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Two modes for concentrating urine in rat inner medulla

Anita T. Layton,1 Thomas L. Pannabecker,2 William H. Dantzler,2 and Harold E. Layton3
1Department of Mathematics, University of North Carolina, Chapel Hill 27759-3250; 2Department of Mathematics, Duke University, Durham, North Carolina 27708-0320; and 3Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona 85724-5051

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The means by which mammals produce an osmolality gradient along the corticomedullary axis of the inner medulla (IM) of the kidney remains undetermined despite many decades of sustained investigation (27, 57). Most researchers believe that the IM gradient, like the outer medullary (OM) gradient, is generated by means of a countercurrent multiplication mechanism involving the renal tubules and that washout of the gradient is prevented by means of vascular countercurrent exchange.

The most frequently cited explanation for the IM urine concentrating mechanism (UCM) is the passive hypothesis proposed in 1972 by Kokko and Rector (31) and by Stephenson (63). However, this hypothesis depends on specialized loop of Henle transepithelial transport properties that appear to be inconsistent with measured values from perfused tubule experiments: when these experimental values are used in mathematical models, a significant axial osmolality gradient cannot be generated (35, 39, 46, 66, 77). Attempts to salvage the passive hypothesis, by means of models using discrete, distributed loops of Henle (35, 39) or by means of representation of the preferential interactions that arise from three-dimensional medullary structure (67, 69, 78), have not been generally successful.

Recently, a new hypothesis involving the generation of an external osmolyte, namely, lactate, has been proposed (13, 68). In principle, such a mechanism can be highly effective (19), and model studies suggest that such a mechanism could contribute to the IM gradient (13, 68). Also recently, hypotheses involving pelvic peristalsis have been revived (27); functional significance for the peristalsis is suggested by major time-dependent alterations of flow patterns in loops of Henle and vasa recta (VR) and, in antidiuretic states, by bolus flow of tubular fluid along the inner medullary collecting duct (IMCD) (54, 62).

New experimental studies combining the techniques of immunohistochemical localization and computerized three-dimensional reconstruction are providing new insight into the transport properties of long loops of Henle in rat IM (49, 50). These studies, which are summarized below (see hypotheses: two concentrating modes), suggest that in the IM, descending thin limbs (DTLs) have terminal segments of significant length that have limited water permeability; that these DTLs are NaCl impermeable except for a short prebend segment; and that most loop of Henle segments are nearly urea impermeable.

The goal of this study is to describe and evaluate, by means of mathematical modeling, two hypothetical concentrating modes for the IM that are based on the new studies. Both modes are similar to the passive hypothesis previously described by Kokko and Rector (31) and by Stephenson (63), in that NaCl absorption from loops of Henle is driven by interstitial urea that diffuses from the collecting duct (CD) system, and the mixing of this NaCl and urea raises the interstitial osmolality. However, both modes depend on locally high rates of NaCl absorption around the bends of the long loops of Henle, and both modes depend on active NaCl absorption from the CDs, which ensures that urea-rich fluid is absorbed from the portion of the CD system that is deep in the IM.

Both hypothetical modes appear to be capable of producing a significant corticomedullary osmolality gradient along the IM while maintaining reasonable urine flow and free water absorp-

1 A quantity or process distributed along the corticomedullary axis is frequently said to be “axial.”
tion rates. We call the two modes the “pipe mode” and the “solute secretion mode” (SS mode), based on differing assumptions about their urea transport properties in terminal portions of descending limbs. In the pipe mode, low solute permeabilities of the terminal descending limb allow little solute transport, and the principal solute delivered to the loop bend is NaCl. In the SS mode, substantial urea enters the terminal descending limb, and most of that urea is absorbed along the ascending thin limb (ATL); i.e., the loop of Henle acts as a countercurrent urea exchanger.

HYPOTHESES: TWO CONCENTRATING MODES

Experimental Findings on Which Our Modes Are Based

It is our objective to perform a computer-assisted, three-dimensional functional reconstruction of the rat renal IM, with emphasis on the thin limbs of Henle’s loops (51). The reconstruction is based on serial transverse sections in which cross sections of tubules are labeled and distinguished by means of antibodies to appropriate transport molecules; the labeled molecules are detected by indirect immunofluorescence (49). Currently, we are using antibodies raised against 1) the water channel aquaporin-1 (AQP1), for DTL identification and function; 2) the ClC-K1 chloride channel, for ATL identification and function; 3) the water channel aquaporin-2 (AQP2), for CD identification and function; and 4) the heat shock-related protein αB-crystallin, for identification of segments that do not express these channels. The initial reconstruction has involved the central region of the IM.

While this functional reconstruction was being performed, several findings involving the thin limbs of Henle’s loops (49) have suggested to us the modes for urine concentration that are described herein. First, the DTLs of Henle’s loops that have their bends within the first millimeter below the OM-IM border lack AQP1 and presumably are largely impermeable to water (Fig. 1A). Second, all the DTLs of loops that have their bends below this first millimeter express AQP1 for about the first 40% of their length below the OM-IM border. After this point, for about the last 60% of their length, they lack AQP1 expression (Fig. 1B). Thus the longer the long loop, the longer the segment that does not express AQP1. Presumably, this 60% of each DTL is impermeable to water or has a moderate water permeability as found in some perfusions of rat DTLs from the deep IM (5), although it is not certain that these measurements involved only the AQP1-null segments. Third, expression of ClC-K1 chloride channels begins abruptly with a prebend segment on the descending side of the loop and continues uniformly along the entire length of the ATL. The length of the prebend segment is nearly uniform and is thus independent of the length of the loop: the prebend segment always begins ~165 μm before the loop bend (Fig. 1C) (49). AQP1 and ClC-K1 are not colocalized in any segment or subsegment of the loops of Henle within the IM (49). We assume, based on previous studies with isolated, perfused rat ATLs (14), that the entire region expressing ClC-K1 and lacking expression of AQP1 (prebend segment and entire ATL) has a high permeability to Cl⁻ (and Na⁺) and virtually no permeability to water.

