

# New insights into urea and glucose handling by the kidney, and the urine concentrating mechanism

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The mechanism by which urine is concentrated in the mammalian kidney remains incompletely understood. Urea is the dominant urinary osmole in most mammals and may be concentrated a 100-fold above its plasma level in humans and even more in rodents. Several facilitated urea transporters have been cloned. The phenotypes of mice with deletion of the transporters expressed in the kidney have challenged two previously well-accepted paradigms regarding urea and sodium handling in the renal medulla but have provided no alternative explanation for the accumulation of solutes that occurs in the inner medulla. In this review, we present evidence supporting the existence of an active urea secretion in the pars recta of the proximal tubule and explain how it changes our views regarding intrarenal urea handling and UT-A2 function. The transporter responsible for this secretion could be SGLT1, a sodium-glucose cotransporter that also transports urea. Glucagon may have a role in the regulation of this secretion. Further, we describe a possible transfer of osmotic energy from the outer to the inner medulla via an intrarenal Cori cycle converting glucose to lactate and back. Finally, we propose that an active urea transporter, expressed in the urothelium, may continuously reclaim urea that diffuses out of the ureter and bladder. These hypotheses are all based on published findings. They may not all be confirmed later on, but we hope they will stimulate further research in new directions.

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Efficient water conservation is a challenge for terrestrial mammals. Concentrating urine several fold above plasma osmolality (up to 4- to 5-fold in humans and to 15- to 20-fold in some rodents) is an important feature of water conservation, allowing the excretion of soluble wastes in a relatively limited amount of fluid. The loop shape of the mammalian nephrons and the unique vascular-tubular relationships of the renal medulla are key factors in this concentrating function.<sup>1</sup> However, the mechanism by which urine is concentrated remains incompletely understood and is not satisfactorily simulated by the most sophisticated mathematical models designed so far.<sup>2–5</sup> The active transport that occurs in the thick ascending limb of Henle's loops and in the collecting duct (CD) in the cortex and outer medulla (OM) provide osmotic energy that accounts for the rise in urine osmolality within these zones, but the further concentration that is observed in the inner medulla (IM) remains largely unexplained.

Urea, a small water-soluble molecule, is the major end product of protein metabolism in mammals and is the most abundant solute in the urine (at least in omnivores and carnivores). Because its concentration in the blood plasma is relatively low (4–10 mmol/l) compared with that of other solutes such as sodium (140 mmol/l), urea represents <2% of the filtered solutes but becomes about 40–50% of all solutes in the urine (for a western-type diet in humans). To excrete this daily load, urea is concentrated up to 100 times in urine with respect to plasma, and the bulk of water reabsorbed by the kidney to concentrate urine is actually devoted to the concentration of urea.<sup>6,7</sup> Moreover, urea has long been known to improve the concentration of other solutes in the urine.<sup>8–11</sup>

In the past two decades, several facilitated urea transporters (UTs) have been cloned, and their localization in the kidney and contribution to the concentrating mechanism have been described (see reviews by Sands<sup>12</sup> and Bagnasco<sup>13</sup>). Mouse models with knockout (KO) of several of these transporters have brought some new insights into urea movements in the kidney<sup>11,14–17</sup> but provided no adequate explanation for the progressive rise in osmolality that occurs in the IM. Moreover, they have challenged at least two previously well-accepted paradigms regarding urea and sodium handling in the renal medulla (see further).

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Several other nitrogenous end products, including ammonia, uric acid, and (to a modest extent) creatinine, are known to be secreted actively in the nephron, i.e., against an unfavorable transepithelial concentration difference. In contrast, urea is usually not considered to be actively secreted in the mammalian kidney in spite of a few sporadic publications in favor of such a secretion.<sup>7,18–21</sup> Recent findings in mice have given a new impulse for the likelihood of an active secretion of urea because two studies found excretion rates of urea that exceed 100% of the filtered load.<sup>22,23</sup>

Active urea transport has been well characterized functionally in many different living beings, from unicellular organisms to mammals. It may serve three different functions. One is nitrogen uptake from the external milieu, as occurs in certain unicellular organisms that possess the enzyme urease and are thus able to break down urea.<sup>24,25</sup> Another is urea conservation in elasmobranchs (through gills and kidneys<sup>26</sup>) and some terrestrial amphibians (through the ventral skin in some frogs or toads<sup>27–32</sup>) that use urea as an ‘osmotic buffer’ in plasma and extracellular fluids to compensate for the hyperosmolality of the sea water (elasmobranchs) or prevent excessive water loss through the skin (terrestrial amphibians). Renal urea conservation may also be important in mammals with a low protein intake (herbivores and especially ruminants,<sup>33–38</sup> or rats fed a protein-poor diet<sup>39</sup>), in association with a bacterial microflora able to hydrolyze urea in their digestive tract. This allows the reuse of urea nitrogen that would otherwise be lost in the urine.<sup>7</sup> Finally, active urea secretion may contribute to more efficient urea excretion, and especially to urea concentration in the urine, as a means to get rid of nitrogen wastes without excessive water demand. This occurs in the kidney of some frogs<sup>40–42</sup> and probably also in the mammalian kidney, as will be explained below.

Until now, a few studies have provided convincing evidence for both active urea reabsorption and active urea secretion in the CD of the mammalian kidney. Significant Na-coupled active urea reabsorption from lumen to peritubular space appears in the upper third of the inner medullary CD (IMCD) of rats after sustained low protein intake.<sup>39,43–45</sup> From a different perspective, a small active urea secretion has also been demonstrated functionally in the very terminal part of the IMCD of normal rats,<sup>46</sup> but this could account only for an extremely small addition of urea to the urine (see section ‘Does active urea reabsorption in the lower urinary tract contribute to maintain high urea concentration in the urine?’).

The molecular structure of several different active urea transporters has been identified in plants,<sup>47</sup> bacteria,<sup>48–50</sup> yeast,<sup>25,51</sup> and fungi,<sup>52,53</sup> but no homology with the corresponding genes has been found in the mammalian genome. It is somewhat surprising that, with so much recent progress in the identification of membrane transporters, the molecular structure of the transporters responsible for active urea transport in vertebrates has not yet been identified.

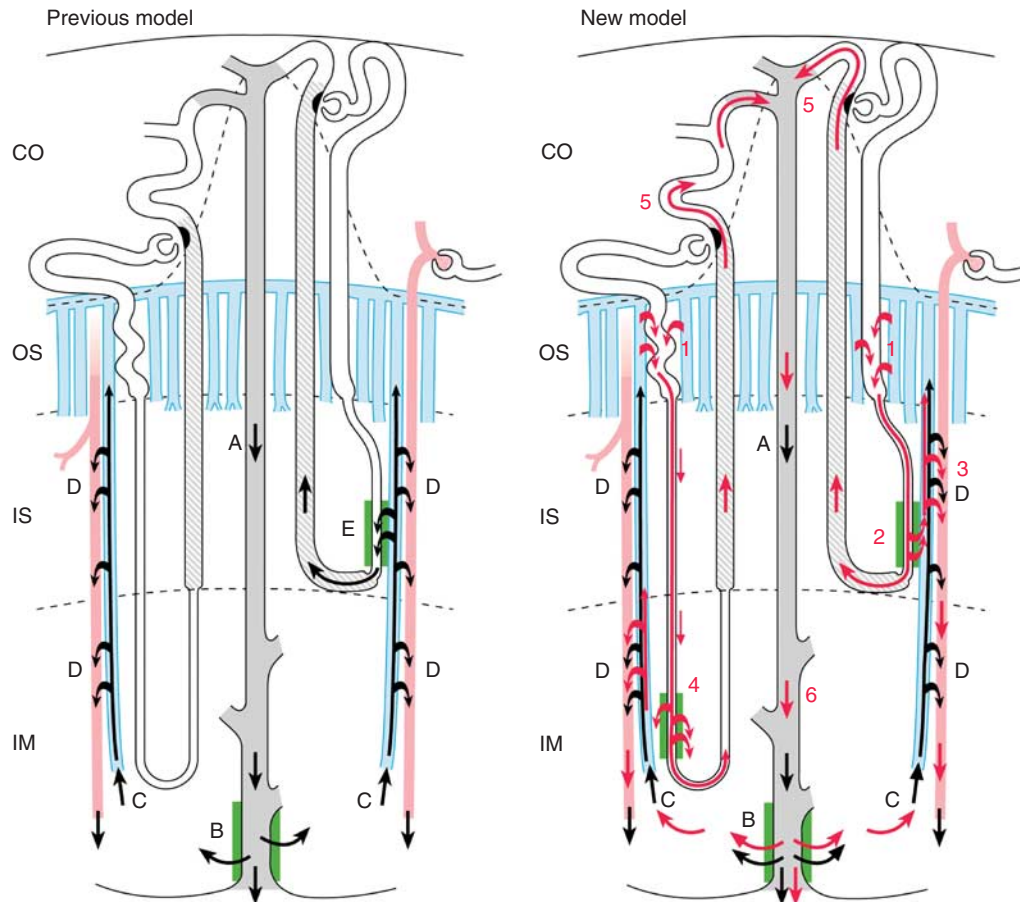
In this review, we will present the evidence supporting an active urea secretion in the pars recta of the mammalian proximal tubule and explain how it changes our views

regarding urea handling in the thin descending limbs (TDLs). We will also propose several new hypotheses. The first two concern the transporter that could be responsible for this urea secretion, and a possible transfer of osmotic energy from the outer to the IM, taking advantage of the unique vascular–tubular spatial relationships in the medulla. The last hypothesis deals with urea handling in the urinary tract. These hypotheses are based on a number of original findings reported in the literature. Maybe these hypotheses will turn to be wrong in a more or less distant future, as have many previous hypotheses. Our aim in writing this review is to stimulate further research in new directions by providing novel provocative insights into poorly explored mechanisms and pathways that could be involved in the production of concentrated urine in mammals.

### ACTIVE SECRETION OF UREA IN THE PARS RECTA AND TRANSFER TO THE INNER MEDULLA

The mechanism by which urea is concentrated in the urine is only partially understood. In the past four decades, it has been understood that it depends (1) on several facilitated UTs expressed in some limited portions of the TDL, CD (see note 1 below), and descending vasa recta, all located in the medulla (see reviews by Sands,<sup>12</sup> Bagnasco,<sup>13</sup> Bankir,<sup>22</sup> and Smith and Fenton<sup>54</sup>), allowing the delivery of concentrated urea to the most inner part of the IMCD, and (2) on a complex intrarenal recycling that continuously brings back to the IM part of the urea that tends to escape through the venous blood in ascending vasa recta<sup>22</sup> (*Note 1*: Although the CD is often considered to be part of the ‘distal nephron’, this structure does not belong to the ‘nephron’ proper as it has a different embryonic origin (ectoderm for the CD and mesoderm for the nephron)).

