Hydration status affects urea transport across rat urothelia

David A. Spector,1 Jie Deng,1 and Kerry J. Stewart2

1Division of Renal Medicine and 2Division of Cardiology, Johns Hopkins Bayview Medical Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

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Spector DA, Deng J, Stewart KJ. Hydration status affects urea transport across rat urothelia. Am J Physiol Renal Physiol 301: F1208–F1217, 2011.—Although mammalian urinary tract epithelium (urothelium) is generally considered impermeable to water and solutes, recent data suggest that urine constituents may be reabsorbed during urinary tract transit and storage. To study water and solute transport across the urothelium in an in vivo rat model, we instilled urine (obtained during various rat hydration conditions) into isolated in situ rat bladders and, after a 1-h dwell, retrieved the urine and measured the differences in urine volume and concentration and total quantity of urine urea nitrogen and creatinine between instilled and retrieved urine in rat groups differing by hydration status. Although urine volume did not change >1.9% in any group, concentration (and quantity) of urine urea nitrogen in retrieved urine fell significantly (indicating reabsorption of urea across bladder urothelia), by a mean of 18% (489 mg/dl, from an instilled 2,658 mg/dl) in rats receiving ad libitum water and by a mean of 39% (2,544 mg/dl, from an instilled 6,204 mg/dl) in water-deprived rats, but did not change (an increase of 15 mg/dl, P = not significant, from an instilled 300 mg/dl) in a water-loaded rat group. Two separate factors affected urea nitrogen reabsorption rates, a urinary factor related to hydration status, likely the concentration of urea nitrogen in the instilled urine, and a bladder factor(s), also dependent on the animal’s state of hydration. Urine creatinine was also absorbed during the bladder dwell, and hydration group effects on the concentration and quantity of creatinine reabsorbed were qualitatively similar to the hydration group effect on urea transport. These findings support the notion(s) that urinary constituents may undergo transport across urinary tract epithelia, that such transport may be physiologically regulated, and that urine is modified during transit and storage through the urinary tract.

Although the lower urinary tract epithelial cell layer (urothelium) contributes to final urine formation in fish, amphibians, and reptiles, it has long been held that bladder and ureter tissues in mammals function solely as transit and storage vehicles for urine produced by the kidneys (13). This paradigm has recently been supported by in vitro studies showing very low permeability to water, urea, and ammonia in intact mammalian bladder epithelium (30) and apical membrane-derived “endosomes” (7). However, a number of factors, including the relatively long urine-to-epithelial cell contact time, enormous and changing water, solute, and hydrogen ion transepithelial gradients, and mechanical and pressure stimuli resulting from bladder filling and emptying, might well promote vectoral water and solute transport across urinary tract epithelia. In support of this idea, in vivo studies in several mammalian species have shown that under certain circumstances water and solutes might traverse urothelia (in either direction) and consequently modify final urine. Thus, using various test solutions and “artificial” urines in a variety of settings and experimental conditions, investigators have shown net transport of urea and other urine constituents, including sodium, chloride, potassium, water, phosphate, and calcium, across rabbit, dog, rat, and human urothelia (15–17, 27, 32, 35, 44). In studies of dogs in which the ureter and bladder were perfused with either the dog’s own urine or an artificial urine, Levinsky and Berliner (25) showed an up to 10% increase in perfusate volume, 20% decrease in perfusate urea, potassium and chloride concentrations, and a 10% increase in perfusate sodium concentrations during 30-min perfusion. Similarly, in carefully performed micropuncture studies, Walser and colleagues (47) showed up to 7% reabsorption of urea, potassium, and creatinine from urine and 9% secretion of sodium into urine perfusing a rat ureter for as little as 3-min duration. In all cases, the direction of transport was down the direction of the concentration gradient between urine and blood. In the most dramatic example of mammalian urothelial transport of water and solutes, Nelson and coworkers (31) showed that the bladder of hibernating black bears reabsorbs the bear’s entire daily urine production during winter hibernation, thus ensuring species survival by conservation of water and solutes.

