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THE RESPONSE OF THE KIDNEY OF THE FRESHWATER RAINBOW TROUT TO TRUE METABOLIC ACIDOSIS

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SUMMARY

Infusion of lactic acid into the bloodstream of trout produced a short-lived depression of blood pH and a long-lasting elevation of blood lactate. The lactate injected was distributed in a volume of 198 ml/kg. Renal excretion of lactate anion and total acid increased by approximately equal amounts during the period of high blood lactate levels, but total renal loss over 72 h accounted for only 2% of the lactate load and 6% of the proton load. Comparable differences in the time courses of blood lactate and pH changes occurred when lactacidosis was induced endogenously by normocapnic hypoxia. The immediate response of the kidney was similar to that with lactic acid infusion, but there was a long-lasting (12–72+ h) elevation of urinary acid efflux that was not associated with lactate excretion. Following hypoxia, renal excretion over 72 h accounted for 1% of the estimated lactate load and 12–25% of the proton load. A renal lactate threshold of 4–10 μequiv/ml prevents significant urinary lactate excretion. The response of the trout kidney to true metabolic acidosis is similar to that of the mammalian kidney.

INTRODUCTION

Acid-base regulation in fish has been traditionally attributed to ionic exchange mechanisms (Na^+ *v.* H^+ or NH_4^+ and Cl^- *v.* HCO_3^- or OH^-) on the gills (cf. Cameron, 1976). However, recently we have shown that the teleost kidney also plays a role in acid-base balance (Cameron & Wood, 1978; Wood & Caldwell, 1978). Indeed, in freshwater rainbow trout, *Salmo gairdneri*, renal acid excretion was totally responsible for the elimination of the proton load associated with the infusion of a dilute mineral acid (HCl) into the bloodstream (Wood & Caldwell, 1978). Under this treatment, the response of the teleost kidney was essentially identical to that of the mammalian kidney (Pitts, 1974).

The infusion of mineral acid produces a 'metabolic' acidosis of the fixed acid type, i.e. one which ultimately can only be corrected by excretion. While this type of acidosis may occur naturally as a result of oxidation of S and P groups in foodstuffs, it is probably not the major form of metabolic acidosis which the animal regularly encounters. The metabolic production and release into the bloodstream of organic acids (e.g. lactic) which are fully dissociated at physiological pH is a common occur-

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rence in fish after exercise or hypoxia (Black *et al.* 1959, 1962; Piiper, Meyer & Drees, 1972; Johnston, 1975; Wood, McMahon & McDonald, 1977). The latter represents a true metabolic acidosis: one which could be corrected either by excretion or by metabolic removal of the acid (i.e. conversion to a neutral intermediate). In mammals the metabolic acidosis associated with exercise or hypoxia is almost entirely due to lactic acid release into the bloodstream (Keul, Keppler & Doll, 1967; Osnes & Hermansen, 1972), and it is corrected almost entirely by metabolic removal of the acid which is reconverted to glycogen or oxidized in the Krebs cycle (Hills, 1973). Renal excretion of lactic acid is negligible (Liljestrand & Wilson, 1925; Jervell, 1928; Yudkin & Cohen, 1975) because of the short time course of lactate elevation in the blood (Bang, 1936; Crescitelli & Taylor, 1944), the high renal lactate threshold (Miller & Miller, 1949), and the reduction of renal blood flow and glomerular filtration rate, at least during severe stress (White & Rolf, 1948). Indeed Yudkin & Cohen (1975) have shown that renal lactate clearance is actually decreased during severe metabolic acidosis in rats. As lactate is an incompletely oxidized product, its metabolic removal rather than excretion would be energetically advantageous.

In fish, the ultimate fates of both the proton and anion components of endogenously generated metabolic acid are unknown. However there are some reasons to suspect that the situation may differ from that in the mammal. For example, blood buffer capacity is much lower in fish (Albers, 1970), the time course of blood lactate elevation is much more prolonged (Black *et al.* 1959, 1962), lactate and H^+ ions do not enter and leave the blood at equal rates (Piiper *et al.* 1972; Wood *et al.* 1977), and other acidic end-products of anaerobic metabolism besides lactic acid may be produced (Johnston, 1975; Hughes & Johnston, 1978). Nothing is known about the renal handling of lactic acid in fish, though the presence of lactate in the urine after stress has been reported (Hunn, 1969; Cameron & Wood, 1978).

The aim of the present work was to examine the response of the kidney in the freshwater trout to a true metabolic acidosis produced either exogenously (by infusion of lactic acid) or endogenously (as a result of normocapnic hypoxic stress). Quantitative comparisons could then be made between the renal handling of protons and lactate, between the renal responses to fixed acid (Wood & Caldwell, 1978) and true metabolic acid, and between the overall compensations of the fish and the mammal to true metabolic acidosis.

MATERIALS AND METHODS

I. *Experimental animals*

Rainbow trout (*Salmo gairdneri*) were purchased from Spring Valley Trout Farm, Petersburg, Ontario, and acclimated for at least 3 weeks to the experimental temperature in dechlorinated, aerated fresh water. While in the holding tanks, fish were fed *ad libitum* on commercial trout pellets, but were starved for at least 7 days prior to an experiment to remove the influence of diet on renal acid output (Wood & Caldwell, 1978).

Chronic cannulae were implanted in the dorsal aorta (Smith & Bell, 1964; Holeton & Randall, 1967) and urinary bladder (Wood & Randall, 1973) while the fish were on an operating table under 1:15000 MS-222 (Sigma) anaesthesia. Trout were allowed to

recover for 36–48 h before the start of an experiment to permit stabilization of renal acid output (Wood & Caldwell, 1978). During recovery and the subsequent experimental period, fish were kept in darkened plexiglass boxes provided with a continuous flow (300–450 ml/min) of aerated, dechlorinated water at acclimation temperature. The chambers confined but did not physically restrain the fish. Blood samples were drawn periodically from the dorsal aortic cannula without disturbing the fish and were replaced with an equal volume of heparinized (52 USP units/ml) Cortland saline (Wolf, 1963). The urinary bladder was drained by a siphon of 7 cm into covered vials, allowing continuous urine collection.

