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Reabsorption of urea by the kidney of the freshwater rainbow trout

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Abstract

In light of recent evidence that carrier-mediated transport of urea occurs in the mammalian kidney, this study examined the renal handling of urea in freshwater rainbow trout (*Oncorhynchus mykiss*). Fish were fitted with indwelling arterial and urinary bladder catheters for the measurement of plasma and urine composition (urea, Na^+, Cl^-, glucose, H_2O), glomerular filtration rate ([^{3}H]PEG-4000), and urine flow rate, thereby allowing quantification of tubular reabsorption rates. The fractional reabsorption of urea (72%) was greater than that of H_2O (50%) but less than that of Na^+, Cl^-, or glucose (95–100%) and occurred against an apparent concentration gradient, suggesting active reabsorptive transport; [urea] in the urine was only 59% of that in blood plasma. When fish were infused with exogenous urea loads, these patterns remained largely unchanged, and urea reabsorption increased in direct proportion to the filtered urea load. There was no evidence for saturation of the reabsorptive transport mechanism at urea filtration rates up to 4-fold above the normal range, representing urea loading rates that proved toxic. Extra-renal excretion, presumably through the gills, increased markedly, almost keeping pace with urea loading. This evidence suggests that carrier-mediated reabsorptive transport of urea occurs in the kidney and that plasma urea levels are normally subject to tight homeostatic control in freshwater trout.

Introduction

The traditional textbook view of urea as a highly diffusable molecule that moves across biological membranes by simple passive diffusion through the lipid phase has undergone substantial revision in recent years. Considerable evidence has now accumulated demonstrating facilitated or even active carrier-mediated transport of urea in several mammalian, reptilian and amphibian tissues (Marsh and Knepper 1992). Renal systems have been a particular focus. The complex cycling of urea in the countercurrent system of the mammalian kidney, long thought to be explained by simple diffusion, is now known to involve both a hormonally controlled facilitated diffusion carrier and a Na^+-coupled active transport carrier (Knepper and Star 1990; Gillin and Sands 1993). Both have been physiologically characterized (Chou and Knepper 1989; Isozaki et al. 1994a,b; Knepper and Chou 1995; Ashkar et al. 1995), and the former ('UT2') has now been cloned in two mammalian species (You et al. 1993; Smith et al. 1995). The latter appears to be expressed only in animals kept on low protein diets (Isozaki et al. 1994a) and has not yet been cloned.

In general, urea transport in fish has received relatively sparse attention (reviewed by Wood 1993 and Korsgaard et al. 1995), apart from a number of fairly early studies on the remarkable ability of marine elasmobranchs to retain urea at the kidney for the purposes of osmoregulation (reviewed by Smith 1936 and Hickman and Trump 1969). A few later studies suggested that

urea was actively reabsorbed by a carrier-mediated mechanism in sharks (Kempton 1953; Schmidt-Nielsen and Rabinowitz 1964; Schmidt-Nielsen et al. 1972; Hays et al. 1976). To date, this mechanism remains uncharacterized. In teleost fish, plasma urea levels are much lower than in elasmobranchs, and there is little evidence that urea plays any significant role in osmoregulation (Wright et al. 1995b). It seems particularly unlikely that freshwater teleosts would 'wish' to retain urea, but both Wood (1993) and Korsgaard et al. (1995) have pointed out the curious fact that plasma urea levels are 5–50 times higher than ammonia levels, even though most teleosts produce only 10–30% as much urea as ammonia.

If there is a 'need' to retain urea in freshwater teleosts, then the kidney would clearly be a possible site of regulation. To our knowledge, to date there have been no detailed studies of urea handling by the freshwater teleost kidney. However, Wood (1993) re-examined plasma and urine urea data from several trout studies directed at other questions, and from this analysis suggested that urea might well be actively reabsorbed by the kidney of freshwater salmonids.

The goal of the present study was to characterize the renal handling of urea in the freshwater rainbow trout relative to other substances (Na^+, Cl^-, glucose, H_2O) whose behaviors are relatively well understood (Hickman and Trump 1969; Wood 1995). Plasma composition, urine composition, glomerular filtration rate, and urine flow rate were quantified, thereby allowing quantification of tubular reabsorption rate of these substances by standard methods (e.g. Wood and Patrick 1994; Wood 1995). Trout were studied under both resting conditions, and conditions where the rate of urea filtration by the glomeruli was experimentally elevated. The latter was achieved by infusion of exogenous urea into the bloodstream, with the objective of characterizing the kinetics of the reabsorptive mechanism. This protocol also allowed characterization of extrarenal urea excretion under conditions of urea loading.

Materials and methods

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*; 250–450g) were obtained from Humber Springs Trout Farm, Orangeville, Ontario, acclimated to seasonal temperature (13–16 °C), and fed daily with commercial trout pellets until the time of the surgery. Both acclimation and experimentation were carried out in dechlorinated Hamilton tapwater [Ca^{2+} = 1.8; Cl^- = 0.8; Na^+ = 0.6; Mg^{2+} = 0.5; K^+ = 0.04; titration alkalinity (to pH 4.0) = 1.9 mequiv l^{-1}; total hardness = 140 mg l^{-1} as $CaCO_3$; pH 8.0]. Following surgery, the fish were kept in darkened individual 'flux' boxes (McDonald and Rogano 1986) that were continually aerated and supplied with flowing water (200 ml min^{-1}).

