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# ACID–BASE REGULATION FOLLOWING ACUTE ACIDOSIS IN SEAWATER-ADAPTED RAINBOW TROUT, *SALMO GAIRDNERI*: A POSSIBLE ROLE FOR CATECHOLAMINES

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## SUMMARY

A fall in blood pH was induced by intra-arterial infusion of HCl in seawater-adapted rainbow trout (*Salmo gairdneri*). The acute acidosis resulting from HCl infusion caused a short-lived decrease in plasma bicarbonate concentration ($[HCO_3^-]$) and an increase in arterial $CO_2$ tension ($Pa_{CO_2}$). Erythrocyte pH and bicarbonate concentrations were not significantly altered by the infusion of acid. Injection of acid did, however, stimulate a branchial net $H^+$ efflux which could be primarily accounted for by a net uptake of bicarbonate equivalent ions from the environmental water.

Acid infusion of animals pre-treated with the $\beta$-adrenergic blocking agent, propranolol, induced a similar pattern of change in plasma acid–base status. However, the recovery of plasma pH and restoration of plasma $[HCO_3^-]$ were slower than in animals infused with acid alone. Red cell pH fell significantly in the face of plasma acidosis in the $\beta$-blocked animals. Erythrocyte $[HCO_3^-]$ showed a similar pattern of change to that of erythrocyte pH. Branchial net $H^+$ efflux increased to a lesser extent following acid infusion in animals treated with propranolol.

We conclude that catecholamines released into the bloodstream during periods of acute acidosis may play an important role in facilitating branchial $H^+$ efflux in seawater-adapted rainbow trout.

## INTRODUCTION

Strenuous exercise in fish often results in an extracellular acidosis of metabolic origin (Holeton, Neumann & Heisler, 1983; Turner, Wood & Clark, 1983). Recent studies on rainbow trout have shown that red cell pH is regulated at a constant level during and following periods of extracellular acidosis brought on either by anaerobic exercise (Primmett, Randall, Mazeaud & Boutilier, 1986) or by direct acid infusion

Key words: acid–base regulation, acidosis, rainbow trout, catecholamines, ammonia excretion, ion transfer, red cell pH.

(Boutilier, Iwama & Randall, 1986). In both instances catecholamines are released into the blood where, through $\beta$-adrenergic mechanisms at the red cell membrane, they are responsible for turning on cellular ion exchange processes which ultimately lead to the regulation of the erythrocyte pH (Nikinmaa, 1982, 1983; Cossins & Richardson, 1985) and therefore blood oxygenation (Boutilier *et al.* 1986).

Several studies have shown that following exhaustive exercise a transient net $H^+$ flux occurs between the animals and the environmental medium and that the transfer takes place mainly across the gills (Holeton *et al.* 1983; Holeton & Heisler, 1983; Milligan & Wood, 1986). Accordingly, the present experiments set out to examine whether catecholamines play a significant role in proton equivalent flux across the gills. The experiments were carried out on seawater-adapted rainbow trout (*Salmo gairdneri*) subjected to acute extracellular acidosis by infusion of HCl. The possible role of the $\beta$-agonist adrenaline in regulating these bicarbonate equivalent movements was determined by pre-treating a second group of fish with propranolol ($\beta$-adrenergic antagonist) before acid infusion.

## MATERIALS AND METHODS

### *Animals and preparation*

Seawater-adapted rainbow trout (*Salmo gairdneri*) of either sex, weighing between 150 and 300 g, were obtained from Ocean Farmers Ltd, St Margaret's Bay, NS. They were kept in one of the Aquatron seawater laboratories in a 4 $m^3$ fibreglass tank supplied with a continuous flow of aerated sea water (7–12°C) for at least 3 weeks before the experiment. Fish were fed daily with commercial trout pellets, but feeding was suspended 2 days prior to experimentation.

Following anaesthetization in a 1:10000 seawater solution (pH 7·0) of MS-222 (Sigma), the dorsal aorta of each fish was chronically cannulated with Clay-Adams polyethylene (PE 50) tubing. Throughout all surgical procedures, the gills of the fish were continuously irrigated with a lower dose of the anaesthetic solution (1:15000 MS-222). Fish were allowed to recover for at least 48 h in a darkened Plexiglas box of the water recirculation system described below. At all stages prior to experimentation, the animals were supplied with continuously flowing aerated sea water, kept at $10 \pm 0{\cdot}5$°C.

### *Experimental set-up*

Animals were contained within a water recirculation system maintained at $10 \pm 0{\cdot}5$°C. The water in the system was pumped ($2{\cdot}5$ l $min^{-1}$) through an aeration column (bubbled with air), through a Plexiglas box containing the fish, and then returned to the aeration column.

Water samples from the recirculation system were taken according to the time schedule of the experiment (see below) and used to measure water ammonia and total $CO_2$ concentrations. Measurement of total $CO_2$ in water was made after each sample had been equilibrated at a constant $P_{CO_2}$ (1 % $CO_2/N_2$) and constant temperature

(30 ± 0·1 °C). The equilibration vessels in which the samples were contained were fitted with scintered glass bottoms for the introduction of humidified and temperature-regulated gases (Wösthoff, FRG). The elevated temperature ensured a more rapid equilibration time, reducing sample processing time. After complete equilibration (temperature and $P_{CO_2}$), samples were transferred to a 5 ml Hamilton gas-tight syringe for subsequent determination of total $CO_2$ concentration by a chromatography method detailed below. In principle, our system is the same as the 'delta-bicarbonate' system described by Heisler (1984, 1987), except that total $P_{CO_2}$, rather than pH, is determined at a fixed $P_{CO_2}$ and temperature. In both cases, bicarbonate concentrations can be estimated by known additions of solid bicarbonate to the recirculation system.

