THE EFFECTS OF PROLONGED EPINEPHRINE INFUSION ON THE PHYSIOLOGY OF THE RAINBOW TROUT,
SALMO GAIRDNERI

I. BLOOD RESPIRATORY, ACID–BASE AND IONIC STATES

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SUMMARY

Rainbow trout were infused continuously for 24 h with epinephrine in order to elevate circulating levels of this hormone to those measured during periods of acute extracellular acidosis (approximately $5 \times 10^{-8} \text{mol}^{-1}$). Concomitant effects on selected blood respiratory, acid–base and ionic variables were evaluated. Infusion of epinephrine caused a transient respiratory acidosis as a result of hypoventilation and/or inhibition of red blood cell (RBC) bicarbonate dehydration. The acidosis was regulated by gradual accumulation of plasma bicarbonate. Even though whole blood pH ($pH_c$) was depressed by 0.16 units, RBC pH ($pH_r$) remained constant, thereby causing the transmembrane pH gradient ($pH_e - pH_r$) to decrease. A similar effect of epinephrine on RBC pH was observed in vitro, although the response required a higher concentration of epinephrine ($2 \times 10^{-7} \text{mol}^{-1}$). We speculate that the release of epinephrine during periods of depressed blood pH is important for preventing excessive shifts in RBC pH and for initiating a series of responses leading to plasma $\text{HCO}_3^-$ accumulation and eventual restoration of blood acid–base status.

INTRODUCTION

The circulating levels of catecholamines, particularly epinephrine, have been shown to increase during periods of stress in fishes (see review by Mazeaud & Mazeaud, 1981). This observation has prompted numerous investigations on the various physiological effects of catecholamines in a variety of fish species. From these studies it is apparent that catecholamines are important modulators of cardiovascular function (see reviews by Nilsson, 1984a,b) as well as branchial gas transfer and blood gas transport (Peyraud-Waitzenegger, 1979; Pettersson, 1983; Perry, Daxboeck & Dobson, 1985; see also reviews by Randall & Daxboeck, 1984; Wood & Perry, 1985). Thus, the release of catecholamines into the circulation during periods of stress is thought to promote a complex series of physiological responses leading to an augmentation of oxygen uptake and delivery to the tissues. Such responses are important because stress-related states are normally associated with a requirement to

Key words: Salmo gairdneri, blood, epinephrine, catecholamines, acid–base, erythrocyte.
enhance the efficiency of O₂ transfer and delivery (e.g. during environmental hypoxia or exhaustive exercise).

Another common feature of stress in fishes is extracellular acidosis. Recently, it has been shown that plasma epinephrine levels rise during periods of reduced blood pH, induced either by intravascular acid infusion (Boutilier, Iwama & Randall, 1986), external hypercapnia (Perry, 1986), exhaustive exercise (Primmett, Randall, Mazeaud & Boutilier, 1986) or acute hypoxia (Fievet, Motais & Thomas, 1986). However, little is known about the involvement of catecholamines in acid–base regulation, although epinephrine appears to play a crucial role in stabilizing red blood cell (RBC) pH during disturbances of plasma pH (Nikinmaa, 1982a).

In these three papers, we examine the hypothesis that the mobilization of epinephrine during periods of acute extracellular acidosis performs an important role in (1) preventing pH-related disturbances of blood O₂ transport by stabilizing RBC pH and (2) restoring acid–base balance by interacting with branchial and renal acid–base regulatory mechanisms. The experimental protocol involves simulating the circulating levels of epinephrine measured during acid–base disturbances by continuous intra-arterial infusion. This paper describes the effects of epinephrine on blood respiratory, acid–base and ionic variables. The following two papers (Vermette & Perry, 1986a,b) present data on branchial and renal function.

**Materials and Methods**

**Experimental animals**

Rainbow trout (*Salmo gairdneri*) of either sex weighing between 171 and 442 g [mean mass = 272.4 ± 11.4 g (s.e.); N = 42] were obtained from Thistle Springs Trout Farm (Ashton, Ontario) and transported to the University of Ottawa. Fish were held indoors in large rectangular fibreglass tanks (Living Stream, Toledo, Ohio) supplied with flowing, aerated and dechlorinated Ottawa City tapwater ([Na⁺] = 0.10 mmol l⁻¹; [Cl⁻] = 0.10 mmol l⁻¹; [Ca²⁺] = 0.35 mmol l⁻¹; [K⁺] = 0.03 mmol l⁻¹; pH 7.0–8.0). The temperature of the holding and experimental water varied between 8 and 14°C during the 4-month span of the experiments (June–September, 1985). Fish were fed a daily diet of dried commercial trout pellets (Purina Trout Chow) but were not fed for 48 h before experimentation.

