Fish Physiology and Biochemistry (2004) 30: 1–12

Does urea reabsorption occur via the glucose pathway in the kidney of the freshwater rainbow trout?

Carol Bucking and Chris M. Wood
McMaster University, 1280 Main St. West, Hamilton, Ont. Canada L8S 4K1
(E-mail: Buckincp@mcmaster.ca)

Accepted: 1 October 2004

Key words: Na^+ reabsorption, *O. mykiss*, phlorizin, renal function, urea transporters, urine

Abstract

This study tested the hypothesis that the renal reabsorption of urea occurs via the glucose transport pathway in the freshwater rainbow trout (*Oncorhynchus mykiss*). The relationship between glucose transport and urea transport was examined by experimentally elevating the rate of renal glucose reabsorption via infusion of the fish with exogenous glucose, and by inactivating the glucose transporters via the administration of phlorizin. Under all treatments, urea was reabsorbed against a concentration gradient, with plasma levels of urea being higher than urine levels. Glucose was almost completely reabsorbed (88%) whereas urea was reabsorbed less efficiently (47%) but to a greater extent than water (22%). Glucose and urea reabsorption were both found to be correlated with Na^+ and Cl^- reabsorption, though the latter were 20 fold and 200–300 fold higher than glucose and urea transport rates, respectively. Glucose infusions greatly increased glucose reabsorption but urea reabsorption was unaffected. Phlorizin treatment completely blocked glucose reabsorption, but urea reabsorption was again unaffected. We conclude that there is no relationship between glucose and urea handling in the trout kidney, thus disproving the hypothesis.

Introduction

Freshwater fish have highly advanced renal filtration–reabsorption mechanisms in their nephrons, creating the most monovalent ion free urine of any vertebrate (Hickman and Trump 1969). The urine is also nearly free of other solutes, and on average its volume balances the quantity of water entering from the external environment, as the fish are subject to hyperosmotic regulation (Hickman and Trump 1969). Glucose is a solute that is essentially completely reabsorbed, with normal urine levels being close to detection limits in fish. It is assumed that the proximal tubule is the primary site of glucose absorption in the vertebrate kidney (Dantzler 1989). Work by Friere et al. (1995) to isolate and prepare brush-border membrane vesicles (BBMV) from the proximal tubule of the rainbow trout has led to considerable insight into the glucose reabsorption pathway. The BBMVs displayed a single Na^+ dependent D-glucose co-transport system, which appeared to use the energy from Na^+ and voltage gradients to transport glucose. The transporter was also found to bind Na^+ and glucose in a 1:1 stoichiometry, with phlorizin inhibiting the transport system.

Phlorizin (phloretin-2′-B-glucoside) is a flavonoid found exclusively in apples and apple-derived products, where it occurs naturally as phloridzin (phloretin-2′-O-glucose) (Crespy et al. 2002). The most frequently described biological effect of phlorizin is its competitive inhibition of glucose uptake via Na^+–D-glucose co-transporters, specifically SGLT1 (a human renal glucose transporter) (Alvarado and Crane 1964). In the case of SGLT1, the binding affinity of phlorizin is dependent on the conformational

changes induced by the binding of Na^+ to the transporter (Leung et al. 2000).

In mammals, the physiological effect of phlorizin appears to be primarily the inhibition of renal tubular reabsorption of filtered glucose. This is accomplished by phlorizin binding to the transporter and inhibiting the conformational change necessary for glucose transport (Horsburhg et al. 1977). This blockade of glucose transport by phlorizin occurs mainly on the luminal membrane of proximal cells (Horsburhg et al. 1977).

Urea is another plasma solute that is transported by the kidney. In fish, urea can be excreted or reabsorbed at two main locations, the gill and the kidney. In the rainbow trout, branchial excretion of urea generally accounts for >90% of total urea excretion (McDonald and Wood 2003). A facilitated diffusion transporter has been suggested for this transport mechanism (McDonald and Wood 1998, 2003), an idea that is strongly supported by studies on other species such as the gulf toadfish (Wood et al. 1998; Walsh et al. 2000), the Magadi tilapia (Walsh et al. 2001b), and the plainfin midshipman (Walsh et al. 2001a, McDonald and Wood 2002).

The pathway of urea transport in the fish kidney is currently unclear. The transporters in the kidney show a differential handling of urea analogues compared to those in the gills, suggesting that a different transporter is present in each organ (McDonald and Wood 2003). McDonald and Wood (1998) found that the concentration of urea in the plasma was greater than the concentration in the urine, and therefore that urea was reabsorbed against an apparent concentration gradient. However in a later study on the same species, McDonald and Wood (2003) found that urea was secreted in some cases and reabsorbed in others. Reasons for these differences are unclear but may involve seasonal, nutritional, age or genetic differences in fish stocks.

Neither McDonald and Wood (1998) nor McDonald and Wood (2003) showed any evidence for saturation of the transporter. Thus as the amount of urea loaded into the trout increased, net renal reabsorption increased in direct proportion even up to urea levels that proved toxic to the fish (McDonald and Wood 1998). The relationship with Na^+ reabsorption was the same in both studies; with an increase in Na^+ reabsorption came a linear increase in urea reabsorption. This directs attention to a possible Na^+-coupled transport mechanism for renal handling of urea as reported in marine elasmobranchs (Schmidt-Nielson et al. 1972; Morgan et al. 2003), though facilitated type diffusion transporters have also been reported in elasmobranchs (Smith and Wright 1999; Hyodo et al. 2004).

McDonald and Wood (1998) put forth three possible explanations for the retention of urea by the freshwater trout. First, urea retention is a developmental legacy from embryonic life. This is supported by findings of Wright et al. (1995), who found that embryonic trout synthesize and retain urea. The second is that urea may be acting homeostatically in an unknown pathway, and hence urea retention is intentional. Thirdly, urea retention is "accidental" with urea acting as an alternative substrate for a transport mechanism. This has also been suggested by Kaplan et al. (1974) for the marine dogfish and was the subject of the present project.