*Experimental protocols*

Parallel experiments were performed on three separate groups. The first group ($N = 8$) was subjected to acid infusion. The second group ($N = 8$) was subjected to both propranolol and acid infusion. The third group ($N = 6$) was subjected to injection of the saline vehicle, and served as a control for handling and sampling effects.

The experiments with the first group focused on post acid-infusion changes in arterial plasma and red cell acid–base status, as well as net $H^+$ flux with the environment (see below). One hour prior to acid infusion, the inflow to the fish box was closed, and the volume standardized to 1 l 100 $g^{-1}$ body mass. Two water samples were taken 1 h apart for total $CO_2$ (at fixed $P_{CO_2}$) and ammonia measurements. Ten minutes before acid infusion, fish were infused through the dorsal aortic cannula with 0·35 ml 100 $g^{-1}$ body mass of saline. At the time of acid infusion, fish were infused with 0·25 ml 100 $g^{-1}$ body mass of 0·1 mol $l^{-1}$ HCl in 140 mmol $l^{-1}$ NaCl, subsequently washed in with 0·1 ml 100 $g^{-1}$ body mass of 140 mmol $l^{-1}$ NaCl. Infusion times ranged from 7 to 9 min. The time when the acid infusion finished was defined as time zero for all succeeding measurements. Arterial blood samples (500 $\mu$l) were taken before acid infusion and at 5, 30, 60, 120 and 240 min post-infusion. The volume of blood sampled was replaced with heparinized (20 i.u. $ml^{-1}$) saline. Portions of each blood sample were analysed for plasma pH (pHe), red cell pH (pHi), total $CO_2$ (in both whole blood and true plasma), and haematocrit. Water samples (10 ml) from the delta-bicarbonate system were taken at 0, 60, 120, and 240 min for determination of ammonia concentration and total $CO_2$ content.

The second group of animals was used to examine the effect of pre-treatment with propranolol on the above acid–base parameters after acid infusion. The experimental protocol was the same as in the first group except that fish were infused with 0·25 ml 100 $g^{-1}$ body mass of $2 \times 10^{-4}$ mol $l^{-1}$ propranolol in saline followed by 0·1 ml 100 $g^{-1}$ body mass of saline to clear the cannula of propranolol 10 min before acid infusion.

The third group acted as a control. The same experimental protocol (as the first group) was employed except that fish were infused with 0·35 ml 100 $g^{-1}$ body mass of 140 mmol $l^{-1}$ NaCl alone at the time of acid infusion.

*Analytical procedures*

Blood pH was determined using a Radiometer G279/G2 glass capillary electrode and K497 calomel electrode coupled with a PHM84 pH meter. The freeze–thaw method of Zeidler & Kim (1977) was used to measure red cell haemolysate pH using the same electrodes as above. Haematocrit values were obtained by centrifugation. Total $CO_2$ in both whole blood and true plasma was measured on anaerobically obtained 50 $\mu$l samples using a gas chromatography method (Boutilier, Iwama, Heming & Randall, 1985; Iwama *et al.* 1987). $Pa_{CO_2}$ and $[HCO_3^-]$ in blood and plasma were calculated using a rearrangement of the Henderson–Hasselbalch equation, with values of plasma pK′ and $CO_2$ solubility for rainbow trout at 10°C (Boutilier, Heming & Iwama, 1984). Red cell $[HCO_3^-]$ was calculated as:

$$\text{cell value} = \frac{(\text{blood value}) - (\text{plasma value})\,(1 - \text{haematocrit})}{\text{haematocrit}}.$$

Total $CO_2$ content measurements of water from the recirculation system were made using a gas chromatography technique similar in principle to that detailed by Lenfant & Aucutt (1966). Our system used a Carle Series 100 chromatograph (Carle Instruments Inc., USA) fitted with $CO_2$-discriminating columns (Porapak Q). The sample (1 ml) was acidified with 50 $\mu$l of 0·1 mol $l^{-1}$ HCl in a 5 ml Hamilton gas-tight syringe whose headspace was filled with pure nitrogen. The syringe was agitated for 20 min at room temperature to ensure complete equilibration of $CO_2$ between gas and liquid phases. The headspace gas was then introduced into the chromatograph (fixed 1 ml loop) through a drying filter. The $CO_2$ of the gas sample was detected by differential thermal conductivity and output peaks recorded and compared with peaks from $NaHCO_3$ standard solutions treated identically to the water samples.

Water ammonia concentration was determined by a micro-modification of the salicylate–hypochlorite method of Verdouw, van Echteld & Dekkers (1978). The net fluxes of ammonia and bicarbonate (in $\mu$mol $kg^{-1}$ $h^{-1}$) were calculated from changes in their respective concentrations in water. Net $H^+$ flux was determined as the difference between the net ammonia flux and net bicarbonate flux (delta ammonia minus delta bicarbonate, signs considered).