Dorsal aortic and urinary catheterization were performed simultaneously while the fish were anaesthetized with MS-222 (0.07 g l^{-1}; Sigma, St. Louis, MO) and artificially ventilated on an operating table. Dorsal aortic catheters (Clay-Adams PE50 tubing) were implanted as described by Soivio et al. (1972) and were filled with Cortland saline (Wolf 1963) containing 50 i.u. per ml ammonium heparin (Sigma, St. Louis, MO). The internal urinary catheterization technique described by Curtis and Wood (1991) and Wood and Patrick (1994) was used, in which the catheter (heat molded Clay-Adams PE 60 tubing) is placed in the urinary bladder so as to drain urine continuously as it is produced from the ureters. Any reabsorptive/secretory role of the bladder is negated so as to examine the function of the kidney alone. During recovery and experimentation, urine was collected continuously with the catheter emptying into a vial approximately 3.0 cm below the water level of the box. The urine flow rate (UFR) was determined gravimetrically.

Following the procedure outlined by Curtis and Wood (1992), [^{3}H]polyethylene glycol (PEG, Mr4000) was used as a marker for glomerular filtration rate (GFR). PEG-4000 was chosen because it is considered a more accurate indicator of GFR in fish than other commonly used markers (e.g. inulin derivatives), as it undergoes minimal radioautolysis, metabolic breakdown, or post-filtration reabsorption across tubules and bladder (Beyenbach and Kirschner 1976; Erickson and

Gingrich 1986; Curtis and Wood 1991). In order to economize on isotope usage, prior to PEG-4000 injection the effectiveness of the catheters was evaluated over the first 12 h of recovery based on UFR and ease of blood sampling. In fish where both catheters were deemed successful, a dose of 17 μCi of [1.2–^{3}H] polyethylene glycol (PEG-4000; New England Nuclear) in 0.66 ml of Cortland saline was then injected via the dorsal aortic catheter, and washed in with an additional 0.3 ml of Cortland saline. The [^{3}H]PEG-4000 was then allowed to equilibrate throughout the extracellular space for 24 h (i.e. 36 h of recovery) before experiments were begun.

Experimental series

Series 1. This series evaluated the basic renal handling of urea relative to substances known to be actively reabsorbed (Na^+, Cl^-), passively reabsorbed (H_2O), or not reabsorbed (PEG-4000, the GFR marker) in the trout kidney (Hickman 1969; Curtis and Wood 1992). Starting at 36 h of recovery, urine was collected over successive 24 h intervals, while blood samples (300 μl, with saline replacement) were taken at the 12 h midpoints of each interval. The sampling continued for up to 5 days; a fish had to meet the criterion of at least two matched samples of urine and plasma to be included in the study. Blood samples were centrifuged at 10,000 G for 2 min, and the plasma decanted. Plasma and urine were stored at –20° C for later analysis of Na^+, Cl^-, urea, and [^{3}H]PEG-4000 concentrations.

Series 2. This series examined the response of the fish to infusions of urea. The specific goal was to examine the pattern of renal reabsorption in the face of elevated plasma urea levels, and therefore elevated filtered loads of urea. In addition to the handling of urea, Na^+, Cl^-, H_2O, and PEG-4000, the handling of glucose by the kidney was also monitored as an indicator of renal function. The protocol also allowed an examination of the role of the remainder of the body surface, relative to that of the kidney, in excreting a urea load. Extra-renal urea excretion was measured by closing the flux box and recording the appearance of urea in the external water.

Following the [^{3}H]PEG-4000 injection and recovery period, three successive 24 h infusions via the dorsal aortic catheter were performed with isosmotic solutions (280 mOsm kg^{-1}) containing 0, 5, and 20 mmol l^{-1} urea respectively in balancing NaCl. The 0 mmol l^{-1} urea infusion served as a control for the effect of volume loading alone. In each case one channel of a Gilson 4 or 8 channel peristaltic pump was used at an infusion rate of 3 ml kg^{-1} h^{-1}; the rate was checked by periodic measurements of the weight of the infusion reservoir. At the start of each infusion period, a fresh urine collection was started, the water flow to the fish box was stopped, the water level set to an exact volume mark of approximately 4 l, and a water sample was taken for measurement of urea concentration. Vigorous aeration maintained P_{O_2} close to air saturation. At 12 h, urine was sampled and a fresh urine collection started, a blood sample was taken (300 μl, with saline replacement), together with a second water sample, and the box was then rapidly flushed over a 15 min period. The box was then closed again for a further 12 h, with water sampling at the beginning and end, and blood and urine sampling at the end (i.e. 24 h). The infusion solution was then changed to a higher urea concentration, and the same protocol of box closure, water, urine and blood sampling repeated over the next 24 h. An identical protocol was employed during the final 24 h infusion period at the highest urea concentration.

Thus for each infusion, two blood samples were taken, and two 12 h measurements of renal and extra-renal urea excretion were recorded. Blood samples were centrifuged as above; plasma and urine were stored at –20 °C for later analysis of Na^+, Cl^-, urea, glucose and [^{3}H]PEG-4000 concentrations. Water samples were analyzed for urea only.

Series 3. Due to high rates of extra-renal urea excretion by infused fish, it proved surprisingly difficult to elevate plasma urea concentrations by infusion. Therefore, another infusion series was performed, using an identical protocol to Series 2, but with much higher concentrations of urea (0, 80, and 140 mmol l^{-1}), again in balancing NaCl.

Analytical techniques

Urea concentrations in plasma, urine, and water were measured using the diacetyl monoxime method of Rahmatullah and Boyde (1980), with appropriate adjustments of reagent strength for the different urea concentration ranges in the three media. Na^+ and Cl^- concentrations in plasma and urine were measured using a Varian 1275 Atomic Absorption Spectrophotometer and a Radiometer CMT10 Chloridometer respectively. Glucose in plasma and urine was measured enzymatically (hexokinase, glucose-6-phosphate dehydrogenase) using a commercial kit (Sigma). For measurements of [^{3}H]PEG-4000 radioactivity, plasma and urine samples (25 µl made up to 5 ml with distilled water), and water samples (5 ml) were added to 10 ml of ACS fluor (Amersham) and analyzed by scintillation counting on an LKB Rackbeta 1217 Counter. The external standard method was used for quench correction.

