Urea and Renal Function in the 21st Century: Insights from Knockout Mice

Robert A. Fenton* and Mark A. Knepper†

**Water and Salt Research Center, Institute of Anatomy, University of Aarhus, Aarhus, Denmark; and †Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung and Blood Institutes, National Institutes of Health, Bethesda, Maryland*

Since the turn of the 21st century, gene knockout mice have been created for all major urea transporters that are expressed in the kidney: the collecting duct urea transporters UT-A1 and UT-A3, the descending thin limb isoform UT-A2, and the descending vasa recta isoform UT-B. This article discusses the new insights that the results from studies in these mice have produced in the understanding of the role of urea in the urinary concentrating mechanism and kidney function. Following is a summary of the major findings: (*1*) Urea accumulation in the inner medullary interstitium depends on rapid transport of urea from the inner medullary collecting duct (IMCD) lumen *via* UT-A1 and/or UT-A3; (*2*) as proposed by Robert Berliner and colleagues in the 1950s, the role of IMCD urea transporters in water conservation is to prevent a urea-induced osmotic diuresis; (*3*) the absence of IMCD urea transport does not prevent the concentration of NaCl in the inner medulla, contrary to what would be predicted from the passive countercurrent multiplier mechanism in the form proposed by Kokko and Rector and Stephenson; (*4*) deletion of UT-B (vasa recta isoform) has a much greater effect on urinary concentration than deletion of UT-A2 (descending limb isoform), suggesting that the recycling of urea between the vasa recta and the renal tubules quantitatively is less important than classic countercurrent exchange; and (*5*) urea reabsorption from the IMCD and the process of urea recycling are not important elements of the mechanism of protein-induced increases in GFR. In addition, the clinical relevance of these studies is discussed, and it is suggested that inhibitors that specifically target collecting duct urea transporters have the potential for clinical use as potassium-sparing diuretics that function by creation of urea-dependent osmotic diuresis.

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Urea is a small molecule, only 60 Da, that constitutes the major form of waste nitrogen that is excreted in mammals. Its extraordinarily high solubility in water (saturation at >6 M) makes it ideal for excretion by the concentrating kidney. Studies by James Shannon in the 1930s demonstrated that the rate of urea excretion is determined chiefly by its rate of filtration through the glomerulus and tubular reabsorption (1,2). Despite this seemingly simple view of renal urea handling, multiple hypotheses that ascribe more complex roles to urea have accrued during the course of the 20th century. The molecular era, shepherded by genome projects in various species, has opened the door for new tools that are capable of addressing these hypotheses, namely, mouse lines in which various molecular urea carriers have been genetically deleted. Here we review the new light that has been shed on renal urea handling through experimentation in these knockout mouse lines.

In mammals, approximately 90% of waste nitrogen is excreted by the kidney as urea. The majority of this urea is generated in the liver as a byproduct of protein metabolism. Under most circumstances, the dietary protein intake of humans and animals greatly exceeds that necessary for the support of anabolic processes; therefore, a large excess of urea is generated (Figure 1). Excretion of this urea constitutes a large osmotic load to the kidney. Most solutes that are excreted in such large amounts, for example mannitol (3), would obligate large amounts of water excretion by causing an osmotic diuresis. However, as first determined by Gamble and colleagues (4,5) in the 1930s, the kidney possesses specialized mechanisms that allow large amounts of urea to be excreted without obligating water excretion.

Evidence for specialized urea transport in the kidney, mediated by molecular urea carriers, has been provided in numerous studies. It is not the purpose of this article to review all of these, but an overview is informative. Urea reabsorption by the mammalian nephron occurs by two different mechanisms: A constitutive process that occurs in the proximal nephron and accounts for reabsorption of nearly 40% of the filtered load of urea and a regulated process that occurs in the distal nephron and depends on the level of antidiuresis (1,2) among other factors. In addition, the use of the isolated perfused tubule technique has determined that (*1*) the cortical collecting duct has a very low urea permeability that is not increased by vasopressin (AVP) (6); (*2*) the terminal part but not the initial

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Address correspondence to: Dr. Robert A. Fenton, Water and Salt Research Center, Institute of Anatomy, Building 233/234, University of Aarhus, DK-8000 Aarhus, Denmark. Phone: +45-894-23008; Fax: +45-861-98664; E-mail: rofe@ana.au.dk