**Animal preparation**

Fish were anaesthetized in a 1:10000 (w/v) solution of ethyl m-aminobenzoate (MS 222, Sigma) buffered to approximately pH 7.0 with NaHCO₃, and placed onto an operating table (Smith & Bell, 1967) that permitted continuous irrigation of the gills with the same solution. To permit blood withdrawal and epinephrine infusion, indwelling cannulae were implanted into the dorsal aorta (Smith & Bell, 1964) using flexible polyethylene tubing (Clay Adams PE 50, i.d. = 0.58 mm, o.d. = 0.965 mm). The trout were also fitted with urinary catheters (Wood & Randall, 1973) by inserting a heat-flared flexible polyethylene tube (Clay Adams PE 60, i.d. = 0.76 mm, o.d. = 1.22 mm) through the urinary papilla and advancing it so that the catheter tip
was located within the urinary bladder. The catheter was secured by a ligature around the papilla and was sutured to the anal fin.

To determine plasma catecholamine levels during dorsal aortic epinephrine infusion, a second cannula was implanted in the caudal artery/vein of four fish according to the method of Wood, McMahon & McDonald (1977), using PE50 tubing. Urinary bladder catheterizations were not performed on these fish.

Following surgery, fish were placed into opaque Perspex boxes (volume = 3 litres) supplied with flowing water and allowed to recover for at least 48 h prior to experimentation. The urinary catheters drained continuously into plastic vials that were secured outside the fish boxes approximately 7 cm below water level. Dorsal aortic and caudal artery/vein cannulae were flushed at least once daily with approximately 0·3 ml of freshwater teleost saline solution (modified from Payan & Matty, 1975) containing (in mmol l⁻¹) NaCl, 113; NaHCO₃, 13; KCl, 4·2; CaCl₂, 1·3; MgSO₄, 1·2; Na₂HPO₄, 1·0; KH₂PO₄, 0·4; (NH₄)₂SO₄, 0·1; and 10 units ml⁻¹ heparin (ammonium salt, Sigma).

In vivo protocol

Initially, fish were infused via the dorsal aorta cannulae with saline (further modified so that [HCO₃⁻] = 4 mmol l⁻¹, pH 7·8–8·0) for a 4-h period using syringe pumps (Sage Model 352) at a flow rate of 0·6 ml h⁻¹. This 4-h preliminary period was considered essential to re-establish steady-state conditions with respect to urine flow rate (UFR) and other sensitive variables. Fish then were subjected to a further 3 h of saline infusion at the same rate, followed by 24 h of L-epinephrine (experimental group) or saline (control group) infusion, and finally an additional 12-h interval of saline infusion (experiments and controls). L-Epinephrine (bitartrate salt, Sigma) was added to saline immediately prior to infusion to yield a final concentration of 2×10⁻⁵ mol l⁻¹ (pH 7·8–8·0). Based on approximations of internal epinephrine distribution volume (assumed to be equivalent to extracellular fluid volume; about 300 ml kg⁻¹ body mass), biological half-life [15 min; from unpublished personal observations of blood pressure changes in trout following bolus injections of epinephrine (S. F. Perry)] and an infusion rate of 0·6 ml h⁻¹, plasma epinephrine levels were estimated to be 5·0×10⁻⁸ mol l⁻¹ throughout the experimental infusion period. These estimated levels were confirmed after 4 h of infusion (see below) by high-pressure liquid chromatography (HPLC) measurements. In order to impede the oxidation of epinephrine during infusion, syringes and cannula tubing were blackened to prevent light penetration.

Urine was collected continuously over 3-, 6- or 9-h intervals and analysed to provide measurements of UFR, pH, total CO₂ (C₄O₂), [Na⁺], [Cl⁻], [K⁺], [Ca²⁺], inorganic phosphate [Pi], [NH₄⁺], flux rates (excretion) of these solutes and net acid excretion [see Vermette & Perry (1986b) for details]. Water samples were removed at appropriate times for determination of branchial solute exchanges [see Vermette & Perry (1986a) for details]. In this study, 0·7-ml blood samples were withdrawn from the dorsal aortic cannula midway through the first 3-h saline infusion period, then at 1·5 h, 4·5 h, 10·5 h and 21·5 h of the 24-h epinephrine or saline infusion period, and
finally at 1.5 h, 4.5 h and 10.5 h of the final 12-h terminal saline infusion interval. Measurements were made of haematocrit (Hct), \( \text{CO}_2 \), oxygen partial pressure (\( \text{PO}_2 \)), whole blood pH (\( \text{pH}_w \)) and RBC pH (\( \text{pH}_r \)) as described below. The remaining blood was centrifuged and the plasma removed; 10% (v/v) of the anti-oxidant sodium bisulphite (5 mmol l\(^{-1}\)) was added to the plasma samples destined for catecholamine analysis. Samples were then quickly frozen in liquid nitrogen and stored at \(-70^\circ\text{C}\) for subsequent ion and/or catecholamine determinations (see below). Remaining blood cells (approximately 0.2 ml) were resuspended in saline and reinjected into fish \textit{via} the dorsal aortic cannulae.

