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ACID–BASE AND IONIC EXCHANGES AT GILLS AND KIDNEY AFTER EXHAUSTIVE EXERCISE IN THE RAINBOW TROUT

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Summary

Unidirectional Na^+ and Cl^- fluxes, net fluxes of Na^+, Cl^-, other ions, titratable acid (TA), ammonia and acidic equivalents (net H^+) across the gills, together with the comparable renal fluxes, were monitored throughout a 24-h period after exhaustive exercise (simple chasing) in the rainbow trout. The gills were the major site of flux. The renal excretion of $[TA-HCO_3^-]$, ammonia, lactate and most electrolytes increased after exercise, coincident with diuresis. Relative to the gills, the kidney accounted for only 8 % of net H^+ flux, 0–15 % of net electrolyte losses and 50 % of lactate loss, though the latter was negligibly small.

Approximately 1000 μequiv kg^{-1} of net H^+ were transported across the gills to the water during the first 4 h, and then fully recovered over the subsequent 8 h, coincident with periods of extracellular acidosis and alkalosis recorded in previous studies. Ammonia efflux increased during the first 4 h; changes in titratable acid flux and extracellular P_{NH_3} and NH_4^+ levels suggest that this elevation occurred partially as NH_3 diffusion in the first hour, and thereafter mainly as NH_4^+ exchange. Small net Na^+ losses ($\approx$300 μequiv kg^{-1}), moderate net K^+ losses ($\approx$600 μequiv kg^{-1}) and large net Cl^- losses ($\approx$1200 μequiv kg^{-1}) correlated well with previously reported plasma changes; only the Na^+ deficit was fully corrected by 24 h. Na^+ influx was stimulated and Cl^- influx inhibited during the 0–4 h period of net H^+ excretion, whereas Na^+ influx returned to control levels and Cl^- influx increased during the 4–12 h period of net H^+ uptake. These data indicate dynamic modulation of Na^+/NH_4^+,H^+ and Cl^-/HCO_3^-,OH^- exchanges; however, an excess of Cl^- over Na^+ efflux also contributed to net H^+ excretion. Acidic equivalent flux correlated well with $[Na^+-Cl^-]$ net flux, in accord with strong ion difference theory.

Introduction

The gills and kidney are the principal sites of ion and acid–base regulation in freshwater fish, with the former being quantitatively more important under most circumstances. The presence of Na^+/acidic equivalent and Cl^-/basic equivalent

Key words: gills, kidney, trout, acid–base regulation, ionoregulation, ammonia excretion.

exchanges on the gills, as originally proposed by Krogh (1939), Garcia-Romeu & Maetz (1964) and Maetz & Garcia-Romeu (1964), has now been experimentally substantiated. Unidirectional flux analysis with radiotracers has confirmed that such exchanges can be dynamically adjusted by the fish so as to achieve acid–base balance (Cameron, 1976; McDonald, Walker & Wilkes, 1983; Wood, Wheatly & Hōbe, 1984; Perry, Malone & Ewing, 1987*a*). However, the mechanistic details remain obscure – e.g. the importance of influx *vs* efflux modulation, coupled fluxes *vs* permeability changes, H^+ *vs* NH_4^+ exchanges etc. Previous studies have all induced acid–base disturbances *via* step changes in the environment (hypercapnia, acid exposure, hyperoxia), whereas the system may well be designed to deal with dynamic, endogenously induced disturbances. The goal of the present study was to employ strenuous exercise and recovery as a tool to probe the roles of gills and kidney in dynamic acid–base and ion adjustment, and help elucidate the mechanisms involved.

The internal acid–base, respiratory, hormonal and metabolic consequences of strenuous exercise have been well characterized in trout (e.g. Black, Chiu, Forbes & Hanslip, 1959; Black, Robertson, Lam & Chiu, 1962; Turner, Wood & Clark, 1983; Primmett, Randall, Mazeaud & Boutilier, 1986; Milligan & Wood, 1986*a*,*b*, 1987; Dobson & Hochachka, 1987). Much less is known about the ionoregulatory consequences and their obligatory interactions with acid–base exchange. The most detailed study to date, that of Holeton, Neumann & Heisler (1983), demonstrated a net uptake of Na^+ and net loss of Cl^- coincident with a net excretion of acidic equivalents for the first few hours after the trout had been electrically stimulated to exhaustion. Thereafter, these net fluxes reversed, coincident with a net uptake of acidic equivalents. These results are in accord with strong ion difference theory (SID, Stewart, 1978, 1983), but as unidirectional fluxes were not measured, the mechanisms were unclear. An earlier radiotracer study (Wood & Randall, 1973*a*) found no change in unidirectional Na^+ influx at the gills, but marked alterations of efflux during exercise and recovery. However, since the fish were chased rather than electrically stimulated, water chemistry was very different and acidic equivalent fluxes were not measured, the comparability of these two studies is uncertain. Nothing is known about the possible renal response. Therefore, the present investigation has incorporated simultaneous measurements of unidirectional Na^+ and Cl^- fluxes, net fluxes of Na^+, Cl^-, other ions, titratable acid, ammonia and acidic equivalents across the gills, together with the comparable renal fluxes, throughout a 24-h period after strenuous exercise induced by simple chasing.

Materials and methods

Experimental animals

Adult rainbow trout (*Salmo gairdneri*; 212–321 g) were obtained from Spring Valley Trout Farm, Petersburg, Ontario, during June, July and August, and

acclimated to experimental temperature (15 ± 1°C) for 7–10 days in flowing, dechlorinated Hamilton tap water. The fish were not fed during this period. Acclimation and experimental water had the following composition (in mequiv l^{-1}): Na^+, 0·6; Cl^-, 0·8; Ca^{2+}, 1·8; Mg^{2+}, 0·5; K^+, 0·04; titration alkalinity (to pH = 4·0), 1·9; total hardness, ≈140 mg l^{-1} as $CaCO_3$; pH 8·0.

Repetitive blood sampling *via* cannulae should be avoided during flux experiments, for it disturbs ion and acid–base exchanges. Whenever blood data were needed for comparison with the flux data, they were taken from our previous blood-sampling studies in which the exercise regime and water quality were identical (Turner *et al.* 1983; Milligan & Wood, 1986*a*,*b*, 1987; van Dijk & Wood, 1988). In the present study, fish were fitted only with urinary bladder catheters (Wood & Randall, 1973*c*), and then allowed to recover for 72 h in black Perspex flux chambers of the design described by McDonald & Rogano (1986). The chambers were aerated so as to maintain $P_{O_2} \geq 130$ mmHg (1 mmHg = 133·3 Pa), and temperature was kept at 15 ± 1°C by an external bath. As the urine flow was collected outside the chambers (siphon = 7 cm), this study assumes, in the usual manner, that all measured exchanges with the water were branchial in origin.

During the recovery period, the flux boxes were operated as closed systems at maximum volume (≈4·8 l), and the water was changed by thorough flushing every 12 h. The objective was to accustom the fish to the conditions used during the actual experiments and to keep water ammonia levels below 200 μmol l^{-1}, so as to minimize the inhibitory effects of water ammonia on ammonia, acid–base and ion fluxes (Wright & Wood, 1985; C. M. Wood, unpublished results). Urine flow was monitored at 12-h intervals, and had stabilized by 72 h in all fish.

