

# Transport Physiology of the Urinary Bladder in Teleosts: A Suitable Model for Renal Urea Handling?

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**ABSTRACT** The transport physiology of the urinary bladder of both the freshwater rainbow trout (*Oncorhynchus mykiss*) and the marine gulf toadfish (*Opsanus beta*) was characterized with respect to urea, and the suitability of the urinary bladder as a model for renal urea handling was investigated. Through the use of the in vitro urinary bladder sac preparation urea handling was characterized under control conditions and in the presence of pharmacological agents traditionally used to characterize urea transport such as urea analogues (thiourea, acetamide), urea transport blockers (phloretin, amiloride), and hormonal stimulation (arginine vasotocin; AVT). Na<sup>+</sup>-dependence and temperature sensitivity were also investigated. Under control conditions, the in vitro trout bladder behaved as in vivo, demonstrating significant net reabsorption of Na<sup>+</sup>, Cl<sup>-</sup>, water, glucose, and urea. Bladder urea reabsorption was not affected by pharmacological agents and, in contrast to renal urea reabsorption, was not correlated to Na<sup>+</sup>. However, the trout bladder showed a threefold greater urea permeability compared to artificial lipid bilayers, a prolonged phase transition with a lowered E<sub>a</sub> between 5°C and 14°C, and differential handling of urea and analogues, all suggesting the presence of a urea transport mechanism. The in vitro toadfish bladder did not behave as in vivo, showing significant net reabsorption of Na<sup>+</sup> but not of Cl<sup>-</sup>, urea, or water. As in the trout bladder, pharmacological agents were ineffective. The toadfish bladder showed no differential transport of urea and analogues, consistent with a low permeability storage organ and intermittent urination. Our results, therefore, suggest the possibility of a urea transport mechanism in the urinary bladder of the rainbow trout but not the gulf toadfish. While the bladders may not be suitable models for renal urea handling, the habit of intermittent urination by ureotelic tetrapods and toadfish seems to have selected for a low permeability storage function in the urinary bladder. *J. Exp. Zool.* 292:604–617, 2002. © 2002 Wiley-Liss, Inc.

The teleost urinary bladder is a morphological extension of the kidney, originating from the mesonephric duct (Nishimura and Imai, '82). The urinary bladder, like the kidney, selectively reabsorbs salt and water on a net basis (Fossat and Lahlou, '77, '79; Renfro, '77; Marshall, '86). For this reason, the urinary bladder has been used extensively as a model for renal tubular function in fish, though some difference in mechanisms between the two organs appear to exist. For example, the urinary bladders of both the freshwater rainbow trout (*Oncorhynchus mykiss*) and the marine gulf toadfish (*Opsanus beta*) have been documented to actively reabsorb Na<sup>+</sup> and Cl<sup>-</sup> in vivo (Howe and Gutknecht, '78; Curtis and Wood, '91), complementing kidney function in both fish and aiding in salt conservation in the case of the trout while contributing to water recovery in the case of the toadfish. However, this trend does not

necessarily hold true for other solutes: in both trout and toadfish, for example, Mg<sup>++</sup> is actively secreted into the kidney tubule but not into the bladder (Beyenbach and Kirschner, '75; Howe and Gutknecht, '78).

In amphibians, the urinary bladder, being of endodermal origin (Phillips, '75), has a different embryological origin than in teleosts. Urea is an important osmolyte in these animals, and in many species of frogs and toads, facilitated diffusion urea transporters (UT), similar to those found in

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mammalian models (Brahm, '83; Chou and Knepper, '89; Chou et al., '90), have been discovered in the urinary bladder and characterized both on a physiological level and a molecular level (Eggena, '73; Levine et al., '73a,b; Ardizzone and Lippe, '82; Shpun and Katz, '90; Martial et al., '91; Zhang and Verkman, '91; Couriaud et al., '99). In the toads *Bufo marinus*, *Bufo viridis*, and *Bufo bufo*, urea transport by the urinary bladder is stimulated by the hormone arginine vasotocin (AVT; analogous to the mammalian arginine vasopressin), which is thought to induce or activate more urea transporters or channels of the facilitated diffusion type (i.e., increasing  $V_{max}$ ) without changing the affinity ( $K_m$ ) to urea (Levine et al., '73a; Shpun and Katz, '89). Such UT-type transporters are typically difficult to saturate, although saturation kinetics have been observed in some cases (Levine et al., '73a; Imai et al., '88; Shpun and Katz, '89; Shpun and Katz, '90; Chou et al., '90). Facilitated diffusion of urea in the amphibian urinary bladder does not depend on the presence of  $Na^+$  and is inhibited both by the urea transport blocker, phloretin, and by urea analogues, which are thought to compete for a common site on the membrane carrier molecule (Eggena, '73; Levine et al., '73a,b; Shpun and Katz, '90).

In contrast, urea transport by the mesonephros-derived urinary bladder of teleost fish has yet to be studied. However, recent evidence suggests that carrier-mediated transport of urea occurs in the kidneys of both rainbow trout, an ammoniotelic species (McDonald and Wood, '98), and gulf toadfish, a species which is facultatively ureotelic (McDonald et al., 2000). In the kidney of the trout, which forms primary urine by glomerular filtration, urea is reabsorbed against a concentration gradient, suggesting an active transport mechanism (McDonald and Wood, '98). When infused with exogenous urea loads, net renal urea reabsorption in the trout increases in direct proportion to the filtered urea load, and saturation of the reabsorptive mechanism is not apparent, even at urea filtration rates well beyond physiological (McDonald and Wood, '98). Furthermore, analysis of in vivo data (M.D. McDonald and C.M. Wood, unpublished results) from the same study suggests that urea reabsorption and sodium reabsorption are closely correlated in the kidney of the trout. In the kidney of the toadfish, which forms primary urine largely by secretion, a similar net uphill movement of urea is observed, but in the direction of secretion (McDonald et al., 2000).

Moreover, in both fish, the kidneys have been shown to preferentially transport urea rather than analogues, namely acetamide and thiourea (McDonald et al., 2000; M.D. McDonald and C.M. Wood, unpublished results). However, the patterns of renal handling of these substances show distinct interspecific differences: the trout kidney transports urea and acetamide similarly, but not thiourea, while the toadfish kidney transports urea and thiourea similarly but not acetamide. As a consequence of the similarities in embryonic development and function between the urinary bladder and kidney in teleosts, the presence of a urea transport mechanism(s) in the urinary bladder, similar to that found in the kidney, is a distinct possibility.

The objectives of this study were twofold. Our first goal was to characterize the transport physiology of the urinary bladder of both the rainbow trout and the gulf toadfish with respect to urea. Secondly, we set out to determine whether the urinary bladder is a suitable model for renal handling of urea. Our results provide evidence for a urea transport mechanism in the urinary bladder of the rainbow trout but not the gulf toadfish and indicate that the urinary bladders of neither fish are suitable models for renal urea handling.

## MATERIALS AND METHODS

### *Experimental animals*

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Humber Springs Trout Farm in Mono Mills, Ontario. The fish were acclimated to seasonal water temperatures (11°C–14°C) and were fed with commercial trout pellets every second day until the time of surgery. Acclimation was carried out in dechlorinated Hamilton tap water [in mmol  $l^{-1}$ :  $Ca^{++} = 1.8$ ;  $Cl^- = 0.8$ ;  $Na^+ = 0.6$ ;  $Mg^{++} = 0.5$ ;  $K^+ = 0.04$ ; titration alkalinity (to pH 4.0) = 1.9; total hardness = 140 mg  $l^{-1}$  as  $CaCO_3$ ; pH 8.0]. Gulf toadfish (*Opsanus beta*) were caught by commercial shrimpers in Biscayne Bay, Florida, in November and December. The toadfish were held in outdoor tanks at the shrimpers' holding facility with running seawater (ambient seasonal conditions; 30‰–32‰, 22°C–26°C) for no longer than 24 hours following capture, then transferred to the laboratory. Fish were treated with a dose of malachite green (final concentration 0.5 mg  $l^{-1}$ ) in formalin (15 mg  $l^{-1}$ ) (Aquavet, Hayward, CA) on the day of transfer to the laboratory in order to prevent infection by the ciliate, *Cryptocaryon irritans* (Stoskopf, '93). Initially the fish were kept in 50L

glass aquaria with flowing, aerated seawater. Fish were maintained in this relatively crowded environment (> 15 fish per tank) in order to initiate a switch to ureotelism (Walsh et al., '94). Fish were fed squid weekly up until surgery.