The method used to determine $H^+$ movements in the present study, as with other similar methods (reviewed by Heisler, 1984, 1987), cannot separate $H^+$ uptake from $HCO_3^-$ excretion, or *vice versa*, or between ammonia movement in the $NH_3$ or $NH_4^+$ forms. Rather, it gives a valid measure of *net* acidic equivalent flux. Various *descriptive terms* to denote such $H^+$ movements are used interchangeably in the current literature (e.g. acidic equivalent flux, net $H^+$ flux, bicarbonate equivalent flux, amongst others) and *should not be mistaken for* statements about *the mechanisms* involved in transepithelial ion transfers.

*Statistical analysis*

Means ± 1 S.E.M. are reported throughout. Student's two-tailed *t*-tests (paired design) were used to assess significant differences ($P < 0·05$) within groups, using

each fish as its own control. A non-paired *t*-test was used for between-group comparisons.

## RESULTS

### *Blood acid–base status*

*Acid infusion*

Intra-arterial infusion of HCl resulted in a pronounced arterial blood acidosis, with plasma pH maximally depressed 5 min after infusion (Fig. 1A). The recovery of blood pH following acid infusion was rapid, taking only 60 min to reach pre-treatment control levels. When experiments were terminated, after 240 min of recovery, blood pH was slightly greater than the pre-infusion value. The infused acid load led to a mixed respiratory and 'metabolic' acidosis, as indicated by the rapid elevation of $Pa_{CO_2}$ (Fig. 2) and the marked depression of plasma [$HCO_3^-$] (Fig. 3A). The metabolic component dissipated quickly, as plasma [$HCO_3^-$] returned to rest levels by 30 min. However, over the same period the respiratory component of the acidosis prevailed, as reflected by the significantly higher than normal $Pa_{CO_2}$ levels

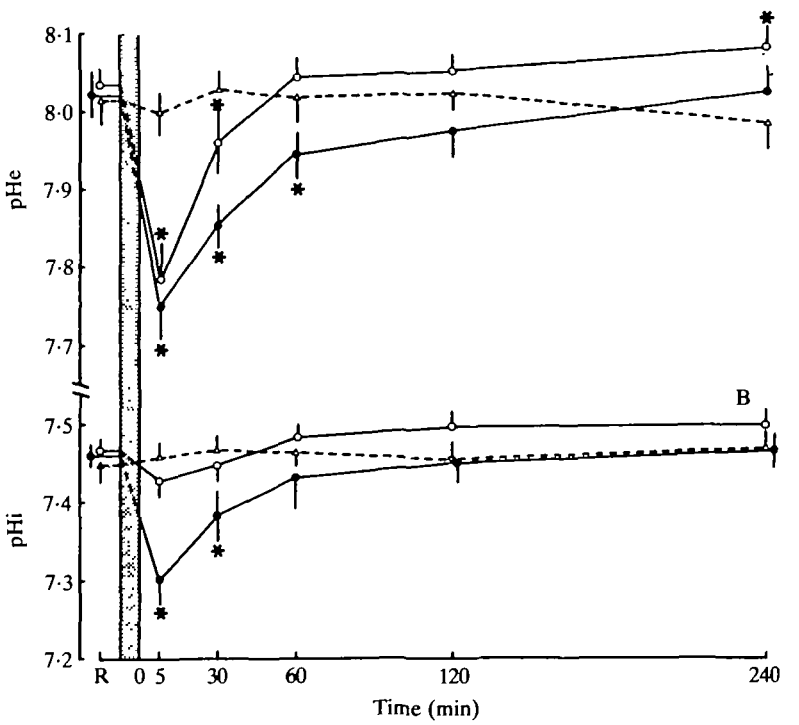


Fig. 1. Effects of acid infusion (○), propranolol plus acid infusion (●) and sham injection (△) on (A) arterial plasma pH (pHe) and (B) red cell pH (pHi). Means ± 1 S.E.M. Experimental group, $N = 8$; sham injection group, $N = 6$; R = rest, bar indicates period of infusion, 0 = immediately after infusion; * indicates a significant difference ($P < 0{\cdot}05$) from rest. Temperature, 10°C.

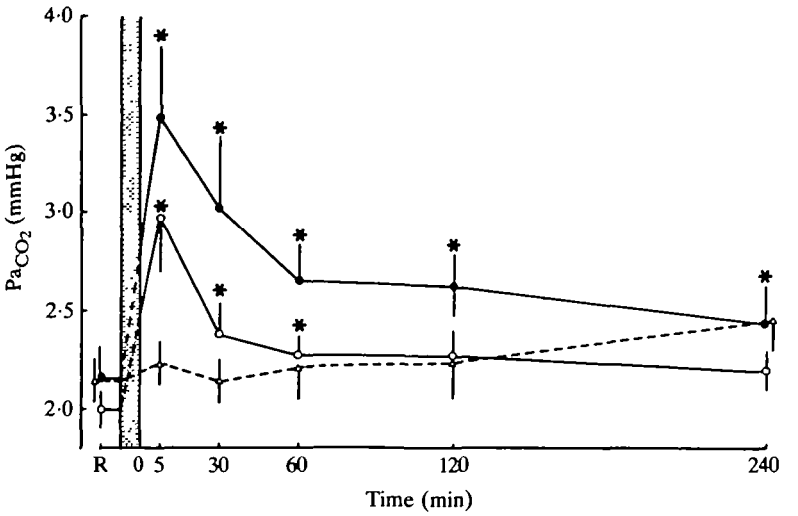


Fig. 2. Arterial $CO_2$ tension ($Pa_{CO_2}$) following acid infusion, propranolol plus acid infusion, and sham injection. Statistical testing for $Pa_{CO_2}$ differences between acid-infused and propranolol plus acid-infused animals revealed that there were significant differences ($P < 0{\cdot}10$) at 30, 60 and 120 min post-infusion. Other details as in Fig. 1.