The hypothesis tested in the present study is that urea may be acting as an alternative substrate for the reabsorptive glucose transporter in the trout kidney. This is supported by several studies on the mammalian glucose transporter, SGLT1. Heirmann and Wright (2001) reported that SGLT1 transported water and urea, and identified transmembrane segments 10–14 (known as C5) as being integral to this function. Water and urea channel activity in SGLT1 have been reported in other studies as well (Loo et al. 1999; Leung et al. 2000), and water and urea influx have been coupled to its co-transport cycle (Loo et al. 1996; Meinld et al. 1998)

In the present study, we examined the relationship between renal glucose transport and the renal handling of urea by the rainbow trout. We aimed to increase the rate of renal tubular glucose reabsorption by exogenous glucose loading and decrease it by phlorizin treatment. Since glucose loading is known to increase tubular glucose reabsorption in fish, we hypothesized two possible outcomes if urea was transported by the same mechanisms as glucose. Specifically, urea reabsorption would decrease if urea and glucose were competing for the same site on the transporter, or urea reabsorption would increase if urea flux

were coupled to the co-transport cycle of glucose transport. Furthermore, we hypothesized that in either case, blockade of glucose transport by phlorizin would likely decrease urea reabsorption. Finally, in view of the likely coupling of glucose reabsorption to Na^+ reabsorption, the effects of altering the glucose transporter's level of functioning on Na^+ and Cl^- reabsorption were explored, and possible correlations with urea and glucose transport were investigated.

Materials and methods

Experimental animals

Rainbow trout 242 ± 25 g ($N = 7$) (ranging from 200–350 g) were obtained from Humber Springs Trout Farm, Orangeville Ont. They were acclimated to seasonal temperatures and dechlorinated Hamilton tap water [$Ca^{2+} = 1.8$; $Cl^- = 0.8$; $Na^+ = 0.6$; $Mg^{2+} = 0.5$; $K^+ = 0.04$; titration alkalinity (to pH 4.0) = 1.9 mequiv l^{-1}; total hardness = 140 mg l^{-1} as $CaCO_3$; pH 8.0]. The fish were fed daily with 5 pt commercial trout pellets (Martin Mills, Ontario, Canada) until 1 week before surgery. The experimental temperature was 10–13 °C.

Experimental protocol

Dorsal aortic and urinary catheters were implanted simultaneously in the fish while they were anaesthetized with MS-222 (0.07 g l^{-1}; Sigma) and artificially ventilated on an operating table. The dorsal aortic catheters (Clay-Adams PE-50) were implanted according to Soivio et al. (1972) and filled with 0.3 ml of Cortland saline (Wolf 1963) containing 50 i.u ml^{-1} of lithium heparin (Sigma). The internal urinary catheters (heat-molded Clay-Adams PE-60) were implanted in the urinary bladder according to Curtis and Wood (1991) and Wood and Patrick (1994). The catheters drained into glass vials placed 3 cm below the water line to allow for gravimetric collection of urine from the kidneys. This method negates any reabsorptive/secretory role of the bladder and therefore solely examines the function of the kidney.

Following the surgery the fish were placed in individual darkened flux boxes and allowed to recover for 24 h, during which the functionality of the catheters was assessed by measuring the urine volume collected and taking a blood sample. A fish was deemed to be experimentally viable if both catheters were functional. Such fish were then injected with 17 μCi of [1,2 ^{3}H] polyethylene glycol (PEG-4000) (New England Nuclear) in 0.66 ml of Cortland saline, which was washed through the catheter with an additional 0.3 ml of Cortland saline. [^{3}H] PEG-4000 was chosen to monitor GFR because it undergoes the least radioautolysis, metabolic breakdown or post-filtration reabsorption across tubules of freshwater fish relative to other GFR markers (Beyenbach and Kirschner 1976; Erickson and Gingrich 1986; Curtis and Wood 1991). The [^{3}H] PEG-4000 was allowed to equilibrate for 12 h before experimentation was begun.

The infusions, at a rate of 0.5 ml kg^{-1} h^{-1}, were begun after allowing the [^{3}H] PEG-4000 to equilibrate. Blood samples (100 μl) were taken every 12 h and centrifuged at 13 000 g for 2 min to separate plasma and red blood cells, and urine samples were collected over 12 h periods. Plasma and urine samples were immediately frozen in liquid nitrogen and then stored at −80 °C. Each fish was subjected to three 24 h infusions in sequence. The first infusion solution contained 10 mM glucose, the second 15 mM glucose and the third 5 mM phlorizin. The solutions for the infusions were changed every 24 h after consecutive urine and blood samples had occurred. The solutions were administered in a 140 mM NaCl solution which was pH balanced to 7.4–7.5 using 1 M NaOH. Previous tests demonstrated that infusion of saline of the pH at this rate had no effect on blood pH.

Analytical techniques

Glucose in plasma and urine was measured enzymatically (hexokinase, glucose-6-phosphate dehydrogenase) using a commercial kit (Sigma, 301A). Urea in plasma and urine was measured using the diacetyl monoxime method of Rahmatullah and Boyde (1980). The radioactivity of the plasma and urine was measured by taking 25 μl of plasma or 1 ml of urine, and then adding enough double distilled water to make 5 ml of solution. Then 10 ml of ACS scintillation fluor (Amersham) was added and the samples

were counted in a LKB Rackbeta 1217 Counter. Tests demonstrated that quenching was uniform and therefore no correction was necessary. Na^+ and Cl^- concentrations in plasma and urine were measured using a Varian 1275 Atomic Absorption Spectrophotometer and the colorimetric spectrophotometric assay of Zall et al. (1956), respectively.

Calculations

All rates were related to fish body mass e.g. ml kg^{-1} h^{-1}.

Urinary excretion (U) rates of any substance (X) were calculated as:

$$U_x = [X]_u \cdot UFR \quad (1)$$

using measured values of urine flow rates (UFR) and urine concentrations of the substance ($[X]_u$). Glomerular filtration rates were calculated as the clearance of [^{3}H] PEG-4000 – i.e. the excretion of radioactivity in the urine (cpm_u) relative to its concentration in the blood plasma (cpm_p):

$$GFR = \frac{cpm_u \cdot UFR}{cpm_p} \quad (2)$$

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

$$FR_x = [X]_p \cdot GFR \quad (3)$$

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

$$TR_x = U_x - FR_x \quad (4)$$

The clearance ratio of a substance (CR_x; Wood 1995) relates the clearance of X to the GFR:

$$CR_x = \frac{[X]_u \cdot UFR}{[X]_p \cdot GFR} \quad (5)$$

A CR_x of more than 1 indicates that there has been net secretion of X; less than 1, then net reabsorption has occurred.

Statistics

Data have been reported as means ± S.E.M (N = number of fish), unless otherwise stated. Regression lines were fitted by method of least squares, and the significance ($p < 0.05$) of the slope assessed. The significance ($p < 0.05$) of differences between two regression lines was calculated according to Zar (1974). The significance of differences between means was evaluated using Student's paired and unpaired two-tailed t-test as appropriate ($p < 0.05$) with Bonferroni correction for multiple comparisons (Nemenyi et al. 1977).