Experimental protocol

The experimental design consisted of a 24-h control period, 6 min of exhaustive exercise, and then experimental measurements over the following 24 h of recovery. The protocol was designed to maximize the precision of branchial flux measurements over short periods in the critical first 12 h after exercise, while ensuring the water ammonia levels remained low and that the external to internal specific activity ratios remained high for greatest accuracy in radioisotopic flux determinations.

For exercise, the urinary catheter was plugged (to avoid blockage by air bubbles), and the trout was transferred in its chamber to the 500-l exercise tank, and released under water. Exhaustive exercise was induced by 6 min of vigorous chasing, as described previously (Turner *et al.* 1983). The fish, which at this point was refractory to further stimulation, was then returned to its flux chamber, again without air exposure, the urinary catheter unplugged, and the urine collection restarted. Fluxes could not be measured during the 6-min exercise period.

Unidirectional fluxes of Na^+ and Cl^-, and net fluxes of ammonia, titratable acid and acidic equivalents at the gills were measured over 0–4, 4–8 and 8–12 h of the control period, over consecutive 1-h intervals for the first 12 h after exercise, and

then over 12–24 h. Net fluxes of all substances were measured over these same intervals, and also over 12–24 h of the control period. The flux boxes were operated at maximum volume (≈4·8 l) during the control period, and at minimum volume (≈2·2 l) during the first 12 h of the experimental period, and then returned to 4·8 l from 12 to 24 h. Thorough flushes were performed at 0, 12 and 24 h of the control period, and 0, 6, 12 and 24 h of the experimental period. Radioisotopes (4 μCi ^{22}Na, 10 μCi ^{36}Cl; New England Nuclear) were added immediately after the flushes at 0 h of the control period, and at 0, 6 and 12 h of the experimental period.

Water samples (50 ml) were withdrawn at each sampling time and analysed for ^{22}Na (counts min^{-1}), ^{36}Cl (counts min^{-1}), and Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, ammonia levels, and titratable acidity. Urine flows were collected over 0–12 h and 12–24 h of the control period, and 0–4, 4–8, 8–12 and 12–24 h of the experimental period. Urine samples were analysed for volume, pH, and Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, lactate, [$TA-HCO_3^-$] (see below) and ammonia levels. At the end of the experiment, the fish was rapidly killed by an overdose of anaesthetic (1 g l^{-1}, MS-222) and a blood sample taken by caudal puncture for analysis of plasma ^{22}Na (counts min^{-1}), ^{36}Cl (counts min^{-1}) and Na^+ and Cl^- levels.

Analytical methods and calculations

^{36}Cl is a pure β-emitter, but ^{22}Na is a mixed γ- and β-emitter. Dual-labelled water (5 ml) and plasma (50 μl) samples were prepared in duplicate, with ^{22}Na alone measured by γ-counting in a well-counter (Nuclear Chicago model 1085), and ^{22}Na plus ^{36}Cl by scintillation counting (LKB Rackbeta 1217). ^{36}Cl counts min^{-1} were obtained by subtraction after correcting for differences in efficiency of ^{22}Na counting by the two instruments. Water, plasma and urine Na^+, K^+, Ca^{2+} and Mg^{2+} levels were determined by atomic absorption spectroscopy (Varian AA-1275), and Cl^- level by coulometric titration (Radiometer CMT10). For the branchial flux measurements, it was necessary to detect very small changes in water ion levels against high backgrounds. Therefore, rather than diluting to a common range, samples were read directly for cations on the AA-1275 without dilution, employing bracketting standards. For Cl^-, the chloridometer was recalibrated for direct reading of 1-ml water samples over the range of interest (0·6–1·0 mequiv l^{-1}).

Ammonia in water and urine was determined by the phenolhypochlorite method of Solorzano (1969). Titratable alkalinity in water was determined by titration of 10-ml samples to pH 4·00 with 0·02 mol l^{-1} HCl, using Radiometer GK2401C electrodes and PHM 72 or PHM 82 meters, as described by McDonald & Wood (1981). The concentration of titratable acidity minus bicarbonate ([$TA-HCO_3^-$]) in urine was determined as a single value in the double-titration procedure recommended by Hills (1973), using a Radiometer pH microelectrode (Type E5021) thermostatted to the experimental temperature. The endpoint of the titration was taken as the normal arterial pH (7·80, Milligan & Wood, 1986*a*). The HCl and NaOH titrants were both 0·02 mol l^{-1}, and 500-μl urine samples were

used. Lactate in urine was determined on 275-μl samples deproteinized by the addition of 25 μl of 70 % $HClO_3$ and then assayed enzymatically (L-lactate dehydrogenase/NADH; Turner *et al.* 1983).

Branchial net flux rates (J^x_{net}) of each substance (e.g. x) over any interval were calculated as:

$$J^x_{net} = \frac{([x]_i - [x]_f)V_{ext}}{Wt}, \quad (1)$$

where i and f refer to initial and final concentrations (in μequiv ml^{-1} or μmol ml^{-1}), V_{ext} is the volume of the system (in ml), corrected for sampling deficits, t is the elapsed time (in h), and W is the body mass (in kg). Thus net losses by the animal have a negative sign, net gains a positive sign. By reversing the i and f terms, the net titratable acid flux was calculated from the titratable alkalinities. The sum of titratable acid and ammonia fluxes gives the net flux of acidic equivalents (J^x_{net}), which derives from the original principles outlined by Maetz (1973). As McDonald & Wood (1981) point out, this method does not distinguish between ammonia movement in the NH_3 and NH_4^+ forms, nor between the net excretion of acidic equivalents and the net uptake of basic equivalents, or *vice versa*. Fortunately this does not matter in terms of the net acid–base budget of the fish.

Unidirectional influxes (J^x_{in}) of Na^+ and Cl^- across the gills were calculated as outlined by Maetz (1956), employing backflux correction, for by the end of the experiment internal specific activity was about 30 % of external specific activity. In brief, influx was calculated as:

$$J^x_{in} = \frac{([R]_i - [R]_f)V_{ext} - SA_{int}([x]_i - [x]_f)}{(SA_{ext} - SA_{int})Wt}, \quad (2)$$

where $[R]_i$ and $[R]_f$ are initial and final radioactivities (in counts min^{-1} ml^{-1}), SA_{int} and SA_{ext} are the mean internal and external specific activities (in counts min^{-1} μequiv^{-1}) over the period, and the other symbols are as in equation 1. SA_{int} at each time was estimated as described by Maetz (1956). In the calculation of SA_{int}, values of 280·4 ± 12·7 (10) and 252·7 ± 12·7 (10) ml kg^{-1} were employed for the internal distribution volumes of Na^+ and Cl^-, respectively, and 42 053 ± 1904 (10) and 32 845 ± 1646 (10) μequiv kg^{-1} for the total exchangeable internal pools of these ions, based on the terminal plasma measurements. Unidirectional efflux rates (J^x_{out}) were obtained by the conservation equation:

$$J^x_{out} = J^x_{net} - J^x_{in}. \quad (3)$$

The urinary efflux rate of each substance (e.g. x) was calculated as the product of urine flow rate (UFR, in ml kg^{-1} h^{-1}) and urinary concentration ($[x]_u$, in μequiv ml^{-1}). Total renal output of acidic equivalents was taken as the sum of the $[TA-HCO_3^-]$ and NH_4^+ components (Hills, 1973). At the urine pH values recorded in the present study, NH_4^+ accounted for >98·8 % of the total ammonia present and was taken to be equal to the latter.