Evidence for urea and creatinine reabsorption across urothelia is also suggested by recent findings of high concentrations of both substances in rat and dog bladder tissues (42). In both species, the bladder tissue concentrations of urea nitrogen and creatinine were two and one-half to three times plasma levels and similar to concentrations in the renal cortex. In rats, urea nitrogen concentration in bladder tissues was directly related to whole animal hydration status, with highest tissue urea concentrations in water-deprived animals and lower values in ad libitum and water-loaded animals, respectively. These data seem to explain previous findings by Kwon and coworkers (24), who had demonstrated the presence of the “protective” organic osmolytes inositol, GPC, and sorbitol in bladder tissues and who further showed, in a pattern analogous to the findings for urea and creatinine concentrations, that tissue osmolyte concentrations were higher in rats than in rats undergoing diuresis (24). Taken together, these data support the notion that, under certain circumstances, net urothelial transport of water and solutes may occur and may be regulated physiologically by animal hydration status. If this is the case, the lower urinary tract must therefore contribute to formation of final urine and might play an unappreciated role in solute, ion, and water homeostasis. To study rat urothelial transport, we developed an in vivo model designed to closely mimic physiological conditions. We instilled rat urine into isolated in situ bladders of rats and, after a 1-h bladder dwell, retrieved the urine and measured changes in urine volume and concentrations and quantities of urine urea nitrogen, creatinine, and other solutes in groups of...
rats during various whole animal hydration conditions including water loading, ad libitum water intake, and water deprivation. Herein, we describe, for the first time, net transport of water, urea, and creatinine across bladder epithelia in rats studied under physiological conditions, the effect on such transport of whole animal hydration status, and urine and bladder factors which likely mediate urothelial water and solute transport.

METHODS

All research reported herein adheres to the Guiding Principles in The Care and Use of Laboratory Animals and was approved by the Johns Hopkins University Animal Care and Use Committee.

Animals, diets, and 24-h urine collection. Female Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 200–240 g, were maintained on an ad libitum intake of chow (2018, Harlan Teklad Quality Lab Products) containing 18% protein, 0.23% sodium, 0.68% potassium, and 0.40% chloride and assigned to an experimental group. Rats were then placed in standard Nalgene metabolic cages and initially assigned to one of three groups for the 48 h immediately before the experimental procedure: ad libitum water intake (group A), water deprivation (group D), or water loading by provision of 3% sucrose in water as the sole drinking fluid (group W). For each rat, a 24-h urine collection was obtained immediately before the experimental procedure for purposes of confirming hydration status of rats and obtaining urine to be (re)instilled into the (same) rat’s bladder. All urine collections were filtered twice, first with qualitative filter paper (Whatman) to remove debris, than with a 0.2-μm pore size syringe filter (Corning) to remove bacteria. Preliminary experiments in which filtered urine was cultured using standard microbiological techniques confirmed that the resultant filtrates were sterile.

Animal groups. Rats were initially divided into three groups differing by state of hydration (group A = ad libitum water, group D = water deprived, and group W = water loaded). To determine whether factors associated with the bladder or the urine (either or both of which might be altered by animal hydration status) might be the cause for differences in urea transport noted in the first three groups, three additional groups of rats were subsequently studied. Thus another group of rats was water-deprived (group A–D) and another water loaded (group A–W) for 48 h before the experimental procedure, but rats in these groups had their bladders instilled with urine previously (3–4 days before) obtained during ad libitum water intake. An additional group of rats was studied during ad libitum conditions (group D–A) but with urine collected during water-deprivation conditions 10 days earlier (animals had fully recovered from water deprivation as assessed by several 24-h urine collections in the ad libitum state).

Experimental procedure. On the day of the experimental procedure, the 24-h urine was completed and filtered as described above. Then, rats were weighed, anesthetized with intraperitoneal Nembutal (60 mg/kg body wt), placed on a heated operating board, and a midline abdominal incision was made, and both ureters were double ligated near their insertion into the bladder. Ureters were then cut proximal to the ligation, and subsequent urine was diverted exteriorly. After a 30- to 40-min equilibration time, the urinary bladder was emptied of any residual urine with gentle finger pressure on the bladder. The external urethral orifice was then catheterized with a soft tapered 22-gauge vascular catheter (Protect IV Plus Jelco Catheter, Smiths Medical) attached to a 1-ml syringe containing filtered urine (previously collected as part of the 24-h urine) warmed to body temperature. The tip of the catheter was carefully inserted into the proximal urethra at its exit from the bladder to avoid damage to the bladder urothelium. To rinse the bladder of any residual urine, 0.1 ml or 0.15 ml of the twice-filtered 24-h urine previously collected wasinstilled into the bladder over 10 (0.1 ml) or 15 (0.15 ml) min. This rate is within the range at which urine enters the bladder in rats drinking water ad libitum [24-h urine volume, 4–20 ml ± 1.440 min (per 24 h) = 0.03 – 0.14 ml/10 min]. Then, the syringe was disconnected and the bladder was emptied through the catheter with gentle finger pressure on the bladder. To prevent contamination by rinse solution effluent in the catheter dead space (0.033 ml), the urethral catheter was removed and gently replaced with a second urethral catheter connected to a syringe containing the same urine for instillation. This urine-filled syringe and catheter had been weighed to the nearest 0.001 mg using either Mettler Toledo AB54-S or Acculab V1 analytic scales. Approximately 0.3 ml of urine warmed to body temperature was then instilled into the bladder over 30 min. This volume is within, or less than, the reported measured average bladder micturation volume of 0.3–0.7 ml (8), bladder capacity of 0.39–0.66 ml (22, 28), and filling volume of >0.4 ml (11) previously reported in rats. The urethral catheter was then removed while the external urethral appendage was double ligated to prevent loss of bladder urine. The syringe and catheter were then weighed, and the quantity of urine instilled was taken as the weight of the urine-filled catheter and syringe minus the weight of the catheter, syringe, and residual urine remaining in the syringe after instillation. The residual urine in the syringe was then aliquoted and stored for subsequent chemical analysis as the “Instilled urine” (Inst-u). The abdominal incision was then approximated and covered with warmed saline-soaked gauze. After a 60-min dwell time, the urine in the bladder was removed by puncture of an avascular site on the bladder using a 25-gauge needle attached to a 1-ml syringe. The urine so retrieved was measured gravimetrically (as syringe and urine minus empty syringe weight) and stored for subsequent chemical analysis as the “Retrieved urine” (Retr-u). Whole blood was then obtained by cardiac puncture, centrifuged, and aliquotted, and the serum was stored for subsequent chemical analysis.