II. *Acid Injection Experiments*

Sixteen rainbow trout, 99–301 g, at acclimation temperature (4·0–12·5 °C) were infused through the dorsal aortic cannula with 5·0 ml/kg fish body weight of an l-(+)-lactic acid (Sigma) solution (272 ± 4 μM/ml) in 120 mM-NaCl. The lactic acid solution was freshly prepared from crystalline lactic acid for each experiment, and therefore varied slightly in concentration between experiments because of the hygroscopic and unstable nature of the compound. Lactate concentration in the infusate was measured directly in each experiment by enzymic assay (see below). Titration with strong base gave identical figures, indicating that the compound was entirely in the free acid form. Mean infused lactic acid load was 1360 ± 20 μM/kg ($\bar{x}$ ± S.E.) with an absolute range of 1270–1640 μM/kg. Preliminary experiments indicated that this lactic acid load approximated the maximum tolerable dose. The infusate was washed in with 2·0 ml/kg of 120 mM-NaCl. Total infusion time (lactic acid plus NaCl wash) was 15 min, the end of infusion being time t_0.

Arterial blood samples (200–300 μl) were withdrawn at t_0-45 min (control) and at t_0+10 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 24 h and every 12 h thereafter until t_0+72 h. Urine was continuously collected for a 12 h pre-infusion period (control) and post-infusion as follows: 0–4 h, 4–8 h, 8–12 h and subsequently for 12 h periods until t_0+72 h. Blood samples were analysed for pH and lactate. Urine samples were analysed for pH, lactate, NH_4^+, TA-HCO_3^-, Na^+, K^+ and Cl^- (see below).

III. *Hypoxia experiments*

Nine rainbow trout, 107–269 g, at acclimation temperature (12·0–16·0 °C) were subjected to normocapnic hypoxia stress in the following manner. At time t_0, water supplied to the fish was switched from an aerated source (P_{O_2} = 135–160 torr; $P_{CO_2} \approx$ 0·5 torr) to an hypoxic source (P_{O_2} = 25–40 torr; $P_{CO_2} \approx$ 0·5 torr) from a nitrogen stripping column, without change in absolute flow rate (300–450 ml/min). The aim of these experiments was to impose maximum tolerable hypoxic stress. Therefore the endpoint of the hypoxic period was determined by visual inspection of the stressed condition of the fish. It varied from t_0+20 min to t_0+35 min. The P_{O_2} of the water in the anterior end of the fish boxes before, during, and after hypoxia was 150 ± 5, 30 ± 3 and 140 ± 3 torr ($\bar{x}$ ± S.E.) respectively.

Since preliminary experiments had demonstrated a slow time course for acidosis incurred under these conditions, blood samples ($\sim$ 200 μl) were taken at the following times: t_0-45 min (control), t_0+30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 24 h, and every 12 h thereafter until t_0+72 h. Urine was collected for the same periods as in

the injected group. Blood and urine samples were analyzed for the same parameters as above.

IV. *Analytical techniques*

Arterial blood pH (pH_a) was determined by drawing a 30 μl aliquot into a Radiometer microelectrode (type E5021) thermostatted to the experimental temperature and coupled to a Radiometer PHM71 MK2 or PHM72 MK2 acid-base analyser. Whole blood lactate was determined by fixing blood samples in 2 vols. 8% ice-cold perchloric acid, centrifuging, and analysing the supernatant by enzymic assay according to a micro-modification of the Sigma (1977) method. In the infusion experiments, duplicate aliquots of the lactic acid solution were similarly fixed at the exact time of infusion for later analysis.

Urine pH and lactate were determined immediately after collection as described above for blood samples. Addition/recovery tests indicated that there was no loss of lactate from the urine during the collection periods. As in mammalian renal physiology (Hills, 1973), total urinary acid output was calculated as urinary [NH_4^+ + titratable acid (TA)-HCO_3^-] x urine flow rate. This approach appears valid for fish (C. M. Wood, in preparation). NH_4^+ was determined by colorimetric assay using a micro-modification of Solorzano's method (1969). TA-HCO_3^- was determined as a single value in the double titration procedure recommended by Hills (1973) using a microelectrode (type E5021 or GKS73041, Radiometer) thermostatted to the experimental temperature and coupled to one of a PHM28, a PHM71 MK2 or a PHM72 MK2 acid-base analyser (all Radiometer). Titrants used were 0·02 N-HCl and 0·02 N-NaOH, the final endpoint of the titration being either the blood pH measured at the middle of the urine collection (4 h periods) or the mean of the blood pH's measured at the beginning and end of the collection (12 h periods). Urinary Na^+ and K^+ concentrations were determined by flame photometry (EEL, Mark II), appropriate swamping being used to remove the interference of Na^+ on K^+ emission. Urinary Cl^- levels were determined by coulometric titration (Radiometer CMT10).

V. *Statistical analysis*

Blood and urine parameters varied considerably between fish but were relatively consistent in individuals over the course of the experimental period. Therefore, all results were analysed employing the paired Students' two-tailed t test ($P \leqslant 0{\cdot}05$), using each fish as its own control. All data are presented as the mean $\pm$ 1 S.E. (N), where N equals number of fish contributing to the mean. Some experiments were terminated prematurely due to catheter displacement or dechlorinator failure, so N values tend to decline at later stages of each experiment.