### *Bladder sac experiments*

Rainbow trout and gulf toadfish were terminally anaesthetized using MS-222 ( $1\text{g l}^{-1}$ ). The peritoneal cavity was cut open, the ureter end of the bladder was tied with one 3.0 silk ligature, and the urinary bladder was removed carefully from the body cavity. In the case of the gulf toadfish, which has a bi-lobed bladder, the larger of the two lobes was used for all experiments. A heat-flared catheter, made out of Clay-Adams PE 50 tubing, was advanced through the small opening at the papilla-end of the bladder and held in place by two or three 3.0 silk ligatures secured tightly around the bladder. The ligatures were tested for leaks by injecting a small amount of saline. The urinary bladder was then filled with saline (Cortland saline for trout; Wolf, '63 (in  $\text{mmol l}^{-1}$ ): 124.0 NaCl, 5.1 KCl, 1.6  $\text{CaCl}_2$ , 0.9  $\text{MgSO}_4$ , 11.9  $\text{NaHCO}_3$ , 2.9  $\text{NaH}_2\text{PO}_4$ , and 5.6 Glucose; Hank's saline for toadfish; Walsh, '87 (in  $\text{mmol l}^{-1}$ ): 151.0 NaCl, 5.9 KCl, 0.9  $\text{MgSO}_4$ , 0.46  $\text{Na}_2\text{HPO}_4$ , 0.48  $\text{KH}_2\text{PO}_4$ , 5.0  $\text{NaHCO}_3$ , 11.0 Hepes, 1.0  $\text{CaCl}_2$ , and 3.0 Glucose), containing typical plasma urea concentrations for each respective fish (1.8  $\text{mmol l}^{-1}$  urea for trout under resting conditions; McDonald and Wood, '98; 7.0  $\text{mmol l}^{-1}$  urea for toadfish held in crowded conditions; Wood et al., '95) plus 1  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]-urea (57.0  $\text{mCi mmol}^{-1}$ ; Amersham, Quebec, Canada). The radio-labeled urea on the mucosal side allows unidirectional urea movement to be monitored. Every bladder was suspended individually in a 20 ml glass vial containing 10 ml of an identical non-radioactive saline and left to incubate 3–4 hours. The solution bathing the serosal surface of the bladder contained the same concentration of urea and was bubbled with a 99.7%  $\text{O}_2$ :0.3%  $\text{CO}_2$  mix gas ( $P_{\text{CO}_2} = 1.7$  torr) and left at ambient holding water temperature for each respective fish. Typical saline pH was 7.6 for both trout and toadfish.

Control series were performed simultaneously with all experimental treatments. Experimental treatments involved filling the bladder with the aforementioned saline plus a given amount of urea analogue (thiourea = 10  $\text{mmol l}^{-1}$  for trout and 20  $\text{mmol l}^{-1}$  for toadfish; acetamide = 10  $\text{mmol l}^{-1}$  for trout), hormone (AVT, the fish homologue of arginine vasopressin =  $10^{-9}$   $\text{mol l}^{-1}$ ) or inhibitor (phlo-

retin = 0.1  $\text{mmol l}^{-1}$  in 0.04% ethanol for trout and 0.250  $\text{mmol l}^{-1}$  in 0.5% DMSO for toadfish; amiloride = 0.1  $\text{mmol l}^{-1}$  in 0.02% DMSO for trout). All reported concentrations of analogue, hormone, inhibitor, and vehicle were final concentrations in saline. Analogue concentrations were much higher than urea concentrations to ensure a competitive interaction between them and urea. In the inhibitor experiments, the vehicle alone (ethanol, DMSO) was tested at the same concentration in the respective control series. The effect of AVT ( $10^{-9}$   $\text{mol l}^{-1}$ ) on thiourea-exposed (10  $\text{mmol l}^{-1}$ ) bladders of trout was investigated by combining these two treatments. In the previously mentioned experimental groups, both mucosal and serosal salines were identical, aside from [ $^{14}\text{C}$ ]-urea on the mucosal side.

The possibility of a  $\text{Na}^+$ -dependent urea transport mechanism was investigated by removing  $\text{Na}^+$  from the saline on the mucosal side (trout  $\text{Na}^+$ -free saline (in  $\text{mmol l}^{-1}$ ): 124.0 N-methyl-D-glucamine, 2.1 KCl, 1.6  $\text{CaCl}_2$ , 0.9  $\text{MgSO}_4$ , 11.9  $\text{C}_5\text{H}_{14}\text{NO}\cdot\text{HCO}_3$ , 2.9  $\text{K}_2\text{HPO}_4$ , 5.6 Glucose, 1.8 Urea, pH balanced to 7.6 with HCl; toadfish  $\text{Na}^+$ -free saline (in  $\text{mmol l}^{-1}$ ): 156.9 N-methyl-D-glucamine, 0.9  $\text{MgSO}_4$ , 0.46  $\text{K}_2\text{HPO}_4$ , 0.48  $\text{KH}_2\text{PO}_4$ , 5.0  $\text{KHCO}_3$ , 11.0 Hepes, 1.0  $\text{CaCl}_2$ , 3.0 Glucose, 7.0 Urea, pH balanced to 7.6 with HCl), ensuring that the  $\text{Na}^+$ -free solution on the mucosal side had the same concentration of urea and the same osmolality as the serosal bath. The effect of temperature was examined by altering the incubation temperature of separate groups of bladders. The effect of a concentration gradient was investigated by placing the bladder in a serosal bath with the urea replaced by NaCl, ensuring the same osmolality on both sides of the bladder.

The handling of thiourea and acetamide by the urinary bladder of trout and toadfish was evaluated by replacing the cold and radio-labeled urea (1.8  $\text{mmol l}^{-1}$  in trout, 7.0  $\text{mmol l}^{-1}$  in toadfish plus 1  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]-urea) in the saline with analogue (thiourea or acetamide) at the same concentration plus 1  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]-thiourea (19.8  $\text{mCi mmol}^{-1}$ ; Sigma, Aldrich, Canada) or [ $^{14}\text{C}$ ]-acetamide (50  $\text{mCi mmol}^{-1}$ ; American Radioactive Chemicals, St. Louis, MO).

Net urea flux ( $J_{\text{net urea}}$ ) was measured following a procedure similar to that outlined by Hirano et al., ('73) where  $J_{\text{net urea}}$  was calculated from the difference in initial and final mucosal urea concentrations and mucosal saline volume. Unidirectional urea flux ( $J_{\text{in urea}}$ ) in the direction of reabsorption was measured following a protocol by Eggena ('73)

and Levine et al., ('73a,b). Briefly, serosal samples were taken at 0.5 hr intervals throughout the incubation period and monitored for the appearance of [<sup>14</sup>C]-urea. Unidirectional analogue flux rate was monitored in a similar fashion in experiments using [<sup>14</sup>C]-acetamide or [<sup>14</sup>C]-thiourea. Net water movement was measured gravimetrically by weighing the bladders (after blotting away excess fluid) at the beginning and end of the 3 hr–4 hr experiments.

For measurements of unidirectional urea flux ( $J_{\text{out urea}}$ ) in the direction of secretion, 2  $\mu\text{Ci}$  of [<sup>14</sup>C]-urea was placed in the serosal bath and initial and final mucosal samples were taken to monitor the appearance of [<sup>14</sup>C]-urea at the inner surface.

#### Analytical techniques and calculations

Urea concentrations in serosal and mucosal solutions were measured using the diacetyl monoxime method of Rahmatullah and Boyde ('80) with appropriate adjustments of reagent strength for the different urea concentration ranges. For measurements of [<sup>14</sup>C]-urea, [<sup>14</sup>C]-thiourea, or [<sup>14</sup>C]-acetamide, samples (25  $\mu\text{l}$ ) were added to 4 ml of Ecolume fluor and analyzed by  $\beta$ -scintillation counting (LKB Rackbeta 1217 counter). Tests demonstrated that quench was constant because the samples were counted in the same matrix, so cps were used directly in all calculations. Bladder surface area was determined by making a ventral cut along the bladder, spreading it on graph paper, and tracing it carefully. Osmolality was determined using a Wescor Inc. 5100C Vapor Pressure Osmometer.  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations were measured using a Varian 1275 atomic absorption spectrophotometer and a Radiometer CMT10 chloridometer, respectively. Glucose was measured enzymatically (hexokinase, glucose-6-phosphate dehydrogenase) using a commercial kit (Sigma).

The concentration ratio of  $X$  ( $\text{CR}_X$ ) was calculated from experiments where [<sup>14</sup>C]-urea was placed initially on both sides of the bladder in equal concentrations:

$$\text{CR}_X = \frac{[X]_m}{[X]_s}, \quad (1)$$

where  $[X]_m$  and  $[X]_s$  are the final mucosal and serosal concentrations of  $X$ , respectively, after 3–4 hours of incubation. A final ratio that was significantly less than 1.0 indicated net reabsorption independent of water movement, a ratio greater than 1.0 indicated net secretion independent of water movement, and a ratio equal to 1.0 indi-

cated no net transport independent of water movement. Only in calculating  $\text{CR}_{\text{urea}}$  was [<sup>14</sup>C]-urea used on both sides of the bladder for increased sensitivity.

Net flux rates of water, urea, and other substances were determined by an approach similar to that of Hirano et al., ('73). The net flux rate of water ( $J_{\text{net water}}$ ) in  $\text{ml cm}^{-2} \text{h}^{-1}$  for bladder sacs was calculated using:

$$J_{\text{net water}} = \frac{\Delta V}{A \times t}, \quad (2)$$

where  $\Delta V$  is the change in volume estimated by the change in weight between the beginning and end of the experiment in ml,  $A$  is the surface area of the urinary bladder in  $\text{cm}^2$ , and  $t$  is the time in hours.

