

THE PROMOTION OF CATECHOLAMINE RELEASE IN RAINBOW TROUT, *SALMO GAIRDNERI*, BY ACUTE ACIDOSIS: INTERACTIONS BETWEEN RED CELL pH AND HAEMOGLOBIN OXYGEN-CARRYING CAPACITY

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SUMMARY

A fall in blood pH was generated either by infusion of HCl or by reducing gill ventilation and raising blood P_{CO_2} in rainbow trout, *Salmo gairdneri* Richardson. The acute acidosis resulting from HCl infusion caused an increase in plasma adrenaline and noradrenaline concentrations, the adrenaline increase being proportional to the decrease in blood pH. Fish subjected to a prolonged respiratory acidosis, caused by a reduction in gill ventilation, showed no increase in catecholamines 24 h after the change in gill ventilation. We suggest that catecholamine levels increase in response to a pH decrease, but if acidotic conditions are maintained, circulating catecholamines return to low levels.

There was a much smaller decrease in erythrocytic pH with a fall in plasma pH when catecholamine levels were high. This ameliorating effect of catecholamines on erythrocytic pH during a plasma acidosis maintains the oxygen-carrying capacity of the haemoglobin. If erythrocytic pH was decreased by increasing blood P_{CO_2} *in vitro*, then there was a fall in haemoglobin oxygen-carrying capacity which was proportional to the reduction in pH.

We conclude that catecholamines are released into the blood in proportion to the fall in blood pH but if the pH is maintained the circulating catecholamines return to their initial low levels. The elevated catecholamine concentrations in blood safeguard against any impairment of haemoglobin oxygen-carrying capacity by maintaining erythrocytic pH in the face of a plasma acidosis.

INTRODUCTION

Strenuous exercise in rainbow trout leads to an extracellular acidosis of metabolic origin (Black, 1957; Holeyton, Neumann & Heisler, 1983; Turner, Wood & Clark, 1983). Recent measurements have shown that catecholamines are released into the

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circulation following periods of burst swimming and that these substances aid in regulating erythrocytic pH at a near constant level in the face of extracellular acidoses (Primmatt, Randall, Mazeaud & Boutilier, 1986). Though protons are normally passively distributed across the erythrocytic membrane of rainbow trout (Heming *et al.* 1986), the catecholamines liberated following a burst swim are thought to react with beta-receptors on the red cell membrane (Nikinmaa, 1982; Nikinmaa & Huestis, 1984; Primmatt *et al.* 1986) and prevent the acidotic conditions in the plasma being transferred into the erythrocyte. The observed adrenergic regulation of erythrocytic pH has vital consequences in these animals owing to the pH-dependent Bohr and Root effects, both of which would exacerbate the transport of blood oxygen during and following periods of metabolic acidosis. In the present study we set out to determine whether the catecholamine release is unique to the conditions associated with muscular activity or whether it is a more widespread response to conditions of extracellular acidosis.

MATERIALS AND METHODS

Animals and preparation

Rainbow trout (*Salmo gairdneri*), weighing 221.5–460 g, were obtained from a commercial hatchery and housed outdoors in large fibreglass tanks supplied with flowing dechlorinated Vancouver tapwater (pH 6.9–7.1; hardness, 4 p.p.m. CaCO₃; temperature, 8–11°C). Fish were fed *ad libitum* from automatic feeders containing dried trout pellets. In the laboratory, the fish were kept in blackened aquaria at 10°C and feeding was suspended 2 days prior to experimentation.

Following anaesthetization in a 1:10 000 solution of MS-222 (buffered to pH 7.5 with sodium bicarbonate) the dorsal aorta of each fish was chronically cannulated with PE 50 tubing (Smith & Bell, 1964). In addition, some animals were fitted with a latex rubber dam which was sewn behind the mouth and in front of the eyes and opercular openings (van Dam, 1938; Cameron & Davis, 1970). The rubber dam was secured to the divider of a two-chambered 'van Dam' apparatus which ensured that all water passing from the front chamber to the back chamber occurred by way of the mouth and gills of the fish. Throughout all surgical procedures, the gills of the fish were continuously irrigated with a lighter dose of the anaesthetic solution (1:15 000 MS-222). Following operations, fish were transferred to darkened Perspex boxes supplied with flowing aerated water (10°C) and artificially ventilated until they regained consciousness. Throughout the post-operative recovery period (48–60 h), the cannula of each fish was flushed twice a day with 0.1 ml of physiological saline (Wolf, 1963) containing 10 i.u. ml⁻¹ heparin.