(Fig. 2). The recovery of blood pH over 60 min could be attributed primarily to the continuing elevation of plasma [$HCO_3^-$]. At 60, 120 and 240 min post-infusion, plasma [$HCO_3^-$] was significantly elevated above the resting level, causing the observed alkalosis at these times (Figs 1, 3), despite the slow branchial washout of $CO_2$ (Fig. 2). The pH–[$HCO_3^-$] diagram (Fig. 4) illustrates the partitioning of the initial acidosis and the subsequent mode of recovery.

Following acid-infusion, the small but associated perturbations in red cell pH and [$HCO_3^-$] were not significantly different from pre-treatment values (Figs 1B, 3B).

*Acid infusion with propranolol*

Propranolol injection followed by acid infusion caused an extracellular acidosis similar in magnitude to that seen following acid infusion alone. However, the recovery of pHe in $\beta$-blocked animals was significantly slower than in the untreated animals (Fig. 1A), taking 4 h to reach pre-treatment levels (3 h more than the untreated animals). This prolonged recovery was also evident in the restoration of plasma bicarbonate concentrations in $\beta$-blocked animals (Fig. 3A). Associated with the decline in pHe was a sharp rise in $Pa_{CO_2}$, similar to the pattern seen in untreated animals (Fig. 2). The magnitude of the changes in $\beta$-blocked animals were slightly higher than in animals infused with acid alone (Fig. 4). The extracellular acidosis in $\beta$-blocked animals induced a significant drop in red cell pH (Fig. 1A), falling 0·16 units below that of pre-infusion levels 5 min following acid infusion, and gradually returning to control values within 2 h. Associated with the red cell disturbance was a similar change in red cell [$HCO_3^-$] (Fig. 3B) which was depressed

significantly 5 min following acid infusion and, thereafter, gradually increased over the remainder of the experimental recovery period.

*Saline infusion*

Control experiments were conducted to examine the influence of the saline vehicle on blood and water parameters. These experiments were performed under conditions identical to those for the experimental groups. Of the variables that were measured, none showed significant changes over the entire experimental period (Figs 1, 2, 3).

*Net ammonia and proton fluxes across the gills*

In both the control group and the pre-infusion period of the experimental group, a net $H^+$ excretion of 17–103 $\mu$mol $kg^{-1}\,h^{-1}$ occurred as a result of net ammonia release at a rate of 270–327 $\mu$mol $kg^{-1}\,h^{-1}$ and net bicarbonate release ($OH^-$ release, or equivalent $H^+$ uptake) of 167–262 $\mu$mol $kg^{-1}\,h^{-1}$. These rates did not change

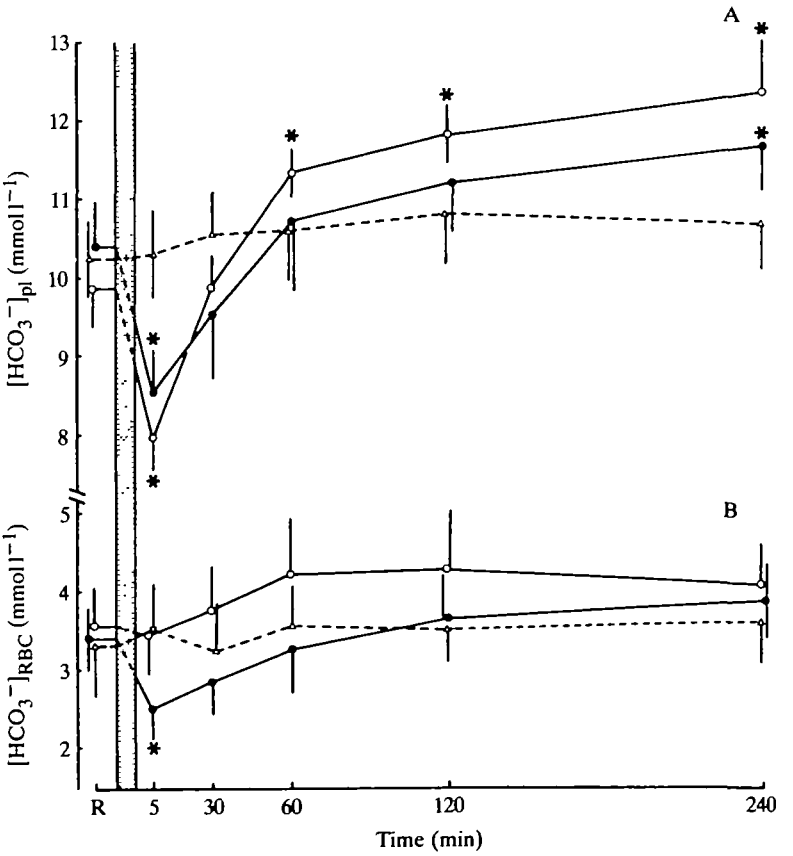


Fig. 3. Changes in (A) plasma $[HCO_3^-]$ and (B) red cell $[HCO_3^-]$ following acid infusion, propranolol plus acid infusion, and sham injection. Other details as in Fig. 1.