Analysis of serum and urine specimen. The osmolality of urine was performed by a vapor pressure osmometer (Wescor). Serum and urine concentrations of urea nitrogen, creatinine, and other analytes were measured using a Hitachi 917 Analyzer (Roche Diagnostics).

Statistical analysis. Mean weight, mean arterial blood pressure, serum urea nitrogen, and 24-h urine volumes and osmolality were determined for each rat group, and group differences were analyzed using one way ANOVA followed by the Tukey-Kramer honestly significant difference (HSD) post hoc test. For each rat, the volume (determined gravimetrically) of instilled and retrieved urine and the concentrations of instilled and retrieved urine urea nitrogen and creatinine were determined, and the total number of milligrams transported into, or out of, the bladder urine was obtained by multiplying the (instilled or retrieved) urine volume by the (instilled or retrieved) concentrations of each analyte. Between-group differences between instilled and retrieved urine volumes, urine analyte concentrations, and total volume and analyte transport (in ml or mg) were calculated using one was ANOVA followed by the Tukey-Kramer HSD post hoc tests. Within-group differences of instilled and retrieved concentrations and quantities of each analyte were analyzed using a paired Student’s t-test. A P value <0.05 was considered statistically significant.

RESULTS

Characteristics of rat groups. Characteristics of groups of ad libitum water, water-loaded, and water-deprived rats are shown in Table 1, panel A. As expected, after a 2-day dehydration, water-deprived rats weighed less than the other groups, had higher serum urea nitrogen concentration, higher urine osmo-
Table 1. Characteristics of rat groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Panel A</th>
<th>Panel B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group W (Water-Loaded)</td>
<td>Group A (Ad Libitum Water)</td>
</tr>
<tr>
<td>Rat hydration status</td>
<td>Water-loaded</td>
<td>Ad libitum</td>
</tr>
<tr>
<td>No. of animals</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Weight, g</td>
<td>226 ± 2</td>
<td>225 ± 2</td>
</tr>
<tr>
<td>Blood pressure, mean mmHg</td>
<td>112 ± 4</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>Serum urea nitrogen, mg/dl</td>
<td>23.4 ± 1.3</td>
<td>24.4 ± 0.7</td>
</tr>
<tr>
<td>24-h Urine volume, ml</td>
<td>53.4 ± 5.6†</td>
<td>8.4 ± 0.7</td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH₂O</td>
<td>244 ± 25†</td>
<td>1,859 ± 136</td>
</tr>
<tr>
<td>Instilled urine osmolality, mosmol/kgH₂O</td>
<td>244 ± 25</td>
<td>1,859 ± 136</td>
</tr>
</tbody>
</table>

Values are means ± SE. Weight, blood pressure, and serum urea nitrogen were obtained on the day of experiment for all groups. Urines were collected for 24 h before the experiment for validation of hydration status in all groups, and for immediate bladder instillation in groups W, A, and D. Additional 24-h urines were collected for subsequent bladder instillation during ad libitum water conditions 3-4 days before experiment for groups A-D and A-W and during water-deprived conditions 9-11 days prior to experiment for group D-A. *P < 0.0001 vs. non-water-deprived groups. †P < 0.0001 vs. non-water-loaded groups.

Changes in urine urea nitrogen concentration and total quantity of urine urea nitrogen after bladder dwell. Urine urea nitrogen concentrations in Inst-u and Retr-u for each individual rat (solid lines) and for the group mean (dotted line) in the three hydration groups are shown in Fig. 1. In 24 water-loaded rats, concentrations of urea nitrogen were higher after the 1-h bladder dwell in 18 rats, and lower in 6 rats (Fig. 1, comparison of Inst-u vs. Retr-u urea nitrogen concentrations, P = 0.0001 vs. non-water-deprived groups. †P < 0.0001 vs. non-water-loaded groups).