Acid infusion experiments

Fourteen animals were infused through the dorsal aortic cannula with 5 ml kg⁻¹ body weight of a 0.05 mol l⁻¹ HCl solution made up in 120 mmol l⁻¹ physiological saline. Five fish treated in an identical fashion were infused with saline alone and these animals acted as controls. Infusion times ranged from 7 to 9 min. Arterial blood

samples (500 μ l) were taken before the infusion and at 5, 30, 60 and 120 min postinfusion. Portions of each blood sample were analysed for plasma pH (pHe), total oxygen content and haemoglobin concentration; the remainder of the blood sample was centrifuged anaerobically. The resulting plasma was taken up into a chilled syringe and transferred to an Eppendorf vial for storage at -40°C (subsequently analysed for catecholamines). The red cell fractions were twice frozen and thawed prior to pH measurement of the slurry (Zeidler & Kim, 1977).

van Dam experiments

Changes in gill ventilation were brought about by adjusting the water height in the inspired water chamber of nine animals fitted with rubber dams. Each fish was subjected to positive, negative and zero pressure heads separated by 24-h intervals. Blood samples taken after steady-state ventilatory responses to changes in the pressure head had occurred (24 h) were analysed for pHe and P_{CO_2} . In five animals, larger blood samples were withdrawn so that measurements of red cell pH and plasma catecholamines could also be performed.

Root effect determination

Blood samples were drawn from quiescent fish which had been chronically cannulated in the dorsal aorta and allowed to recover as before. The blood samples were immediately pooled, transferred to an intermittently rotating tonometer, and equilibrated against humidified gas mixtures containing either 0.2 or 1.0% CO_2 in air (Wösthoff pumps). After 30–40 min of tonometry at 10°C , blood was taken up into a positive displacement gas-tight syringe (Hamilton) and measurements were made of blood pH, red cell pH, haemoglobin concentration and oxygen content. Adrenaline and noradrenaline concentrations were measured in the plasma of a 1-ml sample of the blood pool taken during the initial stages of the equilibration procedure.

Analytical procedures

Measurements of whole blood pH and red cell lysate were made using a Radiometer G279/G2 glass capillary electrode and K497 calomel electrode coupled with a PHM 84 pH meter. The electrodes were calibrated with Radiometer precision buffer solutions S1500, S1510 and S1336, each sample being referenced to the S1500 buffer (Boutilier, Iwama, Heming & Randall, 1985). Carbon dioxide partial pressures were measured with Radiometer electrodes and meters according to the recommendations of Boutilier, Randall, Shelton & Toews (1978) and Boutilier *et al.* (1985). Haemoglobin concentrations were determined spectrophotometrically (Sigma bulletin no. 525), blood oxygen contents were measured by the Lex- O_2 -Con apparatus (Lexington Instruments, MA). Plasma adrenaline and noradrenaline levels were measured using high pressure liquid chromatography (HPLC) as described by Woodward (1982).

Statistical significance between mean data sets was determined by Student's *t*-test (paired or unpaired as appropriate) with a fiducial level of probability of 5 %.

RESULTS

Two series of experiments were carried out in attempts to find whether the release and subsequent action of catecholamines on red cell pH observed during a burst swim (Primmitt *et al.* 1986) could be brought about by an extracellular acidosis produced in the absence of strenuous muscular activity. The object of the 'acid infusion' series of experiments was to subject the animals to an acute extracellular acidosis of the magnitude experienced following a period of burst swimming (Primmitt *et al.* 1986). In the second 'van Dam' series, the plasma pH (pHe) of rainbow trout was experimentally manipulated *in vivo* by altering the water flow over the gills and therefore the CO₂ washout. In these latter experiments, we were concerned with changing the pHe to new steady-state levels so as to compare the development of an acidosis over a prolonged time course with that of the acute situation (i.e. acid infusion) characteristic of a burst swim.

Acute acidoses through acid infusion

Five minutes following an intra-arterial infusion of HCl in rainbow trout, plasma pH fell 0.167 units below that of the pre-infusion controls (Fig. 1). At the same time, plasma adrenaline concentrations increased by sixfold, whereas plasma noradrenaline exhibited a slight but significant rise. A small, but significant, decline in red cell pH also occurred at the 5-min postinfusion period: however, in four out of 14 instances, red cell pH actually increased despite a fall in extracellular pH. There was no significant change in the oxygen content per gram of haemoglobin (relative to control animals) throughout the postinfusion period (Fig. 1). During the 2-h period following the infusion, plasma pH and catecholamine levels gradually returned towards their pre-infusion levels. Red cell pH and haemoglobin oxygen-carrying capacity were, on the other hand, less affected by the acid-infusion procedure and varied little (Fig. 1). Five animals infused with similar volumes of physiological saline showed no significant postinfusion changes in any of the measured variables relative to either their own pre-infusion values or those of the experimental animals (Table 1; Fig. 1).