For several decades, the conventional paradigm has been that this recycling process involves the reentry of inner medullary urea along the descending vasa recta (through UT-B) and in specialized sections of the loops of Henle (through UT-A2) as illustrated in Figure 1 (left panel). The close association of descending and ascending vasa recta and the paucity of interstitial tissue in the vascular bundles of the inner stripe of the OM favor countercurrent exchange of urea between arterial and venous blood (going to and coming from the IM). Evidence for intrarenal urea recycling through the loops of Henle came from micropuncture experiments that showed that, in all species studied, during ‘antidiuretic’ conditions, more urea flows through the early distal tubule (at the exit of the loop of Henle) than in the late proximal tubule (the last accessible segment before the loop of Henle).<sup>55–60</sup> In the rat, the close proximity of the TDLs of short-looped nephrons with the vascular bundles in the inner stripe suggests intense countercurrent exchange of urea between ascending vasa recta and TDLs. In the mouse and in some desert-adapted rodents, TDLs are even intermingled among descending and ascending vasa recta within ‘complex’ vascular bundles, making these exchanges even more efficient.<sup>61–63</sup> A parallel has been made between these structural adaptations



**Figure 1 | Intrarenal urea movements according to the classical theory or to the new concept that assumes an active secretion of urea in the pars recta.** Common features to both panels. The course through the four kidney zones of a short-looped and a long-looped nephron and a collecting duct (CD) is depicted, along with arterial (light red) and venous (light blue) vasa recta (descending and ascending vasa recta, DVR and AVR, respectively). CO, cortex; OS and IS, outer and inner stripe of the outer medulla, respectively; IM, inner medulla. In the OS, ascending vasa recta are very numerous and make close contact with the pars recta (see Figure 2). In the 'vascular bundles' of the IS and in the IM, ascending vasa recta run in parallel with blood flowing in opposite directions. Descending vasa recta express UT-B and AQP1 along their entire length (not shown), and ascending vasa recta are fenestrated. Thus, urea can diffuse rapidly through these two types of vessels that form a 'countercurrent exchanger'. Simultaneously, water can be short-circuited from the descending vasa recta to the ascending vasa recta, so as to minimize water delivery to the IM. Note that ascending vasa recta are more numerous than descending vasa recta in the IS and IM, although only one of each is drawn here for clarity. Expression of UT-A2 (in thin descending limbs) and UT-A1/3 (in terminal IMCD) along discrete regions of these structures is shown as a green lining.

Left (previous model): Concentrated urea (A) is delivered to the IM through the terminal IMCD via UT-A1/3/4 and diffuses in the inner medullary interstitium (B). It is taken up by ascending vasa recta (C) and is then recycled through two different routes: a vascular route (D) by countercurrent exchange between ascending and descending vasa recta, and a tubular route (E) via the thin descending limbs of short-looped nephrons expressing UT-A2.

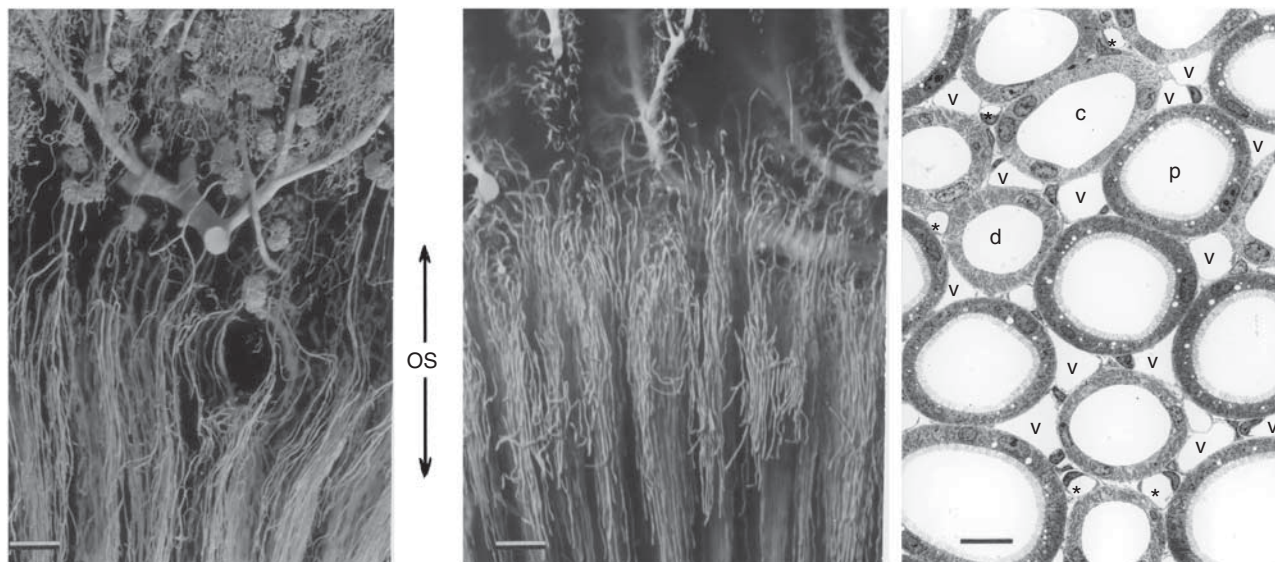
Right (new model): New features are shown by red arrows and numbers. Urea is assumed to be actively secreted in the medullary pars recta of both short- and long-looped nephrons in the OS (1) (and possibly in the medullary rays of the cortex, not shown). This provides a flow of concentrated urea in the thin limbs. When facing a segment expressing UT-A2 in short-looped nephrons, some urea diffuses into the nearby ascending vasa recta (2) and is then trapped into the countercurrent exchanger (3). It is then taken down to the IM. In long-looped nephrons, some of the urea flows directly toward the IM where it brings concentrated urea that may diffuse into the interstitium (4), thus contributing to enhance urea accumulation in the IM. In both types of loops, the urea that remains in the lumen (having escaped diffusion through UT-A2) flows in the nephron, down to the IMCD along a relatively long tubular route (5 and 6).

and the urine concentrating ability in different rodents.<sup>63</sup> For detailed description of the vasculo-tubular relationships of the mammalian renal medulla, see reviews by Bankir *et al.*,<sup>1</sup> Bankir and de Rouffignac,<sup>61</sup> Kriz,<sup>62</sup> Beuwkes and Bonventre,<sup>64</sup> Kriz.<sup>65</sup> However, in spite of this favorable anatomical configuration, the magnitude of the observed urea addition in the loops<sup>66</sup> seems difficult to reconcile with the models

proposed for generating hyperosmolality in the IM by passive mechanisms.

Most of the studies conducted in transgenic mice with deletion of one or more of the facilitated UTs have confirmed and validated many previous concepts about urea handling in the kidney. However, two previous features, assumed to be important steps in the concentrating mechanism, have been





**Figure 2 | Vascular supply of the outer medulla.** Microfil filling of arterial (left) and venous vasculature (middle) and a cross-section of the kidney at the level of the outer stripe (right), illustrating the high surface area of contact between pars recta and venous vasa recta. In the outer stripe (OS), the arterial blood supply is scarce because the efferent arterioles of the juxtamedullary glomeruli do not form abundant capillary networks as they do further down in the inner stripe. The blood supply of the proximal tubule pars recta (the nephron segment occupying the largest volume in the OS) is almost exclusively provided by the ascending vasa recta (shown by 'v' in the right panel) and well visible in the middle panel. \*, descending vasa recta. c, collecting duct; d, distal tubule (thick ascending limb); p, proximal tubule (pars recta).

challenged. (1) Urea recycling through UT-A2 in the TDLs of short-looped nephrons is not important for urine concentration.<sup>17</sup> (2) Sodium chloride can accumulate in the IM, even in the absence of urea delivery at the tip of the papilla.<sup>23,67</sup> Thus, the classical views about how urine gets concentrated in the kidney and how urea contributes to this concentration need to be revised, and new hypotheses are needed to reconcile former observations with the new knowledge acquired from transgenic mice models.

Results observed in UT-B KO mice confirmed the major role of efficient countercurrent exchange of urea between ascending and descending vasa recta, but also suggested that the recycling of urea in the loops of Henle through UT-A2 may be much less important than previously assumed.<sup>11</sup> Subsequent results in UT-A2 KO mice showed that it is indeed the case because these mice showed no urine concentrating defect.<sup>17</sup> Moreover, the suppression of UT-A2 expression in UT-B knockout mice (double UT-B/UT-A2 KO) improved their urine concentrating ability almost to the level seen in wild-type mice, instead of worsening it, as could have been expected from previous concepts<sup>67</sup> (see further). These observations suggest (1) that a functional UT-A2, in the absence of countercurrent exchange in the vasa recta, contributes to dissipate urea instead of bringing it back to the medulla, and (2) that the well-established urea addition in Henle's loops must occur in another segment of their descending branch.

#### Urea secretion in the pars recta

We propose that urea is added to the nephron lumen by active secretion in the pars recta. Uphill urea transport has

been well characterized in the renal tubule of amphibians, in which it allows urea to be concentrated in the urine five- to sevenfold above the plasma level, despite the inability of their kidney to raise urine osmolality as a whole above that of plasma.<sup>40,68</sup> In mammals, this secretion likely occurs in the pars recta of the proximal tubule, and is probably most intense in the portion of the pars recta located in the outer stripe of the OM. In this region, the arterial capillary network is scarce and most of the blood supply is provided by abundant venous vasa recta that ascend from the IM and share a large surface area of contact with the pars recta (Figure 2). In addition to the urea that is present in the blood entering the medulla (issued from the efferent arterioles of the juxtamedullary glomeruli), ascending vasa recta also carry some of the urea brought by the CD's into the inner medullary interstitium. Note that the medullary pars recta is already known to secrete a number of organic compounds including uric acid, hippurate, cAMP and cGMP, xenobiotics, and so on.<sup>69-74</sup> In aglomerular fishes, a segment analogous to the mammalian pars recta generates a flow of fluid and solutes in the nephron by secretion from blood to lumen.<sup>75,76</sup>

Assuming that urea, similar to other nitrogenous end products, is actively secreted in the pars recta of the proximal tubule helps understand the large addition of urea to the loops of Henle observed in a number of micropuncture studies of superficial and deep nephrons.<sup>55,56,59,66,77-79</sup> Because UT-A2 is expressed only in the deepest 30% of the short-loop TDL and only weakly in the long-loop TDL,<sup>80-82</sup> it seems unlikely that large amounts of urea could enter passively in the descending branch of the loops, as had been assumed in previous models. The finding that mice with

UT-A2 deletion exhibit no urine concentrating defect in normal conditions<sup>17</sup> reinforces the hypothesis that urea addition in the loops of Henle does not occur in the TDLs and thus has to occur upstream. Using a mathematical model of the renal medulla, Layton and Layton<sup>83</sup> have shown that urea secretion in the pars recta of either the short loops alone, or the short and long loops, significantly improves the efficiency of the urine concentrating mechanism.

#### **New role for UT-A2 in the transfer of urea to the IM through long and short loops**

The consequences of an active urea secretion in the pars recta are illustrated in Figure 1, right panel. Because AQP1 is not expressed in the TDLs of most short loops and not at all in the TDLs of long loops in the OM,<sup>82</sup> no water can dilute the urea that has been secreted upstream. As a result, urea is most probably more concentrated in the thin-limb lumen than in the surrounding tissues.

