

THE EFFECTS OF PROLONGED EPINEPHRINE INFUSION ON THE PHYSIOLOGY OF THE RAINBOW TROUT

SALMO GAIRDNERI

III. RENAL IONIC FLUXES

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SUMMARY

Rainbow trout were infused continuously for 24 h with epinephrine in order to evaluate the effects of elevated circulating levels of epinephrine on selected renal variables. Pronounced effects of epinephrine included elevation of urine flow rate and concomitant increases in the excretion of all measured electrolytes (Na^+ , Cl^- , K^+ , Ca^{2+} , inorganic phosphate) with the exception of ammonium and bicarbonate ions. Significant reductions in the tubular reabsorption of Na^+ and Cl^- also contributed to enhanced excretion of these ions. Similarly, epinephrine affected the tubular handling of NH_4^+ and HCO_3^- with NH_4^+ secretion decreasing and HCO_3^- reabsorption increasing. We speculate that the stimulation of HCO_3^- reabsorption was a consequence of elevated tubular H^+ secretion. Such a mechanism may be important to permit plasma HCO_3^- retention during periods of internal acidosis. The results are discussed with reference to the role of the fish kidney in regulating acid–base disturbances and the possible interactive effects of elevated epinephrine.

INTRODUCTION

Dynamic manipulations of both branchial and renal acid excretory mechanisms have been postulated as important means of acid–base regulation in fish (see reviews by Cameron, 1978; Heisler, 1984). Indeed, direct modulation of these pathways during acid–base disturbances was demonstrated. The magnitude of the branchial/renal partitioning of acid or base excretion during correction of acid–base disturbances, however, has not been clearly established and may be related to species differences as well as to the nature of the acid–base disturbance (Wood & Caldwell, 1978; Cameron, 1980; Kobayashi & Wood, 1980; McDonald & Wood, 1981; Cameron & Kormanik, 1982; McDonald, Walker, Wilkes & Wood, 1982; Holeyton & Heisler, 1983; Høbe *et al.* 1983; Wheatly, Høbe & Wood, 1984). The variable nature of the involvement of the fish kidney in acid–base regulation is indicated by reports of complete renal compensation (Wood & Caldwell, 1978), minor renal

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compensation (Wheatly *et al.* 1984) or complete lack of renal compensation (Evans, 1982) following imposed internal acid loads. Regardless of the contribution of the kidney to whole body acid excretion, it is likely to play an important role in preventing filtered HCO_3^- from being excreted in the urine by linking acid secretion to HCO_3^- reabsorption (Wood & Jackson, 1980; Wheatly *et al.* 1984) as in mammals (Pitts, 1974). The prevention of urinary HCO_3^- loss is crucial for successful acid–base regulation in fishes because of the reliance on plasma $[\text{HCO}_3^-]$ adjustments to correct pH disturbances (Heisler, 1984).

Perturbations of blood acid–base status induced by external hypercapnia (S. F. Perry, S. Malone & D. Ewing, in preparation), intravascular acid infusion (Boutilier, Iwama & Randall, 1986), exhausting exercise (Opdyke, Carrol & Keller, 1982; Primmatt, Randall, Mazeaud & Boutilier, 1986) or acute hypoxia (Fievet, Motais & Thomas, 1986) have been shown to cause elevated plasma levels of epinephrine. Such elevations of epinephrine are thought to be involved in stabilizing red blood cell (RBC) pH and hence arterial O_2 content in the face of plasma pH reductions (Nikinmaa, 1982; Nikinmaa & Huestis, 1984; Nikinmaa, Cech & McEnroe, 1984; Boutilier *et al.* 1986; Primmatt *et al.* 1986; Perry & Vermette, 1986) and appear to interact with branchial acid extrusion mechanisms to enhance net acid excretion (Vermette & Perry, 1986), thereby elevating plasma pH. The interactions of epinephrine with renal ionic and acid–base excretory mechanisms, however, have not been evaluated. In the present investigation, we have conducted a detailed examination of the effects of continuous intra-arterial epinephrine infusion on renal excretion of electrolytes and acid–base equivalents in order to assess the role of epinephrine in controlling renal compensation of acid–base disturbances.

MATERIALS AND METHODS

The experimental conditions and treatment of animals before and during experimentation were as described in Perry & Vermette (1986). Fish were fitted with dorsal aortic cannulae and urinary catheters and only the fish in which both cannula and catheter were patent were used for experimentation. Continuous urine collection for the duration of the experiments was accomplished by allowing the urinary catheters to drain, by gravity, into plastic vials located below the Perspex holding boxes.

Urine was collected during a preliminary 3-h saline infusion period; during a 24-h epinephrine or saline infusion period (between 0–3 h, 3–6 h, 6–9 h, 9–21 h and 21–24 h) and during a final 12-h period of post-epinephrine saline infusion (0–3 h, 3–6 h, 6–9 h and 9–12 h). Urine samples were analysed immediately upon collection for pH, total CO_2 (C_{CO_2}) and volume and were then diluted ten times, acidified (1% v/v) with 1 mol l^{-1} nitric acid and left frozen at -20°C until further assays could be performed. Samples then were thawed and total ammonia, phosphate, Na^+ , Cl^- , K^+ and Ca^{2+} concentrations were determined.

Urine from a separate group of five fish was titrated immediately upon collection to determine the titratable component ($\text{TA} - \text{HCO}_3^-$) and the non-titratable component (NH_4^+) of urinary acid excretion. Total urinary acid excretion was calculated as the sum of urinary $[\text{TA} - \text{HCO}_3^-]$ and total [ammonia] multiplied by urine flow rate (UFR).

Electrolyte analysis

Urine pH was measured using a microcapillary pH electrode (Radiometer G299A) in conjunction with a Radiometer PHM 71 acid-base analyser and BMS3 MK2 blood micro system. Carbon dioxide was measured by injecting evolved gas into a gas chromatograph (Carle AGC Series 100) following acidification of 100 μl of urine with 2 ml of 1 mol l^{-1} HCl in a gas-tight syringe (Hamilton) that had previously been purged with nitrogen (ultra-high purity). The total ammonia concentration of urine was determined using a micro-modification of the salicylate-hypochlorite technique (Verdouw, vanEchteld & Dekkers, 1978). Urine phosphate and calcium levels were measured using commercially available kits (Sigma). Potassium and sodium concentrations were determined by flame photometry (EEL Flame Photometer) and chloride concentrations were measured by amperometric titration (Buchler-Cotlove Chloridometer).

Urinary $[\text{TA} - \text{HCO}_3^-]$ was measured by adding a known volume of 0.2 mol l^{-1} HCl to 200 μl of urine in order to lower the final pH below 5.0, then vigorously aerating and agitating the sample for 15 min to remove CO_2 . NaOH was then gradually added using a microburette (Gilmont) to restore urine pH to blood pH representative of the particular sampling period (see Perry & Vermette, 1986). The difference between the quantities of acid and base added to the urine yielded the titratable component of urinary acid excretion.