Rainbow trout blood does exhibit a Root shift, for if blood *in vitro* is subjected to various CO₂ levels such that red cell pH is reduced, then the amount of oxygen bound to haemoglobin decreases (Fig. 2). We assume that similar changes in haemoglobin oxygen-carriage would have occurred if the changes in red cell pH had been brought about by addition of HCl. The plasma catecholamine concentrations in the blood pool used to examine the Root effect (adrenaline, 0.98 nmol l⁻¹; noradrenaline, 1.01 nmol l⁻¹) were similar to those from control fish *in vivo* (Fig. 1; Table 1).

Comparison of *in vivo* and *in vitro* relationships between plasma pH and red cell pH are made throughout the present paper. The *in vitro* data are the subject of a

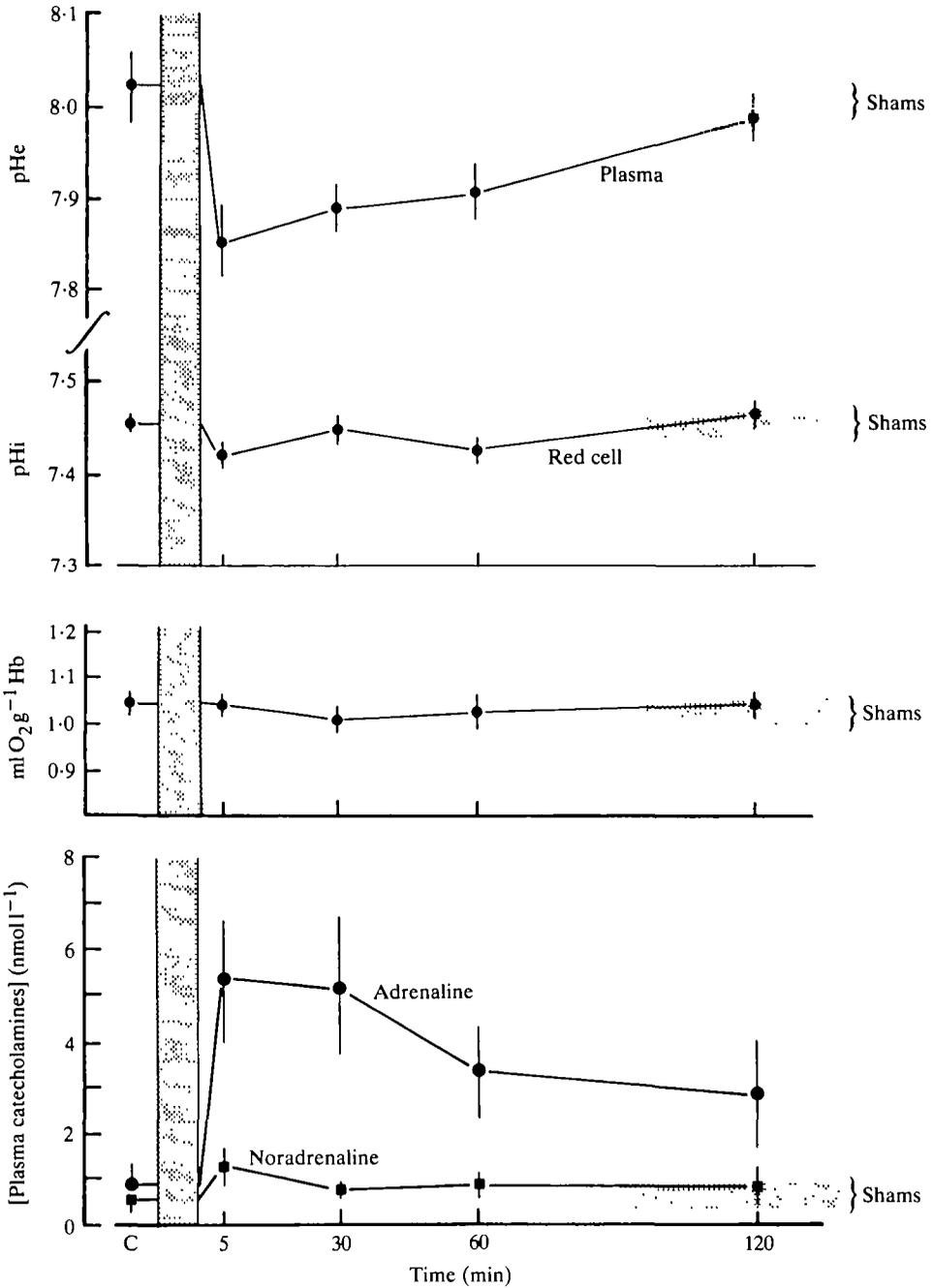


Fig. 1. Arterial plasma pH (pHe), red cell pH (pHi), ml O₂ bound per gram of haemoglobin (ml O₂ g⁻¹ Hb) and plasma catecholamine concentrations following intra-arterial infusion of a 5 ml kg⁻¹ body weight 0.05 mol l⁻¹ HCl solution of 120 mmol l⁻¹ saline in 14 rainbow trout (shaded vertical bars represent the infusion period). Shaded horizontal bars (labelled 'shams') represent ±1 standard error of the combined mean data of five control experiments (Table 1) designed to examine the influence of the saline vehicle alone (i.e. 5 ml kg⁻¹ of 120 mmol l⁻¹ saline injections). The shading is representative of ±1 S.E.M. for both adrenaline and noradrenaline levels in the bottom panel. C = pre-infusion control. All data are means ± 1 S.E.M. (N = 14). Temperature = 10°C throughout.

separate study (Heming *et al.* 1986) in which blood was acidified during tonometry, either by addition of HCl or by varying the CO₂ content of the equilibration gases. Blood for the latter experiments was obtained from indwelling dorsal aorta cannulae

