Theoretical effects of UTB urea transporters in the renal medullary microcirculation

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Zhang, Wensheng, and Aurélie Edwards. Theoretical effects of UTB urea transporters in the renal medullary microcirculation. Am J Physiol Renal Physiol 285: F731–F747, 2003. First published June 24, 2003; 10.1152/ajprenal.00172.2003.—A mathematical model of transport in the renal medullary microcirculation was used to investigate the role of the UTB urea transporter expressed in descending vasa recta (DVR) endothelia and red blood cell (RBC) membranes. Our simulations suggest that UTB raises RBC and plasma and interstitial urea concentrations by facilitating radial diffusion of the solute and therefore serves to increase the contribution of urea to the corticomedullary osmolality gradient, assuming no secondary effects on tubular transport. However, by lowering transmural urea concentration gradients, UTB reduces water efflux from DVR through aquaporin-1 (AQP1) water channels, thereby decreasing plasma sodium concentration. The net result of these competing effects on the osmolality gradient depends on the fraction of filtered urea that is reabsorbed by vasa recta. We also found that the contribution of UTB to water transport across DVR and RBCs is negligible, even in the absence of AQP1. Our model predicts that UTB plays a significant role, however, in reducing the shrinking and swelling of RBCs as blood flows along the medulla.

kidney; vasa recta; aquaporin-1 water channels; mathematical model; transport

The microcirculation of the renal medulla plays a fundamental role by supplying oxygen and nutrients to the medulla, removing water and solutes deposited into the interstitium by reabsorption from the loops of Henle and the collecting ducts, and preserving the corticomedullary concentration gradients that are essential for the formation of concentrated urine. These functions are made possible by the countercurrent arrangement of descending vasa recta (DVR) and ascending vasa recta (AVR) and by ultrastructural differences between DVR and AVR walls.

In the last decade, two families of urea transporters have been identified in the renal medulla, UTa and UTB. UTa isoforms are present in the collecting duct and the descending limb of Henle’s loop, whereas UTB is found in DVR and erythrocytes (17, 24, 32, 34). The erythrocyte urea transporter is most likely a complex channel with modifier sites on the extracellular surface, rather than a carrier (28).

Experimental studies with UTB knockout mice suggest that the urea transporter plays a significant role in the urinary concentrating mechanism. Urinary osmolality was measured to be 25% lower in UTB-deficient mice than in wild-type mice (35); UTB was found to contribute significantly to the capacity of the kidney to concentrate urine and even more greatly to its ability to concentrate urea itself (35). UTB allows red blood cells and vasa recta walls to rapidly exchange urea, thereby preventing it from being carried away from the medulla by the microcirculation and enhancing the corticomedullary osmolality gradients (17, 28, 29). Experimental observations suggest that some of the urea delivered to the tip of the papilla by UTAs and carried up by the blood through AVR is not recycled by DVR lacking UTB but is returned to the general circulation (35). In addition, the urea transporter is thought to play an important role in preventing large volume changes in red blood cells during their transit in the medullary microcirculation, a hypothesis first developed by Macey and Yousef (12).

Yang and Verkman (36) reported a significant osmotic water permeability in Xenopus laevis oocytes expressing UT3 (a UTB isoform), suggesting the existence of a continuous aqueous channel through the UT3 protein for both water and urea transport. Sidoux-Walter et al. (29) suggested that at physiological expression levels in erythrocytes, UTB does not transport water. Whether UTB significantly contributes to transmural fluxes of water in DVR in vivo remains unknown.

Questions also remain regarding possible differences between outer medullary (OM) and inner medullary (IM) urea transporters. Although antibodies to UTB label the continuous endothelium of rat DVR (32, 34), in vivo DVR permeability measurements suggest that inner medullary DVR may lack functional urea transporters. Whereas the permeability of OMDVR to urea (P_u) is five times higher than that to sodium (P_Na), and is inhibited by addition of thiourea, phloretin, and p-chloromercuribenzenesulfonate, in IMDVR P_u and P_Na are closely correlated as a straight line with a slope of 1 originating from the origin and are unaffected by...
thiourea or phloretin (17, 24). It is possible, albeit unlikely, that unstimred layers in the renal interstitium might have obscured the $P_u$ and $P_{Na}$ of IMDVR in vivo.

The objective of this study was to use a mathematical model of transport in the medullary microcirculation to gain some insight into the specific function of UTB in descending vasa recta walls and red blood cells (RBCs).

MODEL AND NUMERICAL METHODS

Glossary

$A_{int}$ Cross-sectional area of medullary interstitium  
AVR Ascending vasa recta  
$C_{iP}$, $C_{iR}$, $C_{iI}$ Concentration of solute $i$ in plasma, red blood cells, and interstitium, respectively  
$D$ Diameter of vasa recta  
DVR Descending vasa recta  
f Equilibrium distribution coefficient of urea between red blood cells and plasma  
f$_i$ Fraction of filtered load of water or solute that is reabsorbed into the microcirculation  
IM Inner medulla  
$J_{i(k)}^f$ Flux of component $i$ through pathway $k$ in compartment $j$  
$J$ Length of renal medulla  
$L_i$ Hydraulic conductivity of pathway $k$ in compartment $j$  
$N$ No. of vasa recta  
OM Outer medulla  
$\Delta P$ Hydraulic pressure difference  
$\Pe$ Peclet number  
P$_i^f(k)$ Permeability of pathway $k$ in compartment $j$ to component $i$  
$q_i^j$, $Q_i^j$ Volume flow rate in compartment $j$ in a single vas rectum and in all vasa recta, respectively  
RBC Red blood cell

Greek Symbols

$\Gamma$ Red blood cell-to-vessel surface area ratio  
$\gamma_i$ Activity coefficient of solute $i$  
$\Pi_i$ Oncotic pressure due to solute $i$  
$\sigma_i$ Reflection coefficient to solute $i$  
$\psi_i$ Generation rate of component $i$ per unit volume of interstitium

Subscripts and Superscripts

A Ascending vasa recta  
AQP1 Aquaporin-1 water channels  
B Blood  
D Descending vasa recta  
I Interstitium  
LM Lipid membrane of red blood cells  
Na Sodium chloride  
p, para Paracellular pathway  
pr Proteins  
P Plasma  
R Red blood cell  
u Urea  
UTB UTB urea transporter  
v Volume

General Description of Model

The renal medullary microcirculation is a countercurrent exchange system with blood flowing down along DVR from the corticomedullary junction to the papillary tip and looping back to the cortical veins along AVR. During transit, water and solutes in blood can diffuse radially between vasa recta and the medullary interstitium. This countercurrent flow configuration helps to maintain corticomedullary osmolarity gradients, which are essential for the formation of concentrated urine.

Our model, which has been described and applied earlier (2, 4, 39, 40), consists of steady-state conservation equations for water and solutes in vasa recta and the interstitium, coupled with expressions for fluxes across vasa recta walls and RBC membranes through paracellular and transcellular pathways. We only consider those vasa recta that are destined for the IM, i.e., those that lie in the center of the vascular bundles and do not perfuse the capillary plexus in the OM.

DVR and AVR exchange water, sodium chloride, urea, and proteins via several routes. Vasa recta walls are perforated by nonselective paracellular pathways, across which fluid transport is driven by Starling forces (i.e., transmembrane hydraulic and oncotic pressure differences). In addition, two transcellular pathways have been identified in DVR endothelium and RBC membranes: aquaporin-1 (AQP1) water channels, which allow for water movement to the exclusion of all solutes, and UTB urea transporters.

One of the functions of the medullary microcirculation is to carry away water and solutes (such as sodium chloride and urea) reabsorbed from the loops of Henle and collecting ducts. In this model, reabsorption into the interstitium is accounted for by interstitial generation rates that undergo spatial variation (2). In the OM, we assume that exchanges occur only between vasa recta and the interstitium because DVR and AVR form vascular bundles from which nephron loops are excluded, so that generation rates are taken to be zero.

Conservation Equations

If $x$ is the axial coordinate along the corticomedullary axis, conservation of volume in plasma and RBCs can be expressed as

$$\frac{dQ_P}{dx} = \pm (J_P^v - \Gamma_P J_P^0) N \pi D + \frac{Q_P}{N} \frac{dN}{dx}$$  \hspace{1cm} (1)

$$\frac{dQ_R}{dx} = \pm \Gamma R J_R^0 N \pi D + \frac{Q_R}{N} \frac{dN}{dx}$$  \hspace{1cm} (2)

where $Q_P$ and $Q_R$ are the plasma and RBC flow rates, respectively, and $J_P^0$ and $J_R^0$ are the volume fluxes...
(per unit membrane area) across vasa recta walls and RBC membranes, respectively. The parameter $\Gamma$ represents the cell-to-vessel surface area ratio, $N$ denotes the number of vasa recta, $D$ their diameter, and $+$ and $-$ apply to AVR and DVR, respectively. The second term on the right-hand side of Eqs. 1 and 2 accounts for the fact that at various depths in the medulla, DVR break up to form a capillary plexus, from which AVR are formed and ascend. Hence, part of the flow is directly shunted from DVR to AVR at various levels.