The urinary clearance ratio (CR_x) for each substance was calculated as described by Wheatly, Hōbe & Wood (1984):

$$CR_x = \frac{[x]_u \times UFR}{[x]_p \times GFR}, \quad (4)$$

using the appropriate plasma data at each time after exercise from Turner *et al.* (1983), Milligan & Wood (1986*a*,*b*) and van Dijk & Wood (1988). GFR (in $ml\,kg^{-1}\,h^{-1}$) was estimated as $1{\cdot}77 \times UFR$ (Holmes & Stainer, 1966). A proportional relationship between UFR and GFR has been demonstrated in *S. gairdneri* over a wide range of UFR (Hofmann & Butler, 1979); thus the conversion factor 1·77 was assumed to be constant.

Data have been expressed as means ± 1 S.E.M. (N) throughout. Statistical comparisons employ Student's paired t-test (two-tailed), using each animal as its own control, with a significance level of $P \leq 0{\cdot}05$. Regression lines were fitted by the method of least squares, and the significance of the simple correlation coefficient (r) assessed.

Results

There was no significant variation in any measured branchial or renal flux throughout the 24-h control period, indicating that the fish had fully recovered from the operation and were in a steady-state condition. Thus, the data of the various control intervals were combined to yield a single control value for each parameter.

Branchial responses

Fig. 1 summarizes the components of the net branchial flux of acidic equivalents (net H^+ flux). At rest, net ammonia excretion slightly exceeded titratable acid uptake, resulting in a net H^+ flux of about $-80\,\mu equiv\,kg^{-1}\,h^{-1}$. This probably reflected an endogenous generation of acidic equivalents by protein catabolism in these non-feeding animals. Net H^+ excretion increased significantly during each of the first 4 h after exhaustive exercise, as did ammonia efflux. However, titratable acid uptake was elevated only during hour 1. After 4 h, net H^+ excretion changed abruptly to net H^+ uptake which remained significantly above control levels for hours 5–10. Titratable acid uptake was elevated significantly throughout this period of net H^+ uptake, while ammonia efflux remained close to resting levels. Control values for all three parameters were re-established at 12–24 h. The cumulative acidic equivalent excretion ($\approx 1000\,\mu equiv\,kg^{-1}$) over the first 4 h after exercise was closely balanced by the subsequent uptake from 4 to 12 h. These intervals corresponded to the periods of plasma acidosis and subsequent alkalosis recorded in previous studies (Turner *et al.* 1983; Milligan & Wood, 1986*a*,*b*, 1987).

At rest, the net flux of Na^+ at the gills was slightly positive ($+65\,\mu equiv\,kg^{-1}\,h^{-1}$) while Cl^- and K^+ net fluxes were not significantly different from zero (Fig. 2). Ca^{2+} and Mg^{2+} fluxes (not shown) were also not significantly

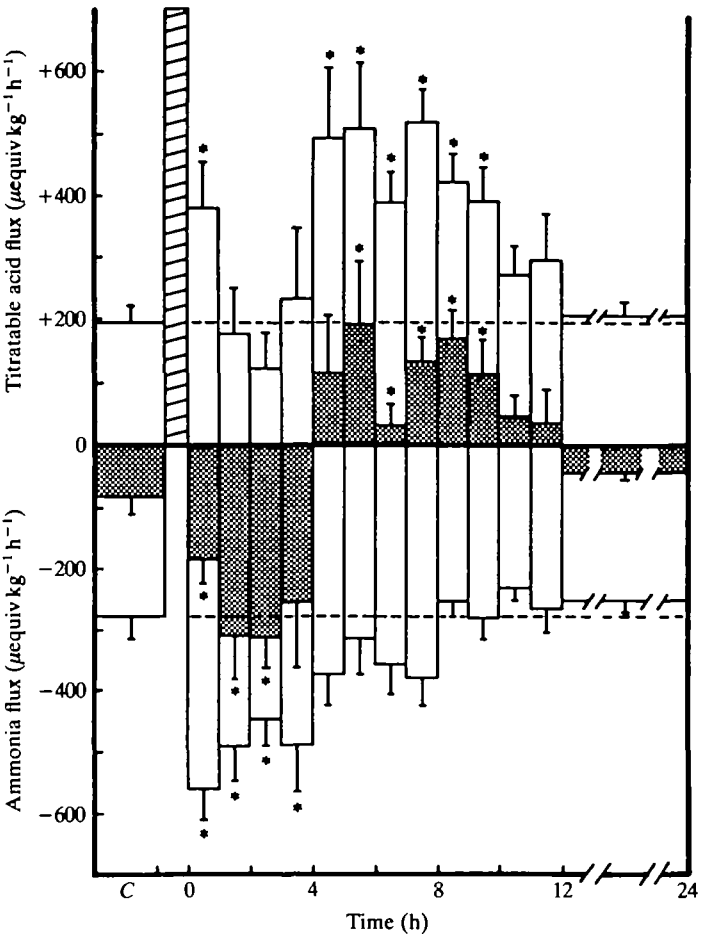

Fig. 1. Changes in the branchial flux of acidic equivalents (net H^+ flux, stippled bars) and its components, titratable acid flux (upward-pointing bars) and ammonia flux (downward pointing bars), after 6 min of exhaustive exercise in rainbow trout. Net gains by the fish have a positive sign, net losses a negative sign. Means ± 1 S.E.M. ($N = 10$). *C*, control; 0 h, immediately after exercise, the dotted line indicates the control level, the cross-hatched bar indicates 6 min of exhaustive exercise, and * indicates a mean value significantly different ($P < 0{\cdot}05$) from the control level.

different from zero, and showed no significant variation over the 24-h post-exercise period. Although this conclusion is reliable for Mg^{2+}, where the detection limit was a flux of about 10 μequiv $kg^{-1} h^{-1}$, it is uncertain for Ca^{2+} where the detection limit was at least five-fold higher because of the much greater background levels of this ion in the water. Exhaustive exercise drove Na^+, Cl^- and K^+ fluxes into negative balance, but thereafter their patterns differed considerably (Fig. 2).

Net Na^+ flux was the first to recover, regaining positive balance by the third hour, and a value significantly above the control level by the fifth hour (Fig. 2A).

The cumulative Na^+ loss in the first 2 h ($\approx$300 μequiv kg^{-1}) was regained by 6 h. In contrast, net Cl^- flux was almost three times more negative initially, and stayed negative far longer (Fig. 2B). Positive Cl^- balance was not regained until 8 h, and significant elevation above control levels occurred only at hour 10. Cumulative Cl^- loss ($\approx$1200 μequiv kg^{-1}) was approximately four times greater than Na^+ loss, and only about 25 % was regained by the end of the experiment. These branchial flux patterns agreed well with previously recorded plasma Na^+ and Cl^- changes. In particular, they explained why measured increases in plasma Na^+ and Cl^- levels (10–15 %; Turner *et al.* 1983; van Dijk & Wood, 1988) were less than the 25–30 % haemoconcentration and contraction of extracellular fluid volume (ECFV) (Milligan & Wood, 1986*a*), and why plasma Cl^- fell significantly below control levels later in recovery.