Table 1. Arterial plasma pH (pHe), red cell pH (pHi), ml O₂ bound per gram of haemoglobin (ml O₂g⁻¹ Hb), adrenaline concentration ([A]) and noradrenaline concentration ([NA]) before and following intra-arterial infusion of a 5 ml kg⁻¹ 120 mmol l⁻¹ saline solution in five animals

	Pre-infusion		Postinfusion		
	Control	+5 min	+30 min	+60 min	+120 min
pHe	7.948 (±0.034)	7.963 (±0.033)	7.954 (±0.028)	7.947 (±0.026)	7.947 (±0.030)
pHi	7.413 (±0.027)	7.418 (±0.032)	7.400 (±0.018)	7.382 (±0.019)	7.415 (±0.029)
ml O ₂ g ⁻¹ Hb	1.04 (±0.03)	1.04 (±0.03)	1.03 (±0.04)	1.03 (±0.05)	1.04 (±0.04)
[A] (nmol l ⁻¹)	0.31 (±0.11)	0.45 (±0.12)	0.34 (±0.11)	0.76 (±0.36)	0.74 (±0.17)
[NA] (nmol l ⁻¹)	0.27 (±0.04)	0.23 (±0.05)	0.22 (±0.03)	0.17 (±0.03)	0.23 (±0.03)

These animals are the controls (i.e. sham injections) for the acid-infusion experiments in Fig. 1. All data are means ± 1 s.e.m. Temperature = 10°C.

At all stages of postinfusion, the mean values are not significantly different from those of pre-infusion controls (Student's paired *t*-test).

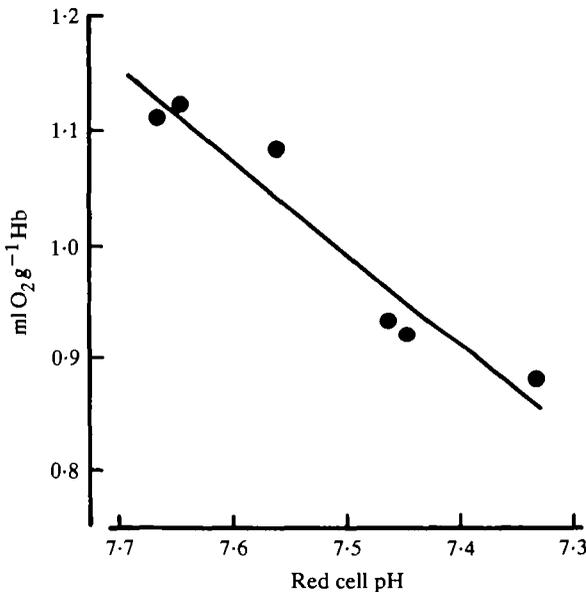


Fig. 2. Relationship between oxygen capacity per gram of haemoglobin and red blood cell pH in rainbow trout blood equilibrated *in vitro* (10°C) with gas mixtures having an oxygen partial pressure of 152 Torr and a carbon dioxide partial pressure of 2.5 Torr. Oxygen bound to haemoglobin was determined by subtracting the dissolved fraction of oxygen in plasma which would be present at 152 Torr P_{O₂} (Boutilier, Heming & Iwama, 1984) from the measurements of oxygen-carrying capacity of whole blood. Data are individual measurements on a single blood pool from three animals. Haemoglobin concentration of the blood pool = 8.4 g Hb 100 ml⁻¹. The line of best fit was generated by linear regression analysis where ml O₂g⁻¹ Hb = -5.005 + 0.800pHi (correlation coefficient, *r* = 0.94).