Because the RBC membrane is impermeable to sodium chloride, proteins, and hemoglobin, conservation of sodium chloride and proteins in plasma and hemoglobin and other nonurea solutes in RBCs, yields, respectively:

$$\frac{d(Q_i^p)}{dx} = \pm J_i^p(\text{para})N\pi D + \left(\frac{Q_i^p}{N}\right)\frac{dN}{dx} \quad (3)$$

$$i = \text{NaCl and proteins}$$

$$\frac{d(Q_i^b)}{dx} = \left(\frac{Q_i^b}{N}\right)\frac{dN}{dx} \quad (4)$$

$$i = \text{hemoglobin, other nonurea solutes}$$

where $J_i^p(\text{para})$ is the paracellular molar flux of solute $i$ (per unit membrane area) from plasma to interstitium, and $C_i^p$ and $C_i^b$ are the plasma and RBC concentration of solute $i$, respectively. Conservation of urea, which traverses vasa recta walls as well as RBC membranes, can be written as

$$\frac{d(Q_i^u)}{dx} = \pm (J_i^u - \Gamma J_i^b)N\pi D + \left(\frac{Q_i^u}{N}\right)\frac{dN}{dx} \quad (5)$$

$$\frac{d(fQ_i^u)}{dx} = \pm J_i^uN\pi D + \left(f\frac{Q_i^u}{N}\right)\frac{dN}{dx} \quad (6)$$

where $J_i^u$ and $J_i^b$ are the molar fluxes of urea through vasa recta walls and RBC membranes, respectively, and $f$ is the equilibrium distribution coefficient of urea between RBCs and plasma, taken to be 0.86 (1).

Order-of-magnitude analysis suggests that axial diffusion in the interstitium is negligible relative to radial transport (39). If reabsorption from the loops of Henle and collecting ducts is accounted for by interstitial generation rates, conservation of volume, sodium chloride, urea, and proteins in the interstitium can be written (2) as

$$[J_i^p(x)N(x)\pi D]_{\text{DVR}} + [J_i^p(x)N(x)\pi D]_{\text{AVR}} + A_{\text{int}}(x)\psi_\text{i}(x) = 0 \quad (7)$$

$$[J_i^p(x)N(x)\pi D]_{\text{DVR}} + [J_i^p(x)N(x)\pi D]_{\text{AVR}} + A_{\text{int}}(x)\psi_\text{v}(x) = 0 \quad (8)$$

where $i$ denotes sodium chloride, urea, and proteins, and $J_i^p$ and $J_i^b$ are the net fluxes of water and solute $i$ through the vasa recta wall. $A_{\text{int}}$ is the cross-sectional area of the medullary interstitium, and $\psi_\text{v}$ and $\psi_\text{i}$ are the local generation rates of volume and solute $i$, respectively, per unit volume of interstitium. The generation rate for proteins is zero, assuming that proteins are exclusively exchanged between vasa recta and interstitium (39). Calculations related to $\psi_\text{v}$ and $\psi_\text{i}$ are described immediately below.

Reabsorption of Water and Solute from Tubular System

Water and sodium chloride are reabsorbed from the loops of Henle and the collecting ducts through the interstitium; urea is reabsorbed mostly from terminal interstitium (28). The reabsorbed water and solutes must then be removed by the medullary microcirculation to avoid accumulation in the medulla. The fractions of filtered volume, sodium, and urea that are recovered by vasa recta from the interstitium are denoted by $f_v$, $f_{\text{Na}}$, and $f_u$, respectively. Baseline values for $f_v$, $f_{\text{Na}}$, and $f_u$ are taken as 1, 1, and 40%, respectively (2). If $C_i^0$ is the systemic concentration of solute $i$ and $\text{GFR}$ is the glomerular filtration rate, the filtered load of solute $i$ is $C_i^0 \times \text{GFR}$. Hence, the overall amount of water or solute recovered by vasa recta (VRR) may be expressed (2) as

$$\text{VRR}_i = f_v \times C_i^0 \times \text{GFR} = A_{\text{IM}}(x_{\text{IM}} = 0) \int_0^{x_{\text{IM}}} (0.25x_{\text{IM}} + 0.05)\psi_\text{i}(x_{\text{IM}})dx_{\text{IM}} \quad (9)$$

where $x_{\text{IM}}$ is the axial position along the IM normalized by its length, $A_{\text{IM}}(x_{\text{IM}} = 0)$ is the medullary cross-sectional area at the OM-IM junction, and $C_i^0$ should be set equal to one for water.

Koepsell et al. (7) observed that sodium concentration increases exponentially along the corticomedullary axis. Because the fraction of osmolality due to urea increases from ~2 to 50% between the corticomedullary junction and the papillary tip (19), urea concentration must increase even more rapidly. To yield a profile consistent with those observations, the baseline expressions for the interstitial area-weighed generation rates are taken (2) as

$$\psi_\text{v}(x_{\text{IM}}) = \psi_\text{v}^0(1 - x_{\text{IM}}) \quad (10)$$

$$\psi_\text{Na}(x_{\text{IM}}) = \psi_\text{Na}^0x_{\text{IM}} \quad (11)$$

$$\psi_\text{i}(x_{\text{IM}}) = \psi_\text{i}^0\exp[6(x_{\text{IM}} - 1)] \quad (12)$$

where $\psi_\text{v}^0$, $\psi_\text{Na}^0$, and $\psi_\text{i}^0$ are constants obtained by substituting Eqs. 10–12 into Eq. 9. The baseline value of GFR is taken as 784 $\mu l/s$, and the systemic concentrations of sodium and urea are 150 and 5 mmol/l, respectively (3). Thus we estimate that water, sodium, and urea are reabsorbed into vasa recta at a rate of $1.31 \times 10^{-4}$ ml/s, $1.96 \times 10^{-5}$ mmol/l, and $2.61 \times 10^{-5}$ mmol/l, respectively.
Flux Equations

As described earlier, there are three different pathways for water transport across DVR endothelium. Hence, in Eq. 1, \( J_p^v \) is the sum of three contributions, the fluxes through paracellular pathways, AQP1 water channels, and UTB urea transporters, respectively

\[
J_p^v(\text{para}) = J_p^v(\text{AQP1}) + J_p^v(\text{UTB})
\]

where \( L_p^v, L_A^v, \) and \( L_D^v \) represent the hydraulic conductivities of the paracellular pathway, AQP1, and UTB, respectively. Superscript \( D \) signifies that, because AQP1 and UTB are expressed in DVR but not AVR endothelia, the corresponding hydraulic conductivities in AVR are taken to be zero. \( \Delta P \) is the transmural hydraulic pressure difference, \( \Delta \Pi_{pr} \) is the transmural oncotic pressure difference due to plasma proteins, and \( \sigma_{pr} \) is the reflection coefficient of the paracellular pathway to proteins. The interstitial concentration and the activity coefficient of solute \( i \) are denoted by \( C^i \) and \( \gamma^i \), respectively, and \( \sigma_i \) is the reflection coefficient of the UTB urea transporter to urea.

**Equations 13–15** indicate that the driving force for water movement differs for paracellular and transcellular pathways. Indeed, the reflection coefficient of paracellular pathways to small hydrophilic solutes is negligible (i.e., there is no sieving of urea and sodium chloride across these pathways), whereas that of AQP1 water channels and UTB urea transporters is >0. Transmural sodium and urea concentration differences contribute significantly to water flux across AQP1 and UTB, given the large value of \( RT \) (19.3 mmHg/mM).

Because small solutes are more concentrated in the interstitium than in DVR plasma, there is water efflux from DVR across AQP1 and UTB; across paracellular pathways, however, sodium and urea exert no effect, and Starling forces favor volume reabsorption into vasa recta (essentially because proteins are more concentrated in plasma than in interstitium). Previous simulations in which water fluxes across UTB were neglected suggested that there is a net efflux of water across DVR walls throughout the medulla. We found that a net amount of \( 1.7 \times 10^{-4} \) cm/s is transported from interstitium to lumen across paracellular pathways, but twice that amount (i.e., \( 3.4 \times 10^{-4} \) cm/s) is carried from lumen to interstitium across AQP1 water channels, so that the overall amount of water exiting DVR is \( 1.7 \times 10^{-4} \) cm/s (39).

The volume flux across the RBC membrane, \( J^v \), is the sum of three terms, corresponding to AQP1 water channels, the lipid membrane, and UTB urea transporters, respectively

\[
J^v = J^v_{\text{AQP1}} + J^v_{\text{LM}} + J^v_{\text{UTB}}
\]

where \( J^v_{\text{AQP1}}, J^v_{\text{LM}}, \) and \( J^v_{\text{UTB}} \) are the hydraulic conductivities of AQP1, the lipid membrane, and UTB in RBC, respectively, and \( \Pi_{pr} \) and \( \Pi_{hb} \) are the oncotic pressures due to plasma proteins and to hemoglobin in RBCs, respectively.

The paracellular flux of solute \( i \) (i.e., sodium, protein, urea) across vasa recta walls can be written as

\[
J^v_i(\text{para}) = J^v_i(\text{para}) \times (1 - \sigma_i) \frac{C^i - C^i \exp(-Pe)}{1 - \exp(-Pe)}
\]

where \( P^v_i(\text{para}) \) is the permeability of the paracellular pathway to solute \( i \), and the Peclet number, Pe, is a measure of the importance of convection relative to diffusion.

