

KIDNEY AND URINARY BLADDER RESPONSES OF FRESHWATER RAINBOW TROUT TO ISOSMOTIC NaCl AND NaHCO₃ INFUSION

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Summary

The relative roles of the kidney and urinary bladder in ion, fluid and acid–base regulation were examined in freshwater rainbow trout chronically infused with either 140 mmol l⁻¹ NaCl or 140 mmol l⁻¹ NaHCO₃ (3 ml kg⁻¹ h⁻¹) for 32 h. NaCl had a negligible effect on blood ionic and acid–base status, whereas NaHCO₃ induced a metabolic alkalosis characterized by a rise in arterial pH and [HCO₃⁻] and an equimolar fall in [Cl⁻]. Urine was collected *via* either an internal catheter, which bypassed bladder function, or an external urinary catheter, which collected naturally voided urine. As a percentage of the infusion rate, glomerular filtration rate increased by about 135 %, but urine flow rate (UFR) by only 80 %, reflecting increased tubular reabsorption of H₂O. During NaCl infusion, virtually all of the extra Na⁺ and Cl⁻ filtered was reabsorbed by the kidney tubules, resulting in an increased UFR with largely unchanged composition. During NaHCO₃ infusion, tubular Na⁺ and Cl⁻ reabsorption again kept pace with filtration. HCO₃⁻ reabsorption also increased, but did not keep pace with filtration; an increased flow of HCO₃⁻-rich urine resulted, which excreted about 10 % of the infused base load. At rest, fish fitted with external catheters voided in discrete bursts of about 0.85 ml kg⁻¹ at 25 min intervals. During infusion, burst frequency increased by about 40 % and burst volume by about 20 %. Reabsorption by the bladder reduced UFR by 25 %, the excretion of Na⁺ and Cl⁻ by 50 %, of K⁺ by 44 % and of urea by 25 %. These differences persisted on a relative basis during NaCl and NaHCO₃ infusion despite the decreased residence time. However, HCO₃⁻ was neither secreted nor reabsorbed by the bladder. We conclude that the freshwater kidney functions to remove as much NaCl as possible from the urine, regardless of the NaCl load, and this role is supplemented by bladder function. The bladder plays no role in acid–base regulation during metabolic alkalosis.

Introduction

The mucosal to serosal transport of Na⁺, Cl⁻ and water by the urinary bladder of the freshwater rainbow trout (*Oncorhynchus mykiss*) has been well established *in vitro* (e.g.

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Lahlou and Fossat, 1971, 1984; Hirano *et al.* 1973). More recently, a role in acid–base regulation as well has been suggested by evidence of Na^+/H^+ , NH_4^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchanges in an *in vitro* bladder preparation of the freshwater brook trout (*Salvelinus fontinalis*) (Marshall, 1986; Marshall and Bryson, 1991). Comparable *in vivo* studies have been impeded by the fact that the traditional internal urinary bladder catheter (Holmes and Stainer, 1966; Beyenbach and Kirschner, 1975) collects ureteral urine, thereby bypassing any regulatory role of the bladder. However, through the development of a new external urinary catheterization technique, which allows the bladder sphincters to operate normally, we have recently demonstrated that transport processes also occur *in vivo* (Curtis and Wood, 1991). The role of the bladder is quantified by comparing the flow and composition of urine collected by the traditional internal bladder catheter with those of urine collected by the new external catheter. In resting trout, reabsorption across the bladder supplements the ionoregulatory role of the kidney in a hypotonic environment; urinary Na^+ and Cl^- losses are reduced by 40–50%, while urine flow rate (UFR) is reduced by only 20–25%. Several other substances may also be reabsorbed. The bladder holds urine for 20–30 min to allow this reabsorption to occur, after which the urine is released in a discrete burst (Curtis and Wood, 1991).

In the present study, we have used this same approach to examine the responses of the bladder, relative to those of the kidney, to two experimental treatments designed to disturb normal renal function. The first was simple volume expansion *via* chronic infusion of isotonic 140 mmol l^{-1} NaCl, designed to increase the Na^+ , Cl^- and water load presented to the tubular and bladder reabsorptive sites. The kidney response to isotonic saline loading has been examined in only a few studies (e.g. Vermette and Perry, 1987; Goss and Wood, 1990), and nothing is known about the possible bladder responses. Nevertheless, this situation may be encountered routinely when salmonids migrate from sea water to fresh water, especially if drinking or H_2O ingestion with the food occurs (Shehadeh and Gordon, 1969; Miles, 1971; Talbot *et al.* 1989). The second was chronic infusion with isotonic 140 mmol l^{-1} NaHCO_3 at the same rate. Base loading was employed to evaluate the relative roles of the kidney and bladder in acid–base regulation. The pure metabolic alkalosis induced was comparable to that seen in trout recovering from the HCO_3^- retention that accompanies chronic respiratory acidosis (Höbe *et al.* 1984; Perry *et al.* 1987a). The kidney is known to make a small but significant contribution to HCO_3^- excretion under these conditions (Wheatly *et al.* 1984; Perry *et al.* 1987b), but again nothing is known about the possible contribution of the bladder.

Materials and methods

Experimental animals

Adult rainbow trout (*Oncorhynchus mykiss*; 230–320 g), were obtained from Spring Valley Trout Farm, Petersburg, Ontario, and acclimated to $15 \pm 1^\circ\text{C}$ in dechlorinated Hamilton tapwater for at least 7 days prior to experimentation. The acclimation and experimental water had the following composition: Ca^{2+} , 1.8; Cl^- , 0.8; Na^+ , 0.6; Mg^{2+} , 0.5; K^+ , 0.04; and titration alkalinity to pH 4.0, 1.9 (all in mequiv l^{-1}). Total hardness was

approximately 140 mg l^{-1} as CaCO_3 , and pH was approximately 8.0. Unless otherwise stated, methods were similar to those described by Curtis and Wood (1991).

To allow for infusion and blood collection during the experiments, all trout were fitted with dorsal aortic catheters (Soivio *et al.* 1972) while under MS-222 anaesthesia. At the same time, either the traditional internal bladder catheter (e.g. Holmes and Stainer, 1966; Beyenbach and Kirschner, 1975) or the newly developed external urinary catheter (Curtis and Wood, 1991) was implanted. Trout were then placed in darkened aerated Perspex boxes served with flowing tapwater ($>300 \text{ ml min}^{-1}$) at experimental temperature. Approximately 12 h after catheterization, each fish was given a bolus injection ($17 \mu\text{Ci}$ in 0.66 ml of Cortland saline, Wolf, 1963) of [^3H]PEG-4000 ([1,2- ^3H]polyethylene glycol, $0.5\text{--}1.0 \text{ mCi g}^{-1}$, New England Nuclear) through the dorsal aortic catheter, followed by an additional 0.3 ml of Cortland saline. [^3H]PEG-4000 served as marker for calculation of glomerular filtration rate (GFR). A further 20 h recovery period prior to the start of experiments allowed equilibration of the label throughout the extracellular space and clearance of the volume load.

Experimental series

NaCl infusion

This series was designed to compare directly the patterns of urination, UFR, GFR, urine composition and urinary ion excretion rates between trout fitted with internal ($N=6$) and external ($N=7$) catheters during infusion of 140 mmol l^{-1} NaCl. The osmotic pressure of 140 mmol l^{-1} NaCl was $276 \text{ mosmol kg}^{-1}$ measured on a vapour pressure osmometer (Wescor 5100C) compared to $282 \pm 2 \text{ mosmol kg}^{-1}$ (12) for trout plasma. This allowed observation of changes in urinary dynamics during plasma volume loading without a change in osmolality.

At 20 h after [^3H]PEG-4000 injection, a $300 \mu\text{l}$ blood sample ('control') was taken and replaced with $300 \mu\text{l}$ of Cortland saline. Arterial pH, haematocrit (500 g for 5 min) and plasma total CO_2 were measured immediately. The remaining whole blood was then centrifuged ($10\,000 \text{ g}$ for 2 min) and the plasma decanted and frozen for later analysis of [^3H]PEG-4000, Na^+ and Cl^- concentrations.

Also at 20 h post-injection (i.e. after about 32 h of recovery), the 'control' urine collection period was started, using methods described previously (Curtis and Wood, 1991). In brief, quantity and pattern of urination from fish fitted with either external or internal urinary catheters were measured by passing the freely venting catheter outflow across an infrared optical switch connected to a chart recorder. A drop passing through the beam was shown as a single spike on the chart record. Using measured average drop volumes of $16.75 \mu\text{l}$ for external and $16.10 \mu\text{l}$ for internal catheters, UFR was calculated from the cumulative number of drops over time. Urine was collected over 4 h to ensure sufficient volume for analysis, but urination dynamics were recorded only over the initial 2 h portion of each collection period. 1 ml of urine was decanted, stored at 4°C and analysed within 24 h of collection for titratable acidity minus bicarbonate ($\text{TA} - \text{HCO}_3^-$). The remaining urine was stored frozen for later analysis of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , NO_3^- , phosphate, SO_4^{2-} , ammonia and urea.