RESULTS

I. *Acid injection experiments*

Control experiments ($N = 6$) to examine the influence of the saline vehicle alone (7·0 ml/kg of 120 mM-NaCl) on blood and urine parameters were performed under identical conditions to the lactic acid injection experiments. The acid-base results

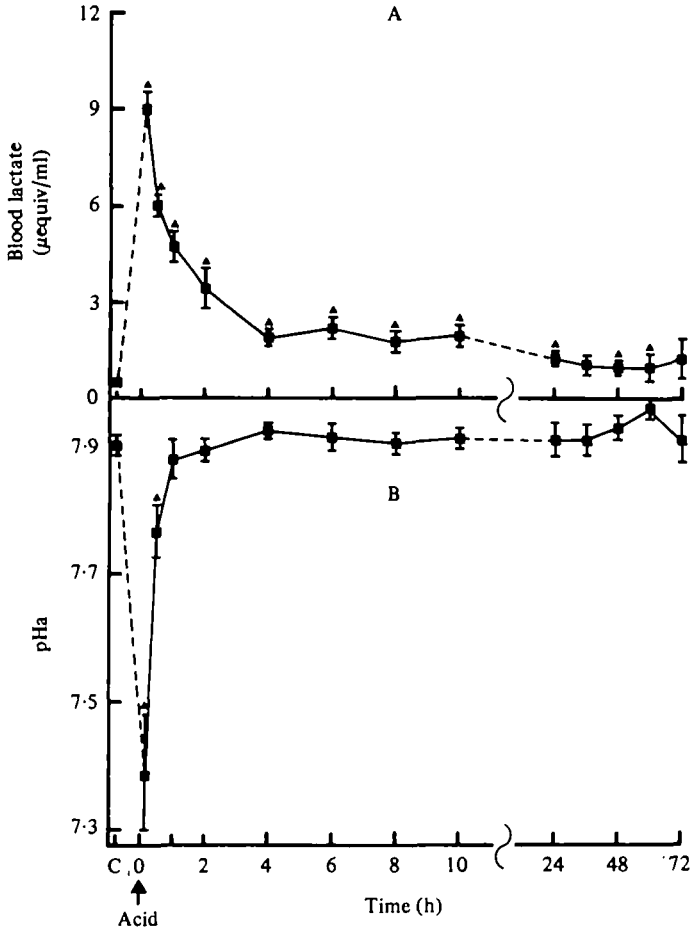

Fig. 1. (A) Blood lactate concentration and (B) arterial pH in rainbow trout following intra-arterial infusion of 1360 ± 20 μM/kg of l-(+)-lactic acid in 7·0 ml/kg of 120 mM-NaCl at time 0. C = pre-infusion control. Means ± S.E. (N = 16 to 7 fish at each point.) Triangles indicate means significantly different ($P \leqslant 0{\cdot}05$) from pre-infusion controls.

from these experiments have been reported elsewhere (Wood & Caldwell, 1978). In brief, the saline infusion had no effect on blood acid-base status. The only disturbance of urinary acid efflux was a short-lived decrease in total renal acid excretion to negative values (i.e. net base efflux) during the first 4 h post-infusion. This was entirely due to a depression of the TA-HCO_3^- component; the NH_4^+ component remained constant. This depression of TA-HCO_3^-, as discussed by Wood & Caldwell (1978), was probably associated with the slight diuresis caused by the volume load. All other parameters of urinary acid excretion remained unchanged at the pre-injection control levels for the entire experimental period (72 h).

In the lactic acid injection experiments, blood lactate concentration rose from the very low control value, 0·4 ± 0·1 (16) μequiv/ml, to a peak of 9·0 ± 0·6 (15) μequiv/ml at 10 min post-infusion, followed by an exponential decline to about 2 μequiv/ml over

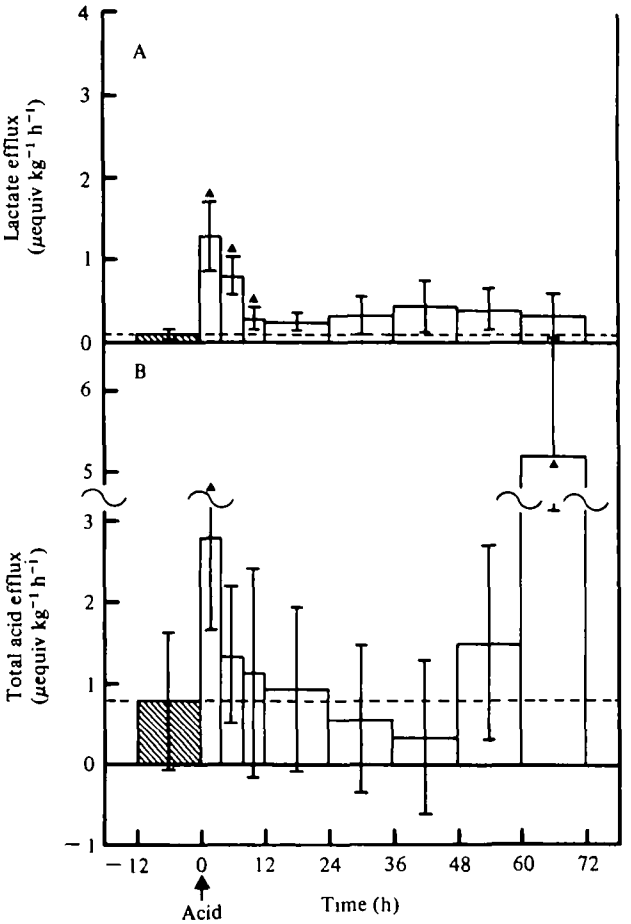

Fig. 2. (A) Urinary lactate efflux and (B) urinary total acid efflux in rainbow trout following intra-arterial infusion of lactic acid at time 0. The period −12 h to 0 h represents the pre-infusion control (cross-hatched area, dashed line). Other details as in Fig. 1.

the following 4 h (Fig. 1A). Thereafter, lactate levels fell only slightly, remaining significantly higher than control until at least 60 h. This continuing slight elevation was possibly an endogenous response to the stress of repetitive blood sampling rather than a persistence of the exogenous lactate load. Arterial pH fell by about 0·5 units in concert with the lactate peak, but rapidly returned to the control value within 1 h (Fig. 1B). There were no further significant fluctuations in pHa over the experimental period.