Urinary effluxes for ammonia, phosphate, Na^+ , Cl^- , K^+ and Ca^{2+} were taken as the product of urinary concentrations of the electrolytes multiplied by UFR. Renal clearance ratios for these electrolytes (C_x) were calculated using the following equation:

$$C_x = \frac{[\text{X}]_u \times \text{UFR}}{[\text{X}]_p \times \text{GFR}}, \quad (1)$$

where $[\text{X}]_u$ and $[\text{X}]_p$ represent the concentrations of the particular electrolyte in the urine and plasma, respectively (for plasma concentrations, see Perry & Vermette, 1986), and glomerular filtration rate (GFR) is assumed to be $1.77 \times \text{UFR}$ (Holmes & Stainer, 1966). A linear relationship between UFR and GFR has been demonstrated (Hoffmann & Butler, 1978); thus the conversion factor of 1.77 is assumed to be constant over the range of experimental urine flow rates.

Statistical analysis

Due to variability between the control and experimental groups (although not statistically different), a lot of the data have been presented as absolute changes from

the initial control or experimental value during the initial period of saline infusion. For simplicity and clarity, only the absolute values of the experimental group have been indicated. The results were statistically analysed by comparing the sample means of control and experimental animals at identical time periods using two-tailed paired and unpaired Student's *t*-tests. 5% was accepted as the fiducial limit of significance.

RESULTS

Considerable differences were observed between control and experimental values during the initial period of saline infusion for many of the measured renal variables. Such differences, although never statistically significant, tended to obscure actual changes during epinephrine infusion. Thus a lot of the data have been presented as absolute changes from the initial saline infusion values. For simplicity, in such instances, only the absolute pre-epinephrine infusion values from the experimental group have been reported.

An immediate and pronounced effect of epinephrine infusion was a transient increase in urine flow rate (UFR) that returned to normal levels following 9 h of infusion (Fig. 1). Upon return to saline infusion, there was a significant reduction of UFR. In a similar manner, epinephrine caused a significant depression of urine pH during the first 9 h of administration, whereas return to saline infusion caused urine pH to increase (Fig. 1). Associated with the reduction of urine pH was a short-lived elevation of urinary acid excretion, primarily due to a stimulation of the titratable component, $J_{TA} - J_{HCO_3^-}$ (Fig. 2), although $J_{NH_4^+}$ also increased in a non-significant fashion. Similarly, renal acid excretion was reduced during the post-epinephrine period as a consequence of both altered $J_{TA} - J_{HCO_3^-}$ and $J_{NH_4^+}$ (Fig. 2). Urinary CCO_2 was constant throughout the epinephrine infusion period, but was consistently elevated during the 12-h post-epinephrine period (Fig. 2).

Large and persistent increases in urinary concentrations of Na^+ and Cl^- were observed during epinephrine treatment with $[Cl^-]$ increasing to a greater extent than $[Na^+]$ (Fig. 3). The changes in urine $[Na^+]$ and $[Cl^-]$ were due to decreased tubular reabsorption as indicated by increased renal clearance ratios (Fig. 4). Urine K^+ concentration also increased (Fig. 3), but the renal clearance ratio for K^+ was not significantly elevated except at one time period (between 9–12 h; Fig. 4), suggesting that the increase of urine $[K^+]$ was probably not due to altered tubular functioning, but simply due to the increased K^+ levels in the blood (Perry & Vermette, 1986). Urine Ca^{2+} levels were unaffected by epinephrine infusion, although renal Ca^{2+} efflux was markedly elevated, primarily due to the increase in UFR (Fig. 5). Renal efflux rates of Na^+ , Cl^- and K^+ (Fig. 5) were greatly stimulated due to the combined effects of decreased tubular reabsorption (Na^+ and Cl^- only) and increased UFR. The effects of epinephrine on urinary ionic excretion were most evident during the first 12 h of infusion (Fig. 5).

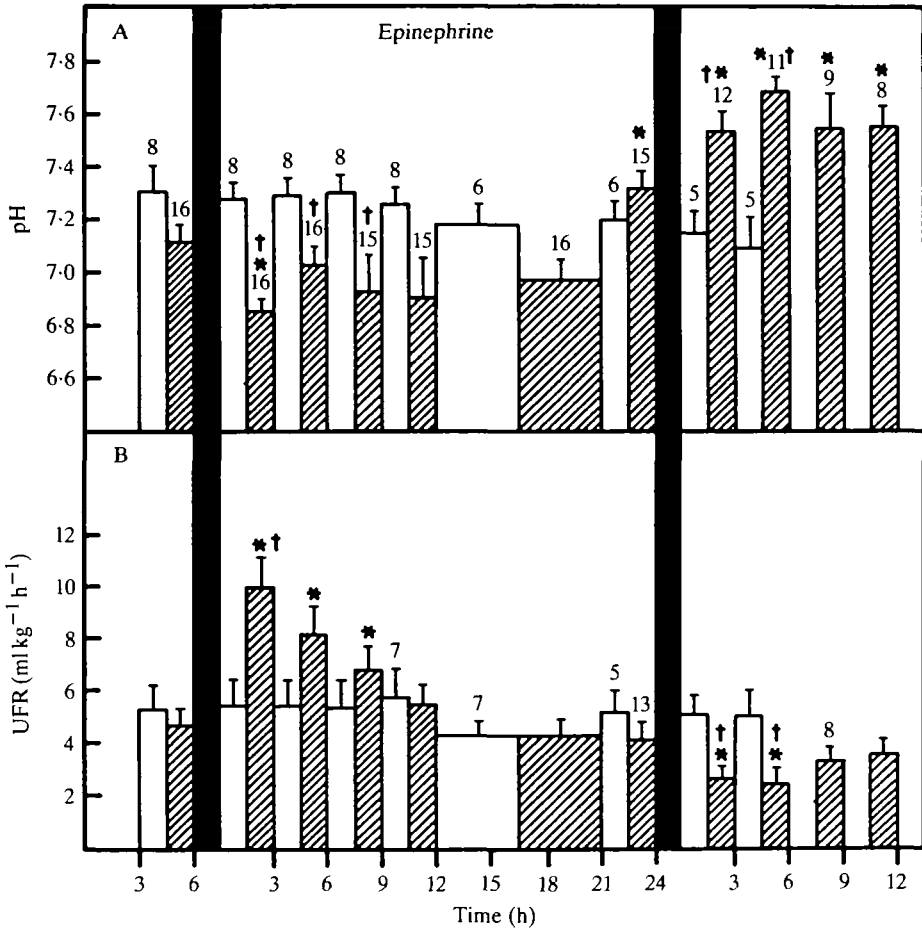


Fig. 1. The effects of prolonged intra-arterial infusion of epinephrine on (A) urine pH and (B) urine flow rate (UFR). Control fish (□) were infused continuously with saline for 42 h while the epinephrine-treated (▨) fish were infused initially for 7 h with saline followed by 24 h of epinephrine infusion and finally 12 h of saline infusion. * Significantly different from pre-epinephrine value; † significantly different from corresponding value in control group. *N* is indicated.