Calculations

All rates were related to fish body weight – e.g. ml kg^{-1} h^{-1}.
Urinary excretion rates (U) of any substance (*X*) were calculated as:

$$U_X = [X]_u \times UFR \quad (1)$$

using measured values of urine flow rates (UFR) and urine concentrations $[X]_u$.

Clearance rates (*C*) were calculated as:

$$C_X = \frac{[X]_u \times UFR}{[X]_p} \quad (2)$$

where $[X]_p$ was the measured plasma concentration.

Glomerular filtration rates (GFR) were calculated as the clearance of [^{3}H]PEG-4000 – i.e. the excretion of radioactivity in the urine (dpm_u) relative to its concentration in the blood plasma (dpm_p):

$$GFR = \frac{dpm_u \times UFR}{dpm_p} \quad (3)$$

The filtration rate (FR) of a substance *X* at the glomeruli was calculated as:

$$FR_X = [X]_p \times GFR \quad (4)$$

and the tubular reabsorption rate (TR) of *X* as:

$$TR_X = FR_X - U_X \quad (5)$$

The concept of clearance ratio (CR_X; see Wood 1995) relates the clearance of a substance *X* to the GFR (i.e. to the clearance of [^{3}H]PEG-4000):

$$CR_X = \frac{C_X}{GFR} = \frac{[X]_u \times UFR}{[X]_p \times GFR} = \frac{U_X}{FR_X} \quad (6)$$

Assuming that a substance *X* is filtered at the glomeruli with the same efficiency as [^{3}H]PEG-4000, then the clearance ratio provides quantitative information on the tubular handling of *X*; this is a relatively safe assumption with the small neutral molecules (urea, glucose, H_2O) and monovalent ions (Na^+, Cl^-) of the present study. If the clearance ratio is greater than 1, then there has been net secretion of *X*; if less than 1, then net reabsorption has occurred. For example, CR_X = 0.05 would indicate 95% net reabsorption of the filtered load of *X*.

In Series 2 and Series 3, the rate of extra-renal urea excretion into the water by catheterized trout was calculated from the change in water urea concentration, factored by water volume, fish weight, and time.

Unless otherwise stated, data have been reported as means ± 1 SEM (*N* = number of fish). Regression lines have been fitted by the method of least squares, and the significance ($p < 0.05$) of the Pearson's correlation coefficient r assessed. The significance of differences between means was evaluated using Student's paired or unpaired two-tailed t-test ($p < 0.05$) as appropriate, with the Bonferroni correction (Nemenyi et al. 1977) for multiple comparisons.

Table 1. Average values for plasma and urinary composition, urine flow rate, glomerular filtration rate and the calculated clearance ratios for water, sodium, chloride and urea from each resting fish in Series 1

UFR (ml kg^{-1} h^{-1})	GFR (ml kg^{-1} h^{-1})	$[Na^+]p$ (mmol l^{-1})	$[Na^+]u$ (mmol l^{-1})	$[Cl^-]p$ (mmol l^{-1})	$[Cl^-]u$ (mmol l^{-1})	[urea]p (mmol l^{-1})	[urea]u (mmol l^{-1})
3.3 ± 0.4	7.3 ± 0.8	154.6 ± 2.4	8.8 ± 1.4	131.1 ± 2.6	9.3 ± 1.6	1.9 ± 0.1	1.1 ± 0.1

CR_{H_2O}	CR_{Na^+}	CR_{Cl^-}	CR_{urea}
0.500 ± 0.032	0.0288 ± 0.006	0.034 ± 0.004	0.282 ± 0.038

Data are shown as mean ± SEM (N = 7); UFR = urine flow rate, GFR = glomerular filtration rate, p = plasma, u = urine, CR = clearance ratio.

Results

Series 1. Seven trout met the criterion of at least two matched samples of urine and plasma (i.e. 48 h of continuous recording after the initial 36 h of recovery). Mean data for plasma and urine composition, UFR, GFR, and the calculated clearance ratios for water, urea, Na^+ and Cl^- are reported in Table 1. In these resting fish, the concentration of urea in the urine was only about 59% of that in blood plasma, despite the fact that UFR averaged only 45% of GFR, whereas the urinary concentrations of Na^+ and Cl^- were less than 10% of plasma levels (Table 1). Clearly urea was reabsorbed in the renal tubules, against an apparent concentration gradient. Clearance ratio analysis demonstrated that this pattern was consistent in all fish. CR_{Urea} ranged from 0.111 to 0.875 in the 7 fish, averaging 0.282 ± 0.038 overall, indicating 72% urea reabsorption in the tubules, significantly greater than the mean CR_{H2O} of 0.500 ± 0.032 (7), representing 50% reabsorption of the filtered water load. Clearance ratios for Na^+ and Cl^- were consistently much lower – CR_{Na} = 0.028 ± 0.006 (7) and CR_{Cl} = 0.034 ± 0.004 (7) respectively. Thus, these two major plasma electrolytes underwent approximately 97% tubular reabsorption. Urea was reabsorbed to a greater extent than water but to a lesser extent than Na^+ and Cl^-.

In these resting fish, there was a linear, almost proportional relationship between the filtration rate (FR) and the tubular reabsorption rate (TR) of urea over the observed FR_{Urea} range of 6–27 μmol kg^{-1} h^{-1} (Figure 1). Thus, there was no evidence for a transport maximum for the urea reabsorption process (which would be seen as a plateau of TR_{Urea}). Therefore, the mechanism could not be saturated in this range, and a more or less constant fraction of the filtered load was reabsorbed.