Net K^+ fluxes were much smaller, and remained negative throughout the first 12 h of recovery (Fig. 2C). The pattern of K^+ loss closely paralleled the previously recorded pattern of K^+ elevation in the blood plasma (Turner *et al.* 1983; van Dijk & Wood, 1988), suggesting that the former was driven by the latter. Cumulative K^+ loss was about 600 μequiv kg^{-1} and was not restored over the period of the experiment.

The general pattern of the net fluxes in relation to acid–base balance was an excess of Cl^- over Na^+ loss during the period of net H^+ excretion (0–4 h), followed by an excess of Cl^- over Na^+ gain during the subsequent period of net H^+ uptake. If Na^+ and Cl^- were the only two strong electrolytes involved in modulating acidic equivalent flux across the gills, then SID theory (Stewart, 1978, 1983) would predict a 1:1 relationship between net $[Na^+-Cl^-]$ flux and net H^+ flux in the opposite direction. Fig. 3 shows that agreement of the mean values with a 1:1 relationship was reasonable, but the slope of the true regression line was only $-0{\cdot}66$. Inclusion of K^+ in the analysis (i.e. $[Na^+ + K^+ - Cl^-]$) improved agreement at positive values of net H^+ flux and worsened it at negative values; the overall effect was a decrease in slope to $-0{\cdot}55$.

The unidirectional flux measurements (Fig. 2A,B) demonstrated a significant stimulation of Na^+ influx during each of the first 4 h after exercise, coincident with the period of net H^+ excretion. There was an accompanying inhibition of Cl^- influx during the second hour. During the subsequent period of net H^+ uptake, there was a pronounced elevation of Cl^- influx (significant from hours 5 to 12), while Na^+ influx returned to control levels.

Na^+ efflux was also elevated during the first 4 h after exercise; over 0–2 h, these increases in efflux were larger than those in influx, resulting in the net negative balance. Cl^- efflux was similarly elevated during the first 4 h. However, since there was no compensating increase in influx, Cl^- balance became highly negative throughout this period. Later, during the period of net H^+ uptake (4–12 h), Cl^- efflux remained elevated. These later elevations in Cl^- efflux were not as large as those in influx, allowing restoration of positive Cl^- balance. Na^+ efflux stayed at control levels during this period. Both Na^+ and Cl^- efflux were elevated from 12 to 24 h.

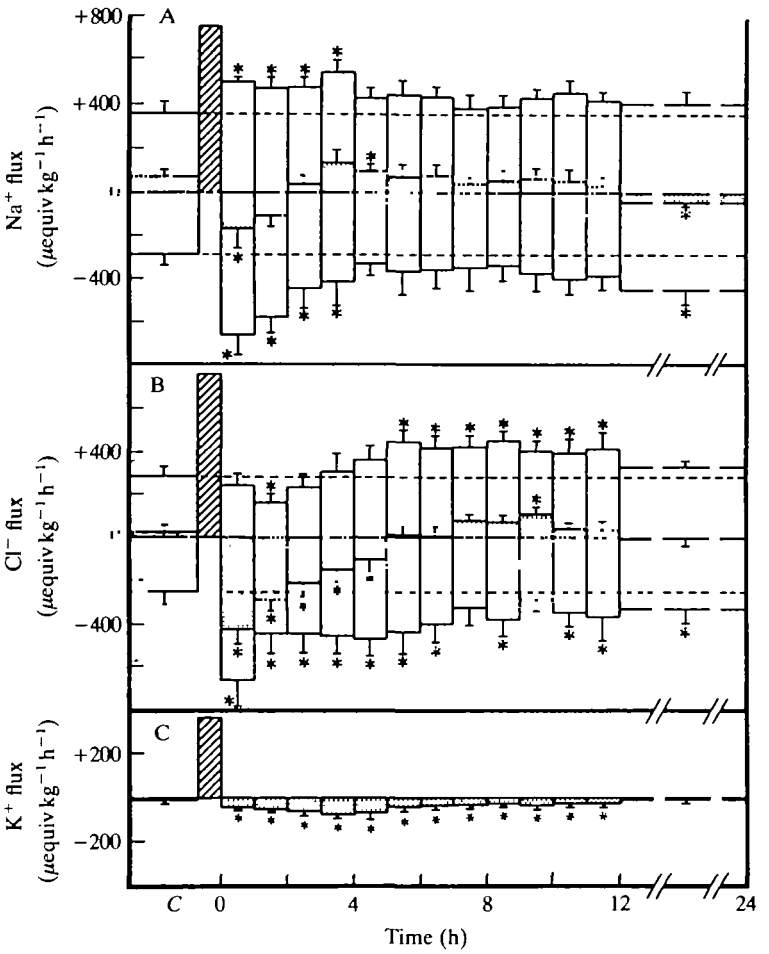

Fig. 2. Changes in the unidirectional and net flux rates of (A) sodium, (B) chloride and (C) potassium (net fluxes only) across the gills of rainbow trout after 6 min of exhaustive exercise. Upward-pointing bars represent influx, downward-pointing bars represent efflux, and the stippled bars represent net flux. Means ± 1 S.E.M. ($N = 10$). Other details as in legend of Fig. 1.

Renal responses

Urine flow rate increased approximately 50 % after exhaustive exercise and remained elevated for 12 h (Fig. 4C). At rest, there was a small net excretion of acidic equivalents ($\approx 5\,\mu$equiv $kg^{-1}\,h^{-1}$) through the kidney (Fig. 4A). This increased approximately four-fold during the first 4 h after exercise. The response comprised a large elevation in [TA−HCO_3^-] efflux (Fig. 5A), reflected in a fall in urine pH (Fig. 5C), and a smaller but longer-lasting rise in NH_4^+ efflux (Fig. 5B). However, cumulative acidic equivalent efflux (Fig. 4A) through the kidney was small relative to that through the gills, amounting to only 8 % of the total. Increases in lactate efflux through the kidney were much larger on a relative basis