The effects of epinephrine on the renal handling of the two most prevalent urinary buffers, inorganic phosphate (Pi) and ammonia (NH₄⁺), are shown in Figs 6 and 7. Urine Pi levels increased throughout epinephrine infusion (Fig. 6A) but due to the large variability of the data, no individual value could be shown to be significantly different from control values. A statistical comparison between the overall mean value of the epinephrine-treated group and the overall mean value of the control group during this 24-h period, however, proved to be highly significant. During the post-epinephrine period, when urine pH was significantly elevated, urine Pi was greatly reduced due to an increase of tubular Pi reabsorption by a factor of three (Fig. 4). Renal excretion of Pi changed in a similar fashion to urine Pi concentration.

During epinephrine infusion, the urinary concentration of NH_4^+ decreased (Fig. 6), but due to the increase of urine flow rate, total ammonia efflux remained relatively constant (Fig. 7) even though renal tubular secretion of ammonia decreased significantly (Fig. 4).

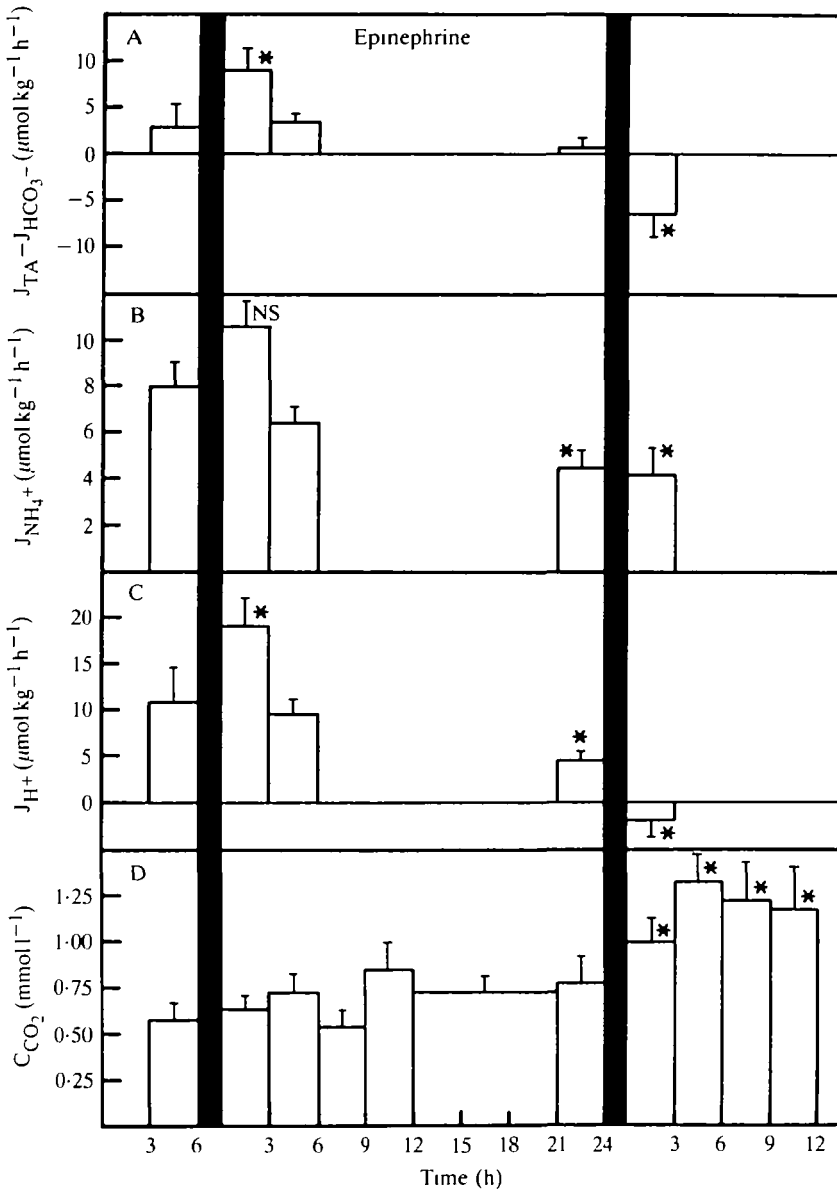


Fig. 2. The effects of prolonged intra-arterial infusion of epinephrine on the renal efflux rates of (A) titratable acid minus bicarbonate ($J_{TA} - J_{HCO_3^-}$; measured as a single component), (B) ammonium ($J_{NH_4^+}$), (C) acidic equivalents (J_{H^+}) and (D) total urinary CO_2 levels (C_{CO_2}). * Significantly different from pre-epinephrine value; $N = 5$ throughout; NS, not significant.

Fig. 8 summarizes the effects of epinephrine on renal electrolyte excretion. It is evident that prior to epinephrine infusion, a state of charge balance existed in the urine with only a minor cationic component of the urine unaccountable (<8%).