**In vitro protocol**

Approximately 3 ml of blood was withdrawn slowly (1 ml min\(^{-1}\)) from the dorsal aortic cannula of fish that had been allowed to recover from surgery for at least 48 h. Blood from each fish (\( N = 12 \)) was added to individual 50-ml tonometer flasks containing ammonium heparin (10 units ml\(^{-1}\)) that were immersed in a constant-temperature water bath (10°C). Flasks were shaken continuously and gassed for approximately 1 h with 0.3% \( \text{CO}_2 \) in \( \text{O}_2 \) (\( \text{P}_{\text{CO}_2} = 2.3 \text{Torr} \)) before the experiments commenced. Experiments consisted of monitoring \( \text{pH}_w \), \( \text{pH}_r \) and \( \text{Hct} \) at various \( \text{P}_{\text{CO}_2} \) values, and epinephrine levels using a paired experimental design. Gas mixtures of 0.3%, 0.5% and 1% \( \text{CO}_2 \) in \( \text{O}_2 \) were provided by mixing \( \text{CO}_2 \) and \( \text{O}_2 \) using a gas mixing pump (Wösthoff Model M301 A-F). 25 \( \mu \)l of L-epinephrine (in saline) was added directly to the tonometer flasks from freshly prepared stock solutions (\( \text{pH} = 7.8-8.0 \)) to yield estimated final concentrations of \( 5 \times 10^{-8} \), \( 5 \times 10^{-7} \) and \( 5 \times 10^{-6} \) mol l\(^{-1}\). Again, actual epinephrine levels were later determined by HPLC (see below). Blood samples were withdrawn and analysed 30 min after the addition of epinephrine or the switching of the gases.

**Analytical procedures**

\( \text{pH}_w \) and arterial \( \text{PO}_2 \) (\( \text{P}_{\text{AO}_2} \)) were determined by using a microcapillary pH electrode (Radiometer G299A) and \( \text{PO}_2 \) electrode (E5046) maintained at ambient temperature in conjunction with a Radiometer PHM 71 acid–base analyser and BMS3 Mk2 blood micro system. \( \text{pH}_r \) was determined with the same apparatus, utilizing the fast freeze–thaw technique of Zeidler & Kim (1977). Blood \( \text{CO}_2 \) was determined on 100-\( \mu \)l samples using a total \( \text{CO}_2 \) analyser (Corning Model 905). \( \text{Hct} \) was determined by centrifuging 80 \( \mu \)l of blood in a heparinized capillary tube for 8 min at 5000 \( \text{g} \). \( \text{P}_{\text{CO}_2} \) and bicarbonate concentration ([\( \text{HCO}_3^- \)]) were calculated using a reorganization of the Henderson–Hasselbalch equation. The operational pK values of carbonic acid (pK') at various temperatures and pH values and the solubility coefficients of \( \text{CO}_2 \) (\( \alpha_{\text{CO}_2} \)) for trout plasma were obtained from Boutilier, Heming & Iwama (1984).

Plasma [\( \text{Na}^+ \)] and [\( \text{K}^+ \)] were determined on diluted plasma (200\( \times \)) using flame photometry (EEL Flame Photometer), plasma [\( \text{Cl}^- \)] by coulometric titration on 100 \( \mu \)l of undiluted sample using a chloridometer (Buchler–Cotlove), plasma [\( \text{Ca}^{2+} \)] and [\( \text{Pi} \)] by spectrophotometry using commercial assay kits (Sigma), and plasma
Epinephrine infusion in trout I

[ammonia] by a micro-modification of the salicylate–hypochlorite reaction (McDonald & Wood, 1981). Pi and ammonia determinations were performed on samples deproteinized with 12% trichloracetic acid.

Plasma catecholamines were determined using HPLC (Waters Chromatograph) in conjunction with electrochemical detection (Bioanalytical Systems LC-4B Electrochemical Detector) following the extraction procedures of Woodward (1982). A reverse phase column (Waters, Nova Pak C18) was used with a 0·15 mol l\(^{-1}\) monochloroacetate buffer (pH 3·0; containing 2 mmol l\(^{-1}\) Na\(_2\)EDTA, 200 mg sodium octyl sulphate) mobile phase at a flow rate of 1·0 ml min\(^{-1}\). The applied potential to the LC-4B oxidative flow cell was +0·65 V versus the Ag\(^+\)/AgCl reference electrode.

Statistical analysis

In Figures and Tables, variability of the data is indicated by ±1 s.e. Results have been statistically analysed using paired and unpaired Student's t-test where appropriate between sample means; 5% was taken as the fiducial limit of significance.

RESULTS

Maintaining the patency of dorsal aortic cannulae throughout the course of experimentation was a major problem in the present study. As a consequence of cannula failure, values for \(N\) declined during the latter stages of infusion. In order to offset the attrition-related lack of values at 22·5 h of epinephrine infusion and during post-epinephrine infusion, results from two separate groups of fish were pooled. In one group, blood was sampled throughout the entire experiment, while in the other blood sampling commenced after 10·5 h of epinephrine infusion. These are the reasons for variable \(N\) numbers in the Figures and Tables. As variability in both groups was similar, we believe such a change in the experimental design did not alter the results. Cannula failure was not a problem in control animals.

