in plasma acid-base status between this "simulated" sample and the real in vivo exercised sample either before or after the assay (Table 3). Note however that the exercised sample exhibited a lower pHe (not significant), a significantly higher RBC pHi, and therefore a significantly smaller trans-membrane pH gradient (pHe – pHi) relative to the simulated sample, all indicative of adrenergic activation of Na^+/H^+ exchange in the former. HCO_3^- dehydration rate (about 72 μmol·ml^{-1}.h^{-1}) was more than twice as high in the simulated sample as in the exercised sample (30 μmol·ml^{-1}.h^{-1}) at the post-exercise P_{CO_2}. Thus the true *relative* inhibition in vivo may be even greater than indicated by the data of Fig. 3B.

4. Discussion

The present results clearly demonstrate that a *relative* inhibition of HCO_3^- dehydration occurs in blood sampled from rainbow trout subjected to exhaustive exercise in vivo (Fig. 3B; Table 3). The phenomenon appears similar to that seen in acidotic blood in vitro when Na^+/H^+ exchange is activated by β_1-adrenergic stimulation (Wood and Perry, 1991; Perry et al., 1991; Wood and Simmons, 1994). For in vivo samples, the effect occurs during a period characterized by a large mobilization of plasma catecholamines (Fig. 2B), intense extracellular acidosis (Fig. 2A), and obvious evidence of Na^+/H^+ exchange activation in the RBCs as manifested in a decreased pHe – pHi gradient and RBC swelling (Table 2). Based on the in vitro dose-response curves presented by Perry et al. (1991), the measured plasma noradrenaline and adrenaline levels (both about 40 nmol·L^{-1}) occurring in vivo after exercise should have inhibited the HCO_3^- dehydration by about 25% when assessed at resting $P_{CO_2} \simeq 2$ Torr. The observed inhibition for in vivo samples assayed at this P_{CO_2} was 15–35% (Fig. 3B).

However Pa_{CO_2} in vivo was also greatly elevated during this period, and Wood and Perry (1991) demonstrated that the measured rate is very responsive to P_{CO_2}. This phenomenon explains the *absolute* ~50% increase in rates at in vivo Pa_{CO_2} after exercise (Fig. 1B) despite the *relative* inhibition. It also likely explains the larger increase measured in exhaustively exercised trout with the "boat" assay by Tufts et al. (1988), although these workers neither measured nor controlled P_{CO_2}. The overall increase of 50% appears quite reasonable relative to whole animal in vivo measurements which show that total $\dot{M}_{CO_2}$ increases by 100%–200% in salmonids (Steffensen et al., 1987; Milligan and McDonald, 1988; Scarabello et al., 1992) and cardiac output (i.e. gill blood flow) by ~55% (Neumann et al., 1983) during the first 30 min after exhaustive exercise. An increase in blood HCO_3^- dehydration of ~50% would be needed to explain these whole animal data.

The data of Table 3 suggest that the increases in HCO_3^- dehydration rates could have been over twice as high at elevated post-exercise P_{CO_2} had this *relative* inhibition not occurred. In this regard, it may be noted that the absolute rate in the resting samples simulated to post-exercise conditions by P_{CO_2} elevation was more than twice as high

as reported for trout blood samples at the same P_{CO_2} by Wood and Perry (1991). Much of this difference was likely due to the higher temperature (15 °C vs 10 °C), which also elevated control rates in the present study by about 70% relative to the data of Wood and Perry (1991).

In agreement with the original suggestion of Wood and Perry (1985), we conclude that an adrenergically mediated *relative* inhibition of HCO_3^- dehydration through the RBCs likely contributes to post-exercise P_{CO_2} elevation in the blood of salmonids. This build-up of P_{CO_2} will ensure that $\dot{M}_{CO_2}$ across the gills keeps pace with the elevated CO_2 production in the tissues, but the system is reset to higher blood P_{CO_2} levels. Wood and Perry (1985) suggested that the adaptive significance of the P_{CO_2} elevation may be to promote post-exercise hyperventilation, and Wood and Munger (1994) have presented experimental evidence in favour of this idea.

Nevertheless, several important *caveats* about the present conclusion must be noted. Firstly, as pointed out by Randall and Perry (1992) and Thomas and Perry (1992), it is unlikely that relative inhibition of HCO_3^- dehydration through the RBCs is the sole factor contributing to post-exercise elevation in blood P_{CO_2}. The direct production of CO_2 in the plasma due to the titration of extracellular HCO_3^- by H^+ ions extruded from acidotic muscle, and from adrenergically stimulated RBCs, is probably an additional contributing factor. Secondly, the assay conditions do not fully duplicate in vivo conditions, in terms of either time or oxygenation status. *In vivo*, blood is likely in contact with the gills for a few seconds at most which may introduce transit time limitations, effects which would not be seen in an assay run for 3 min. Furthermore, blood will enter the gills in the partially deoxygenated state and become oxygenated as plasma HCO_3^- is dehydrated through the RBCs and CO_2 is excreted. Recent studies have shown that both the starting state of partial deoxygenation (Wood and Simmons, 1994) and the availability of Bohr protons during oxygenation (Perry and Gilmour, 1993) will accelerate the HCO_3^- dehydration rate. While none of these effects should negate the *relative* inhibition caused by catecholamines, they would all tend to introduce quantitative discrepancies between in vivo rates and rates measured with the present assay.

To conclude, while some questions about magnitude remain, it is clear that catecholamine mobilization induced by exhaustive exercise causes a *relative* inhibition of HCO_3^- dehydration by trout RBCs and contributes to respiratory acidosis during the recovery period. The respiratory acidosis may play an adaptive role in driving post-exercise hyperventilation (Wood and Munger, 1994). As discussed by Perry et al. (1991) and Wood and Simmons (1994), the mechanism is linked to β_1-activation of Na^+/H^+ exchange and probably reflects an alteration in the electrochemical gradient for HCO_3^- entry on band 3 and/or CO_2 recycling from plasma to RBC (Motais et al., 1989). Direct effects of catecholamines or associated consequences of exercise (e.g. acidosis, lactate, dilution by RBC swelling) on carbonic anhydrase activity itself appear unlikely, but cannot be eliminated at the present time.

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