Series 2. Urea infusions were performed with the goal of raising the plasma urea concentration and therefore the filtration rate of urea at the glomeruli, to see whether the reabsorptive process could be saturated. The first two 12 h periods (infusion with isosmotic NaCl only, 0 mmol l^{-1} urea) served to evaluate the effects of the volume loading alone. All 9 fish survived the infusions, though the N number tended to fall later in the experiment due to catheter failure.

Table 2 summarizes mean values of UFR, GFR, and plasma and urine compositions, most of which exhibited minimal change over the 72 h infusion protocol. Plasma urea concentration (Figure 2C) remained approximately stable throughout infusions with 0 and 5 mmol l^{-1} urea (loading rate = 15 μmol kg^{-1} h^{-1}), but rose significantly when the infusate concentration was raised to 20 mmol l^{-1} (loading rate = 60 μmol kg^{-1} h^{-1}) in the final two 12 h periods. Urine urea concentration also increased significantly in the final 12 h. However, the expected stimulatory effect on FR_{Urea} was minimized by a non-significant fall in GFR at this time.

Volume loading alone appeared to stimulate extra-renal urea excretion (Figure 2A), which doubled in the second 12 h period prior to any exogenous urea loading. Extra-renal excretion continued to increase throughout the experiment. Thus in the second 12 h period of urea loading at 15 μmol kg^{-1} h^{-1}, it was significantly greater than in the second period of volume loading

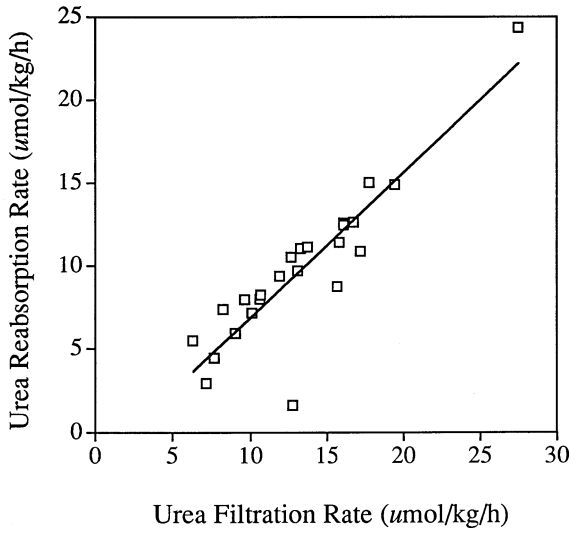


Figure 1. A plot of the rate of urea reabsorbed versus the rate of urea filtered in the resting, non-infused fish of Series 1 demonstrating a correlation between the two variables and the lack of a transport maximum (N = 24 data points from 7 fish). The equation of the regression line and the significance of the correlation are y = 0.877x–1.924 (r^2 = 0.801, $p < 0.001$) where y is the urea reabsorption rate (μmol kg^{-1} h^{-1}) and x is the urea filtration rate (μmol kg^{-1} h^{-1}).

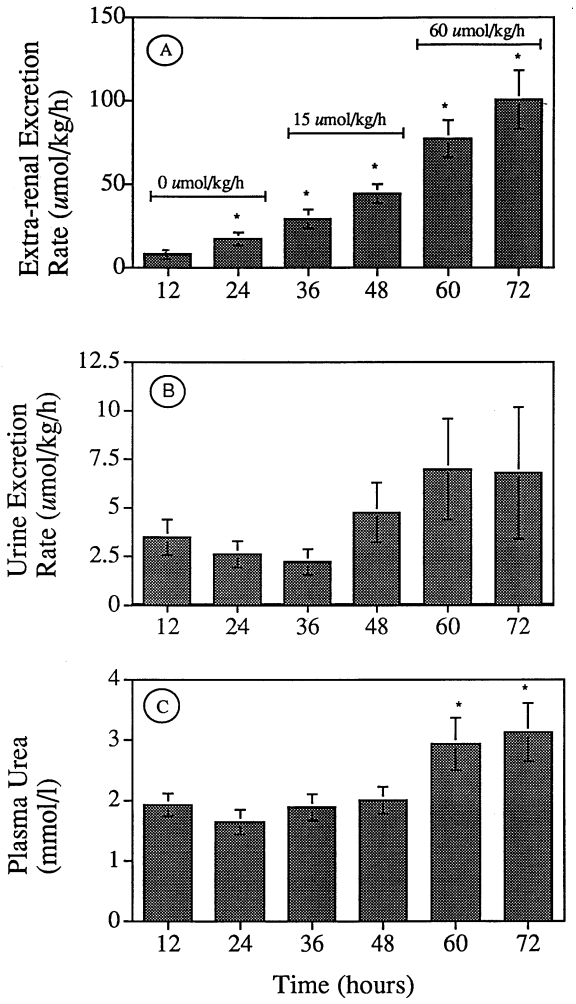


Figure 2. (A) The extra-renal excretion rate (μmol kg^{-1} h^{-1}) of urea, (B) the urinary excretion rate (μmol kg^{-1} h^{-1}) of urea and (C) the concentration of urea in the plasma (mmol l^{-1}) in the fish of Series 2 infused with urea at a rate of either 0, 15, or 60 μmol kg^{-1} h^{-1}. Means ± 1 SEM (N = 9). * indicates a significant difference ($p < 0.05$) relative to the first 12 h.