Table 1. Plasma levels of epinephrine and norepinephrine during continuous intrarterial infusion of teleost saline

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>[Epinephrine] (nmol l(^{-1}))</th>
<th>[Norepinephrine] (nmol l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-infusion (6)</td>
<td>4·65 ± 1·24</td>
<td>7·93 ± 3·01</td>
</tr>
<tr>
<td>4·5 (6)</td>
<td>6·90 ± 1·26</td>
<td>3·10 ± 0·25</td>
</tr>
<tr>
<td>7·5 (6)</td>
<td>7·31 ± 2·55</td>
<td>8·48 ± 1·89</td>
</tr>
<tr>
<td>10·5 (6)</td>
<td>5·11 ± 0·99</td>
<td>6·11 ± 0·17</td>
</tr>
<tr>
<td>16·5 (6)</td>
<td>5·38 ± 0·99</td>
<td>4·83 ± 0·50</td>
</tr>
<tr>
<td>28·5 (5)</td>
<td>5·74 ± 0·85</td>
<td>4·92 ± 0·51</td>
</tr>
<tr>
<td>31·5 (5)</td>
<td>3·79 ± 1·58</td>
<td>5·75 ± 2·36</td>
</tr>
<tr>
<td>34·5 (5)</td>
<td>2·55 ± 0·68</td>
<td>5·86 ± 1·14</td>
</tr>
<tr>
<td>40·5 (5)</td>
<td>2·54 ± 0·45</td>
<td>6·22 ± 0·99</td>
</tr>
</tbody>
</table>

Infusion rate = 0·6 ml h\(^{-1}\).

Means ± s.e.; values of \(N\) are shown in parentheses.
Table 2. Plasma catecholamine levels after 4 h of continuous intra-arterial infusion of $2 \times 10^{-5}$ mol$^{-1}$ epinephrine

<table>
<thead>
<tr>
<th>Condition</th>
<th>[Epinephrine] (nmol$^{-1}$)</th>
<th>[Norepinephrine] (nmol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline infusion</td>
<td>3.87 ± 2.2</td>
<td>3.38 ± 1.2</td>
</tr>
<tr>
<td>Epinephrine infusion</td>
<td>62.09 ± 16.7</td>
<td>2.21 ± 0.7</td>
</tr>
</tbody>
</table>

Means ± s.e. $(N = 4)$.

Continuous intra-arterial infusion of saline was without significant effect on the circulating levels of epinephrine or norepinephrine (Table 1); thus any differences between control and experimental groups presumably reflects physiological effects of exogenous rather than endogenous epinephrine. Infusion of $2 \times 10^{-5}$ mol$^{-1}$ epinephrine for 4.5 h at a flow rate of 0.6 ml h$^{-1}$ caused plasma epinephrine levels to rise by approximately 16-fold to $6 \times 10^{-8}$ mol$^{-1}$ (Table 2). This value is similar to the estimated and desired value of $5 \times 10^{-8}$ mol$^{-1}$. It is assumed that plasma epinephrine remained elevated at this level throughout the remainder of the infusion period.

Epinephrine infusion induced a pronounced but transient blood acidosis that had disappeared by 10.5 h of infusion, and thereafter blood pH remained constant. The acidosis was respiratory in origin as indicated by the elevation of arterial $P_{CO_2}$ ($P_{aCO_2}$) that persisted throughout the experimental period (Fig. 1B). The apparent elevation of $P_{aCO_2}$ during the post-epinephrine saline infusion may be artefactual and related to the unusually low $P_{aCO_2}$ during the initial saline infusion. Indeed, experimental and control $P_{aCO_2}$ values were not significantly different during the final 12 h of infusion. Plasma $[HCO_3^-]$ levels increased as a consequence of epinephrine infusion (Fig. 1C) and correlated well with the restoration of blood pH to pre-epinephrine values. Plasma $[HCO_3^-]$ gradually returned towards initial and control levels during the final 24 h of infusion. The changes in $[HCO_3^-]$ induced by epinephrine treatment were over and above those predicted by non-bicarbonate carbon dioxide buffering (Fig. 2). $P_{aO_2}$ was depressed temporarily by epinephrine and became significantly elevated during the 12-h post-epinephrine period (Fig. 1B). Epinephrine elicited an immediate rise in Hct that returned to control values by 4.5 h (Fig. 3A). Serial blood sampling led to gradual but significant reductions in Hct in both experimental and control groups (Fig. 3A), even though a considerable quantity of removed RBCs were resuspended and reinjected into fish after each sampling period. Mean Hct never decreased below 12% in either group.