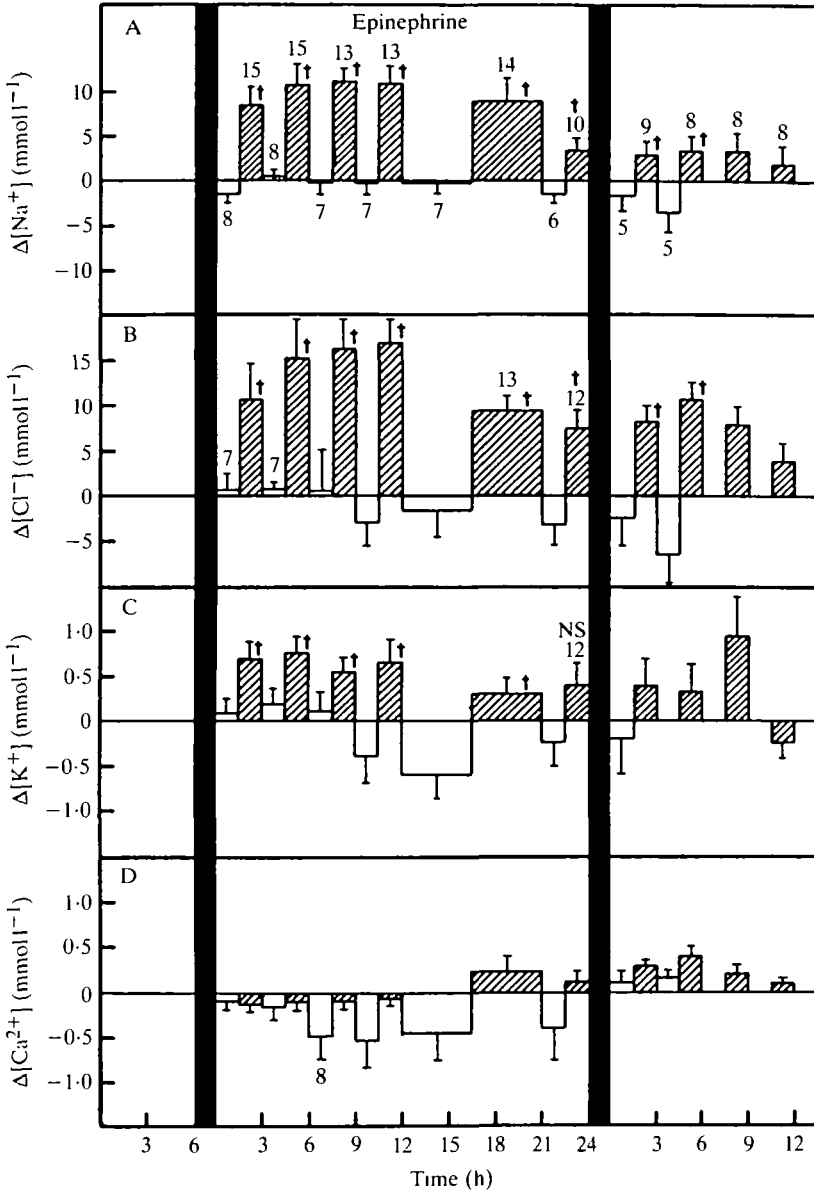


Fig. 3. The effects of prolonged intra-arterial infusion of epinephrine (■) on urinary concentrations of (A) sodium, (B) chloride, (C) potassium and (D) calcium. Data have been plotted as absolute changes from the values during an initial period of saline infusion (sodium 7.40 ± 0.86 , chloride 12.2 ± 2.7 , potassium 1.42 ± 0.16 and calcium $2.20 \pm 0.11 \text{ mmol l}^{-1}$). † Significantly different from corresponding value in control group (□). N is indicated. All other details are as in Fig. 1; NS, not significant.

Epinephrine infusion caused large increases in overall electrolyte excretion; however, the excretion of measured anions increased to a greater extent than measured cations such that charge balance was not possible without the addition of an unknown cation. We speculate that this cation is magnesium.

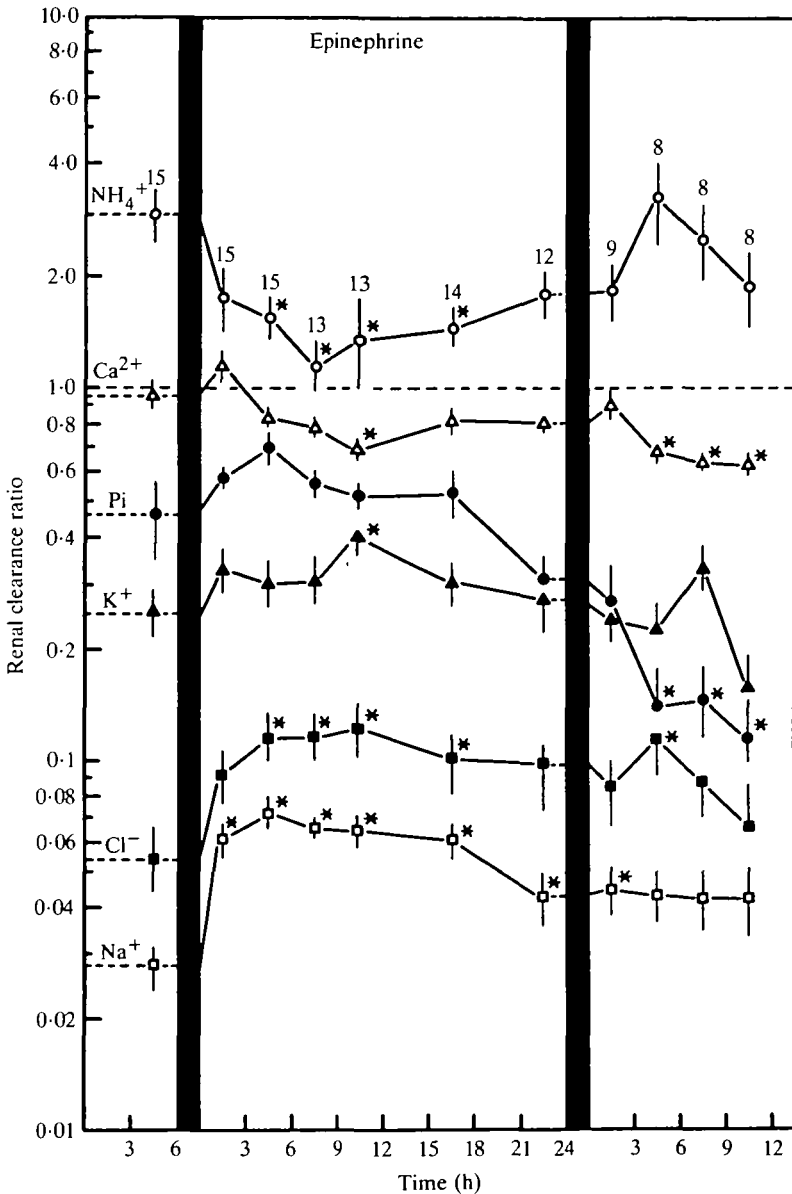


Fig. 4. The effects of prolonged intra-arterial infusion of epinephrine on the apparent renal clearance ratios for major urinary electrolytes. Renal clearance ratios were calculated using an estimated glomerular filtration rate (GFR) of $1.77 \times \text{UFR}$ and have been plotted on a logarithmic scale. * Significantly different from pre-epinephrine value. *N* numbers as indicated for NH_4^+ (top). See text for further details.

DISCUSSION

The values noted in the present study for renal electrolyte and acid excretion in control fish are considerably greater (approximately 1.5–2 times) than those