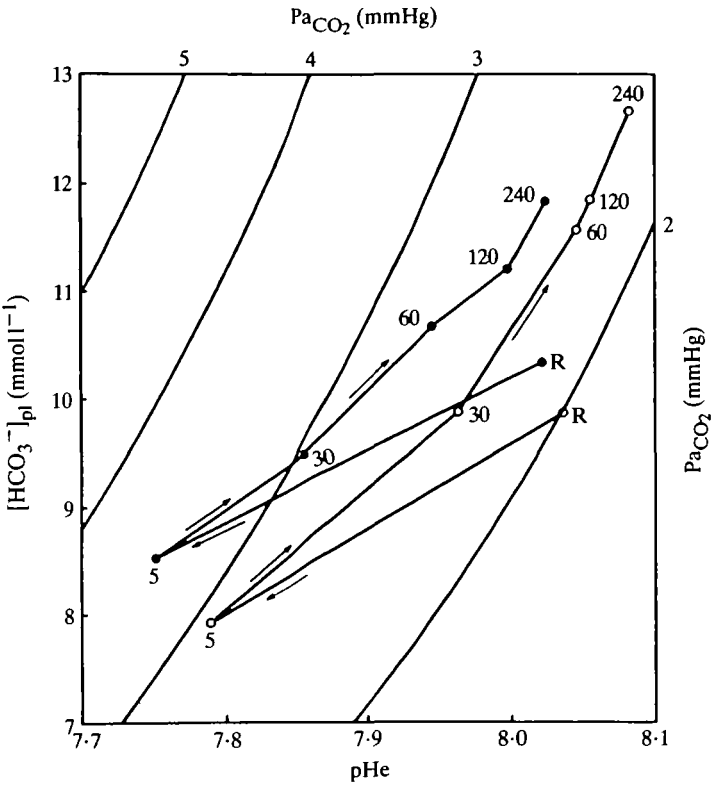


Fig. 4. pH–$[HCO_3^-]$ diagram showing changes in blood acid–base status following infusion of either acid (○) or propranolol plus acid (●). Times (in min) of later blood samples are indicated adjacent to each data point. R = rest.

significantly following the sham injection in the control group (Fig. 5A). Upon infusion of acid, however, net $H^+$ excretion rose significantly in the first 2 h (Fig. 5B). The response consisted of an immediate significant increase in net bicarbonate influx and a small but non-significant increase in ammonia efflux (Fig. 5B). Acid infusion following propranolol treatment induced similar changes in the above parameters (Fig. 5C). However, as indicated by an unpaired Student's *t*-test of the corresponding values, propranolol-treated fish excreted significantly less $H^+$ (as compared with untreated fish) in the first hour following acid infusion ($P < 0{\cdot}05$). This difference in $H^+$ efflux was primarily due to the discrepancy in bicarbonate equivalent flux between the two groups (Fig. 5B,C).

## DISCUSSION

### *Catecholamines and red cell pH*

Acute extracellular acidoses, whether of endogenous or exogenous origin, cause an increase in the circulating levels of catecholamines in salmonid fish, with the release

of the catecholamine adrenaline being proportional to the fall in blood pH (Primmett *et al.* 1986; Boutilier *et al.* 1986). It has also been repeatedly observed that the erythrocyte pH of certain teleosts increases when the cells are incubated in the presence of adrenaline (reviewed by Nikinmaa, 1986). Indeed, red cell pH is regulated at a constant level *in vivo* during periods of acute extracellular acidosis brought on by exercise (Primmett *et al.* 1986), acid infusion (Boutilier *et al.* 1986), hypercapnia (Perry, 1986) and hypoxia (Boutilier, Dobson, Hoeger & Randall, 1987). According to the relationship between the change in plasma adrenaline and the change in blood pH reported by Boutilier *et al.* (1986), the acidosis in the present study (0·25 and 0·27 units drop in mean blood pH) should have promoted an approximately 10-fold increase in circulating adrenaline levels.

The exogenously induced extracellular acidosis of the present experiments was not transmitted to the erythrocyte, whose pH was regulated instead at a constant level in the face of changes in plasma pH (Fig. 1). As indicated by the decline in erythrocyte