The reduction of whole blood pH ($pH_e$) induced by epinephrine, in vivo, was not accompanied by a parallel reduction of RBC pH ($pH_i$) (Fig. 3B). In fact, $pH_i$ actually increased slightly during epinephrine infusion, although the changes were not statistically significant. The dissimilar patterns of $pH_e$ and $pH_i$ changes during the course of epinephrine infusion caused the pH gradient across the red cell membrane ($pH_e - pH_i$) to become significantly reduced (Fig. 3C), especially during the first 4.5 h of infusion. The relationship between $pH_e$ and $pH_i$ observed in vivo,
Epinephrine infusion in trout

during epinephrine infusion, was also very different from the relationship quantified in vitro as shown in Fig. 4A. In vitro levels of epinephrine were \(9.0 \pm 2.5 \text{ nmol} \text{l}^{-1}\) \((N = 6)\), approximately an order of magnitude lower than the level measured during

![Epinephrine effect diagram]

Fig. 1. The effects of prolonged intra-arterial epinephrine infusion on measured (\(\text{pH}_e, \text{P}_o_2\)) and calculated (\(\text{pCO}_2, [\text{HCO}_3^-]\)) blood respiratory and acid–base variables. Control fish (●—●) were infused with saline continuously for 42 h while the epinephrine-treated fish (○—○) were infused initially for 7 h with saline followed by 24 h of epinephrine infusion (between the two vertical bars) and finally 12 h of saline infusion. *Significantly different from pre-epinephrine value; †significantly different from corresponding value in the control group. \(N\) values are indicated at each data point; NS, not significant.
epinephrine infusion. The strikingly different relationships between pH_e and pH_t in vitro and during epinephrine infusion are illustrated in Fig. 4B. It is clear that epinephrine caused pH_t to deviate far from the in vitro pH_e versus pH_t relationship and this deviation can be quantified as the vertical distance BB' (0.20 pH units). Following 4.5 h of epinephrine infusion (point C, Fig. 4B), pH_t was still significantly elevated above the predicted value by 0.14 pH units, but had returned to the pre-epinephrine value by 10.5 h (point D). Deviation from the 'normal' pH_e versus pH_t relationship was also observed in vitro when epinephrine levels were progressively elevated to $1.2 \times 10^{-6}$ mol l$^{-1}$ during equilibration with either 0.5% or 1.0% CO$_2$ (Figs 4B, 5C). Significant reductions in the RBC transmembrane pH gradient were noted only at epinephrine levels of $2.0 \times 10^{-7}$ mol l$^{-1}$ or higher (Fig. 5C) and were due to reciprocal changes in both pH_e and pH_t, although only the changes in pH_e were statistically significant. The epinephrine-induced reductions of pH_e−pH_t in vitro were similar in magnitude to those noted in vivo, although the effect in vivo occurred at a lower epinephrine level (10 times lower). Epinephrine in vitro caused RBCs to swell as indicated by increasing Hct (Fig. 5A). This effect was minor compared to the elevation of Hct observed in vivo (Fig. 3A) and was only statistically

![Fig. 2](image-url)

Fig. 2. Measured (O—O) and predicted (□—□) changes in blood [HCO$_3^-$] during prolonged intra-arterial epinephrine infusion. Predicted [HCO$_3^-$] was calculated using the experimentally determined buffer value ($\beta$) of $-9.81 \pm 0.9$ mmol l$^{-1}$ whole blood ($N = 6$) according to the relationship $[\text{HCO}_3^-] = -\beta \times \Delta \text{pH}$. *Significantly different from predicted value. $N$ values are indicated at each data point.
significant at an epinephrine concentration of $1.2 \times 10^{-6}$ mol l$^{-1}$ (20 times greater than during epinephrine infusion).

Epinephrine infusion had no apparent effect on plasma levels of Na$^+$ or Cl$^-$, but did induce significant elevations of plasma [K$^+$] during the first 4.5 h of epinephrine infusion.

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**Fig. 3.** The effects of prolonged intra-arterial epinephrine (○—○) infusion on (A) haematocrit and (B) whole blood pH ($pH_w$), red blood cell (RBC) pH ($pH_r$) and the pH gradient across the RBC membrane ($pH_w - pH_r$). *Significantly different from pre-epinephrine value; † significantly different from corresponding value in the control group (●—●). N values are indicated at each data point. All other details are as in Fig. 1; NS, not significant.
infusion (Fig. 6). Plasma Ca\(^{2+}\) levels increased significantly throughout the epinephrine infusion period and remained elevated during the first 12 h of saline infusion (Fig. 7A). The effects of epinephrine on plasma [Pi] and [ammonia] were somewhat variable and difficult to interpret, although plasma [Pi] tended to decrease during the initial 4·5 h of epinephrine treatment and rise during the latter stages of infusion (Fig. 7B). It is unclear why plasma ammonia levels were significantly elevated in the control group.
**DISCUSSION**