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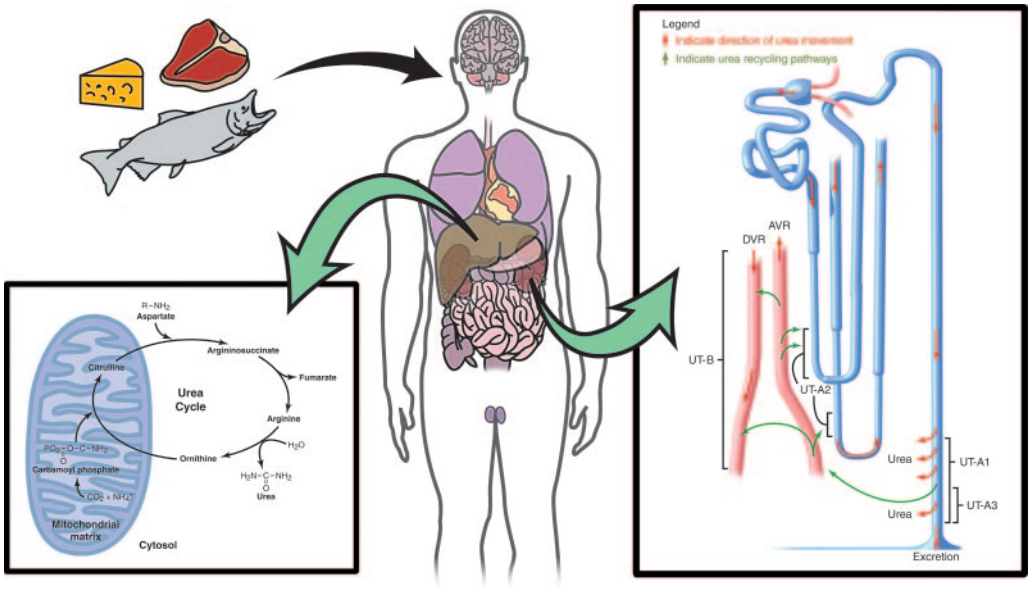

Figure 1. Urea handling in mammals. The majority of mammals consume diets that are high in protein. Under most circumstances, this dietary protein intake greatly exceeds that which is necessary for the support of anabolic processes. Excess protein is catabolized by the liver, which results in the formation of large amounts of urea by the ornithine-urea cycle. Urea is freely filterable by the kidney and the excretion of this urea constitutes a large osmotic load to the kidney. Most solutes excreted in such large amounts would obligate large amounts of water excretion by causing an osmotic diuresis. However, along the nephron, the specialized urea transporters UT-A1, UT-A2, UT-A3, and UT-B are involved in complex urea reabsorption and recycling pathways that allow large amounts of urea to be excreted without obligating water excretion. Illustration by Josh Gramling—Gramling Medical Illustration.

part of the inner medullary collecting duct (IMCD) possesses extraordinarily high urea permeability (7); (*3*) AVP increases urea permeability only in the terminal IMCD (8); (*4*) urea transport in the IMCD is inhibited by phloretin and urea analogues and is a saturable process, consistent with a transporter-mediated (facilitated) mechanism (9,10); and (*5*) urea and water transport across the IMCD occur by two distinct pathways, but the time course of increased urea and water permeability changes in the IMCD in response to AVP are virtually indistinguishable (11–14). Combined, this rich body of descriptive physiologic observations facilitated the cloning of urea transporters from the kidney as detailed in what follows.

Urea Transporters

There are two distinct but closely related urea transporter genes: UT-A (*Slc14a2*) and UT-B (*Slc14a1*) (15–17). Several urea transporter isoforms are derived from the UT-A gene *via* alternative splicing and alternative promotors (Figure 2) (15). Multiple cDNAs (Figure 2) that encode urea transporters have been isolated and characterized (18–25). UT-A1 is expressed exclusively in IMCD cells (Figure 3) (19,26). UT-A1 activity is regulated acutely by AVP (27), but the long-term effects of AVP on UT-A1 abundance are still a subject of controversy. UT-A2 is expressed in the inner stripe of the outer medulla, where it is localized to the lower portions of the thin descending limbs (tDL) of short loops of Henle (Figures 1 and 3) (19,28,29) and, under prolonged antidiuretic conditions, in the inner medulla, where it is localized to the tDL of long loops of Henle (29). AVP increases UT-A2 abundance (19,29,30), and recent studies have determined that UT-A2–mediated urea transport can be regulated acutely by cAMP (31). In a similar manner to UT-A1, expression of UT-A3 is restricted to the terminal IMCD (Figures 1 and 3), where, in mouse, it is both intracellular and in the

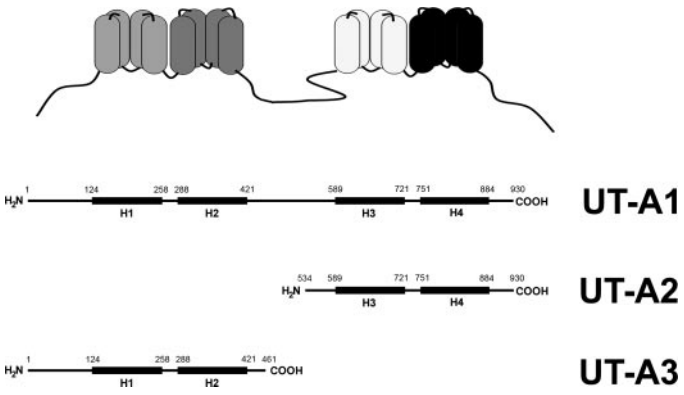

Figure 2. Urea transporters derived from mouse UT-A gene. At the top is a schematic representation of the largest isoform, UT-A1, with putative membrane-spanning domains represented as barrels. Below, H1 through H4 represent hydrophobic domains, with amino acid numbers indicated. UT-A1 and UT-A3 are driven by the same promoter and are identical through amino acid 459. Use of an alternative exon inserts a stop codon that terminates UT-A3 after amino acid 460 (an aspartic acid). UT-A2 is identical to the terminal 397 amino acids of UT-A1 and is driven by an alternative promoter in intron 13 of the mouse gene (15). Illustration by Josh Gramling—Gramling Medical Illustration.

basolateral membrane domains (32). UT-A3 mRNA abundance can be upregulated by the prolonged action of AVP (15). In contrast to the multiple UT-A isoforms, the mouse UT-B gene encodes only a single protein that is expressed throughout the kidney medulla in the basolateral and apical regions of the descending vasa recta (DVR) endothelial cells (Figures 1 and 3) (33–35). Long-term treatment with the type II vasopressin receptor agonist dDAVP causes downregulation of UT-B protein abundance (36).