Table 2. Values for plasma and urinary variables determined from each sample period in Series 2

Infusion (μmol kg^{-1} h^{-1})	Time (h)	UFR (ml kg^{-1} h^{-1})	GFR (ml kg^{-1} h^{-1})	[urea]p (mmol l^{-1})	[urea]u (mmol l^{-1})	[Cl^-]p (mmol l^{-1})	[Cl^-]u (mmol l^{-1})	[Na^+]p (mmol l^{-1})	[Na^+]u (mmol l^{-1})	[glucose]p (mmol l^{-1})	[glucose]u (mmol l^{-1})
0	12	3.6±0.7 (9)	6.3±1.2 (9)	1.9±0.2 (9)	1.1±0.2 (9)	132.0±1.0 (9)	6.7±1.1 (9)	140.5±2.0 (9)	7.5±1.2 (9)	2.7±0.1 (9)	n.d.
0	24	3.4±0.7 (9)	5.7±1.3 (9)	1.6±0.2 (9)	1.0±0.2 (9)	127.5±1.0[a] (9)	7.6±2.1 (9)	146.2±3.1 (9)	9.9±1.6 (9)	2.5±0.2 (9)	n.d.
15	36	2.4±0.6 (9)	4.6±1.0 (7)	1.9±0.2 (7)	0.8±0.1 (8)	130.6±0.6 (7)	4.6±0.8[a] (7)	139.0±1.5 (8)	8.6±1.9 (9)	2.8±0.2 (7)	n.d.
15	48	2.8±0.7 (8)	5.7±1.4 (7)	2.0±0.2 (7)	1.3±0.3 (8)	127.4±1.4[a] (7)	8.4±1.7 (8)	139.9±2.9 (7)	6.7±1.5 (9)	2.7±0.1 (7)	n.d.
60	60	3.2±1.1 (6)	5.7±1.8 (4)	2.9±0.4[a] (6)	1.7±0.4 (7)	130.9±1.1 (6)	7.3±1.2 (6)	142.7±4.8 (7)	9.3±2.0 (6)	3.0±0.2 (6)	n.d.
60	72	3.1±1.3 (5)	4.4±1.4 (5)	3.1±0.5[a] (7)	2.0±0.2[a] (5)	130.7±1.2 (7)	6.3±0.9 (5)	140.4±4.1 (7)	6.6±2.9 (4)	2.7±0.2 (7)	n.d.

Data are shown as mean±SEM (N); UFR = urine flow rate, GFR = glomerular filtration rate, p = plasma, u = urine; [a]indicates a significant difference ($p < 0.05$) relative to the first 12 h; n.d. indicates not detectable (< 0.05 mmol l^{-1}).

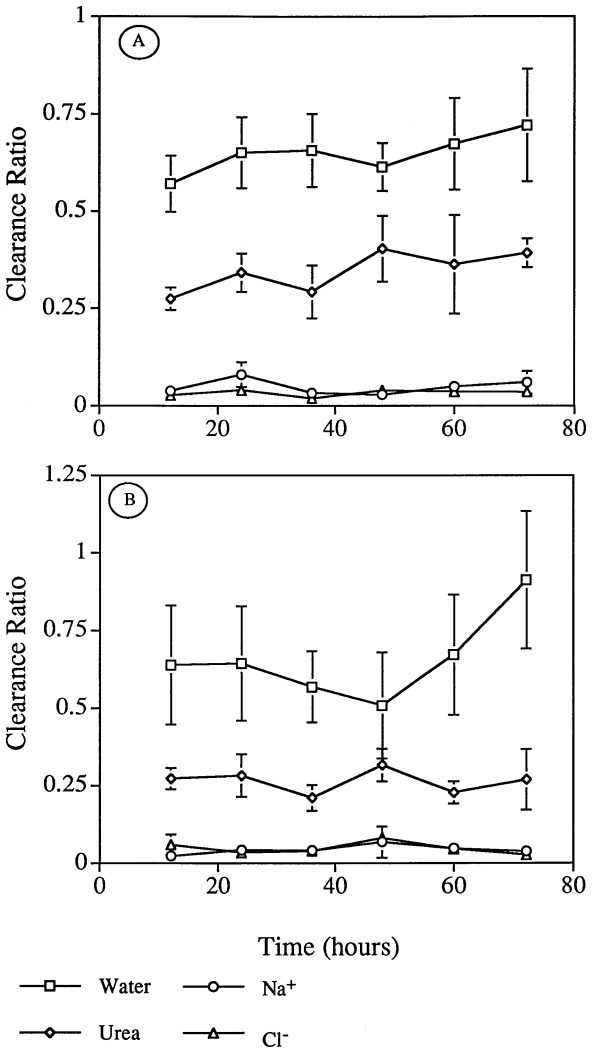


Figure 3. Clearance ratios for water, urea, Na^+ and Cl^- in the fish of (A) Series 2 (N = 9) and (B) Series 3 (N = 7). Means ± 1 SEM. See legends of Figures 2 and 4 for details on the infusion treatment at each time interval.

alone. Similarly in the second 12 h period of urea loading at 60 μmol kg^{-1} h^{-1}, it was significantly elevated again. On a quantitative basis, these increases in extra-renal urea excretion more or less kept pace with the loading rate, thereby explaining the relative stability of plasma urea concentrations.

In contrast, urinary excretion of urea, while increasing in some fish, exhibited no significant elevation overall (Figure 2B). Excretion of urea through the kidney remained less than 10% of excretion through the extra-renal pathway.

Clearance ratio analysis (Figure 3A) demonstrated that the renal handling of water, urea, Na^+, and Cl^- was not greatly affected by infusion with increasing loads of urea. Overall, patterns were similar to those seen in the resting fish of Series 1 (cf. Table 1), with clearance ratios averaging approximately 0.6 (40% reabsorption) for H_2O, 0.3 (70% reabsorption) for urea, and 0.05 (95% reabsorption) for Na^+ and Cl^-, and remaining more or less stable throughout the 72 h of infusion. Since glucose concentrations in urine were not detectable (Table 3; i.e. < 0.05 mmol l^{-1}), $CR_{Glucose}$ could not be calculated, but the reabsorption of glucose was obviously close to 100%.