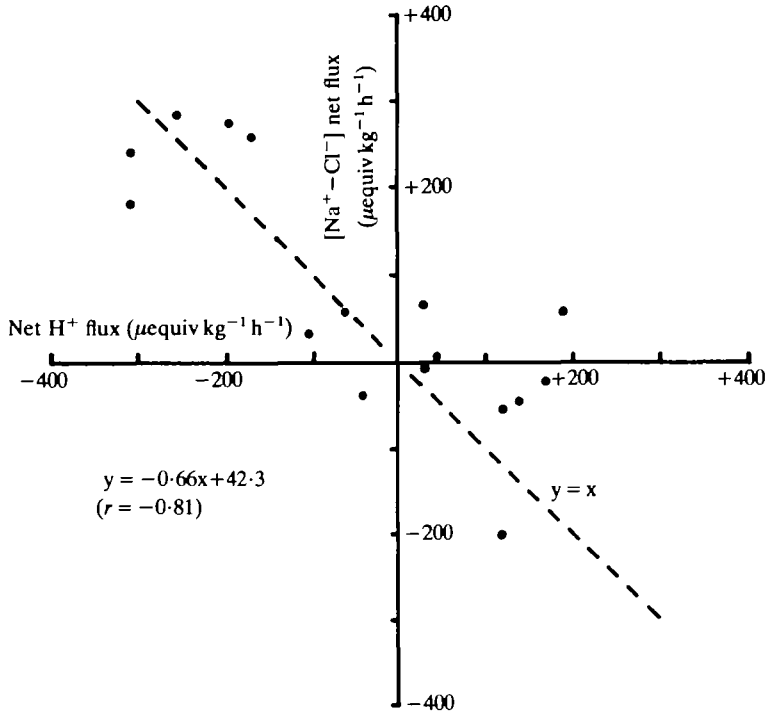

Fig. 3. The relationship between the branchial flux rate of acidic equivalents (x: net H^+ flux) and the simultaneously measured difference between the net branchial flux rates of sodium and chloride (y: $[Na^+-Cl^-]$ net flux) in rainbow trout, based on mean values for 10 fish in 16 measurement periods of the experimental regime. The dotted line (y = x) is the line of equality. The equation of the true regression line is also given ($r = -0{\cdot}81$, $P < 0{\cdot}01$).

(Fig. 4B) and accounted for 50 % of the whole-body lactate excretion recorded by Milligan & Wood (1986*a*). Note, however, that the scale of Fig. 4B is only one-tenth that of Fig. 4A, so lactate efflux was negligible relative to net H^+ excretion. Renal lactate efflux paralleled previously recorded blood lactate changes (Turner *et al.* 1983; Milligan & Wood, 1986*a*,*b*).

At rest, urinary effluxes of Na^+ and Cl^- ($\approx 30\,\mu equiv\,kg^{-1}\,h^{-1}$; Fig. 6A,B) were about five- to 10-fold higher than those of K^+, Ca^{2+} and Mg^{2+} (Fig. 6C–E), and tended to balance net uptake at the gills (cf. Fig. 2). Renal excretion rates of all electrolytes except Cl^- increased significantly during the first 4 h after exercise, but remained small (<15 %) in comparison with those through the gills. The relative increases were larger and longer lasting for K^+ and Ca^{2+} (12 h; Fig. 6C,D) than for the other ions, in concert with the prolonged plasma elevations of these ions (Turner *et al.* 1983; van Dijk & Wood, 1988).

Values of $CR_x > 1$ indicate net tubular secretion, values < 1 indicate net tubular reabsorption, and variations in CR_x indicate alterations in tubular processing (see

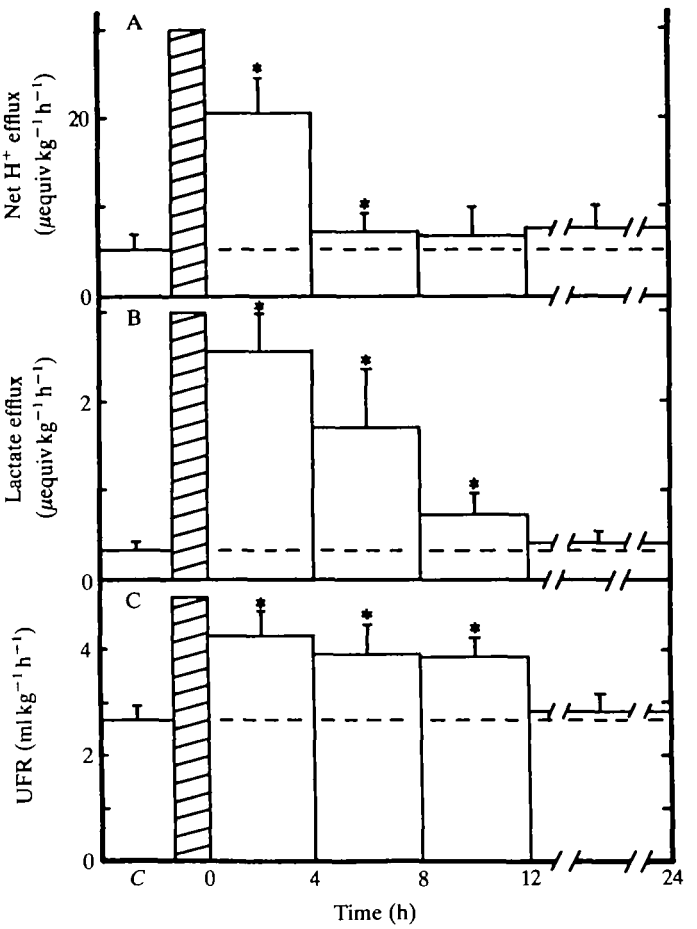

Fig. 4. Changes in the renal excretion rates of (A) acidic equivalents (net H^+ efflux), (B) lactate and (C) urine flow rate (UFR) in rainbow trout after 6 min of exhaustive exercise. Note the 10-fold difference in scale between A and B. Means ± 1 S.E.M. ($N = 10$). Other details as in legend of Fig. 1.

Wheatly *et al.* 1984) (Fig. 7). Under resting conditions, all ions were reabsorbed on a net basis, except for NH_4^+, which was clearly secreted. Net reabsorption was much greater for Na^+ and Cl^- than for Ca^{2+}, K^+ and lactate. CR_{Mg} could not be calculated because there were no plasma data. Increased urinary effluxes during the first 4 h after exhaustive exercise were associated with increased values of CR_K, CR_{Ca} and CR_{NH_4} but not of CR_{Na} or CR_{Cl}. Thus for K^+, Ca^{2+} and NH_4^+, increased effluxes were due to decreased percentage tubular reabsorption (or increased secretion), in addition to elevated plasma concentrations and GFR. $CR_{lactate}$ showed a very different pattern, falling dramatically from 0 to 12 h after exercise, indicating a greatly increased percentage reabsorption during this period.