Because UTB serves as a common channel for water and urea, the UTB-mediated transmural and transmembrane fluxes of urea are given by, respectively

\[
J^u_{\text{UTB}} = J^u_{\text{UTB}} \times (1 - \sigma_u) \times C^u + P^u_{\text{UTB}}(\text{UTB}) \times (C^u - C^u)
\]

where in Eq. 21, \( C^u = C^u_{\text{UTB}} \) if \( J^u_{\text{UTB}} > 0 \) (i.e., the volume flux through UTB is directed from DVR to the interstitium), and \( C^u = C^u_{\text{RBC}} \) otherwise. Similarly, in Eq. 22, \( C^u = C^u_{\text{RBC}} \) if \( J^u_{\text{UTB}} > 0 \) (i.e., the volume flux through UTB is directed from RBC to the lumen), and \( C^u = C^u_{\text{UTB}} \) otherwise. \( P^u_{\text{UTB}}(\text{UTB}) \) and \( P^u_{\text{UTB}}(\text{RBC}) \) are the urea permeability of UTB in DVR walls and RBC membranes, respectively. In Eq. 5, the overall flux of urea through the DVR wall, \( J^u_{\text{DVR}} \), is the sum of two terms: the flux...
through the paracellular pathway (i.e., Eq. 19) and that through the UTB urea transporter in DVR (i.e., Eq. 21). Similarly, the overall flux of urea across the RBC membrane, \( J_{	ext{R}}^{(LM)} \), is the sum of the urea flux through the UTB urea transporter in RBC (i.e., Eq. 22) and that through the RBC lipid membrane. The latter can be expressed as

\[
J_{	ext{R}}^{(LM)} = P_{	ext{R}}^{(LM)} C_{	ext{R}}^{\infty} - P_{	ext{R}}^{(LM)} C_{	ext{R}}
\]  

(23)

where \( P_{	ext{R}}^{(LM)} \) is the urea permeability of the RBC lipid membrane.

Expressions for the cell-to-wall surface area ratio, the number of vasa recta, the cross-sectional area of the interstitium, and the relationship between protein concentration and oncotic pressure are summarized in the APPENDIX. Transport and morphological parameters, as well as initial values, are given in Table 1. Parameters specifically related to the transport of urea and water across UTB, also shown in Table 1, are further described below.

### Table 1. Model parameter values

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>DVR</th>
<th>AVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydraulic conductivity of vasa recta, cm/s/100 Pa</td>
<td>1.8 x 10^-6</td>
<td>12.5 x 10^-6</td>
</tr>
<tr>
<td>Through paracellular pathways, ( P_{\text{LM}}^{(LM)} )</td>
<td>1.0 x 10^-7</td>
<td>2.46 x 10^-9</td>
</tr>
<tr>
<td>Through AQPI, ( L_{\text{R}}^{(D)} )</td>
<td>1.8 x 10^-8</td>
<td>1.8 x 10^-8</td>
</tr>
<tr>
<td>Through UTB, ( L_{\text{R}}^{(L)} )</td>
<td>0.34 x 10^-8</td>
<td>0.34 x 10^-8</td>
</tr>
<tr>
<td>Paracellular permeability of vasa recta, cm/s</td>
<td>1.35 x 10^-9</td>
<td>1.35 x 10^-9</td>
</tr>
<tr>
<td>Permeability of UTB to urea ( P_{\text{R}}^{(UTB)} )</td>
<td>285 x 10^-5</td>
<td>285 x 10^-5</td>
</tr>
<tr>
<td>Permeability of RBC to urea, cm/s</td>
<td>3 x 10^-5</td>
<td>3 x 10^-5</td>
</tr>
<tr>
<td>Through lipid membrane, ( P_{\text{R}}^{(LM)} )</td>
<td>156 x 10^-5</td>
<td>156 x 10^-5</td>
</tr>
<tr>
<td>Reflection coefficient of vasa recta to proteins</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Reflection coefficient of UTB to urea</td>
<td>1.86</td>
<td>1.86</td>
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<tr>
<td>Activity coefficient of urea</td>
<td>0.9</td>
<td>0.9</td>
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<tr>
<td>Activity coefficient of nonurea solutes in RBCs</td>
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<td>0.9</td>
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<tr>
<td>Physical dimensions</td>
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<tr>
<td>Diameter, ( \mu )</td>
<td>15.6</td>
<td>20</td>
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<tr>
<td>Total length of medulla, mm</td>
<td>7.8</td>
<td>5.9</td>
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<tr>
<td>Length of inner medulla, mm</td>
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<td></td>
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</table>

**Initial conditions in DVR at corticomedullary junction**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-vessel blood flow rate, nl/min</td>
<td>10</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.25</td>
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<tr>
<td>Plasma protein concentration, g/dl</td>
<td>6.8</td>
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<tr>
<td>Plasma sodium concentration, mmol/l</td>
<td>150</td>
</tr>
<tr>
<td>Plasma urea concentration, mmol/l</td>
<td>5</td>
</tr>
<tr>
<td>RBC urea concentration, mmol/l</td>
<td>5</td>
</tr>
<tr>
<td>RBC hemoglobin concentration, mmol/l</td>
<td>5.1</td>
</tr>
<tr>
<td>RBC concentration of nonurea solutes, mosmol/kgH2O</td>
<td>292</td>
</tr>
<tr>
<td>Whole kidney glomerular filtration rate, ( \mu )l/min</td>
<td>784</td>
</tr>
</tbody>
</table>

Unless otherwise specified, see Refs. 3 and 4. DVR and AVR, descending and ascending vasa recta, respectively; AQPI, aquaporin-I; UTB, urea transporter; RBC, red blood cells. UTB parameters are discussed in the text. *Protein transport parameters are given in Ref. 39. † The value of the activity coefficient is taken as twice that for sodium to implicitly account for chloride in the osmotic pressure term.

### UTB Transport Parameters

**UTB permeability to urea.** Yang et al. (35) reported that, at 10°C, the urea permeability of the RBC membrane was 45-fold lower in UTB knockout mice than in wild-type mice. In the absence of data at higher temperature, we assumed that the urea permeability of UTB transporters, \( P_{\text{R}}^{(UTB)} \), and that of the lipid membrane, \( P_{\text{R}}^{(LM)} \), are equal to 44/45 and 1/45 of the overall permeability of RBC to urea, respectively, taken as 160 x 10^-5 cm/s (1). Hence, \( P_{\text{R}}^{(UTB)} = 156 \times 10^{-5} \) cm/s and \( P_{\text{R}}^{(LM)} = 3 \times 10^{-5} \) cm/s in our simulations. The former estimate is close to the measured urea permeability of the human RBC urea transporter hUT-B1 (i.e., HUT11), 1.2 x 10^-3 cm/s (14). In DVR, the urea permeability of UTB transporters, \( P_{\text{R}}^{(UTB)} \), was taken as 285 x 10^-5 cm/s, as reported by Pallone et al. (24).

**UTB permeability to water.** Yang and Verkman (37) estimated that the water permeability of UTB transporters in RBCs, \( P_{w}^{(UTB)} \), is \( \sim 0.145 \times 10^{-2} \) cm/s at
10°C; because their results suggest a weak dependence of that permeability on temperature, we assumed the same value at 37°C.

Direct measurements of the water permeability of UTB transporters in DVR walls, \( P^D_{V}(UTB) \), have not been reported until now. Yang and Verkman (37) found that, in RBCs, the single-channel permeability of UTB to water is similar to that of AQP1 water channels. We assumed that the single-channel permeability of UTB to water, as well as that to urea, is identical in RBC membranes and DVR endothelia. In addition, we assumed that the overall water and urea permeabilities in RBC and DVR are the products of their single-channel value and the channel density, respectively. Comparing the overall permeabilities of DVR and RBC to water and urea, we obtain

\[
P^D_{V}(UTB) = P^D_{V}(UTB) \times \frac{P^R_{D}}{P^R_{0}(UTB)}
\]

(24)

where, as described above, \( P^D_{V}(UTB) \) and \( P^0_{V}(UTB) \) are the permeabilities of UTB in DVR endothelia to water and urea, respectively, and \( P^D_{V}(UTB) \) and \( P^0_{V}(UTB) \) are the permeabilities of UTB in RBC membranes to water and urea, respectively. With the use of this approach, the overall water permeability of UTB in DVR walls was estimated as \( 2.64 \times 10^{-3} \text{ cm/s} \).

The water permeability and hydraulic conductivity of UTB in DVR endothelia \( [P^D_{V}(UTB) \text{ and } L^D_{U}] \), respectively) can be related (36) knowing that

\[
J^D_{V}(UTB) = P^D_{V}(UTB) \cdot u_w \cdot A \cdot \Delta C = L^D_{U} \cdot A \cdot RT \cdot \Delta C
\]

(25)

where \( A \) is the surface area across which transport occurs, \( \Delta C \) is the transmural solute concentration difference, and \( u_w \) the partial molar volume of water, taken as 18 cm³/mol. Hence, \( L^D_{U} \) is given by

\[
L^D_{U} = \frac{P^D_{V}(UTB) \times u_w}{RT} = \frac{L^D_{V}(UTB) \times 18 \times 10^{-3}}{62.4 \times 310}
\]

(26)

yielding \( L^D_{U} = 2.46 \times 10^{-9} \text{ cm·s}^{-1}·\text{mmHg}^{-1} \). Similarly, \( L^R_{U} \) was calculated as \( 1.35 \times 10^{-9} \text{ cm·s}^{-1}·\text{mmHg}^{-1} \).