Series 3. Much higher concentrations of urea in the infusate were employed in an attempt to raise plasma urea concentrations to a greater extent than in Series 2. This strategy was successful; plasma urea concentration approximately tripled after the first 12 h of infusion with 80 mmol l^{-1} urea (loading rate = 240 μmol kg^{-1} h^{-1}), relative to levels at the end of 24 h infusion with the volume load alone (Figure 4C; Table 3). Further modest increases occurred during the final periods of infusion with 140 mmol l^{-1} (loading rate = 420 μmol kg^{-1} h^{-1}). However, as 3 of the 7 fish died during these final 24 h, this very high loading rate was likely pathological. Significant declines in plasma Na^+, Cl^-, and glucose concentrations at this time reinforce this conclusion (Table 3).

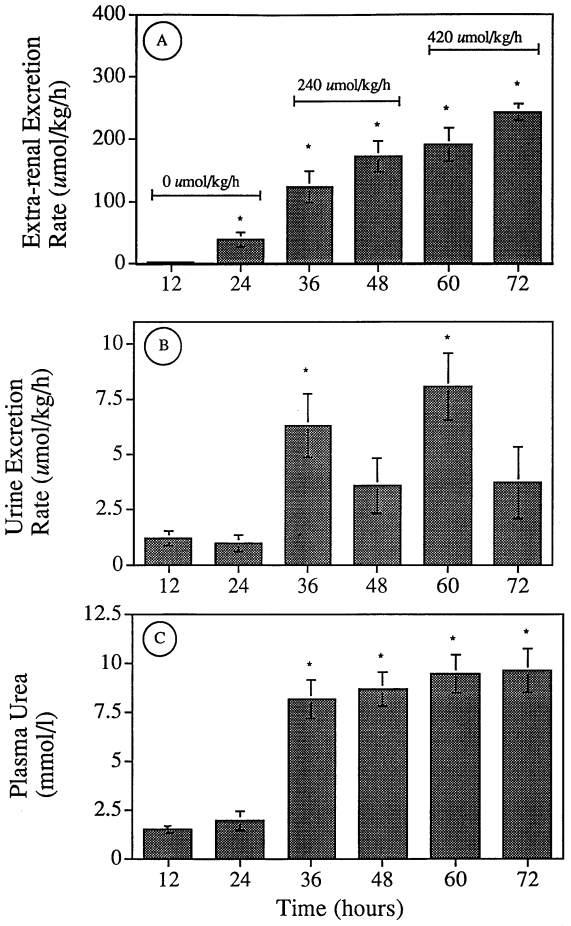
Initial mean GFR and UFR values were much lower than in Series 2 for unknown reasons. Both tended to increase during infusion with 80 mmol l^{-1} urea (Table 3), before falling again during infusion with 140 mmol l^{-1} urea, though only the latter effect (for GFR) was significant. Except during the final 12 h when GFR fell precipitously in surviving fish, FR_{Urea} increased substantially, largely due to the increases in plasma urea concentrations. Urine urea concentrations were significantly elevated by both levels of urea infusion.

As in Series 2, volume loading alone again appeared to stimulate extra-renal urea excretion (Figure 4A), and thereafter the rate continued to increase significantly throughout the experiment. By the second 12 h period of infusion with 80 mmol l^{-1} urea, the rate of urea excretion through

Table 3. Values for plasma and urinary variables determined from each sample period in Series 3

Infusion (μmol kg^{-1} h^{-1})	Time (h)	UFR (ml kg^{-1} h^{-1})	GFR (ml kg^{-1} h^{-1})	[urea]p (mmol l^{-1})	[urea]u (mmol l^{-1})	[Cl$^-$]p (mmol l^{-1})	[Cl$^-$]u (mmol l^{-1})	[Na$^+$]p (mmol l^{-1})	[Na$^+$]u (mmol l^{-1})	[glucose]p (mmol l^{-1})	[glucose]u (mmol l^{-1})
0	12	1.7 ± 0.6 (6)	3.0 ± 0.8 (6)	1.5 ± 0.1 (7)	0.8 ± 0.1 (6)	127.0 ± 1.5 (7)	6.7 ± 1.5 (6)	146.5 ± 6.2 (7)	7.0 ± 2.2 (6)	3.0 ± 0.1 (7)	n.d.
0	24	1.4 ± 0.5 (5)	2.1 ± 0.6 (5)	2.0 ± 0.5 (7)	0.6 ± 0.1 (5)	125.3 ± 2.1 (7)	5.7 ± 1.3 (6)	143.8 ± 6.3 (7)	9.3 ± 2.6 (5)	3.3 ± 0.3 (7)	n.d.
240	36	2.4 ± 0.5 (7)	3.9 ± 0.8 (7)	8.2 ± 1.0[a] (7)	2.6 ± 0.3[a] (7)	119.9 ± 3.0[a] (7)	9.2 ± 2.7 (5)	138.7 ± 6.1 (7)	10.7 ± 3.7 (7)	3.0 ± 0.2 (7)	n.d.
240	48	2.7 ± 1.1 (6)	5.6 ± 3.8 (6)	8.6 ± 0.4[a] (7)	4.1 ± 0.3[a] (6)	118.4 ± 2.0[a] (7)	9.2 ± 3.1 (5)	130.0 ± 5.8[a] (7)	4.9 ± 1.5 (6)	2.6 ± 0.1[a] (7)	n.d.
420	60	2.9 ± 0.7 (5)	4.2 ± 0.7 (5)	9.5 ± 0.6[a] (6)	3.0 ± 0.3[a] (5)	111.4 ± 5.3[a] (6)	8.4 ± 3.4 (5)	128.0 ± 10.6[a] (6)	10.6 ± 7.3 (4)	2.6 ± 0.2[a] (6)	n.d.
420	72	1.2 ± 0.7 (4)	1.1 ± 0.7[a] (3)	9.6 ± 1.1[a] (3)	2.6 ± 0.2[a] (3)	110.8 ± 7.4[a] (3)	3.6 ± 2.1 (2)	132.5 ± 12.1 (3)	5.6 ± 1.7 (4)	1.6 ± 0.6[a] (3)	n.d.