Recently, several mouse models with selective deletion of different urea transporter isoforms have been created. The remainder of this article summarizes the studies from these mice and discusses the conclusions of these studies with respect to the role of urea in the urinary concentrating mechanism.

UT-A1 and UT-A3 Knockout Mice

The two UT-A isoforms that are expressed in the IMCD are UT-A1 and UT-A3—the so-called "collecting-duct urea transporters." Recently, by knocking out both collecting duct urea transporters in tandem, we developed a mouse model that allowed us to assess specifically the role of IMCD urea transport in kidney function (37). These mice (termed *UT-A1/3*$^{-/-}$ mice) were generated by replacing 3 kb of the UT-A gene, which contains a single 148-bp exon (exon 10), with a neomycin selection cassette. Exon 10 of the UT-A gene codes for amino acids 291 to 339 of UT-A1 or UT-A3 and is situated in a large, hydrophobic, membrane-spanning region (38); therefore, it is thought to be of functional significance. Immunoblotting and immunocytochemistry with several isoform-selective polyclonal antibodies demonstrated successful deletion of the transporters from the IMCD. Importantly, a functional assessment of *UT-A1/3*$^{-/-}$ mice was performed using isolated perfused tubule studies and showed a complete absence of phloretin-sensitive and AVP-regulated urea transport in IMCD segments, whereas AVP-stimulated water permeability was unaffected. We concluded from these studies that UT-A1 and/or UT-A3 is responsible for the high urea permeability of the IMCD that was observed previously and that water and urea are transported by different mechanisms.

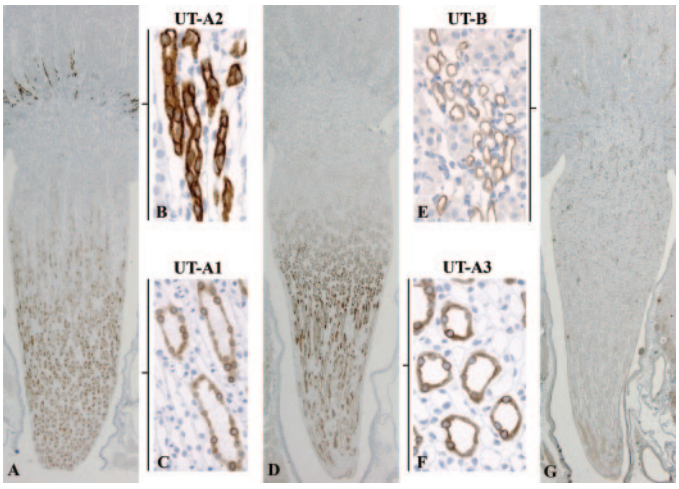

Figure 3. Localization of UT-A urea transporters. UT-A1 is localized to the terminal portion of the inner medullary collecting duct (IMCD), whereas UT-A2 is localized to the thin descending limbs of Henle's loop in the inner stripe of the outer medulla (A). Higher magnification shows that both UT-A2 (B) and UT-A1 (C) predominantly are intracellular. UT-A3 is localized to the terminal portion of the IMCD (D) and is both intracellular and in the basolateral membrane domains (F). UT-B is expressed in the descending vasa recta (G), where it is localized to the basolateral and apical regions (E).

Role of IMCD Urea Transporters in the Urinary Concentrating Mechanism

Much of our fundamental understanding of the contribution of urea transporters to the urinary concentrating mechanism is based on a model of urea handling proposed in the 1950s by Berliner *et al.* (39). The following is a brief explanation undated with subsequent observations. The concentration of urea in the tubule fluid that enters the collecting duct system in the renal cortex is relatively low. During antidiuresis, water is osmotically absorbed from the urea-impermeable parts of the collecting duct system *via* aquaporin water channels, causing a progressive increase in luminal urea concentration along the collecting duct system. Subsequently, when the tubule fluid reaches the highly urea-permeable terminal IMCD, urea can exit rapidly from the lumen to the inner medullary interstitium. This urea is trapped in the inner medullary interstitium because the effective blood flow is very low owing to countercurrent exchange by the vasa recta (40,41). In the presence of AVP, the urea permeability of the terminal IMCD is extremely high, and, under steady-state conditions, urea accumulates to very high concentrations in the interstitium and nearly equilibrates across the IMCD epithelium. This allows urea in the interstitium to almost completely balance osmotically the high urea concentration in the collecting duct lumen, preventing the osmotic diuresis that would otherwise occur in association with the large amounts of urea that are present in the urine.