NaCl infusion was started 36 h after [^3H]PEG-4000 injection. Fish were infused at a rate of 3.07 ± 0.23 (13) $\text{ml kg}^{-1} \text{h}^{-1}$ with 140 mmol l^{-1} NaCl ($430 \pm 32 \mu\text{mol kg}^{-1} \text{h}^{-1}$) via the arterial catheter for 32 h using one channel of an eight-channel Gilson peristaltic pump for each fish. Urine was collected and stored, and urination dynamics were measured, as above in five periods: 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 16 h, and 24 to 32 h after the beginning of NaCl infusion. Blood samples were taken as above at 6 h, 12 h, 24 h and 32 h.

NaHCO₃ infusion

This series was designed to compare directly the urination patterns, UFR, GFR, urine composition and urinary ion and acid-base excretion rates between fish fitted with internal ($N=7$) and external ($N=7$) urinary catheters during infusion with 140 mmol l^{-1} NaHCO₃ (measured osmotic pressure = $261 \text{ mosmol kg}^{-1}$). The average infusion rate was $2.98 \pm 0.30 \text{ ml kg}^{-1} \text{h}^{-1}$ ($N=14$). This allowed observation of urinary dynamics during base loading ($417 \pm 42 \mu\text{mol kg}^{-1} \text{h}^{-1}$) via a solution with only a slightly lower osmotic pressure ($261 \text{ mosmol kg}^{-1}$ vs $276 \text{ mosmol kg}^{-1}$) and identical Na⁺ concentration to that used during plasma volume loading (140 mmol l^{-1} NaCl).

NaHCO₃-loaded fish were run simultaneously with NaCl-loaded fish, with blood sampling and urine collection occurring at the same intervals. Blood and urine samples were analysed in a similar manner.

Analytical methods and calculations

Titrateable acidity minus bicarbonate ($[\text{TA} - \text{HCO}_3^-]$) was measured as a single value in the double titration procedure recommended by Hills (1973). Urine samples were titrated below pH 5.0 with 0.02 mol l^{-1} HCl to neutralize HCO₃⁻, and stirred for at least 20 min to remove remaining CO₂. They were then titrated back to pH_a using 0.02 mol l^{-1} NaOH (exact molarity tested against Sigma reference standard 2 mol l^{-1} HCl). The difference between the acid and base yielded $[\text{TA} - \text{HCO}_3^-]$. All pH measurements were made using a Radiometer micro-pH electrode (E5021) thermostatted to experimental temperature.

Burst volumes (V_{burst} in ml kg^{-1}) were calculated by multiplying the number of drops in a burst by the average drop volume. Urine flow rate (UFR) was calculated from the cumulative collected volume of urine (ΣV_{total}):

$$\text{UFR} = \frac{\Sigma V_{\text{total}}}{\text{mass} \times \text{time}}, \quad (1)$$

and GFR was then calculated as:

$$\text{GFR} = \frac{\text{UFR} \times \text{cpm}_u}{\text{cpm}_p}, \quad (2)$$

using activities of PEG-4000 ($\text{cts min}^{-1} \text{ ml}^{-1}$) in urine (cpm_u) and plasma (cpm_p). The measurements of PEG-4000 activity in plasma did not coincide with the midpoints of the urine collection periods, so the values of cpm_p used in equation 2 were interpolated to the midpoints. The error in this procedure and its influence on calculated GFR were

negligible, because the rate at which PEG-4000 activity changed in plasma was very low – about $0.35\% \text{ h}^{-1}$ in an approximately exponential fashion.

The urinary excretion rate (U) of any substance (X) was given by:

$$U_X = [X]_u \times \text{UFR}, \quad (3)$$

using measured urine concentration values ($[X]_u$). Ammonia and urea (colorimetric assays) and all anion (by HPLC) and cation (by atomic absorption) measurements in plasma and urine were made using equipment and techniques described previously (Curtis and Wood, 1991).

Following haematocrit measurement, plasma was removed anaerobically from the capillary tube, and plasma total CO_2 was determined using a Corning 965 CO_2 analyser. Plasma P_{CO_2} and bicarbonate concentrations were calculated using the Henderson–Hasselbalch equation and appropriate values of α_{CO_2} and pK' for rainbow trout plasma from Boutilier *et al.* (1984).

Statistical analysis

Results were expressed as means ± 1 S.E.M. (N) throughout. Statistical significance ($P \leq 0.05$) of differences was assessed using Student's two-tailed paired or unpaired t -tests as appropriate, with the Bonferroni procedure for multiple comparisons.

Results

Blood responses

Plasma Na^+ concentrations, initially about 145 mmol l^{-1} , did not change significantly throughout the infusion with 140 mmol l^{-1} NaCl in either internally or externally catheterized trout (Fig. 1A). Plasma Cl^- levels, initially about 132 mmol l^{-1} , rose slightly (Fig. 1C), though the only significant increase occurred at 6 h after the start of infusion in externally catheterized fish. No change in plasma Na^+ concentrations occurred during NaHCO_3 infusion (Fig. 1A). However, plasma Cl^- levels dropped by about 8 mmol l^{-1} during the infusion period (Fig. 1B). Since urinary Cl^- excretion rates did not change throughout the NaHCO_3 infusion period (see Fig. 5C), the change in plasma Cl^- levels was not due to changes in renal Cl^- excretion. No significant differences in plasma Na^+ or Cl^- concentrations were apparent between fish fitted with internal catheters and those fitted with external catheters at the control sample or during either NaCl or NaHCO_3 infusion (Fig. 1).

In all groups, control acid–base status was $\text{pH} \approx 7.9$, plasma $[\text{HCO}_3^-] \approx 9 \text{ mmol l}^{-1}$ and $P_{\text{aCO}_2} \approx 0.4 \text{ kPa}$. NaCl infusion resulted in no change in blood pH (Fig. 1B), plasma $[\text{HCO}_3^-]$ (Fig. 1D) or P_{aCO_2} (not shown) in either internally or externally catheterized fish. In contrast, NaHCO_3 infusion caused significant increases in arterial pH in both groups, to a maximum of 8.13 at 32 h (Fig. 1B). These were caused entirely by significant elevations in plasma HCO_3^- levels to about 16.5 mmol l^{-1} (Fig. 1D) since arterial P_{aCO_2} levels remained unchanged during the infusion period. The increase in plasma $[\text{HCO}_3^-]$ (Fig. 1D) was approximately equivalent to the decrease in plasma Cl^- concentration

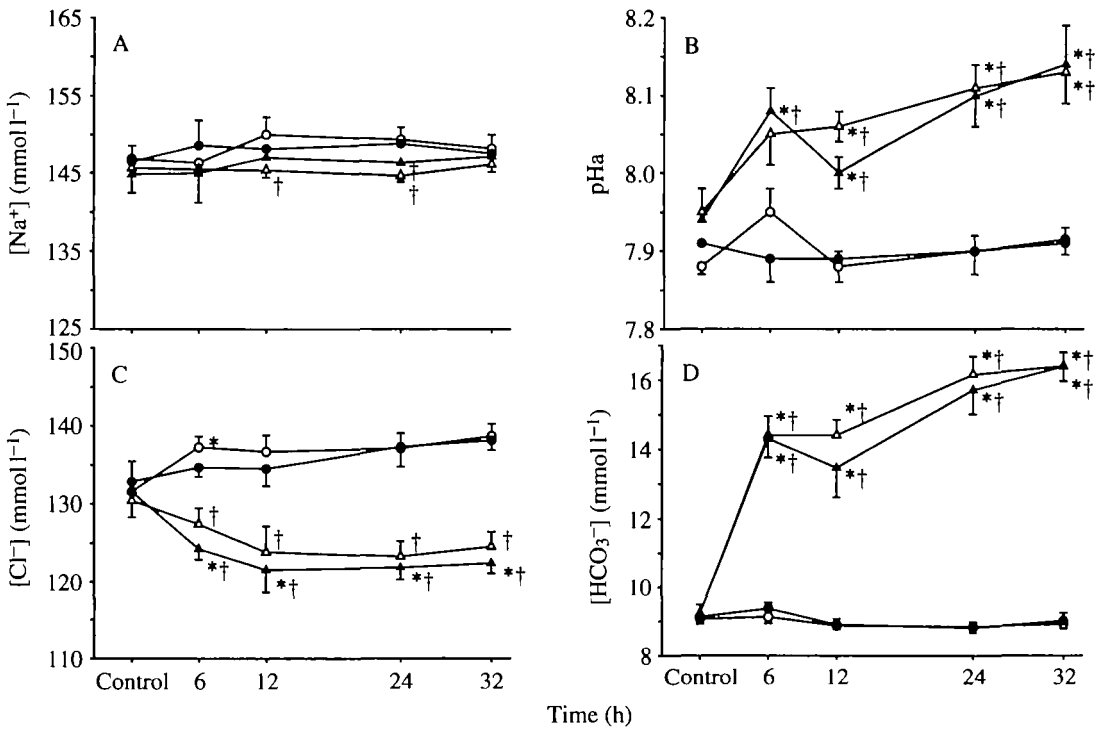


Fig. 1. (A) Plasma sodium concentration, (B) arterial blood pH, (C) plasma chloride concentration and (D) arterial true plasma bicarbonate concentration in rainbow trout infused with isosmotic NaCl (circles) or NaHCO₃ (triangles) and fitted with either internal (filled symbols) or external (open symbols) bladder catheters. Means \pm 1 S.E.M. ($N=6-7$ in each treatment group). * denotes a significant difference ($P \leq 0.05$) from the pre-infusion control value in that treatment group; † denotes a significant difference ($P \leq 0.05$) in NaHCO₃-infused fish from the corresponding value in NaCl-infused fish; there were no significant differences ($P > 0.05$) between values in externally catheterized fish and corresponding values in internally catheterized fish.