**RBC lipid membrane permeability.** Yang and Verkman (37) estimated that AQP1 water channels and the lipid membrane account for 90 and 2% of all the water that passes through the RBC membrane, respectively, at 10°C; at 37°C, they account for 79 and 15% of the water exchanged, respectively. Hence, we assumed that the lipid membrane-to-AQP1 water channel hydraulic conductivity ratio, \( L^R_{LM}/L^R_{A} \), is equal to 15/79 at 37°C. Because the combined hydraulic permeability of AQP1 and the lipid membrane has been reported as \( 22.8 \times 10^{-3} \text{ cm/s at 37°C} \) (13), and it may be converted to the hydraulic conductivity as \( 2.1 \times 10^{-8} \text{ cm·s}^{-1}·\text{mmHg}^{-1} \) from Eq. 26, \( L^R_{LM} \) and \( L^R_{A} \) were estimated as \( 0.34 \times 10^{-8} \) and \( 1.8 \times 10^{-8} \text{ cm·s}^{-1}·\text{mmHg}^{-1} \), respectively, at 37°C. The validity of these assumptions was confirmed by using the same approach at 10°C. At that temperature, if the ratio \( L^R_{LM}/L^R_{A} \) is taken as 2/90, \( L^R_{LM} \) and \( L^R_{A} \) are calculated as \( 0.46 \times 10^{-9} \) and \( 2.05 \times 10^{-8} \text{ cm·s}^{-1}·\text{mmHg}^{-1} \), respectively. The former value is in excellent agreement with the measured water permeability of RBC in AQP1/UTB null erythrocytes at 10°C, reported as \( 4.5 \times 10^{-4} \text{ cm/s} \) (37), that is, \( 0.42 \times 10^{-9} \text{ cm·s}^{-1}·\text{mmHg}^{-1} \) in units of hydraulic conductivity.

**Numerical Methods**

Ordinary differential equations (ODEs) described by Eqs. 1–6 were solved along DVR and AVR to obtain the plasma and RBC volume flow rates \( (Q^P \text{ and } Q^R) \) and solute concentrations \( (C^P \text{ and } C^R) \), with initial values (i.e., in DVR at the corticomedullary junction) for all variables as specified in Table 1. Solving Eqs. 1–6 requires determination of the fluxes (as expressed in Eqs. 13–19), which are themselves a function of solute concentrations in DVR, AVR, and the interstitium. Hence, the conservation equations cannot be simply solved along DVR first and AVR afterward. Thus we first assumed values for all variables throughout AVR, integrated Eqs. 1–6 along DVR and then looped back along AVR, where “guess” values were replaced with new calculated values as integration proceeded. This integration process was iterated until the values for all variables along AVR converged. At every step, hydraulic pressure and solute concentrations in the interstitium were obtained by solving interstitial conservation equations (Eqs. 7 and 8).

The ordinary differential equations were integrated along vasa recta using Gear’s method, which is a self-adaptive, multistep, predictor-corrector method for stiff ODEs. At each value of \( x \), the system of four nonlinear algebraic equations (Eqs. 7 and 8) was solved using a modified Powell hybrid method, as described more fully in our previous work (38).

**RESULTS**

In this model of the medullary microcirculation, the reabsorption of water, sodium chloride, and urea from the loops of Henle and the collecting ducts is accounted for by interstitial generation rates, which vary in magnitude along the corticomedullary axis. The amount reabsorbed is expressed as the fraction of the filtered load that is removed by the medullary microcirculation, denoted by \( f_v, f_{Na}, \) and \( f_u \), for water, sodium, and urea, respectively. The baseline values for \( f_v, f_{Na}, \) and \( f_u \) are taken to be 1, 1, and 40%, respectively.

Changes in the expression of UTB urea transporters and AQP1 water channels in vasa recta will likely affect the entire urinary concentrating mechanism, including the reabsorption of water and solutes into the interstitium. Nevertheless, given the absence of specific experimental data and the constraint that our model does not explicitly take into consideration tubular transport, the amount of filtered load that is reabsorbed into vasa recta was assumed to remain the same with and without UTB in the simulations described below.

In our baseline case, both AQP1 water channels and UTB urea transporters are expressed in DVR walls
been reported as 1.6 to urea, in the limit of zero solute concentration, has Maximal Urea Transport Capacities

interstitium, then to interstitium. diffuses in the opposite direction, from RBCs to AVR interstitial urea concentrations decrease, so that urea blood ascends back to the corticomedullary junction, with a lag because permeabilities are not in raising concentrations in both compartments, albeit cotic gradients) (38, 39), and from DVR into RBCs, DVR across paracellular pathways by transmural on-
tality. Urea thus diffuses from the interstitium into along DVR, it encounters regions of increasing osmo-
terstitial generation rates of water, sodium, and urea transport asymmetry across UTB in the DVR wall is unlikely, because urea moves through both the apical and abluminal membranes of endothelial cells, and the two sides are mirror images of each other with respect to the urea diffusion pathway. In vitro measurements of the permeability of DVR to urea based on diffusion from bath to lumen and lumen to bath were indeed found to be similar (17). We therefore simulated transport asymmetry only across the RBC membrane. In the absence of specific measurements of UTB permeability to urea according to the direction of transport, we investigated the effect of higher urea efflux permeabilities by either increasing $P_u^{R}(UTB)$ 10-fold during urea efflux (with $P_u^{R}(UTB)$ remaining equal to its baseline value during urea influx) or decreasing $P_u^{R}(UTB)$ 10-fold during urea influx (with $P_u^{R}(UTB)$ equal to its baseline value during urea efflux). Although there was a slight decrease in the contribution of urea to osmo-
alitlality at the papillary tip in the latter case (from 52 to 49%), the effects of such variations in $P_u^{R}(UTB)$ were otherwise negligible.

If the direction of asymmetry is reversed, that is, if influx $P_u^{R}(UTB)$ is increased 10-fold, or if efflux $P_u^{R}(UTB)$ is decreased 10-fold, the effects on medullary transport remain negligible. Because there seems to be no significant effect of UTB transport asymmetry on transport across vasa recta and erythrocytes, and given the lack of specific experimental data, we did not take this asymmetry into account in the remainder of our simulations.

Fig. 1. Variations in urea concentration in descending vasa recta (DVR) and ascending vasa recta (AVR) plasma, DVR and AVR red blood cells (RBCs), and interstitium along the corticomedullary axis toward the papillary tip. a, Baseline case; b, urea transporter (UTB) deleted from RBC membrane; $x$, position along the corticomedullary axis; $L$, total length of the medulla.

and RBC membranes. Illustrated in Fig. 1a are the baseline urea concentrations in RBCs, plasma, and interstitium near the papillary tip. As blood flows along DVR, it encounters regions of increasing osmo-
lality. Urea thus diffuses from the interstitium into DVR (there is also a smaller contribution from conv-
ction, as the model predicts that water is driven into DVR across paracellular pathways by transmural on-
ctotic gradients) (38, 39), and from DVR into RBCs, raising concentrations in both compartments, albeit with a lag because permeabilities are not infinite. As blood ascends back to the corticomedullary junction, interstitial urea concentrations decrease, so that urea diffuses in the opposite direction, from RBCs to AVR lumen, then to interstitium.

Maximal Urea Transport Capacities

The maximum permeability of the RBC membrane to urea, in the limit of zero solute concentration, has been reported as $1.6 \times 10^{-3}$ cm/s (14). In addition, measurements of the maximum urea flux through UTB in the RBC membrane range from 0.8 to $2.5 \times 10^{-7}$ mol·cm$^{-2}$·s$^{-1}$, as reviewed by Sands et al. (28). Using Eq. 24, we estimated that the maximum urea flux through UTB in the DVR wall is between 1.4 and $4.4 \times 10^{-7}$ mol·cm$^{-2}$·s$^{-1}$. In our baseline results, the urea fluxes through UTB in RBC membranes and DVR walls are $<0.5 \times 10^{-8}$ and $<0.2 \times 10^{-8}$ mol·cm$^{-2}$·s$^{-1}$, respectively, that is, at least 10 times lower than saturated fluxes. Hence, urea transport through UTB does not appear to reach its maximal capacities under the conditions we considered.

Asymmetry of Urea Transport by UTB Channels

Several investigators have reported that the net ef-

function difference is greater than the net influx for an equal, but oppositely directed, gradient (14, 28). While there could be asymmetry in the RBC membrane, transport asymmetry across UTB in the DVR wall is unlikely, because urea moves through both the apical and abluminal membranes of endothelial cells, and the two sides are mirror images of each other with respect to the urea diffusion pathway. In vitro measurements of the permeability of DVR to urea based on diffusion from bath to lumen and lumen to bath were indeed found to be similar (17). We therefore simulated transport asymmetry only across the RBC membrane. In the absence of specific measurements of UTB permeability to urea according to the direction of transport, we investigated the effect of higher urea efflux permeabilities by either increasing $P_u^{R}(UTB)$ 10-fold during urea efflux (with $P_u^{R}(UTB)$ remaining equal to its baseline value during urea influx) or decreasing $P_u^{R}(UTB)$ 10-fold during urea influx (with $P_u^{R}(UTB)$ equal to its baseline value during urea efflux). Although there was a slight decrease in the contribution of urea to osmo-
alitlality at the papillary tip in the latter case (from 52 to 49%), the effects of such variations in $P_u^{R}(UTB)$ were otherwise negligible.

If the direction of asymmetry is reversed, that is, if influx $P_u^{R}(UTB)$ is increased 10-fold, or if efflux $P_u^{R}(UTB)$ is decreased 10-fold, the effects on medullary transport remain negligible. Because there seems to be no significant effect of UTB transport asymmetry on transport across vasa recta and erythrocytes, and given the lack of specific experimental data, we did not take this asymmetry into account in the remainder of our simulations.

Function of UTB as an Urea Transporter

To examine the effect of UTB on urea transport in the renal medullary microcirculation, we first investi-
gated the effects on osmolality of eliminating the urea transporter from DVR endothelia, RBC membranes, and both. As stated above, we assumed that the interst-
itial generation rates of water, sodium, and urea remained unaffected by deletion of UTB or AQP1. Shown in Table 2 are plasma osmolality and $u_{\%}$ (that is, the fraction of osmolality attributable to urea) at the papillary tip, with and without UTB. Our results sug-
gest overall that by making transport barriers more permeable, urea transporters significantly increase urea concentrations throughout the medulla. However, the UTB-mediated decrease in transmembrane urea concentration differences is also predicted to reduce water efflux from AQP1 water channels, thereby in-
creasing blood flow, decreasing plasma sodium concen-
trations, and possibly reducing osmolality.