The net flux rate of  $X$  ( $J_{\text{net } X}$ ) in  $\mu\text{mol cm}^{-2} \text{h}^{-1}$  in isolated bladder sacs was found by calculating the change in concentration of substance  $X$  before and after incubation with respect to mucosal volume as follows:

$$J_{\text{net } X} = \frac{([X]_i \times V_i) - ([X]_f \times V_f)}{A \times t}, \quad (3)$$

where  $[X]_i$  and  $[X]_f$  are the initial and final concentrations in  $\mu\text{mol ml}^{-1}$ ,  $V_i$  and  $V_f$  are the initial and final mucosal volumes in ml,  $A$  is the bladder surface area in  $\text{cm}^2$ , and  $t$  is the time in hours.

Preliminary tests showed that there were small but detectable changes in the internal specific activity (SA) that followed a simple exponential decline over the course of the experiment. Unidirectional flux rate of  $X$  ( $J_{\text{in } X}$ ) in the direction of reabsorption in  $\mu\text{mol cm}^{-2} \text{h}^{-1}$  in isolated bladder sacs was therefore determined by first calculating the specific activity (SA) on the mucosal side of the bladder at different times during the experiment. The initial and final SA could be calculated directly by dividing the measured amount of radioactivity (cpm) on the mucosal side at the beginning and end of the incubation period by the respective unlabelled  $X$  concentration at those two time periods.

$$\text{SA} = \frac{\text{cpm}_m}{[X]}, \quad (4)$$

where  $\text{cpm}_m$  is the radioactivity in  $\text{cpm ml}^{-1}$  measured on the mucosal side, and  $[X]$  is the concentration of unlabelled substance  $X$  on the mucosal side in  $\mu\text{mol ml}^{-1}$ . The mucosal SAs at intermediate times during the experiment (be-

tween the initial and final mucosal sampling periods when mucosal samples were not taken) were approximated by fitting an exponential regression against time to the initial and final mucosal SAs (Shaw, '59).

Unidirectional flux rate of  $X$  ( $J_{inX}$ ) in the direction of reabsorption in isolated bladder sacs for any period was then calculated as follows:

$$J_{inX} = \frac{(\text{cpm}_s^b \times V^b) - (\text{cpm}_s^a \times V^a)}{t \times \text{SA} \times A}, \quad (5)$$

where  $\text{cpm}_s^a$  and  $\text{cpm}_s^b$  are the radioactivities per ml measured at time 1 and time 2, respectively, in serosal samples;  $V^a$  and  $V^b$  are the volumes of the serosal bath at times 1 and 2 (taking removed sample volumes into account) in ml;  $t$  is the time between samples in hours; SA is the specific activity in  $\text{cpm} \mu\text{mol}^{-1}$  at that given sample period; and  $A$  is the bladder surface area in  $\text{cm}^2$ .

The permeability ( $P$ ) of the bladder to a substance  $X$  in  $\text{cm s}^{-1}$  was calculated using the following equation:

$$P_X = \frac{\Delta \text{cpm}_s \times V_s}{\text{cpm}_m \times T \times A}, \quad (6)$$

where  $\Delta \text{cpm}_s$  is the change of radioactivity between two successive samples measured on the serosal side (cold side) in  $\text{cpm ml}^{-1}$ ,  $\text{cpm}_m$  is the mean radioactivity measured on the mucosal side (hot side) in  $\text{cpm ml}^{-1}$ ,  $V_s$  is the volume in ml (or  $\text{cm}^3$ ) on the serosal side,  $T$  is the time between the two samples in seconds, and  $A$  is the bladder surface area in  $\text{cm}^2$ .

The transient urea concentration ( $T_{\text{urea}}$ ) in  $\text{mmol l}^{-1}$  was calculated by dividing  $J_{\text{net urea}}$  in  $\mu\text{mol cm}^{-2} \text{h}^{-1}$  by  $J_{\text{net water}}$  in  $\text{ml cm}^{-2} \text{h}^{-1}$  at a given sample period as follows:

$$T_{\text{urea}} = \frac{J_{\text{net urea}}}{J_{\text{net water}}}. \quad (7)$$

The  $Q_{10}$  factor is defined as the ratio of two rates for a  $10^\circ\text{C}$  difference in temperature and was calculated as follows:

$$Q_{10} = \frac{J_{K2}^{(10/(K2-K1))}}{J_{K1}}, \quad (8)$$

where  $J_{K1}$  and  $J_{K2}$  are flux rates at a low and high temperature, respectively, in  $\mu\text{mol cm}^{-2} \text{h}^{-1}$  and  $K1$  and  $K2$  are the low and high temperatures, respectively, in  $^\circ\text{C}$ . The  $Q_{10}$  factor for a physical

process such as diffusion is about 1.  $Q_{10}$  factors for biochemical reactions including carrier-mediated transport are typically 2 to 3 (Withers, '92).

An Arrhenius plot displays the natural logarithm of transport rate versus the inverse of temperature in degrees Kelvin ( $\text{K}^{-1}$ ), the slope of which is equal to:

$$m = \frac{-E_a}{R}, \quad (9)$$

where  $m$  is the slope of the line,  $E_a$  is the activation energy in  $\text{J mol}^{-1}$ , and  $R$  is the gas constant ( $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ ). Using this equation, the activation energy of a biological system can be calculated.

### Statistical analysis

Data have been reported as means  $\pm$  SEM ( $N$  = number of bladders). 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 difference between means was evaluated using Student's paired, unpaired, or one-sample two-tailed  $t$ -test ( $P < 0.05$ ) as appropriate, with the Bonferroni correction (Nemenyi et al., '77) for multiple comparisons.

## RESULTS

### Rainbow trout

Under control conditions, the isolated urinary bladder of the rainbow trout incubated in vitro demonstrated net flux ( $J_{\text{net}}$ ; positive values signifying reabsorption) of urea ( $0.019 \pm 0.003$  (39)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) and water ( $0.012 \pm 0.002$  (39)  $\text{ml}^{-2} \text{h}^{-1}$ );  $J_{\text{net urea}}$  and  $J_{\text{net water}}$  were both significantly different from zero (Fig. 1A). Unidirectional urea flux ( $J_{\text{in urea}}$ ;  $0.098 \pm 0.029$  (39)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) from the bladder was fivefold greater than simultaneous  $J_{\text{net urea}}$  ( $0.019 \pm 0.003$  (39)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ), indicating a substantial backflux component to urea movement. Similar to observations in vivo, the isolated bladder demonstrated substantial net reabsorption of  $\text{Na}^+$  ( $2.17 \pm 0.17$  (20)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ),  $\text{Cl}^-$  ( $1.92 \pm 0.28$  (11)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) and glucose ( $0.71 \pm 0.26$  (8)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ; Fig. 1B). Calculated concentration ratios for urea ( $0.98 \pm 0.01$  (6)) and glucose ( $0.91 \pm 0.07$  (6)) at the end of the 3–4 hr flux periods were not significantly different than 1.0, indicating that there was no net movement of these two substances independent of water movement under symmetrical conditions (Fig. 1C). In contrast, the final concentration ratios for both

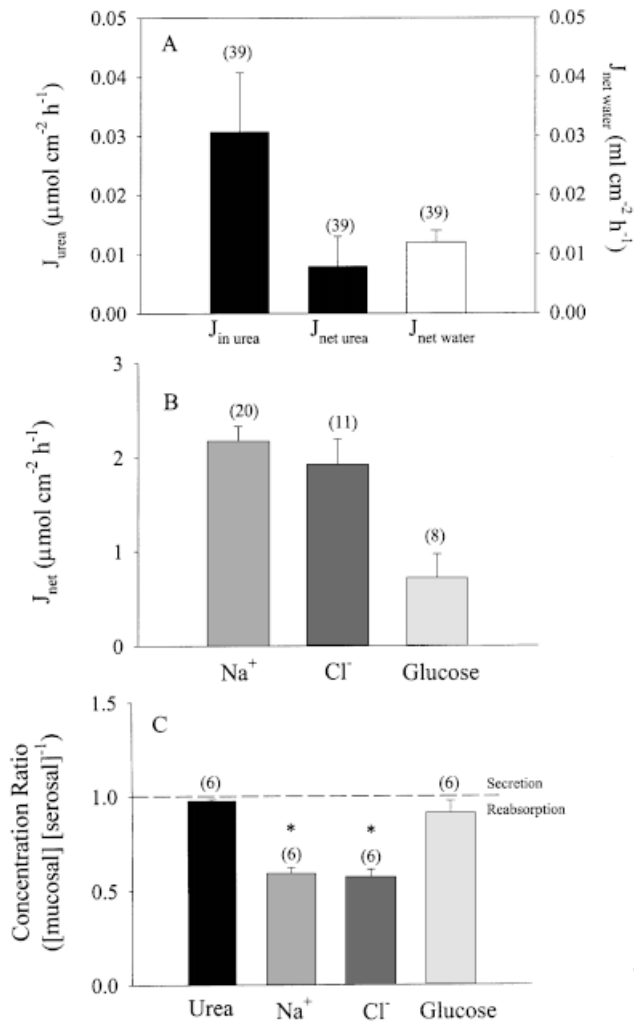


Fig. 1. Control rates for (A) unidirectional ( $J_{\text{in urea}}$ ) and net fluxes of urea ( $J_{\text{net urea}}$ ) and water ( $J_{\text{net water}}$ ) and (B) Na<sup>+</sup>, Cl<sup>-</sup> and glucose in isolated bladders of the rainbow trout, showing unidirectional flux of urea in the direction of reabsorption plus significant net reabsorption of urea, water, Na<sup>+</sup>, Cl<sup>-</sup> and glucose. (C) Final concentration ratios at the end of 3hr–4hr flux experiments indicate a significant reabsorption of Na<sup>+</sup> and Cl<sup>-</sup> independent of water movement but not of urea or glucose. Values are means  $\pm$  1 SEM (N); \*  $P < 0.05$  significantly different than 1.0.