Results

Seven trout were deemed viable after the initial recovery period and [^{3}H] PEG-4000 equilibration period (i.e. 36 h after surgery). Glucose levels in the plasma increased over time in response to glucose loading, but they declined at 72 h (i.e. 24 h after the start of the phlorizin infusion) when they dropped to become not significantly different from the starting values (Table 1). Glucose levels in the urine were much lower then the levels in the plasma, indeed close to the limits of analytical detection, until phlorizin was administered when they rose to levels close to those found in the blood plasma (at 60 and 72 h). Plasma and urine urea levels did not change significantly over time, but urine urea levels were significantly lower than plasma levels (the average value was 59% of that in the plasma). Plasma Na^+ and Cl^- levels fluctuated with a significant decrease during the phlorizin infusion for both. Urine Na^+ and Cl^- levels were much lower than those in plasma (averaging 8.8% and 11% of the plasma values, respectively), and exhibited comparable fluctuations over time. There was a significant increase in UFR during the 15 mM glucose infusion, while there was no change in GFR throughout the experiment (Table 1). The average overall GFR value was 4.53 ± 0.29 ml kg^{-1} h^{-1} ($N = 7$), and the average UFR value was 3.58 ± 0.11 ml kg^{-1} h^{-1} ($N = 7$).

There was a significant increase in glucose excretion during the phlorizin infusion compared to both the 10 mM glucose infusion and the 15 mM glucose infusion, as would be expected by the blockade of the renal glucose reabsorptive transporters (Figure 1). However there was no significant change in the urea excretion rates during the various infusions (Figure 1B). Note that urinary excretion rates of urea exceeded those of glucose prior to phlorizin treatment, whereas glucose excretion rates rose to many fold higher than urea excretion rates thereafter.

Table 1. Comparison of mean plasma and urine concentrations, and UFR and GFR values, means ± SEM ($N = 7$)

Infusion		Glucose				Phlorizin	
		10 mM	10 mM	15 mM	15 mM	5 mM	5 mM
Time	(h)	12	24	36	48	60	72
[glucose]p	(mM)	3.5 ± 1.4	4.8 ± 0.7	5.5 ± 1.9[1]	6.2 ± 1.7[1]	7.4 ± 2.0[1]	5.4 ± 1.8[1]
[glucose]u	(mM)	0.25 ± 0.04[2]	0.45 ± 0.03[2]	0.26 ± 0.06[2]	0.18 ± 0.08[2]	8.5 ± 1.2[1]	7.5 ± 1.3[1]
[urea]p	(mM)	1.0 ± 0.1	0.88 ± 0.07	0.89 ± 0.06	0.77 ± 0.08	0.85 ± 0.05	0.88 ± 0.06
[urea]u	(mM)	0.65 ± 0.04[2]	0.60 ± 0.03[2]	0.50 ± 0.10[2]	0.48 ± 0.08[2]	0.43 ± 0.03[2]	0.43 ± 0.07[2]
$[Na^+]$p	(mM)	160 ± 3	150 ± 3	140 ± 2[1]	150 ± 3[1]	140 ± 1[1]	140 ± 2[1]
$[Na^+]$u	(mM)	12.0 ± 0.6[2]	8.5 ± 1.0[2]	11.0 ± 0.8[2]	8.6 ± 1.1[2]	6.9 ± 0.9[2]	7.0 ± 0.9[2]
$[Cl^-]$p	(mM)	140 ± 1	120 ± 1	110 ± 1[1]	130 ± 2[1]	95 ± 2[1]	96 ± 1[1]
$[Cl^-]$u	(mM)	10.0 ± 1.4[2]	9.1 ± 0.7[2]	21.1 ± 2.9[1,2]	13.0 ± 1.8[2,1]	11.1 ± 2.0[2]	7.9 ± 1.6[2]
UFR	(ml kg^{-1} h^{-1})	2.4 ± 0.1	3.0 ± 0.8	5.6 ± 0.9[1]	4.6 ± 0.6[1]	3.0 ± 0.8	2.9 ± 0.6
GFR	(ml kg^{-1} h^{-1})	5.2 ± 2.5	3.2 ± 0.6	6.1 ± 0.8	5.1 ± 0.9	3.5 ± 0.8	4.1 ± 0.7

[1]indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 10 mM glucose infusion values.
[2]Indicates a significantly lower urine value than plasma value. $N = 7$.

Further support of the action of phlorizin on renal glucose reabsorptive transporters is shown in Table 2. The rate of glucose reabsorption rose significantly from the 10 mM infusion to the 15 mM infusion in concert with an increase in the filtration rate of glucose, and then fell significantly as the glucose transporters were blocked by phlorizin. The reabsorption rates during the phlorizin infusion were not significantly different from zero, indicating that the filtration rate of glucose was equal to the excretion rate of glucose. Urea reabsorption was not affected by the infusions, indicating that urea filtration and excretion rates are not affected by the alterations in glucose transport or by the blockade of glucose transporters. Similarly neither Na^+ nor Cl^- reabsorption were significantly affected by the various infusions.

The clearance ratio of glucose was close to zero [0.12 ± 0.05 (7), indicating 88% reabsorption] until the phlorizin treatment was begun (Figure 2a). At this point the clearance ratio became not statistically different from 1.0, showing that glucose was being filtered with the same efficiency as the [^{3}H] PEG-4000 and was subject to neither net secretion nor net reabsorption. However the urea clearance ratios were significantly less than one and the average value was 0.53 ± 0.01 (7), meaning that 47% of the urea was reabsorbed (Figure 2b) against an apparent concentration gradient (Table 1). This did not change over time indicating that the infusions did not affect urea reabsorption.

Na^+ and Cl^- clearance ratios (Table 3) were also not affected by the treatments either, averaging 0.041 ± 0.007 (7) for Na^+ (96% reabsorption) and 0.08 ± 0.02 for Cl^- (7) (92% reabsorption). The clearance ratio for water averaged 0.78 ± 0.19 (7) indicating that 22% of the filtered water was reabsorbed.