We have not yet examined in detail the expression of possible urea transporters along the thin limbs of Henle’s loops in the IM. However, a few preliminary sections show no evidence of expression of the urea transporters UT-A1, UT-A2, or UT-A4 in thin limbs below the first millimeter of the IM (Pannabecker TL and Dantzler WH, unpublished observations). These observations appear to agree in general with the expression pattern indicated in other types of immunocytochemical studies (48, 71), although Wade et al. (71) reported colabeling of UT-A type protein and AQP1 in DTLs from the base of the IM. Preliminary studies also show no expression of the urea transporter UT-B1 in IM thin limbs (Pannabecker TL and Dantzler WH, unpublished observations). This was expected because other data indicate that this transporter is found only in descending VR, not in thin limbs (3). These preliminary observations suggest that the AQP1-null segment of the DTLs does not express any of these known urea transporters, although a full reconstruction needs to be completed. Moreover, these observations suggest that the AQP1-null segment of the DTLs has a very low permeability to urea. However, this urea permeability has yet to be measured directly, and a high urea permeability could be found in these DTL segments and in the ATLs as the result of an unidentified transporter.

Pipe Mode Hypothesis

The pipe mode corresponds to the low urea permeability limit of the loop of Henle: those portions of the loop of Henle that are AQP1 null are assumed to have nearly zero urea permeability. The key elements of the pipe mode are these:

1) The DTL AQP1-null segment is assumed to be nearly impermeable to NaCl and urea and to have only a low-to-moderate permeability to water.
2) Because permeabilities to NaCl and urea are low, their transepithelial fluxes are small, and therefore the DTL AQ1-p
null segment functions much like a conduit or a "pipe" with respect to NaCl and urea. However, this segment’s moderate
water permeability allows it to maintain an approximation to interstitial osmolality, as indicated by micropuncture experi-
ments (8, 52).

3) At the prebend segment, the DTL permeability to NaCl increases greatly, and that high permeability is sustained along
the ATL. The prebend segment and ATL are assumed imper-
meable to water.

4) Because of urea absorbed from the IMCD (and especially urea absorbed from the innermost IMCD), the interstitial urea
concentration is large and the concentrations of electrolytes small. Thus a large transepithelial gradient favors NaCl ab-
sorption around the loop bend. A fortiori, this is the case for those loops that reach deep into the papilla.

5) Along the ATL, NaCl continues to be absorbed; indeed, the ATL serves as a near-equilibrating segment for NaCl, but
as fluid ascends the ATL, the gradient favoring its absorption diminishes, owing to NaCl that is absorbed from loop bends
that turn nearer the OM-IM boundary.

6) This mode concentrates principally by the vigorous net absorption of solute, namely, NaCl, from loop bends, which is
unaccompanied by water absorption from loop bends.

7) The VR carry away solutes and water absorbed from CDs and loops of Henle. The VR form a countercurrent exchange
configuration that maintains a high interstitial urea concentra-
tion and that prevents washout of the axial interstitial osmo-
ality gradient.

8) Active absorption of NaCl from the IMCD, accompanied
by water absorption, raises CD tubular fluid urea concentration
and reduces the load presented to the concentrating mechanism
in the inner portions of the IM. More generally, active NaCl
absorption from the IMCD serves to promote, modulate, and
spatially distribute urea absorption from the CD.

9) Even with low urea permeabilities (~1 × 10⁻⁵ cm/s),
substantial urea enters the loop of Henle, perhaps sufficient to
account for micropuncture data.

**SS Mode Hypothesis**

The SS mode corresponds to the high urea permeability limit
of the loop of Henle: those portions of the loop of Henle that
are AQ1 null are assumed to have very high urea permeability
so that near-equilibration with the interstitium can be main-
tained. The key elements of the SS mode are these:

1) The DTL AQ1-null segment is assumed to be nearly impermeable to water and NaCl; however, in this mode, the
segment is assumed highly permeable to urea.

2) Because permeability to water and NaCl along the AQ1-p
null portion of the DTL is low, NaCl concentration changes
little along the DTL; however, because urea permeability is
high, urea enters the DTL and tubular fluid urea nearly equili-
brates with the interstitial urea concentration. Thus substantial
urea is secreted into the DTL, and DTL tubular fluid osmolality
increases substantially. Indeed, if interstitial NaCl concentra-
tion is less than DTL NaCl concentration, DTL osmolality may
exceed interstitial osmolality.

3) At the prebend segment, the DTL permeability to NaCl
increases greatly, and the high permeability to urea is sustained
along the prebend segment and ATL. Thus the prebend seg-
ment and ATL are assumed to be highly permeable to both
NaCl and urea, but they are assumed to be impermeable to
water.

4) Because of urea absorbed from the IMCD (and especially urea absorbed from the innermost IMCD), the interstitial urea
concentration will be large and the concentrations of electrolytes small. Thus a large transepithelial gradient will favor
NaCl absorption around the loop bend. A fortiori, this is the case for those loops that reach deep into the papilla.

5) Along the ATL, NaCl continues to be absorbed; indeed, the ATL serves as a near-equilibrating segment for NaCl, but
as fluid ascends the ATL, the gradient favoring its absorption diminishes, owing to NaCl that is absorbed from loop bends
that turn nearer the OM. Also, as urea-rich fluid flows up the
ATL, it is opposed by a decreasing interstitial urea concentra-
tion, and urea is absorbed, thus decreasing the ATL urea
concentration and maintaining a near-equilibrium with the
interstitial urea concentration.

6) As in the pipe mode, the SS mode concentrates princi-
pally by the vigorous net absorption of a solute, i.e., NaCl, from
loop bends, which is unaccompanied by water absorption.
However, unlike the pipe mode, the loop of Henle functions as
a highly effective countercurrent urea exchanger.

7) The VR carry away solutes and water absorbed from
CDs and loops of Henle. The VR form a countercurrent exchange
configuration that maintains a high interstitial urea concentra-
tion and prevents washout of the interstitial osmo-
lality gradient.

8) As in the pipe mode, active absorption of NaCl from the
IMCD raises CD tubular fluid urea concentrations and reduces
the load presented to the concentrating mechanism in the inner
portions of the IM.

**Transport and Structural Properties That May Support
the Hypothesized Modes**

**AQ1-positive DTL segment.** As noted above, our studies
have shown that loops of Henle reaching beyond the first
millimeter of the IM have an AQ1-positive DTL segment that
makes up ~40% of the IM portion of the DTL. We hypothe-
size that water absorbed from this segment will tend to raise
the tubular fluid NaCl concentration before that fluid reaches
the AQ1-null DTL segment. A resulting higher NaCl concentra-
tion in tubular fluid at the loop bend will favor more vigorous
absorption of NaCl from the loop bend and ATL. Although our
base-case model studies (in Results) show no signiﬁcant net
water absorption from the AQ1-positive DTL segment, we
consider it likely that more complete experimental information
and more detailed model formulations will support net water
absorption from that segment.