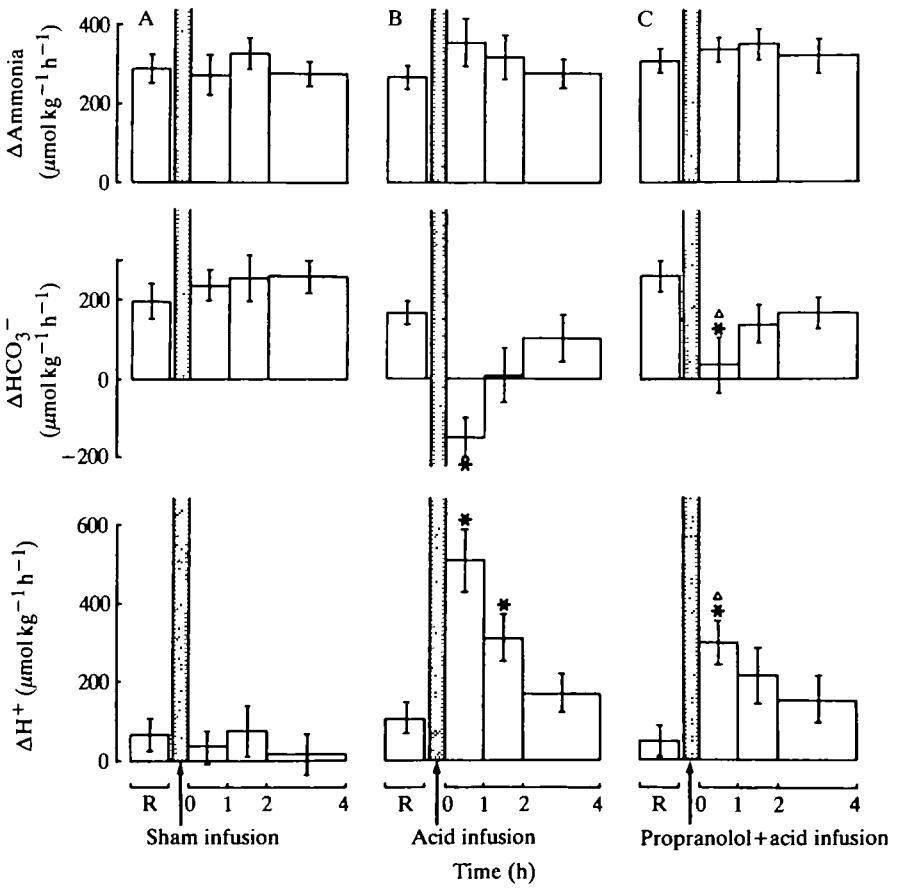


Fig. 5. Net ammonia, bicarbonate and $H^+$ fluxes in sham-injected (A), acid-infused (B) and propranolol plus acid-infused animals (C). Δ indicates a significant difference ($P < 0{\cdot}05$) from corresponding value for fish infused with acid alone. Other details as in Fig. 1.

pH during the acute plasma acidosis of animals treated with propranolol, the regulation of red cell pH appears to be *via* $\beta$-adrenergic mechanisms.

### *Extracellular acid–base status*

The titration of blood bicarbonate immediately following the acid infusion resulted in a decline in plasma [$HCO_3^-$] and an associated rise in $Pa_{CO_2}$ (Figs 2, 3, 4). A short-lived respiratory acidosis following strenuous exercise is a phenomenon observed in all fish species examined to date (reviewed by Wood & Perry, 1985). Heming (1984), Wood & Perry (1985) and Perry (1986) reported that $\beta$-adrenergic stimulation inhibits bicarbonate flux through trout red cells *in vitro*, and that this could, under certain circumstances, lead to a transient reduction or inhibition of $CO_2$ excretion *in vivo*. Since adrenaline is released to the circulation during acute acidoses (Boutilier *et al.* 1986), it follows that the acid-infused animals in the present study might be expected to have incurred an even greater respiratory acidosis than their $\beta$-blocked counterparts. On the contrary, our data indicate that the respiratory acidosis in the propranolol-treated group is slightly greater than that of the untreated acid-infused animals (Fig. 2). Indeed, the sustained $Pa_{CO_2}$ elevation appears to play an important role in the much longer term acidosis of $\beta$-blocked animals (Fig. 1). Similar results between propranolol *versus* untreated animals have also been observed in seawater-adapted trout following exhaustive exercise (Y. Tang & R. G. Boutilier, unpublished results). Certainly, these data indicate that adrenaline probably enhances overall $CO_2$ excretion as demonstrated by the comparatively greater build-up of $Pa_{CO_2}$ in the propranolol-treated animals (Fig. 2). Although many studies have shown that elevated levels of adrenaline stimulate $O_2$ uptake across the gills of fish (Petterson, 1983; Perry, Payan & Girard, 1984; Peyraud-Waitzenegger, 1979), far less is known about $CO_2$ exchange. Even so, factors such as adrenaline which enhance $O_2$ uptake should simultaneously favour $CO_2$ flux in the opposite direction. To attribute such effects to the actions of the red cells themselves would be over simplistic. Clearly, several limitations apply to whole-animal studies of this kind and the conclusions drawn must be rather speculative. For instance, administration of a $\beta$-blocker to an intact animal may evoke numerous effects, including changes in ventilation, cardiac output, patterns of blood flow through the gill, or modulation of membrane/epithelial permeabilities and/or transporter functions (Randall & Daxboeck, 1984; Evans, 1986). Any or all of these effects may modify the response of the animal and lead to the differences in $Pa_{CO_2}$ and pH observed between $\beta$-blocked and unblocked animals (Figs 1–5).