The phlorizin treatment significantly altered renal glucose handling across a wide range of glucose filtration rates (Figure 3a). During the phlorizin infusions reabsorption was not occurring, while during the glucose infusions the glucose reabsorption was equal to the filtration (i.e. the slope of the line was 1.0), showing that all the filtered glucose was reabsorbed. In contrast, phlorizin did not affect the relationship between urea reabsorption and urea filtration (Figure 3b). The linear relationships for the glucose treatments, indicate a lack of a transport maxima for both glucose (Figure 3a) and urea reabsorption (Figure 3b) at these experimental concentrations, which would be indicated by a leveling off of the lines at high values.

While Na^+ reabsorption and glucose reabsorption were well correlated, Figure 4a illustrates that Na^+ reabsorption rates were almost 20 fold higher than glucose reabsorption rates in

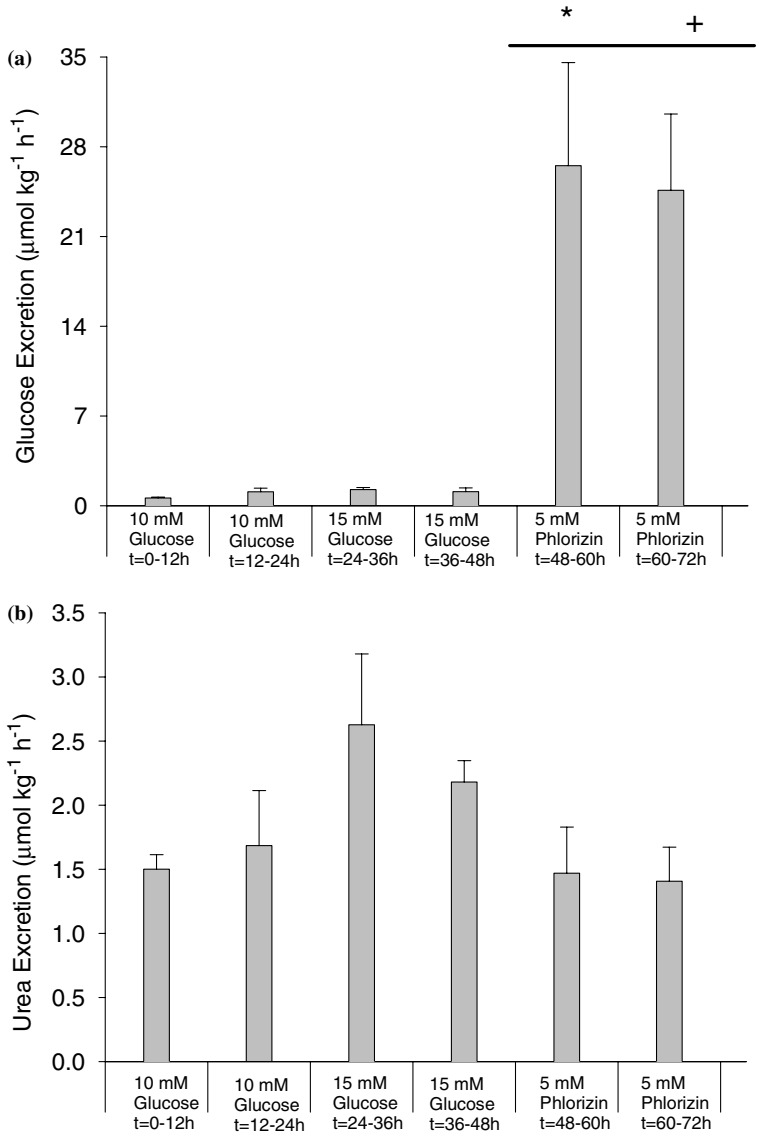

Figure 1. (a) Urinary glucose excretion rates (μmol kg^{-1} h^{-1}). (b). Urinary urea excretion rates (μmol kg^{-1} h^{-1}). The values are shown above the infusion that the fish received as well as the time period over which the sample was collected. Values are means $\pm$ 1 S.E.M ($N = 7$). * indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 10 mM glucose infusion values. + indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 15 mM infusion values.

glucose-loaded fish. Urea reabsorption was also correlated with Na^+ reabsorption (Figure 4b), but here the discrepancy in the relative rates was even greater, approximately 200–300 fold. During the phlorizin treatment, the reabsorption of glucose was blocked, eliminating the relationship with Na^+ (Figure 4a). However Na^+ continued to be reabsorbed, presumably by other transporters. In contrast, phlorizin treatment did not affect the relationship between urea and Na^+ reabsorption (Figure 4b).

Discussion

It is evident that the phlorizin infusion at 2.5 μmol kg^{-1} h^{-1} was effective in blocking the reabsorption of glucose. The reabsorption of

Table 2. Reabsorption rates of glucose, urea, Na^+, and Cl^-, means ± SEM ($N = 7$)

Infusion		Glucose				Phlorizin	
		10 mM	10 mM	15 mM	15 mM	5 mM	5 mM
Time	(h)	12	24	36	48	60	72
Reabsorption rates							
Glucose	(μmol kg^{-1} h^{-1})	9.8 ± 1.9	8.9 ± 1.9	34 ± 7[1]	32 ± 4[1]	−5.0 ± 3.4[1,2]	−4.3 ± 4.0[1,2]
Urea	(μmol kg^{-1} h^{-1})	0.85 ± 0.25	1.40 ± 0.35	3.10 ± 0.79	2.30 ± 1.00	1.10 ± 0.22	2.00 ± 0.29[1]
Na^+	(μmol kg^{-1} h^{-1})	280 ± 25	420 ± 86	700 ± 110[1]	500 ± 39[1]	450 ± 110	460 ± 60
Cl^-	(μmol kg^{-1} h^{-1})	390 ± 95	480 ± 150	890 ± 180[1]	630 ± 190[1]	490 ± 102	430 ± 96
H_2O	(ml kg^{-1} h^{-1})	2.6 ± 1.7	0.20 ± 0.09	0.30 ± 0.08	0.32 ± 0.09	0.51 ± 0.07	0.98 ± 0.79

[1]Indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 10 mM glucose infusion values.
[2]Indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 15 mM infusion values. $N = 7$.

glucose was complete in the absence of phlorizin as evidenced by the extremely low clearance ratio of glucose (Figure 4a), the nearly undetectable urine concentrations (Table 1) as well as the low excretion rates for glucose (Figure 1a). Glucose excretion rates increased in response to phlorizin (Figure 1a), becoming equal to filtration rates. As the excretion rates were rising, the reabsorption rates were falling to zero creating a clearance ratio of glucose that was not significantly different from one (Figure 1a), indicating glucose was subject only to filtration to the same extent as [^{3}H] PEG-4000 (Wood 1995).