If the AQ1-positive DTL segment is sufﬁciently permeable
to urea, transepithelial gradients may favor urea secretion into
this segment. Such secretion could support the pipe mode by
contributing to a urea-cycling pathway that conveys the se-
creted urea to the IMCD by means of tubular fluid advection
down the distal nephron. Urea secretion into the AQ1-posi-
tive DTL segment could support the SS mode by contributing
to the accumulation of urea in the papilla; indeed, the DTL and
ATL may sequester urea and, by participating in countercur-
rent urea exchange with the VR, the DTL and ATL may help
maintain the axial interstitial urea gradient and thereby help promote NaCl absorption from loop bends and ATLs.

**Loop of Henle distribution.** The loop of Henle distribution, with bends at all levels of the IM, will tend to distribute vigorous near-bend NaCl absorption all along the corticomedullary axis, thus providing a concentrating effect distributed all along that axis. Those water-impermeable loops that turn within the first millimeter below the OM-IM boundary present no load on the concentrating mechanism if no net solute is secreted into them. However, Na⁺ absorption from these loops may promote water absorption from longer loops, which have an initial AQP1-positive segment, and thus raise the NaCl concentration in the longer loops (as described above). Moreover, the approximately exponential decrease in loop population, as a function of depth, will balance the local load presented by the CD and VR with a degree of NaCl absorption that is sufficient to produce a concentrating effect at each medullary level.

**The interstitium.** The interstitium is the medium for communication among the tubules and VR; however, the extracellular gelatinous interstitial matrix and the lipid-laden cells that predominate in the interstitium of the IM may hinder axial fluid and solute movement and may thereby effectively eliminate axial advection and diffusion in the interstitium (41).

**MATHEMATICAL MODEL.**

Our mathematical model of the IM, which is based on the central core (CC) formulation introduced by Stephenson (63), includes loops of Henle and a CD; the loops of Henle and the CD interact in a common tubular compartment, the CC. The DTLs, ATLs, CD, and CC are represented by rigid tubules that are oriented along the corticomedullary axis, which extends from $x = 0$ at the OM-IM boundary to $x = L$ at the papillary tip (see Fig. 2). The model is formulated for three solutes: NaCl, urea, and a nonreabsorbable solute; NaCl is represented by Na⁺. The nonreabsorbable solute, denoted NR, is assumed to be present only in significant amounts in the tubular fluid of the CD; therefore, in the model, NR is represented only in CD tubular fluid. The model predicts fluid flow, solute concentrations, transepithelial water and solute fluxes, and fluid osmolality as a function of medullary depth, in the tubules and in the CC.

Because short loops of Henle turn in the inner stripe of the OM, mostly within a narrow band near the OM-IM boundary (12), only long loops are included in our model. We assume that 12,667 long loops of Henle (one-third of a total of 38,000 loops of Henle) and 7,300 CDs (12) extend into the IM. The long loops appear to form loop bends at all levels of the IM; thus the population of loops decreases as a function of increasing medullary depth. That decreasing loop population can be represented by a model formulation having continuously distributed loops (34) (see Figs. 2 and 3A); in such a formulation, tubular concentration profiles in loops turning at differing levels are assumed to differ, and transmural fluxes are weighted at each medullary depth according to the number of loops of a particular length remaining at that depth (see Eq. A8 in the APPENDIX). Measurements in the rat (12) indicate that the fraction of long loops of Henle decreases nearly exponentially along the IM; based on those measurements, we approximated the fraction $w_l(x)$ of loops remaining at IM depth $x$ by

$$w_l(x) = \left[1 - 0.95 \left(\frac{x}{L}\right)^2\right] e^{-2.8(x/L)}.$$  \hfill (1)

Because the CDs undergo successive coalescences along the IM, the population of CDs also decreases as a function of increasing medullary depth. In the model, all CDs are merged into a single composite tubule, and the effect of the coalescences on tubular surface area is represented by decreasing the tubular radius as a function of increasing medullary depth. That radius is decreased through multiplication by the fraction of CDs $w_{CD}$ remaining at medullary level $x$. Based on measurements in the rat (12), that fraction was approximated by

$$w_{CD}(x) = \left[1 - 0.9616 \left(\frac{x}{L}\right)^2\right] e^{-2.5(x/L)}.$$  \hfill (2)

The loop of Henle and CD population fractions, as approximated by Eqs. 1 and 2, are shown in Fig. 3A.

The model equations, which are summarized in the APPENDIX, embody the principle of mass conservation of both solute and water and represent transmural transport processes, which are described by single-barrier model equations that approximate double-barrier transepithelial transport. Transmural solute diffusion is characterized by solute permeabilities, and active transport is approximated by a saturable expression having the form of Michaelis-Menten kinetics. Transport equations for water represent osmotically driven fluxes. Boundary conditions prescribe flows and concentrations in the DTLs and the composite CD at the OM-IM boundary, i.e., at $x = 0$.  

---

**Fig. 2.** Schematic diagram of IM model, showing loops of Henle, collecting duct (CD) and central core (CC). The number of loops decreases as a function of medullary depth. Six representative loops are shown, but the numerical formulation of the model uses 300 discrete loops of Henle to approximate a continuingly decreasing distribution. Each DTL that reaches beyond the first millimeter of the IM is divided into the LDL2 (which makes up 40% of the DTL), LDL3, and a prebend segment. A DTL that turns within the first millimeter of the IM is divided into LDL2S (S denoting short) and a prebend segment. Flow from each model DTL enters directly into its associated ATL. The CD system is represented by a composite CD with decreasing luminal surface area. The DTLs, ATLs, and composite CD interact with a common compartment, the CC.
Base-case parameters for transtubular transport are given in Table 1. The model DTL of a loop of Henle that reaches beyond the first millimeter of the model IM was divided structurally and functionally into three segments. The first segment, which we call LDL2 and which spans the initial 40% of the DTL, was assumed to be highly water permeable but NaCl impermeable. (We reserve the notation LDL1 for the portion of a long descending limb that passes through the OM, a segment that is not represented in this model.) The second IM segment, which we call LDL3 and which corresponds to the AQP1-null segment of the DTL, was assumed to be impermeable to NaCl but to have a water permeability of 400 μm/s in the pipe mode, based on micropuncture experiments in rat DTLs from the deep the IM (5), and to have no water permeability in the SS mode, consistent with our finding of no AQP1 expression and with micropuncture experiments in chinchilla indicating a water permeability of 50 μm/s in DTLs from the deep IM (5). The third segment, which corresponds to the prebend segment, is a 166.7-μm-long terminal portion of the DTL that was assigned the transport properties of the ATL (the length of 166.7 μm, rather than the length of ~165 μm found in experiments, ensures that each prebend segment begins at a numerical grid point; a transition at a grid point allows the accurate representation of an abrupt change in tubular properties). The ATL was assumed to be water impermeable but highly NaCl permeable.