Table 2. Urine volumes instilled into and retrieved from bladders of ad libitum water, water-loaded, and water-deprived groups of rats

<table>
<thead>
<tr>
<th>Urine Volume</th>
<th>Water-Loaded</th>
<th>Ad Libitum</th>
<th>Water-Deprieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instilled, ml</td>
<td>0.301 ± 0.001</td>
<td>0.305 ± 0.002</td>
<td>0.303 ± 0.001</td>
</tr>
<tr>
<td>Retrieved, ml</td>
<td>0.307 ± 0.001*</td>
<td>0.309 ± 0.002*</td>
<td>0.308 ± 0.002*</td>
</tr>
<tr>
<td>Retrieved - instilled, ml</td>
<td>0.006 ± 0.001</td>
<td>0.004 ± 0.001</td>
<td>0.005 ± 0.001</td>
</tr>
<tr>
<td>Retrieved - instilled, %</td>
<td>1.85 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. There is no significant difference for any urine volume parameter between rat groups, *P < 0.002 retrieved vs. instilled.
urea nitrogen fell significantly in urine during the bladder dwell in both ad libitum water and in water-deprived rats, but did not significantly change in water-loaded rats. The fall in urea nitrogen in Retr-u, expressed both as the concentration difference and as percentage of Inst-u concentration, was greater ($P < 0.0001$) in water-deprived rats than the other two hydration groups. Urea nitrogen concentration in ad libitum water and water-deprived animals fell by 18 and 39% of instilled urea nitrogen concentration, respectively (Table 3, and Fig. 2A) (differences between groups, $P = <0.0001$). Similarly, there were significant differences in the percent change in total quantity of urea nitrogen after the 1-h bladder dwell between the groups (Table 3, bottom). Thus ad libitum water animals and water-deprived animals lost 16 and 38% of total instilled urea nitrogen, respectively. In contrast, water-loaded animals had an insignificant rise in concentration (12%), and quantity (14%) of urea nitrogen.

To evaluate whether the changes in urine urea nitrogen concentration after the 1-h bladder dwell in the three animal groups were determined, at least in part, by the urea nitrogen concentration (or by an associated urine factor) in the Inst-u, we performed regression analysis by plotting Instilled urea nitrogen concentration on the $x$-axis and change in urea nitrogen concentration on the $y$-axis using all animals in the three hydration groups. Figure 3 shows a significant association of the concentrations of urea nitrogen in the Inst-u with the change in urine urea nitrogen concentration in the Retr-u after the 1-h bladder dwell. The solid black line in the figure represents the overall linear regression model of all groups combined. The regression equation was the following: slope of the urea nitrogen concentration change (mg/dl) $= -0.448$, Interception $= 2,544$.

Table 3. Concentration and quantity of urea nitrogen in instilled and retrieved urine and differences in concentration and quantity of urea nitrogen in urine dwelling for 1 h in bladders of groups of rats differing in hydration status and instilled urine

<table>
<thead>
<tr>
<th>Rat hydration status</th>
<th>Panel A</th>
<th>Panel B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group W</td>
<td>Group A</td>
</tr>
<tr>
<td></td>
<td>Water-loaded</td>
<td>Ad libitum water</td>
</tr>
<tr>
<td>Concentration of urea nitrogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instilled urine, mg/dl</td>
<td>$300 \pm 40^\dagger$</td>
<td>$2,658 \pm 220^\dagger$</td>
</tr>
<tr>
<td>Retrieved urine, mg/dl</td>
<td>$315 \pm 37^\dagger$</td>
<td>$2,169 \pm 181^\dagger$</td>
</tr>
<tr>
<td>Concentration difference (retrieved – instilled), mg/dl</td>
<td>$+15 \pm 12^\dagger$</td>
<td>$-489 \pm 70^* + -2,544 \pm 355^\dagger$</td>
</tr>
<tr>
<td>Concentration difference (retrieved – instilled): percentage of instilled</td>
<td>$+12 \pm 5^\dagger$</td>
<td>$-18 \pm 2$</td>
</tr>
</tbody>
</table>

| Quantity of urea nitrogen (volume × concentration) |
|----------------------|---------|---------|
|                            | Group W | Group A | Group D | Group W | Group A-W | Group D-A |
| Instilled urine, mg        | $0.9 \pm 0.1^\dagger$ | $8.3 \pm 0.7^\dagger$ | $18.8 \pm 0.9^\dagger$ | $7.7 \pm 0.5^\dagger$ | $7.7 \pm 0.6^\dagger$ | $16.4 \pm 0.8^\dagger$ |
| Retrieved urine, mg        | $1.0 \pm 0.1^\dagger$ | $6.9 \pm 0.6^\dagger$ | $11.3 \pm 0.9^\dagger$ | $5.9 \pm 0.4^\dagger$ | $6.3 \pm 0.5^\dagger$ | $13.9 \pm 0.7^\dagger$ |
| Difference in quantity (retrieved – instilled), mg | $+0.1 \pm 0.0^\dagger$ | $-1.4 \pm 0.2^* - 7.5 \pm 1.1^* + -1.8 \pm 0.3^* - 1.4 \pm 0.2^* - 2.5 \pm 0.3^*$ | $-21.5 \pm 3.2^\dagger - 17.2 \pm 1.9 - 14.9 \pm 1.7$ |
| Percentage of instilled    | $+14 \pm 5^\dagger$ | $-16 \pm 2$ | $-38 \pm 4^\dagger$ | $-21.5 \pm 3.2^\dagger - 17.2 \pm 1.9 - 14.9 \pm 1.7$ |