This was expected as previous *in vitro* studies on fish encounter complete inhibition of glucose transport at plasma phlorizin concentrations of 0.1–10 μM. Pritchard et al. (1976) found that 2.5 μmol kg^{-1} (the amount we infused in each hour) of phlorizin injected into the caudal vein of the winter flounder (creating an estimated plasma concentration of 5–10 μM of phlorizin) created an increase in mannitol clearance ratios to 0.8–1.0 from a starting value of 0.3. Phlorizin appears to be acting on the single glucose transporter located in the rainbow trout kidney, which was identified by Friere et al. (1995) as a Na^+-dependent D-glucose co-transport system. The data of Figure 4a are in accord with Na^+ dependency, though they do not prove it. Stimulation and inhibition of renal glucose reabsorption by glucose and phlorizin infusion, respectively had negligible effects on the active reabsorption of Na^+ (and Cl^-) (Tables 2 and 3). However it is likely that any such effect would be so small as to be undetectable against Na^+ (and Cl^-) transport rates 20 fold greater than those of glucose (Figure 4a).

Contrary to the hypothesis behind this study (see Introduction), urea transport is not related to glucose transport in any way in the trout kidney. There was no obvious effect of either glucose loading or phlorizin on any of the indices of urea reabsorption leading to the conclusion that urea transport is not associated with the glucose transporter, and that urea is transported via a different mechanism than proposed. These results must be interpreted against the background of literature on glucose–urea coupling which is somewhat clouded at present. Heirmann and Wright (2001) found that urea actually passed through a channel that was a part of the glucose transporter but some distance from the glucose and Na^+ binding sites on the rabbit renal glucose transporter (rbSGLT1). The channel was not affected by phlorizin because it was not involved with the binding of phlorizin to the transporter and hence urea transport was not affected by phlorizin or glucose binding as in this study. However Heirmann and Wright (2001) also found that urea transport was passive in this system which is in contradiction to present and previous findings (McDonald and Wood 1998, 2003) that urea was being transported via an active transporter against its concentration gradient in the trout kidney, as sometimes also reported in the mammalian kidney (Kawamura and Kokko 1976; Kato and Sands 1998).

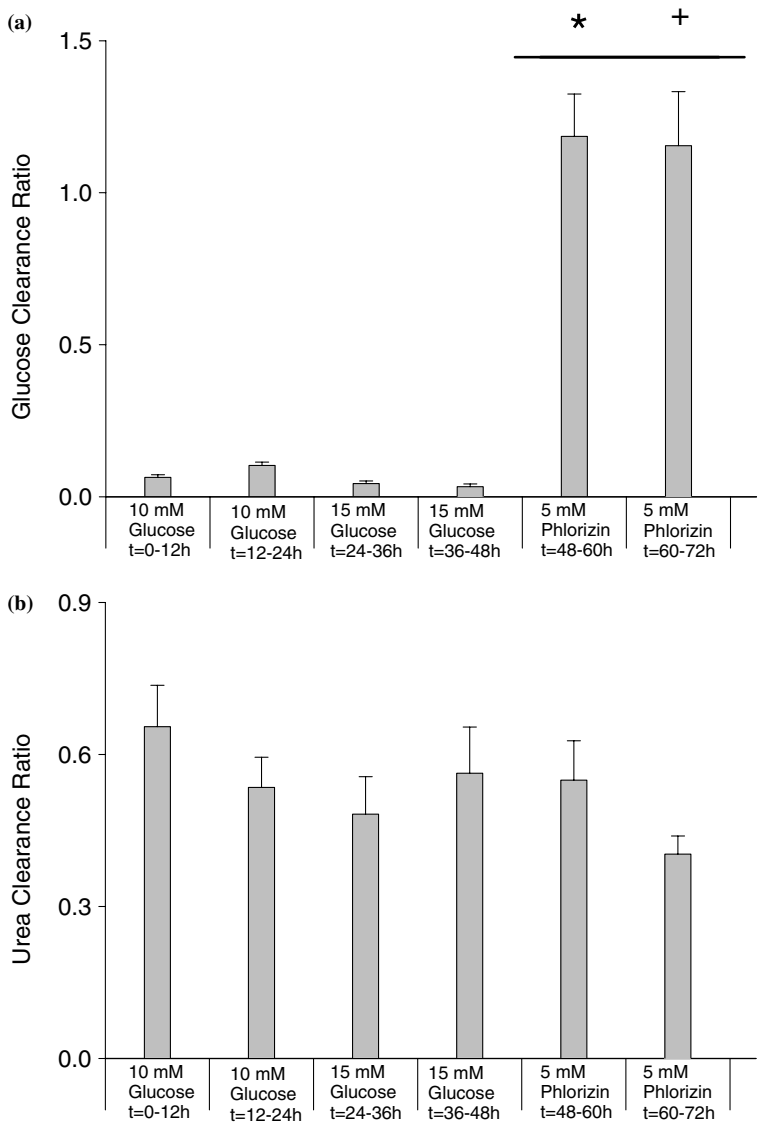

Figure 2. (a) Renal clearance ratios of glucose. (b). Renal clearance ratios of urea. The values are shown above the infusion that the fish received as well as the time period over which the sample was collected. Values are means ± 1 S.E.M ($N = 7$). *indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 10 mM glucose infusion values. + indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 15 mM infusion values.

Leung et al. (2000) presented evidence that the co-transporters studied (rbSGLT1, the low affinity Na^+–glucose co-transporter (pSGLT3), the Na^+–iodide co-transporter (rNIS), and the Na^+–Cl^-–GABA co-transporter (hGAT1)) behave as passive urea channels in the absence of substrate, and under substrate-transporting conditions the same co-transporters behave as active urea co-transporters. They go on to further propose that water and urea are transported by conformational changes, which the co-transporters undergo during substrate transport, an idea that is supported by many (Zeuthen 1991, 1994; Loo et al. 1996, 1999; Zeuthen et al. 1996, 1997; Meinld et al. 1998, 2000). However, Leung et al. (2000) also found that phlorizin was effective in blocking urea transport and that the addition of sugars to the medium stimulated the

Table 3. Clearance ratios for Na^+, Cl^-, and H_2O, means ± SEM ($N = 7$)

Infusion	Glucose				Phlorizin	
	10 mM	10 mM	15 mM	15 mM	5 mM	5 mM
Time (h)	12	24	36	48	60	72
Clearance ratio						
Na^+	0.071 ± 0.019	0.061 ± 0.021	0.097 ± 0.022	0.089 ± 0.024	0.043 ± 0.010[2]	0.039 ± 0.007[2]
Cl^-	0.141 ± 0.071	0.085 ± 0.035	0.122 ± 0.036	0.109 ± 0.029	0.077 ± 0.013	0.084 ± 0.017
H_2O	0.75 ± 0.15	0.87 ± 0.09	0.92 ± 0.09	0.91 ± 0.08	0.85 ± 0.07	0.67 ± 0.04

[1]Indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 10 mM glucose infusion values.
[2]Indicates a significant difference ($p < 0.05$) between the mean of the indicated infusion values and the mean of the 15 mM infusion values. $N = 7$.