(Fig. 1C). Arterial pH values were significantly higher in NaHCO₃- than NaCl-infused fish throughout the infusion period (Fig. 1B) entirely because of higher plasma [HCO₃⁻] (Fig. 1D). No differences in blood pH, plasma HCO₃⁻ concentration or plasma P_{CO_2} , at the same sample times, were measured between fish fitted with internal catheters and those fitted with external bladder catheters during either NaCl or NaHCO₃ infusion.

In all groups, haematocrits started at about 28%, falling gradually to 12% after 32 h as a result of repetitive blood sampling. There were no significant differences between groups. A haematocrit of 12% is above the thresholds reported to cause acid-base disturbance (Wood *et al.* 1982) and catecholamine release (Iwama *et al.* 1987) in rainbow trout, and therefore probably had a negligible influence on the results of this study.

Urinary dynamic responses

Glomerular filtration rates (GFR), initially about 5.3 ml kg⁻¹ h⁻¹, increased significantly in internally and externally catheterized fish infused with both NaCl and

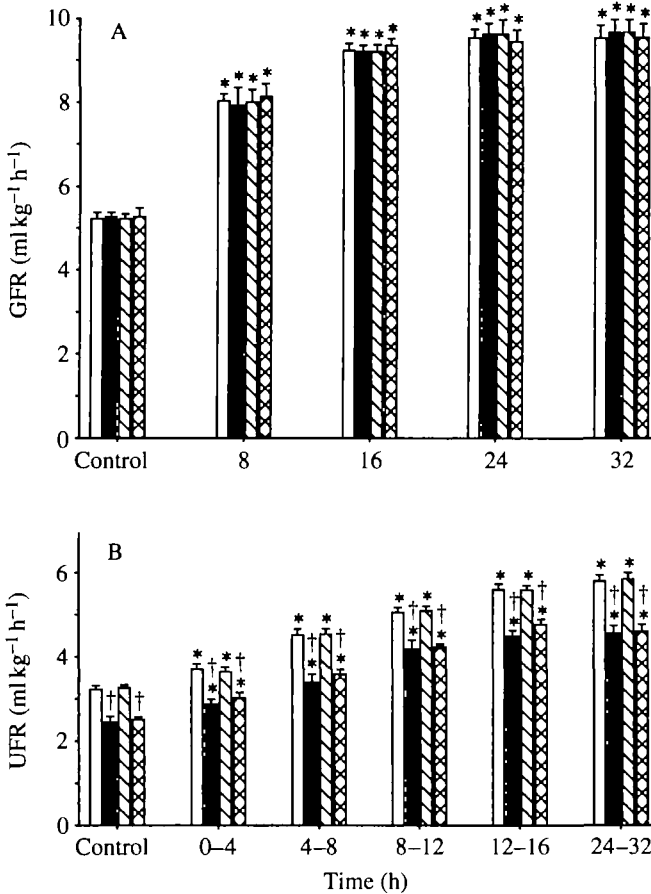


Fig. 2. (A) Glomerular filtration rates (GFR) and (B) urinary flow rates (UFR) in rainbow trout infused with isosmotic NaCl and fitted with internal bladder catheters (open bars), infused with isosmotic NaCl and fitted with external bladder catheters (filled bars), infused with isosmotic NaHCO₃ and fitted with internal bladder catheters (hatched bars) or infused with isosmotic NaHCO₃ and fitted with external bladder catheters (cross-hatched bars). Means \pm 1 s.e.m. ($N=6-7$ in each treatment group). * denotes a significant difference ($P \leq 0.05$) from the pre-infusion control value in that treatment group; † denotes a significant difference ($P \leq 0.05$) in externally catheterized fish from the corresponding value in internally catheterized fish; there were no significant differences ($P > 0.05$) between corresponding values in NaHCO₃-infused and NaCl-infused fish.

NaHCO₃ (Fig. 2A). The final increases were slightly more than $4 \text{ ml kg}^{-1} \text{ h}^{-1}$, which was greater than the infusion rate of $3 \text{ ml kg}^{-1} \text{ h}^{-1}$. GFRs were very similar amongst experimental groups during both the control and infusion periods, indicating no effect of either catheter type or NaCl vs NaHCO₃ infusion.

In parallel with GFR, urinary flow rates (UFR) increased significantly during the infusion period in both internally and externally catheterized fish (Fig. 2B), though the absolute changes were only about half those in GFR (Fig. 2A). The increases were

similar in both NaCl- and NaHCO₃-infused fish, amounting to about 2.6 ml kg⁻¹ h⁻¹ in internally catheterized and 2.2 ml kg⁻¹ h⁻¹ in externally catheterized fish. The elevations in UFR were lower than the infusion rates, suggesting that fluid was being retained within the fish. During the control period, UFRs were significantly lower in externally catheterized fish, indicating an important role for the bladder in fluid reabsorption. Externally catheterized fish excreted only 75% as much urine as internally catheterized fish. This difference persisted throughout the infusion period as the absolute UFRs of both groups increased. At 24–32 h, externally catheterized fish were excreting 79% as much urine as internally catheterized fish.

All fish fitted with internal bladder catheters exhibited similar urination patterns, with urine simply dripping from the catheters in a continuous manner. No internally catheterized trout showed any clumping of drips to indicate bursts of urination. This indicates that internal catheterization rendered the urinary bladder storage function essentially incompetent. During infusion with NaCl or NaHCO₃ the drip rate increased, but there was no evidence of any patterning. In contrast, fish fitted with external bladder catheters urinated in an extremely uniform pattern of 0.8–0.9 ml kg⁻¹ bursts (Fig. 3B) at about 25 min intervals (Fig. 3A) during the control period. About 90% (range 85–97%; *N*=14) of the total UFR occurred in discrete bursts. Interestingly, during infusion the intervals between bursts decreased while average burst volumes increased (Fig. 3). The pattern was independent of the infusion solution. This response clearly indicates the competence of the urinary bladder in urine storage and intermittent release.

Urinary acid–base fluxes

During NaCl infusion, no change in urine pH (approximately 7.3; Fig. 4C) or renal excretion of TA–HCO₃⁻ (very slightly negative at about -2 μmol kg⁻¹ h⁻¹; Fig. 4B) occurred. Similarly, renal ammonia excretion rates (+1 μmol kg⁻¹ h⁻¹) remained unchanged during NaCl infusion (Fig. 4D) and consequently net H⁺ excretion rates (the sum of TA–HCO₃⁻+ammonia fluxes) remained constant at -1 μmol kg⁻¹ h⁻¹ (Fig. 4A).

NaHCO₃ infusion produced a highly significant increase in urine pH (Fig. 4C), which eventually stabilized at about 8.15 after 16 h of infusion. This alkalization was associated with a change in TA–HCO₃⁻ excretion from -2 to -38 μmol kg⁻¹ h⁻¹ (Fig. 4B); ammonia excretion (Fig. 4D) did not change. At the urine pH values measured in the present study, most urinary phosphate would be in the form HPO₄²⁻. Since net phosphate excretion, which normally constitutes the major component of urine TA (Hills, 1973; Wheatly *et al.* 1984), remained negligible and largely unchanged throughout base infusion (Table 1), the change in TA–HCO₃⁻ excretion to highly negative values must have resulted entirely from an increase in the HCO₃⁻ component. Mean urine TA–HCO₃⁻ concentrations became over tenfold more negative between pre-infusion control measurements and 2–32 h of infusion (-0.47±0.16 *vs* -6.26±1.01 mequiv l⁻¹, internal; -0.83±0.33 *vs* -8.18±1.61 mequiv l⁻¹, external), making HCO₃⁻ the most concentrated ion in the urine.