Eliminating UTB from DVR walls. Our simulations indicate that if urea transporters are eliminated from DVR walls, urea can only enter DVR through the more restrictive (i.e., less urea-permeable) paracellular pathway, urea influx is significantly reduced, and plasma urea concentrations decrease. Because the inter-
stitial-to-plasma urea concentration gradient is sig-

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The variation in papillary tip osmolality itself depends on which of these two competing effects dominates, the reduction in urea influx into DVR or the increase in water efflux, as illustrated in Table 2. If \( f_u \) remains equal to 40 or 60%, osmolality increases with the removal of UTB in DVR walls, because urea concentrations are high overall and the increase in transmural concentration gradients has a significant effect on water efflux and thus on plasma sodium concentration. If \( f_u \) is taken as 20% both before and after deletion of the transporter, the reduction in urea influx into DVR more than compensates for the increase in vas recta sodium chloride concentration, and osmolality at the papillary tip slightly decreases.

**Eliminating UTB from RBC membranes.** Our model indicates that if UTB is selectively removed from erythrocytes, RBC urea concentrations are significantly reduced given the lower membrane permeability. Due to diffusional (radial) equilibration between all compartments, plasma and interstitial concentrations decrease as well, as illustrated in Fig. 1b. However, there is a competing effect. Because DVR-to-RBC urea concentration gradients increase significantly, there is initially more water efflux from RBCs to DVR (Fig. 2A), which in turn translates into a higher efflux from DVR to interstitium to preserve the water balance. The net result is a more significant volume efflux from DVR, as shown in Fig. 2B, which raises plasma concentrations. Overall, the increase in the plasma concentration of sodium chloride is greater than (or equal to, if \( f_u = 20\% \)) the decrease in that of urea, so that osmolality at the papillary tip increases (or remains unchanged, if \( f_u = 20\% \)), whereas the contribution of urea is significantly reduced, as shown in Table 2. With \( f_u = 40\% \), papillary tip osmolality rises slightly from 1,077 to 1,106 mosmol/kgH\(_2\)O when UTB is removed from RBC membranes, whereas \( u\% \) decreases from 51 to 42%.

Even though the value of \( P_u^R(LM) \) remains uncertain, sensitivity analysis suggests that this parameter has a very small effect on results. \( P_u^R(LM) \) was measured as 1/45 of the overall urea permeability of the RBC membrane at 10°C (35), but there are no reported measurements at 37°C. Because the permeability of the RBC lipid membrane to water increases about sevenfold from 10 to 37°C (35), it is possible that the permeability to urea increases by a similar factor. We found that in the presence of UTB, multiplying the baseline lipid membrane-to-overall urea permeability ratio by 10 does not affect papillary tip osmolality or the fraction due to urea. If UTB is eliminated from RBCs, variations in \( P_u^R(LM) \) have a small effect on osmolality. As

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**Table 2. Effect of UTB urea transporters on papillary tip osmolality**

<table>
<thead>
<tr>
<th>Urea Reabsorption Ratio, ( f_u^* )</th>
<th>UTB in Both RBC and DVR (Baseline)</th>
<th>UTB Deletion From DVR</th>
<th>UTB Deletion From RBC</th>
<th>UTB Deletion From Both RBC and DVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>756 (34%)</td>
<td>720 (23%)</td>
<td>756 (27%)</td>
<td>725 (20%)</td>
</tr>
<tr>
<td>40%</td>
<td>1,077 (51%)</td>
<td>1,097 (38%)</td>
<td>1,106 (42%)</td>
<td>1,121 (33%)</td>
</tr>
<tr>
<td>60%</td>
<td>1,438 (60%)</td>
<td>1,643 (48%)</td>
<td>1,521 (51%)</td>
<td>1,675 (42%)</td>
</tr>
</tbody>
</table>

Shown are the osmolality at the papillary tip (1st term; in mosmol/kgH\(_2\)O) and the percentage accounted for by urea (2nd term; \( u\% \)).

\( f_u \) is the fraction of filtered urea that is reabsorbed from loops of Henle and collecting ducts into the interstitium. The baseline value is 40%.

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**Fig. 2.** Water fluxes with and without UTB in RBC membrane. The junction between the outer (OM) and the inner medulla (IM) corresponds to \( x/L = 0.24 \). A: across RBC membrane in DVR. Water efflux through UTB is negligible compared with that through non-UTB pathways (i.e., aquaporin-1 (AQP1) and lipid membrane). In the absence of UTB in RBC membrane, water efflux through non-UTB pathways is much higher near the corticomedullary junction. B: across AQP1 in DVR wall. In the absence of UTB in RBC membrane, the larger water efflux from RBC to DVR also increases water efflux from DVR to the interstitium, thereby concentrating plasma.
described above, removing UTB in erythrocytes raises the plasma concentration of sodium chloride and lowers that of urea. However, the larger the \(P_r(LM)\), the smaller these effects because the contribution of the RBC lipid membrane to urea transport is comparatively larger. Hence, if the lipid membrane-to-overall urea permeability ratio is 10 times the baseline value, osmolality at the papillary tip is 1,082 mosmol/kgH\(_2\)O (with u\% = 50\%) without UTB in RBCs, and 1,077 mosmol/kgH\(_2\)O (with u\% = 51\%) with UTB.

Eliminating UTB from both RBC membranes and DVR walls. Not surprisingly, when UTB is removed from both DVR walls and RBC membranes, the combined effects are predicted to lead to a further decrease in u\% at the papillary tip. Whether osmolality increases or decreases depends on the urea reabsorption ratio, as described above. With our baseline value (\(f_u = 40\%\) before and after deletion), eliminating UTB urea transporters leads to a 4\% increase in papillary tip osmolality, whereas the fraction that is due to urea decreases more significantly, from 51 to 33\%.

Our results suggest that UTB transporters greatly increase the diffusive exchange of urea across vasa recta and erythrocytes and therefore raise medullary urea concentrations in RBC, plasma, and interstitium, assuming that reabsorption from the loops of Henle and the collecting ducts remains unaffected; because the high permeability of UTB confers to membranes lowers transepithelial urea concentration gradients, the transporter also reduces water efflux from DVR through AQP1 water channels, thereby lowering medullary sodium concentrations. If \(f_u\) is at least equal to 40\%, the increase in \(C_u\) imparted by UTB is predicted to be smaller than the decrease in \(C_{Na}\), so that osmolality at the papillary tip decreases overall.

Function of UTB Without AQP1 Water Channels

As our previous simulations indicated, AQP1 water channels in DVR favor the shunting of water from DVR to AVR in the OM, therefore reducing blood flow rate to the deep medulla and increasing osmolality (18). More specifically, without AQP1 in DVR walls there appears to be no water efflux from DVR into the interstitium (except near the corticomedullary junction) because the balance of forces (i.e., oncotic vs. hydraulic pressure differences) favors water influx across the paracellular pathway throughout most of the medulla (38). Plasma concentrations thus remain lower. Eliminating AQP1 water channels from RBC membranes also decreases osmolality, because reducing water efflux from RBCs to DVR lowers solute concentrations in RBCs, and therefore in plasma and interstitium as well. Indeed, given the countercurrent arrangement of vasa recta and the diffusive transport of urea, the concentration of urea in the interstitium (almost) always lies between that in AVR plasma and that in DVR plasma, whereas plasma urea concentrations are themselves bounded by RBC urea concentrations (see Fig. 1).

Yang and Verkman (37) generated mice lacking both UTB and AQP1 and reported that the single-channel water permeability of the urea transporter is similar to that of AQP1, suggesting that UTB could play a role in facilitated water transport. Our simulations also make it possible to selectively eliminate AQP1 water channels and/or UTB urea transporters from DVR walls, RBC membranes, or both. Results are summarized in Table 3. In the absence of AQP1 water channels, small-solute concentration differences have no direct effect on volume fluxes in this model, so that there is no UTB-mediated reduction in water efflux, and therefore no decrease in sodium concentration. On the assumption that interstitial generation rates remain unaffected, our model predicts that without AQP1, UTB transporters increase medullary urea concentrations in vasa recta and interstitium by enhancing the diffusive (radial) transfer of urea. As a result, both the osmolality at the papillary tip and the fraction due to urea increase. In the complete absence of water channels, the expression of UTB results in a 35\% increase in papillary tip osmolality, and u\% increases from ~30 to >50\%, assuming that \(f_u = 40\%\) (Table 3).

Function of UTB as a Water Channel

Verkman and colleagues (36, 37) have shown that UTB urea transporters also function as water channels. Whether the contribution of UTB to transmural water fluxes is significant under physiological conditions has been a matter of debate among investigators (29, 36, 37). Our simulations indicate that the water flux across UTB in DVR walls and in RBC membranes is negligible relative not only to the transcellular flux across AQP1 water channels but also compared with the water flux across the paracellular pathway in DVR walls (Figs. 2A and 3). This is not surprising given that the hydraulic conductivity of UTB in DVR endothelia is estimated as \(2.46 \times 10^{-9} \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}\), as described above, whereas that of the paracellular pathway has been measured as \(1.8 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1}\).