Previous studies examining the physiological effects of exogenous catecholamines in fishes have utilized single, rapid intravascular injections (e.g. Peyraud-Waitzenegger, 1979; Peyraud-Waitzenegger, Barthelemy & Peyraud, 1980; Nikinmaa, 1982a,b; Hughes, Peyraud, Peyraud-Waitzenegger & Soulier, 1982; Epple & Nibbio, 1985; Epple & Kahn, 1985) to simulate circulating plasma levels as measured during a variety of stressful conditions. In nearly all instances, plasma catecholamine levels were not measured but were calculated on the basis of the concentration of the solution injected and estimates of biological half-life and internal catecholamine distribution volume. Based on the recent catecholamine measurements of Epple & Nibbio (1985), following single injections of epinephrine into American eels (*Anguilla rostrata*) (1–8 μg kg⁻¹), it is likely that the actual circulating epinephrine levels in previous studies using such estimations (see above) ranged between 10⁻⁶ and 10⁻⁷ mol L⁻¹. These levels are considerably higher than the recently reported values in trout during or following a variety of stresses including external hypercapnia (2·4×10⁻⁸ mol L⁻¹; S. F. Perry, D. Ewing & S. Malone, in preparation), intravascular acid infusion (6·0×10⁻⁹ mol L⁻¹; Boutilier et al. 1986), exhaustive exercise (7×10⁻⁸ mol L⁻¹; Primmett et al. 1986) and acute hypoxia (Fievet et al. 1986). In the present study, 'resting' values of epinephrine and norepinephrine were 4·7 and 7·9×10⁻⁹ mol L⁻¹, respectively, and are similar to the values reported by Ristori, Rehm & Laurent (1979), Woodward (1982) and Primmett et al. (1986) for intact, catheterized rainbow trout. These recently measured values are significantly lower than in earlier reports ([epinephrine] = 2·7×10⁻⁸ mol L⁻¹; Nakano & Tomlinson, 1967; [norepinephrine] = 1·2×10⁻⁸ mol L⁻¹; Rich, 1979) due to refinements in blood sampling techniques and methodology for measuring catecholamines. During continuous infusion of epinephrine, the plasma level increased to 6·2×10⁻⁸ mol L⁻¹ (Table 2), which is similar to the levels measured during physiological stresses (see above). Due to the rapid degradation of epinephrine following a single bolus injection, we believe that continuous infusion permits long-term evaluation of elevated epinephrine levels, thereby simulating more

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**Fig. 4.** The relationships between whole blood pH (pHₑ) and red blood cell (RBC) pH (pHᵢ) and the interactive effects of epinephrine both (A) *in vitro* and (B) *in vivo*. The pHₑ versus pHᵢ relationship was determined *in vitro* by equilibrating blood obtained from six chronically catheterized fish in separate tonometer flasks with three different CO₂ tensions (0·3, 0·5 and 1·0 % CO₂). The average epinephrine concentration in the *in vitro* experiment was 9·0±2·5×10⁻⁹ mol L⁻¹. The *in vitro* pHₑ versus pHᵢ relationship has been reproduced as the solid line in Fig. 6B with various *in vitro* and *in vitro* values superimposed. Point A represents the values during initial saline infusion while points B, C and D represent the pH values after 1·5, 4·5 and 10·5h of epinephrine infusion, respectively. Point B' signifies the predicted pHᵢ value at 1·5h, given the same change in pHₑ as at point B, according to the *in vitro* relationship. Thus the vertical distance AB' is the predicted decrease in pHᵢ at 1·5h, AB is the actual measured increase in pH, at 1·5h, and BB' is the deviation from the *in vitro* relationship that we attribute to elevated epinephrine. For comparative purposes, the *in vitro* effects of epinephrine on pHₑ versus pHᵢ have been illustrated (open squares). See text for further details.
closely the prolonged elevation of plasma epinephrine induced by some stresses (LeBras, 1982; see above also). Nikinmaa (1982b) reported that the effect of epinephrine on increasing Hct was dissipating within only 10 min of a single injection. The technique of intra-arterial infusion used in the present study (0.6 ml h⁻¹) did not appear to stress the fish unduly, as indicated by stable catecholamine levels (Table 1) and other physiological variables. The only parameter to be affected by infusion of saline was urine flow rate (see Vermette & Perry, 1986b) which increased presumably as a result of vascular hypervolaemia or a related elevation of blood pressure. We do recommend, however, that a significant period (4–6 h) be allowed for fish to adjust to the process of infusion itself, before experimentation commences. The limitations of prolonged intra-arterial infusion

![Fig. 5. In vitro effects of epinephrine on (A) haematocrit, (B) whole blood pH (pHₐ) and red blood cell pH (pHᵢ) and (C) the pH gradient across the red blood cell membrane (pHₑ — pHᵢ). Blood was collected from cannulated trout (N = 6) and equilibrated in separate flasks with 1% CO₂. C, control blood (9·0 × 10⁻⁵ mol l⁻¹ epinephrine); where not indicated, s.e. values lie within the mean. *Significantly different from control values.](image-url)
of epinephrine include possible desensitization of the cyclic AMP response and subsequent blunting of physiological responses.