The significant change to highly negative TA–HCO₃⁻ excretion rates during NaHCO₃ infusion resulted in highly significant differences between NaHCO₃- and NaCl-infused fish over the experimental period (Fig. 4B). This, combined with the lack of significant

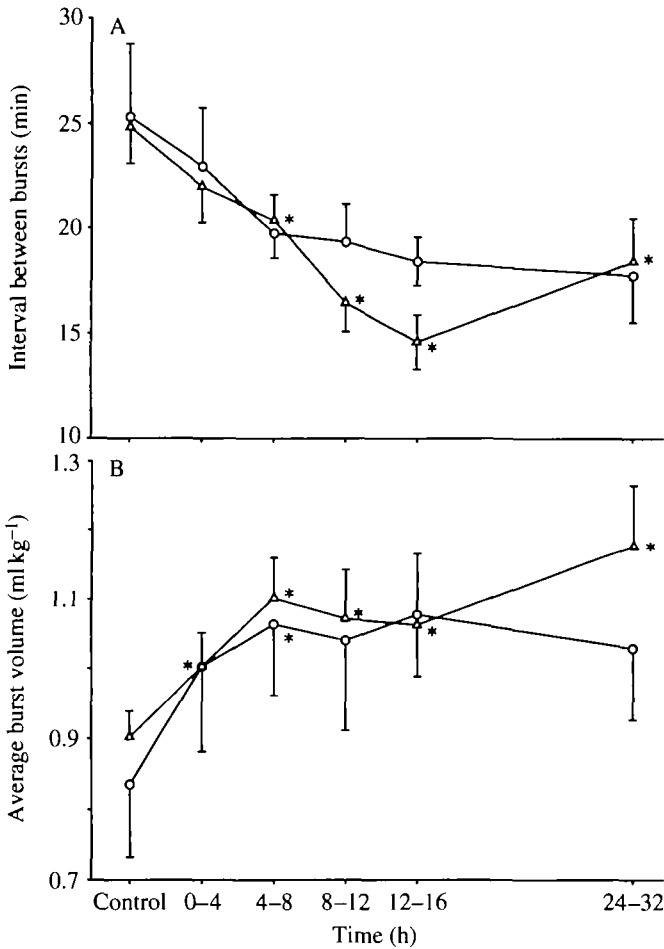


Fig. 3. (A) Intervals between bursts of urination and (B) average urination burst volumes in rainbow trout fitted with external bladder catheters and infused with either isosmotic NaCl (circles) or isosmotic NaHCO₃ (triangles). Means \pm 1 S.E.M. ($N=7$ in each treatment group). * denotes a significant difference ($P \leq 0.05$) from the pre-infusion control value in that treatment group; there were no significant differences ($P > 0.05$) between values in NaHCO₃-infused fish and corresponding values in NaCl-infused fish.

variation in ammonia excretion rates between infusion groups (Fig. 4D), resulted in much more negative net H⁺ excretion rates (Fig. 4A) and associated higher urine pH values in the NaHCO₃-infused trout (Fig. 4C).

No significant differences were found in renal TA-HCO₃⁻ excretion between internally and externally catheterized fish during either NaCl or NaHCO₃ infusion (Fig. 4B). Similarly, ammonia excretion rates did not differ between internally and externally catheterized fish during either type of infusion (Fig. 4D) and, consequently, net H⁺ excretion rates were equal in internally and externally catheterized fish (Fig. 4A).

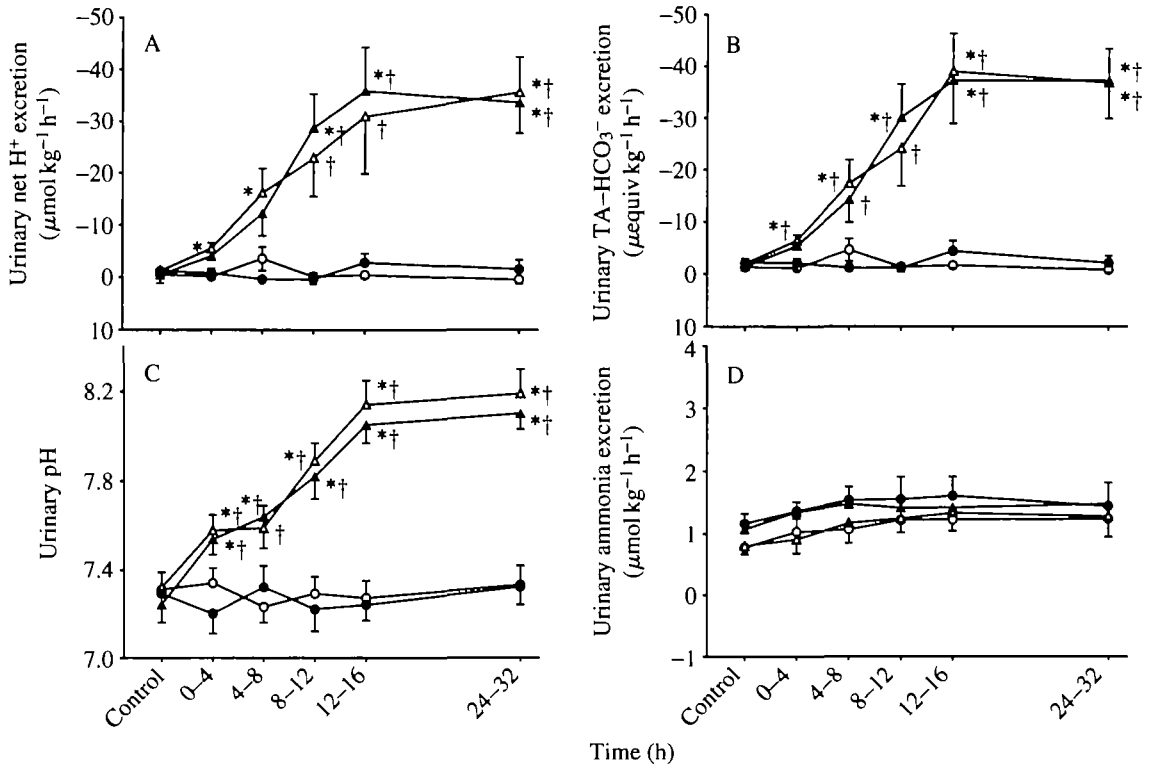


Fig. 4. (A) Urinary net H^+ excretion rates, (B) urinary titratable acid minus bicarbonate excretion rates, (C) urinary pH and (D) urinary ammonia excretion rates in rainbow trout infused with isosmotic NaCl (circles) or $NaHCO_3$ (triangles) and fitted with either internal (filled symbols) or external (open symbols) bladder catheters. Means \pm 1 S.E.M. ($N=6-7$ in each treatment group). * denotes a significant difference ($P \leq 0.05$) from the pre-infusion control value in that treatment group; † denotes a significant difference ($P \leq 0.05$) in $NaHCO_3$ -infused fish from the corresponding value in NaCl-infused fish; there were no significant differences ($P > 0.05$) between values in externally catheterized fish and corresponding values in internally catheterized fish.

These results suggest that the bladder played no role in acid-base regulation during $NaHCO_3$ loading.

Urinary ion fluxes

Prior to infusion, Na^+ and Cl^- were excreted in equimolar amounts (Fig. 5A,C) within all groups, though there were substantial differences in absolute rates between the internally and externally catheterized fish (see below). During NaCl infusion, Na^+ and Cl^- excretion rates increased by more than $10 \mu\text{mol kg}^{-1} \text{h}^{-1}$ but remained equimolar in both externally and internally catheterized fish. These elevated excretion rates resulted entirely from increased UFRs since urinary Na^+ (7.28 ± 1.50 vs $6.98 \pm 0.68 \text{ mmol l}^{-1}$, internal; 4.62 ± 0.48 vs $4.33 \pm 0.53 \text{ mmol l}^{-1}$, external) and Cl^- (6.41 ± 0.78 vs $6.45 \pm$

Table 1. Excretion rates ($\mu\text{mol kg}^{-1} \text{h}^{-1}$) of various substances and under control conditions and after 24–32 h of infusion