The DTL of a loop of Henle that turns within the first millimeter of the model IM was assumed to be water impermeable; it is divided functionally and structurally into two segments. The first, which we call LDL2S (S denoting short) and which we assumed to be NaCl impermeable, extends to the second segment, the prebend segment, which was assumed to be functionally like its associated ATL, which we designate ATL-S.

Our base-case urea permeabilities in loops of Henle differ substantially between the two modes. Our preliminary experimental results (see above, hypothesis: two concentrating modes) suggest that urea permeability in most segments is essentially zero. However, in the pipe mode we used a value of $1 \times 10^{-5}$ cm/s to allow some urea entry, because micropuncture studies suggest urea entry into DTL (52). Based on findings by Wade et al. (71) of apparent colocalization of AQP1 and a UT-A urea transporter, we assumed that the LDL2 segment has a moderate permeability of $13 \times 10^{-5}$ cm/s, a value suggested by microperfusion measurements (47).

In the SS mode, we assumed that all IM loop segments have at least a moderate permeability to urea and that the urea permeabilities of LDL3, the prebend segment, and the ATL are very large. The LDL2S, ATL-S, and LDL2 were assigned a permeability of $13 \times 10^{-5}$ cm/s (47). The urea permeabilities of the LDL3, the prebend segment, and the ATL were suggested to us by the high permeabilities measured in the long loops of Henle of chinchilla (6). The permeability of the

### Table 1. Base-case transtubular transport parameters

<table>
<thead>
<tr>
<th></th>
<th>LDL2s</th>
<th>LDL2</th>
<th>LDL3</th>
<th>ATL-S</th>
<th>ATL-S</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_w$, μm/s</td>
<td>0 (49)</td>
<td>2200 (14)</td>
<td>400 (5)</td>
<td>0 (14)</td>
<td>0 (14)</td>
<td>750 (58)</td>
</tr>
<tr>
<td>Pipe mode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS mode</td>
<td>0 (49)</td>
<td>2200 (14)</td>
<td>0 (49)</td>
<td>0 (14)</td>
<td>0 (14)</td>
<td>750 (58)</td>
</tr>
<tr>
<td>$P_{Na}$, $10^{-5}$ cm/s</td>
<td>0 (49)</td>
<td>2200 (14)</td>
<td>0 (49)</td>
<td>0 (14)</td>
<td>0 (14)</td>
<td>1 (60)</td>
</tr>
<tr>
<td>$P_{\text{urea}}$, $10^{-5}$ cm/s</td>
<td>0 (49)</td>
<td>2200 (14)</td>
<td>0 (49)</td>
<td>0 (14)</td>
<td>0 (14)</td>
<td>1 (60)</td>
</tr>
<tr>
<td>Pipe mode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS mode</td>
<td>13 (47)</td>
<td>13 (47, 71)</td>
<td>13 (47)</td>
<td>13 (47)</td>
<td>13 (47)</td>
<td>13 (47)</td>
</tr>
<tr>
<td>$V_{\text{max}, \text{Na}}$, mmol/cm²s⁻¹</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100 (6)</td>
<td>150 (6)</td>
</tr>
<tr>
<td>$V_{\text{max}, \text{urea}}$, mmol/cm²s⁻¹</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100 (6)</td>
</tr>
</tbody>
</table>

Parameters are given by, based on, estimated from, or suggested by references given in parentheses. $P_w, P_{\text{urea}}, P_{\text{Na}}$: permeability to water, Na, and urea, respectively; $V_{\text{max}, \text{Na}}, V_{\text{max}, \text{urea}}$: maximum transport rate. *A prebend segment is assumed to have the same transport parameters as its contiguous short ATL (ATL-S) or ATL. †Parameter increases exponentially as medullary depth $x$ increases from 2.5 to 5.0 mm. ‡Arrows indicate that parameter varies linearly between the given values as medullary depth $x$ increases; see details in text.
TWO MODES FOR CONCENTRATING URINE

prebend segment and ATL was taken to be $150 \times 10^{-5}$ cm/s, lower than the measured value of $170 \times 10^{-5}$ cm/s in chinchilla ATL, but about an order of magnitude larger than reported values in rat of $14-23 \times 10^{-5}$ cm/s (14, 47). The permeability for LDL3 was taken to be $100 \times 10^{-5}$ cm/s, about twice the value of $48 \times 10^{-5}$ cm/s reported in chinchilla for the lower DTL (6). Our value for urea permeability in LDL3 supports the effective function of the SS mode and may not be unreasonable, because results from microperfusion studies of DTLs may have been skewed by tubules that spanned more than one functional segment.

The CD urea permeability was assumed to be 1 (in units of $10^{-5}$ cm/s) for the first half of the model IM (i.e., for $x \in [0, L/2]$); for the second half of the IM ($x \in [L/2, L]$), CD urea permeability was assumed to increase exponentially, according to the formula

$$P_{CD, urea}(x) = P_0 + (P_1 - P_0) \frac{e^{\alpha(x/L/2)} - 1}{e^{\alpha(L/2)} - 1},$$

where $P_0$ and $P_1$ are the initial and terminal CD urea permeabilities, 1 and 110, respectively, and $\alpha = 7$; the CD urea permeability profile is shown in Fig. 3B. This profile was constructed to be consistent with experiments in antidiuretic rats showing high urea permeabilities in the terminal CD (22, 59) and to ensure sufficient urea delivery to the deep medulla to support the hypothesized modes (see above).