Total urinary acid efflux was negligible in the control state (Fig. 2B), reflecting a low NH_4^+ output (Fig. 3B) and a net TA-HCO_3^- excretion which was not significantly different from zero (Fig. 3A). Lactate excretion by the kidney was similarly negligible in the control state (Fig. 2A). Renal efflux rates of both total acid and lactate rose sharply immediately after infusion, but returned to control values by 12 h (Fig. 2A, B). There was a second large increase in urinary total acid output at 60–72 h

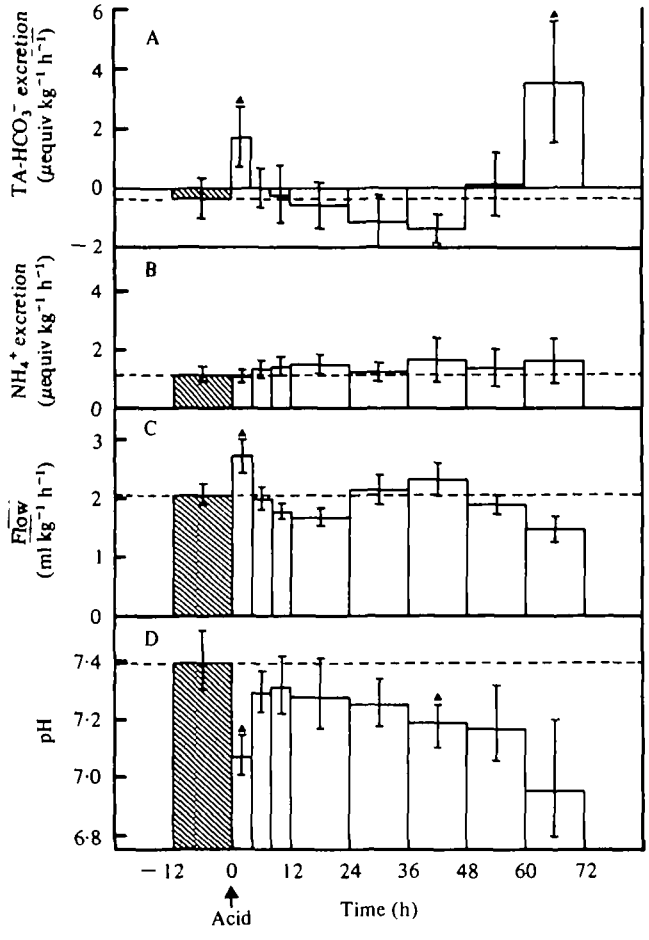

Fig. 3. (A) Urinary TA-HCO_3^- excretion, (B) urinary NH_4^+ excretion, (C) urine flow ,and (D) urine pH in rainbow trout following intra-arterial infusion of lactic acid at time 0. The period −12 h to 0 h represents the preinfusion control (cross-hatched area, dashed line). Other details as in Fig. 1.

unaccompanied by any change in lactate efflux. These variations in total acid efflux were accounted for, almost entirely, by variations in the TA-HCO_3^- component (Fig. 3A). Renal NH_4^+ excretion remained remarkably uniform throughout the experimental period (Fig. 3B). Urine flow also remained constant except for a slight diuresis during the 4 h immediately following infusion (Fig. 3C), as in the saline controls. Urine pH was significantly depressed during the peak acid excretion at 0–4 h post-infusion, but then returned to normal (Fig. 3D). There appeared to be a secondary decline in urine pH later in the experiment, but data variability precluded significance at all but the 36–48 h period.

Changes in renal lactate output were quantitatively similar to changes in total acid output during the first 12 h after infusion, but thereafter showed no correlation (Fig. 2A, B). The greatest increase in urinary lactate efflux, occurring 0–4 h after infusion, was coincident with the highest blood lactate levels. However, Table 1

Table 1. *Percentages of total lactate and proton load, $\bar{x}$ (N), excreted by the kidney of trout over different time periods*

	0–24 h	0–48 h	0–72 h
Lactic acid injection			
Lactate load	0·7 (13)	1·4 (7)	1·9 (7)
Proton load	0·9 (13)	−0·5 (7)	5·8 (7)
Hypoxia			
Lactate load (estimated)	1·1 (5)	1·2 (4)	1·4 (3)
Proton load (estimated)	4·6 (6)	10·2 (5)	25·3 (3)

Only animals for which complete data sets were available are included in the analysis.

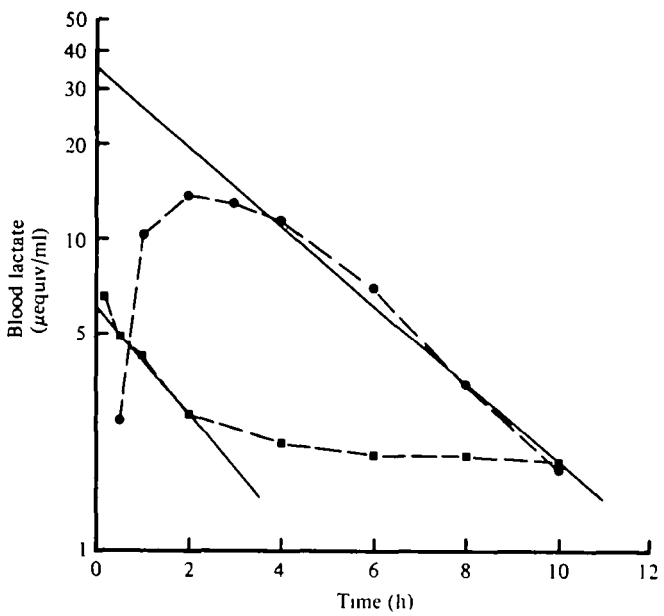

Fig. 4. Representative plots of log blood lactate concentration versus time in one trout (■, wt = 249 g) injected with lactic acid (load = 1330 μequiv/kg) and another trout (●, wt = 269 g) treated with hypoxia. The blood lactate values have been corrected for the pre-treatment control levels. Extrapolations (solid lines) of the linear regions of these curves back to time 0 gave estimates of lactate t_0 (■ = 6·1 μequiv/ml; ● = 34·8 μequiv/ml) used in calculating lactate space in the injection experiments, and endogenous lactate load in the hypoxia experiments. Lactate space in ■ was 54·3 ml and endogenous lactate load in ● was 6910 μequiv/kg assuming a lactate space of 198 ml/kg.

shows that in those fish for which all data were available for 72 h (N = 7), elevated renal lactate efflux over 72 h accounted for less than 2% of the infused lactic acid load. Similarly, increased urinary total acid efflux over 72 h accounted for less than 6% of the infused lactic acid load.