Two additional problems associated with studying the effects of catecholamines in fishes that are unrelated to the method of administration (i.e. injection versus

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**Fig. 6.** The effects of prolonged intra-arterial epinephrine (○—○) infusion on plasma potassium levels (A), sodium levels (B) and chloride levels (C). *Significantly different from pre-epinephrine value; † significantly different from corresponding value in the control group (●—●). N = 9 for both groups except where otherwise indicated. All other details are as in Fig. 1.
continuous infusion) are seasonality (see Peyraud-Waitzenegger et al. 1980) and the possibility of catecholaminotropic effects (Epple & Nibbio, 1985). In the present study, experiments were conducted between 13 June and 18 September, thus it is

Fig. 7. The effects of prolonged intra-arterial epinephrine (O—O) infusion on plasma calcium levels (A), inorganic phosphate (Pi) levels (B) and ammonia levels (C). *Significantly different from pre-epinephrine value; †significantly different from corresponding value in the control group (●—●). N = 9 for both groups except where otherwise indicated. All other details are as in Fig. 1.
unlikely that the results were affected significantly by changing seasons. Norepinephrine levels remained constant during infusion of epinephrine but the possibility of catecholaminotropic effects cannot be dismissed since dopamine levels were not monitored. Epple & Nibbio (1985) reported significant elevations of dopamine following injections of 2-8 μg kg⁻¹ of epinephrine in American eels.

We have here presented evidence that infusion of epinephrine induces a condition of respiratory acidosis along with an elevation of plasma [HCO₃⁻], which ultimately corrects the extracellular acidosis. The rise in plasma [HCO₃⁻] initially is due to non-bicarbonate H⁺ buffering but ultimately results from enhanced net acid excretion at the gills and kidney (see Vermette & Perry, 1986a,b). There are two possible causes for retention of CO₂ during epinephrine infusion; these are hyperventilation and inhibition of erythrocytic bicarbonate dehydration. Hypoventilation has been shown to induce hypercapnic acidosis by inhibiting CO₂ excretion (Boutilier et al. 1986; G. K. Iwama, R. G. Boutilier, T. A. Heming, P. A. Wright & D. J. Randall, in preparation) and may result from epinephrine administration (Peyraud-Waitzenegger et al. 1980) via stimulation of α-adrenergic receptors. In European eels (Anguilla anguilla), the hypoventilatory effect of epinephrine has been observed only during winter months whereas identical treatment during summer causes hyperventilation, a result of β-adrenergic receptor stimulation (Peyraud-Waitzenegger et al. 1980). Thus, based on seasonality, one would predict hyperventilation and elevation of PAO₂ (Peyraud-Waitzenegger, 1979) in the present investigation, in the absence of species- or dose-related differences. Since ventilation volumes were not measured in the present study, the ventilatory effects of epinephrine in trout remain unclear. However, the results are not consistent with hyperventilation. Indeed, the initial reduction of PAO₂ and the pronounced elevation of PAO₂ following the removal of epinephrine support the hypothesis of epinephrine-induced hypoventilation. Restoration and maintenance of 'normal' PAO₂ values during the final 22.5 h of epinephrine infusion may be related to adrenergic enhancement of gill diffusing capacity as demonstrated by Pettersson (1983) and Perry et al. (1985), which would tend to offset the effects of hypoventilation on arterial oxygenation. However, any factor which enhances gill O₂ diffusing capacity should have a similar effect on CO₂ diffusing capacity, yet Paco₂ did not change in a reciprocal fashion to the changes in PAO₂, except at 1.5 h of infusion (Fig. 1). For similar reasons, the prolonged elevation of Paco₂ cannot be attributed to epinephrine-induced increase in metabolism (Larsson, 1973).

The prolonged elevation of Paco₂ in the face of unaltered PAO₂ provides evidence for a reduction in the rate of RBC HCO₃⁻ dehydration, perhaps due to inhibition of RBC Cl⁻/HCO₃⁻ exchange, a process which has been postulated as the rate-limiting step in piscine CO₂ excretion (Perry, Davie, Daxboeck & Randall, 1982; see also review by Perry, 1986). This hypothesis is supported by in vitro data on rainbow trout blood showing inhibition of Cl⁻/HCO₃⁻ exchange (Heming, 1984), an increase in the Donnan Cl⁻ ratio (Cl⁻/Cl₋e) and a concomitant increase in plasma [HCO₃⁻] (T. A. Heming, D. J. Randall & M. M. Mazeaud, in preparation) following epinephrine addition (10⁻⁶ mol l⁻¹). It must be emphasized that a direct
comparison between the results of the present study and those of Peyraud-Waitzenegger (1979) are probably not justified given the likelihood of higher circulating levels of epinephrine (estimated to be $10^{-7}$ mol$^{-1}$), the short duration of the experiments (measurements were made within 5 min of epinephrine injection) and markedly lower initial $P_{aO_2}$ levels (30–50 Torr versus 100–110 Torr).