The CD Na+ maximum transport rate ($V_{max, Na}$) was assumed to be 9 nmol·cm⁻²·s⁻¹ for the initial three-tenths of the model IM; it linearly decreases to 2.5 nmol·cm⁻²·s⁻¹ for the next two-tenths, and linearly decreases to 0 nmol·cm⁻²·s⁻¹ along the remainder of the IM; the $V_{max, Na}$ profile is shown in Fig. 3C. Substantial evidence, recently summarized by Weinstein (75), indicates that the IMCD is capable of brisk active Na⁺ absorption. The profile for $V_{max, Na}$ was chosen to ensure that substantial urea was absorbed from the CD (by means of maintaining a sufficient transepithelial urea gradient) and that the solute load reaching the terminal CD was consistent with experimental evidence for moderately antidiuretic rats (see below). The Michaelis constant for CD Na⁺ active transport was set to 40 mM (10). All tubules were assumed to have no active urea transport: although active urea transport has been found in the CD (23), the rate of such transport appears to be small relative to passive fluxes.

The osmotic coefficients $\phi_k$ were set to be 1.84 for NaCl and NR and 0.97 for urea (74). The reflection coefficients $r_{k,k}$ for all solutes were set to 1 for all tubules (56). The partial molar volume of water $V_w$ was set to 0.018136 cm³/mM for 37°C (74).

Axial length $L$ of the model IM was taken to be 5 mm, an appropriate value for the rat kidney (26). Tubular diameters were assumed to vary as a function of medullary depth. Luminal diameters for loops of Henle were based on measurements by Koepsell et al. (29): DTL diameter decreases linearly, starting at the OM-IM boundary (i.e., at $x = 0$), from 15 to 11 μm, and then abruptly increases to 13 μm at the prebend segment and remains at that value to the point of loop bend; along the ATL, the diameter increases linearly from 13 to 20 μm at the return to the OM-IM boundary. CD diameter, based on measurements by Knepper et al. (26), increases from 20 to 25 μm. CC diameter was assumed to be 20 μm at $x = 0$, and CC cross-sectional area decreases with medullary depth at the same fractional rate as the CD population decreases (Eq. 2).

The boundary water flows and solute concentrations that were specified for the DTL and CD at the OM-IM boundary (i.e., $x = 0$) are given in Table 2. DTL boundary water flow was assumed to increase linearly as a function of the length of the loop, based on evidence that juxtamedullary glomeruli have higher SNGFRs than glomeruli that are likely to give rise to short-looped nephrons (55). Because the boundary values have not been measured directly by experiment, our choices (especially those for the CD) were influenced by general considerations based on relevant measurements (e.g., the composition of urine and of tubular fluid in distal tubule) (1, 52) and were chosen to provide urea delivery rates sufficient for the concentrating modes. Indeed, CD inflow rate and concentrations at the OM-IM border, and the magnitudes and spatial distribution of Na⁺ and urea transport along the IMCD, were chosen, in part, by means of an informal optimization procedure. Many trial calculations were conducted using various combinations of the CD boundary conditions and transport properties. Based on the experience gained, the conditions and properties were chosen to obtain model urine osmolalities and flow rates that are consistent with experimental findings. We believe that this optimization procedure is justified by the substantial regulatory control exhibited by the CD system in response to physiological needs.

The SLSI-Newton method, which was previously developed and tested for models of the UCM (32, 33), was used to obtain steady-state numerical solutions for this model study. A spatial discretization of 300 subintervals was used; thus 300 discrete loops of Henle were represented, each having a loop bend at a distinct numerical grid point. All calculations were performed by means of computer programs written in FORTRAN and executed in double precision on a computer system with two Intel Pentium IV 1.8-GHz processors and with 1 GB of RAM.

RESULTS

The model equations (APPENDIX) were solved, using the base-case model configuration, parameter sets (Table 1), and boundary conditions (Table 2) to obtain steady-state model solutions for the pipe and SS modes. Key results are displayed graphically in Figs. 4 and 5, and they are compared with micropuncture measurements in Table 4 (see DISCUSSION). A quantity expressed in units “per nephron” is the total of that quantity per kidney (or the total of that quantity per kidney at a specified medullary level), divided by the number of nephrons per kidney.

Table 2. Boundary conditions at OM-IM boundary

<table>
<thead>
<tr>
<th>Tubule</th>
<th>$F_{V_C}$</th>
<th>$C_{Na}$</th>
<th>$C_{urea}$</th>
<th>$C_{NR}$</th>
<th>Osmolality, mosmol/kg H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTL</td>
<td>5 – 7.5*</td>
<td>350</td>
<td>30</td>
<td>0</td>
<td>673.1</td>
</tr>
<tr>
<td>CD, pipe mode</td>
<td>2.4†</td>
<td>180</td>
<td>314.54</td>
<td>20</td>
<td>673.1</td>
</tr>
<tr>
<td>CD, SS mode</td>
<td>2.2†</td>
<td>120</td>
<td>428.35</td>
<td>20</td>
<td>673.1</td>
</tr>
</tbody>
</table>

OM, outer medulla; IM, inner medulla; $F_{V_C}$, water flow rate; $C_{Na}$, $C_{urea}$, $C_{NR}$: Na⁺, urea, and nonreabsorbable solute (NR) concentration, respectively; DTL, descending thin limb; CD, collecting duct; SS, solute secretion. *Inflow rate increases linearly between the values given as IM loop length increases from 0 to 5 mm. †Flow per CD; corresponding flows per nephron are −0.461 nl/min and −0.423 nl/min for the pipe and SS modes, respectively.

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Base-Case Pipe Mode Model Results

The pipe mode model predicts a urine osmolality of 1,265 mosmol/kg H2O and urine flow rate of 0.0780 nl/min per nephron (2.96 µl/min per kidney), assuming 38,000 nephrons per kidney). Model urine concentrations were 254, 597, and 118 mM for Na+, urea, and NR, respectively. These values are consistent with experimental measurements from moderately antidiuretic rats (52).

Fundamental results for the pipe mode, as a function of IM depth, are shown in Fig. 4. Figure 4A shows that osmolality increases, with increasing depth, in the CD, CC, and longest DTL (except near the OM-IM boundary and in the prebend segment). In the CD, the osmolality increases from 673 to 1,265 mosmol/kg H2O, i.e., by a factor of 1.88. Along the longest DTL, osmolality increases from 673 to 1,218 mosmol/kg H2O at the beginning of the prebend segment, and then it decreases to 1,073 mosmol/kg H2O at the loop bend.