### *Transepithelial $H^+$ and ammonia excretion*

Proton equivalent exchanges with the environment have been examined in freshwater trout following exercise (Holeton *et al.* 1983; Milligan & Wood, 1986), during continuous adrenaline infusion (Vermette & Perry, 1987) and in seawater-adapted fishes subjected to acid infusion (Evans, 1982; McDonald, Walker, Wilkes & Wood, 1982; Fig. 5) or hypercapnia (Evans, 1982; Toews, Holeton & Heisler, 1983).

All these studies showed that net $H^+$ efflux increased in the face of an extracellular acidosis. Following acid infusion in the present study, trout exhibited a minor but non-significant increase in ammonia excretion and a reversal of bicarbonate flux from net excretion to net uptake, the combined effects of which resulted in an increase in net $H^+$ efflux (Fig. 5B,C). In freshwater trout, large increases in net $H^+$ efflux following exercise could be attributed mostly to a rise in ammonia excretion (Milligan & Wood, 1986). The minor contribution of ammonia to net $H^+$ efflux in the present study may be related to the mode of induction of the acidosis. The exhaustive exercise protocol in Milligan & Wood's study is likely to have induced an increase in nitrogen metabolism in white muscle (Dobson & Hochachka, 1987), resulting in an elevation of ammonia excretion. In the present study, however, the acidosis was exogenously induced, and increased nitrogen metabolism is unlikely under these circumstances. Since excretion of ammonium ions is thought to be performed by carrier-mediated ion transfer mechanisms in exchange for $Na^+$ (Maetz, 1973), an elevated $NH_4^+$ excretion in a marine fish would add to the osmoregulatory load of the $Na^+$ extrusion mechanism. Our results are similar to those of Toews *et al.* (1983), who found a hypercapnia-induced acidosis in the marine teleost fish *Conger conger* was partially compensated by a net uptake of bicarbonate from the sea water, with no increase in ammonia excretion.

The relative importance of gills and kidneys in acid–base regulating processes is not yet well established for fishes. It may be related to species differences as well as to the nature of the acid–base disturbance. For freshwater rainbow trout, Vermette & Perry (1987) reported an increased branchial acid efflux and renal acid excretion during continuous adrenaline infusion, accounting for 85 % and 15 % of total acid efflux, respectively. Kobayashi & Wood (1980) found that only a few percent of an infused lactate load was excreted renally. Wood & Caldwell (1978), however, reported that all of an infused HCl load was excreted renally. Although renal acid efflux was not examined in the present study, considering the relatively small throughput of urine in marine teleosts, a significant renal contribution to acid excretion in our seawater-adapted fish would not be expected. Therefore, the results presented here (Fig. 5) suggest an important role for adrenaline in facilitating branchial extrusion of acid equivalents to the external environment. Lack of a renal response to acid challenge has been reported for the marine teleost *Parophrys vetulus* (McDonald *et al.* 1982) and a marine elasmobranch *Scyliorhinus stellaris* (Heisler, 1980). Evans (1982) has also reported that all the $H^+$ efflux subsequent to mineral acid loading is *via* branchial pathways in the marine teleost *Opsanus beta*.

Our results indicate that the elevation in net $H^+$ excretion following acid infusion is facilitated by adrenaline, as demonstrated by the 37 % reduction in net $H^+$ efflux in the propranolol-treated animals (Fig. 5). To calculate the net clearance of proton equivalents from acidotic fish to environmental water, we assumed the average $H^+$ excretion of the sham-treated animals (Fig. 5A) to be representative of basal $H^+$ excretion following infusion. Therefore, for each interval concerned, the 'sham' values were subtracted from the corresponding post-infusion measurements in each treatment group (i.e. Fig. 5B,C). When this was done, we found that the fish

apparently 'overcompensate' for the infused acid load. Thus, whereas 25 $\mu$mol $100\,g^{-1}$ of acid was infused, 86 $\mu$mol $100\,g^{-1}$ was excreted in the 0–4 h post-infusion period in the acid-infused fish and 54 $\mu$mol $100\,g^{-1}$ in those treated with propranolol. Whether this excess $H^+$ clearance represents the stimulation of a secondary metabolic acidosis or is related to a bicarbonate uptake mechanism is not yet known. Regardless, the overcompensation of the infused acid load is not observed in all species.

In the freshwater catfish, *Ictalurus punczatus*, only 20–30 % of an infused acid load was subsequently cleared by the gills over 2 h; after that time there was little further excretion (Cameron, 1980). Such results imply that the acid load can be buffered intracellularly and slowly cleared from the body over a prolonged period. However, the amount of $H^+$ excreted over the first 4 h following acid infusion in the marine species *Opsanus beta* and *Squalus acanthias* was approximately twice that of the infused load (Evans, 1982), similar to the results presented here. McDonald *et al.* (1982) also found that the marine teleost, *Parophrys vetulus*, was able to clear an infused acid load by 5 h in one group and by 16 h in another. In the present study, the continuation of acid excretion beyond the point at which the extracellular acidosis is corrected (Fig. 5 *versus* Fig. 1) evidently accounts for the eventual overshoot of arterial pH (Fig. 4) by 2–4 h post-infusion. It is noteworthy that the overshoot only occurred in the unblocked acid-infused animals (Fig. 4). Such overcompensation may be aimed at restoring bicarbonate concentrations in all parts of the $CO_2$ storage system (e.g. Fig. 3).