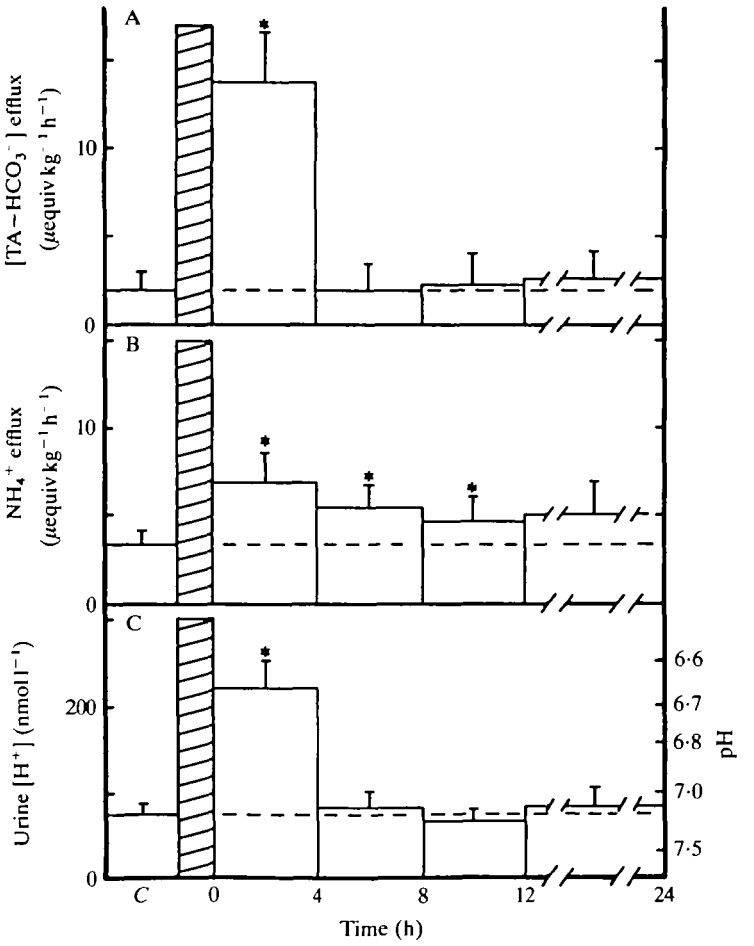

Fig. 5. Changes in the renal excretion rates of (A) urinary titratable acidity minus bicarbonate and (B) urinary ammonia in rainbow trout after 6 min of exhaustive exercise. (C) shows the simultaneously measured activity of H^+ or pH in the urine. Means ± 1 S.E.M. ($N = 10$). Other details as in legend of Fig. 1.

Discussion

The importance of branchial H^+ flux after exercise

In the present study, both the magnitude and pattern of net H^+ excretion to the environment after exhaustive exercise were very similar to those reported by Milligan & Wood (1986*a*) in rainbow trout. Holeton *et al.* (1983) and Holeton & Heisler (1983) measured four- to six-fold greater fluxes in trout and dogfish, respectively, perhaps due to the different method used to induce exhaustive exercise – electric shocks in contrast to simple chasing. Based on the pHi and tissue buffer capacity measurements of Milligan & Wood (1986*a*,*b*), the cumulative flux of acidic equivalents to the external environment ($\approx$1000 μequiv kg^{-1}) was less than 10 % of net whole-body production by glycolysis and ATP degradation.

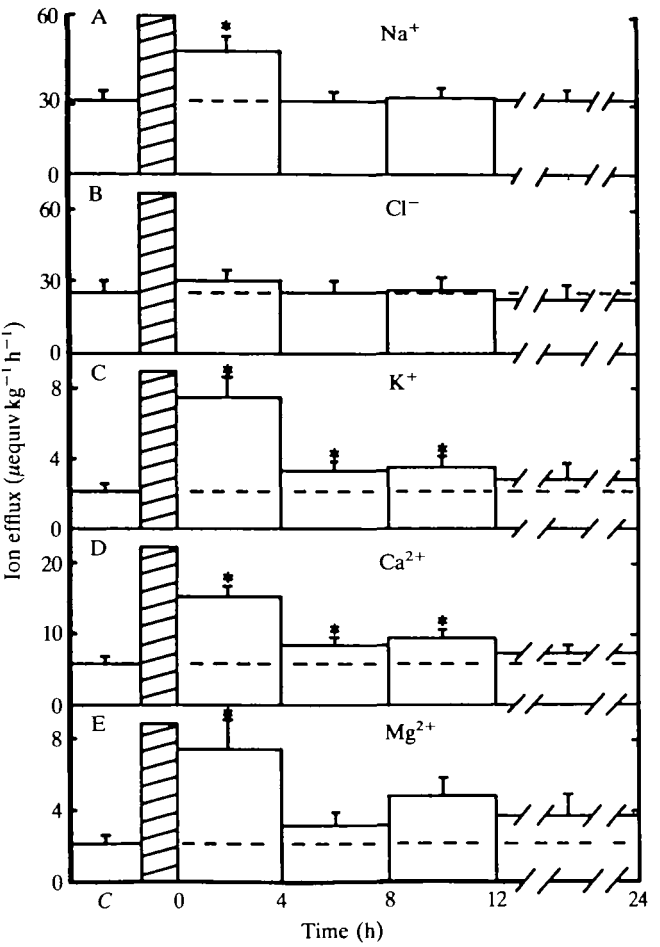

Fig. 6. Changes in the renal excretion rates of electrolytes after 6 min of exhaustive exercise in the rainbow trout: (A) sodium; (B) chloride; (C) potassium; (D) calcium; and (E) magnesium. Means ± 1 S.E.M. ($N = 10$). Other details as in legend of Fig. 1.

Nevertheless, this transient 'storage' in the water over the first 12 h played a crucial role in the recovery of extracellular acid–base balance. In essence, the acidic equivalent load released from muscle to ECFV was subsequently transferred to the environment, thereby expediting pHe correction (with overshoot) long before correction of muscle pHi, blood lactate or muscle lactate. The subsequent re-uptake of these acidic equivalents from the water allowed final acid–base homeostasis *via* H^+ and lactate metabolism.

The role of the kidney after exercise

The gills accounted for 92 % and the kidney for only 8 % of net H^+ efflux after exercise. This partitioning was in accord with previous studies employing different acidotic treatments – e.g. 68 %:32 % after NH_4Cl loading in catfish (Cameron &

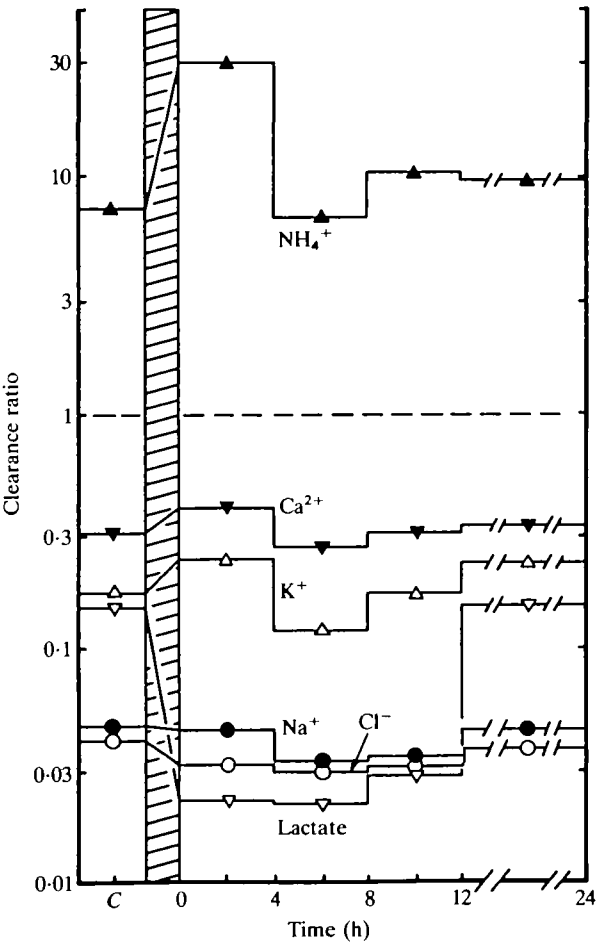

Fig. 7. Changes in the apparent renal clearance ratios for various substances in the urine after 6 min of exhaustive exercise in the rainbow trout. The values are based on mean urinary excretion rates and plasma concentrations; see text for details of calculation. Note that the clearance ratio scale is logarithmic; ratios below 1·0 represent net tubular reabsorption, and above 1·0 net secretion. Other details as in legend of Fig. 1.