Values are means ± SE *$P < 0.0001$ retrieved vs. instilled. †$P < 0.0001$ water-deprived (group D) and group D-A vs. all other groups, and groups A, A-D, and A-W vs. all other groups. ‡$P < 0.0001$ group D vs. all other groups; $P < 0.02$ group W vs. groups A–D and D–A; $\S P < 0.0001$ group W vs. all other groups; $P < 0.006$ group D vs. all other groups.
loaded group, the change in urea nitrogen concentration = 
\begin{align*}
\text{from the bladder after a 1-h dwell, and the actual and percent} \\
volume difference between the urine volume retrieved and 
\text{and instilled in the additional three rat groups were not statistically} \\
different from each other (data not shown) or from the original 
\text{rat groups.} \\
\end{align*}

As was the case for the initial rat groups, for each of the 
additional rat groups there was a greater volume of urine 
retrieved from the bladder than instilled, averaging 0.005 ml, a 
1.6% increase over the instilled volume. There were no 
significant differences in any volume parameter between these 
additional rat groups or between these additional rat groups and 
the original three rat groups.

Changes in urine urea nitrogen concentration and total 
quantity of urine urea nitrogen after bladder dwell in the 
additional rat groups. Urine urea nitrogen concentration and 
total quantity of urea nitrogen in Inst-u and Retr-u, and differences 
in concentration and quantity of urea nitrogen between 
Inst-u and Retr-u in the three additional animal groups, are 
shown in Table 3, panel B and Fig. 2B. Concentration 
and quantity of instilled urea nitrogen in groups A–D and A–W 
were similar to the ad libitum group (group A) and in group 
D–A were similar to the water-deprived group (group D) (all 
\( P = \text{NS} \)).

In all additional groups, there was a significant reduction of 
concentration and quantity of urea nitrogen in the Retr-u 
compared with that in the Inst-u with the quantitative and 
percent reductions of concentration and quantity of urea nitrogen 
in the additional groups similar to those of the ad libitum 
water rats (all \( P = \text{NS} \)). As was the case for the ad libitum rats, 
the reduction in concentration and quantity of urea nitrogen in 
these three additional groups were significantly less than the 
original water-deprived group (Fig. 2, Table 3). The additional 
groups studied with either water-deprived urine or during
water-deprived condition, groups D–A and A–D, but not group A–W and the ad libitum water group (group A), had greater reduction in urea concentration and quantity of urea following the bladder dwell than the original water-loaded group (P ≤ 0.02). The similar reductions of urinary urea nitrogen concentration and quantity in rats instilled with urine obtained during ad libitum water conditions, but studied during water-loaded (group A–W), water-deprived (group A–D), and ad libitum water (group A) conditions, suggested that the urea reabsorption rate must not be regulated by bladder tissue factors alone. Conversely, since reabsorption rates of urinary urea nitrogen from urine obtained during water deprivation were different in rats studied during water-deprived (group D) and ad libitum water conditions (group D–A), urine urea reabsorption must be regulated, at least in part, by a bladder tissue factor associated with hydration.

Thus data from the original and the additional rat groups suggested that two factors, a urinary factor (likely the concentration of instilled urea nitrogen) and a bladder factor, both influenced by the animal hydration status, might affect the transport of urea nitrogen from (or into) bladder-dwelling urine. To examine this hypothesis, we performed regression analysis by plotting Inst-u urea nitrogen concentration on the x-axis and the change in urea nitrogen concentration in the Retr-u on the y-axis and compared all rats studied in the ad libitum water condition (groups A and D–A) and all rats studied during water-deprived conditions (groups D and A–D), as shown in Fig. 4. The regressions of both groups of animals suggest that urine urea nitrogen concentration changes are dependent on instilled urinary urea nitrogen concentration, but the slope of the regression line in all rats studied during ad libitum water conditions [combined groups W and A-W, the change in urea nitrogen concentration (mg/dl) = -0.240 × Instilled urea nitrogen concentration, 95% confidence interval for the slope, -0.356, -0.123] was similar to that obtained during ad libitum water conditions, but was also significantly different than that for the combined water-deprived groups (groups D and A–D) (P < 0.001) (data and slope not shown). Thus these data are consistent with the notion that change in urine urea nitrogen concentration after the bladder dwell is dependent not only on the concentration of instilled urea nitrogen, but also on an additional bladder factor or factors related to the hydration status of the animal. These effects seem to be additive since both urine obtained during water deprivation (and thereby containing high concentrations of urea nitrogen) and the water-deprived state are required for maximal rates of urine nitrogen reabsorption (observed only in the water-deprived rat group), and both urine obtained during the water-loaded state (and thereby containing low concentrations of urea nitrogen) and the water-loaded state are required for the minimal rate of urine urea nitrogen reabsorption (observed only in the water-loaded rat group).