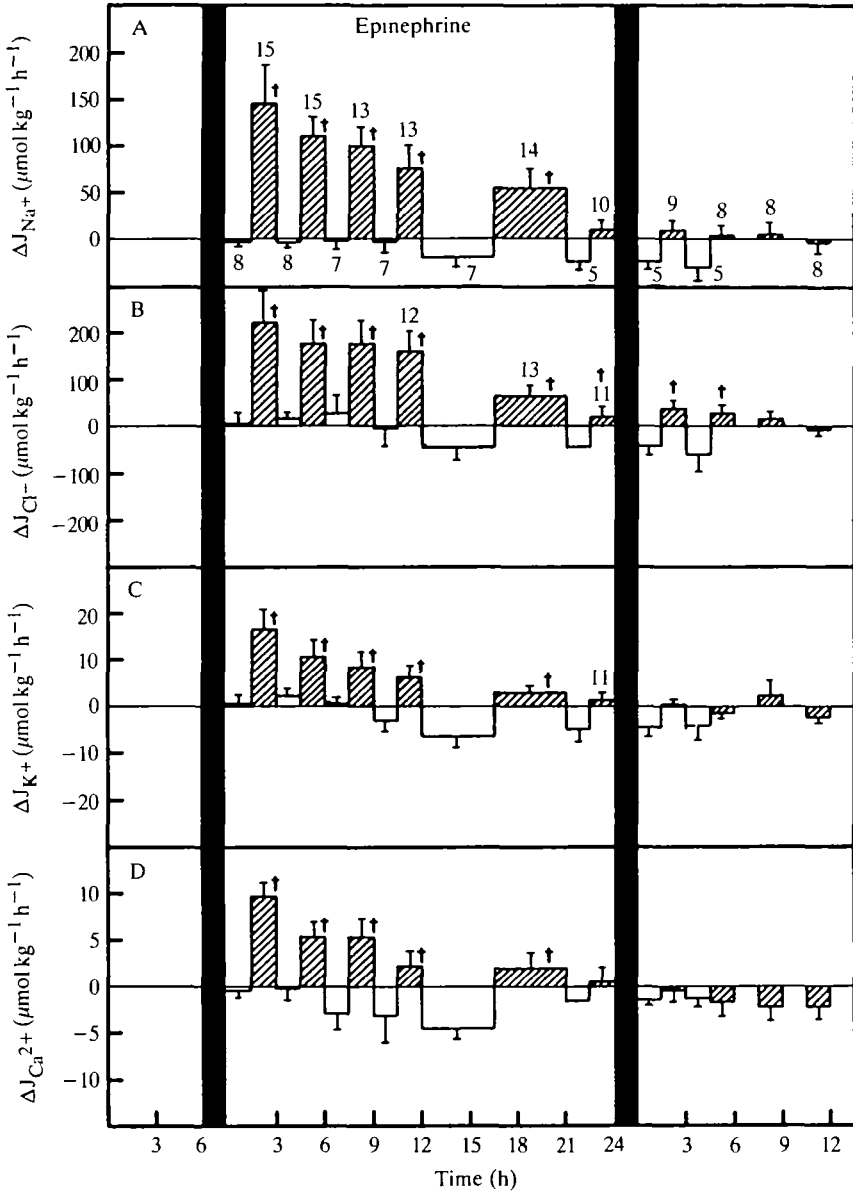


Fig. 5. The effects of prolonged intra-arterial infusion of epinephrine (■) on renal efflux rates of (A) sodium (J_{Na^+}), (B) chloride (J_{Cl^-}), (C) potassium (J_{K^+}) and (D) calcium ($J_{\text{Ca}^{2+}}$). Data have been plotted as absolute changes from the values during the period of initial saline infusion (sodium 36.1 ± 5.8 , chloride 54.5 ± 11.2 , potassium 7.18 ± 1.21 and calcium $10.7 \pm 1.0 \text{ mmol l}^{-1}$). For clarity, only the absolute values of the experimental group are given. † Significantly different from corresponding value in control group (□). *N* is indicated. All other details are as in Fig. 1.

associated with resting and non-stressed rainbow trout (e.g. Wood & Jackson, 1980; McDonald & Wood, 1981; McDonald, 1983; Wheatly *et al.* 1984). These differences were not related to altered tubular reabsorption or secretory mechanisms because

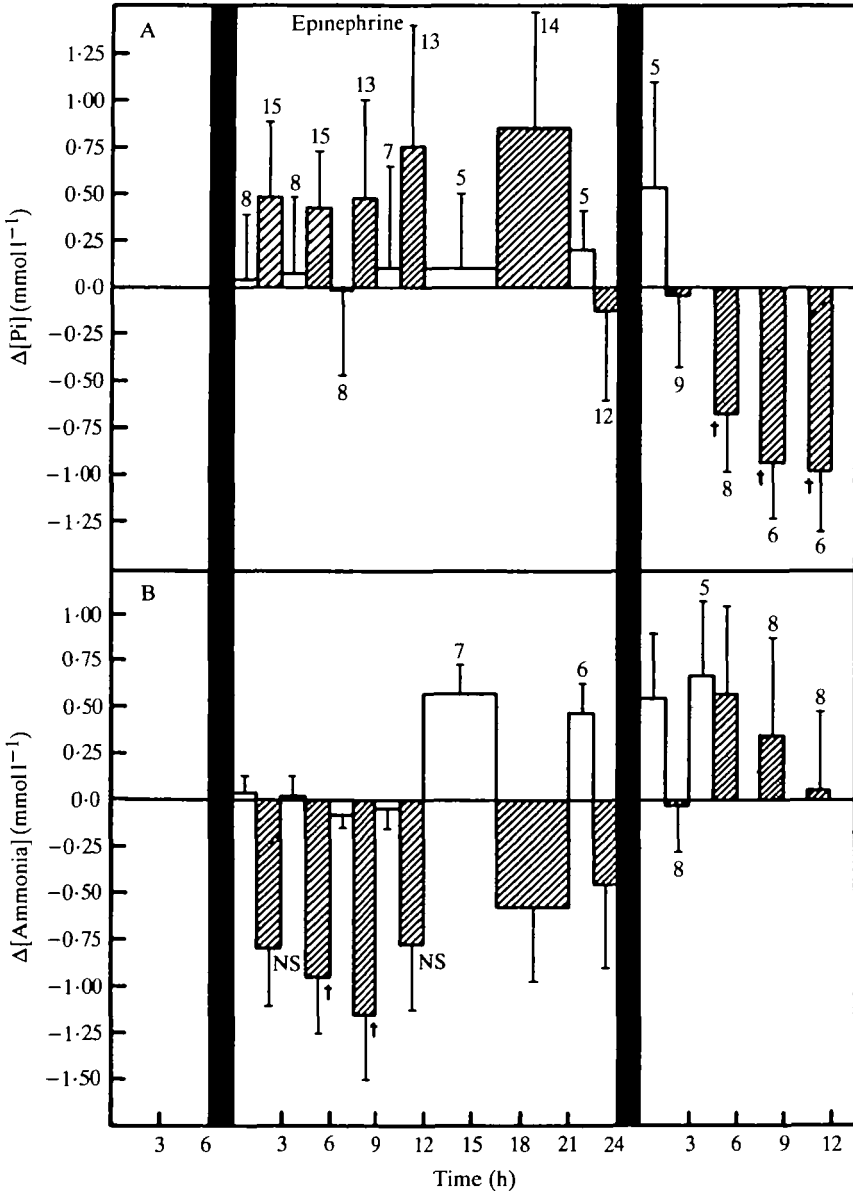


Fig. 6. The effects of continuous intra-arterial infusion of epinephrine (■) on urinary concentrations of (A) inorganic phosphate (Pi) and (B) ammonia. Data have been plotted as absolute changes from the values of the experimental group during the initial saline infusion (Pi 2.21 ± 0.46 and ammonia $2.46 \pm 0.42 \text{ mmol l}^{-1}$). For clarity, only the absolute values of the experimental group during the initial saline infusion are given. † Significantly different from corresponding value in control group (□). N numbers as indicated.

urine electrolyte concentrations and renal clearance ratios were similar to values reported in earlier studies. Instead, it is likely that elevated renal excretion rates resulted from abnormally high urine flow rates (approximately 1.5–2 times greater