### *Control mechanisms for acid–base regulation*

There are two possible ways that adrenaline could effect branchial $H^+$ efflux. First, adrenaline may affect the ionic exchange systems. Beta-adrenergic stimulation of branchial $Na^+/H^+(NH_4^+)$ exchange with simultaneous inhibition of $Cl^-/HCO_3^-$ exchange has been reported for the isolated perfused head preparation of freshwater trout (Payan & Girard, 1978; Perry *et al.* 1984). In a recent study, Vermette & Perry (1987) found that adrenaline infusion in freshwater trout caused a significant elevation of the arithmetic difference between $Na^+$ and $Cl^-$ net fluxes, and that a significant correlation existed between this difference and net branchial $H^+$ excretion. This kind of effect is more difficult to approach experimentally in seawater-adapted fish. Second, adrenaline may affect the ionic fluxes by modulating gill permeability. For example, Isaia, Maetz & Haywood (1978) demonstrated that there was an increase in gill permeability to water in response to catecholamines. This indicates that there may be a general increase in permeability to ions as well.

Since transepithelial ion exchange mechanisms at the gill are the primary way in which fish regulate acid–base status (Heisler, 1984), their utility in responding to an acidosis may be limited by the concentrations of $Na^+$ and $Cl^-$ in the external medium. Obviously, marine fishes are not faced with such limitations. Comparisons of pH compensation following environmentally induced hypercapnic acidoses in marine fish (Toews *et al.* 1983; Heisler, Weitz & Weitz, 1976) and freshwater trout (Eddy, Lomholt, Weber & Johansen, 1977; Janssen & Randall, 1975; Perry,

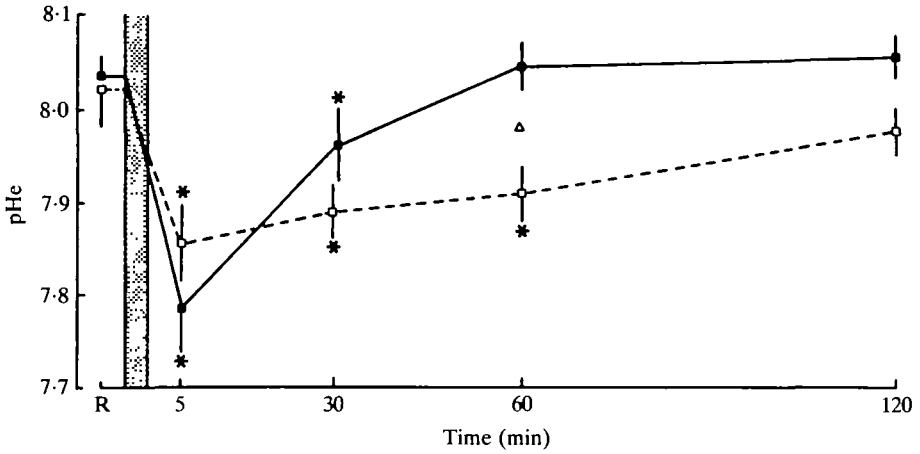


Fig. 6. Changes of arterial plasma pH (pHe) following HCl infusion in seawater-adapted rainbow trout (solid line) ($N = 8$, present study) and freshwater-adapted rainbow trout (dashed line) ($N = 14$, Boutilier, Iwama & Randall, 1986). * indicates a significant difference ($P < 0{\cdot}05$) from rest. △ indicates a significant difference ($P < 0{\cdot}05$) from corresponding freshwater-adapted animals. Other details as in Fig. 1.

Haswell, Randall & Farrell, 1981) show that pH compensation takes longer and is less complete in fresh water than in water of greater ionic strength or sea water. Recently, Iwama (1986) also demonstrated that water concentrations of $Na^+$ and $Cl^-$ have a positive effect in correcting the extracellular acidosis in trout acclimated to higher salinities as well as in marine conger eel conditioned to dilute waters. It is evident that this phenomenon also occurs in pH regulation of trout subjected to acid infusion (Fig. 6). In the present study with seawater-adapted trout, pHe recovered by 1 h following acid infusion. However, in a very similar investigation with freshwater-adapted trout (Boutilier *et al.* 1986), the restoration of pHe took at least 2 h. The faster pH compensation in the seawater-adapted animals might also be explained by the difference in concentrations of $Na^+$ and $Cl^-$ in external water as discussed above. However, for seawater-adapted fish, additional factors may be involved in this faster pH compensation. First, sea water contains higher concentrations of bicarbonate than fresh water. This buffer pool may provide bicarbonate for fish during acidosis *via* a 180°-rotated $Cl^-/HCO_3^-$ exchange system across the gill epithelium of marine fish. This rotated $Cl^-/HCO_3^-$ exchange is indicated by the finding that $Cl^-$ efflux from the marine toadfish is increased by increasing external $[HCO_3^-]$ (Kormanik & Evans, 1979). Second, since the branchial epithelium of marine fishes is decidedly more permeable to ions than that of freshwater fishes (Evans, 1979), the relatively higher permeabilities for marine fishes may necessitate that their ionic exchange systems should be more effective than those of their freshwater counterparts. In addition, the hyporegulating marine fish probably faces greater ionoregulatory pressures than the hyper-regulating freshwater fish, since the osmolality of fish plasma is about one-third that of sea water. Therefore, marine fish

may be better equipped to regulate those ions which are related to acid–base regulation.

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