**Urea handling in short and long loops.** In the short loops, urea secreted upstream may diffuse out of the TDL in the short subsegment expressing UT-A2 (Figure 1, right panel) rather than be added to the TDL, as assumed previously (Figure 1, left panel). This could seem counterproductive for the urine concentrating mechanism, but it is not so because urea should be rapidly trapped in the nearby countercurrent exchanger formed by the ascending and descending vasa recta. In the vascular bundles of the inner stripe, these vessels run in parallel and in close contact, with virtually no interstitium. The close association of TDLs with the vascular bundles in the inner stripe in rodents with a high urine concentrating ability favors countercurrent exchanges between TDLs and ascending vasa recta.<sup>61–63,65</sup> These exchanges are further facilitated by the fact that UT-A2 is expressed only in the lowest third of the TDL. This allows urea entry in the deepest portion of the vascular bundles in the inner stripe and subsequent countercurrent exchange between ascending and descending vasa recta in the more superficial part of the inner stripe (Figure 1, right panel).

In the long loops, urea secretion in the pars recta will bring concentrated urea down to the IM. This explains the massive addition of urea observed by micropuncture at the tip of Henle's loops in the rat renal papilla (three to five times the filtered load), an addition that, as stated by the authors, could not be explained by passive mechanisms.<sup>66</sup> Part of this urea will diffuse out of the thin limbs toward the interstitium in the portions of the long-loop TDLs expressing UT-A2. It will be taken up by ascending vasa recta and will undergo countercurrent exchange between ascending and descending vasa recta in the IM and inner stripe of OM.

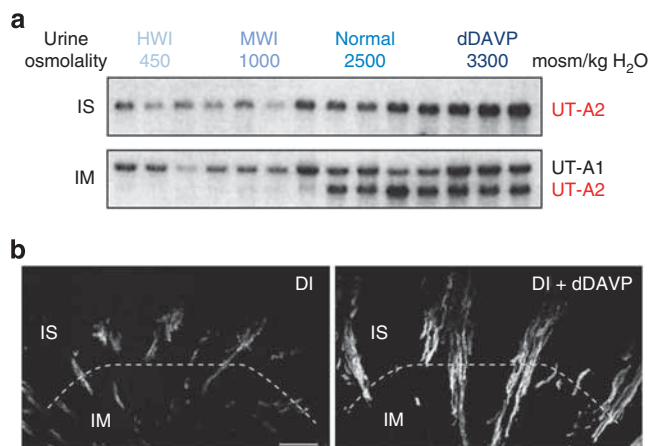
In both short and long loops, only a fraction of the urea will diffuse out in the subsegments expressing UT-A2. The urea remaining in the lumen will continue its way in the loop, pass the macula densa, the distal tubule, and will follow to the CD, thus bringing more urea to the deep IM. In this way, the short and long loops contribute to deliver the secreted urea to the deep medulla via both the countercurrent

exchanger (vascular route) and the CDs (tubular route). This pathway also results in an increased urea concentration in the fluid passing by the macula densa. A higher urea concentration in the thick ascending limb probably allows this segment to bring the luminal sodium concentration to lower values (for the same total osmolality) and could thus participate indirectly in the feedback control of glomerular filtration rate as proposed earlier.<sup>6,7,84</sup>

The vascular route is shorter and is probably more efficient when the inner medullary blood flow remains relatively slow. Countercurrent exchange is less efficient and urea escape from the IM is more significant if the medullary blood flow is increased even modestly, e.g., by vasodilation induced by prostaglandins (produced by inner medullary interstitial cells<sup>85</sup>) or by other vasodilatory mediators. The fact that an increase in medullary blood flow reduces the urine concentrating ability has been known for a long time. It 'washes out' the corticomedullary osmotic gradient, mainly by compromising countercurrent exchange of solutes and short circuit of water in the IM.

In summary, urea secretion in the pars recta brings a relatively large amount of urea in the nephron lumen that adds to the filtered urea remaining after proximal tubule reabsorption. This allows a greater urea delivery to the medullary CD. Some of the secreted as well as filtered urea is short-circuited when flowing in the portions of the TDLs expressing UT-A2 and is carried to the IM through a shorter, vascular route.

**Regulation of UT-A2 abundance by vasopressin and glucagon.** A long-term regulation of the abundance of UT-A2 mRNA and/or protein has been observed in relation to urine osmolality. In normal rats, the intensity of UT-A2 mRNA in the OM was found to decrease dose dependently when urine osmolality was reduced by feeding rats a water-rich gel diet, or to increase when urine osmolality was raised by chronic infusion of dDAVP, a selective vasopressin V2 receptor agonist. Moreover, UT-A2 mRNA appeared in the IM (probably in TDLs of long loops) when urine osmolality rose above 2000 mosm/kg H<sub>2</sub>O (Figure 3a).<sup>86,87</sup> In Brattleboro rats, unable to secrete vasopressin because of a single point deletion in the vasopressin gene, UT-A2 mRNA and UT-A2 protein are only weakly expressed in the inner stripe and not at all in the IM. After a chronic treatment with dDAVP, the abundance of UT-A2 mRNA<sup>88</sup> and protein<sup>81</sup> was markedly increased in the outer stripe and appeared in the long-loop TDLs in the IM (Figure 3b). These changes do not seem to result from a direct V2 receptor-mediated action of vasopressin on the TDL, because this nephron segment does not express V2 receptors. However, autoradiography of rat kidney sections with a radiolabeled specific V1a agonist suggests that V1a receptors may be colocalized with UT-A2 in the TDLs.<sup>89</sup> As dDAVP infusion is known to suppress the endogenous release of AVP (by allowing more water reabsorption and thus decreasing plasma sodium concentration), this observation raises the interesting possibility that V1a receptor stimulation may downregulate UT-A2. This is in good agreement with the

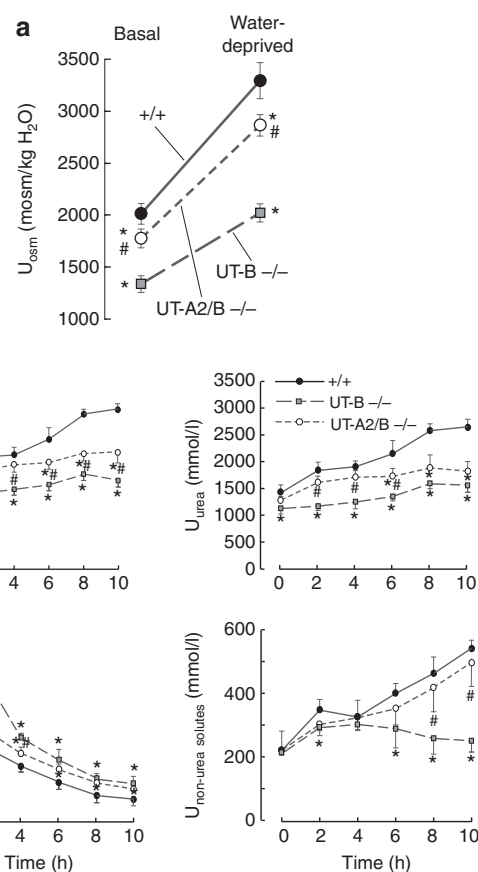


**Figure 3 | Abundance and localization of UT-A2 in the outer medulla.** (a) Northern blot showing the influence of urine concentration on the abundance of UT-A2 mRNA in the inner stripe and inner medulla of rats. To increase or reduce their spontaneous urine osmolality, some rats received a chronic infusion of dDAVP and others were offered their daily food moderately or highly enriched with water in the form of an agar gel (MWI and HWI, respectively). Reproduced with permission from Trinh-Trang-Tan and Bankir.<sup>86</sup> UT-A2 was present in the inner stripe (IS) in all situations, whereas it was present in the inner medulla (IM) only after normal or high V2 receptor stimulation (by endogenous AVP or by dDAVP). (b) *In situ* localization of UT-A2 protein in the IS and in the upper IM of Brattleboro rats with diabetes insipidus (DI), untreated (left) or treated chronically with dDAVP. UT-A2 protein abundance was increased in the IS and appeared in the upper IM under the influence of V2 stimulation by dDAVP. Reproduced with permission from Wade *et al.*<sup>81</sup> MWI and HWI, moderate and high increases in water intake, respectively.

observation that AVP infusion in rats (combining V2 and V1a effects) induced almost no change in UT-A2 mRNA expression, whereas infusion of dDAVP (a pure V2 agonist) induced a marked increase.<sup>88</sup> Other yet unknown mechanisms are probably involved in the upregulation of UT-A2 mRNA and protein expression that has been described in mice with deletion of UT-B, AQP2, or AQP3,<sup>90</sup> because AVP is most probably elevated in these mice that exhibit urine concentrating defects.

A role for glucagon in renal urea handling may be assumed, as this hormone has been shown to increase significantly the fractional excretion of urea ( $FE_{urea}$ ).<sup>91-93</sup> In this context, it is interesting to observe that chronic glucagon infusion in normal rats induced no change in the abundance of UT-A1 and UT-B mRNA in the kidney but doubled the abundance of UT-A2 mRNA in the inner stripe and the upper IM.<sup>94</sup> Moreover, studies in isolated rat IMCD showed that the addition of glucagon to the medium decreased urea reabsorption dose dependently (in microperfused IMCD) and decreased UT-A1 expression (in IMCD suspensions).<sup>95</sup>

Glucagon has been shown to influence the expression of the facilitated UTs. A chronic infusion of glucagon increases the expression of UT-A2 mRNA in the inner stripe and the upper IM (see above) and that of UT-A1 in the IMCD.<sup>95</sup>



**Figure 4 | Effect of an acute urea load in wild-type mice and in mice with deletion of either UT-B alone or UT-B and UT-A2.** Adapted from Lei *et al.*<sup>67</sup> (a) Urine osmolality in basal condition and after 18 h water deprivation. Mice with combined UT-B/UT-A2 KO concentrate urine almost as well as do wild-type mice in both situations. (b) Urine osmolality and flow rate, and urinary concentration of urea and non-urea solutes in conscious mice before (time 0) and 2–10 h after an acute urea load. In the late phase of the study (6–10 h), the concentration of non-urea solutes rose progressively in the urine of the double KO mice, as in wild-type mice, but that of urea did not. This suggests that UT-A2 has a role in the progressive accumulation of urea in the inner medulla. It also shows that urea accumulation is not a prerequisite for accumulation of sodium chloride (the most abundant ‘non-urea solute’) in the medulla. #, significantly different from UT-B KO mice; \*, significantly different from wild-type mice. KO, knockout.