Renal excretion rates of Na^+, Cl^- and K^+ rose significantly during the first 4–8 h after lactic acid injection and thereafter returned to normal (Table 2). However these changes in general paralleled those seen in fish injected with the saline vehicle alone (Table 2). Indeed there were no significant differences at any time between the

Table 2. *Urinary excretion rates, μequiv kg^{-1} h^{-1} ($\bar{x} \pm$ 1 S.E.), of potassium, sodium and chloride ions in trout infused with saline vehicle alone (7·0 ml/kg of 120 mM-NaCl) or lactic acid (1360 ± 20 μM/kg) in saline vehicle.*

	Control	Time post-infusion (h)							
	−12–0	0–4	4–8	8–12	12–24	24–36	36–48	48–60	60–72
Potassium									
Vehicle (N = 6)	2·0	4·2*	2·8	2·1	1·5*	1·9	2·6	2·5	2·1
	±0·1	±0·5	±0·4	±0·2	±0·2	±1·2	±1·1	±1·1	±0·3
Lactic acid (N = 16–8)	1·6	4·7*	2·3*	2·3	1·5	2·1	2·0	2·0	1·6
	±0·1	±1·0	±0·4	±0·6	±0·2	±0·5	±0·4	±0·2	±0·2
Sodium									
Vehicle (N = 6)	26·6	34·0*	19·2	19·0	17·2*	16·8*	14·3*	15·8	17·7
	±2·5	±1·4	±3·5	±4·2	±2·4	±1·6	±2·0	±3·3	±2·0
Lactic acid (N = 16–8)	26·1	40·0*	23·5	23·6	21·9	25·5	25·7	26·8	20·5
	±4·3	±6·8	±6·7	±4·7	±4·0	±6·5	±7·4	±7·7	±4·2
Chloride									
Vehicle (N = 6)	20·7	34·6*	16·5	18·8	13·6*	13·6*	12·0*	15·9	14·6
	±3·0	±6·8	±2·0	±2·9	±1·7	±1·1	±1·3	±3·8	±1·0
Lactic acid (N = 16–8)	21·3	36·1	19·3	19·1	19·5	24·0	19·8	27·3	19·1
	±3·1	±6·9	±4·9	±3·6	±3·8	±5·1	±5·9	±6·2	±3·9

* = Significantly different ($P \leqslant 0{\cdot}05$) from pre-infusion control.
There are no significant differences between the means of the vehicle and lactic acid groups at any time.

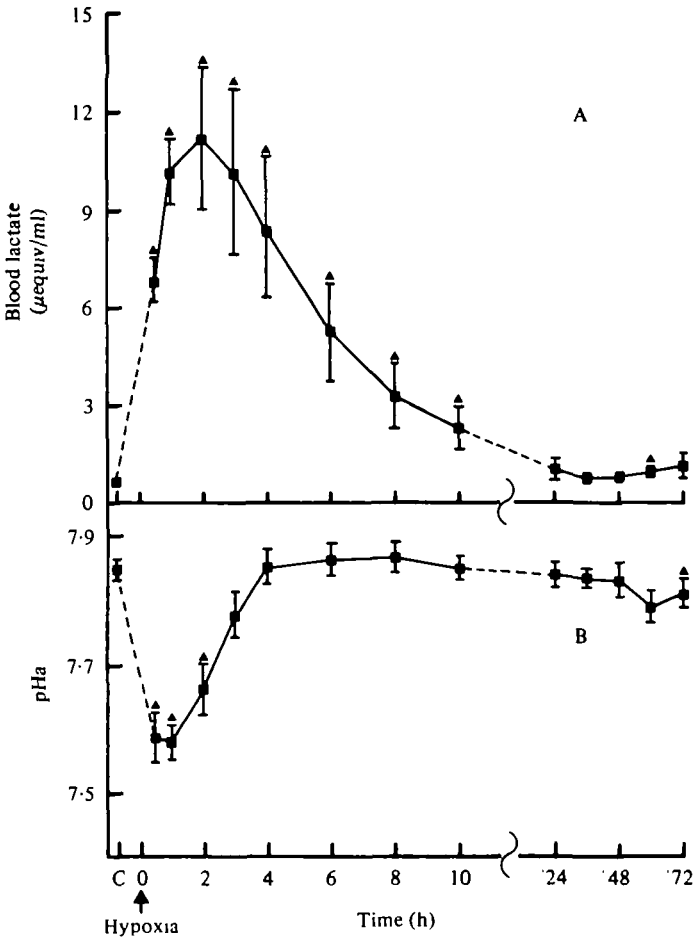

Fig. 5. (A) Blood lactate concentration and (B) arterial pH in rainbow trout following exposure to 20–35 min of normocapnic hypoxia (P_{O_2} = 25–40 torr) starting at time 0. C = pre-hypoxia control. Means ± 1 S.E. (N = 9 to 5 fish at each point.) Triangles indicate means significantly different ($P \leqslant 0{\cdot}05$) from pre-hypoxia controls.

excretion rates in the two groups. Therefore the increased urinary efflux of these ions appears to be an effect of the volume load and unassociated with the elevated renal acid excretion at this time.

The theoretical equilibrium distribution volume of lactate, or lactate space, was estimated using the formula:

$$\text{lactate space} = \frac{\text{injected lactic acid load } (\mu\text{equiv/kg})}{\text{lactate } t_0\ (\mu\text{equiv/ml}) - \text{lactate } t_0 - 45 \text{ min } (\mu\text{equiv/ml})}.$$

Lactate t_0 represents the theoretical concentration of lactate in whole blood at t_0 when the injected load is fully mixed throughout the lactate space. As blood lactate appeared to follow a simple exponential decline in all fish prior to the stabilization at 4 h, lactate t_0 was estimated by extrapolation of log lactate versus time curves (Fig. 4) back to t_0 after correction for control blood lactate levels. Mean lactate space in the injected group was 198 ± 13 (16) ml/kg.