**Effect of animal hydration status on changes in creatinine concentration and quantity of creatinine in urine instilled in rat bladders.** To determine whether the concentration of other urine solutes might also be altered during the 1-h bladder dwell, and whether hydration status might affect the concentration changes of other solutes, we measured creatinine concentration and calculated the amount of creatinine in the same Inst-u and Retr-u samples utilized for urea nitrogen measurements (Table 4). Similar to urine urea nitrogen, concentration and quantity of urine creatinine instilled into and retrieved from the bladder were highest in water-deprived animals and lowest in water-loaded animals. Urine creatinine concentration fell significantly in both the water-deprived and ad libitum groups and rose in the water-loaded group. When expressed as a percentage of instilled urine creatinine, these changes were similar in direction to the changes in urinary urea nitrogen (Table 4, Fig. 5), suggesting that both concentration and quantity of urine creatinine may be altered during a 1-h dwell in the urinary bladder and that such alterations may be affected by an animal’s hydration status.

**DISCUSSION**

Herein, we report for the first time significant net reabsorption of urea from urine dwelling in the urinary bladder in rats studied under physiological conditions. These findings must result from net reabsorption of urea across the bladder epithelium and are unexpected since they contrast with the widely held view that the mammalian genitourinary tract is impermeable to urinary constituents (13), a view supported by recent Ussing chamber data reporting very low (though finite) mammalian urothelial permeability to urea (7, 30). Our data are supported, however, by older in vivo data in rats demonstrating 7% urea (and other solutes) reabsorption from urine perfusing
the rat ureter for a 3-min duration (47) and up to 20% urea (and other solutes) reabsorption from artificial urine perfusing the dog ureter and bladder over 30 min (25). We further show for the first time that whole animal hydration status is a determinant of the magnitude of bladder urea reabsorption, with 2-day water deprivation doubling the total urea reabsorptive rate from 16% in rats receiving water ad libitum to 39% and with water loading blocking urea nitrogen reabsorption entirely. Data derived from rat groups including those whose bladders were instilled with urine obtained under various hydration conditions indicated that the magnitude of the absolute and percent changes in urea transport across urothelia was a function of both the concentration of the instilled urea nitrogen and the animal’s hydration state. The maximum urea nitrogen reabsorption rate was achieved only in water-deprived rats whose bladders were instilled with urine obtained during water deprivation, and an absence of urea nitrogen reabsorption occurred only in water-loaded rats whose bladders were instilled with urine obtained during water loading. These novel findings thus show that at least two factors linked to an animal’s hydration status seem to independently mediate the effect(s) of that status on urothelial urea reabsorption, a urine factor, likely the concentration of urinary urea nitrogen (but possibly urine osmolality or other constituent factor associated with changes in urine concentration), and a factor(s) associated with the bladder itself, presumably an alteration of one or more of the bladder urothelial permeability “barriers,” resulting in increased urothelial reabsorption rate in the water-deprived state and an absence of reabsorption in the water-loaded state. The finding in water-loaded animals that urine urea nitrogen concentration is unchanged after a 1-h bladder dwell, despite the still steep urine (300 mg/dl)-to-blood (23 mg/dl) urea nitrogen concentration gradient, likely reflects the limit on passive reabsorptive processes for urea for the rat bladder urothelium, but one cannot entirely rule out the possibility than an active urea transporter, demonstrated in elasmobranches (9) and amphibians (36) and long-sought in the mammalian renal collecting duct (20), might exist in mammalian urothelia as previously suggested (49). Finally, we show that urothelial permeability to urine constituents and regulation by an animal’s hydration status is not limited to urine urea alone since urine creatinine concentration and quantity also fell in rats drinking water ad libitum and in water-deprived rats. In this respect, our data are supported by prior data demonstrating 3.5% reabsorption of creatinine from urine briefly perfusing the rat ureter (49), and up to 10% reabsorption from artificial urine perfusing the dog bladder for 30 min (25). Our present findings are also consistent with previous data demonstrating high concentrations of urea (and creatinine) in bladder tissues of rats and dogs (42). Rats in that study and in the present study were identically treated and grouped in terms of hydration status, and bladder tissue urea nitrogen concentrations were highest in the water-deprived rats and lowest in the water-loaded rats, consistent with the transport data for urea described herein and with the notion that water deprivation increases and water loading decreases urothelia urea transport.