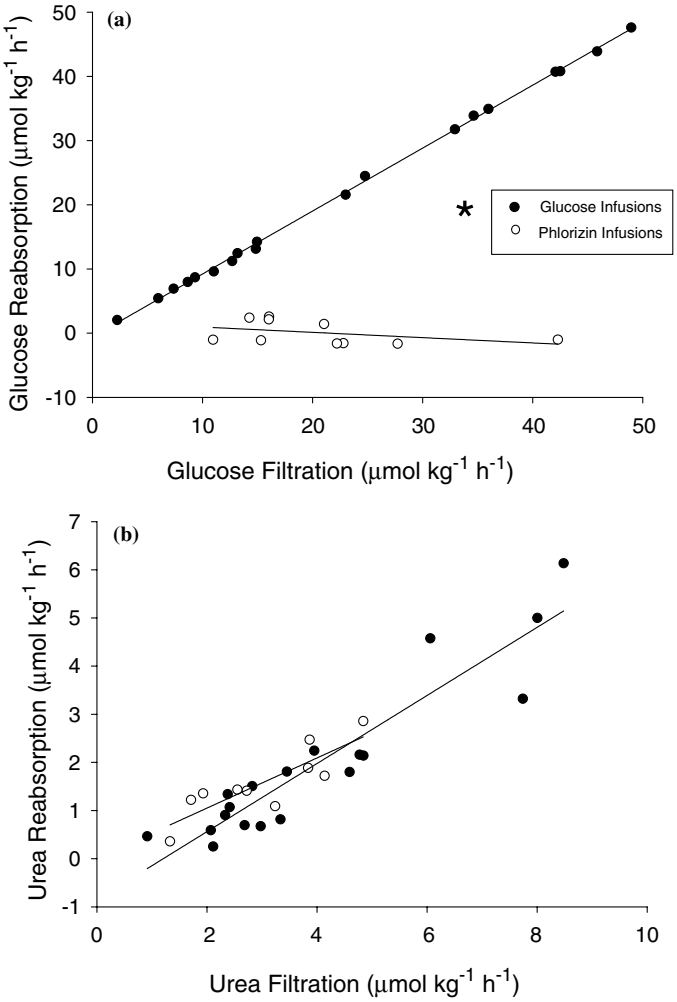

Figure 3. (a). Glucose filtration (μmol kg^{-1} h^{-1}) vs. glucose reabsorption (μmol kg^{-1} h^{-1}) in fish infused with glucose or phlorizin. (b) Urea filtration (μmol kg^{-1} h^{-1}) vs. urea reabsorption (μmol kg^{-1} h^{-1}). $N = 7$, *indicates significantly different ($p < 0.05$) regression lines. Points are values for individual fish at different times, not mean values.

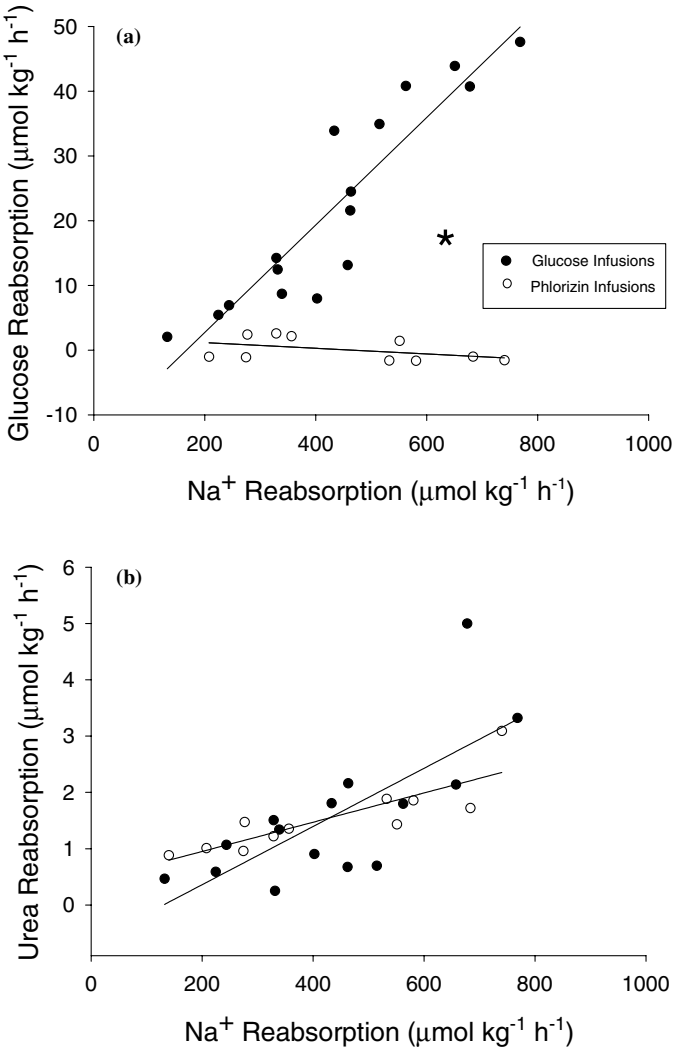

Figure 4. (a). Glucose reabsorption (μmol kg^{-1} h^{-1}) vs. Na^+ reabsorption (μmol kg^{-1} h^{-1}) in fish infused with glucose or phlorizin. (b) Urea reabsorption (μmol kg^{-1} h^{-1}) vs. Na^+ reabsorption (μmol kg^{-1} h^{-1}). $N = 7$, *indicates significantly different ($p < 0.05$) regression lines. Points are values for individual fish at different times, not mean values.

uptake of urea, both of which are in contradiction to the findings of the present study. As well, Heirmann and Wright (2001) found that urea transport through rbSGLT1was Na^+-independent, which again is contrary to this study as well as others (McDonald and Wood 1998, 2003). Overall, these findings suggest that the model proposed for urea transport in SGLT1 is not appropriate for trout renal handling of urea.