On the basis of this model, one would predict that the deletion of specialized urea transporters from the IMCD should result in an impaired capacity to conserve water, owing to urea-dependent osmotic diuresis. For testing of this hypothesis, the urinary concentrating function of *UT-A1/3*$^{-/-}$ mice on three different levels of dietary protein intake was examined in a series of metabolic cage studies (Figure 4) (37,42). With free access to water, *UT-A1/3*$^{-/-}$ mice that were fed either a standard-protein (20% protein by weight) or high-protein (40%) diet had a significantly greater fluid intake and urine flow than wild-type controls, resulting in a decreased urine osmolality. However, on a low-protein diet (4% protein) *UT-A1/3*$^{-/-}$ mice did not show a substantial degree of polyuria. With this low protein intake, hepatic urea production is low and urea delivery to the IMCD is low, thereby preventing urea-induced osmotic diuresis. Further studies examined the maximal concentrating ability of *UT-A1/3*$^{-/-}$ mice after 18 h of water restriction (Figure 4). Knockout mice on a 20 or 40% protein intake were unable to reduce their urine flow to levels below those that were observed under basal conditions, resulting in severe volume depletion and loss of body weight. In contrast,

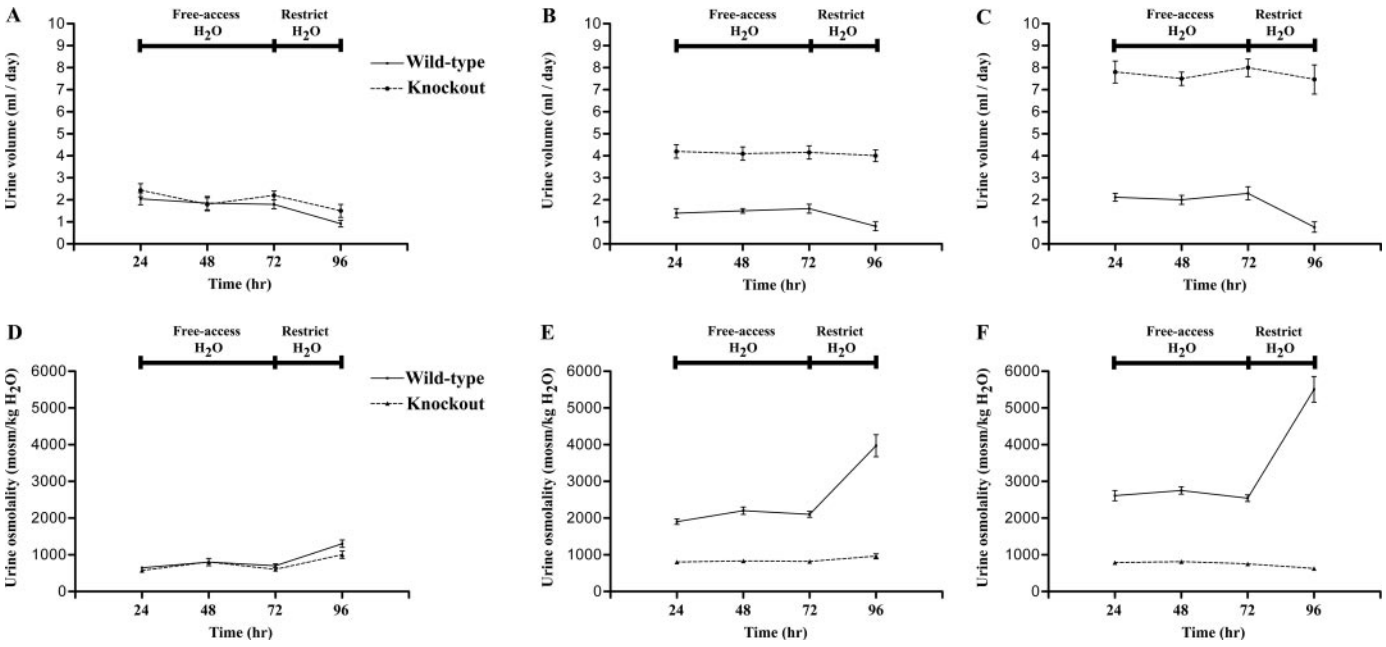

Figure 4. Water conservation and urinary concentrating ability of *UT-A1/3*$^{-/-}$ mice. For all graphs, data are means ± SEM; wild-type mice are indicated by solid lines, and knockout mice are represented by dashed lines. Mice received 4, 20, or 40% protein intake for 7 d before and throughout the duration of the study. Graphs show either the urine output under basal conditions (free access to drinking water) for 3 consecutive days, followed by a 24-h water restriction on a 4% (A), 20% (B) or 40% (C) protein diet or the corresponding urine osmolality on a 4% (A), 20% (B), or 40% (C) protein diet. The conclusion from these data is that the role of IMCD urea transporters in water conservation is to prevent a urea-induced osmotic diuresis. Adapted from data in references (37,42).

UT-A1/3$^{-/-}$ mice that were on a low-protein diet were able to maintain fluid balance without a marked loss of body weight.