Na<sup>+</sup> ( $0.59 \pm 0.04$  (6)) and Cl<sup>-</sup> ( $0.57 \pm 0.05$  (6)) were significantly less than 1.0 ( $P < 0.05$ ), indicating a significant reabsorption that is independent of water movement.

The average urea permeability of the sample population of control bladders was  $10.8 \pm 0.8$  (40)  $\times 10^{-6} \text{cm s}^{-1}$ . This permeability is threefold higher than the theoretical permeability of urea through artificial lipid bilayers ( $3.7 \times 0.6$  (6)  $\times 10^{-6} \text{cm s}^{-1}$ ; Galluci et al., '71) and suggests the presence of a urea transport mechanism in the bladder of the rainbow trout.

Despite this higher permeability, none of the pharmacological agents traditionally used to characterize urea transport mechanisms had any significant effect on  $J_{\text{in urea}}$ ,  $J_{\text{net urea}}$ , or  $J_{\text{net water}}$  (Table 1). The pharmacological agents used in this study were placed on both sides of the bladder and can be grouped into three categories. The first category consisted of urea analogues (thiourea; 10 mmol l<sup>-1</sup> and acetamide; 10 mmol l<sup>-1</sup>) in fivefold to sixfold excess of urea concentrations. The second category contained potential urea transport blockers (amiloride; 0.1 mmol l<sup>-1</sup> and phloretin; 0.1 mmol l<sup>-1</sup>), and the third category consisted of a hormone (AVT; 10<sup>-9</sup> mol l<sup>-1</sup>) that could potentially stimulate facilitated diffusion of urea. The effect of AVT when urea movement was being "inhibited" by the presence of thiourea was also tested. None of the treatments had significant effects.

In agreement with the lack of amiloride influence, there was also no change in  $J_{\text{in urea}}$ ,  $J_{\text{net urea}}$ , or  $J_{\text{net water}}$  when Na<sup>+</sup> was removed from the mucosal side of the isolated bladder, indicating that urea movement is not dependent on Na<sup>+</sup> (Table 1).

Isolated bladders from fish acclimated to 11°C–14°C appeared to have a range of homeostasis for the transport of urea and water with respect to acute changes in temperature, with  $J_{\text{in urea}}$ ,  $J_{\text{net urea}}$ , and  $J_{\text{net water}}$  remaining relatively unchanged from 5°C–20°C (Fig. 2). However, the bladders were sensitive to extreme temperatures, showing a significant decrease in  $J_{\text{in urea}}$  when exposed to 0°C ( $0.049 \pm 0.005$  (6)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) and a significant increase in  $J_{\text{in urea}}$  when exposed to 30°C ( $0.150 \pm 0.015$  (6)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) when compared to  $J_{\text{in urea}}$  at control temperatures ( $0.080 \pm 0.011$  (6)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ; Fig. 2A). The  $Q_{10}$  from 0°C to 5°C was 2.4 and the  $Q_{10}$  from 20°C to 30°C was only 1.5. An Arrhenius plot of these data showed a prolonged phase transition with a lowered  $E_a$  of 0.4 KJ mol<sup>-1</sup> between 5°C and 14°C (Fig. 2B). A similar trend in temperature sensitivity was also observed in measured  $J_{\text{net urea}}$  and  $J_{\text{net water}}$  values but was only significant for fluxes at 30°C (Fig. 2C,D).

Isolated bladders exposed to a 1.8 mmol l<sup>-1</sup> urea concentration gradient achieved by removing urea on the serosal side showed a significantly greater  $J_{\text{net urea}}$  ( $0.054 \pm 0.009$  (6)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) compared to controls ( $0.011 \pm 0.003$  (6)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ; Fig. 3A). There was no significant change in  $J_{\text{in urea}}$ . The gradient conditions essentially removed the backflux component of urea movement (urea was unavailable on the serosal side to move back into the bladder), consequently  $J_{\text{in urea}}$  ( $0.052 \pm 0.011$  (6)  $\mu\text{mol cm}^{-2} \text{h}^{-1}$ ) and  $J_{\text{net urea}}$  ( $0.054 \pm 0.009$  (6)

TABLE 1. The influence of various pharmacological treatments on urea flux rates in *in vitro* urinary bladder sac experiments from the rainbow trout ( $J_{in}$  unidirectional flux in the direction of reabsorption;  $J_{net}$  net flux; positive values signify reabsorption from the bladder; values are means  $\pm$  1 SEM (N))

Experiment	$J_{in\ urea}$ ( $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ )	$J_{net\ urea}$ ( $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ )	$J_{net\ water}$ ( $\text{ml cm}^{-2}\ \text{h}^{-1}$ )
Control (6)	0.087 $\pm$ 0.010	0.048 $\pm$ 0.009	0.030 $\pm$ 0.006
Thiourea (6)	0.072 $\pm$ 0.014	0.027 $\pm$ 0.004	0.014 $\pm$ 0.002
Control (9)	0.127 $\pm$ 0.023	0.009 $\pm$ 0.003	0.007 $\pm$ 0.001
Acetamide (6)	0.098 $\pm$ 0.016	0.011 $\pm$ 0.005	0.006 $\pm$ 0.003
Control (6)	0.077 $\pm$ 0.020	0.011 $\pm$ 0.003	0.007 $\pm$ 0.001
Amiloride <sup>†</sup> (6)	0.099 $\pm$ 0.017	0.020 $\pm$ 0.008	0.011 $\pm$ 0.004
Control (6)	0.193 $\pm$ 0.031	0.026 $\pm$ 0.014	0.014 $\pm$ 0.006
Phloretin <sup>‡</sup> (6)	0.142 $\pm$ 0.018	0.015 $\pm$ 0.004	0.009 $\pm$ 0.001
Control (6)	0.056 $\pm$ 0.016	0.015 $\pm$ 0.005	0.008 $\pm$ 0.002
AVT (6)	0.058 $\pm$ 0.011	0.014 $\pm$ 0.004	0.007 $\pm$ 0.002
Control (6)	0.056 $\pm$ 0.016	0.015 $\pm$ 0.005	0.008 $\pm$ 0.002
AVT + Thiourea (6)	0.053 $\pm$ 0.007	0.009 $\pm$ 0.002	0.006 $\pm$ 0.001
Control (6)	0.031 $\pm$ 0.010	0.008 $\pm$ 0.005	0.120 $\pm$ 0.002
Na <sup>+</sup> -free (6)	0.041 $\pm$ 0.010	0.011 $\pm$ 0.002	0.007 $\pm$ 0.002

<sup>†</sup>DMSO alone had no effect on  $J_{in\ urea}$ ,  $J_{net\ urea}$ , or  $J_{net\ water}$ .

<sup>‡</sup>Ethanol alone had no effect on  $J_{in\ urea}$ ,  $J_{net\ urea}$ , or  $J_{net\ water}$ .

Urea concentration in all experiments was 1.8 mmol l<sup>-1</sup>.

There was no significant difference ( $P > 0.05$ ) between experimental and simultaneous control treatments.

$\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ), although measured independently, became equal. There was no effect on the movement of water.

The "transient urea concentration" ( $6.47 \pm 1.04$  (6) mmol l<sup>-1</sup>) of these gradient-exposed bladders was threefold greater than the concentration of urea measured initially in the mucosal solution ( $2.11 \pm 0.02$  (6) mmol l<sup>-1</sup>; Fig. 3B). This difference in urea concentration between the "transient solution" and the mucosal solution was not evident in isolated bladders from any other treatment group (Fig. 3B).

In support of carrier-mediated transport, when radiolabelled analogues were all tested at the same concentration (1.8 mmol l<sup>-1</sup>) in separate experiments, the urinary bladder preferentially transported urea over both acetamide and thiourea.  $J_{in\ urea}$  ( $0.098 \pm 0.029$  (39)  $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ) was significantly greater than  $J_{in\ acetamide}$  ( $0.030 \pm 0.007$  (6)  $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ) and to an even greater extent  $J_{in\ thiourea}$  ( $0.014 \pm 0.003$  (6)  $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ; Fig. 4). Correspondingly, the permeability of the bladder to urea ( $P_{urea}$ ;  $10.8 \pm 0.8$  (40)  $\times 10^{-6}\ \text{cm s}^{-1}$ ) was 2.5-fold greater than  $P_{acetamide}$  ( $4.3 \pm 0.9$  (6)  $\times 10^{-6}\ \text{cm s}^{-1}$ ) and 3.4-fold greater than  $P_{thiourea}$  ( $2.3 \pm 0.5$  (6)  $\times 10^{-6}\ \text{cm s}^{-1}$ ).