Substance	NaCl infused						NaHCO ₃ infused					
	Internal			External			Internal			External		
	Control	24–32 h	Control	24–32 h	Control	24–32 h	Control	24–32 h	Control	24–32 h	Control	24–32 h
Na ⁺	23.4±1.5	34.3±5.8*	11.4±1.2†	20.6±2.7*†	22.4±1.3	39.8±1.3*	11.4±0.8†	20.5±0.5*†	11.4±0.8†	39.8±1.3*	11.4±0.8†	20.5±0.5*†
K ⁺	2.80±0.40	4.33±0.93*	1.80±0.30	3.78±0.45*	3.48±0.61	5.46±0.80*	1.69±0.23†	3.16±0.61*	1.69±0.23†	5.46±0.80*	1.69±0.23†	3.16±0.61*
Ammonia	1.16±0.16	1.45±0.37	0.78±0.11	1.24±0.29	1.07±0.23	1.48±0.34	0.80±0.14	1.28±0.37	0.80±0.14	1.48±0.34	0.80±0.14	1.28±0.37
Ca ²⁺	4.76±0.48	6.80±1.44*	3.86±0.51	5.74±0.70*	5.28±0.62	7.50±0.66*	4.16±0.40	5.65±0.50*	4.16±0.40	7.50±0.66*	4.16±0.40	5.65±0.50*
Mg ²⁺	2.54±0.71	1.67±0.39*	1.56±0.26	0.99±0.20*	2.56±0.47	1.32±0.37*	1.87±0.49	0.90±0.26*	1.87±0.49	1.32±0.37*	1.87±0.49	0.90±0.26*
Cl ⁻	20.2±1.84	32.4±2.20*	11.5±1.54†	21.5±1.21*†	21.1±1.93	23.3±2.67‡	11.7±1.63†	11.9±1.87†‡	11.7±1.63†	23.3±2.67‡	11.7±1.63†	11.9±1.87†‡
SO ₄ ²⁻	2.99±0.90	2.17±0.92	2.58±0.61	2.37±0.44	3.17±0.84	2.67±0.60	2.58±0.51	1.85±0.52	2.58±0.51	2.67±0.60	2.58±0.51	1.85±0.52
NO ₃ ⁻	0.59±0.32	1.44±0.76	0.41±0.29	1.40±0.58	0.29±0.25	0.71±0.45	0.10±0.08	0.32±0.26	0.10±0.08	0.71±0.45	0.10±0.08	0.32±0.26
Phosphate	0.16±0.05	0.46±0.33	0.40±0.20	1.02±0.43*	0.48±0.23	0.94±0.32	0.24±0.20	1.11±0.60	0.24±0.20	0.94±0.32	0.24±0.20	1.11±0.60
TA-HCO ₃ ⁻	-2.10±0.88	-2.14±1.36	-1.34±0.68	-0.78±0.52	-1.45±0.58	-37.2±6.17	-1.97±0.82	-36.8±7.00	-1.97±0.82	-37.2±6.17	-1.97±0.82	-36.8±7.00
Urea	2.89±0.21	4.17±0.94*	2.15±0.33	3.77±0.54*	2.82±0.12	4.90±0.40*	2.26±0.23	3.81±0.39*	2.26±0.23	4.90±0.40*	2.26±0.23	3.81±0.39*

Means ± 1 S.E.M. (N=6–7).

* denotes a significant difference from corresponding control value ($P \leq 0.05$).† denotes a significant difference from corresponding value in internally catheterized fish ($P \leq 0.05$).‡ denotes a significant difference from corresponding value in NaCl-infused fish ($P \leq 0.05$).TA-HCO₃⁻, titratable acidity minus bicarbonate.

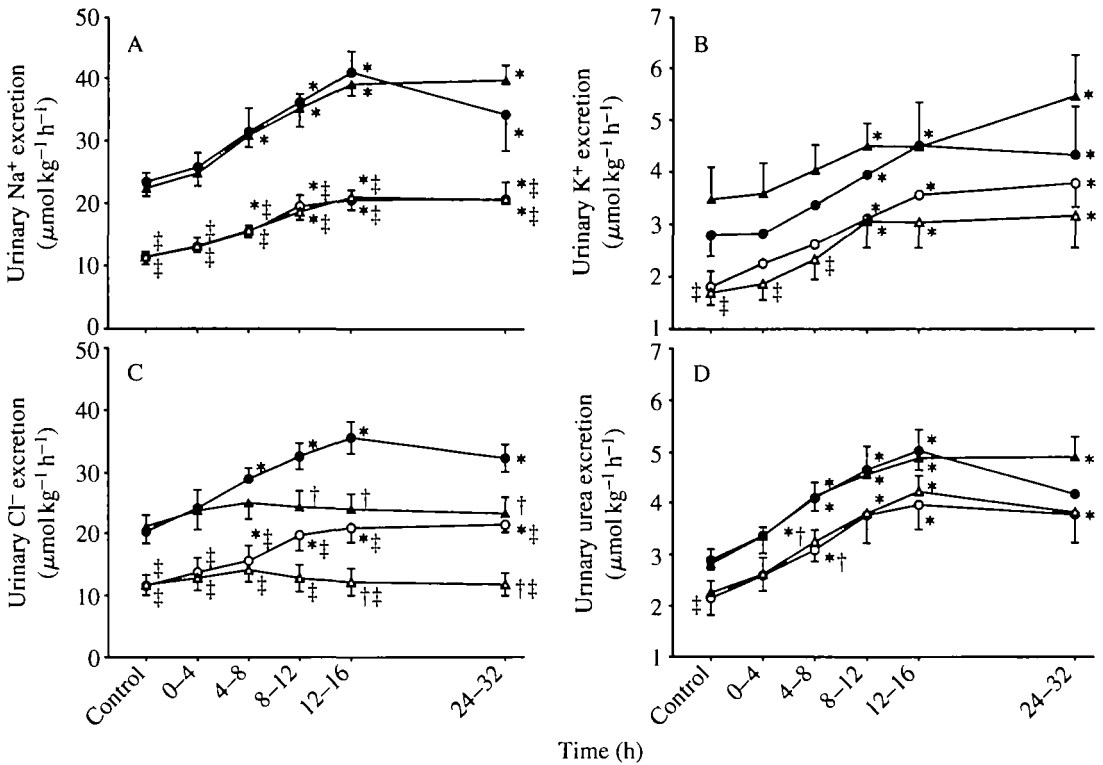


Fig. 5. (A) Urinary sodium, (B) potassium, (C) chloride and (D) urea excretion rates in rainbow trout infused with isosmotic NaCl (circles) or NaHCO₃ (triangles) and fitted with either internal (filled symbols) or external (open symbols) bladder catheters. Means \pm 1 S.E.M. ($N=6-7$ in each treatment group). * denotes a significant difference ($P \leq 0.05$) from the pre-infusion control value in that treatment group; ‡ denotes a significant difference ($P \leq 0.05$) in externally catheterized fish from the corresponding value in internally catheterized fish; † denotes a significant difference ($P < 0.05$) in NaHCO₃-infused fish from the corresponding value in NaCl-infused fish.

0.57 mmol l⁻¹, internal; 4.67 \pm 0.61 vs 4.58 \pm 0.33 mmol l⁻¹, external) concentrations remained relatively unchanged throughout the experiment.

During NaHCO₃ infusion, renal Na⁺ excretion rates increased in a very similar fashion to those in NaCl-infused fish (Fig. 5A). However, renal Cl⁻ excretion rates did not increase significantly over control values in either internally or externally catheterized fish (Fig. 5C) because Cl⁻ concentration in the urine decreased significantly (6.52 \pm 0.57 vs 3.99 \pm 0.45 mmol l⁻¹, internal; 4.62 \pm 0.65 vs 2.68 \pm 0.48 mmol l⁻¹, external). Therefore, by 12–16 h of infusion, the Cl⁻ excretion rates of NaHCO₃-infused fish were significantly lower than those of comparably catheterized NaCl-infused trout.

Renal Na⁺ and Cl⁻ excretion rates were both significantly lower in externally catheterized (about 11 μ mol kg⁻¹ h⁻¹) than internally catheterized fish (about 22 μ mol kg⁻¹ h⁻¹) under control conditions, indicating an important contribution of the

bladder to Na^+ and Cl^- reabsorption (Fig. 5A,C). These 50% lower excretion rates were associated with approximately 35% lower Na^+ and Cl^- concentrations (see above) and 25% lower UFRs (Fig. 2B). These differences were maintained on a relative basis, and therefore increased on an absolute basis, during infusion with NaHCO_3 and NaCl , with the exception of Cl^- during NaHCO_3 infusion (Fig. 5C). Na^+ and Cl^- transport by the bladder epithelium clearly did not diminish during the infusion period.

Renal K^+ excretion rates increased equally in both NaCl - and NaHCO_3 -infused fish (Fig. 5B), becoming significantly higher after 8 h of infusion. Again, these increases were entirely due to higher UFRs without any accompanying change in urine K^+ concentrations (approximately 0.9–1.0, internal; 0.7–0.8 mmol l^{-1} , external). When control K^+ excretion rates for the two externally catheterized treatments were grouped (about 1.7 $\mu\text{mol kg}^{-1} \text{h}^{-1}$), they were significantly lower (by 44%) than those in the similarly grouped internally catheterized fish (about 3.2 $\mu\text{mol kg}^{-1} \text{h}^{-1}$; Fig. 5B). This difference persisted but did not increase over the infusion period. Infusion of NaCl or NaHCO_3 did not have a differential effect on K^+ excretion in internally vs externally catheterized fish.