Data are shown as mean ± SEM (N); UFR = urine flow rate, GFR = glomerular filtration rate, p = plasma, u = urine; [a]indicates a significant difference ($p < 0.05$) relative to the first 12 h; n.d. indicates not detectable (< 0.05 mmol l^{-1}).



the extra-renal pathway had increased to about 75% of the rate of urea loading (240 μmol kg^{-1} h^{-1}). However, despite further absolute increase during the final two periods, this amounted to only about 55% of the final loading rate (420 μmol kg^{-1} h^{-1}).

Urinary urea excretion increased significantly by 2–4-fold in a somewhat irregular fashion during both periods of urea loading (Figure 4B). However, as in Series 2 the renal route was of minimal contribution (less than 10%) relative to the extra-renal pathway.

Clearance ratio analysis (Figure 3B) revealed a similar overall pattern to that seen in Series 1 (Table 1) and Series 2 (Figure 3A), but with greater variability, due perhaps in part to the toxic effects of the high urea loading rates used. Again CR_{Urea} was about 0.3 while CR_{H2O} was more than twice as large (0.65), CR_{Na} and CR_{Cl} remained less than 0.05, and $CR_{Glucose}$ was effectively zero. CR_{H2O} tended to increase in the final 12 h period in concert with other developing abnormalities.

An analysis of all individual data pairs for urea filtration rate (FR) and tubular reabsorption rate

←

Figure 4. (A) The extra-renal excretion rate (μmol kg^{-1} h^{-1}) of urea, (B) the urinary excretion rate (μmol kg^{-1} h^{-1}) of urea and (C) the concentration of urea in the plasma (mmol l^{-1}) in the fish of Series 3 infused with urea at a rate of either 0, 240, or 420 μmol kg^{-1} h^{-1}. Means ± 1 SEM (N = 7). * indicates a significant difference ($p < 0.05$) relative to the first 12 h.

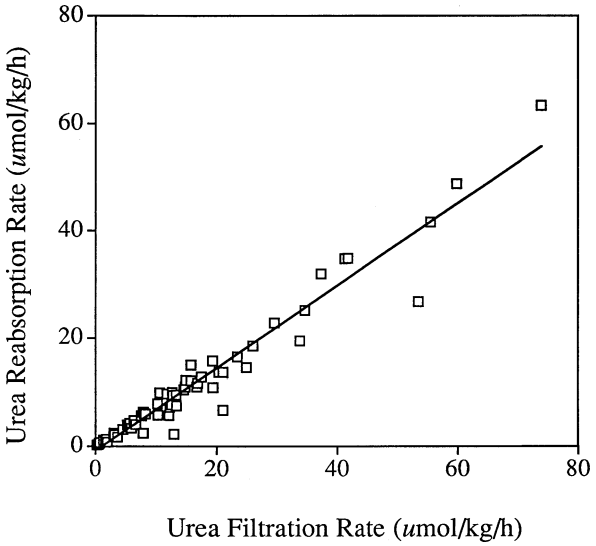


Figure 5. A plot of the rate of urea reabsorbed versus the rate of urea filtered in the infused fish of Series 2 and Series 3 demonstrating a correlation between the two variables and the lack of a transport maximum (N = 69 data points from 16 fish). The equation of the regression line and the significance of the correlation are y = 0.766x–0.879 (r^2 = 0.945, $p < 0.001$) where y is the urea reabsorption rate (μmol kg^{-1} h^{-1}) and x is the urea filtration rate (μmol kg^{-1} h^{-1}).

(TR) from all infused fish from both Series 2 and Series 3 was performed (Figure 5). This provided a much wider range of FR_{Urea} than in the resting trout of Series 1 (c.f. Figure 1), from close to 0 up to almost 80 μmol kg^{-1} h^{-1}, far above the normal physiological range. These particularly high FR_{Urea} values came mainly from the fish in Series 3 loaded with urea at the lower rate of 240 μmol kg^{-1} h^{-1}. Over this wide range, there was again a linear, proportional relationship between FR_{Urea} and TR_{Urea} (Figure 5), and the slope was very similar to that seen earlier in the resting fish (Figure 1). Thus the reabsorptive mechanism could not be saturated even at supra-physiological levels, concentrations which caused toxicity to the fish, and a constant fraction of the filtered urea load was always reabsorbed.

Discussion

The urinary bladder catheterization technique collects ureteral urine unmodified by residence in the bladder (Wood and Patrick 1994), so the function of the kidney alone was investigated in this study. Values of GFR, UFR, plasma Na^+ and Cl^-, urinary excretion rates, and the tubular reabsorption rates of these ions and of H_2O were similar to those measured previously in our laboratory using similar methods on rainbow trout of similar size (Wheatly et al. 1984; Wood 1988; Curtis and Wood 1991, 1992).