In accordance with the Berliner model, these studies showed that the concentrating defect in *UT-A1/3*$^{-/-}$ mice largely is a result of urea-dependent osmotic diuresis. However, collecting duct urea transport has been proposed to play an additional role in the urinary concentrating mechanism, acting as the first step of the so-called "passive model" in which accumulation of NaCl in the inner medullary interstitium depends indirectly on urea reabsorption from the IMCD (43,44). As discussed in the next section, studies in *UT-A1/3*$^{-/-}$ mice provided a direct test of the passive model.

Accumulation of NaCl in the Inner Medulla: Role of IMCD Urea Transporters

Early experiments that used tissue slice analysis determined that a corticomedullary osmolality gradient exists in the kidney with maximum osmolality at the tip of the inner medulla (45). This gradient was found to be due mainly to accumulation of NaCl in the outer medulla and urea in the inner medulla. The cause of the gradient in the outer medulla is well understood on the basis of the classical countercurrent multiplier model (46). This model relies on active NaCl reabsorption in the water-impermeable thick ascending limb of the loop of Henle (TAL) (47,48) for an energy source. In the inner medulla, the urea gradient is due to passive urea reabsorption from the IMCD *via* UT-A urea transporters, aided by countercurrent exchange (see Urea Recycling). In addition to the urea gradient, the inner medulla generates a NaCl gradient that is not as steep as the NaCl gradient in the outer medulla but nevertheless is important in the production of a concentrated urine. The mechanism that is responsible for this NaCl gradient in the inner medullary interstitium has been an important focus of research in the past 35 yr, and no clear consensus has emerged. Repeated studies of thin ascending limbs have failed to show evidence for an active NaCl transport process in the inner medulla (49,50). Therefore, another process presumably is responsible for the energy that is needed to concentrate NaCl in the inner medulla. One hypothesis was the "passive model" that was introduced in the previous section. This model was proposed independently by Stephenson (44) and by Kokko and Rector in 1972 (43) and also is referred to as the "passive countercurrent multiplier mechanism." In this mechanism, rapid urea reabsorption from the IMCD generates and maintains a high urea concentration in the inner medullary interstitium and causes the osmotic withdrawal of water from the thin descending limb, concentrating NaCl in the lumen. This highly concentrated NaCl then is proposed to exit passively from the thin ascending limb. If the urea permeability of the ascending limbs is extremely low, then any NaCl that has been reabsorbed from the ascending thin limb will not be replaced by urea and the ascending limb fluid will become dilute relative to the surrounding interstitial fluid. This dilutional process is proposed to constitute a "single effect" for countercurrent multiplication, similar to that in the outer medulla but generated by passive transport processes within the inner medulla.

The passive countercurrent multiplier mechanism in the inner medulla relies on rapid urea transport from the IMCD, facilitated by the urea transporters UT-A1 and UT-A3. If the passive model is correct, then we would predict that in *UT-A1/3*$^{-/-}$ mice, the lack of urea transport across the epithelium of the IMCD would impair the ability to concentrate NaCl in the inner medulla. Direct tests of this prediction were made by measurement of inner medullary solute concentrations in inner medullary tissue in two different studies (37,42). In one study, the mean urea, Na^+, Cl^-, and K^+ concentrations were measured in whole inner medullary tissue isolated from *UT-A1/3*$^{-/-}$ mice and wild-type littermates (37). *UT-A1/3*$^{-/-}$ mice displayed a marked decrease in inner medullary urea concentration, but there was no reduction in the mean Na^+, Cl^-, or K^+ concentrations. In a separate study, the osmolality and urea and Na^+ concentrations were measured in the cortex, outer medulla, and two levels of the inner medulla from *UT-A1/3*$^{-/-}$ and wild-type mice that were fed either a low-protein (4%) or a high-protein (40%) diet (42). In *UT-A1/3*$^{-/-}$ mice that were on either protein intake, there was a substantially reduced corticomedullary osmolality gradient and no urea gradient. However, the observed corticomedullary sodium gradients in wild-type and knockout mice that were on either level of dietary protein intake were indistinguishable. Furthermore, in wild-type mice, decreasing the dietary protein intake resulted in a decrease in tissue osmolality that was caused solely by reduced urea accumulation in the inner medulla. That is, sodium concentrations along the corticomedullary axis were unchanged by the change in protein intake and the resulting change in medullary urea accumulation. Therefore, a marked depletion in medullary urea concentration had no effect on the ability of the medulla to accumulate NaCl in the inner medulla, whether the depletion was caused by dietary protein restriction or by deletion of IMCD facilitative urea transporters. On the basis of these two studies, it seems that NaCl accumulation in the inner medulla does not depend on either IMCD urea transport or the accumulation of urea in the medullary interstitium. These findings, therefore, contradict an essential prediction of the passive concentrating model, namely that elimination of passive urea absorption from the IMCD and the resulting depletion of inner medullary urea would decrease inner medullary NaCl accumulation. These results in *UT-A1/3*$^{-/-}$ mice and their wild-type controls seem to refute the passive countercurrent multiplier hypothesis for inner medullary NaCl accumulation. That is, the passive model is not the chief mechanism by which NaCl is concentrated in the inner medulla. It should be emphasized that previous objections have been raised to the validity of the passive model largely on the basis of the high urea permeabilities that have been measured in thin descending and ascending limbs (summarized in reference [51]), but these studies in *UT-A1/3*$^{-/-}$ mice provide the most direct test of the hypothesis to date.