Osmolality profiles for loops of Henle lengths of 1.0, 2.0, 3.5, and 5.0 mm are shown in Fig. 4B. The loops of Henle that turn within the first millimeter of the IM are assumed to be entirely water impermeable, and thus the osmolality in the LDL2S of the 1-mm-long loop shows only a small increase, which is due to diffusive urea entry. Beginning at the transition to the prebend segment, osmolality decreases abruptly in all loops, and a rapid rate of decrease continues around the loop bend. Osmolality continues to decrease (as considered in the tubular flow direction) along the contiguous ATL for a portion of that ATL that is about one-third to one-half of its length. In sufficiently long loops, ATL tubular fluid osmolality eventually exceeds that of its corresponding DTL at the same level; however, because of the overlapping of shorter loops that have an ATL osmolality that is lower than corresponding DTL osmolality, the osmolality of ATL fluid, taken as a whole at each medullary level, is dilute relative to DTL fluid. Thus the ATLs, taken as a whole, carry dilute fluid out of the IM. This dilute ascending flow is a necessary mass-balance requirement for concentrating urine in our modes, because the osmolarities of flows entering DTLs and the CD at the OM-IM boundary are fixed at a common boundary value and because the CC carries fluid out of the IM that has only a very slightly lower osmolality at the OM-IM boundary than DTL and CD tubular fluid at the boundary.

CC fluid is very slightly dilute at the OM-IM boundary (672 mosmol/kg H2O) relative to DTL and CD fluid (673 mosmol/kg H2O) because urea and Na+ secretion into ATLs is sufficiently large near the boundary to have a net diluting effect on CC fluid; this diluting effect is apparent in Fig. 4A along the first millimeter to the right of x = 0: CC osmolality is initially decreasing with increasing medullary depth, but ATL tubule fluid osmolality, along its flow direction, is increasing. Electron microprobe measurements performed at a corresponding medullary levels in antidi-
uretic rat kidneys detected a similar local nonmonotonicity in Na⁺ and Cl⁻ concentrations (30).

The change in the patterns of osmolality in the loops of Henle can be understood by consideration of the transepithelial Na⁺/H⁺, urea, and water fluxes, which can be inferred from their concentrations relative to the CC and CD. The Na⁺, urea, and NR concentrations for the longest loop, CD, and CC are shown in Fig. 4, C and D. Because the LDL2 is assumed to be impermeable to Na⁺, its nearly constant Na⁺ concentration indicates little net reduction in water flow along the LDL2. Thus its osmolality is increased by the entry of urea, which continues, although at a slower rate, along the LDL3, because the LDL3 is assumed to have a lower urea permeability than the LDL2. Water absorption from the moderately water-permeable, but Na⁺-impermeable, LDL3 segments contributes to the concentrating mechanism by increasing tubular fluid Na⁺ concentration significantly before that fluid reaches the prebend segments.

The vigorous absorption of Na⁺ around the loop bend is a consequence of the abrupt increase in Na⁺ permeability and a transepithelial gradient that favors Na⁺ absorption. That gradient arises because urea absorbed from the CD results in a high CC urea concentration relative to the CC Na⁺ concentration. Indeed, the resulting CC Na⁺ concentration is below that of the loop Na⁺ concentration, and thus a substantial transepithelial outward-directed Na⁺ concentration gradient is maintained around the loop bend and along part of the ATL. This effect is particularly marked for loops that reach deep into the IM, because of the high CD urea permeability and thus large CD urea absorption rate along the terminal CD. However, the secretion of some urea into the loops of Henle tends to somewhat reduce the concentrating effect generated by Na⁺ absorption from the loops: the osmolality of core fluid is directly reduced by the urea secretion, and, in addition, this reduction has the indirect effect of discouraging water absorption from LDL2 segments, which tends to reduce loop Na⁺ concentration and thus the gradient favoring Na⁺ absorption from Na⁺-permeable loop segments.

Fluid flow rates, along the CD and CC, and composite fluid flow rates along the DTLs and ATLs, per nephron, are shown in Fig. 4E; the flow rates are taken to be positive when flow is in the direction of increasing medullary depth. Because of loops turning back along the IM, the DTLs-to-CD flow ratio (i.e., the ratio of total flow in all DTLs to total flow in all CDs at a given medullary level) rapidly decreases, from 4.12 at the OM-IM boundary to 0.141 at the papillary tip, where the longest loops are assumed to turn back.

Solute flow rates, per nephron, along the composite CD are shown in Fig. 4F. The CD loses 76% of its Na⁺ in the first 2 mm of the IM, because, by our modeling assumptions, Na⁺ is vigorously actively absorbed (with a V_{max,Na} of 9 nmol·cm⁻²·s⁻¹). This active absorption promotes water absorption from the early CD, because the low CD urea permeability (1 × 10⁻⁵ cm/s) restricts the participation of urea flux
in transepithelial osmotic equilibration. Moreover, the active Na\(^+\) absorption raises the relative concentration of urea in CD tubular fluid (see Fig. 4D), which ultimately promotes vigorous urea absorption from the terminal CD, where urea permeability greatly increases. In the inner 60\% (approximately) of the CD, active Na\(^+\) transport is only sufficient to nearly balance diffusive Na\(^+\) backleak.

**Base-Case SS Mode Model Results**

The SS mode model predicts a urine osmolality of 1,031 mosmol/kgH\(_2\)O and urine flow rate of 0.0889 nl·min\(^{-1}\)·nephron\(^{-1}\) (3.38 µl·min\(^{-1}\)·kidney\(^{-1}\), assuming 38,000 nephrons/kidney); model urine concentrations were 110, 430, and 95 mM for NaCl, urea, and NR, respectively. These values are consistent with experimental measurements in moderately antidiuretic rats (52).

Fundamental model results for the SS mode are shown in Fig. 5. Figure 5A shows that osmolality increases, with increasing depth, in the CD, CC, and longest DTL (except near the OM-IM boundary and in the prebend segment). In the CD, the osmolality increases from 673 to 1,031 mosmol/kgH\(_2\)O, i.e., by a factor of 1.53. The osmolality increase along most of the DTLs is large, due to the large urea permeability of the LDL3, which results in substantial urea entry; indeed, the osmolality near the bend of the longest loop exceeds the osmolality of all other IM model structures, and even after an abrupt decrease of osmolality around the loop bend, the ATL has an osmolality that exceeds that of the CC and CD.