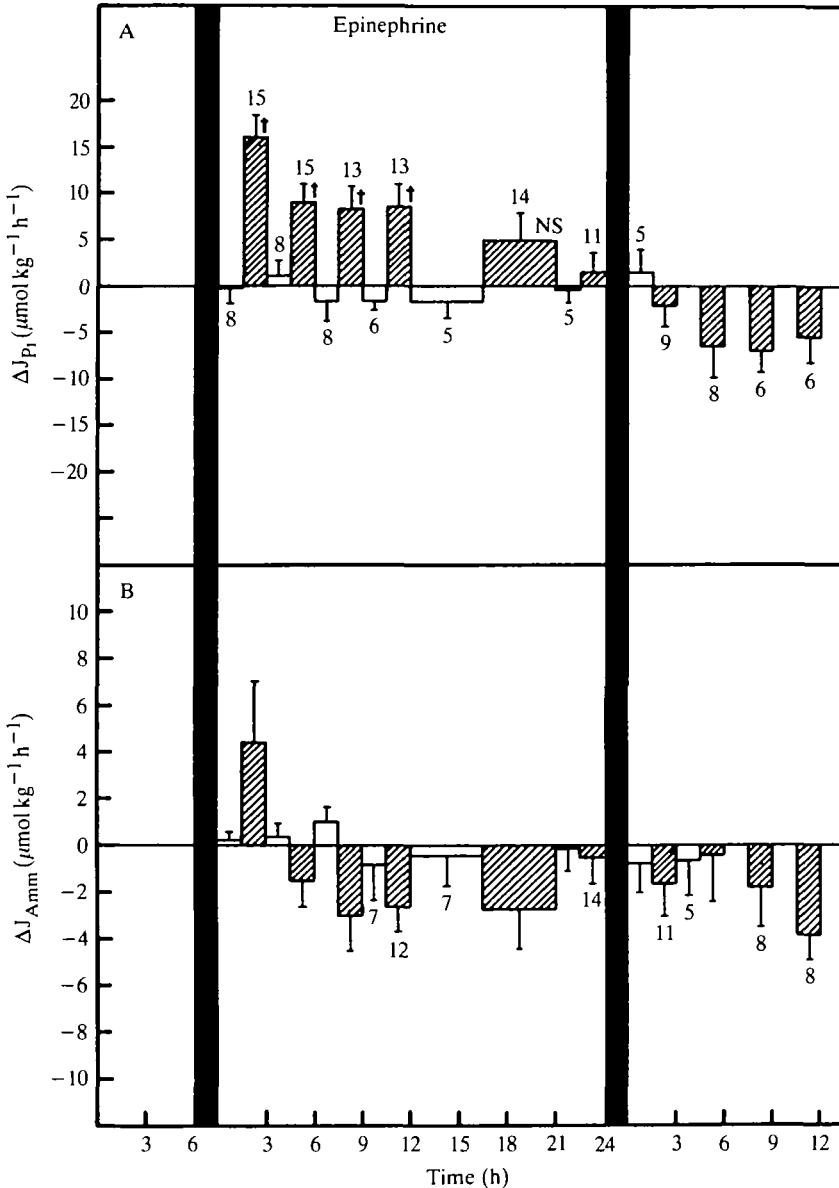


Fig. 7. The effects of prolonged intra-arterial infusion of epinephrine (■) on renal efflux rates of (A) inorganic phosphate (J_{Pi}) and (B) ammonia (J_{Amm}). Data have been plotted as absolute changes from the values during the period of initial saline infusion (Pi 11.50 ± 2.58 and ammonia $11.84 \pm 2.22 \mu\text{mol kg}^{-1} \text{h}^{-1}$). For clarity, only the absolute values of the experimental group during the initial saline infusion are given. † Significantly different from corresponding value in control group (□). N is indicated; NS, not significant.

than usually reported; e.g. Wood & Jackson, 1980; Wheatly *et al.* 1984; Fig. 1) that in turn were a consequence of the saline infusion process. Previous studies (Wood & Caldwell, 1978; Kobayashi & Wood, 1980), as well as our own unpublished observations, have indicated that urine flow rate is increased rapidly by plasma

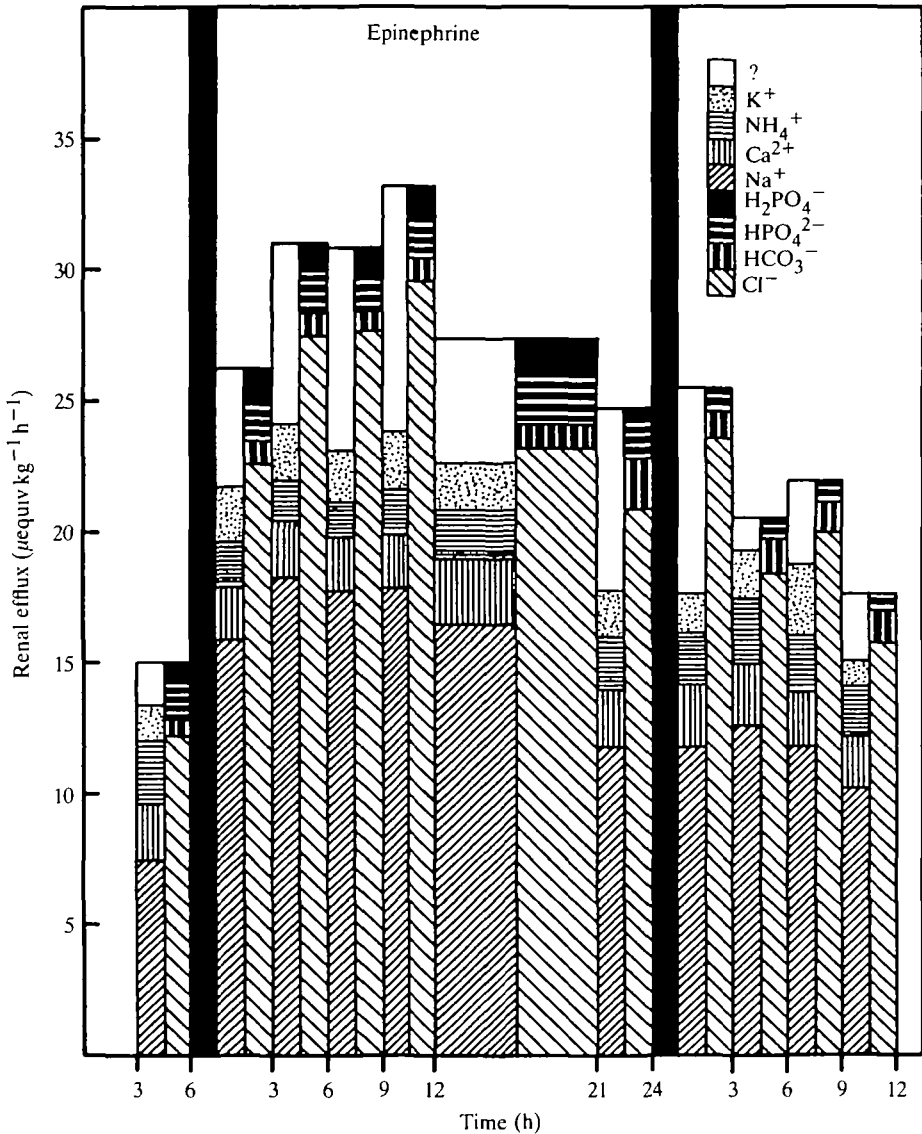


Fig. 8. The effects of prolonged intra-arterial infusion of epinephrine on renal excretion charge balance. $[\text{HCO}_3^-]$ was calculated from $[\text{TA} - \text{HCO}_3^-]$ assuming that H_2PO_4^- accounted for all titratable acidity (TA). HPO_4^{2-} and H_2PO_4^- concentrations were calculated from the Henderson-Hasselbalch equation using the measured levels of total inorganic phosphate, urine pH and a pK value of 6.86. Urine anions and cations were sampled at identical times; note that concentrations are given in $\mu\text{equiv l}^{-1}$. See text for further details.

volume loading following either single intravascular injections or continuous infusion of saline. In the present study, an attempt was made to minimize plasma volume changes by infusing at a low rate (0.6 ml h^{-1}), but clearly even this low rate of infusion was capable of elevating glomerular filtration rate. Thus, we make no claim that renal excretion rates reported here are indicative of true resting values, but nonetheless the methodology employed did allow a valid assessment of the relative effects of elevated plasma levels of epinephrine on renal function.