Kormanik, 1982); 93 %:7 % during hyperoxia in trout (Wood *et al.* 1984); and 85 %:15 % during adrenaline infusion in trout (Vermette & Perry, 1987*b*).

Renal excretion of acidic equivalents after exercise was largely due to an elevation of $[TA-HCO_3^-]$ efflux over the first 4 h, with a smaller and more persistent elevation of NH_4^+ efflux (Figs 4, 5). This was similar to the pattern after environmental hypoxia in trout (Kobayashi & Wood, 1980). However, in that study there was a secondary stimulation of acidic equivalent excretion of unknown origin from 12 to 72 h post-hypoxia. At least up to 24 h, this was not seen in the present investigation. The NH_4^+ response was partially due to increased tubular

secretion of ammonia, as shown by the clearance ratio analysis (Fig. 7). The $[TA-HCO_3^-]$ response probably resulted from a combination of decreased HCO_3^- filtration accompanying plasma metabolic acidosis, and increased tubular H^+ and phosphate secretion. The latter is suggested by the fact that the excretion rates of all cations increased, while that of Cl^- remained constant (Fig. 6). The missing negative charge was probably phosphate (see Wheatly *et al.* 1984; Perry *et al.* 1987*b*; Vermette & Perry, 1987*b*). This constancy of urinary Cl^- efflux is presumably advantageous at a time of greatly elevated losses of Cl^- across the gills (Fig. 2B).

The prolonged post-exercise diuresis (Fig. 4C) probably resulted from increased branchial water entry and associated increases in blood pressure and GFR (Wood & Randall, 1973*a*,*c*; Hofmann & Butler, 1979). This water entry is due to the enhanced diffusive conductance of the gills which facilitates respiratory gas exchange during and after exercise (Randall & Daxboeck, 1984). The increased electrolyte excretion rates (Fig. 6) were caused by a combination of increased GFR and plasma concentrations (i.e. greater filtered load) with largely unchanged tubular reabsorption efficiency. However, the clearance ratio analysis indicated initial decreases in K^+ and Ca^{2+} reabsorption (Fig. 7), effects which are not uncommon during acidotic stresses (Wheatly *et al.* 1984; Vermette & Perry, 1987*b*), especially when plasma catecholamine levels are elevated as after exhaustive exercise (Primmett *et al.* 1986; Milligan & Wood, 1987). The greatly increased tubular reabsorption of lactate (Fig. 7) at a time of elevated blood lactate undoubtedly reflected the operation of the tubular threshold identified in the trout kidney by Kobayashi & Wood (1980). This threshold system minimizes urinary lactate excretion. Together with the very low branchial permeability to lactate (Milligan & Wood, 1986*a*), it ensures that excretory losses of this valuable energy source are negligible.

Branchial ammonia excretion

Although it is now generally accepted that ammonia can move across the gills of fish as either NH_3 or NH_4^+, it remains controversial which is the dominant mechanism and whether NH_4^+ flux (which represents acidic equivalent efflux) is dynamically modulated during acid–base adjustment (Cameron & Heisler, 1983; Wright & Wood, 1985; Evans & Cameron, 1986; Cameron, 1986; Randall & Wright, 1987). The present flux data (Fig. 1), in combination with previously unpublished information on arterial plasma ammonia levels after identical exercise (Fig. 8), cast some light on the issue. P_{NH_3} and $[NH_4^+]$ were calculated from the measured pHa and total ammonia data of Turner *et al.* (1983), and the values for pK and αNH_3 determined by Cameron & Heisler (1983).

During the first hour after exercise, at least some of the elevated ammonia efflux (Fig. 1) undoubtedly occurred as NH_3 diffusion, because the plasma P_{NH_3}, and therefore the gradient, were elevated (Fig. 8) and titratable acid uptake increased simultaneously (Fig. 1). By the same rationale, from hours 1 to 4 the flux was mainly as NH_4^+, because titratable acid flux and plasma P_{NH_3} returned to resting

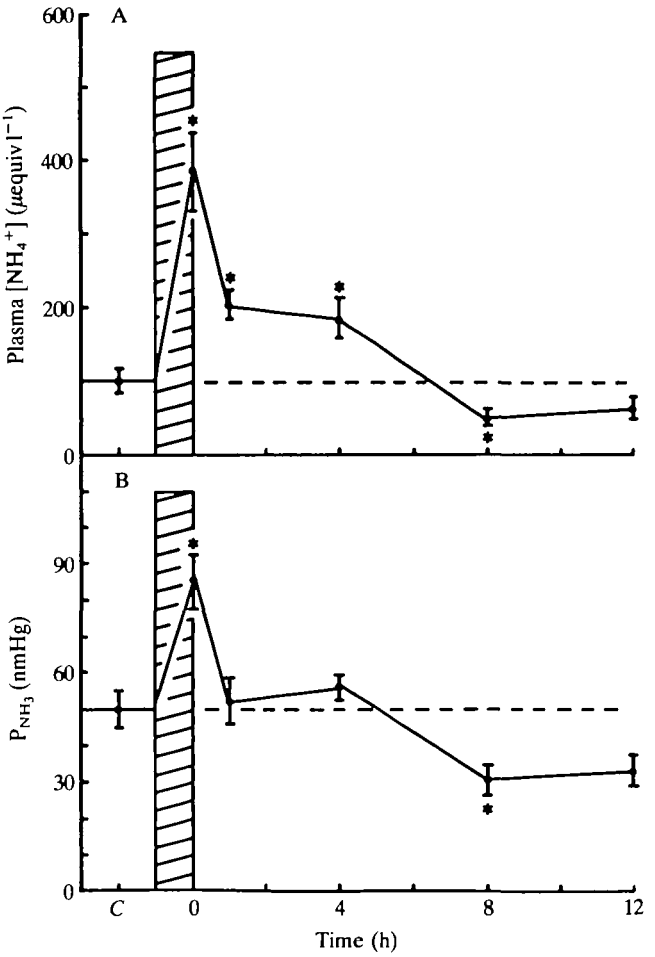

Fig. 8. Changes in plasma ammonia levels after 6 min of exhaustive exercise in rainbow trout, as determined by arterial blood sampling from chronically catheterized fish (previously unpublished data from the experiments of Turner, Wood & Clark, 1983): (A) NH_4^+ concentration; and (B) the partial pressure of NH_3. Means ± 1 S.E.M. ($N = 4$–7). Other details as in legend of Fig. 1.

levels, while plasma NH_4^+ remained elevated. NH_4^+ efflux would thereby account for most of the acidic equivalent efflux. In contrast, Holeton *et al.* (1983) proposed that NH_3 excretion predominated throughout post-exercise recovery in trout, and that acidic equivalent efflux occurred mainly as H^+. Their argument was based on simultaneously measured Na^+, Cl^- and 'HCO_3^-' fluxes, the estimated diffusing capacity of the gills for NH_3, and the premise that ammonia production by amino acid degradation for the purposes of acid–base regulation would be unnecessarily costly. In contrast, we interpret their data as indicating that the efflux was mainly as NH_4^+, for ammonia excretion was well correlated with net Na^+ uptake and 'HCO_3^-' excretion actually declined (equivalent to a decline in titratable acid

uptake in our system). The metabolic cost argument is somewhat teleological. In any event, excess ammonia production after exercise is largely an unavoidable consequence of anaerobic AMP deamination in white muscle, rather than amino acid breakdown (Dobson & Hochachka, 1987). Even when there is no apparent metabolic reason for elevated ammonia production, ammonia excretion increases on a long-term basis in freshwater teleosts during compensation of acidoses – e.g. hypercapnia in carp (Claiborne & Heisler, 1984) and trout (Perry *et al.* 1987*a*), and hyperoxia in trout (Wood *et al.* 1984).