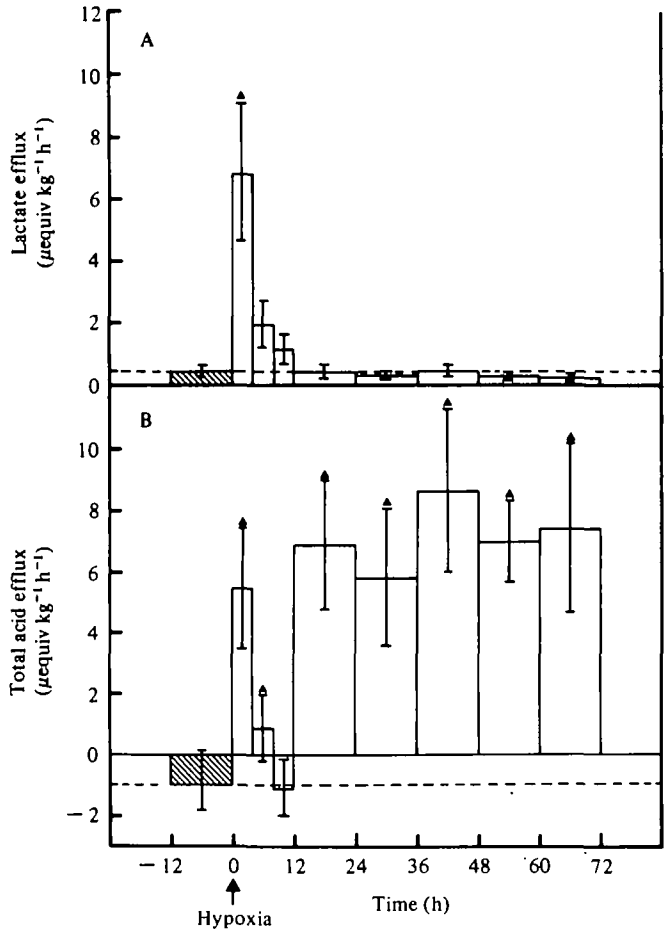

Fig. 6. (A) Urinary lactate efflux and (B) urinary total acid efflux in rainbow trout following exposure to 20–35 min of hypoxic stress starting at time 0. The period −12 h to 0 h represents the pre-hypoxia control (cross-hatched area, dashed line). Other details as in Fig. 5.

II. *Hypoxia experiments*

Despite the higher temperature range of these experiments, the only significant difference in control values between this group and the acid-injected group was a slightly lower mean pHa in the former [7·849 ± 0·014 (9) *v.* 7·903 ± 0·015 (16)].

Blood lactate levels rose much more slowly than in the injection series to a maximum of 11·2 ± 2·2 (9) μequiv/ml at 2 h post-hypoxia (Fig. 5A). Thereafter lactate concentrations decreased exponentially until 24 h, by which time the values were not significantly different from the pre-hypoxia control levels. A slight but significant secondary rise in blood lactate was seen at 60 h. Changes in pH_a also occurred much more slowly than in the injection series. The overall decline (about 0·3 units) was smaller, but of much longer duration, pH_a reaching a minimum at 1 h and returning to normal by 4 h (Fig. 5B). As with blood lactate, there was a slight but significant

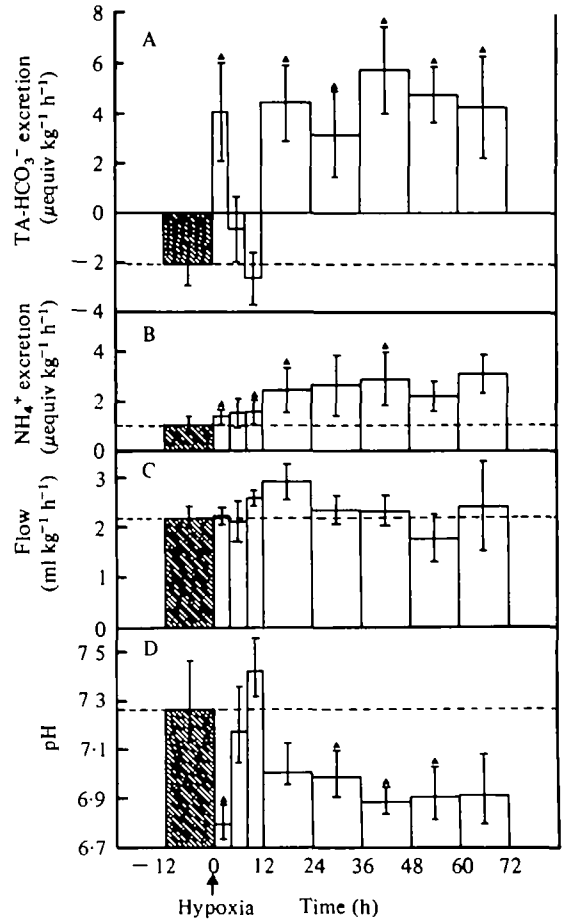

Fig. 7. (A) Urinary TA-HCO_3^- excretion, (B) urinary NH_4^+ excretion, (C) urine flow, and (D) urine pH in rainbow trout following exposure to 20–35 min of hypoxic stress starting at time 0. The period −12 h to 0 h represents the pre-hypoxia control (cross-hatched area, dashed line). Other details as in Fig. 5.

disturbance of pHa during the last 12 h of the experiment, which may have been a response to repetitive blood sampling.

At 0–4 h post-hypoxia, renal lactate excretion (Fig. 6A) rose dramatically to a level approximately fivefold that in the injected fish for the same period (cf. Fig. 2B), followed by a return to control levels by 12 h. As in the injected group, the period of highest renal lactate output coincided with the highest blood lactate levels (Fig. 5A). These changes in lactate efflux were again quantitatively paralleled by alterations in total renal acid excretion over the first 12 h (Fig. 6B). However there occurred a second large increase in urinary total acid efflux which was maintained from 12 h until 72 h. The latter was not accompanied by any change in renal lactate output. Elevations of TA-HCO_3^- efflux (Fig. 7A) were again the major source of the observed changes in total acid efflux. However, unlike the injection series, increases in NH_4^+ output also made significant contribution (Fig. 7B). Over most of the post-hypoxia period,

Table 3. *Urinary excretion rates, μequiv kg^{-1} h^{-1} ($\bar{x} \pm 1$ S.E.), of potassium, sodium and chloride ions in trout subjected to normocapnic hypoxia.*