**Building the urea gradient vs. steady state.** Urea secretion in the pars recta and urea recirculation in the countercurrent exchanger initiated by urea escape from the TDLs through UT-A2 are probably especially important for the building of the medullary urea gradient and restoring a high urine osmolality after a period of water diuresis, which washed out this gradient. This secretion and recirculation are probably less important for maintaining the gradient in steady state, once it is established. Recent findings in double UT-B and UT-A2 KO mice support this interpretation.<sup>67</sup> Invalidation of UT-A2 in UT-B-deficient mice partially corrected the urine concentrating defect seen in these mice and brought urine osmolality to values close to those observed in wild-type mice, even after water deprivation (Figure 4a). However, when

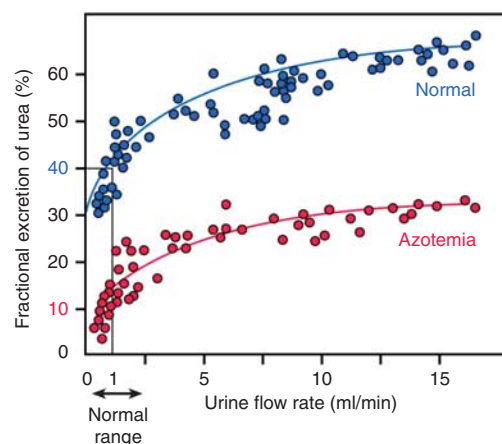


challenged with an acute urea load, it became obvious that both urine osmolality and urine urea concentration could not rise as much in the double KO mice as in wild-type mice. In contrast, the concentration of all other solutes ('non-urea solutes' calculated from the difference between urine osmolality and urine urea concentration) rose to the same level as in wild-type mice (Figure 4b). These observations suggest that UT-A2 contributes to the accumulation of urea in the IM after an acute challenge, but not in steady state,<sup>67</sup> thus explaining why single UT-A2 KO mice do not exhibit a urine concentrating defect.<sup>17</sup> It would be interesting to test whether urine concentrating ability in UT-A2 KO mice would be restored less rapidly after an acute episode of water diuresis, or whether their urine osmolality would be improved after an acute urea load, as performed in other mouse models.<sup>11,96</sup> These results also confirm that the accumulation of NaCl (which represent a large fraction of the 'non-urea solutes') does not depend on the prior accumulation of urea, as already deduced from observations in UT-A1/3 KO mice.<sup>23</sup> In any case, this hypothesis was not compatible with previous observations in rats during water diuresis and in Brattleboro rats with central diabetes insipidus, because in both cases a near-normal NaCl concentration was observed in the IM in the absence of any urea gradient.<sup>97-99</sup>

AQP1 is abundantly expressed along the entire proximal tubule and accounts for most of the high water permeability of this nephron segment. However, an additional aquaporin, AQP7, is expressed only in the pars recta. This is an aquaglyceroporin that, in addition to water, facilitates the transmembrane transport of glycerol and urea. Mice lacking AQP7 do not exhibit a urine concentrating defect and show normal plasma urea level and normal urea concentration in the IM.<sup>100</sup> However, the selective expression of AQP7 in the pars recta suggests that it could have a role in the dynamic phase of restoration of the urea gradient after a period of high diuresis. This hypothesis deserves to be explored in experiments evaluating the time course of recovery of urine concentrating ability and medullary urea concentration after an acute water diuresis in AQP7 KO mice.

### Observations supporting active urea secretion in the pars recta

**Direct evaluation of urea transport in isolated pars recta.** Because micropuncture experiments in rats showed a net urea addition in the loops of Henle, two groups attempted, several decades ago, to evaluate, by *in vitro* micropuncture experiments, whether urea was secreted in the pars recta of the proximal tubule. Both groups used rabbit tubules because this was the only species in which microdissection and micropuncture were possible in those early times. A very modest but significant active urea secretion was observed in the first study (using cortical and medullary pars recta),<sup>101</sup> but this was not confirmed in the second study (using only cortical pars recta).<sup>102</sup> Although a recent study suggests that urea secretion could occur in the rabbit kidney,<sup>103</sup> it seems clear that this animal is not the best-suited species for



**Figure 5 | Urea handling in normal subjects and subjects with familial azotemia.** In normal subjects, the fractional excretion of urea ( $FE_{urea}$ ) is relatively high at high urine flows but decreases with declining urine flow and varies around 40% for urine flows within the normal range. This fall in  $FE_{urea}$  at reduced urine flows is due to urea reabsorption in the collecting duct that increases with a longer contact time and is favored by the direct action of vasopressin on UT-A1/3/4. In subjects with familial azotemia, the handling of urea in the collecting duct seems to be normal, as the usual flow-dependent decline in  $FE_{urea}$  is observed, but the whole relationship is shifted toward lower values. This reduces  $FE_{urea}$  to one-third of normal for urine flows within the normal range. The difference between the two situations may be accounted for by a lack of urea secretion in patients with familial azotemia. Adapted from Bankir and Trinh-Trang-Tan.<sup>7</sup>

demonstrating this secretion because of its protein-poor diet, making urea-nitrogen conservation far more important than efficient urea excretion. To our knowledge, no attempt has been made to evaluate *in vitro* a possible active urea secretion in isolated perfused pars recta of rats, mice, or other mammals.

**Familial azotemia without renal failure.** Unexplained high plasma urea concentration ( $P_{urea}$ ) in the absence of any other sign of renal dysfunction has been occasionally reported in a few human families. Three different papers described in detail such cases of familial azotemia without renal failure.<sup>104-106</sup> The phenotype consists in a three- to fourfold elevation in  $P_{urea}$  due to a markedly decreased  $FE_{urea}$ . Interestingly, the sensitivity of  $FE_{urea}$  to urine flow rate in these subjects exactly paralleled that in healthy individuals, but was largely shifted down (Figure 5). This suggests that the vasopressin-dependent influence on  $FE_{urea}$ , due to flow-dependent reabsorption in the distal nephron and CD, is unaltered in these subjects. The defect was attributed to an excessive urea reabsorption in the pars recta of the proximal tubule, but an editorial accompanying one of these studies suggested that it might be due to a genetic anomaly that interferes with urea secretion in the pars recta.<sup>107</sup>

At present, familial azotemia could adequately be explained by a loss-of-function mutation in the transporter responsible for this active urea secretion. The observations reported in these patients provide a mean to quantify the magnitude of this secretion. Comparison with healthy subjects in the same study shows that urea secretion is responsible for the excretion

**Table 1 | Studies in which fractional excretion of urea was increased by various protocols and reached values exceeding 0.50**

Intervention and condition A and B	Index of GFR Urine collection	FE <sub>urea</sub> Condition A	FE <sub>urea</sub> Condition B	Statistical significance	Reference
<i>Humans</i>					
Increase in diuresis A=low; B=high (after water load)	Inulin	0.32	<b>0.69</b>		104
Single protein meal A=before; B=1–2 h after the meal	Inulin	<b>0.60</b>	<b>0.73</b>	<i>P</i> <0.001	111
Pyrazinoylguanidine (3 days) A=before; B=during treatment	Creatinine	0.45 ± 0.12	<b>0.67 ± 0.16</b>	<i>P</i> <0.001	112
Sickle cell anemia A=healthy subjects; B=sickle cell patients	Inulin	<b>0.59</b>	<b>0.68</b>	<i>P</i> <0.05	113
Dehydration in three patients with central DI A=control; B=dehydrated	Creatinine	0.47, <b>0.60</b> , <b>0.56</b>	<b>1.65</b> , <b>1.10</b> , <b>1.28</b>		114
<i>Dogs</i>					
Meat meal (24 experiments in 3 dogs) (N) A=Before the meal; B=4 h After the meal	Creatinine	0.44 ± 0.03	<b>0.57 ± 0.02</b>	<i>P</i> <0.001	115
<i>Rats</i>					
Change in salt and protein Intake (N) A=normal; B=high protein and NaCl diet	Inulin	<b>0.55</b>	<b>0.80</b>		116
Hypertonic urea infusion (GA) A=control; B=during infusion	Inulin	0.37 ± 0.04	<b>0.67 ± 0.02</b>	<i>P</i> <0.001	21
Change in protein intake (N) A=low protein; B=high protein	Creatinine 24-h urine	<b>0.60 ± 0.07</b>	<b>1.26 ± 0.05</b>	<i>P</i> <0.001	117
Change in urine flow rate (1 week) (N) A=low (dDAVP); B=high (hydrated food)	Inulin 24-h urine	0.43 ± 0.02	<b>1.18 ± 0.18</b>	<i>P</i> <0.001	84
<i>Mice</i>					
UT-A1/3/4 KO on high-protein diet (N) A=wild type; B=UT-A1/3/4 KO	Inulin 24-h urine	<b>0.68 ± 0.05</b>	<b>1.02 ± 0.09</b>	<i>P</i> <0.05	23
UT-B/UT-A2 KO (N) A=wild type; B=UT-B/UT-A2 KO	Creatinine 24-h urine	<b>2.00 ± 0.10</b>	<b>1.50 ± 0.10</b>	<i>P</i> <0.05	67

Abbreviations: FE, fractional excretion; GA, glycine-alanine; GFR, glomerular filtration rate; KO, knockout. All values of FE<sub>urea</sub> >0.50 are shown in bold. For animal studies: N, no anesthesia. UT-A1/3/4 KO: mice with KO of UT-A1, UT-A3, and UT-A4. UT-B/UT-A2 KO: mice with KO of UT-B and UT-A2.

of about half of the excreted urea in water diuresis, and even more at urine flows falling in the physiological range (Figure 5). Such a large urea secretion is fully compatible with an extraction of less than half of the urea entering the medulla via the efferent arterioles of juxtamedullary glomeruli, as explained elsewhere (page 681 in the article by Bankir and Trinh-Trang-Tan<sup>7</sup>).