Osmolality profiles for loops of Henle with lengths of 1.0, 2.0, 3.5, and 5.0 mm are shown in Fig. 5B. The loops of Henle that turn within the first millimeter of the IM are assumed to be water impermeable, and thus the osmolality in the DTL of the 1-mm-long loop shows a small increase due to urea influx, which arises from a higher urea permeability than in the pipe mode. Each of the longer loops of Henle exhibits a very substantial increase in osmolality along its LDL3, which is due to the very high urea permeability (100 × 10\(^{-5}\) cm/s) assigned to the LDL3 in this mode. At the transition to the prebend segment, each loop has a substantial decrease in osmolality, and that osmolality continues to decrease, in the flow direction, along much of the ATL. Although, at some levels, the osmolality of an ATL may exceed that of its corresponding DTL, the net effect, as in the pipe mode, is that the overlapping loops, taken as a whole, at each level carry a fluid that is osmotically dilute relative to DTL, and thus the mass-balance requirement for a concentrated urine is met. (A small mass-balance difference in this mode, however, relative to the pipe mode, is that the CC carries fluid from the IM that is slightly higher in osmolality than the inflow osmolarities of the DTLs and the CD at the OM-IM boundary.)

The Na\(^+\), urea, and NR concentrations for the longest loop, CD, and CC are shown in Fig. 5, C and D. As in the pipe mode, the LDL2 is assumed to be Na\(^+\) impermeable, and its nearly constant Na\(^+\) concentration indicates little net change in water flow along the LDL2; indeed, water flow slightly increases (detailed results not shown). Thus tubular fluid osmolality in LDL2 is increased only by urea entry, a process that is accelerated along the LDL3 by its yet higher urea permeability.

As in the pipe mode, the vigorous absorption of Na\(^+\) around the loop bend is a consequence of the abrupt increase in Na\(^+\) permeability and a transepithelial gradient that favors Na\(^+\) absorption, and that gradient is sustained by urea absorption into the interstitium from the CD. Also, as in the pipe mode, the effect of near-bend Na\(^+\) absorption is particularly prominent for loops that reach deep into the IM. Unlike the pipe mode, however, the high urea permeabilities that are assumed in the SS mode for the water-impermeable loop segments result in the loops of Henle acting as countercurrent urea exchangers: at each IM level, much urea enters DTLs and much urea leaves ATLs, resulting in a urea cycle that is closed by advection of urea in tubular fluid around the loop bend and by transfer of urea from ATLs to DTLs through the CC. Although the addition of urea to loops is ultimately dissipative, because the ATLs carry more urea out of the IM than the DTLs carry in (detailed results not shown), urea entry into DTLs nonetheless results in the advection of fluid that is hyperosmotic, relative to fluid in other structures, toward the papillary tip. This effect is made possible by the water-impermeable segments of the DTLs, which permit a decoupling of loop tubular fluid osmolality from interstitial osmolality.

Fluid flow rates along the CD and CC, and composite fluid flow rates along the DTL and ATL, per nephron, are shown in Fig. 5E. As in the pipe mode, the distributed loop configuration yields a small DTLs-to-CD flow ratio at the papillary tip, where the longest loops turn. Na\(^+\), urea, and NR flow rates along the CD are shown in Fig. 5F. As in the pipe mode, a high urea concentration is maintained in the CD via a vigorous active NaCl absorption near the OM-IM boundary. However, for the SS mode, we used a higher urea inflow at the OM-IM boundary, and a lower Na\(^+\) inflow, relative to the pipe mode, to ensure that sufficient urea was available to attain a urine osmolality closer to that attained in the pipe mode.

These base-case model results, for the pipe and SS modes, are generally consistent with our hypotheses (as described above in HYPOTHESES: TWO CONCENTRATING MODES), with the exception that, in the model results, significant water is not absorbed from the LDL2.

**Parameter Studies**

By means of parameter studies, we investigated the impact of transmural transport properties, structural assumptions, CD inflow rate, and our CC configuration on simulated urine osmolality, urine flow, and the free water absorption rate (FWA). FWA, which is the volume, per unit time, of blood plasma that could be considered to be completely cleared of solutes by the production of urine that is more concentrated than blood plasma (18, 76), is given by

\[
FWA = F_{\text{urine}, v} (U/P - 1),
\]

where \(F_{\text{urine}, v}\) is the urine flow rate, and U/P is the urine-to-plasma osmolality ratio; in our studies we assumed that the plasma osmolality is 310 mosmol/kgH\(_2\)O (11). A typical value for FWA, for a moderately antidiuretic rat having a urine osmolality of ~1,200 mosmol/kgH\(_2\)O and a urine flow of 0.060 nl·min\(^{-1}\)·nephron\(^{-1}\), is ~0.17 nl·min\(^{-1}\)·nephron\(^{-1}\) (52). Our base-case pipe and SS modes yielded FWA rates of 0.240 and 0.207 nl·min\(^{-1}\)·nephron\(^{-1}\), respectively.

In some instances, we assess changes from base-case model urine osmolality by means of a change in relative percent osmolality increase along the IM. The relative percent osmolality increase is defined by
where $U_B$ is the model base-case urine osmolality (1,265 or 1,031 mosmol/kgH$_2$O in the pipe or SS modes, respectively, as appropriate to context); $U$ is the model urine osmolality obtained by a change in parameter value from its base-case value; and $C_{OM}$ is the osmolality of tubular fluid entering the IMCD from the outer medullary collecting duct (OMCD) at the OM-IM boundary (i.e., at $x = 0$), which is 673.1 mosmol/kgH$_2$O. The relative increase given by Eq. 5 provides a measure of osmolality change that is relative only to the base-case increase in osmolality produced along the IM; this measure is appropriate because we assume that the OM always provides a fixed CD tubular fluid osmolality at the OM-IM boundary, and the OM increase above systemic blood plasma osmolality should not contribute, in our parameter studies, to comparisons of osmolality change along the IM. In subsequent text, changes in this measure are called “relative” increases (or decreases or changes) in urine osmolality (or concentrating capability).

**LDL3 water permeability.** Based on perfused tubule studies in rats (14) and other mammals (57), the rat DTL has usually been assumed to be highly water permeable up to the loop bend, or until the beginning of the prebend segment (39, 64, 72; for an exception, see Ref. 66). Indeed, DTL water permeability was considered as an essential element of the passive mechanism (31, 63); as in our pipe mode, water absorption from the DTL was hypothesized to raise the NaCl concentration of tubular fluid entering the ATL and thus promote a larger transepithelial gradient favoring NaCl absorption from the ATL.