If the passive countercurrent multiplier model is not the explanation for the NaCl gradient in the renal inner medulla, then what is? A full answer to this question is beyond the scope of this short review, because the proposed alternatives do not involve urea transporters. The reader is referred to either a chapter by Gamba and Knepper (51) or a recent review article (52) for a discussion of alternative models. Fundamentally, it is safe to conclude at this point that more research is needed to develop a full understanding of the concentrating function of the renal inner medulla.

The "Gamble Phenomenon"

The Gamble phenomenon (described more than 70 yr ago as "an economy of water in renal function referable to urea" [4]) pointed to a special role for urea in the urinary concentrating mechanism and provided part of the original support for the Kokko-Rector-Stephenson passive model. The general features of the Gamble phenomenon are that (*1*) the water requirement for the excretion of urea is less than for the excretion of an osmotically equivalent amount of NaCl, and (*2*) when fed various mixtures of urea and salt in the diet, less water is required for the excretion of the two substances together than the water needed to excrete an osmotically equivalent amount of either urea or NaCl alone. The latter finding suggests that the concentrating mechanism depends in some complex way on an interaction between NaCl and urea. Indeed, the latter finding is what would be predicted by the passive model; therefore, the Gamble phenomenon was viewed as providing support for the validity of the passive model (38). *UT-A1/3*$^{-/-}$ mice were used to investigate the role of collecting duct urea transport in the Gamble phenomenon (53). Indeed, in *UT-A1/3*$^{-/-}$ mice, both elements of the Gamble phenomenon were absent, indicating that IMCD urea transporters play a critical role. A titration study in which wild-type mice were given progressively increasing amounts of urea or NaCl showed that both substances can induce osmotic diuresis at high enough levels of excretion (6000 μosmol/d for urea; 3500 μosmol/d for NaCl). It is interesting that mice were unable to increase urinary NaCl concentrations to beyond approximately 420 mM. The second component of the Gamble phenomenon derives from the fact that both urea and NaCl excretion are saturable, presumably a result of the ability to exceed the respective reabsorptive capacity for urea and NaCl. Thus, conservation of water with mixtures of NaCl and urea *versus* pure NaCl or urea occurs simply as a result of lowering the concentration of each to levels that avoid osmotic diuresis, rather than to any specific interaction of urea transport and NaCl transport at an epithelial level.

Urea Recycling

In addition to countercurrent exchange, urea recycling is believed to provide an important means of maintaining a high level of urea in the renal inner medulla (54). Recycling occurs when urea that is reabsorbed from the IMCD is re-secreted into the loop of Henle, causing it to be returned to the collecting duct lumen with the flow of tubule fluid (Figure 1). Recycling first was demonstrated by Lassiter *et al.* (55), who showed that the mass flow rate of urea in the superficial tubule exceeds the filtered load of urea. It has been proposed (54) that a major element of urea secretion into Henle's loop is *via* transfer from the vasa recta to the thin descending limbs of short-loop nephrons in the vascular bundles of the outer medulla, where these two structures are in close apposition (56,57). The chief

urea transporter of the vasa recta is UT-B, whereas the chief urea transporter in the thin descending limbs is UT-A2 (Figures 1 and 3). Therefore, it would be predicted that the deletion of either of these transporters would impair significantly urea accumulation in the inner medulla, resulting in increased water excretion *via* urea-induced osmotic diuresis. However, it came as a surprise when Uchida *et al.* (58) showed that deletion of UT-A2 did not affect substantially the concentrating ability or water excretion with a normal level of protein intake. Only when urea excretion was diminished by administration of a low-protein diet was there a decrease in medullary urea accumulation relative to wild-type control mice. Therefore, urea secretion into the thin descending limb of Henle's loop does not seem to be as important in medullary urea accumulation as previously believed.

In contrast to the results that were observed with UT-A2 knockout mice, deletion of UT-B, the major urea transporter of the DVR and of erythrocytes, resulted in a substantial impairment in renal water conservation. UT-B knockout mice were developed in 2002 (59), and their physiology recently was discussed extensively elsewhere (60). On a normal-protein diet, UT-B null mice have a significantly higher daily urine output, resulting in lower urine osmolality, compared with wild-type mice. However, when UT-B knockout mice are subjected to water deprivation for 36 h, they are able to concentrate their urine, although to a lesser extent than controls. Knockout mice have a significantly higher plasma urea, and their urine-to-plasma urea ratio is reduced more severely than that of other solutes, indicating that the UT-B null mice have a "urea-selective" urinary concentrating defect (61). This diminished ability to concentrate urea is highlighted by a lower inner medullary urea concentration compared with other solutes.