Familial azotemia could also result from the dysfunction of other urea transporters. (1) A loss of function of a putative active urea transporter expressed in the bladder urothelium (see further) could also explain a selective rise in plasma urea in the absence of renal dysfunction. (2) Theoretically, a reduced efficiency of countercurrent exchange between ascending and descending vasa recta due to a loss of function of UT-B should result in a greater return of urea through venous blood into the peripheral circulation and a higher P<sub>urea</sub>. This is the case in UT-B KO mice,<sup>11,16</sup> which show a P<sub>urea</sub>-value 50% higher than that of wild-type mice. This difference is much more modest than the threefold difference seen in humans with familial azotemia. Moreover, blood urea nitrogen is not significantly altered in UT-B-deficient subjects.<sup>108</sup> This species difference in phenotype between UT-B-deficient mice and humans probably reflects the fact that countercurrent urea exchange between ascending and

descending vasa recta is much less important in humans than in mice, because of their much lower urine concentrating ability and much shorter relative length of the IM.<sup>22</sup>

**High fractional excretion of urea observed in some situations.** Because about 50% of the filtered urea is invariably reabsorbed in the proximal convoluted tubule<sup>19,55,56,59,78,109,110</sup> and escapes the kidney through the venous cortical blood flow, at least 50% of filtered urea cannot be excreted in final urine. Accordingly, values of FE<sub>urea</sub> above 50%—not 100% as often believed—reveal net urea secretion. Table 1 lists several reports in which FE<sub>urea</sub> was found to exceed 50% (and even occasionally 100%) in humans, dogs, or rats, either in basal conditions or after a single meat meal, a high-protein diet for a few days, or specific experimental maneuvers such as increases in diuresis, or infusion of glucagon (Table 1). Other situations in which high FE<sub>urea</sub> was observed include water diuresis,<sup>84,118,119</sup> saline diuresis,<sup>120</sup> chronic renal failure,<sup>121,122</sup> sickle cell anemia,<sup>113,123</sup> or infusion of prostaglandins<sup>124,125</sup> or glucocorticoids.<sup>93</sup> The low P<sub>urea</sub> and high FE<sub>urea</sub> observed in sickle cell disease may be due to a more efficient urea secretion in the pars recta probably favored by the longer transit time of sickled red cells in outer medullary vasa recta. FE<sub>urea</sub> exceeding 100% was observed in rats and dogs during intense osmotic diuresis,<sup>120,126</sup> after providing hyperosmotic urea as sole



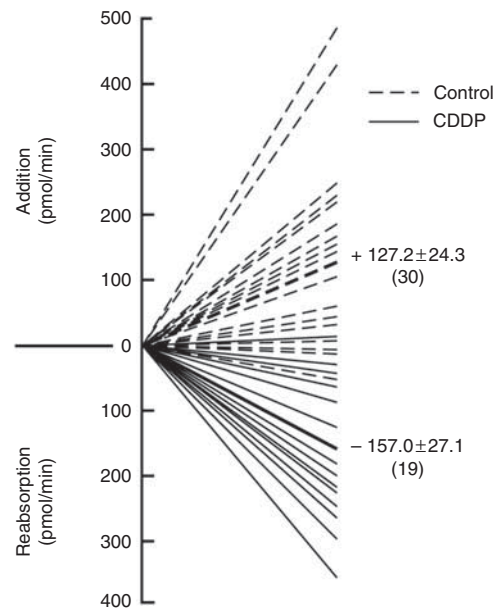
drinking fluid,<sup>21</sup> or after the combination of high protein and high salt intake.<sup>116</sup> Other situations with  $FE_{urea}$  higher than 50% are shown in Table 1.<sup>111,112,114,115</sup>

During an episode of dehydration in normal subjects, a fall in  $FE_{urea}$  and a rise in serum urea were observed, as expected from the known action of vasopressin on urea reabsorption in the CD. In contrast, in patients with diabetes insipidus, dehydration induced changes in the opposite direction:  $FE_{urea}$  rose above 100% and serum urea fell markedly.<sup>117</sup> This high  $FE_{urea}$  observed in the absence of vasopressin suggests that urea is abundantly secreted in the nephron but that, in healthy subjects, this secretion is largely compensated by a strong vasopressin-dependent urea reabsorption.

Recent studies in mice suggests that  $FE_{urea}$  is higher in this species than in other mammals and could even exceed 100%, whether filtration rate is evaluated by inulin<sup>23</sup> or creatinine clearance, with a reliable creatinine assay (Table 1).<sup>11,67</sup> As explained elsewhere, this high  $FE_{urea}$  in mice is probably related to the huge turnover of urea that imposes on the kidney a considerably larger load of urea to excrete, because of the more intense metabolism and much greater food intake per unit body weight observed in smaller animals.<sup>22</sup>

$FE_{urea}$  can be altered pharmacologically by pyrazinoylguanidine (a drug that shares some homology with pyrazinamide, an inhibitor of urate secretion). This drug increased the urea/creatinine clearance ratio in mongrel dogs and Dalmatian coach hounds.<sup>18</sup> In patients with chronic renal failure, oral treatment with pyrazinoylguanidine for 3 days increased the urea/creatinine clearance ratio from  $0.45 \pm 0.12$  to  $0.67 \pm 0.16$  ( $P < 0.001$ ).<sup>112</sup> Stop-flow experiments in dogs showed that urea secretion, inhibitable by pyrazinoylguanidine and analogs, probably occurs in the most distal portion of the proximal tubule.<sup>127</sup>

**Consequences of impaired pars recta function.** *Cisplatin*, an antineoplastic drug, is known to be nephrotoxic and to impair the urine concentrating ability. Pathological changes are localized to the S3 segment of the proximal tubule in the outer stripe.<sup>128,129</sup> Safirstein *et al.*<sup>130</sup> described in detail the renal handling of the different solutes and the urine concentrating defect observed in rats 3 days after cisplatin administration.<sup>130</sup>  $P_{urea}$  rose about threefold. Solute concentrations in the renal medulla were markedly reduced, but that of urea was reduced to a much greater extent than that of sodium, suggesting a selective defect in medullary urea accumulation. Although urine flow rate was markedly increased,  $FE_{urea}$  decreased (a change opposite to what is usually observed). Micropuncture studies in the late proximal and early distal tubule on the surface of the renal cortex of control and cisplatin-treated rats allowed the evaluation of solute movements in the loops of Henle of superficial nephrons. The fraction of filtered urea delivered to the late convoluted proximal tubule was closely comparable in the two groups. However, as shown in Figure 6, the usual net addition of urea, known to occur in the loop of Henle, was observed only in control rats, whereas net urea reabsorption



**Figure 6 | Influence of cisplatin on urea handling in the loop of Henle.**

Results obtained during micropuncture study in control rats and rats treated previously with cisplatin, a drug that impairs the function of the pars recta. Lines represent the calculated difference between the rate of urea delivered to the early distal tubule and that measured in the late proximal tubule in the same nephron. In control rats (dotted lines,  $n = 30$  nephrons), significant amounts of urea are added to the loop of Henle, whereas in cisplatin-treated rats (continuous lines) there is a net reabsorption of urea ( $n = 19$  nephrons). This (passive) reabsorption probably also occurs in control rats and is overridden by a higher urea secretion. It may thus be assumed that urea secretion amounts to  $157 + 127 = 284$  pmol/min per nephron and  $8.5$   $\mu$ mol/min per kidney (assuming 30,000 nephrons per kidney, with similar secretory capacity). Adapted from Safirstein *et al.*<sup>130</sup>

was found in cisplatin-treated rats.<sup>130</sup> These results indicate that the pars recta is responsible for the urea addition observed in control rats and that cisplatin-induced damage impaired this secretion. Cisplatin administration in dogs lead to similar observations.  $P_{urea}$  rose 3-fold, whereas plasma creatinine was increased by only 1.8-fold,<sup>131</sup> suggesting a marked fall in  $FE_{urea}$ , likely due to impaired secretion by the damaged pars recta, as shown in rats.

The data provided in the micropuncture study of Safirstein *et al.*<sup>130</sup> allow a quantitative evaluation of the magnitude of urea secretion in rats. In controls, the net addition of urea between the late proximal and early distal tubule of a punctured superficial nephron was 127 pmol/min, whereas the net urea reabsorption in a nephron of cisplatin-treated rat was 157 pmol/min. Thus, the total amount of urea secreted per superficial nephron in control rats should amount  $127 + 157 = 284$  pmol/min. Urea secretion in juxta-medullary nephrons is likely even higher because of the tortuous course of their pars recta that lengthens the contact area with ascending vasa recta in the inner stripe. Factoring 284 pmol/min by 30,000 nephrons per kidney suggests that pars recta in one kidney of control rats secreted urea at a rate of about  $8.5$   $\mu$ mol/min, a value close to that of glomerular

urea filtration (9.8  $\mu\text{mol}/\text{min}$ ). These results indicate that, in rats, urea secretion probably contributes to about half of the urea excreted in the urine. Interestingly, this is also the conclusion drawn from comparison between healthy subjects and subjects with familial azotemia (see above). This is fully compatible with the fact that medullary blood flow, issued from postglomerular blood of the juxtamedullary glomeruli, carries in plasma and erythrocytes more urea than that delivered by the glomerular filtrate to the pars recta (see calculations in Table 2).

*Cyanide* is a drug that induces a reversible inhibition of oxidative metabolism. Elegant studies conducted in dogs by *in situ* kidney perfusion showed that, after infusion of cyanide in the left renal artery, urea clearance decreased dramatically to 1/10 of the previous value, in spite of a marked rise in urine flow rate and unchanged inulin and creatinine clearances. The right vehicle-infused kidney showed no change.<sup>132</sup> The author, in 1949, assumed that kidney cells have 'to perform work involving the use of enzyme systems, inhibited by cyanide, to prevent urea from diffusing back from the concentrated tubular urine'. In the light of our present knowledge, the fall in urea clearance, in

**Table 2 | Quantitative aspects related to urea filtration and secretion**

*The gross calculations below show that the amount of urea potentially available for secretion from the vasa recta into the pars recta is quite significant compared to luminal urea issued from filtration and urea issued from recycling from the collecting duct.*

*Amount of urea flowing in vasa recta blood (issued from efferent arterioles of juxtamedullary glomeruli) and available for uptake in case of active pumping from surrounding cells.*

*Vasa recta urea=15% of urea in renal blood flow (RBF), assuming that medullary blood flow is 15% of total RBF. Note that both urea in plasma and red cells is available for rapid use because red cells express the facilitated urea transporter UT-B.*

*Amount of urea flowing in the pars recta lumen of all nephrons.*

*Pars recta urea=urea in RBF  $\times$  0.55 (if hematocrit=45%)  $\times$  0.25 (if filtration fraction is 25%)  $\times$  0.50 (if half of the urea is reabsorbed in the convoluted proximal tubule)=7% of the urea present in the blood perfusing the whole kidney.*

*Amount of urea possibly added to ascending vasa recta blood as a result of urea delivery at the tip of the papilla by UT-A1 in the terminal inner medullary CD.*

Fractional urea excretion in antidiuresis is about 30–40%. Assuming that half of the urea is recycled through UT-A1 instead of being excreted means that <20% of the filtered urea can be returned in the ascending vasa recta, i.e., about 7%. And less than that should reach the outer stripe and be available for secretion because of countercurrent exchange with descending vasa recta in the inner medulla and inner stripe of outer medulla.

*Amount of urea produced locally by hydrolysis of arginine.*

The pars recta can hydrolyze arginine into urea plus ornithine. But the arginine supply in this area is relatively low because arginine concentration in the blood is only about 200  $\mu\text{mol}/\text{l}$ . Thus, the ability to form urea in the pars recta is probably relatively modest. But it may have a significant contribution in some situations, or if unknown pathways can bring to the pars recta some arginine produced in the convoluted part of the proximal tubule.

spite of a marked increase in urine flow rate (which usually increases urea clearance), may be best explained by an inhibition of active urea secretion in the pars recta.

*Acute renal failure* may depend on either prerenal hemodynamic factors or on acute tubular necrosis. Several studies have shown that values of  $P_{\text{urea}}$ ,  $FE_{\text{urea}}$ , or the ratio of urine-to-plasma urea concentration (U/P ratio) can be used to differentiate these two conditions.<sup>133–135</sup> In the case of acute tubular necrosis, the U/P urea ratio is largely reduced and  $P_{\text{urea}}$  rises significantly more than does plasma creatinine concentration. This may be easily explained by an inability of the damaged pars recta to secrete urea, thus resulting in a greater reduction in urea clearance than in creatinine clearance, the latter being sensitive only to the reduction in glomerular filtration rate.