In contrast to the dramatic change in urine urea nitrogen and creatinine concentrations after a 1-h bladder dwell time, we found only a very small though consistent increase in urine volume (averaging a 1.6%) in retrieved urine compared with instilled urine, regardless of hydration status or experimental condition (all 6 group comparisons, \( P = \text{NS} \)). Although it is possible that this reflects similar (small) net gain of urine volume (resulting from net water transport flowing across the urothelium into the bladder urine) in each of the six groups, our finding of an almost identical net volume increase in a separate group of 10 rats in which the instilled urine was immediately retrieved by bladder puncture (rather than after a 60-min dwell) suggests the likelihood that the 1.6% volume increase reflects a technical artifact, possibly resulting from a urinary bladder dead space. If such is the case, our data indicate that, at least for the experimental conditions in this model,
there is little or no net urothelial transport of water in either direction. There are few prior studies reporting volume changes in urine perfusing the genitourinary tract, but in an early in vivo rat bladder model Hohbbruger et al. (17) reported water entry into the bladder following bladder instillation of various sodium chloride solutions, while Levin and Berliner (25) showed up to a 10% increase in urine volume during in vivo perfusion of the dog ureter and/or bladder. In the latter studies, the percentage of urine volume increase was inversely related to the rate of perfusion of the ureter and/or bladder by the artificial urine solutions. Finally, in studies in hibernating black bears, Nelson and coworkers (31) reported absorption of the entire (albeit reduced) daily urine volume produced during the 3- to 5-mo winter hibernation.

To study urothelial permeability of urine, water, and solutes, we designed and utilized an in vivo isolated rat bladder model, derived in part from in vivo bladder preparations described by prior investigators (16, 17). However, our model is significantly different from previous preparations in a number of important aspects. First, the animal’s own urine, rather than an artificial solute solution, was used as the instilled solution, thus reducing the possibility that an absence of one or more urinary factors might predispose in some way to altered epithelial cell barrier integrity.

The volume of the instilled urine (0.3 ml) was chosen because it is below the previously described filling volume and capacity of rat bladders, and the rate of instillation of the urine into the bladder (0.3 ml/30 min) is within the range of bladder urine accumulation occurring in rats receiving ad libitum water (and an ad labium diet). Finally, except for gentle finger pressure to ensure complete bladder emptying, the bladder itself was not instrumented until the end of the experiment, when it was punctured by a needle to obtain the retrieved urine. Thus our model more closely reflects the normal physiological state of the bladder than previous ex vivo and in vivo models of urothelial transport. Similarly, the chosen bladder dwell time of 1 h is within the range of reported micturition intervals for rats under ad libitum conditions. Although we do not have data regarding micturition rates in water-deprived and water-loaded rats, it is possible that micturition intervals are lengthened in water-deprived and reduced in water-loaded conditions. If so, this might magnify the hydration-related effects we describe herein.

We utilized a widely used protocol of sucrose in drinking water to establish the water-loaded condition in our water-loaded group of rats. While the sucrose might result in increased caloric intake, decreased food (protein) intake, and consequent metabolic and hormonal perturbations, we believe it unlikely that it affected urothelial tissue metabolism and/or solute transport in this group.