Table 3. Combined effect of UTB urea transporters and AQP1 water channels on osmolality

<table>
<thead>
<tr>
<th>UTB in Both DVR and RBC (Baseline)</th>
<th>UTB Deletion From DVR</th>
<th>UTB Deletion From RBC</th>
<th>UTB Deletion From Both RBC and DVR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP1 in RBC and DVR</td>
<td>1,077(51%)</td>
<td>1,097(38%)</td>
<td>1,106(42%)</td>
</tr>
<tr>
<td></td>
<td>(Baseline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AQP1 deletion from DVR</td>
<td>831(52%)</td>
<td>626(37%)</td>
<td>796(43%)</td>
</tr>
<tr>
<td>AQP1 deletion from RBC</td>
<td>951(53%)</td>
<td>908(40%)</td>
<td>897(43%)</td>
</tr>
<tr>
<td>AQP1 deletion from both RBC and DVR</td>
<td>802(53%)</td>
<td>605(38%)</td>
<td>731(43%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Shown are the osmolality at the papillary tip (1st term; in mosmol/kgH\(_2\)O) and the percentage accounted for by urea (2nd term; u\%). The urea reabsorption ratio and the reflection coefficient of UTB to urea are taken as 40\% and 0.3, respectively.
Our model predicts that across the RBC membrane, the fraction of water transported through UTB transporters, the lipid membrane, and AQP1 water channels is 6, 15, and 79%, respectively, in close agreement with the values calculated by Yang and Verkman (37). Thus eliminating the water transport property of UTB urea transporters in RBC membranes also has an insignificant effect on water flux through the RBC membrane in the presence of AQP1 water channels. Without AQP1, our simulations suggest that nearly 30% of the water carried across erythrocytes goes through UTB transporters and 70% through the lipid membrane, so that setting the water permeability of UTB to zero significantly decreases water fluxes across RBCs. However, the effect on papillary tip osmolality is small, as the latter decreases by <1%, and the fraction due to urea increases slightly. Hence, our results indicate that eliminating water transport through UTB in erythrocytes has a negligible effect on osmolality, with or without AQP1 water channels.

The simulations above were based on a reflection coefficient of UTB to urea ($\sigma_u$) equal to 0.3, as estimated by Yang and Verkman (36). A zero reflection coefficient would indicate that urea has no effect on water flux across UTB (see Eq. 15), whereas $\sigma_u = 1$ would mean that UTB is impermeable to urea. Because this parameter is uncertain, we varied it between 0 and 0.6.

If $\sigma_u$ increases from 0.3 to 0.6, the net amount of water transported across UTB from RBCs into the lumen throughout DVR increases from $5.2 \times 10^{-6}$ to $5.5 \times 10^{-6}$ cm$^3$/s, and that transported from the lumen into the interstitium throughout DVR increases from $5.5 \times 10^{-6}$ to $7.1 \times 10^{-6}$ cm$^3$/s; the latter figure represents only 2 and 4% of the net amount of water transported across AQP1 and the paracellular pathways, respectively. It is not surprising that changes in $\sigma_u$ have small effects because the high permeability to urea imparted by UTB results in small transmural osmotic pressure gradients due to urea; most of the driving force for water transport across UTB stems from other solute concentration differences. Thus if $\sigma_u$ is either increased twofold or set to zero, variations in the amount of water transported through UTB in DVR walls have little effect: the osmolality at the papillary tip and $u%$ remain the same (1,078 mosmol/kgH$_2$O and 51%, respectively, with $\sigma_u = 40\%$).

We assumed in our baseline case that the reflection coefficient of UTB to nonurea small solutes (i.e., sodium chloride and other RBC nonurea solutes) is equal to 1; that is, the transporter is impermeable to these other solutes. It is possible, however, that UTB constitutes a shared pathway for water, urea, and other solutes. If the reflection coefficient of UTB to nonurea small solutes is taken as 0.3 (that is, equal to that to urea), our simulations indicate that the net amount of water transported across UTB from lumen to interstitium throughout DVR decreases significantly, from $5.5 \times 10^{-6}$ to $2.5 \times 10^{-6}$ cm$^3$/s. The direction of water movement through UTB is even reversed across the RBC membrane, because small-solute concentration differences then play a lesser role and oncotic pressure differences constitute the main driving force; whereas the net amount of water transported across erythrocyte UTB throughout DVR is calculated to be $5.2 \times 10^{-6}$ cm$^3$/s from RBC into the lumen in the baseline case, it is $2.8 \times 10^{-5}$ cm$^3$/s from the lumen into RBC if the reflection coefficient of UTB to nonurea solutes is taken as 0.3. The overall amount of water transported across RBC membranes is predicted to drop by only 1% nevertheless, as nonurea solutes in RBCs exert a smaller osmotic pressure and more water is then carried out across AQP1 as a result; osmolality at the papillary tip and the contribution of urea are found to remain the same as in the baseline case. It should be noted that varying the reflection coefficient of the transporter to nonurea solutes has a greater effect on water fluxes than varying that to urea because DVR walls and RBC membranes are much less permeable to these other solutes, so that corresponding transmural concentration differences are significantly larger and more important as a driving force.

**UTB and the RBC Osmotic Balance**

Several investigators have proposed that erythrocyte UTB urea transporters play an important role in balancing the osmotic pressure on each side of the barrier.
(i.e., RBC and plasma), thereby limiting the extent to which RBCs shrink along DVR and swell along AVR (11, 12).

To examine this assumption, we predicted relative RBC flow rate variations along the corticomedullary axis in a single DVR and AVR, with and without the presence of UTB in RBC membranes. Our results suggest that UTB indeed reduces the magnitude of RBC volume changes along vasa recta, as illustrated in Fig. 4. In the presence of AQP1, as blood flows from the corticomedullary junction to the papillary tip along DVR, RBC volume decreases to 63 and 55% of its initial value with and without UTB, respectively. It then increases back to 100 and 104% of its initial value, respectively, as blood returns to the cortex along AVR. The effects of UTB on RBC volume are slightly smaller in the absence of AQP1 in the RBC membrane, because water efflux across erythrocytes is reduced without the high osmotic water permeability imparted by AQP1 water channels. In this case, we found that along DVR, RBC volume decreases to 73 and 65% of its initial value with and without UTB, respectively; it then returns to 100 and 102% of its initial value, respectively, as blood flows back to the cortex.

Urea vs. Sodium Chloride DVR Permeability

Pallone et al. (22) suggested that a tradeoff may have evolved in the medullary microcirculation. The authors noted that whereas the urea gradient across the DVR wall is probably small due to the high permeability imparted by expression of the UTB transporter, the permeability of at least some DVR to sodium chloride is low. Relatively low DVR sodium chloride permeability would favor the bypassing of water from DVR to AVR via AQP1, the purpose of which may be to lower blood flow rate toward the papillary tip. A reduced blood flow rate to the deepest portions of the medulla is expected to enhance the efficiency of microvascular exchange in the IM by reducing solute washout.

Our model indicates that both sodium chloride and urea contribute significantly to water efflux across DVR. As illustrated in Fig. 5, interstitial-to-DVR concentration gradients are higher for sodium chloride than for urea in parts of the IM but lower in the OM. Indeed, because the initial concentration of urea is 50-fold lower than that of sodium, because the fraction of filtered urea that is reabsorbed by vasa recta is 40 vs. 1% for sodium in our baseline case, and given that the interstitial area-weighted generation rate in the IM is assumed to increase exponentially for urea but only linearly for sodium (2), the concentration of urea increases much faster than that of sodium in the OM and transmural gradients are correspondingly higher. In the IM, the high DVR permeability to urea plays a more dominant role and reduces interstitial-to-DVR urea concentration gradients.

Thus the contribution of sodium to water efflux across AQP1 water channels appears to be more significant than that of urea in the IM, but the opposite is true in the OM. Decreasing the urea reabsorption ratio to 20% reduces transmural urea concentration differences (Fig. 5), but the latter still play a larger role in driving water out of DVR in the OM.

If the permeability of DVR to sodium were as high as that to urea, our simulations indicate that water efflux from DVR to the interstitium through AQP1 would be lowered, blood flow at the papillary tip would significantly increase as shown in Fig. 6, and plasma urea concentration would decrease. However, because a higher permeability to sodium increases transmural (i.e., radial) transport of sodium and thereby significantly raises sodium concentration, the osmolality at the papillary tip would increase from 1,077 to 1,134
mosmol/kgH₂O if fₖ remained equal to 40% (and from 756 to 830 mosmol/kgH₂O with fₖ = 20%). The contribution of urea to papillary tip osmolality would fall from 51 to 45% with fₖ equal to 40% (and from 34 to 29% with fₖ equal to 20%). Although it may first appear surprising that the presence of UTB (i.e., a large DVR permeability to urea) slightly lowers osmolality at the papillary tip if fₖ is at least 40% whereas increasing the DVR permeability to sodium has the opposite effect, the results here again depend on the fraction of filtered solute that is reabsorbed by vasa recta. If the fraction corresponding to sodium is decreased from 1 (i.e., our baseline value) to 0.5%, increasing DVR permeability to sodium as described above would have an insignificant effect on osmolality at the papillary tip. Our model shows that increasing the permeability of DVR to a given solute i gives rise to two competing effects: higher plasma concentration of solute i due to more efficient radial transport, and lower concentration of all other solutes due to reduced transmural gradients and thus less water efflux from DVR. Which effect dominates depends on the amount of solute reabsorbed into the microcirculation.

In summary, our results confirm the hypothesis of Pallone et al. (22) that the low permeability to sodium chloride measured in some DVR may serve to enhance water transport from DVR to AVR across AQP1, thereby lowering blood flow to the papillary tip. Assuming that interstitial generation rates remained unchanged, we found that if permeability of DVR to sodium were equal to that to urea, blood flow rate at the papillary tip would indeed increase slightly (by ~10% compared with our baseline case). It is likely, however, that varying permeability of DVR to sodium affects reabsorption from the loops of Henle and the collecting ducts, so that the in vivo effects of changes in sodium permeability are difficult to predict.