### Species differences in renal urea handling

In studies conducted several decades ago, Schmidt-Nielsen<sup>136</sup> had already proposed that urea excretion was regulated because  $FE_{\text{urea}}$  could vary largely with changes in the protein content of the diet, especially in ruminants.<sup>136</sup> Actually, marked differences in urea handling are observed in different mammalian groups in relation to their widely different natural diets and habits. Renal urea reabsorption takes place especially in herbivores for the purpose of nitrogen conservation, whereas renal urea secretion might be more intense in carnivores that eat a protein-rich diet and consume large amounts at distant intervals. Interestingly, urea permeability in red cells (which also express UT-B) also differs according to the diet in different groups of mammals, being far higher in carnivores than in herbivores and intermediate in humans, pigs, and rodents.<sup>137</sup>

In sheep (and probably other ruminants),  $FE_{\text{urea}}$  can fall to extremely low levels when their protein intake is strongly restricted.<sup>138,139</sup> This can be explained by the active urea reabsorption that has been characterized in the initial IMCD<sup>39,43,140</sup> in rats fed a low-protein diet for several weeks. The reabsorbed urea is then broken down by urease-possessing bacteria in the rumen (in ruminants) or colon (in other mammals), and the nitrogen can be reused by the mammalian organism.<sup>7</sup> Urea accesses the rumen or colon lumen by facilitated UTs that share homology with those expressed in the kidney.<sup>35,36</sup>

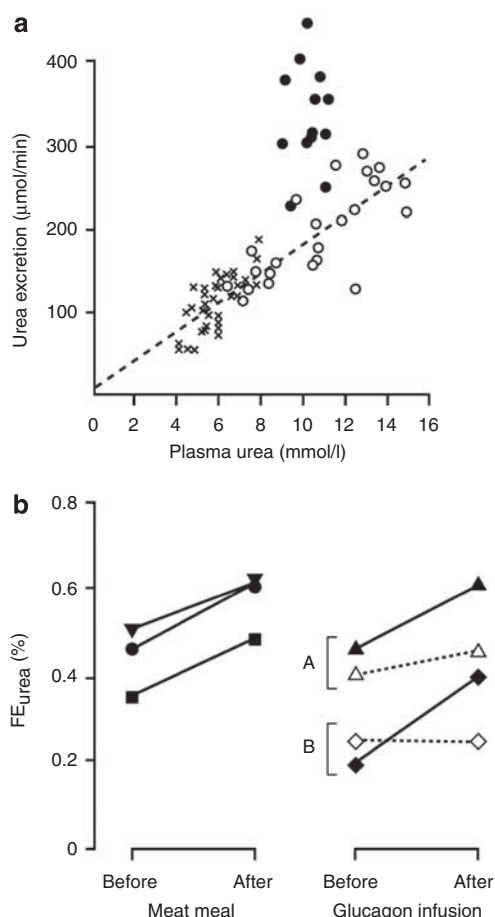
In carnivores, the kidney exhibits a specialized 'S4' segment in the proximal tubule (in addition to the classical S1, S2, and S3 segments) with anatomical features suggesting solute secretion rather than solute reabsorption.<sup>141</sup> This observation could explain why dogs are able to excrete much more urea per gram kidney mass than other mammals.<sup>142</sup> Small rodents in which high  $FE_{\text{urea}}$  has been reported may also exhibit intense urea secretion because of their high food intake associated with their high metabolic rate linked to their small size.<sup>22</sup> In other mammals including humans, this secretion most likely also occurs, as suggested by familial azotemia without renal failure (see above), even if it is more difficult to demonstrate.

### Role of glucagon in the regulation of urea excretion

Within a given species, urea excretion is probably selectively stimulated after a protein meal, as suggested by elegant studies in dogs. O'Connor and Summerill<sup>115</sup> showed that  $FE_{urea}$  increased markedly 4 h after a regular meat meal, whereas it did not change when an equivalent load of urea was given by means of a stomach tube (Figure 7a and b, left). Ingestion of proteins is known to stimulate glucagon secretion, and glucagon is a potent stimulus of ureagenesis in the liver.<sup>143–149</sup> This hormone is considered to have a major role in the disposal of nitrogen wastes.<sup>150,151</sup> Moreover, in the face of this increased solute load, it contributes to water economy. Studies in anesthetized rats have shown that glucagon infusion increases medullary hypertonicity,<sup>152</sup> urine

concentration,<sup>153</sup> and solute-free water reabsorption, along with an increase in urea excretion.<sup>91</sup>

Glucagon has also been shown to increase  $FE_{urea}$  (Figure 7b, right), thus demonstrating a tubular action independent of, and additive to, its influence on glomerular filtration rate.<sup>91,92,154</sup> Glucagon could improve the efficiency of urea excretion by stimulating active urea secretion. An action of glucagon in the pars recta is strongly suggested by micro-puncture studies in rats.<sup>155</sup> This action could possibly be mediated by specific receptors<sup>156</sup> that would not be coupled to adenylate cyclase, contrary to those located in the distal nephron.<sup>157</sup> Alternatively, this stimulation could also be induced by circulating, extracellular cAMP originating from the liver under the influence of glucagon, as suggested previously.<sup>158</sup> In addition, glucagon has been shown to influence the expression of the facilitated UTs (see above). See Notes added in proof (Note 1).



**Figure 7 | Acute regulation of urea excretion.** (a) Relationship between urea excretion and plasma urea concentration in the same dog studied either in control conditions (crosses), after a meat meal (closed circles), or after an infusion of urea bringing the same amount of nitrogen (open circles; 8–10 experiments in each condition). The influence of the protein meal clearly differed from that of the urea infusion. Reproduced with permission from O'Connor and Summerill.<sup>115</sup> (b) Left: influence of a protein meal on  $FE_{urea}$  in three dogs (mean of 8–10 experiments per dog). Drawn after O'Connor and Summerill.<sup>115</sup> Right: influence of an acute infusion of glucagon (solid line) or vehicle (dotted line) in anesthetized rats. Drawn after Ahloulay *et al.*<sup>91</sup> (a), and Knepper *et al.*<sup>154</sup> (b). Both the meat meal and the infusion of glucagon increased  $FE_{urea}$  by about 30%.

### POSSIBLE LINKS BETWEEN UREA AND GLUCOSE HANDLING

Given the multiple studies that support the existence of an active urea secretion in the mammalian kidney, it is surprising that the transporter responsible for this secretion has not yet been identified. As recalled above, active urea uptake has been characterized in unicellular organisms and plants, but no homology with any of the corresponding active urea transporters has been found in the mammalian genome. Actually, the transporter secreting urea in the pars recta may have been cloned already but thought to have another function, as has recently been the case for the protein encoded by the gene *SLC2A9*. This membrane protein, initially identified as a facilitated glucose/fructose transporter (GLUT9), has now been shown to be, in fact, a uric acid transporter.<sup>159,160</sup> Genetic variants or experimental inactivation of this gene are associated with reduced urinary urate clearance and increased serum urate concentration, suggesting impaired urate secretion.<sup>161,162</sup> Interestingly, a secondary active, Na-coupled, glucose transporter, SGLT1, which is abundantly expressed in the mammalian pars recta, has been shown to transport also urea.<sup>163,164</sup> Thus, obviously, some membrane proteins involved in sugar transport (GLUT9 and SGLTs) can also transport nitrogen wastes such as uric acid and urea, respectively.

Actually, the fate of glucose and that of urea are often linked. In the liver, gluconeogenesis and ureagenesis are always associated<sup>165</sup> so as to dispose of the nitrogen atoms of the amino acids used for gluconeogenesis, whether these amino acids come from the food or from endogenous stores (during fasting).<sup>7,150,151</sup> Glucagon, the hormone that promotes glucose formation, is also a potent stimulus for urea synthesis by the liver, as mentioned above.<sup>149,166</sup> In the kidney, the nephron subsegment where urea is probably secreted is also involved in both gluconeogenesis (from various substrates) and formation of urea (by hydrolysis of arginine).<sup>167</sup> It is tempting to assume that glucagon could promote simultaneously gluconeogenesis,<sup>168</sup> urea formation,<sup>169</sup> and urea secretion in the pars recta. See Notes added in proof (Note 1).

Although the pars recta shares a number of characteristics with the convoluted part of the proximal tubule, it exhibits several anatomical and functional features that make this segment quite different (Table 3). Its vascular blood supply is different (Figure 2), and it expresses a few additional enzymes (arginase<sup>167</sup>) and transporters not present in the earlier portion of the proximal tubule, namely SGLT1 (see below) and AQP7 (a urea-permeable aquaporin).<sup>100</sup>

### Could SGLT1 be involved in urea secretion?

Two different secondary active, sodium-dependent glucose transporters are expressed in the luminal membrane of the proximal tubule, SGLT1 and SGLT2. However, their localization along this nephron segment, their affinity for glucose, and their stoichiometry are different.<sup>170</sup> SGLT2 is expressed

**Table 3 | Specific metabolic pathways and membrane proteins in the medullary pars recta and specific environment in the outer stripe of the outer medulla**

#### Metabolic pathways

*Gluconeogenesis* (preferentially from lactate) that may result in increased intracellular glucose concentration.

*Urea formation* (+ ornithine) from arginine hydrolysis by arginase (arginine taken up from the basolateral side of the cells) that may result in increased intracellular urea concentration.

(many other metabolic pathways not mentioned here, e.g., ammoniogenesis)

#### Membrane transporters

*SGLT1* Sodium-glucose transporter, also transporting urea

*AQP7* Aquaglyceroporin also permeable to urea

(many other transporters not mentioned here, e.g., NaPi, organic anion transporter, and so on)

#### Membrane receptors

*Glucagon or cAMP receptors?* Possible regulation either directly through glucagon or through extracellular cAMP released in the blood by the liver under the influence of glucagon?

(many other receptors not mentioned here, e.g., parathyroid hormone receptor)

#### Environment

*Almost no significant direct arterial blood supply* (scarce branching of the efferent arterioles of deep glomeruli).

*Blood supply comes mainly from wide numerous ascending venous vasa recta (AVR).* These vasa recta ascend from the inner medulla through vascular bundles in inner stripe of the outer medulla, allowing intense countercurrent exchange. Blood velocity is low in these AVR because their cross-sectional area is far greater than that of corresponding descending vasa recta. Moreover, there is a large surface area of contact between pars recta and these AVR. Both this long contact time and large contact area favor transmembrane transport along the peritubular side of pars recta cells.

#### Secretion

*The pars recta is known to secrete a number of organic molecules* (uric acid, hippurate, cyclic nucleotides, and so on) and xenobiotics.