Further problems associated with analysing urine collected continuously from gas-permeable urinary bladder catheters have been discussed in detail by Cameron & Kormanik (1982). Briefly, these include the inability (i) to collect urine under anaerobic conditions thereby leading to CO_2 loss from the urine and concomitant pH fluctuations and (ii) to assess the role of the urinary bladder in acid-base/ionic regulation since the catheterization technique only enables the collection of continuously voided tubular urine. Of course, under normal conditions, urine resides in the bladder for variable periods of time before being excreted.

Epinephrine induced numerous and pronounced changes in renal function, many of which suggest a role for epinephrine in the control of renal compensation of acid-base disturbances. The epinephrine-induced effects on renal variables that are consistent with compensation of internal acidosis included transient elevations of urine flow rate, phosphate excretion and net acid excretion, as well as depressed urine pH. However, the persistent reductions of urine NH_4^+ levels and NH_4^+ excretion rate that were observed during epinephrine infusion certainly are counterproductive with respect to compensation of internal acidotic conditions, because NH_4^+ , as well as contributing to net acid excretion, is an important urine buffer. These changes in the renal handling of ammonia were related to pronounced reductions in tubular ammonia secretion and may serve to explain why marked reductions in urine pH during the first 9 h of epinephrine infusion caused only minor and very short-lived increases of net acid excretion. The functional significance of decreased ammonia secretion during conditions of elevated epinephrine is unclear but may be related to the constraints of urine charge balance or an attempt to minimize changes in urine buffering capacity during the period of enhanced phosphate excretion (Fig. 7). The increase in renal phosphate excretion was due to the combined effects of elevated urine flow rate, non-significant decreases in tubular reabsorption, and increased renal filtration in the latter stages of epinephrine infusion due to elevated plasma phosphate levels (Perry & Vermette, 1986).

Although renal net acid excretion was not greatly stimulated by epinephrine infusion, this does not preclude a role for epinephrine in renal compensation of acid-base disturbances. Indeed, our results suggest that epinephrine may be important in reducing the amount of filtered HCO_3^- that is actually excreted in the urine. This suggestion is based on the fact that plasma HCO_3^- levels increased significantly during epinephrine infusion (Perry & Vermette, 1986), yet urine HCO_3^- levels remained constant and subsequently increased during the post-epinephrine infusion period (Fig. 2). Thus, it is likely that tubular H^+ secretion did increase during epinephrine administration, without major impact on net acid

excretion, in order to reabsorb the additional HCO_3^- that must have appeared in the glomerular filtrate. Similarly, we speculate that tubular H^+ secretion was diminished upon return to saline infusion, thereby causing urine $[\text{HCO}_3^-]$ to increase and net acid excretion to decrease significantly (Fig. 2). It is important to point out that the prominent role of the freshwater teleost kidney in the compensation of respiratory acidosis is considered to be the prevention of excess filtered HCO_3^- from being excreted (Wood & Jackson, 1980; Wheatly *et al.* 1984; S. F. Perry, S. Malone & D. Ewing, in preparation), thereby allowing plasma HCO_3^- levels to rise until pH is restored. This scheme also necessitates increased tubular H^+ secretion that may, based on our results, be dependent upon elevated epinephrine levels.

The marked increase in urine flow observed during the initial 9 h of epinephrine infusion was probably not due to plasma volume changes as all fish were pre-infused, at identical infusion rates, with saline. However, the possibility of water movements from interstitial to vascular compartments cannot be discounted. Other possible causes of elevated UFR are decreased tubular reabsorption of water and increased systemic blood pressure. We consider the latter to be the most likely possibility given the known effect of single epinephrine injections in raising systemic blood pressure (Wood & Shelton, 1975; Pettersson & Nilsson, 1980; Farrell, 1981) due to α -adrenergic stimulation (see review by Nilsson, 1984). It is interesting that UFR returned to control values during the latter stages of epinephrine infusion and then decreased significantly below control values upon return to saline infusion (Fig. 1). Various other renal variables (pH , $J_{\text{net}}^{\text{H}^+}$, $[\text{Pi}]$, J_{Pi}) responded in a similar manner. These results suggest that it may not be the absolute levels of plasma epinephrine that ultimately affect physiological function but sudden changes in epinephrine levels. Epple & Nibbio (1985) reached a similar conclusion with respect to the catecholaminotropic and glycaemic effects of catecholamines in the eel (*Anguilla rostrata*).

Due to the constraints of charge balance, urine equivalents of anions and cations (valencies considered) must be equal. Given the number of measurements performed and the errors associated with each, we are satisfied that such a balance did exist during the pre-epinephrine, and throughout most of the post-epinephrine, infusion periods (Fig. 8). The minor 'cation gap' that did exist (i.e. $[\text{Cl}^-] + [\text{HCO}_3^-] + [\text{H}_2\text{PO}_4^-] + 2[\text{HPO}_4^{2-}] - [\text{Na}^+] - 2[\text{Ca}^{2+}] - [\text{K}^+] - [\text{NH}_4^+]$) presumably reflected a ratio of unmeasured cations to unmeasured anions that was greater than unity and relatively constant during the saline infusion periods. The principal unmeasured ions were probably magnesium and sulphate. The mechanisms involved in the renal excretion of sulphate and magnesium in freshwater fishes have not been studied in detail, but recently Oikari & Rankin (1985) have demonstrated that trout are capable of either net secretion or net reabsorption of magnesium depending on plasma Mg^{2+} status. Thus, the pronounced increase in the magnitude of the 'cation gap' during epinephrine infusion may have been due to altered renal handling of magnesium.

It has been proposed that circulating catecholamines are important for mediating osmoregulatory adjustments following transfer between fresh water and sea water,

(Mazeaud & Mazeaud, 1981; Eddy, 1981). Our results suggest that epinephrine may be important in this regard by decreasing the reabsorption of Na^+ and Cl^- ions, thereby stimulating renal excretion of NaCl . Such a response might be beneficial immediately upon transfer to sea water when salt loading is maximal and branchial ion extrusion mechanisms have not yet become activated.

In this series of three papers (Perry & Vermette, 1986; Vermette & Perry, 1986; this paper), continuous infusion of epinephrine was used as a method to increase the circulating levels of epinephrine in rainbow trout, without submitting the animals to other stresses that are normally associated with elevated epinephrine levels (e.g. exercise or hypercapnia). Although distinguishing the effects of epinephrine from the effects of the acid–base disturbance which it incurs is not possible, given the present experimental design, it is apparent that epinephrine can play an important role in the regulation of acid–base disturbances. The epinephrine-induced maintenance of RBC pH in the face of whole blood acidosis serves to ensure adequate arterial oxygenation by preventing the deleterious effects of reduced pH on haemoglobin function (Bohr and Root effects) (Perry & Vermette, 1986). Increased branchial acid efflux (Vermette & Perry, 1986) and renal acid secretion, which occur during epinephrine infusion, correct internal acidosis by allowing the accumulation of HCO_3^- (branchial acid efflux accounts for approximately 85% of total acid efflux, with the kidney accounting for the remaining 15%). It is obvious from this series of experiments that additional work is required to separate the effects of epinephrine from possible pH effects, and to clarify further its role in piscine acid–base regulation.