**UTB Urea Transporters in OM vs. IM**

As discussed by Pallone et al. (22), in vivo DVR permeability measurements suggest that IMDVR may lack a functional urea transporter. We thus examined the effect of selectively removing UTB from IMDVR or OMDVR, assuming here again that deletion of the transporter does not affect reabsorption from the loops of Henle and the collecting ducts. As shown in Table 4, our model predicts that papillary tip osmolality is lowest when UTB is only present in OMDVR and highest when the transporter is only present in IMDVR. These intriguing results stem from the competing effects of the urea transporter on sodium and urea concentrations. As illustrated in Fig. 7A, in the OM, Cₛ is calculated to be the same whether UTB is present throughout DVR or in OMDVR only, and it is also the same (but lower) whether UTB is present in IMDVR only or not at all. In the IM, however, the rate of increase in Cₛ is reduced when UTB is present in OMDVR only, because the permeability of vasa recta to urea is suddenly decreased. Conversely, the rate of increase in Cₛ is augmented in the IM when UTB is present in IMDVR only. As a consequence, urea concentration at the papillary tip is highest when UTB is present in IMDVR only and lowest when it is only found in OMDVR, with intermediate values if the urea transporter is either present throughout the medulla or entirely absent from DVR walls (Fig. 7A). Plasma sodium concentration, meanwhile, increases steadily as the urea transporter is removed first from OMDVR and then from IMDVR as well (Fig. 7B), due to the progressively larger water efflux from DVR. The net result is that, relative to the baseline case, osmolality at the papillary tip decreases if UTB is present in OMDVR only and increases if UTB is present in IMDVR only (Table 4).

Our simulations thus suggest that the effects of the urea transporter are as significant in the IM as in the OM. Assuming that fₖ remains equal to 40%, selectively removing UTB from IMDVR reduces osmolality.

| Table 4. Selective effects of UTB urea transporters in outer and inner medullary DVR |
|--------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                      | UTB in OMDVR and IMDVR (Baseline) | UTB Deletion From IMDVR | UTB Deletion From OMDVR | UTB Deletion From All DVR |
| Osmolality at papillary tip, mosmol/kgH₂O | 1,077 | 1,018 | 1,133 | 1,097 |
| Fraction due to urea, u%              | 51   | 41   | 50   | 38   |

OMDVR and IMDVR, outer medullary and inner medullary DVR, respectively.
at the papillary tip by 5.5% (from 1,077 to 1,018 mosmol/kgH$_2$O), whereas eliminating UTB from OM-DVR only increases it by 5.2% (from 1,077 to 1,133 mosmol/kgH$_2$O).

**DISCUSSION**

The urea transporter UTB has been identified in the membrane of erythrocytes and in the endothelium of renal medullary DVR. The transporter has been reported to play an important role in regulating urea concentration in the kidney, and it appears to function as a common channel for both urea and water (34, 35, 37).

In this study, we used our mathematical model of the renal medullary microcirculation to gain more insight into the role of UTB. Our approach is limited in two important ways. First, to obviate the need to fully simulate the urinary concentrating mechanism, the model specifies interstitial generation rates of water, sodium, and urea to account for reabsorption from the loops of Henle and the collecting ducts. Because it is not known how changes in the expression of UTB and AQP1 in DVR affect tubular transport, we assumed that the fraction of filtered load that is reabsorbed into the medullary microcirculation remains unaffected by deletion of the transporters. However, given that the entire countercurrent system of the medulla acts in an integrated manner, changes in transmural fluxes are bound to have secondary effects on transtubular gradients in vivo and therefore alter supply to the interstitium.

It is likely that deleting UTB affects not only $f_\text{u}$ (and the spatial variations of the interstitial generation rate of urea) but also water and sodium reabsorption, as they are closely linked. As reviewed by Sands (26), hyperosmolality in the IM collecting duct can raise facilitated urea permeability and increase urea reabsorption; water diuresis also appears to enhance urea reabsorption; and urea is actively transported from the IM collecting duct to the interstitium by “sodium-urea cotransporters” in rats on a low-protein diet. In addition, even the baseline value of $f_\text{u}$ is difficult to estimate based on experimental data, as discussed previously (2). Theoretical studies (30, 31) suggest that $f_\text{u}$ is comprised between 20 and 60%, hence the range examined in this work and our choice of 40% for the baseline case.

The predictive ability of our model is further restricted by the nature of the relevant experimental data. Whereas most of the morphological and transport parameters used in this model come from measurements in rats, experiments in which the expression of UTB or AQP1 is deleted are often performed in mice. Observations by Verkman and colleagues (35) suggest that, in mice, UTB-dependent countercurrent exchange of urea in the renal medulla may contribute to one-third of the total capacity of the kidney to concentrate urine and even more greatly to the ability of the kidney to concentrate urea itself. In studies with UTB knockout mice, urine osmolality was 25% lower, plasma urea concentration was 30% higher, and urine urea concentration was 35% lower than in wild-type mice (35). The medullary architecture of mice is different from that of rats, as mice do not have short-looped nephrons, but it has not been thoroughly described in quantitative terms in the literature. Thus defects in the concentrating ability observed in UTB knockout mice cannot be directly compared with the predictions of this model.

Despite these limitations, our approach can help in gaining an understanding of the function of UTB. AQP1 knockout mice have been shown to manifest a severe urinary concentrating defect associated with defective medullary interstitial osmolality (10). Our model, with parameters derived from measurements in rats, accurately predicted that deletion of AQP1 leads to a substantial reduction of interstitial osmolality. Simulations suggested that DVR expression of AQP1 enhances medullary osmolar gradients by providing a route for volume efflux that shunts blood flow from DVR to AVR, secondarily reducing blood flow to the IM (18). The present study, while impaired by the lack of experimental data regarding the effects of UTB deletion on $f_\text{u}$, $f_\text{Na}$, and $f_\text{u}$ in rats, may also provide some insights into the role of UTB as a urea transporter.
Our simulations suggest that, by greatly facilitating transmembrane urea diffusion, UTB significantly increases urea concentrations throughout the medulla. However, by decreasing radial urea concentration gradients, UTB also reduces volume efflux from DVR through AQP1 water channels and thereby lowers the plasma concentration of other solutes such as sodium chloride. The presence of UTB therefore appears to increase the contribution of urea to the corticomedullary gradient. Whether the UTB-mediated increase in the concentration of urea compensates for the decrease in that of sodium chloride depends on the fraction of filtered urea that is reabsorbed by vasa recta, \( f_u \); the net effect on overall concentrating ability (as measured by osmolality at the papillary tip) is not expected to be very significant. In the absence of AQP1, however, the reduction in transmembrane urea concentration differences imparted by UTB has no direct effect on water fluxes, and our model predicts that the urea transporter significantly increases both papillary osmolality and the fraction of total osmolality that is due to urea.

AQP1 water channels, expressed by DVR endothelia (16), have been shown to be a transport pathway across which small hydrophilic solutes such as sodium chloride and urea drive water flux (20). UTB also appears to function as a water channel, with urea and water sharing a common aqueous pathway. Yang and Verkman (36) first reported a significant osmotic water permeability in *X. laevis* oocytes expressing UT3 (a UTB isoform), suggesting the existence of a continuous aqueous channel through the UT3 protein that passes both water and urea. Sidoux-Walter et al. (29) later found that at physiological expression levels, the HUT11A transporter in humans (whose rat homologue is UT3) confers urea permeability to RBCs but not water permeability. They proposed that water transport activity in HUT11A-expressing oocytes occurs when the transporter takes another conformation at high density in the oocyte membrane, allowing for water movement.

In subsequent studies with knockout mice, Yang and Verkman (37) reported that the single-water channel permeability of UTB, \( 7.5 \times 10^{-14} \text{ cm}^2/\text{s} \), is similar to that of AQP1. The authors found that, at 10°C, the erythrocyte osmotic water permeability was significantly reduced in AQP1-UTB-deficient mice compared with AQP1-deficient mice (0.045 \( \times 10^{-2} \) vs. 0.19 \( \times 10^{-2} \) cm/s). There was, however, no significant difference at 35°C; at that temperature, 79% of water was transported through AQP1, 6% through UTB, and the rest through the lipid membrane. The investigators also found that urine osmolality in double knockout mice was similar to that in AQP1 knockout mice. They concluded that UTB functions as an efficient water transporter, but its absolute contribution to total water transport in normal erythrocytes is small because RBCs express many fewer UTB urea transporters than AQP1 water channels (37).

Our theoretical predictions regarding the function of UTB as a water channel confirm the experimental results of Sidoux-Walter et al. (29) and Yang and Verkman (37), namely, that the absolute contribution of UTB transporters to water transport in normal erythrocytes is not significant. In our baseline case (i.e., both AQP1 and UTB are present in erythrocytes and vasa recta), the total amount of water transported across DVR walls through UTB is calculated to be only 2 and 3% of that transported through AQP1 and the paracellular pathway, respectively. Along DVR, the fraction of water transported across RBCs through the UTB transporters, lipid membrane, and AQP1 water channels is predicted to be 6, 15, and 79%, respectively, in close agreement with the values reported by Yang and Verkman. It is thus not surprising that eliminating water transport across UTB should have a negligible effect on small-solute concentrations and osmolality, even in the absence of water channels.