Urea was always reabsorbed against a concentration gradient in support of the original findings of McDonald and Wood (1998). The clearance ratio found for urea in the present study (0.53 ± 0.01) match those (0.40 ± 0.09) reported by McDonald and Wood (1998). McDonald and Wood (1998) also reported water clearance ratios of 0.50 ± 0.04 which were lower than those (0.78 ± 0.19) found in this experiment. However, the clearance ratios of urea in both this study and the report of McDonald and Wood (1998) were lower than those of water, which suggests that urea transport is relatively greater than water transport and is therefore not occurring by solvent drag. The urea clearance

ratio was also higher than the clearance ratios for Na^+ and Cl^-, which suggests that it is not being actively transported as efficiently as these ions. Active secretion of urea was also seen in some of the trout studied by McDonald and Wood (2003), but not in the present study. Reasons for this contradiction are unclear, but unseen variations in the nutritional levels of protein in the fish may have played a part. Both active reabsorption and active secretion of urea have been reported in the mammalian kidney (Kato and Sands 1998). Kato and Sands (1998) found that rats fed a low protein diet expressed active Na^+-dependent urea reabsorption in the initial inner medullary collecting duct, while rats fed a normal protein diet expressed no such activity. Variations in the mass of the fish are not believed to be responsible for the contradiction as both studies had similar values [242 ± 25 g (present study), and 259 ± 17 g (McDonald and Wood 2003)].

Urea transport was active and correlated well with Na^+ transport in the trout kidney in both this and earlier studies (McDonald and Wood 1998, 2003), though correlation does not prove causation. In the spiny dogfish kidney, an active urea transporter was identified (Schmidt-Nielson and Rabinowtiz 1967) where Na^+ and urea were reabsorbed at a much lower fixed ratio of 1.6:1 (Schmidt-Nielson et al. 1972). Active Na^+-dependent "back transport" of urea (of unknown stoichiometry) has also been reported in the gills of the dogfish (Fines et al. 2001), and both active Na^+-coupled reabsorption and secretion of urea (again of unknown stoichiometry) have been described in the mammalian kidney (Kato and Sands 1998). Urea transport in the little skate kidney exhibits a non-saturable uniporter in the dorsal section and a Na^+-linked transporter in the ventral section of the kidney (Morgan et al. 2003). All of the mechanisms appear to be very different from the facilitated diffusion transporters ("UT"s) characterized in teleost gills in recent years (Wood et al. 1998; Walsh et al. 2000; Walsh et al. 2001a, b) which appear to be Na^+-independent and passive, but controlled in different manners in different species by mechanisms which are not yet understood. Clearly there is much more to learn about urea handling in fish.

Acknowledgements

Supported by an NSERC Discovery grant to CMW. CMW is supported by the Canada Research Chair Program.

References

Alvarado, F. and Crane, R.K. 1964. Studies of the mechanism of intestinal absorption of sugars. Biochim. Biophys. Acta 93: 116–135.

Beyenbach, K.W. and Kirschner, L.B. 1976. The unreliability of mammalian glomerular markers in teleostean renal studies. J. Exp. Biol. 64: 369–378.

Brown, J., Oliver, J., Henderson, I. and Jackson, B. 1980. Angiotensin and single nephron glomerular function in the trout. Am. J. Physiol. 239: R509–R514.

Crespy, V., Aprikian, O., Morand, C., Besson, C., Manach, C., Demige, C. and Remesy, C. 2002. Bioavailability of phloretin and phloridzin in rats. J. Nutr. 132: 3227–3230.

Curtis, J.B. and Wood, C.M. 1991. The function of the urinary bladder *in vivo* in the freshwater rainbow trout. J. Exp. Biol. 155: 567–583.

Dantzler, W.H. 1989. Comparative Physiology of the Vertebrate Kidney. Springer-Verlag, Berlin.

Duff, D. and Olson, K. 1986. Trout vascular and renal responses to atrial natriuretic factor and heart extracts. Am. J. Physiol. 251: R639–R642.

Elger, B. and Hentschel, H. 1983. Effects of adrenergic blockade with bretylium and phentolamine on glomerular filtration rates in the rainbow trout, adapting to saline water. Comp. Biochem. Physiol. 75C: 253–258.

Erickson, D.A. and Gingrich, W.H. 1986. Effect of injected rotenone on the production and composition of urine from the rainbow trout (*Salmo. gairdneri*). Aquat. Toxicol. 9: 263–274.

Fines, S.A., Ballantyne, J.S. and Wright, P.A. 2001. Active urea transport and an unusual basolateral membrane composition in the gills of a marine elasmobranch. Am. J. Physiol. 280: R16–R24.

Friere, C.A., Kinne-Saffran, E., Beyenbach, K.W. and Kinne, R.K.H. 1995. Na–D-glucose co-transport in renal brush-border membrane vesicles of an early teleost (*Oncorhynchus mykiss*). Am. J. Physiol. 269: R592–R602.

Heirmann, M.P. and Wright, E.M. 2001. Mapping the urea channel through the rabbit Na^+–glucose cotransporter SGLT1. J. Physiol. 535: 419–425.

Hickman, C.P. and Trump, B.F. 1969. In: Fish Physiology. Chapter 2, pp. 91–227. Edited by W.S. Hoar and D.J. Randall. Academic Press, New York.

Horsburhg, T., Cannon, J.K. and Pitts, R.F. 1977. Action of phlorizin on luminal and antiluminal membranes of proximal cells of kidney. Am. J. Physiol. 234: F485–489.

Hyodo, S., Katoh, F., Kaneko, T. and Takei, Y. 2004. A facilitative urea transporter is localized in the renal collecting tubule of the dogfish, *Triakis scyllia*. Exp. Biol. 207: 347–356.

Kaplan, M.A., Hays, L. and Hays, R.M. 1974. Evolution of a facilitated diffusion pathway for amides in the erythrocyte. Am. J. Physiol. 226: 1327–1332.

Kato, A. and Sands, J. 1998. Evidence for sodium-dependent active secretion in the deepest subsegment of the rat inner medullary collecting duct. J. Clin. Invest. 101: 423–428.

Kawamura, S. and Kokko, J. 1976. Urea secretion by the straight segment of the proximal tubule. J. Clin. Invest. 58: 604–612.