### Gulf toadfish

Under control conditions, the isolated urinary bladder of the gulf toadfish demonstrated unidirectional urea flux rates ( $J_{in\ urea}$ ; positive values signifying reabsorption) of  $0.125 \pm 0.020$  (33)  $\mu\text{mol}$

$\text{cm}^{-2}\ \text{h}^{-1}$ , which were much greater than net urea flux rates ( $J_{net\ urea}$ ;  $-0.039 \pm 0.020$  (32)  $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ; Fig. 5A). In most cases,  $J_{net\ urea}$  was not significantly different than zero, indicating that the backflux component to urea movement was equal to  $J_{in\ urea}$ .  $J_{net\ water}$  ( $-0.003 \pm 0.002$  (32)  $\text{ml cm}^{-2}\ \text{h}^{-1}$ ) was not significantly different from zero. Similar to observations *in vivo*, the isolated bladder demonstrated net reabsorption of Na<sup>+</sup> ( $0.75 \pm 0.33$  (20)  $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ) and Cl<sup>-</sup> ( $0.43 \pm 0.21$  (19)  $\mu\text{mol cm}^{-2}\ \text{h}^{-1}$ ), although the latter was not significantly different than zero (Fig. 5B). Final concentration ratios of urea ( $0.98 \pm 0.01$  (8)), Na<sup>+</sup> ( $1.04 \pm 0.07$  (8)) and Cl<sup>-</sup> ( $1.08 \pm 0.04$  (8)) were not significantly different than 1.0 indicating that there was no net movement of these three substances under symmetrical conditions independent of the movement of water (Fig. 5C).

The average urea permeability of a sample population of control toadfish bladders was  $8.0 \pm 1.2$  (34)  $\text{cm s}^{-1} \times 10^{-6}$ , which was not significantly different from the value for the trout bladder. This measured permeability is 2.2-fold greater than the theoretical permeability of urea through artificial lipid bilayers ( $3.7 \pm 0.6$  (6)  $\times 10^{-6}\ \text{cm s}^{-1}$ ; Galluci et al., '71), which again may suggest carrier-mediated transport of urea by the urinary bladder.

However, similar to the trout bladder, pharmacological agents traditionally used to characterize urea transport mechanisms did not affect  $J_{in\ urea}$ ,  $J_{net\ urea}$ , or  $J_{net\ water}$  in the bladder of the gulf toad-

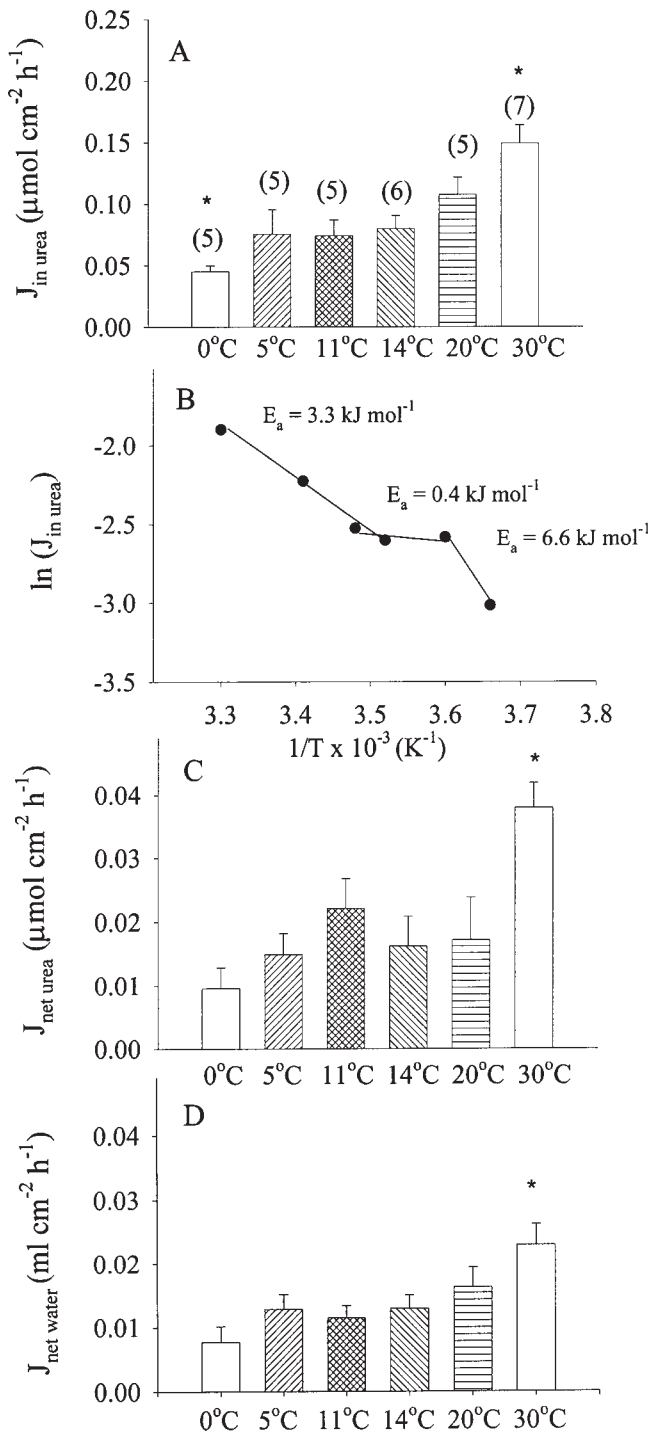


Fig. 2. Flux rates of isolated bladders of the rainbow trout acclimated to 11°C–14°C and then acutely exposed to different temperatures. A significant decrease in (A)  $J_{in\ urea}$ , (C)  $J_{net\ urea}$ , and (D)  $J_{net\ water}$  at 0°C and an increase at 30°C is evident. A lateral phase transition is evident in the Arrhenius plot (B). Values are means  $\pm$  1 SEM (N); \* $P$  < 0.05 significantly different from rates at temperature of acclimation.

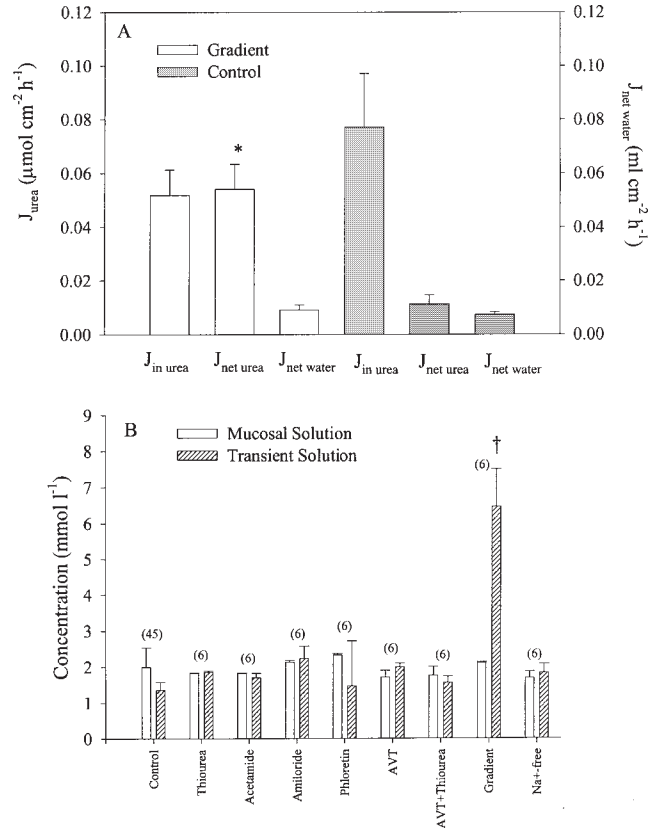


Fig. 3. (A) Flux rates of urinary bladders of rainbow trout exposed to a 1.8 mmol l<sup>-1</sup> urea concentration gradient, from mucosal to serosal surface (urea-free) showing a significant increase in  $J_{net\ urea}$  relative to controls. No increase in  $J_{net\ water}$  is apparent. (B) There is a significant difference between the urea concentration in the initial mucosal solution and that in the transient solution in bladders exposed to a gradient compared to those in all other treatment groups. Values are means  $\pm$  1 SEM (N = 6); \* $P$  < 0.05 significantly different from control flux rates; † $P$  < 0.05 significantly different from mucosal urea concentrations.

fish (Table 2). Again, three main treatments were used. A urea analogue (thiourea; 20 mmol l<sup>-1</sup>) in three-fold excess of urea concentrations, a urea transport blocker (phloretin 0.250 mmol l<sup>-1</sup>), and the hormone AVT (10<sup>-9</sup> mol l<sup>-1</sup>) were investigated. All pharmacological agents were placed on both sides of the bladder. Furthermore, removing Na<sup>+</sup> from the mucosal side of the bladder had no effect on  $J_{in\ urea}$  (0.122  $\pm$  0.038 (13)  $\mu$ mol cm<sup>-2</sup> h<sup>-1</sup>) when compared to controls (0.101  $\pm$  0.037 (13)  $\mu$ mol cm<sup>-2</sup> h<sup>-1</sup>). Na<sup>+</sup>-free conditions did not affect either  $J_{net\ urea}$  or  $J_{net\ water}$ .