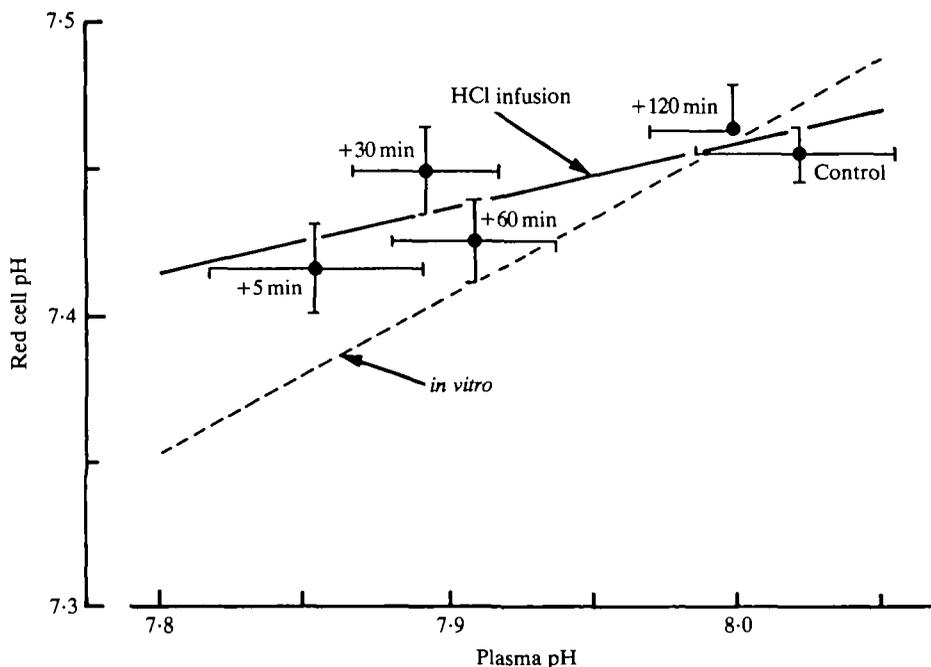


Fig. 3. Relationship between red blood cell pH and plasma pH for animals infused with HCl-containing saline ($N = 14$; mean data as in Fig. 1 at 10°C). Dashed line labelled '*in vitro*' represents that which was observed when HCl was added to rainbow trout blood equilibrated *in vitro* at 10°C (Heming *et al.* 1986). Linear regression analyses of all data points were used to generate the lines (HCl infusion: $\text{pHi} = 5.610 + 0.231\text{pHe}$, $r = 0.79$, $N = 70$; *in vitro*: $\text{pHi} = 3.219 + 0.531\text{pHe}$, $r = 0.95$, $N = 31$). A least squares linear regression program (Hewlett-Packard 41C) which computes and compares the slopes and y-intercepts for two sets of data with a *t*-test showed that the lines were significantly different at $P < 0.05$.

of resting trout, whose levels of endogenous catecholamines were consistently low (Heming *et al.* 1986).

The *in vivo* relationship between red cell pH (pHi) and plasma pH (pHe) for the HCl-infused fish is shown in Fig. 3 and is a replot of the data illustrated in Fig. 1. The '*in vitro*' dashed line (Fig. 3) represents the relationship between pHi and pHe for trout blood at 10°C in which pHe was adjusted using HCl *in vitro* (Heming *et al.* 1986); the relationship is described by the regression equation $\text{pHi} = 3.219 + 0.531\text{pHe}$. The regression equation for the relationship *in vivo* is $\text{pHi} = 5.610 + 0.231\text{pHe}$ (Fig. 3). Statistical comparison of these regression equations by *t*-test (Kleinbaum & Kupper, 1978) showed that the slopes and intercepts are significantly different at the 5% level. This shows that the change in pHi with pHe *in vivo* is less than would be expected to occur on the basis of the data collected *in vitro*. Though the intersection of the two lines should occur in the region of the control fish as observed (Fig. 3) there can be little doubt that there is significant departure of the two lines as pHe declines. The five sham-injected fish (Table 1) showed no change in pHe following saline infusion, but individual fish varied in their steady-state level of pHe. A plot of the mean values of all of the data

from each of the sham-injected fish reveals a relationship between pHe and pH_i which is not significantly different from that predicted by Heming *et al.* (1986) for blood *in vitro*. The control values for pHe and pH_i *in vivo* (Fig. 1) and the data from sham-injected fish (Table 1) correspond with low concentrations of plasma catecholamines, as do the *in vitro* data of Heming *et al.* (1986). Following HCl infusion *in vivo*, however, the comparatively higher pH_i values at any given pHe are associated with elevated levels of circulating catecholamines.