The present results showed clearly that in resting rainbow trout, urea undergoes net tubular reabsorption against an apparent concentration gradient such that urea concentrations in ureteral urine are about 40% below those of plasma. The rate of urea reabsorption, on either a fractional or absolute basis, was less than that of three substances (Na^+, Cl^-, glucose) known to undergo active reabsorption in the freshwater teleost kidney (Hickman and Trump 1969; Wood 1995). However, the fractional reabsorption of urea was significantly greater than that of water, so the phenomenon cannot be explained by solvent drag. Similarly, in the rat inner medullary collecting duct, urea and water move through separate pathways so solvent drag of urea does not occur (Knepper et al. 1989). The results therefore support the suggestions of Wood (1993) that some sort of active reabsorption mechanism is present in the freshwater trout kidney, but it is unclear whether this is primary or secondary transport, or whether a facilitated diffusion system is also involved.

When the rate of urea filtration was increased either naturally or due to exogenous urea loading, the rate of tubular urea reabsorption increased in direct proportion, even when the filtration rate rose to 4 times the normal level (cf. Figure 5 vs. Figure 1) and toxicity to the whole organism was observed, so there was no evidence that the transport system could be saturated. Interestingly, this characteristic of non-saturability is also a property of the Na^+ coupled reabsorptive mechanism (presumably active) in the kidney of the marine elasmobranch (Kempton 1953; Schmidt-Nielsen et al. 1972). Similarly, the facilitated diffusion carrier of the mammalian renal tubule UT-2 does not saturate at submolar concentrations unless the urea concentration is varied simultaneously on both sides, and even under the latter equilibrium conditions, the Km is extremely high (Knepper and Star 1990; Knepper and Chou 1995).

The present study was not designed to evaluate whether Na^+ reabsorption is saturable in the trout kidney. However, Curtis and Wood (1992) in almost identically treated trout, demonstrated that saturation did not occur even at Na^+ filtration rates far greater than those seen in the present study; the same was true of Cl^-. Thus, if urea transport is coupled to Na^+ transport, saturation would not be expected. Absolute rates of Na^+ reabsorption exceeded those of urea by 10–100-fold in the present study, whereas the transport mechanism of the shark kidney exhibits a fixed stoichiometry (Na^+: urea coupling) of about 1:1.6 (Schmidt-Nielsen et al. 1972; Hays et al. 1976). To our knowledge, neither the stoichiometry nor the saturability (or lack thereof) has yet been evaluated for the Na^+-coupled active transport carrier of the mammalian kidney.

Certainly the shark and two mammalian kidney transport systems are not the only possible models. A facilitated diffusion type urea carrier has been found in liver cells of the toadfish (Walsh et al. 1994; Walsh and Wood 1996), in the urinary bladder of the toad (Levine et al. 1973a, b) and in the red blood cells of various species (Kaplan et al. 1974; Marsh and Knepper 1992). Furthermore in various amphibians, evidence has been presented for both H^+-coupled, Na^+-independent (Rapoport et al. 1988, 1989), and H^+-independent, Na^+-independent (Lacoste et al. 1991; Dytko et al. 1993) active transport mechanisms.

In the present urea infusion experiments, the extra-renal pathway proved remarkably efficient at excreting exogenous urea loads at up to 10-fold normal rates, thereby limiting increases in plasma concentration to quite modest levels. It is noteworthy that extra-renal excretion increased initially in response to volume loading alone (*prior* to urea loading) and that subsequent increases in excretion rate were far greater than expected from a simple proportionality to blood urea levels. Although ammonia levels typically increased to approximately 300 μmol l^{-1} during each 12 h period of flux box closure, it has been determined that under the water chemistry conditions used for this study (pH, hardness, temperature), this level of ammonia has negligible effect on urea excretion rate in adult rainbow trout (R.W. Wilson and C.M. Wood, unpublished results). Very likely, this extra-renal route was largely or entirely through the gills, though as Wood (1993) has pointed out, the actual evidence separating urea excretion through the gills from that through the skin is remarkably sparse, relying mainly on the original experiments of Smith (1929). Nevertheless, the present observations are in accord with a very large body of work showing that the extra-renal route is quantitatively far more important than the renal route for urea excretion (reviewed by Wood 1993; Korsgaard et al. 1995). The mechanism of extra-renal urea excretion is unclear. Many workers have simply assumed that urea flux occurs via standard passive diffusion across the gills. Indeed, prior to the recognition that urea carriers may exist, urea flux was commonly used as an index of gill surface area or general permeability (e.g. Bergman et al. 1974; Haywood et al. 1977). The one study directed specifically at the mechanism (Wright et al. 1995b) found no evidence for either facilitated or active carrier mediation in the gills of the tidepool sculpin. On the other hand, urea was reported to accumulate in branchial chloride cells of the eel (Masoni and Romeu 1972) and indirect evidence has been found recently for facilitated diffusion type urea transport in the gills of both toadfish (Wood et al. 1997; Walsh 1997) and the dogfish shark (Part et al. 1998).

Why does the kidney of the trout reabsorb urea? Given the osmotic situation of a freshwater fish, the active retention of a nitrogenous waste osmolyte does not seem to make sense. We offer three possible explanations. The first is that urea retention is a developmental legacy from embryonic life. Recently, Wright et al. (1995a) have demonstrated that embryonic trout synthesize and retain urea prior to hatching. The second, as first suggested by Kaplan et al. (1974) in their consideration of amide transport mechanisms of red blood cells, is that urea transport may be ‘accidental’, because urea simply serves as an alternative substrate for an evolving pathway whose major function is the transport of some other substance (e.g. glucose) The third is that the reabsorption of urea is ‘intentional’ – i.e. maintenance of a certain concentration of urea in the body fluids is of direct adaptive value. Perhaps urea participates in some as yet unknown reaction, or serves to stabilize protein function. In this light, one can

imagine a system whereby a highly flexible excretion rate at the gills is balanced against a fixed fractional reabsorption at the kidney so as to maintain overall homeostasis.

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