Korsgaard, B., Mommsen, T.P. and Wright, P. 1995. Nitrogen excretion in teleostean fish: Adaptive relationships to environment, ontogenesis, and viviparity. *In:* Nitrogen Metabolism and Excretion. pp. 243–258. Edited by P.J. Walsh and P.A. Wright. CRC Press Inc., Boca Raton.

Leung, D., Loo, D., Hirayama, B., Zeuthen, T. and Wright, E. 2000. Urea transport by cotransporters. J. Physiol. 528: 251–257.

Loo, D., Eskandari, S., Meinild, A. and Wright, E. 1999. Passive water and ion transport by contransporters. J. Physiol. 518: 195–202.

Loo, D., Zeuthen, T., Chandy, G. and Wright, E. 1996. Cotransport of water by the Na^+/glucose cotransporter. Proc. Natl. Acad. Sci. USA 93: 13367–13370.

McDonald, M.D. and Wood, C.M. 1998. Reabsorption of urea by the kidney of the freshwater rainbow trout. Fish Physiol. Biochem. 18: 375–386.

McDonald, M.D. and Wood, C.M. 2003. Differential handling of urea and its analogues suggests carrier-mediated urea excretion in the freshwater rainbow trout (*Oncorhynchus mykiss*). Physiol. Biochem. Zool. 76 (6): 791–802.

Meinld, A., Klaerke, D., Loo, D. and Wright, E. 1998. The human Na^+-glucose cotransporter is a molecular water pump. J. Physiol. 508: 15–21.

Meinld, A., Loo, D., Pajor, A., Zeuthen, T. and Wright, E. 2000. Water transport by the renal Na^+–dicarboxylate cotransporter. Am. J. Physiol. 278: F777–F783.

Morgan, R., Wright, P. and Ballantyne, J. 2003. Urea transport in kidney brush border membrane vesicles from an elasmobranch *Raja erinacea*. J. Exp. Biol. 206: 3293–3302.

Nemenyi, P., Dixon, S.K., White, N.B. and Jedstrom, M.L. 1977. Statistics from Scratch. Holden-Day, San Fransisco.

Rahmatullah, M. and Boyde, T.R. 1980. Improvements in the determination of urea using diacetyl monoxime: Methods with and without deproteinization. Clin. Chim. Acta 107: 3–9.

Schmidt-Nielson, B. and Rabinowitz, L. 1967. Renal tubules of the shark actively reabsorb urea. Science 146: 1587–1588.

Schmidt-Nielson, B., Truniger, B. and Rabinowitz, L. 1972. Sodium-linked urea transport by the renal tubule of the spiny dogfish. Comp. Biochem. Physiol. 42A: 13–25.

Smith, C. and Wright, P. 1999. Molecular characterization of an elasmobranch urea transporter. Am. J. Physiol. 276: R622–R626.

Soivio, A., Westman, K. and Nyholm, K. 1972. Improved method of dorsal aorta catheterization: Haematological effects followed for 3 weeks in rainbow trout (*Salmo gairdneri*). Finnish Fish Res. 1: 11–21.

Valtin, H. 1983. Renal Function: Mechanisms Preserving Fluid and Solute Balance in Health. Little, Brown and Company, Boston/Toronto.

Walsh, P., Grosell, M., Goss, G., Bergman, H., Bergman, A., Wilson, P., Laurent, P., Alper, S., Smith, C., Kamunde, C. and Wood, C. 2001b. Physiological and molecular characterization of urea transport by the gills of the Lake Magadi tilapia. J. Exp. Biol. 204: 509–520.

Walsh, P., Heitz, M., Campbell, C., Cooper, G., Medina, M., Wang, Y., Goss, G., Vincek, V., Wood, C. and Smith, C. 2000. Molecular characterization of a urea transporter in the gill of the gulf toadfish. J. Exp. Biol. 203: 2357–2364.

Walsh, P., Wang, Y., Campbell, C., DeBoeck, S. and Wood, C.M. 2001a. Patterns of nitrogenous waste excretion and gill urea transporter mRNA expression in several species of marine fish. Mar. Biol. 139: 839–844.

Wolf, K. 1963. Physiological salines for freshwater teleosts. Prog. Fish Culturist 25: 135–140.

Wood, C.M. 1993. Ammonia and urea metabolism and excretion. *In:* The Physiology of Fishes. pp. 379–425. Edited by D.H. Evans. CRC Press Inc., Baton Rouge.

Wood, C.M. 1995. Excretion. *In:* Physiological Ecology of Pacific Salmon. pp. 381–438. Edited by C. Groot, L. Margolis and W.C. Clarke. UBC Press, Vancouver.

Wood, C.M., Gilmour, K.G., Perry, S.F., Part, P., Laurent, P. and Walsh, P.J. 1998. Pulsatile urea excretion in the gulf toadfish (*Opsanus beta*): Evidence for activation of a specific facilitated diffusion transport system. J. Exp. Biol. 201: 803–817.

Wood, C.M. and Patrick, M.L. 1994. Methods for assessing kidney and urinary bladder function in fish. *In:* Biochemistry and Molecular Biology of Fishes. Vol. 3, pp. 127–143. Elsevier Science, New York.

Wright, P.A., Felskie, A. and Anderson, P.M. 1995. Induction of ornithine–urea cycle enzymes and nitrogen metabolism and excretion in rainbow trout (*Oncorhynchus mykiss*) during early life stages. J. Exp. Biol. 198: 127–135.

Zall, D.M., Fisher, D. and Garner, M.D. 1956. Photometric determination of chloride in water. Anal. Chem. 28: 1665–1678.

Zar, J.H. 1974. Biostatistical Analysis. Prentice Hall Inc., Englewood Cliffs, NJ.

Zeuthen, T. 1991. Secondary active transport of water across ventricular cell membranes of choroid plexus epithelium of *Necturus maculosus*. J. Physiol. 444: 153–173.

Zeuthen, T. 1994. Cotransport of K^+, Cl^-, and H_2O by membrane proteins from choroid plexus epithelium of *Necturus maculosus*. J. Physiol. 478: 203–219.

Zeuthen, T., Hamann, S. and La Cour, M. 1996. Cotransport of H^+, lactate and H_2O by membrane proteins in retinal pigment epithelium of bullfrog. J. Physiol. 497: 3–17.

Zeuthen, T., Meinld, A., Klaerke, D., Loo, D., Wright, E., Belhage, B. and Litman, T. 1997. Water transport by the Na^+/glucose cotransporter under isotonic conditions. Biol. Cell 89: 307–312.