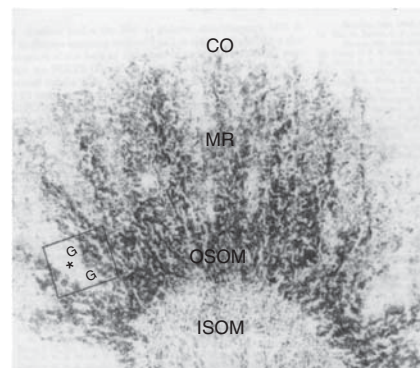
*These molecules are extracted from ascending vasa recta blood* that is issued from the efferent blood of juxtamedullary glomeruli and has flown through the outer and inner medulla before flowing around the pars recta.

*The secretory activity of the pars recta probably exceeds its reabsorptive activity.* Water follows the secreted solutes because the pars recta is freely permeable to water. This means that a net addition of fluid and solutes is likely to occur in this nephron segment.

in most of the proximal tubule. In contrast, SGLT1 is restricted to the pars recta, and its expression is the highest in the outer stripe,<sup>171</sup> as shown in Figure 8. SGLT1 has a higher affinity for glucose than SGLT2; it transports one molecule of sugar along with two Na, whereas SGLT2 transports one sugar with one Na.

Transport studies in amphibian oocytes expressing SGLT1 have shown that this cotransporter can also transport urea in two independent ways. First, besides the sites involved in the coupled glucose and sodium transport, this highly complex membrane protein (over 650 amino acids and 14 transmembrane domains) exhibits a urea channel that allows urea and water to diffuse along their concentration gradient, a process that is inhibitable by phloretin. Second, urea is also transported uphill when the cotransporter is activated by the presence of sugar, and the rate of sugar-dependent urea uptake is directly proportional to the rate of Na<sup>+</sup>-glucose-H<sub>2</sub>O cotransport and is inhibited by phlorizin.<sup>163</sup> Interestingly, Sce-DUR3, the active urea transporter identified in yeast, and the different mammalian SGLTs belong to the same cotransporter family SLC5.<sup>172</sup> This observation further supports the concept of a significant link between urea and glucose through evolution.

It is usually assumed that SGLT1 contributes to reabsorb the small amount of glucose that remains in the lumen of the pars recta after most of the filtered glucose has been reabsorbed upstream through SGLT2. This is confirmed by recent observations in SGLT1 KO mice.<sup>173</sup> Not often mentioned is the fact that glucose concentration within the cells of the pars recta may be relatively higher than in other cells because of intense local gluconeogenesis (see further). SGLT1 can work in both directions, depending on the ligand concentration on each side of the membrane.<sup>174</sup> Thus, it is possible to assume that SGLT1 might transport some glucose from the cell cytoplasm into the lumen, especially in the long loops, and preferentially so at certain times of the diurnal



**Figure 8 | Localization of SGLT1 in the rat kidney.** *In situ* hybridization of SGLT1 showing that it is localized to the medullary rays (MR) of the cortex (CO) and in the outer stripe (OSOM) but not in the inner stripe (ISOM) of the outer medulla, a localization corresponding to the pars recta of the proximal tubule. Reproduced with permission from Lee et al.<sup>177</sup> \*, blood vessel. G, glomeruli.



cycle. In other words, it is conceivable that some glucose may undergo secondary active, sodium-dependent secretion in the pars recta and that some urea may be cotransported along with glucose, as has been demonstrated in oocyte experiments.<sup>164</sup> The fate of the secreted glucose will be discussed in the next section.

### Could gluconeogenesis in the pars recta contribute to urine concentration?

The whole proximal tubule is able to perform gluconeogenesis from various substrates. Some studies however suggest that, in usual conditions, the S3 subsegment is involved more strongly in this metabolic pathway than S1 and S2 subsegments.<sup>175,176</sup> Moreover, PEPCK, the enzyme that is the limiting factor for gluconeogenesis, and/or its mRNA are more abundantly expressed in the pars recta than in the convoluted part of the proximal tubule.<sup>175,177</sup> Lactate appears to be the preferred substrate for gluconeogenesis in this part of the nephron in several species including humans.<sup>178–180</sup>

Why would the S3 segment extract substrates from the peritubular blood and spend six ATP molecules per newly formed glucose in the OM when so much more glucose can be synthesized in the liver? Could this renal-borne glucose serve some function in the kidney itself? Instead of being released in peripheral blood, this glucose might be secreted into the lumen and be carried through the thin limbs down to the inner stripe of the OM, and the IM (through the short-looped and the long-looped nephrons, respectively). In the inner stripe, renal-borne glucose could be used as fuel in the medullary thick ascending limbs, assuming that glucose can be taken up by these cells through their luminal membrane. In the IM, this renal-borne glucose may contribute to the urine concentrating mechanism in several ways as described below.

Countercurrent exchange of oxygen between arterial (descending) and venous (ascending) vasa recta limits oxygen delivery to the IM. In this kidney region, anaerobic glycolysis is used to provide energy. Lactate formed during this process is distributed along a distinct corticomedullary concentration gradient, qualitatively similar to the gradient known to occur for sodium and chloride.<sup>181</sup> Part of the lactate is trapped locally by countercurrent exchange between venous (ascending) and arterial (descending) vasa recta, so that lactate concentration at the tip of the papilla reaches values four- to sixfold higher than in the cortex.<sup>181,182</sup> It has been proposed that anaerobic glycolysis, forming two lactate molecules from one glucose, could contribute to increase interstitial medullary osmolality in two ways: first, by providing two ATP molecules per glucose to support the metabolism, and, second, by generating two osmoles out of one, thus resulting in a 'single osmotic effect' that helps drive water from the surrounding structures. Two independent mathematical models have shown the benefit of this process on urine concentration.<sup>183–185</sup> The most elaborate model,<sup>183</sup> although not integrating the three-dimensional architecture of the IM illustrated in more recent studies,<sup>186</sup> and simulating

nephrons and vessels as single tubes, shows that the conversion of as little as 15% of the medullary blood glucose into lactate improves urine osmolality at the tip of the papilla by about 600 mosm/l, an effect mostly accounted for by accumulation of NaCl.<sup>183</sup>

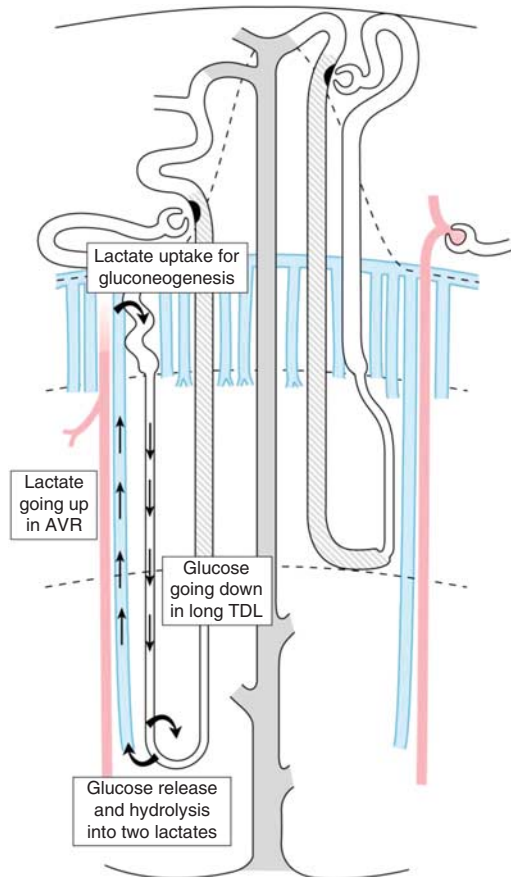
This model,<sup>183</sup> as well as that of Zhang and Edwards,<sup>185</sup> assumed that glucose used in the IM was coming from the blood perfusing the IM through descending vasa recta. We propose that glucose available for conversion into lactate could originate from gluconeogenesis in the pars recta and be delivered directly to the IM via the descending limbs of Henle. Some studies have identified glucose transport in the TDLs of short loops of Henle, possibly along with urea.<sup>187</sup> It is conceivable that a similar transport exists also in long loops. Even if the number of long loops decreases progressively along the IM,<sup>186</sup> this pathway could ensure a functionally significant additional glucose delivery to this region because the luminal glucose, added upstream by an active secretion, would be several fold more concentrated than that present in the descending vasa recta.

Nephrogenic glycosuria in the absence of hyperglycemia,<sup>188</sup> observed in rare circumstances, could, at least in some cases, be due to the failure of secreted glucose to be broken down into lactate when flowing through the IM, and/or to be taken up by the thick ascending limbs.

### Could an intrarenal Cori cycle allow the conversion of chemical energy spent in the outer stripe into osmotic energy carried to the IM?

Because ascending vasa recta are highly fenestrated, the medullary interstitium is always equilibrated with vasa recta blood. As a result, lactate, accumulated in the IM as explained above, should, similar to all other medullary solutes, be continuously taken up by ascending vasa recta and returned to the arcuate veins at the corticomedullary border and back to the general circulation. However, in the outer stripe, some of this lactate can be taken up by the proximal tubule pars recta to serve as a substrate for gluconeogenesis. As recalled earlier, the anatomical arrangement of vessels and tubules in the outer stripe favors a close contact between ascending vasa recta and descending pars recta,<sup>1,62,64,65</sup> especially those of long loops that exhibit a tortuous course that increases S3 epithelium abundance.

The reuse of lactate for gluconeogenesis in the outer stripe after glucose breakdown into lactate in the IM forms an intrarenal Cori cycle (Figure 9). The traditional Cori cycle operates between liver and extrahepatic tissues such as muscles. Glucose is used in these tissues to provide energy through glycolysis, and the resulting lactate diffuses into the blood stream, is reconverted into glucose in the liver, and can be resupplied to extrahepatic tissues. This cycle consumes six ATPs for gluconeogenesis and releases two ATPs during glycolysis. The net cost is thus four ATPs. This cycle has been reported to occur within the rat kidney studied *in vitro*.<sup>189</sup> If such a glucose-lactate-glucose cycling occurs within the kidney, it will allow the conversion of chemical energy spent



**Figure 9 | Intrarenal Cori cycle.** The Cori cycle consists of the breakdown of one glucose into two lactates plus two ATPs, and the resynthesis of one glucose from two lactates, requiring six ATPs. The net energetic cost of this cycle is four ATPs. In the kidney, we propose that glucose (formed by gluconeogenesis in the pars recta) is secreted in the tubule lumen and flows down to the inner medulla where it can diffuse into the interstitium and be broken down into two lactates, thus increasing the osmolality of the inner medulla. Ascending vasa recta (AVR) take up this lactate, which can then, in part, return to the inner medulla by countercurrent exchange with the descending vasa recta. Some lactate, remaining in the ascending vasa recta in the outer stripe, can be taken up by the pars recta and used again as a substrate for gluconeogenesis. TDL, thin descending limb.

in the upper part of the OM, still relatively well oxygenated (lying close to the arcuate arteries), into metabolic plus osmotic energy (two ATPs plus two osmoles) delivered to the deep IM.