Quantitatively, the most important loss of urea from the inner medullary interstitium is thought to occur *via* the vasa recta (54); therefore, the greater concentrating defect in UT-B null mice compared with UT-A2 null mice may not be surprising. UT-B potentially is important for both countercurrent exchange of urea between ascending vasa recta (AVR) and DVR and for transfer of urea from the vasa recta to the thin descending limb. A comparison of the results in UT-B and UT-A2 knockout mice suggests that the former may be more important to the overall process that traps urea in the inner medulla. That is, at face value, the results suggest that the recycling of urea between the DVR and AVR is more important quantitatively than recycling of urea between the AVR and the renal tubules with regard to net effect on water conservation. A key element of data that needs to be provided for full acceptance of this conclusion is micropuncture of the distal tubule to verify that UT-A2 deletion actually eliminates urea recycling. Specifically, it would be of value to repeat the measurements that were made originally by Lassiter *et al.* (55,62) in wild-type and UT-A2 knockout mice to address the hypothesis that UT-A2 is necessary for urea recycling.

In addition to the DVR, UT-B protein is expressed in red blood cells (RBC) (63) and contributes to their high urea permeability. This high urea permeability is thought to have a physiological role; rapid urea transport may help to preserve the osmotic stability and deformability of the RBC (64) and thereby help to prevent dissipation of the urea gradient in the renal medulla (discussed in reference [65]) and overall concentrating ability. Because erythrocytes from UT-B knockout mice have an approximately 45-fold lower urea permeability compared with those from controls, it is important to recognize that the concentrating defects that are observed in the UT-B knockout mice could be due to the loss of urea transport in the vasa recta, in RBC, or both. Furthermore, the loss of UT-B from both the vasculature and RBC also may help to explain the difference in concentrating ability between the UT-A2 and UT-B knockout mice.

Urea Recycling and Regulation of GFR by High-Protein Diets

Consumption of diets that are rich in protein results in increases in whole-kidney GFR (66,67). Microperfusion studies by Seney *et al.* (68) determined that protein-induced increases in GFR result from changes in the tubuloglomerular feedback (TGF) system. Their studies found that the sensing mechanism of the TGF system was rendered less responsive by high protein intake and that the diminished TGF was caused, at least in part, by a reduced early distal NaCl concentration, without a change in early distal tubule osmolality (69). However, the cause of the reduced luminal NaCl concentration remains unknown. One model for this reduced early distal NaCl concentration and the subsequently reduced GFR was proposed by Bankir *et al.* (70,71) and depends on changes in urea concentration in the fluid that is delivered to the TAL. This hypothesis posits that increased luminal concentrations of urea, consequent to a high-protein diet, causes increases in osmotic water secretion in the TAL, thereby lowering luminal NaCl concentration in the fluid that is delivered to the macula densa. A lower NaCl generally is recognized to be a signal that can increase GFR *via* the TGF mechanism.

Increases in urea concentration in the TAL with dietary protein excess are believed to depend on both an increase in the urea concentration of the glomerular filtrate and an increase in the extent of urea recycling. In *UT-A1/3*$^{-/-}$ mice, the drastically reduced urea reabsorption from the collecting duct and the consequent reduction in inner medullary interstitial urea concentration is likely to virtually eliminate urea recycling and therefore is a useful model to address whether the mechanism that was proposed by Bankir *et al.* is correct. In *UT-A1/3*$^{-/-}$ mice, one would predict that the increase in GFR in response to high protein feeding would be attenuated markedly. For examination of this, a series of clearance studies in conscious *UT-A1/3*$^{-/-}$ and wild-type control mice that were fed either a low-protein (4%) or a high-protein (40%) diet were performed. Increasing the protein content of the diet approximately doubled the GFR in both *UT-A1/3*$^{-/-}$ mice and controls. Furthermore, under both dietary conditions, no significant differences were observed in the FITC-inulin clearance between *UT-A1/3*$^{-/-}$ and wild-type mice. The conclusion from these studies is that urea reabsorption from the IMCD and, more specific, the process of urea recycling are not necessary elements of the

overall process that is responsible for protein-induced increases in GFR.

Possible Role of Urea Transporters in Regulation of Extracellular Fluid Volume

As discussed, deletion of the two collecting duct urea transporters UT-A1 and UT-A3 in mice results in a urea-induced osmotic diuresis. It therefore seems plausible that regulating the activity or expression of IMCD urea transporters could regulate indirectly water and NaCl excretion by modulation of the extent of urea-induced osmotic diuresis. Such a regulatory process would require feedback mechanisms that alter collecting duct urea transport in response to changes in extracellular fluid (ECF) volume. Therefore, it is pertinent to ask whether changes in ECF volume or tonicity are associated with regulation of urea transporters. Indeed, urea transporter expression was found to be downregulated in aldosterone-induced ECF volume expansion (72), in ECF volume expansion that is associated with nephrotic syndrome (73), in obese Zucker rats with type 2 diabetes and hypertension (74), in response to hypertension induced by angiotensin II or norepinephrine (75), and in an animal model of the syndrome of inappropriate antidiuresis (76). These decreases in urea transporter expression potentially could be a homeostatic response to ECF volume expansion or hypertension, increasing NaCl and water excretion *via* urea-induced osmotic diuresis. Conversely, in the salt-sensitive Dahl rat, which is another model with ECF volume expansion and hypertension, the higher expression of urea transporters may be responsible in part for the hypertension by reduction of urea-induced osmotic diuresis (77). In this regard, it would be informative to address whether the hypertension that is seen in the salt-sensitive Dahl rat is altered by changes in dietary protein. Beyond this, an important goal for future research is to address the extent to which urea transporter regulation can affect ECF fluid volume and BP by measuring these variables in knockout and wild-type mice on different levels of protein intake.