When urea was removed from the serosal side of six bladders, creating a urea concentration gradient of approximately 7 mmol l<sup>-1</sup>, there was no effect on  $J_{in\ urea}$  (0.244  $\pm$  0.051 (6)  $\mu$ mol cm<sup>-2</sup> h<sup>-1</sup>)



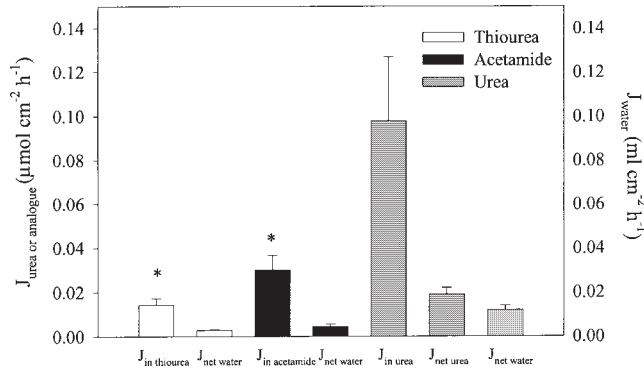


Fig. 4. Measurements of urea ( $1.8 \text{ mmol l}^{-1}$ ), thiourea ( $1.8 \text{ mmol l}^{-1}$ ) and acetamide ( $1.8 \text{ mmol l}^{-1}$ ) unidirectional flux rates and accompanying net water flux rates by the isolated urinary bladder of the rainbow trout. The urinary bladder appears to differentiate between urea and both thiourea and acetamide. Values are means  $\pm$  1 SEM ( $N = 6$  for thiourea and acetamide groups,  $N = 39$  for urea); \*  $P < 0.05$  significantly different from urea flux rate.

when compared to simultaneous controls ( $0.143 \pm 0.066$  (7)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ). However, there was a significant increase in  $J_{net \text{ urea}}$  ( $0.252 \pm 0.102$  (6)  $\text{mmoles cm}^{-2} \text{ h}^{-1}$ ) compared to controls ( $0.004 \pm 0.009$  (7)  $\text{mmoles cm}^{-2} \text{ h}^{-1}$ ). Note that as in trout,  $J_{net \text{ urea}}$  in gradient exposed bladders became essentially equal to  $J_{in \text{ urea}}$ . There was no effect on the movement of water. Consistent with the variability of  $J_{net \text{ urea}}$  and  $J_{net \text{ water}}$  measured in both experimental and control toadfish bladders, the calculated concentrations of urea in the transient solution were also exceedingly variable. For that reason, no significant differences were observed between urea concentrations in the transient fluid (overall control means:  $10.19 \pm 6.12$  (26)  $\text{mmol l}^{-1}$ ) versus those in the initial mucosal solution (control:  $7.07 \pm 0.13$  (27)  $\text{mmol l}^{-1}$ ) in any of the experimental treatments, including the gradient experiment (data not shown).

Isolated bladders from toadfish acclimated to  $26^\circ\text{C}$  were sensitive to temperatures both above and below the temperature of acclimation (Fig. 6A). Bladders exposed to  $15^\circ\text{C}$  and  $20^\circ\text{C}$  showed a increase in  $J_{in \text{ urea}}$  ( $0.458 \pm 0.083$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$  and  $0.254 \pm 0.004$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ , respectively) compared to control bladders incubated at  $26^\circ\text{C}$  ( $0.126 \pm 0.021$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ). Bladders incubated at  $30^\circ\text{C}$  demonstrated an elevated  $J_{in \text{ urea}}$  ( $0.239 \pm 0.035$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ) compared to controls by a  $Q_{10}$  factor of 4.6. In addition, bladders incubated at  $35^\circ\text{C}$  had a greatly elevated  $J_{in \text{ urea}}$  ( $1.690 \pm 0.533$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ), greater than control values by a  $Q_{10}$  factor of 17.3. An Arrhenius

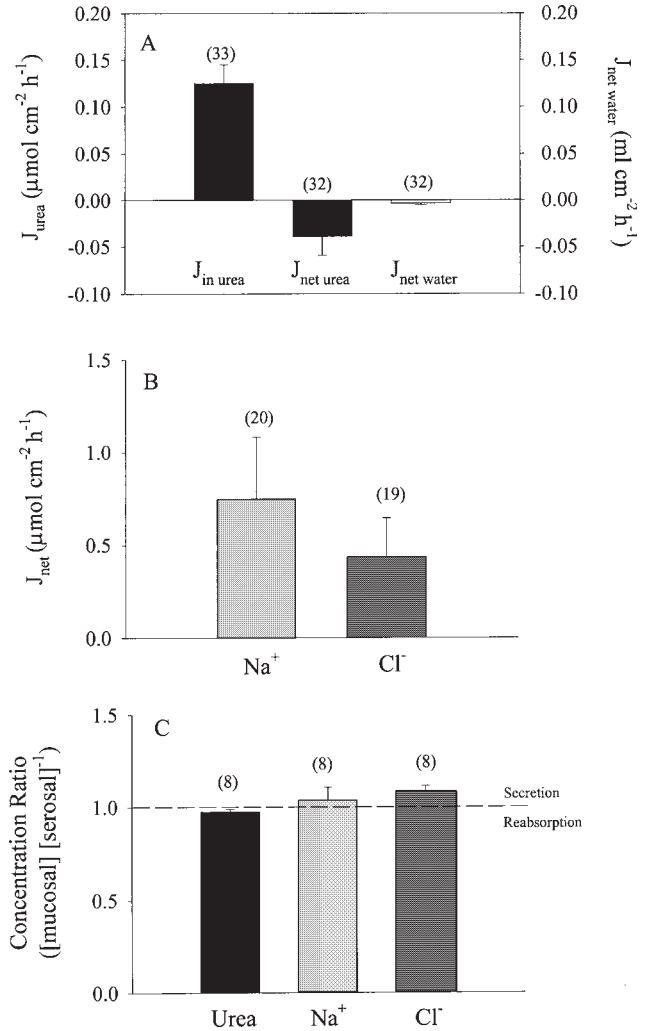


Fig. 5. Control rates for (A) unidirectional ( $J_{in \text{ urea}}$ ) and net fluxes of urea ( $J_{net \text{ urea}}$ ) and water ( $J_{net \text{ water}}$ ) and (B)  $\text{Na}^+$  and  $\text{Cl}^-$  ( $J_{net \text{ ions}}$ ) in isolated bladders of the gulf toadfish showing significant unidirectional flux of urea and net flux of  $\text{Na}^+$  in the direction of reabsorption but values of  $J_{net \text{ urea}}$ ,  $J_{net \text{ water}}$ , and  $J_{net \text{ Cl}^-}$  that are not significantly different from zero. (C) Final concentration ratios at the end of 3 hr–4 hr flux experiments indicate that there is no significant movement of urea,  $\text{Cl}^-$  or  $\text{Na}^+$  independent of the movement of water. Values are means  $\pm$  1 SEM (N).

plot of these data showed a sharp phase transition at  $26^\circ\text{C}$  (Fig. 6B). The same trend in temperature sensitivity is evident in  $J_{net \text{ urea}}$ ; however, these observations were not significant (Fig. 6C,D).

When serosal to mucosal urea flux ( $J_{out \text{ urea}}$ ) was examined in a separate series, measured  $J_{out \text{ urea}}$  ( $-0.041 \pm 0.008$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ) was not significantly different than  $J_{in \text{ urea}}$  ( $0.146 \pm 0.063$  (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ), emphasizing zero net urea movement by the urinary bladder of the toadfish.