This hypothesis is attractive because it fits well with the facts that PEPCK is most abundant in the S3 segment, that lactate is the preferred substrate for gluconeogenesis in the pars recta, that lactate is abundant in the IM, and that vasa recta issued from the IM supply nutrient blood to the OM pars recta (Figure 2). Further studies are needed to evaluate whether this hypothesis holds true. The mathematical model that has evaluated the contribution of anaerobic glycolysis to urine concentration<sup>183</sup> could probably be adapted to simulate the reuse of lactate from ascending vasa recta to pars recta, local gluconeogenesis, and subsequent delivery of glucose to the IM via the long descending thin limbs.

### DOES ACTIVE UREA REABSORPTION IN THE LOWER URINARY TRACT CONTRIBUTE TO MAINTAIN HIGH UREA CONCENTRATION IN THE URINE?

As recalled earlier, urea concentration in the urine may be a 100-fold higher than in plasma and extracellular fluids in humans, and even more in rodents. Even without expressing a facilitated UT, all cell membranes are permeable to urea to some extent. If a marked transepithelial difference in urea concentration is present, urea concentration will equilibrate across an epithelium within minutes to an hour instead of seconds when a facilitated UT protein is present. In the kidney, the expression of epithelial UTs is limited to short portions of the thin limbs and IMCDs, allowing rapid movements of only limited amounts of urea between well-defined juxtaposed tubular and/or vascular structures. In contrast, after urine exits the kidney, it flows and/or stays for hours in structures that all abundantly express UT-B (as well as AQP3) in the basolateral membrane of their urothelium, including the pelvic wall, ureter, and bladder,<sup>190,191</sup> which are in permanent contact with surrounding extracellular fluids iso-osmotic with plasma. Why UT-B is expressed in these structures is not yet understood, and the experiments conducted so far in UT-B-null mice have not contributed information on this matter. See Notes added in proof (Note 2).

Urothelium umbrella cells exhibit a unique morphology that is assumed to contribute to their ability to prevent back diffusion of concentrated solutes into the extracellular space. Nevertheless, it is intriguing that a facilitated UT is abundantly expressed in structures that need to maintain a very high transepithelial urea concentration difference, thus leaving the luminal membrane as the only barrier between urine and the body's extracellular fluids. The presence of UT-B in the basolateral membrane of the urothelium makes more sense if one assumes that an active (or secondary active) urea transporter is expressed on the luminal side, permanently reclaiming urea that would be at risk of escaping the bladder.

Thus, it seems reasonable to hypothesize that the ureter and bladder are equipped with an active urea transporter that reabsorbs urea. This transporter probably has a high affinity and a low capacity, and could be homologous to the transporters that have been functionally characterized (but not identified) in the elasmobranch gills<sup>26</sup> and in the ventral skin of some toads.<sup>27–32,192</sup> It is probably different from the transporter expressed in the upper IMCD, which initiates the urea-nitrogen salvage in ruminants and low protein-fed rats (see above) and from the transporter that is responsible for urea secretion in the pars recta (see above). These latter two transporters probably have a lower affinity and a higher capacity.

As in many studies of transepithelial transport, the small urea leakage observed in the ureter<sup>193</sup> or bladder,<sup>194</sup> may be the net result of two opposite movements almost compensating each other, including a passive escape and a partial active recovery. During bladder infections, the bladder becomes permeable to solutes.<sup>195–197</sup> This could partly result from a significant downregulation of several transporters including this active urea transporter. The same may be true in

hibernating bears.<sup>198,199</sup> Nitrogen conservation during hibernation is ensured by a permanent recycling of urea-nitrogen between the bladder (which becomes permeable to urea) and the digestive tract where urea can be degraded by bacteria and its nitrogen reused by their host, as in herbivores.<sup>35,36</sup>

Interestingly, in rats, a small but significant active Na-dependent, phloretin-insensitive urea secretion has been observed in the deepest subsegment of the IMCD (i.e., along a much shorter length than that expressing UT-A1/3).<sup>46</sup> Because of the very small number of CDs remaining at the tip of the papilla, and of the very short length of CD in which it occurs, this active transport can add only very small amounts of urea in the CD lumen. Moreover, the high UT-A1/3-mediated urea permeability of these ducts and the direction of the transepithelial difference in urea concentration in this region should induce all the secreted urea to diffuse back into the medullary interstitium. This active urea secretion in the very terminal portion of the IMCD is thus puzzling. We assume it could be the early manifestation of an active transport that extends further down along the urothelium of the pelvis, ureter, and bladder, and contributes to prevent the dissipation of the urine-to-blood urea gradient in the lower urinary tract. This hypothesis requires further investigation.

## CONCLUSIONS

In the past few decades, the handling of urea in the kidney has been largely neglected, and few clinical or experimental studies now report data about urea, although this solute represents about 40% of all urinary solutes in normal human urine. Most recent renal textbooks include chapters on water and electrolyte physiology but no chapter on urea. Urea is often omitted in fluid used in *in vitro* experiments, or, if present, the normal proportion between urea and other solutes is not adequately taken into account. The normal urea/Na concentration ratio in the urine is largely minored in most *in vivo* studies in anesthetized animals because of the intravenous infusion of isotonic saline currently administered in such studies. However, including urea in the infusate improves the stability of the results and certainly places the kidney in a more physiologically relevant situation.<sup>200</sup> If nitrogenous wastes were excreted easily, this underrepresentation of urea would not matter much, but several studies have shown that increasing urine concentration (and thus mainly urea concentration) may participate in glomerular hyperfiltration,<sup>84</sup> may accelerate deterioration of renal function,<sup>201–203</sup> and increase albuminuria<sup>204–207</sup> and blood pressure.<sup>208</sup> Moreover, it may be interesting to recall that, in addition to its effect on the kidney, vasopressin has a direct effect in the liver through V1a receptors,<sup>209–211</sup> leading to stimulation of glucose and urea synthesis<sup>212</sup> and increased blood glucose level in humans.<sup>213</sup> Vasopressin is elevated in diabetes mellitus,<sup>214</sup> and several recent studies suggest that it may participate in the incidence of this disease and in other metabolic disturbances.<sup>215,216</sup> Even if these results need to be replicated and confirmed, they strengthen the concept that water conservation—and thus urea concentration in the

urine—depending on vasopressin's multiple actions, may have a significant cost<sup>6</sup> and can become deleterious in certain situations.

This review presents several novel hypotheses in an attempt to provide new directions for research regarding urine concentration and the role of urea in this complex process. The first hypothesis about urea secretion in the medullary pars recta is most likely correct because it is strongly supported by many results accumulated over several decades in humans and various experimental models. However, some direct experimental demonstration of this secretion is missing, except for the study of Kawamura and Kokko<sup>101</sup> in rabbits.<sup>162</sup> Microperfusion experiments of pars recta of normal rats and mice, as well as micropuncture studies comparing urea delivery in late proximal and early distal tubules in UT-A2 KO mice, would provide definitive answers regarding this proposed secretion.

Several polymorphisms of UT-A2 have been identified in humans. Although the functional consequence of these variants on urea transport have not been explored to our knowledge, epidemiological studies in large populations revealed that some of these variants are associated with blood pressure and metabolic syndrome,<sup>217–219</sup> suggesting that UT-A2 and renal urea handling influence fluid balance in humans. New studies should address the function of these variants and attempt to better define the contribution of UT-A2 to overall urea handling.

As already proposed by Schmidt-Nielsen<sup>34,136,139,220,221</sup> in several of her studies, it seems obvious now that urea excretion in mammals is regulated. In addition to being filtered, urea probably undergoes an active reabsorption when protein intake is low, and an active secretion (probably secondary active) on a normal or high protein intake. Moreover, an acute regulation should also take place, reducing urea excretion during a fast and increasing it after protein meals. Glucagon (a hormone secreted after ingestion of proteins) and glucocorticoids may regulate the intensity of urea secretion in the pars recta, either directly or indirectly. This needs to be confirmed by specific experimental designs, either by acute application of these hormones (or cAMP) in the bath of isolated perfused pars recta, or by prior *in vivo* treatment. The prior influence of a high protein intake or of an acute amino-acid load, shortly before pars recta microdissection and perfusion, would also be interesting to evaluate.

All other hypotheses, the actual magnitude of urea secretion through SGLT1 *in vivo*, the possibility that glucose could be secreted in the pars recta at least under certain circumstances, the possibility that this glucose may serve to feed lactate to the IM and be recycled in a Cori-like cycle, are more speculative, although they are all based on several converging indices and seem plausible. They need to be evaluated by appropriate *in vivo* and *in vitro* experiments. Finally, the possibility that urea escape through the bladder wall might be prevented by the operation of an active urea transporter located in the luminal membrane of umbrella cells, opposite to the membrane expressing UT-B, could be tested by measuring transepithelial unidirectional urea fluxes

in the rat or mouse bladder *in situ*. This transporter might share the same functional characteristics as that observed in the very terminal portion of the IMCD.<sup>46</sup>

New theories may turn out to be incorrect. However, they provide new food for thought and possibly additional new ideas that are essential for science to progress. Old hypotheses need to be revisited in the light of new results brought by molecular biology that challenge our previous paradigms. It is our hope that this review will stimulate further research in the field of urea handling and will encourage more colleagues to publish data about urea in renal studies.

#### NOTES ADDED IN PROOF

*Note 1.* A recent study now demonstrates that glucagon does stimulate renal gluconeogenesis.

Mutel E, Gauthier-Stein A, Abdul-Wahed A *et al.* Control of blood glucose in the absence of hepatic glucose production during prolonged fasting in mice. Induction of renal and intestinal gluconeogenesis by glucagon. *Diabetes* 2011; **60**: 3121–3131.

*Note 2.* A recent study described a new case of familial autosomal dominant azotemia segregating with a duplication of the UT-B gene. Affected individuals exhibit a marked increase in UT-B protein abundance in a kidney biopsy and in erythrocytes, and a three-fold acceleration of urea-induced hemolysis. An enhanced permeability to urea in vasa recta and erythrocytes cannot adequately explain the elevated plasma urea and dramatic fall in FE<sub>urea</sub> (1.4%) seen in this family. A more likely interpretation, in our view, is that an overexpression of UT-B in the bladder urothelium is responsible for an increased leakage of urea from bladder urine back to the blood, that exceeds the capacity of the putative active urea transporter to reclaim urea. This hypothesis needs to be confirmed. According to data presented in a poster at the American Society of Nephrology annual meeting, November 2011 (poster 2741), this new pedigree of familial azotemia displays a more severe phenotype (higher plasma urea and lower FE<sub>urea</sub>) than that described in previous studies<sup>104–106</sup> in which we assume a loss of function of an active urea transporter (see above). Alike many other genetic diseases, familial azotemia may probably be due to different genetic defects, with minor but discernable differences in phenotype.

Bokenkamp A, Sands JM, Wigman L *et al.* Familial azotemia is caused by a duplication of urea transporter-B gene. *J Am Soc Nephrol* 2011; **22** (online only): 750A.

#### DISCLOSURE

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