Compensatory Mechanisms

One of the limitations of knockout mouse studies is that other genes/proteins potentially may compensate for the loss of the targeted protein, thereby rendering the observed phenotype less than anticipated. This may be the case in both *UT-A1/3*$^{-/-}$ mice and UT-B knockout mice, in which targeted proteomic studies using an ensemble of antibodies have shown upregulation of other proteins that are involved in the urinary concentrating mechanism. For example, UT-A2 is upregulated in UT-B knockout mice (78), potentially as a means of compensating for the diminished urea recycling, and both aquaporin-2 and aquaporin-3 are upregulated in *UT-A1/3*$^{-/-}$ mice (42). These compensatory mechanisms have to be considered when addressing the role of the deleted gene in biological mechanisms.

Clinical Perspective

Although "science for science's sake" is a broadly held concept among basic scientists, the ultimate objective of studies in basic physiology is to improve medical care. In this section, we discuss the potential relevance of the new findings that have been obtained in urea transporter knockout mice with regard to clinical nephrology. First and foremost, the results presented here predict that drugs that are designed to inhibit urea transporters have considerable promise in the treatment of water and salt imbalance disorders. In the absence of urea transport in the renal collecting duct, urea is rendered an osmotic diuretic with the potential to increase the excretion of both water and NaCl. Therefore, a drug that could inhibit urea reabsorption effectively in the IMCD potentially could be used as an aquaretic and diuretic agent. Such drugs potentially would be useful in the treatment of hypertension or ECF volume–expanded disorders such as congestive heart failure and hepatic cirrhosis. The unique aspect of urea transporter antagonists (unlike thiazides, loop diuretics, epithelial sodium channel blockers, or mineralocorticoid receptor blockers) are that they are unlikely to cause secondary potassium balance and acid-base disorders, owing to the location of the IMCD downstream from the nephron sites where potassium and acid-base transport are regulated. Existing urea transporter inhibitors such as phloretin and mercurial agents are too toxic to be clinically useful, although mercurials once were used clinically as diuretics (79), presumably because of actions at multiple sites to inhibit NaCl reabsorption. Amiloride has been reported to inhibit urea transport in toad bladder (80). However, we have found that amiloride is ineffective as a urea transport inhibitor in isolated perfused IMCD tubules whether added to the luminal perfusate or the peritubular bath (9). Accordingly, we suggest that investigation of potential urea transporter inhibitors would be an appropriate target for drug discovery studies in which automated assays are used to screen thousands of compounds that are present in chemical libraries (81).

As yet, few examples of clinical abnormalities have arisen from genetic defects in urea transporters. Patients with mutations in the UT-B gene have been identified by the absence of the Kidd blood group antigen that normally is present on the surface of erythrocytes (82,83). Clinically, these UT-B null patients are overtly normal, although they exhibit a mild concentrating defect (84), possibly as a result of the absence of urea transporter activity in their vasa recta and/or RBC. The abnormal concentrating defect in UT-B null patients is similar to what is observed in UT-B knockout mice.

No mutations of the UT-A gene have been defined explicitly, although families with "familial azotemia" have been described in Europe (85) and the United States (86), with the disorder potentially due to abnormal activation of collecting duct urea transport. Affected members of the US family exhibited high serum urea concentrations in the setting of normal serum creatinine levels and had normal urinary concentrating and diluting abilities. The high serum urea concentrations are associated with low urea clearances, suggesting an abnormally high rate of urea absorption at some point along the nephron. To our knowledge, none of these patients have undergone genetic testing to identify possible mutations in the UT-A gene that could account for possible hyperactivation of UT-A1 and/or UT-A3 in the collecting duct.

Finally, a number of single-nucleotide polymorphisms that point to a possible relationship between polymorphisms in the UT-A gene and BP regulation have been identified in the UT-A gene (87). Specifically, it was found that two of the seven identified polymorphisms in UT-A1 or UT-A2 (Val/Ile at position 227 and Ala/Thr at position 357) were associated with decreased diastolic BP in men but not women. These findings add credence to the idea that pharmacologic manipulation of UT-A proteins potentially can be effective in treatment of high BP.

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Disclosures

None.

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See the related editorial, "Critical Role of Urea in the Urine-Concentrating Mechanism," on pages 670–671.

This review of current concepts of the role of urea in urinary concentrating mechanisms, and the accompanying editorial by Sands, are related to two papers in the current issue of *CJASN*. The report by Bankir *et al.* (pages 303–311) suggests that ethnic differences in urinary concentration and sodium retension may be causally related to increased blood pressure, and the accompanying editorial by Luft (pages 196–197) provides perspective on these observations and the potential role of vasopressin.