The results of this study demonstrate that epinephrine may play an important role in stabilizing RBC pH during conditions of extracellular acidosis. Indeed, reductions of plasma pH, induced by intra-arterial acid infusion (Boutilier et al. 1986) or external hypercapnia (S. F. Perry, D. Ewing & S. Malone, in preparation) have been shown to initiate the release of epinephrine into the circulation. We have shown here that circulating levels as low as $6 \times 10^{-8}$ mol$^{-1}$ \textit{in vivo} are sufficient to prevent a reduction in pH, during a simultaneous lowering of pH$_e$ by 0.16 units. An identical effect of epinephrine was observed \textit{in vitro} (Figs 4, 5), although significantly greater quantities of epinephrine were required to elicit changes in the transmembrane proton gradient (a significant reduction of pH$_e$–pH$_i$ was observed only at $2.0 \times 10^{-7}$ mol$^{-1}$ epinephrine). Similar \textit{in vitro} changes in the proton distribution across the RBC have been reported by Nikinmaa (1982a) and T. A. Heming, D. J. Randall & M. M. Mazeaud (in preparation), although at considerably higher levels of epinephrine ($5 \times 10^{-6}$ mol$^{-1}$). It is unclear why higher levels of epinephrine are required to initiate effects on the RBCs \textit{in vitro}, but possibilities include the absence of catecholaminotrophic effects and interactions with other humoral/neurohumoral systems. Alternatively, the physiological function of the RBCs \textit{in vitro} may be gradually deteriorating. The mechanism responsible for epinephrine-induced RBC alkalinization apparently involves $\beta$-receptor-mediated stimulation of Na$^+$/H$^+$ exchange (Baroin, Garcia-Romeu, Lamarre & Motais, 1984; Cossins & Richardson, 1985). Stimulation of Na$^+$/H$^+$ exchange is also consistent with RBC swelling, which is known to accompany RBC alkalinization (Nikonmaa, 1982a; Nikonmaa & Huestis, 1984). Recently, Bennett & Rankin (1985) have identified $\beta$-receptors on eel RBC membranes, providing further evidence for their involvement in stabilizing RBC acid–base status. The maintenance of RBC pH during extracellular acidosis is of physiological significance because it allows arterial oxygen content to remain relatively constant by preventing deleterious shifts in the haemoglobin–oxygen dissociation curve (Bohr and Root effects). The importance of epinephrine in the maintenance of arterial oxygen content is indicated by the observations of constant $C_{aO_2}$ following exhaustive exercise in trout (Primmett et al. 1986) and striped bass (\textit{Morone saxatilis}; Nikonmaa, Cech & McEnroe, 1984) and HCl infusion in trout (Boutilier et al. 1986). In these experiments, pH$_e$ decreased by 0.16–0.30 units, epinephrine levels were elevated (not measured in the study of Nikonmaa \textit{et al.} 1984) and the changes in RBC pH were significantly less than those predicted based on a passive transmembrane distribution of protons (from \textit{in vitro} studies). Pre-treatment of the exercising fish with the $\beta$-receptor antagonist propranolol resulted in significant
reductions of CaO₂ which were associated with reduced RBC pH, when compared to 'control' exercising fish.

No changes in plasma levels of Na⁺ or Cl⁻ were observed during epinephrine infusion even though significant changes in branchial net fluxes and renal excretion of these ions were noted (see Vermette & Perry, 1986a, b). These results are in agreement with those of Eppe & Nibbio (1985) following single injections of epinephrine into eels and are not surprising given the large extracellular pool of NaCl in relation to the low rates of the branchial and renal net fluxes (no greater than 200 μmol kg⁻¹ h⁻¹ in the present investigations). The increase of plasma [K⁺] during the early stages of epinephrine infusion may reflect K⁺/H⁺ exchange between intracellular and extracellular compartments (Lade & Brown, 1963) and might be a significant mechanism in the regulation of blood pH during acute extracellular acidosis. A similar, although more prolonged, elevation of plasma [K⁺] was reported by Wheatly, Höbe & Wood (1984) during the regulation of hyperoxic acidosis in rainbow trout. The significant elevation of plasma [Ca²⁺] was not due to increased renal reabsorption (Vermette & Perry, 1986b) but may have been related to Ca²⁺/H⁺ exchange between extracellular fluid and bone and/or mobilization of bone calcium carbonate. Whether or not such mechanisms operate during regulation of acid–base disturbances in rainbow trout has not been examined, although Cameron (1985) concluded that the bone compartment of the Channel catfish (Ictalurus punctatus) does not contribute to the compensation of hypercapnic acidosis.

We have attributed the results presented here and in the following two papers (Vermette & Perry, 1986a, b) to elevated circulating levels of epinephrine. It must be emphasized, however, that the interpretation of these results is somewhat complicated by the simultaneous reductions of plasma pH that were induced by epinephrine. While the possibility that some results are due to depressed pH and not epinephrine cannot be ignored, we consider this unlikely as many of these effects persist even after plasma pH is restored. The problem of separating the effects of epinephrine from the effects of pH is discussed in greater detail in the subsequent paper (Vermette & Perry, 1986a).

Based on the results of this and other studies, we speculate that the release of epinephrine into the circulation during periods of acute extracellular acidosis initiates a series of adrenergic responses that includes stabilization of RBC pH and accumulation of plasma bicarbonate. RBC pH stabilization prevents deleterious reductions of arterial oxygen content whereas plasma HCO₃⁻ accumulation is necessary for restoring extracellular pH. The branchial and renal mechanisms involved in plasma HCO₃⁻ accumulation are discussed subsequently.

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Epinephrine infusion in trout I