The difference between pre-infusion and 5 min postinfusion values for pHe and adrenaline concentration are shown in Fig. 4 for the 14 animals contributing to the mean data in Fig. 1. Fig. 4 shows that the magnitude of the adrenaline concentration increase is proportional to the magnitude of the decrease in pHe following acid

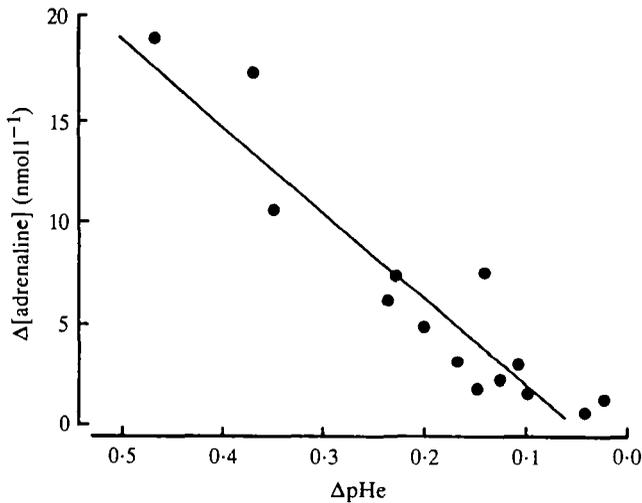


Fig. 4. Relationship showing the change in plasma adrenaline concentrations (Δ [adrenaline]) as a function of the change in plasma pH (Δ pHe) between pre-infusion control samples and +5 min postinfusion samples for each of the 14 animals contributing to the mean data in Fig. 1. Linear regression analyses of the data points were used to generate the line, Δ [adrenaline] = $42.46 - 2.28\Delta$ pH, correlation coefficient, $r = 0.92$.

Table 2. Gill ventilation, arterial plasma P_{CO_2} and pH of nine rainbow trout experiencing various head pressures in a van Dam apparatus

Range of head pressures (mm)	\dot{V}_g (ml min ⁻¹ kg ⁻¹)	P_{aCO_2} (Torr)	pH _a		
-7 to -1	191.3 ± 23.8	4.3 ± 0.2	7.745 ± 0.017	} NS	} S
0	269.2 ± 53.2	3.9 ± 0.2	7.767 ± 0.028		
+5 to +20	393.7 ± 58.3	3.4 ± 0.1	7.826 ± 0.015	} NS	

NS = not significantly different; S = significantly different at $P < 0.05$, Student's *t*-test.

Blood measurements were made 24 h after adjusting the water height in the inspired water chamber, when animals were in a steady state with respect to their ventilation.

Values are means ± 1 S.E.M. of 9-12 measurements. Temperature = 10°C.

infusion; i.e. the greater the initial fall in pHe the greater the increase in adrenaline concentration.

Steady-state acidoses through changes in ventilation

A reduction in gill ventilation in nine rainbow trout, to steady-state levels below $250 \text{ ml min}^{-1} \text{ kg}^{-1}$, led to an increase in arterial blood P_{CO_2} and to a decline in plasma pH (Table 2). Data for plasma catecholamine concentrations as a function of changes in pHe (Table 3), were not as extensive as in the acid infusion series (Figs 1, 4) and measurements of haemoglobin oxygen-carrying capacity were unfortunately not made in the ventilation series. The available data do show, however, that catecholamine concentrations were at the same level both before and 24 h after the reduction in gill ventilation (and therefore pHe) to a new steady state (Tables 2, 3). The values for both adrenaline and noradrenaline were significantly higher than the resting values for our pre-infusion animals in the first experimental series (Fig. 1; Table 1) and may reflect a slightly higher level of stress (Mazeaud & Mazeaud, 1981) associated with the restrictive nature of the van Dam procedure. Nevertheless, pHe was experimentally manipulated, through changes in ventilation, over a broad pH range with no corresponding increase in plasma catecholamines taking place (Table 3). Very similar pHe changes when induced through acid infusion, on the other hand, led to marked increases in adrenaline as shown in Figs 1 and 4. We cannot eliminate the possibility that catecholamines may have increased and decreased in a transient fashion during the 24-h period in which ventilation and pHe were brought to a new steady-state.

The slope of the pHe to pH_i relationship observed in the five artificially ventilated fish for which catecholamine measurements were made ($\text{dpH}_i/\text{dpH}_e = 0.549$ at 10°C) was not significantly different from that observed by Heming *et al.* (1986) when trout blood was equilibrated against various levels of CO_2 *in vitro* ($\text{dpH}_i/\text{dpH}_e = 0.595$ at 10°C ; Fig. 5). The difference between this observation and that of the acid-infused fish (Fig. 3) is that the changes in pHe were brought about by prolonged steady-state adjustment to a ventilatory-induced respiratory acidosis (Figs 5, 6) as opposed to an acute metabolic acidosis (Fig. 1).