	Control	Time post-hypoxia (h)							
	−12 to 0	0–4	4–8	8–12	12–24	24–36	36–48	48–60	60–72
Potassium	1·4	4·2*	1·9	2·3*	2·1*	1·7	1·7	1·8	2·3
(N = 9–5)	±0·2	±0·7	±0·5	±0·4	±0·4	±0·3	±0·3	±0·4	±1·1
Sodium	31·8	68·7	42·6	58·1	44·9	36·6	33·8	27·7	34·4
(N = 9–5)	±7·5	±10·1	±12·9	±11·5	±9·0	±8·3	±7·5	±4·2	±11·9
Chloride	24·6	47·7	33·6	45·4	34·0	30·0	27·5	19·2	20·4
(N = 9–5)	±6·1	±10·1	±9·9	±8·6	±5·0	±5·9	±5·8	±3·4	±6·9

* = Significantly different ($P \leqslant 0{\cdot}05$) from pre-hypoxia control.

NH_4^+ effluxes were more than twice the control value. As expected, changes in urine pH inversely paralleled those in TA-HCO_3^- excretion (Fig. 7A, D). There were no significant variations in urine flow rate over the experimental period (Fig. 7C).

Urinary excretion rates of Na^+, Cl^-, and K^+ all increased markedly after hypoxia and stayed elevated for up to 24 h (Table 3), despite the constancy of urine flow. There was no obvious relationship between the renal effluxes of these ions and any parameter of urine acidity (Figs. 6, 7).

A theoretical lactate t_0 could also be calculated in these hypoxia experiments, based on the exponential nature of the blood lactate decline after the initial peak (Fig. 5A). Plots of log lactate versus time were extrapolated back to t_0 after correction for control levels (Fig. 4). An estimate of total effective lactate load in equilibrium with the blood could then be determined from the formula:

$$\text{lactate load} = \text{lactate } t_0 \ (\mu\text{equiv/ml}) \times \text{lactate space (ml/kg)},$$

where lactate space is the mean value obtained in the injection series, 198 ml/kg. These calculated endogenous loads were extremely variable (range: 690–13020 μequiv/kg), probably reflecting variability in the degree of hypoxic stress, and averaged 5170 ± 1440 (9) μequiv/kg. This represents approximately fourfold the injected load in the previous group.

From these estimates of effective blood lactate load, the overall contribution of renal excretion mechanisms to the ultimate correction of the lactacidosis could be calculated. In individual fish for which all data were available up to 72 h ($N = 3$), the increased urinary efflux of lactate over 72 h accounted for about 1% of the estimated load (Table 1). If equivalence is assumed between lactate load and acid load, increased renal output over the same period accounted for about 25% of the protons associated with the lactate (Table 1). The N number is small in this analysis because of occasional missing data in many fish. If, instead, overall means are used, renal lactate efflux again represents about 1% of the endogenous load, but total proton efflux is reduced to 12%. Whichever figure is used for total proton efflux, it is probably an underestimate, for there is no indication in the data that this renal compensation had reached an end by 72 h (Fig. 6B).

DISCUSSION

Clearly the response of the trout kidney to a true metabolic acidosis was very different from that to a fixed mineral acidosis (Wood & Caldwell, 1978). Whereas all the protons in an HCl load were excreted in the urine, only a small percentage of the protons in a lactic acid load were eliminated in this manner (Table 1). Similarly, only an insignificant fraction of the lactate anion load passed out through the kidney (Table 1). In this regard, the fish again appears identical to the mammal where renal lactic acid excretion is negligible, i.e. < 4% of total load (Liljestrand & Wilson, 1925; Hewlett, Barnett & Lewis, 1926; Jervell, 1928; Johnson & Edwards, 1937; Yudkin & Cohen, 1975). An analysis of the data from the present experiments reveals that a similar mechanism is operating in the fish as in the mammal to minimize renal lactate loss. A plot of urinary lactate excretion rate versus time-averaged lactate concentration in blood (i.e. area means) clearly indicates the presence of a renal lactate threshold

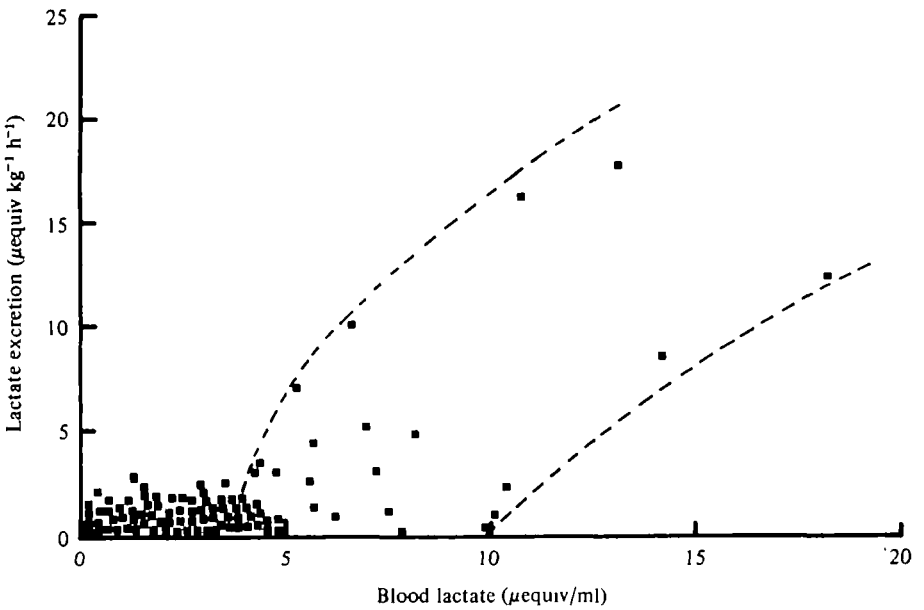

Fig. 8. The relationship between time-averaged blood lactate concentration (i.e. area means) and urinary lactate excretion rate in individual fish over discrete 4 or 12 h time periods. Data from both the lactic acid injection and the hypoxia series were used in the analysis. Of the total. 196 data points, 101 have been omitted for clarity. These all lay in the rectangular area bounded by 0–2·5 μequiv kg^{-1} h^{-1} lactate efflux and 0–3 μequiv/ml blood lactate. The approximate limits of the renal lactate threshold (4–10 μequiv/ml of blood lactate) are indicated by the dashed lines.

lying somewhere between 4 and 10 μequiv/ml (Fig. 8). A similar threshold occurs in the mammal with similar variability (Hewlitt *et al.* 1926; Craig, 1946; Miller & Miller, 1949). Below 4 μequiv/ml, tubular lactate reabsorption appears to be greater than 80%, assuming a normal GFR figure for freshwater trout (Hickman & Trump, 1969).