Urea excretion rates also increased from control values during NaCl and NaHCO_3 infusions (Fig. 5D), becoming significantly higher after 4 h of infusion. At no times were the urea excretion rates significantly different between NaCl - and NaHCO_3 -infused fish. Once again, increased renal urea excretion was due entirely to increased UFR since urea concentrations in the urine did not change. When control rates for the two externally catheterized treatments were grouped and compared to grouped values for internally catheterized fish (as for K^+), urea excretion rates (about 2.2 $\mu\text{mol kg}^{-1} \text{h}^{-1}$) were significantly lower than those of internally catheterized fish (about 2.9 $\mu\text{mol kg}^{-1} \text{h}^{-1}$; Fig. 5D). Again, this trend persisted throughout the period of infusion. As there was no difference in urea concentration (approximately 0.9 mmol l^{-1}), these responses simply resulted from the differences in UFR, suggesting that urea and H_2O are reabsorbed equally by the bladder.

During infusions of both NaCl and NaHCO_3 , renal Ca^{2+} excretion rates increased significantly by 12 h in both internally and externally catheterized fish, and by 24–32 h had reached the values shown in Table 1. Once again, this was due to increased UFR since no significant changes occurred in urinary Ca^{2+} concentrations (approximately 1.5 mmol l^{-1}). There were no differences in Ca^{2+} excretion rates between NaCl - and NaHCO_3 -infused fish. There were also no significant differences between internally and externally catheterized fish, though Ca^{2+} excretion was generally lower in the latter (Table 1).

Renal Mg^{2+} excretion rates were about half those of Ca^{2+} under control conditions (Table 1). Furthermore, renal excretion rates of Mg^{2+} , in contrast to those of Ca^{2+} , decreased significantly from control values over the infusion period, an effect which was significant in all groups by 24–32 h (Table 1). This phenomenon reflected a 67% reduction in urinary Mg^{2+} concentration from about 0.75 to 0.25 mmol l^{-1} . There were no significant differences in Mg^{2+} excretion between NaCl and NaHCO_3 -infused fish, or between internal and external catheterization.

Renal SO_4^{2-} excretion rates were similar to those of Mg^{2+} , and again a slight decrease occurred in all groups in response to chronic infusion, though these changes were not significant (Table 1). However, urinary SO_4^{2-} concentration fell by about 60 % from 1.0 to 0.4 mmol l^{-1} , a difference which was significant in all groups. Again, there were no differences in SO_4^{2-} excretion attributable to either NaCl vs NaHCO_3 infusion or the type of catheterization.

Renal NO_3^- excretion rates were low and rather variable (Table 1). Rates tended to increase with chronic infusion, but the responses were not significant. There were no differences attributable to either NaCl vs NaHCO_3 infusion, or internal vs external catheterization.

Renal phosphate excretion rates for all experimental groups were also very low and increased slightly over the infusion period; the increase was significant only in the externally catheterized fish infused with NaCl (Table 1). Otherwise, there were no differences attributable to either the type of infusion or the style of catheterization.

Discussion

Blood electrolyte and acid-base status

The lack of any marked change in either plasma Na^+ or Cl^- concentrations or acid-base status during chronic isosmotic NaCl infusion (Fig. 1) was in general agreement with several previous investigations on rainbow trout (Perry and Vermette, 1987; Goss and Wood, 1990). The latter study, using a protocol almost identical to that of the present investigation, demonstrated that the major pathways of regulation were a branchial excretion of NaCl at a rate 10- to 20-fold greater than the present renal excretion rates and a penetration of extravascular compartments by the infused electrolytes. These results suggest that changes in GFR, UFR and urination patterns in the present study were due entirely to volume loading and not to any disturbance of plasma ion concentrations. The responses to experimental volume loading resemble those found in salmonids moving through salinity gradients. During migration of *Oncorhynchus kisutch* (Miles, 1971) and *Salmo salar* (Talbot *et al.* 1989) from sea water to fresh water, UFR increased significantly, while plasma Na^+ and Cl^- levels declined. Similarly, smolting juvenile *Salmo salar* exhibited increased UFRs in preparation for migration from fresh water to sea water (Eddy and Talbot, 1985) while concentrations of plasma electrolytes in smolting rainbow trout did not change significantly (Holmes and Stainer, 1966). Freshwater rainbow trout fed a NaCl-enriched diet also increased UFR substantially (Salman and Eddy, 1988).

During chronic infusion of NaHCO_3 , plasma Na^+ concentration remained constant but plasma Cl^- concentration decreased by an extent almost equimolar to the elevation in plasma HCO_3^- concentration, while pH_a rose markedly (Fig. 1). Similar reciprocal depressions of plasma $[\text{Cl}^-]$ and elevations of plasma $[\text{HCO}_3^-]$ have been observed in numerous studies of trout during adaptation to environmental hypercapnia (e.g. Perry *et al.* 1987a) or hyperoxia (Höbe *et al.* 1984), and a similar blood metabolic alkalosis was seen as soon as such fish were returned to normocapnic, normoxic conditions. In this circumstance, the blood becomes alkalotic because the P_{CO_2} drops quickly while

[HCO₃⁻] drops more slowly. The present results were again very similar to those of Goss and Wood (1990), who demonstrated that branchial excretion of Na⁺ and basic equivalents and uptake of Cl⁻ were the major pathways of regulation in response to an almost identical isosmotic NaHCO₃ infusion. Note particularly that plasma [Cl⁻] fell despite net branchial uptake and unchanged renal excretion (Fig. 5C), suggesting considerable internal redistribution of Cl⁻. The elevated renal excretion of basic equivalents measured in the present study (Fig. 4) amounted to only about 10% of the branchial excretion rates recorded by Goss and Wood (1990) and was comparable to those recorded in studies of trout recovering from post-hypercapnic or post-hyperoxic alkaloses (Wheatly *et al.* 1984; Perry *et al.* 1987b).

Filtration and reabsorption calculations

The measurements made in the present study allow calculation of glomerular filtration rates (FR_X) and separate reabsorption rates for the kidney (i.e. tubule system; TR_X) and the urinary bladder (BR_X) using mean values for GFR, plasma concentrations, excretion rates from internally catheterized fish (IER_X) and excretion rates from externally catheterized fish (EER_X):

$$TR_X = FR_X - IER_X, \quad (4)$$

$$BR_X = IER_X - EER_X, \quad (5)$$

$$FR_X = GFR \times [X]_p. \quad (6)$$

TR_X and (FR_X) for a substance X could be calculated directly only for H₂O, Na⁺, Cl⁻ and HCO₃⁻ because only these substances were measured in blood plasma. However, BR_X could be calculated for all measured substances, as these were common to both methods of catheterization (see Table 2).

Glomerular filtration rate and urine flow rate

GFR increases were 30–40% greater than the infusion rate (Fig. 2A); mechanisms responsible could include both 'passive' (e.g. increased blood pressure, reduced plasma oncotic pressure) and 'active' factors (e.g. changes in angiotensin II, Brown *et al.* 1980; sympathetic nervous activity, Elger and Hentschel, 1983; or atrial natriuretic factor, Duff and Olson, 1986). Elevated tubular reabsorption in the kidney reduced the increases in UFR to only about 80% of the infusion rate (Fig. 2B). Fractional H₂O reabsorption remained constant at about 40%; therefore, increases in net tubular reabsorption of H₂O did not keep pace with increases in GFR in either NaCl- or NaHCO₃-infused fish (Fig. 6C,D). In contrast, increases in net tubular reabsorption of Na⁺ and Cl⁻ (Fig. 6A,C) almost kept pace with increased filtration. The calculated Na⁺ and Cl⁻ 'concentrations' of the reabsorbed fluid remained constant over the NaCl infusion period (control=356 mmol l⁻¹, 24–32 h=355 mmol l⁻¹). The net result was the production of an increased volume of dilute urine. The elevation in UFR reached 2.6 and 2.2 ml kg⁻¹ h⁻¹ in internally and externally catheterized fish, respectively, after 24–32 h, and thereby compensated largely but not completely for the volume loading induced by the infusion (3 ml kg⁻¹ h⁻¹). By integration of the data in Fig. 2B, we estimate that the elevated UFR

cleared 66 % of the volume infused over 32 h in internally catheterized fish and 59 % in externally catheterized fish. Thus, 32.5 ml kg^{-1} (internal) to 39.5 ml kg^{-1} (external), which is equivalent to about 15 % of the extracellular fluid volume (Munger *et al.* 1991), was either retained inside the fish or excreted through alternative routes.