DISCUSSION

Acute extracellular acidoses, whether of endogenous or exogenous origin, promote the release of catecholamines in rainbow trout (Nakano & Tomlinson, 1967; Primmatt *et al.* 1986; Figs 1, 4). In both instances, the potentially detrimental effects that an acidosis might bring to blood oxygen transport through Bohr and Root shifts appear to be offset by an adrenergically mediated regulation of the pH environment of the haemoglobin (Nikinmaa, 1983; Fig. 1). The decline in red cell pH following acid infusion (7.454 to 7.416) was less than would be expected on the basis of a passive transmembrane distribution of protons (Fig. 3) and had no measureable effect on haemoglobin oxygen-carrying capacity (Fig. 1) as one

might predict from the relationships in Fig. 2. While we have no evidence for the mechanisms involved in the regulation of red cell pH, there can be little doubt that the magnitude of the catecholamine increase is related to the severity of the acidosis (Fig. 4) so that its beneficial effects on oxygen transport can be realized both in the acute condition as well as throughout protracted periods of recovery (Fig. 1). Responses such as these could be of vital importance during situations when extracellular pH becomes lowered due to environmental challenges such as aquatic hypercapnia and/or acidity.

In experiments in which rainbow trout were exposed to water of pH 4.0 for prolonged periods of time, there was no detectable increase in plasma catecholamine concentrations (measured after 24 h), despite there being a considerable arterial

Table 3. Gill ventilation, arterial plasma pH (pHe), adrenaline concentration ([A]) and noradrenaline concentration ([NA]) of five rainbow trout experiencing various head pressures in a van Dam apparatus (as in Table 2)

Range of head pressures (mm)	\dot{V}_g (ml min ⁻¹ kg ⁻¹)	pHe	plasma catecholamines (nmol l ⁻¹)	
			[A]	[NA]
-8 to -2	215.5 ± 52.8	7.685 ± 0.048	2.18 ± 0.64	2.52 ± 0.71
0 to +11	376.5 ± 48.8	7.856 ± 0.076	2.97 ± 0.47	2.38 ± 0.31

Values are means ± 1 S.E.M. of 5-6 measurements. Temperature = 10°C.

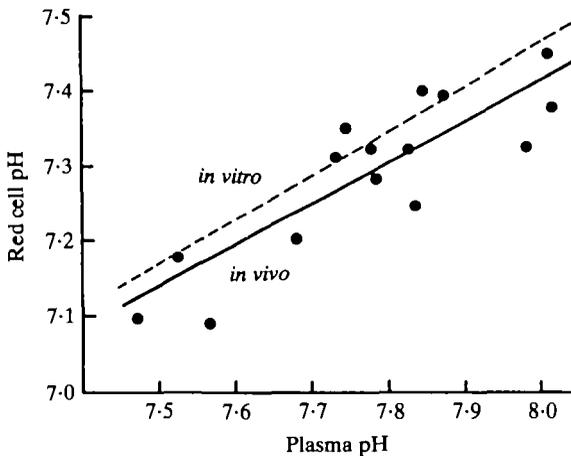


Fig. 5. Relationship between red blood cell pH and plasma pH in experiments in which blood pHe was manipulated *in vivo* by altering water flow over the gills of the five animals shown in Table 3. The dashed line labelled '*in vitro*' represents that which was observed when rainbow trout blood was equilibrated against various concentrations of CO₂ *in vitro* at 10°C (Heming *et al.* 1986). Linear regression analyses of all data points were used to generate the lines (*in vivo*: pHi = 3.023 + 0.550pHe, $r = 0.83$, $N = 15$; *in vitro*: pHi = 2.708 + 0.595pHe, $r = 0.95$, $N = 27$). A least squares linear regression program (Hewlett-Packard 41C) which computes the slopes and y-intercepts for two sets of data with a *t*-test showed that the lines were not significantly different at $P > 0.05$.

blood acidosis (X. He & D. J. Randall, unpublished observations). It seems likely that catecholamines increase in response to the rate of decrease of pHe (i.e. Fig. 4) rather than to the absolute levels of pHe that can be maintained in the steady state. This would explain why the artificially ventilated fish (Tables 2, 3), and those exposed to acid waters, had low levels of catecholamines, even though in some cases the change in steady-state blood pHe was similar in magnitude to that seen in fish following acid infusion (i.e. Figs 1, 5). Evidently, there can be changes in the absolute levels of pHe without any maintained changes in plasma catecholamine concentrations. However, given that catecholamine release into and disappearance from the plasma is rather labile (Mazeaud & Mazeaud, 1981; Fig. 1), it is possible that transient responses (i.e. Fig. 4) will occur during the initial stages of an acidotic challenge so as to provide a safeguard against an impairment of haemoglobin oxygen-carrying capacity. If so, measurements such as the ones made after a 24 h ventilatory adjustment to a lower pHe (Tables 2, 3) could mean that if the initial acidotic conditions are subsequently maintained, blood catecholamine levels will eventually decrease. Whether the higher catecholamine levels measured in artificially ventilated fish (Table 3), relative to unrestricted control animals (Fig. 1; Table 1), can be viewed as 'maintenance levels' to a prolonged acidotic condition, or are simply a measure of the added stress of the van Dam procedure, seems well worth investigating in the future.