In contrast to the trout urinary bladder, the

TABLE 2. The influence of various pharmacological treatments on urea flux rates in *in vitro* urinary bladder sac experiments using gulf toadfish ( $J_{in}$  unidirectional flux in the direction of reabsorption;  $J_{net}$ : net flux; positive values signify reabsorption from the bladder; negative values signify secretion into the bladder; values are means  $\pm$  1 SEM (N))

Experiment	$J_{in \text{ urea}}$ ( $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ )	$J_{net \text{ urea}}$ ( $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ )	$J_{net \text{ water}}$ ( $\text{ml cm}^{-2} \text{ h}^{-1}$ )
Control (6)	0.192 $\pm$ 0.028	0.127 $\pm$ 0.085	0.011 $\pm$ 0.006
Thiourea (5)	0.203 $\pm$ 0.072	-0.048 $\pm$ 0.061	-0.008 $\pm$ 0.009
Control (6)	0.192 $\pm$ 0.028	0.127 $\pm$ 0.085	0.011 $\pm$ 0.006
Phloretin <sup>‡</sup> (6)	0.159 $\pm$ 0.056	0.009 $\pm$ 0.020	0.002 $\pm$ 0.001
Control (8)	0.097 $\pm$ 0.023	-0.015 $\pm$ 0.011	-0.001 $\pm$ 0.001
AVT (12)	0.186 $\pm$ 0.058	0.032 $\pm$ 0.039	0.002 $\pm$ 0.001
Control (7)	0.143 $\pm$ 0.066	0.004 $\pm$ 0.009	0.005 $\pm$ 0.001
Gradient (6)	0.244 $\pm$ 0.051	0.252 $\pm$ 0.102	0.006 $\pm$ 0.002
Control (13)	0.101 $\pm$ 0.037	-0.001 $\pm$ 0.010	0.002 $\pm$ 0.001
Na <sup>+</sup> -free (13)	0.122 $\pm$ 0.038	0.021 $\pm$ 0.024	0.004 $\pm$ 0.004

<sup>‡</sup>DMSO alone had no effect on  $J_{in \text{ urea}}$ ,  $J_{net \text{ urea}}$ , or  $J_{net \text{ water}}$ .

Urea concentration in all experiments was 7 mmol l<sup>-1</sup>.

There was no significant difference ( $P > 0.05$ ) between experimental and simultaneous control treatments.

toadfish urinary bladder sac did not differentiate between urea, thiourea and acetamide.  $J_{in \text{ urea}}$  (0.125  $\pm$  0.020 (33)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ) was not significantly different than either  $J_{in \text{ thiourea}}$  (0.131  $\pm$  0.036 (6)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ) or  $J_{in \text{ acetamide}}$  (0.210  $\pm$  0.075 (7)  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ; Fig. 7). Likewise,  $P_{\text{urea}}$  (8.0  $\pm$  1.2 (34)  $\times 10^{-6} \text{ cm s}^{-1}$ ) was not significantly different than  $P_{\text{thiourea}}$  (5.8  $\pm$  1.3 (6)  $\times 10^{-6} \text{ cm s}^{-1}$ ) nor  $P_{\text{acetamide}}$  (6.8  $\pm$  2.1 (7)  $\times 10^{-6} \text{ cm s}^{-1}$ ).

## DISCUSSION

The objectives of this study were to investigate the transport physiology of the urinary bladder in the gulf toadfish and rainbow trout and to determine whether the urinary bladder would make a suitable model for renal urea handling in these two fish. Our results suggest the presence of a urea transport mechanism in the urinary bladder of the rainbow trout but in not the gulf toadfish and indicate that the urinary bladders of neither fish are suitable models for the handling of urea by the kidney. The bladder of the trout shows a net reabsorption of urea when exposed to symmetrical conditions, i.e., in the absence of osmotic or electrochemical gradients. In addition, when the backflux component was eliminated, it was apparent that urea flux was not associated with water flux; the urea concentration in fluid leaving the bladder ("the transient concentration") was three-fold more concentrated than the initial mucosal solution. Thus, urea reabsorption is not a consequence of solvent drag. Furthermore, the trout urinary bladder demonstrates an ability to differentiate between urea and the urea analogues, thiourea and acetamide, showing a speci-

ficity towards urea transport,  $P_{\text{urea}}$  being greater than  $P_{\text{thiourea}}$  and  $P_{\text{acetamide}}$ .

The urinary bladder of the rainbow trout plays a significant role in salt and urea reabsorption *in vivo*; urinary excretion rates of Na<sup>+</sup>, Cl<sup>-</sup>, and urea are significantly lower for resting fish compared to those whose bladder function is by-passed by internal urinary catheterization (Curtis and Wood, '91). The net rates of reabsorption for Na<sup>+</sup> and Cl<sup>-</sup> determined *in vivo* by Curtis and Wood ('91) were 0.7–2.5  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ , thus comparable to the rates determined using the *in vitro* bladder sac preparation (1.9–2.2  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ) in the present study. However, the net rate of reabsorption of urea *in vivo* (approximately 0.2  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ) was approximately double the rate obtained *in vitro* (0.098  $\mu\text{mol cm}^{-2} \text{ h}^{-1}$ ). Despite this small inconsistency, it appears in the case of the trout that the *in vitro* bladder sac preparation functions similarly to the *in vivo* bladder.

Using this *in vitro* preparation, we determined that the urinary bladder probably does not function similarly to the kidney *in vivo* with respect to urea handling. Renal urea reabsorption measured in resting trout showed a linear proportional relationship to the reabsorption of Na<sup>+</sup> (slope = 0.011  $\mu\text{mol-urea kg}^{-1} \text{ h}^{-1}/\mu\text{mol-Na}^+ \text{ kg}^{-1} \text{ h}^{-1}$ ) that is well correlated ( $r^2 = 0.76$ ; unpublished data from the study of McDonald and Wood, '98). These data suggest that a Na<sup>+</sup>-coupled transport mechanism may exist in the kidney of the rainbow trout, similar to that observed in mammalian inner medullary collecting ducts and elasmobranch kidney tubules (Schmidt-Neilsen et al., '72; Isozaki et al., '94a,b). In contrast, reabsorption of urea by the

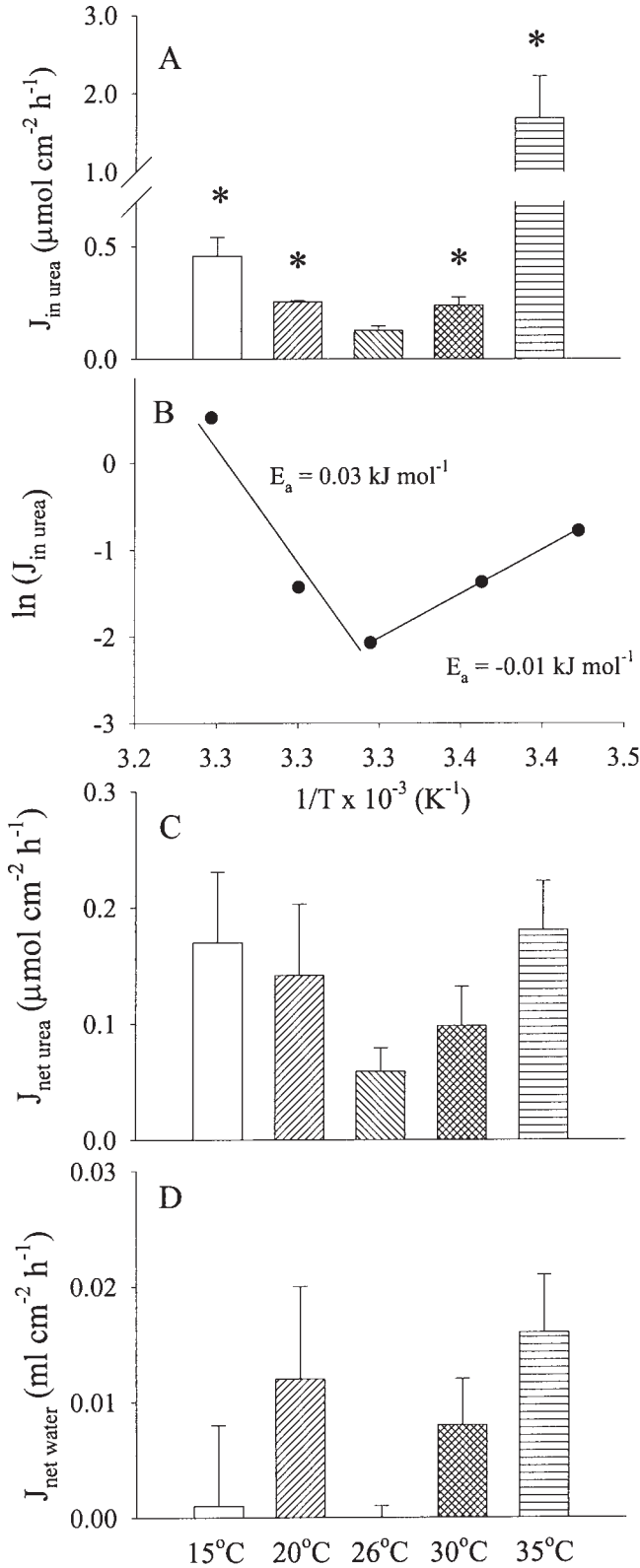


Fig. 6. Flux rates of isolated urinary bladders of the gulf toadfish acclimated to 26°C and then acutely exposed to different temperatures. A significant increase in (A)  $J_{in\ urea}$  is evident with temperatures above and below the temperature of acclimation. A similar trend, though not significant, is also

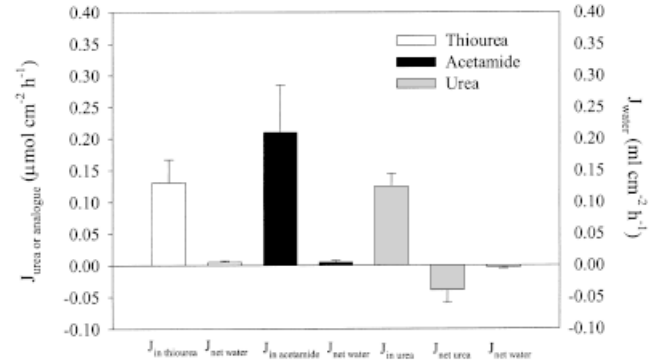


Fig. 7. Measurements of urea (7 mmol l<sup>-1</sup>), thiourea (7 mmol l<sup>-1</sup>) and acetamide (7 mmol l<sup>-1</sup>) unidirectional flux rates, and accompanying net water flux rates by the isolated urinary bladder of the gulf toadfish. The urinary bladder does not appear to differentiate between the three substances. Values are means  $\pm$  1 SEM (N = 6 for thiourea and acetamide groups, N = 33 for urea), no significant differences ( $P > 0.05$ ).

in vitro bladder sac was not affected when Na<sup>+</sup> was removed from the mucosal bath (Table 1). Thus, unlike the kidney, there is no correlation between urea and Na<sup>+</sup> movement through the urinary bladder, thereby eliminating the possibility that urea transport is Na<sup>+</sup>-coupled in this organ.