If lactate is not excreted by the kidney, then what is its ultimate fate? One possibility is branchial excretion, as a slight negative arterial–venous difference in blood lactate concentration has been detected in trout after exercise (Driedzic & Kiceniuk, 1976). However, attempts to measure lactate efflux into the water bathing the gills have been unsuccessful (Piiper *et al.* 1972; G. Holeton & N. Heisler, personal communication). A more likely explanation is metabolic removal by the gill tissues which are known to have a high capacity for lactate oxidation (Bilinski & Jonas, 1972). Indeed, the ability to oxidize lactate is widespread in trout tissues (Bilinski & Jonas, 1972); oxidation, rather than glycogen synthesis, is probably the principal fate of metabolized lactate (Black *et al.* 1962).

Whatever the pathway, metabolism will remove protons and lactate anions in stoichiometrically equivalent amounts. It is interesting, therefore, that the time courses of blood pH correction and blood lactate decline were so very different (Figs. 1, 5). Because of the possibility of respiratory compensation, one cannot conclusively prove that the net loads of lactate anions and 'metabolic' H^+ ions in the blood differed at any one time in the present experiments without performing a complete

Davenport analysis on the blood (cf. Wood *et al.* 1977; McDonald *et al.* 1979). However, at least in the hypoxia experiments, preliminary calculations indicate that even maximum respiratory compensation (reduction in P_{a,CO_2} to 0·5 torr) would have been insufficient to explain the return of blood pH to normal while lactate remained elevated (Fig. 5). Rather, there appears to have occurred a higher rate of proton removal from the blood, or a lower rate of proton entry into the blood, or both, as in the dogfish (Piiper *et al.* 1972) and the crab (McDonald, McMahon & Wood, 1979) after exercise.

Several possible explanations exist, e.g. the production or uptake of metabolic base, differential retention of H^+ ions over lactate ions at the site of production (e.g. white muscle), differential uptake and buffering of H^+ ions by other tissues, or a differential excretion of H^+ ions by the gills or kidney. The present results rule out the latter possibility, for under both treatments the renal excretion rates of protons and lactate anions were approximately equivalent (Figs. 2, 6) during the periods of discrepancy in the blood (0–12 h post-treatment; Figs. 1, 5). However, in the hypoxia trials there was a substantial delayed renal efflux of H^+ ions lasting from 12 h until 72 h which was unassociated with lactate excretion (Fig. 6). A similar effect occurred in the lactic acid-injected fish from 60–72 h post-infusion (Fig. 2). One possible interpretation is that these phenomena represented the eventual release and excretion of some of the 'missing' protons which had been temporarily stored outside the vascular compartment. An alternative explanation, at least in the case of hypoxia, would be delayed excretion of other organic acids produced either by different anaerobic pathways (e.g. succinic acid; Johnston, 1975; Hughes & Johnston, 1978) or as a result of some influence of hypoxia on aerobic metabolic pathways (e.g. a stimulation of keto acid production).

The estimated volume of distribution for injected lactate of 198 ± 13 ml/kg in the trout indicates that the substance is freely exchangeable throughout a compartment much larger than the blood volume ($\approx$ 50 ml/kg) and comparable to that of the extracellular fluid volume (150–200 ml/kg; Holmes & Donaldson, 1969). Yudkin & Cohen (1975) have calculated a lactate space in the rat only slightly higher than the extracellular volume.

The present results imply a restriction on lactate movement between interstitial fluid and cells, for which Wardle (1978) has recently provided some direct evidence in the plaice (*Pleuronectes platessa*). In the hypoxia experiments, this lactate space value has been used to calculate the total effective lactate load which is in equilibrium with the blood. Numerous criticisms can be levelled at this procedure since in these experiments the lactate was produced endogenously by the tissues rather than added exogenously to the blood. Nevertheless, the approach seems far more reasonable than simply using either the blood volume alone (cf. Cameron & Wood, 1978) or the total white muscle volume (cf. Wardle, 1978) in the calculation. The former would obviously be an underestimate as lactate should be distributed between the plasma and the interstitial fluid according to a simple Donnan equilibrium with a value close to unity. The latter would be an overestimate if Wardle's (1978) finding of limited movement of lactate from muscle to E.C.F. is applicable to the trout; the data of Black *et al.* (1962) indicate that this is highly probable.

In terms of the mechanisms involved in renal acid excretion, the present results are

in general agreement with our previous findings in freshwater fish (Wood & Caldwell, 1978; Cameron & Wood, 1978). In particular, the immediate response of the kidney to an acid load is to markedly increase the TA-HCO_3^- component of net acid efflux (e.g. Figs. 3A, 7A). Only during prolonged renal compensation does elevation of the NH_4^+ component become important (e.g. Fig. 7B). The teleost kidney functions like the mammalian kidney in this regard (Hills, 1973; Pitts, 1974). Urinary ion effluxes were measured in an attempt to detect the ionic interactions which characterize the acid secretion process in the mammalian renal tubule: Na^+/H^+ exchange, K^+/H^+ competition, and Cl^-/HCO_3^- reciprocity (Pitts, 1974). No such interactions could be detected, and the variations observed were due to either the volume load of the saline vehicle (Table 2) or an effect of hypoxia on renal function apparently unrelated to acid excretion (Table 3). Hunn (1969) has reported a similar general increase in the urinary excretion rates of most ions following hypoxic stress in trout, probably reflecting a decrease in the efficiency of tubular reabsorption. The mechanisms of urinary acidification in the trout will be considered in greater detail elsewhere (C. M. Wood, in preparation).

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