A complication of all such arguments is that ammonia gradients from the branchial cells to the water, rather than from the blood plasma to the water, may be determinants of NH_3 and NH_4^+ efflux. These have never been measured in teleost gills, but there is reason to believe that they will be strongly influenced by electrical gradients across cell membranes (Wright, Randall & Wood, 1988) and the rate of CO_2 flux into the unstirred layers next to the gills (Randall & Wright, 1987).

H^+ versus *ion exchange at the gills*

The branchial efflux of acidic equivalents after exercise was associated with positive values of net $[Na^+ - Cl^-]$ flux, and their later re-uptake with negative values of net $[Na^+ - Cl^-]$ flux (Figs 1–3). This pattern has now been shown many times in freshwater fish during a variety of different acid–base disturbances (Holeton *et al.* 1983; McDonald *et al.* 1983; Wood *et al.* 1984; Claiborne & Heisler, 1984; Perry *et al.* 1987*a*; Vermette & Perry, 1987*a*). Earlier studies which failed to show any relationship can be attributed to methodological problems, as reviewed by Wood *et al.* (1984). Both SID theory (Stewart, 1978, 1983) and the constraints of electrical neutrality dictate that the difference between the fluxes of all strong cations and all strong anions (i.e. SID flux) will constrain an equal and opposite flux of acidic equivalents, *no matter how these electrolyte fluxes occur.* The $[Na^+ - Cl^-]$ *vs* H^+ net flux relationship is not surprising, inasmuch as Na^+ and Cl^- are the two strong electrolytes present in greatest concentration in the blood plasma, and the two which move in greatest quantities across the gills. The interesting question is therefore not whether a relationship exists between $[Na^+ - Cl^-]$ and H^+ net fluxes, but rather how it occurs, a question which can only be answered by unidirectional flux analysis.

Na^+ and Cl^- are known to move across the gills of freshwater fish by at least three mechanisms: (i) active, independent, carrier-mediated, electroneutral transport, probably as Na^+ *vs* NH_4^+ or H^+, and Cl^- *vs* HCO_3^- or OH^- exchanges; (ii) passive diffusion as a function of the respective electrochemical gradients and permeabilities for Na^+ and Cl^-; and (iii) exchange diffusion – i.e. 1:1 Na^+:Na^+ and Cl^-:Cl^- exchanges which may share the same carrier mechanisms as i (Maetz & Garcia-Romeu, 1964; Garcia-Romeu & Maetz, 1964; Maetz, 1972, 1973; DeRenzis & Maetz, 1973; Wood & Randall, 1973*b*; DeRenzis, 1975; Girard & Payan, 1980; McDonald *et al.* 1983; Payan, Girard & Mayer-Gostan, 1984; Wood *et al.* 1984; McDonald & Rogano, 1986). Although it is obvious that mechanism i

can be involved in acidic equivalent flux, and mechanism iii can have no such role, it should be pointed out that differential Na^+ and Cl^- movement by mechanism ii will also create an acidic equivalent 'flux', without necessary physical movement of H^+ or OH^- across the gills, for water is an infinite source or sink of acidic or basic equivalents.

In the present study, acidic equivalent excretion in the first 4 h after exercise was associated with a stimulation of Na^+ influx, a small inhibition of Cl^- influx and increases in both Na^+ and Cl^- efflux (Fig. 2). The influx changes are best explained as an elevation of Na^+ *vs* NH_4^+ (or H^+) and inhibition of Cl^- *vs* HCO_3^- (or OH^-) transport, which would promote net acidic equivalent efflux. The simultaneous increases in both Na^+ and Cl^- efflux were undoubtedly associated with increased passive losses by diffusion. These would be favoured both by the initially elevated plasma levels of these ions (Turner *et al.* 1983; van Dijk & Wood, 1988) and by the enhanced diffusive conductance of the gills (Randall & Daxboeck, 1984). Averaged over the first 4 h, the increase in Cl^- efflux was significantly greater than that in Na^+ efflux, which would serve as an additional mechanism to promote net acidic equivalent efflux.

Acidic equivalent uptake over 4–12 h post-exercise was associated with a stimulation of Cl^- influx, and a return of Na^+ influx and ammonia excretion to control levels (Figs 1, 2). These changes are best explained as an elevation of Cl^- *vs* HCO_3^- (or OH^-) and a correction of Na^+ *vs* NH_4^+ (or H^+) transport, which would promote net acidic equivalent uptake. The simultaneously elevated Cl^- efflux is somewhat puzzling for it would not favour this process; it perhaps represents increased exchange diffusion associated with stimulated influx. Indeed the same explanation may also partially apply to the elevated Na^+ efflux during the first 4 h. Such changes would be neutral in terms of SID and acidic equivalent flux.

The present results differ from both previous studies on ion exchange after exercise. Wood & Randall (1973*a*,*b*) found changes only in Na^+ efflux and not in Na^+ influx, perhaps because the much lower Na^+ levels in their water (14 % of those used here) limited Na^+ uptake. Holeton *et al.* (1983) measured only net fluxes but found a pronounced stimulation of net Na^+ flux starting immediately after exercise, perhaps because the much higher Na^+ levels in their water (285 % of those used here) facilitated Na^+ uptake. The pattern of Cl^- net flux alterations was qualitatively similar to that of the present study. However, the present results agree reasonably well with those of Wood *et al.* (1984) in water of nearly identical composition. That study found that branchial excretion of acidic equivalents during the respiratory acidosis of hyperoxia was associated with stimulated Na^+ influx, inhibited Cl^- influx and stimulated Cl^- efflux. Acidic equivalent uptake during the metabolic alkalosis of normoxic recovery was associated with inhibited Na^+ influx, stimulated Cl^- influx and elevations of both Na^+ and Cl^- efflux. The data of Cameron (1976) on grayling and Perry *et al.* (1987*a*) on trout during hypercapnia can be interpreted in a similar fashion. A recurring theme from all these studies is that while modulation of Na^+ and Cl^- influxes may be the dominant process in acidic equivalent flux across the gills, it is not the only one.

Efflux modulation may also play an important role, and deserves detailed investigation in future studies.

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