The sites and mechanisms potentially underlying and regulating vectorial transport of urea and other substances in urothelia have not been extensively considered until recently (because the bladder was thought to be functionally impermeable) and are presently unclear. However, the major permeability barriers to water and solute transport likely include one or a combination of the apical (luminal) cell membrane of the so-called “umbrella” epithelial cells comprising the mucosal surface of the urinary tract lumens, the tight junctions between the umbrella cells, and the glycosaminoglycans (GAGs), a mucous-like layer which overlies and is loosely attached to the apical membrane (12, 21, 26). Theoretically, transported water and solutes must pass through one or more of these barriers, with water and different solutes potentially traversing the urothelial barriers at different sites. Although simple diffusion and/or urothelial “leaks” might account for some (perhaps “basal”) transurothelial transport, there have recently been reports of numerous molecular channels and transporters in urothelial membranes in mice, rats, dogs, and humans. To date, channels and/or transporters have been identified for water [aquaporin-1 (AQPI) in subepithelial capillaries, AQP2 and AQP3 in basolateral membranes of the umbrella cells and plasma membranes of the intermediate and basal epithelial cells] (39); urea (urea transporter B on basolateral membranes of the umbrella cells and plasma membranes of the underlying intermediate and basal cell) (29, 40, 48); sodium [epithelial sodium channel (ENaC) on the apical surface of the umbrella cells] (38, 48); and potassium (ROMK Kir 1.1 on the apical surface of the umbrella cells, Kir 6.1 on basolateral surface of the umbrella cells and plasma membrane of the underlying cells, and Kir 2.1 and maxi-K in uncertain sites) (21, 40, 48). These channels/transporters all play important roles in renal epithelial cell water and solute transport and conceivably could play similar roles in urothelia. Urea transporter B and AQP2 and 3 (which may also transport urea), which in urothelia may be regulated by hydration status (29, 39, 41), seem ideally situated to facilitate movement of urea from the umbrella cells lining the urinary tract lumen through the deeper epithelial cell layers and then (down the steep gradient) to the underlying capillary network. Whether urothelial cells might also possess a creatinine transporter, perhaps similar to the secretory transporter found in mammalian renal epithelium, is not known. Although hydration state might regulate urothelial solute transport by directly affecting specific urothelial molecular transporters and channels, it might also regulate transport in a more general manner by altering the permeability of the GAG layer (composed largely of chondroitin sulfate, heparin sulfate, and “ordered water” molecules) lining the apical membrane, the umbrella cell tight junctions, or some component of the umbrella cell apical membrane containing specialized lipid bilayers as well as asymmetric unit membrane proteins composed of uropilaks (both known to participate in apical membrane barrier formation) (14, 18, 50). Although there are little direct data supporting any of these possibilities, it has been shown that the composition of the surface GAG layer is not constant, but a “dynamic variable” (19), and further that bathing the bladder lumen in protamine sulfate or dilute acid (both of which alter the GAG layer) results in a reversible increase in membrane ion conductance in stretched rabbit bladder (45), membrane permeability in vivo (32), and in bladder permeability to urea in humans (27). Interestingly, bladder surface GAGs appear to derive from urine excreted by the kidneys since urine from rats having undergone cystectomy contained quantities and types of GAGs identical to those in urine from the same rats before cystectomy (19). Conversely, hydration status might also affect urothelial paracellular water and solute transport by altering umbrella cell tight junctions. Tight junction proteins, including zona occluden-1, occludin, and Claudin-4, -8, and -12, have recently been described in bladder epithelial cells (1). Since in other epithelial cells Claudins have been reported to be acutely physiologically regulated (3, 4, 34, 46), it is possible that they, or other urothelial tight junction proteins might be regulated by hydration status. In this regard,
prior studies have demonstrated dehydration-induced changes in renal collecting duct paracellular pathways resulting in higher permeability to water and nonelectrolytes (10) as well as altered rat renal papillary and renal pelvis epithelia (known to be similar in appearance and function to lower urinary tract epithelia) morphology in antidiuresis (5). In the latter studies, water deprivation resulted in widely dilated intercellular spaces, suggesting significant fluid movement across the epithelium. Furthermore, it has recently been shown that intraperitoneal injections of hydrocortisone and norepinephrine in mice and spinal cord injury in rats can lead to disruption of umbrella cell tight junctions and increases in lanthanum and urea permeability, respectively (2).

In summary, our data demonstrate net transport of urinary urea and creatinine across bladder epithelia in rats and show that such transport is regulated by urine and bladder factors associated with hydration status. If confirmed, these findings would provide strong evidence against the long-held paradigm that mammalian lower urinary tract functions solely as a transit and storage vehicle and would suggest that the lower urinary tract might play an unappreciated role in water and solute homeostasis. In fact, similar solute transport across urothelia may occur in humans as well since recent studies have shown that the pH as well as concentrations of sodium and potassium in renal pelvic urine are altered as that urine traverses the ureter and bladder in patients being treated for various urinary tract disease (6, 37). While reabsorption and reutilization of urine nitrogenous wastes (urea, creatinine) is critical for survival in hibernating animals, the advantages of such transport in nonhibernating mammals are not clear. It has recently been suggested, however, that urea and creatinine reabsorbed from the urinary bladder might subsequently be metabolized and the nitrogen moiety utilized for protein synthesis in conditions such as starvation, low protein intake, pregnancy, and growth in children (42). Conversely, it is also possible that the lower urinary tract epithelium participates in the pathophysiology of “prerenal azotemia,” a clinical state characterized by relatively high serum levels of urea nitrogen in the setting of systemic volume depletion or dehydration and previously thought largely due to urea reabsorption in the renal proximal tubule and collecting duct. Given the data presented herein, it seems possible that reabsorption of urea across urothelia plays a role in the high concentrations of urea nitrogen in these settings.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.A.S., J.D., and K.I.S. provided conception and design of research; D.A.S. and J.D. performed experiments; D.A.S. and J.D. analyzed data; D.A.S., J.D., and K.I.S. interpreted results of experiments; D.A.S. and J.D. prepared figures; D.A.S. drafted manuscript; D.A.S., J.D., and K.I.S. edited and revised manuscript; D.A.S., J.D., and K.I.S. approved final version of manuscript.

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