The measured permeability of the trout bladder for urea ( $10.8 \pm 0.8\ \text{cm s}^{-1} \times 10^{-6}$ ) was three-fold higher than that observed in artificial lipid bilayers that lack transport proteins to facilitate urea movement (Galluci et al., '71). The permeability of urea through human red blood cell membranes ( $1.2 \times 10^{-3}\ \text{cm s}^{-1}$ ; RBC), in which a UT-B transporter is present, is approximately 1,000-fold higher than that observed in artificial membranes (Mayrand and Levitt, '83). However, the permeability of urea through vasopressin-stimulated, UT-A1 transport mechanisms is generally much lower than that measured in RBCs. In fact, the urinary bladders of amphibians, under control conditions, have measured permeabilities for urea ranging from  $7.7 \times 10^{-6}\ \text{cm s}^{-1}$  to  $12.7 \times 10^{-6}\ \text{cm s}^{-1}$ , in the same range as the current measurements for the trout bladder and at most threefold greater than membranes without transport proteins (Levine et al., '73a,b; Eggena, '73; Levine and Worthington, '76; Sphun and Katz; '90). In addition, only a 2.5-fold rise in permeability has been noted in *Xenopus* oocytes injected with the branchial facilitated diffusion urea transporter (a UT-

apparent in (C)  $J_{net\ urea}$  and (D)  $J_{net\ water}$  values. A sharp phase transition is evident in the Arrhenius plot (B). Values are means  $\pm$  1 SEM (N = 6); \* $P < 0.05$  significantly different from rates at temperature of acclimation.

A type transporter) of the Lake Magadi tilapia (mUT; Walsh et al., 2001). Therefore, the measured permeability of the trout urinary bladder for urea is comparable with permeabilities observed in other systems when UT-A type transporters are present.

In contrast to the transporter of the amphibian bladder, the transporter believed to be present in the trout bladder is not AVT-sensitive. Then again, not all facilitated diffusion mechanisms for urea demonstrate AVT sensitivity. Although UT-A1 transporters (4.0 kDa mRNA) are stimulated by ADH (vasopressin in mammals, vasotocin in amphibians) and cAMP, UT-A2 transporters (2.9 kDa mRNA) are not (You et al., '93; Smith et al., '95; Shayakul et al., '96). In addition, UT-A2 transport mechanisms are less sensitive to inhibition by urea analogues (Chou et al., '90; You et al., '93). The urinary bladder of the trout appeared not to be sensitive to urea analogues, even at analogue concentrations that were 5× greater than urea concentrations. To date, urea transport mechanisms in teleost fish are not well defined. Although UT transporters in teleosts demonstrate a high amino acid sequence homology to mammalian and amphibian UT transporters (> 60%; Walsh et al., 2000, 2001), they show unconventional sensitivity and regulation patterns and may be controlled by entirely different mechanisms than UT transporters in other systems (Wood et al., 2001).

The trout urinary bladder shows a differential permeability toward urea, acetamide and thiourea,  $P_{\text{urea}}$  being 2.5-fold greater than  $P_{\text{acetamide}}$  and 3.4-fold greater than  $P_{\text{thiourea}}$ . Thiourea and urea show similar permeabilities through artificial lipid bilayers while the permeability of acetamide through artificial bilayers is greater than both of these substances (Galluci et al., '71). Differential handling of urea and thiourea is an observation consistent with the handling of these substances by the trout kidney in vivo (M.D. McDonald and C.M. Wood, unpublished results). In the same in vivo experiment, similar handling of acetamide and urea by the trout kidney was observed, contrary to the handling of these substances by the urinary bladder, and giving further evidence that the bladder is not an appropriate model for the kidney. Differential handling of urea versus both thiourea and acetamide has been observed by the branchial facilitated diffusion transporter (tUT) of the gulf toadfish, which has a branchial clearance of urea that is greater than acetamide and

much greater than thiourea (McDonald et al., 2000). The ability of the urinary bladder to distinguish between urea and both these substances that are similar to urea in size and molecular weight suggests the presence of a carrier protein that is specific for urea.

The trout bladder shows a discontinuity in temperature dependence with unidirectional urea flux rate not significantly changing between 5°C and 14°C. Below 5°C and above 14°C,  $Q_{10}$  values were consistent with carrier-mediated transport. Pure phospholipids are known to exhibit sharp phase transitions due to temperature; whereas, more complex phospholipid membranes exhibit prolonged thermal transitions (Linden et al., '73). A prolonged thermal transition such as that observed in the trout bladder from 5°C to 14°C is defined by a distinct beginning and end, marking the beginning ( $t_l$ ) and end ( $t_h$ ) of the process of lateral phase separation. Often a lower activation energy for transport is observed during phase separation, as the energy required for the insertion of a transport-associated protein into a membrane in this phase is small (Linden et al., '73). At temperatures below  $t_l$ , the activation energy for transport is again large.

In contrast to the trout bladder, there is little evidence for a urea transporter in the urinary bladder of the gulf toadfish. The toadfish bladder does not show net transport of urea under symmetrical conditions. Although the permeability of the toadfish bladder ( $8.0 \pm 1.2 \text{ cm s}^{-1} \times 10^{-6}$ ) to urea is higher than in artificial lipid bilayers, this could be due to the higher temperature at which toadfish live. In addition, the urinary bladder of the toadfish does not differentially handle urea, acetamide, or thiourea, (similar permeabilities), suggesting that a urea-specific transport mechanism that preferentially moves urea is not present in the urinary bladder of the toadfish. The toadfish bladder does exhibit temperature sensitivity, namely a sharp phase transition at 26°C; however, this is indicative of a membrane consisting of pure phospholipids (Linden et al., '73). At low temperatures there is a "paradoxical" increase in urea flux rate, which is similar to observations of passive potassium flux in mammalian erythrocytes (Hall and Willis, '86).

In vivo, the urinary bladder of *Opsanus tau*, a close relative of the gulf toadfish *Opsanus beta*, recovers 60% of the fluid excreted from the kidneys by reabsorbing an isosmotic absorbate containing primarily NaCl (Lahlou et al., '69; Howe and Gutknecht, '78; Baustain et al., '97). In con-

trast, the *in vitro* urinary bladder of *Opsanus beta* does not show significant net reabsorption of Cl<sup>-</sup> or water (though Na<sup>+</sup> reabsorption is significant), nor does it significantly transport urea in either the reabsorptive or secretory direction. Ionic measurements suggest that the bladder of *Opsanus beta* *in vivo* reabsorbs Na<sup>+</sup> and Cl<sup>-</sup>, but without knowledge of water movement it is impossible to determine whether urea is also transported (McDonald et al., 2000).

The key conclusion is that in the gulf toadfish, the kidney itself regulates the transport and excretion of urea while the urinary bladder is used as a low permeability storage organ in which urea will not be secreted or absorbed. A secondary conclusion is that the toadfish urinary bladder *in vitro* is not a good model for renal urea handling *in vivo*, where active secretion of urea by the kidney occurs (McDonald et al., 2000). Active secretion of urea does not occur in the bladder *in vitro*. Furthermore, unlike the bladder, the toadfish kidney has the ability to differentiate between urea and the urea analogue acetamide, preferentially transporting urea but not acetamide (McDonald et al., 2000). Because the *in vitro* urinary bladder has identical low permeability to urea and acetamide, it represents an epithelium that does not selectively transport urea.

In conclusion, the data of the present study indicate that the *in vitro* urinary bladder preparations of both species are not suitable models for understanding the handling of urea by the kidney *in vivo*. However, these data do suggest the possibility of a urea transport mechanism in the urinary bladder of the rainbow trout, which holds urine for approximately 30 minutes *in vivo* (Curtis and Wood, '91); however, further investigation is needed to understand this mechanism. This study also demonstrated urinary bladder function in toadfish as being primarily a storage organ of low permeability consistent with long urine holding periods and the intermittent urine release in this species (Wood et al., '95). It is interesting to speculate that the habit of intermittent urine release in tetrapods and toadfish should in both cases be associated with bladder epithelium of low (basal) permeability, a possible example of convergent evolution.

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