Renal electrolyte handling

In contrast to H_2O , virtually all the extra Na^+ and Cl^- filtered was removed from the urine in both NaCl - and NaHCO_3 -infused groups. In consequence, renal Na^+ and Cl^- excretion rates increased by only a relatively small amount during NaCl infusion, while Na^+ excretion rate alone increased slightly during NaHCO_3 infusion. Similar small elevations in Na^+ and Cl^- excretion rates in response to chronic isosmotic infusion were reported by Goss and Wood (1990), while Vermette and Perry (1987) actually found slight decreases during intravascular infusion of Cortland saline (Wolf, 1963). Using the results of the present study, we estimate that, if renal tubular reabsorption had not increased during isosmotic volume loading, urinary Na^+ and Cl^- excretion rates would have increased by approximately $700 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ in all these studies (cf. Fig. 6). It therefore appears that the freshwater teleost kidney is a simple organ, designed to reabsorb as much Na^+ and Cl^- as possible from the urine. Salman and Eddy (1988) reached a similar conclusion based on experiments with freshwater trout fed a NaCl -enriched diet. The mechanism(s) by which tubular NaCl reabsorption keeps pace with increased filtration remains unknown. The same phenomenon is commonly seen in mammals, where it is termed 'glomerulo-tubular balance'. A constant fraction of filtered NaCl is reabsorbed in the proximal tubules regardless of filtered load, leaving only a small additional amount, most of which is reabsorbed by the high-capacity transport systems in the lower part of the tubule (Valtin, 1983). Even in mammals the explanation remains unknown, although alterations in oncotic pressure in the peritubular capillaries, nervous and hormonal influences and linkages of Na^+ transport with organic solute transport may all be involved (Valtin, 1983).

The present results suggest that Na^+ and Cl^- reabsorption are not completely coupled in the renal tubule. During NaCl infusion, increases in Na^+ and Cl^- reabsorption were approximately equal, but during NaHCO_3 infusion, increases in Cl^- reabsorption were less than those in Na^+ (Fig. 6A,B). Increased HCO_3^- reabsorption accounted for only half of this difference (Fig. 6E,F). Nishimura *et al.* (1983) have proposed that coupled Na^+/Cl^- cotransport occurs in the distal tubules of rainbow trout based on transport blocking *in vitro* by furosemide or ouabain or replacement of either Na^+ or Cl^- in the perfusate. However, ion transport in the proximal tubules has not been characterized.

Only qualitative conclusions can be drawn about the renal handling of those substances that were measured in the urine but not in the plasma (i.e. K^+ , Mg^{2+} , Ca^{2+} , ammonia, SO_4^{2-} , NO_3^- , phosphate and urea). Assuming standard literature values (McDonald and Milligan, 1992) for plasma levels in freshwater rainbow trout, with appropriate corrections for protein binding of Ca^{2+} and Mg^{2+} (Björnsson and Haux, 1985), the expected increases in filtration rate were calculated for each substance. With the exception of ammonia, none of the measured excretion rates even approached these values (indeed most were less than half), indicating that increased tubular reabsorption of

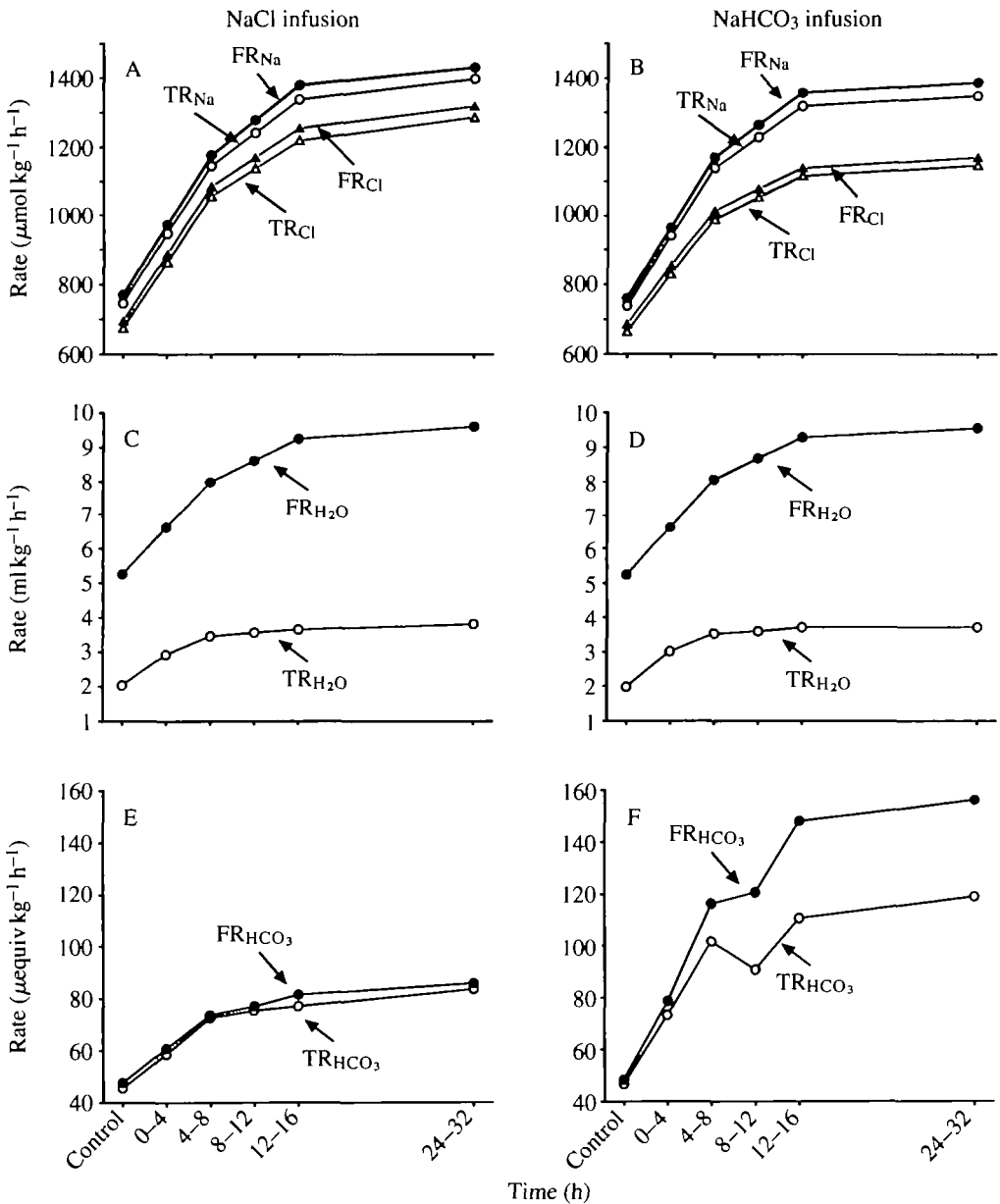


Fig. 6. (A) Net glomerular filtration rates (FR, filled symbols) and net renal tubular reabsorption rates (TR, open symbols) of (A,B) sodium (circles) and chloride (triangles), (C,D) water and (E,F) bicarbonate in rainbow trout infused with either isosmotic NaCl (A,C,E) or isosmotic NaHCO₃ (B,D,F).

K⁺, Mg²⁺, Ca²⁺, SO₄²⁻, NO₃⁻, phosphate and urea probably all occurred during isosmotic volume loading. Clearly, however, the reabsorptive system was more efficient for some substances (Mg²⁺ and SO₄²⁻) than for others (K⁺, Ca²⁺, NO₃⁻, phosphate and

urea) (Table 1). Ammonia was secreted; this process was not markedly affected by isosmotic volume loading.

Renal acid-base balance

During NaCl infusion, renal tubule HCO_3^- reabsorption closely matched glomerular filtration (Fig. 6E), so that net renal acid-base excretion was negligible (Fig. 4A). However, during NaHCO_3 infusion, HCO_3^- filtration greatly increased and tubular reabsorption, while increasing more than during NaCl infusion, did not keep up (Fig. 6F). The net effect was a greatly increased HCO_3^- excretion (Fig. 4A,B), which accounted for about 10% of the base load, in quantitative agreement with previous studies of induced metabolic alkalosis in freshwater trout (Wheatly *et al.* 1984; Perry *et al.* 1987b; Goss and Wood, 1990). During the return to normoxia after hyperoxia, a situation in which plasma acid-base balance is similar to that during NaHCO_3 infusion (see above), Wheatly *et al.* (1984) found a very similar response, with elevated HCO_3^- filtration exceeding elevated HCO_3^- reabsorption.