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REFERENCES

- BOUTILIER, R. G., IWAMA, G. K. & RANDALL, D. J. (1986). Acute extracellular acidosis promotes catecholamine release in rainbow trout (*Salmo gairdneri*): interactions between red cell pH and haemoglobin oxygen-carrying capacity. *J. exp. Biol.* **123**, 145–157.
- CAMERON, J. N. (1978). Regulation of blood pH in teleost fish. *Respir. Physiol.* **33**, 129–144.
- CAMERON, J. N. (1980). Body fluid pools, kidney function and acid–base regulation in the freshwater catfish, *Ictalurus punctatus*. *J. exp. Biol.* **105**, 107–125.
- CAMERON, J. N. & KORMANIK, G. A. (1982). The acid–base responses of gills and kidneys to infused acid and base loads in the Channel catfish, *Ictalurus punctatus*. *J. exp. Biol.* **99**, 143–160.
- EDDY, F. B. (1981). Effects of stress on osmotic and ionic regulation in fish. In *Stress and Fish* (ed. A. D. Pickering), pp. 77–102. London: Academic Press.
- EPPLÉ, A. & NIBBIO, B. (1985). Catecholaminotropic effects of catecholamines in a teleost. *J. comp. Physiol.* **155**, 285–290.
- EVANS, D. H. (1982). Mechanisms of acid extrusion by two marine fishes: the teleost, *Opsanus beta*, and the elasmobranch, *Squalus acanthias*. *J. exp. Biol.* **97**, 289–299.
- FARRELL, A. P. (1981). Cardiovascular changes in the lingcod (*Ophiodon elongatus*) following adrenergic and cholinergic drug infusions. *J. exp. Biol.* **91**, 293–305.

- FIEVET, B., MOTAIS, R. & THOMAS, S. (1986). Role of adrenergic-dependent H^+ release from red cells in acidosis induced by hypoxia in trout. *Am. J. Physiol.* (in press).
- HEISLER, N. (1984). Acid-base regulation in fishes. In *Fish Physiology*, vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 315-400. London: Academic Press.
- HÖBE, H., WILKES, P. R. H., WALKER, R. L., WOOD, C. M. & MCMAHON, B. R. (1983). Acid-base balance, ionic status and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*). *Can. J. Zool.* **61**, 2660-2668.
- HOFFMANN, E. L. & BUTLER, D. G. (1979). The effect of increased metabolic rate on renal function in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **82**, 11-23.
- HOLETON, G. F. & HEISLER, N. (1983). Contribution of net ion transfer mechanisms to acid-base regulation after exhausting activity in the larger spotted dogfish. *J. exp. Biol.* **103**, 31-46.
- HOLMES, W. M. & STAINER, I. M. (1966). Studies on the renal excretion of electrolytes by the trout, *Salmo gairdneri*. *J. exp. Biol.* **44**, 33-46.
- KOBAYASHI, K. A. & WOOD, C. M. (1980). The response of the kidney of the freshwater rainbow trout to true metabolic acidosis. *J. exp. Biol.* **84**, 227-244.
- MCDONALD, D. G. (1983). The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. I. Branchial and renal net ion and H^+ fluxes. *J. exp. Biol.* **102**, 123-140.
- MCDONALD, D. G., WALKER, R. L., WILKES, P. R. H. & WOOD, C. M. (1982). H^+ excretion in the marine teleost, *Parophrys vetulus*. *J. exp. Biol.* **98**, 403-414.
- MCDONALD, D. G. & WOOD, C. M. (1981). Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. *J. exp. Biol.* **93**, 101-118.
- MAZEAUD, M. M. & MAZEAUD, F. (1981). Adrenergic responses to stress in fish. In *Stress and Fish* (ed. A. D. Pickering), pp. 49-75. London: Academic Press.
- NIKINMAA, M. (1982). Effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout, *Salmo gairdneri*. *Molec. Physiol.* **2**, 287-297.
- NIKINMAA, M. & HUESTIS, W. H. (1984). Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose and two teleosts, striped bass and rainbow trout. *J. exp. Biol.* **113**, 215-224.
- NIKINMAA, M., CECH, J. J. & MCENROE, M. (1984). Blood oxygen transport in stressed striped bass (*Morone saxatilis*): role of beta-adrenergic responses. *J. comp. Physiol.* **154**, 365-369.
- NILSSON, S. (1984). Adrenergic control systems in fish. *Mar. Biol. Letts* **5**, 127-146.
- OIKARI, A. O. J. & RANKIN, J. C. (1985). Renal excretion of magnesium in a freshwater teleost, *Salmo gairdneri*. *J. exp. Biol.* **117**, 319-334.
- OPDYKE, D. F., CARROL, R. G. & KELLER, N. E. (1982). Catecholamine release and blood pressure changes induced by exercise in dogfish. *Am. J. Physiol.* **242**, 306-310.
- PERRY, S. F. & VERMETTE, M. G. (1986). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. I. Blood respiratory, ionic and acid-base states. *J. exp. Biol.* **128**, 235-253.
- PETTERSSON, K. & NILSSON, S. (1980). Drug induced changes in cardiovascular parameters in the Atlantic cod, *Gadus morhua*. *J. comp. Physiol.* **137**, 131-138.
- PITTS, R. F. (1974). *Physiology of the Kidney and Body Fluids*. Third edition. Chicago: Yearbook Medical Publishers.
- PRIMMETT, D. R. N., RANDALL, D. J., MAZEAUD, M. M. & BOUTILIER, R. G. (1986). The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout during exercise. *J. exp. Biol.* **122**, 139-148.
- VERMETTE, M. G. & PERRY, S. F. (1986). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. II. Branchial solute fluxes. *J. exp. Biol.* **128**, 255-267.
- VERDOUW, H., VANECHTELD, C. J. A. & DEKKERS, E. M. J. (1978). Ammonia determinations based on indophenol formation with sodium salicylate. *Water Res.* **12**, 399-402.
- WHEATLY, M. G., HÖBE, H. & WOOD, C. M. (1984). The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. II. The role of the kidney. *Respir. Physiol.* **55**, 155-173.
- WOOD, C. M. & CALDWELL, F. H. (1978). Renal regulation of acid-base balance in a freshwater fish. *J. exp. Zool.* **205**, 301-308.

- WOOD, C. M. & JACKSON, E. B. (1980). Blood acid-base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **42**, 351-372.
- WOOD, C. M. & SHELTON, G. (1975). Physical and adrenergic factors affecting systemic vascular resistance in the rainbow trout: a comparison with branchial vascular resistance. *J. exp. Biol.* **53**, 505-524.