The acid loads administered to the animals in the present experiments are certainly far less severe than these fish are capable of tolerating. Infusions of lactic acid into the bloodstream of rainbow trout ($1360 \mu\text{mol kg}^{-1}$; approximately the maximum tolerable dose) caused the mean pHe to decline by 0.5 pH units (Kobayashi & Wood, 1980), this being equal to the values observed when animals were prodded to exhaustion (Turner *et al.* 1983; Holeton *et al.* 1983). When trout were swum to exhaustion at 120% of their critical swimming velocities, the mean decline in pHe was approximately 0.3 units, at which time adrenaline and noradrenaline levels increased by 35- and 25-fold respectively (Primmatt *et al.* 1986). The acidosis caused by HCl infusion in the present experiments (Fig. 1) is approximately half of that observed by Primmatt *et al.* (1986) and correspondingly lower adrenaline levels were observed (as might be expected from the relationship shown in Fig. 4). Both Primmatt *et al.* (1986) and Holeton *et al.* (1983) found that the postexercise acidosis did not alter the blood oxygen-carrying capacity despite the fact that these animals exhibit a Root effect (Fig. 2). Indeed, the 0.5 pHe unit decline reported by Holeton *et al.* could, according to our data in Figs 2 and 3, have accounted for a 2.5 vol% fall in blood oxygen-carrying capacity. Certainly, our measurements suggest that the magnitude of the catecholamine release is matched to the level of acidosis so as to offset the potentially detrimental effects that a Root effect might bring to subsequent aerobic performance (Figs 3, 4). It also seems apparent from our data that the time course of the catecholamine response is linked to the time course of the acid-base disturbance following periods of acute acidosis (Fig. 1). It would appear that the linkage of both the magnitude and time course of the catecholamine response to changes in pHe has evolved to maintain oxygen transport following acidosis induced

by burst exercise. The way in which this is accomplished appears to reside with a β -adrenergic-mediated regulation of erythrocytic pH, the net effect of which is to protect the pH integrity of the cytosol and thereby prevent the offloading of oxygen from haemoglobin (Nikinmaa, 1983; Primmitt *et al.* 1986; Figs 1, 2).

In experiments in which the blood pHe was experimentally manipulated *in vivo* by altering the water flow over the gills and therefore the carbon dioxide wash-out (Table 2), we were unable to detect any increases in plasma catecholamines (Table 3). Despite the fact that several of these animals experienced pHe changes of the order of 0.3–0.4 pH units (i.e. similar to the acute changes seen in the present experiments, Fig. 1), there appeared to be no adrenergic regulation of erythrocytic pH in the long term (Fig. 5). More detailed measurements of transient catecholamine responses to respiratory acidosis, and corresponding influences on haemoglobin oxygen-carrying capacity, will be required in order to substantiate these claims.

The present body of information suggests that catecholamine release may be reserved for those instances when abrupt changes in extracellular pH take place (i.e. stress due to handling, burst exercise or acid infusion; Nakano & Tomlinson, 1967; Mazeaud & Mazeaud, 1981; Primmitt *et al.* 1986; Fig. 1). We suspect that any significant short-term transient in pHe will promote the release of catecholamines as indicated in Fig. 4, but that longer term adjustments to new levels of pHe in the steady state are achieved through alternative means. The evolution of an emergency mechanism which allows these animals to maintain oxygen transport in the face of an acute acidosis means that subsequent levels of aerobic performance will not be impaired. Indeed, recent measurements of critical velocity in coho salmon have shown that these animals can maintain the same levels of aerobic performance both before and after a period of burst swimming (D. J. Randall, D. Mense & R. G. Boutilier, unpublished observations).

Various nucleotide triphosphate compounds are known to affect haemoglobin oxygen affinity in fishes (Weber, 1982) and can be considered as long-term modulators of oxygen-binding characteristics (Randall & Daxboeck, 1984). Little is known, however, about the modulation of blood oxygen transport by such compounds during acute periods of respiratory and/or metabolic acidoses in fishes. Certainly, catecholamines appear to be responsible, at least in the short term, for preserving both haemoglobin oxygen affinity and oxygen-carrying capacity (Nikinmaa, 1983; Figs 1, 2). Longer term solutions to a continuing acidosis may, however, involve the activation of other acclimatory processes which supplant the actions of catecholamines.

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