It is interesting to compare this pattern with that reported during the compensation of hyperoxia (Wheatly *et al.* 1984) or hypercapnia (Perry *et al.* 1987b), where the fish is accumulating HCO_3^- to compensate for acidotic blood pH, rather than excreting HCO_3^- to compensate for alkalotic pH. Under these circumstances, net renal HCO_3^- reabsorption exceeds net filtration (i.e. net H^+ excretion occurs) coincident with increased NH_4^+ and TA (as phosphate) excretion *via* the urine. In both cases, plasma $[\text{HCO}_3^-]$, and therefore HCO_3^- filtration rate greatly elevated, but during acidosis, reabsorption exceeds filtration, while during alkalosis, reabsorption is less than filtration. From this it would appear that the HCO_3^- reabsorption rate is modulated by blood pH. However, almost nothing is known about the mechanism in freshwater fish, apart from the fact that it is probably dependent on carbonic anhydrase, as in higher vertebrates. Acetazolamide impaired renal H^+ secretion/ HCO_3^- reabsorption in two species of catfish (Hodler *et al.* 1955; Nishimura, 1977).

Urinary bladder dynamics

During the control period, urination patterns in externally catheterized fish (burst volume = 0.85 ml kg^{-1} , interburst interval = 25 min; Fig. 3) were reasonably close to those previously estimated by indirect methods in uncatheterized freshwater trout (1.2 ml kg^{-1} , 29.8 min; Curtis and Wood, 1991). In comparison to our previous measurements with the external catheterization technique (0.45 ml kg^{-1} , 21 min; Curtis and Wood, 1991), present burst volumes were higher, interburst intervals were longer and a greater percentage of total UFR occurred in discrete bursts (90% vs 65%). We attribute this difference to an improved placement of the catheter tips in the optical devices, which minimized the chance of siphoning occurring.

During infusion, the increase in burst frequency (approximately 40%; Fig. 3A) was greater than the increase in burst volume (approximately 20%; Fig. 3B), which suggests that, as in higher vertebrates (e.g. humans – Guyton, 1986), the act of urination is triggered by a critical filling volume or pressure in the bladder (i.e. *via* stretch receptors). Using a urinary bladder residence time analysis developed earlier and assuming an

unchanged residual volume of 0.47 ml kg^{-1} (see Curtis and Wood, 1991), we estimate that the average residence time decreased from approximately 25 min during the control period to approximately 17 min after 24–32 h infusion, while the time-averaged urine volume in the bladder increased from 0.9 to 1.0 ml kg^{-1} .

Urinary bladder electrolyte handling

Table 2 summarizes the calculated mean reabsorption rates of the bladder under control conditions and during NaHCO_3 infusion. Results during NaCl infusion (not shown) were essentially identical, with the exception that Cl^- reabsorption increased in proportion to Na^+ reabsorption in NaCl -infused fish, but barely changed in NaHCO_3 -infused fish (cf. Fig. 5A,C). The five substances showing clear evidence of bladder reabsorption (i.e. significantly lower excretion rates in externally catheterized fish) were H_2O , Na^+ , Cl^- , K^+ and urea. Positive bladder reabsorption values were also obtained for all other measured substances in the urine except phosphate and HCO_3^- (i.e. Ca^{2+} , Mg^{2+} , SO_4^{2-} , NO_3^- , ammonia), but these must be interpreted with caution, for they were calculated from excretion rates that were not significantly different between the two catheterization techniques.

During NaCl infusion, relative increases (about 70%) in the rates of bladder reabsorption for H_2O , Na^+ and Cl^- were all very similar, and the same was true for H_2O and Na^+ reabsorption during NaHCO_3 infusion (Table 2). Na^+ and Cl^- reabsorption rates increased on an absolute basis and stayed unchanged on a relative basis, despite a decrease in the mean residence time of the urine in the bladder. The calculated NaCl 'concentration' of the reabsorbed fluid stayed constant at the control level of about 14 mmol l^{-1} throughout the infusion period. The bladder reabsorbate was clearly hypertonic to the ureteral urine entering the bladder but hypotonic to the blood plasma; presumably the benefits of additional ion recovery from the urine are more important than the osmotic 'penalty' incurred. This figure may be compared to the constant value of

Table 2. Net reabsorption rates ($\mu\text{mol kg}^{-1} \text{ h}^{-1}$) of various substances across the urinary bladder under control conditions and after 24–32 h of NaHCO_3 infusion

Substance	Control	24–32 h NaHCO_3 infused
Na^+	11.0	19.3
K^+	1.8	2.3
Ammonia	0.3	0.2
Ca^{2+}	1.1	1.9
Mg^{2+}	0.7	0.4
Cl^-	9.4	11.4
NO_3^-	0.2	0.4
SO_4^{2-}	0.6	0.8
Phosphate	0.2	-0.2
HCO_3^-	-0.5	0.4
Urea	0.6	1.1
H_2O^*	0.76	1.30

* $\text{ml kg}^{-1} \text{ h}^{-1}$.

about 356 mmol l^{-1} (clearly hypertonic to blood plasma) earlier calculated for NaCl 'concentration' of the threefold greater volume of fluid reabsorbed across the renal tubules.

Comparison of the present bladder reabsorption data with previous *in vitro* studies on rainbow trout bladder is difficult, because all have used the unphysiological situation of isotonic Ringer on the mucosal as well as the serosal surface (e.g. Hirano *et al.* 1973; Fossat *et al.* 1974; Fossat and Lahlou, 1979). Not surprisingly, the 'concentration' of the reabsorbate was generally hypertonic under such conditions. Nevertheless it is noteworthy that, despite the large urine-to-plasma concentration gradients opposing NaCl transport *in vivo*, absolute rates of Na^+ and Cl^- transport ($10\text{--}20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) observed in the present study were very comparable to those observed *in vitro* in the absence of opposing gradients.

Both urea and K^+ were clearly reabsorbed across the bladder *in vivo*, thereby supporting our earlier indirect calculations on non-catheterized trout which suggested bladder transport of these two substances (Curtis and Wood, 1991). K^+ reabsorption did not change substantially with infusion, whereas urea reabsorption increased in proportion to H_2O reabsorption (Table 2). For both substances, measured concentrations in urine were considerably below typical plasma levels, suggesting that transport was active, though a passive solvent drag explanation is also possible. We are aware of no *in vitro* data on urea transport across the trout bladder whereas, for K^+ , secretion rather than reabsorption has been demonstrated *in vitro* (Harvey and Lahlou, 1986). Again, however, comparison is difficult because the *in vitro* bladder was bathed in isotonic Ringer with typical plasma levels of K^+ on both mucosal and serosal surfaces.

The role of the urinary bladder in acid–base regulation

The present results show conclusively that the contribution of the urinary bladder to systemic acid–base regulation during NaHCO_3 infusion was negligible. Net HCO_3^- reabsorption or secretion rates in the bladder were effectively zero, and therefore (negative) net H^+ excretion rates through the renal system were identical in internally and externally catheterized fish (Fig. 4A). While this study rules out any role of the bladder in net HCO_3^- secretion during metabolic alkalosis, it remains possible that the bladder could contribute to acid–base regulation under other conditions. For example, during environmental hyperoxia or hypercapnia, respiratory acidosis occurs; net HCO_3^- reabsorption/ H^+ secretion by the renal tubules plays a vital role in the maintenance of elevated plasma HCO_3^- levels at this time (Wheatly *et al.* 1984; Perry *et al.* 1987b). Similarly, during metabolic acidosis induced by exposure to low environmental pH, renal tubular H^+ secretion/ HCO_3^- reabsorption serves as a compensation mechanism of major importance (McDonald and Wood, 1981). Studies examining the possible contribution of the bladder under these acidotic conditions are needed.

The traditional model (e.g. Fossat and Lahlou, 1979; Lahlou and Fossat, 1984; Harvey and Lahlou, 1986) of ion transport across the urinary bladder of the rainbow trout *in vitro* has been a neutral, coupled NaCl cotransport system ('secondarily active Na^+ -dependent Cl^- transport'). More recently, Marshall (1986) and Marshall and Bryson (1991) have

provided evidence for independent $\text{Na}^+/\text{H}^+,\text{NH}_4^+$ and $\text{Cl}^-/\text{HCO}_3^-$ exchanges in the urinary bladder of the brook trout *in vitro*. Similarly, Hirano *et al.* (1973) found higher Na^+ than Cl^- reabsorption rates in the rainbow trout bladder *in vitro*. The present data are equivocal as to which model is most appropriate for the true *in vivo* situation in the rainbow trout. On the one hand, the equimolar reabsorption of Na^+ and Cl^- under control conditions or during NaCl infusion (Fig. 5A,C) and the absence of net acid-base exchange across the bladder (Fig. 4) were in accord with the cotransport model. On the other hand, Na^+ and Cl^- reabsorption became independent during NaHCO_3 infusion (Fig. 5A,C; Table 2), in accord with the exchange model. However, by this model, the excess of Na^+ over Cl^- reabsorption should have acidified the urine, which clearly did not occur. These discrepancies further emphasize the need for *in vitro* studies employing a realistic dilute solution (i.e. synthetic urine; Marshall, 1988) on the mucosal surface, rather than isotonic Ringer on both surfaces. When this approach was applied to the frog skin, a fundamental reinterpretation of the transport model resulted (Kirschner, 1983).

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