Yang and Verkman (36) speculated that the UTB-mediated solvent drag of urea could provide a way for urea to exit from vasa recta to balance the osmotically driven water exit. However, our simulations suggest that the convective efflux of urea from DVR across UTB represents only \( \approx 1% \) of the diffusional influx across the same transporter (i.e., \( 8.9 \times 10^{-7} \) vs. \( 8.5 \times 10^{-5} \) mmol/s). The net amount of urea that enters DVR from the interstitium through UTB is \( 8.4 \times 10^{-5} \) mmol/s, which is also more than twice the amount that enters the lumen across the paracellular pathway, \( 4.1 \times 10^{-5} \) mmol/s. The ability of UTB to transport water, therefore, does not appear to significantly affect urea fluxes across DVR walls. Water movement through UTB is unlikely to be physiologically important in the kidney.

As discussed by Macey and Yousef (12), shrinkage of RBCs to \( <60\% \) of their original volume leads to irreversible damage, whereby the membrane becomes leaky to sodium; conversely, swollen erythrocytes are less deformable and more prone to destruction. Our results confirm that the UTB urea transporter plays a significant role in erythrocytes by reducing the magnitude of RBC shrinkage and swelling along the corticomedullary axis. We found that with UTB, RBC volume decreases to 63% of its initial value along DVR and increases back to 100% of its initial DVR value along AVR; without the transporter, volume would be reduced to 55% of its initial value along DVR and would slightly exceed its initial DVR value on leaving medullary AVR. Those trends agree with the predictions of Macey and Yousef. These investigators estimated that the RBC volume at the papillary tip is \( \approx 65 \) and 55–60% of its initial DVR value along AVR; without the transporter, volume would be reduced to 55% of its initial value along DVR and would slightly exceed its initial DVR value on leaving medullary AVR. These results agree with the predictions of Macey and Yousef. These investigators estimated that the RBC volume at the papillary tip is 65 and 55–60% of its initial DVR value along AVR; without the transporter, volume would be reduced to 55% of its initial value along DVR and would slightly exceed its initial DVR value on leaving medullary AVR. These trends agree with the predictions of Macey and Yousef. These investigators estimated that the RBC volume at the papillary tip would be reduced to 55% of its initial value along DVR and would slightly exceed its initial DVR value on leaving medullary AVR. These trends agree with the predictions of Macey and Yousef. These investigators estimated that the RBC volume at the papillary tip would be reduced to 55% of its initial value along DVR and would slightly exceed its initial DVR value on leaving medullary AVR. These trends agree with the predictions of Macey and Yousef. These investigators estimated that the RBC volume at the papillary tip would be reduced to 55% of its initial value along DVR and would slightly exceed its initial DVR value on leaving medullary AVR. These trends agree with the predictions of Macey and Yousef.
Whether the effect of UTB in reducing swelling and shrinking of RBCs is significant in vivo remains uncertain. Erythrocytes in individuals lacking the Kidd (Jk) antigen also lack UTB expression (5, 27) because the human Kidd blood group and the UTB urea transporter proteins are encoded by the same Jk gene (8, 9). Whereas Woodfield et al. (33) found that some Jk-null individuals have mild hemolytic diseases, other investigators report that Jk-null individuals do not suffer a clinical syndrome except for a reduced capability to concentrate urine (27); similarly, individuals lacking AQP1 water channels do not suffer from hemolytic anemia (25).

Measurements by Pallone and colleagues (17, 18, 24) have shown that the apparent permeability of OMDVR to urea is reduced by the addition of thiourea, methyleneurea, phloretin, or p-chloromercuribenzenesulfonate, and that in the presence of thiourea at maximal inhibitory concentrations, the permeability of OMDVR to urea and sodium is strongly correlated. In IMDVR, however, the investigators found a close correlation between the estimated permeability to urea and sodium in vivo that is unaffected by thiourea or phloretin (24). Pallone et al. (22) therefore speculated that urea transporters may not be functional in IMDVR. To examine how this would affect transport in the medullary microcirculation, we performed simulations in which UTB was selectively removed from parts of DVR. Our model predicts that eliminating UTB transporters from IMDVR only reduces osmolality at the papillary tip by ~5% if interstitial generation rates remain unaffected; conversely, eliminating UTB transporters from OMDVR increases osmolality by a similar percentage. These results suggest that UTB can have as significant an effect in the IM as in the OM. However, it is likely here again that deletion of UTB affects tubular transport and reabsorption rates. Moreover, no conclusion from these simulations can be drawn as to whether IMDVR urea transporters are truly functional in vivo.

What role does asymmetry play in the countercurrent exchange of urea? As described above, investigators have observed that the net efflux of urea across the RBC membrane at a given concentration difference is greater than the net influx for an equal but opposite directed gradient (14, 28). In addition, UTB urea transporters have been found in the continuous DVR endothelium, but they have not been identified in the highly fenestrated AVR endothelium. Our model suggests that a 10-fold increase in the urea efflux permeability of UTB in RBCs (or a 10-fold decrease in the urea influx permeability) has a negligible effect on urea transport in the medullary microcirculation. We also examined how the expression of UTB in AVR endothelium would affect urea countercurrent exchange. If the overall permeability of AVR to urea was identical to that of DVR, our model predicts that the higher efflux of urea from AVR into the interstitium would be compensated for by a higher influx into DVR, so that urea concentrations would increase everywhere. Osmolality at the papillary tip would then increase from 1,077 to 1,337 mosmol/kgH2O and u% from 51 to 58%. As expected, these results indicate overall that the efficiency of countercurrent exchange would be significantly enhanced if transport rates were maximized in both directions. Whereas the absence of UTB in AVR endothelium may stem from the fact that it is fenestrated, a better understanding of the purpose of asymmetric transport across the transporter itself would require more precise measurements of UTB permeability to urea in specific directions.

In summary, our results suggest that the presence of UTB in DVR walls and RBC membranes increases the contribution of urea to the corticomedullary osmolality gradient but not necessarily the magnitude of the gradient itself. In addition, we found that UTB significantly reduces the swelling and shrinking of RBCs as they are carried through the medullary countercurrent exchanger. The role of UTB as a water channel appears to be negligible. Although some experimental reports suggest that UTB may not be functional in the IM, our model predicts that the urea transporter may have as significant an effect in the IM as in the OM.

**APPENDIX**

**Oncotic Pressure**

The oncotic pressure due to proteins is given (3) by

\[ \Pi_{pr} = 2.8C_{pr} + 0.18C_{pr}^2 + 0.012C_{pr}^3 \]  
(A1)

where \( C_{pr} \) denotes protein concentration. The oncotic pressure due to hemoglobin is calculated (2) as

\[ \Pi_{Hb} = RT(C_{Hb} + 0.106C_{Hb}^2 + 0.02C_{Hb}^3) \]  
(A2)

\[ C_{Hb} = \left( \frac{5.1}{34.4} \right) \frac{C_{Hb}}{1 - \frac{V}{C_{Hb}} \times 10^{-m^3}} \]  
(A3)

where \( C_{Hb} \) is the RBC concentration of hemoglobin (in g/dl), \( C_{Hb} \) is the solubility-based molar concentration of hemoglobin (in mmol hemoglobin/l solvent), and \( V = 0.75 \) ml/g is the partial specific volume of hemoglobin.

**Interstitial Cross-Sectional Area and Number of Vasa Recta**

The cross-sectional area of the IM (in cm²) is calculated (3) as

\[ A_{im}(x_{IM}) = 0.175 - 0.3883x_{IM} + 0.2606x_{IM}^2 - 0.04193x_{IM}^3 \]  
(A4)

where \( x_{IM} \) is the axial position along the IM normalized by its length. The cross-sectional area of the IM interstitium is estimated (2) as

\[ A_{int}(x_{IM}) = A_{im}(x_{IM})(0.25x_{IM} + 0.05) \]  
(A5)

In the OM, we assume that \( A_{int} \) is constant and equal to its value at the OM-IM junction (i.e., \( x_{IM} = 0 \)).

The AVR-to-DVR number ratio (\( N_n \)) is assumed to remain constant along the corticomedullary axis and equal to 2.25 (41). Thus the number of vasa recta in the IM is given by

\[ N_{DVR}(x_{IM}) = N_{AVR}(x_{IM}) = N_{AVR}(x_{IM}) \]  
(A6)

\[ N_{AVR}(x_{IM}) = N_{AVR}(x_{IM}) \]  
(A7)

where \( F_{VR} \) is the fraction of cross-sectional area occupied by vasa recta in the IM, taken as 0.3 (6, 41). In the OM, the
number of vasa recta is assumed to be constant (because we only consider those vessels that are destined to, or emanate from, the IM), and equal to its value at the OM-IM junction.

**Cell-to-Wall Surface Area Ratio**

The cell-to-wall surface area ratio for DVR and AVR is given by, respectively (40)

\[
\Gamma_{DVR} = \frac{s_0 D}{4v_0} \left( \frac{Q^R}{Q^4 + Q^R} \right) \left( \frac{Q^R N^R}{Q^R N^W} \right)^{0.5}
\]

\[
\Gamma_{AVR} = \frac{s_0 D}{4v_0} \left( \frac{Q^R}{Q^4 + Q^R} \right) \left( \frac{Q^R N^R}{Q^R N^W} \right)^{0.5}
\]

where \(s_0\) and \(v_0\) are the surface area and volume of a RBC at the corticomedullary junction, taken as 129 \(\mu\text{m}^2\) (21) and 61 \(\mu\text{m}^3\) (15), respectively, and \(N_0\) is the number of DVR at the corticomedullary junction (i.e., at \(x_{IM} = 0\)).

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**DISCLOSURES**

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