We investigated the impact of LDL3 osmotic water permeability by varying it from 0 to 1,000 μm/s; results are shown in Fig. 6, Aa–Ac. In the pipe mode, as LDL3 water permeability increased, urine osmolality increased monotonically from 1,198 to 1,298 mosmol/kgH$_2$O. Thus the pipe mode does not require LDL3 water permeability, but relative osmolality change (as defined by Eq. 5) was reduced 11% when LDL3 was made water impermeable. Reckoned from the base case of 1,265 mosmol/kgH$_2$O obtained at a water permeability of 400 μm/s, relative osmolality change was increased 5.6% from base case when water permeability was increased to 1,000 μm/s. The increased osmolality arises from increased water absorption from LDL3. Because the LDL3 tubular fluid osmolality is hyposmotic relative to the CC, more water was absorbed as the LDL3 became increasingly more water perme-
able. The absorption of water increased DTL tubular fluid Na⁺ concentration and promoted increased rates of loop-bend Na⁺ absorption. However, increased LDL3 water permeability also resulted in reduced urine flow rates: water absorbed from the LDL3 reduced CC urea concentration and increased urea absorption from the CD, and this urea absorption was accompanied by water absorption from the CD. A LDL3 water permeability of 1,000 μm/s resulted in a urine flow rate of 0.075 nl·min⁻¹·nephron⁻¹, a 3.8% decrease. However, FWA decreased little relative to base case, because of the increase in urine osmolality.

In the SS mode, as LDL3 osmotic water permeability increased from 0 to 1,000 μm/s, urine osmolality decreased to 799 mosmol/kgH₂O, a 65% relative decrease (as defined by Eq. 5). In contrast to the pipe mode, tubular fluid osmolality in the LDL3 is hyperosmotic to CC fluid in the base-case SS mode. Thus as LDL3 water permeability increased, water entered the LDL3, diluted its tubular fluid, and thus reduced Na⁺ absorption near the loop bend. Consequently, urine osmolality and FWA decreased, whereas urine flow rate increased.

**LDL3 and ATL Na⁺ permeabilities.** As we previously noted, in both base-case modes high rates of Na⁺ absorption from loops of Henle are localized near loop bends (Figs. 4C and 5C). This absorption pattern arises from our specification of loop Na⁺ permeability: to conform to evidence from our immunohistochemical localization studies, LDL3 Na⁺ permeability was set to zero, and the Na⁺ permeabilities of the model prebend segment and ATL were set to a high value of 80 × 10⁻⁵ cm/s (14). Model results, as LDL3 Na⁺ permeability varied from 0 to 20 × 10⁻⁵ cm/s, are shown in Fig. 6, Ba––Bc. As LDL3 Na⁺ permeability increased, more Na⁺ was absorbed from LDL3, resulting in decreased Na⁺ delivery to the loop bend and thus reduced tubular fluid Na⁺ concentration near the loop bend and along the ATL. As Na⁺ absorption from the LDL3 increased, urine osmolality and FWA decreased, and urine flow increased. These results suggest that Na⁺ absorption from the LDL3 is less effective than Na⁺ absorption from the deeper, loop-bend region.

The effect of variation in ATL Na⁺ permeability is shown in Fig. 6, Ca––Cc. As permeability increased from 1 to 150 × 10⁻⁵ cm/s, urine osmolality increased, FWA increased, and urine flow decreased, effects due to more vigorous Na⁺ absorption around the loop bend and more nearly complete Na⁺ concentration equilibration with the CC along the ATL. However, when ATL Na⁺ permeability is sufficiently high (higher than the base-case value), additional increases have a diminishing effect on the rate of urine osmolality increase.

**LDL3 and ATL urea permeabilities.** The effect of varying urea permeabilities in LDL3 and ATL is shown in Fig. 7. Figure 7, Aa––Ac, shows the impact of increasing LDL3 urea permeability from 1 to 200 × 10⁻⁵ cm/s while keeping all other urea permeabilities at base-case values; Fig. 7, Ba––Bc, shows the impact of increasing ATL urea permeability from 1 to 200 × 10⁻⁵ cm/s, while keeping all other urea permeabilities at base-case values; and Fig. 7, Ca––Cc, shows the impact of simultaneously increasing LDL3 and ATL urea permeabilities from 1 to 200 × 10⁻⁵ cm/s.

In the pipe mode, one of the LDL3 or ATL urea permeabilities increased while the other remained at base-case value, urine osmolality and FWA decreased sharply, whereas urine flow rate increased. When the urea permeability of the LDL3 or ATL was set to 20 × 10⁻⁵ cm/s, relative urine osmolality decreased by 88 or 87% to 745 and 756 mosmol/kgH₂O, respectively; further increases in urea permeability further reduced urine osmolality, but at a slower rate. The increased urea permeabilities increased urea secretion into the LDL3 and the ATL; this flux reduced CC urea concentration but increased loop urea concentration. The secretion of urea in the loop tends to reduce CC osmolality, and when LDL3 urea permeability was increased, the secreted urea tended to be sequestered in the ATL (which has low urea permeability), thus increasing the osmolality of ATL fluid, which, in turn, tends to dissipate the concentrating effect by carrying fluid toward the OM that is not sufficiently dilute. When ATL urea permeability was increased, secretion of urea into the ATL was similarly dissipative.

When LDL3 and ATL urea permeabilities were simultaneously increased, urine osmolality showed an initial dramatic reduction: a urine osmolality of 711 mosmol/kgH₂O was obtained for urea permeabilities of 20 × 10⁻⁵ cm/s. However, as the urea permeabilities were further increased, urine osmolality increased as the model configuration more nearly approximated the SS mode; for urea permeabilities of 200 × 10⁻⁵ cm/s, an osmolality of 927 mosmol/kgH₂O was attained. In this limit, the entry of urea results in loop-bend fluid having an ATL osmolality exceeding that of the adjacent CC, and along the ATL, the high urea permeability supports the effective absorption of urea and limits its dissipative effect.

In the SS mode, when LDL3 or ATL urea permeability was reduced while the other was kept at its base-case value, the model yielded lower urine osmolality, lower FWA, and a higher urine flow rate. Urine osmolalities of 677 and 628 mosmol/kgH₂O were obtained for LDL3 and ATL urea permeabilities of 1 × 10⁻⁵ cm/s, respectively, which correspond to relative osmolality decreases of 99 and 113%, reductions which indicate that the IM osmolality gradient was abolished at these low-permeability limits.

Compared with the pipe mode base-case urine osmolality, however, the SS mode base case is less sensitive to variations in LDL3 and ATL urea permeabilities, inasmuch as small changes in urea permeability resulted in less change in osmolality than that observed in the pipe mode’s dramatic decrease. In the SS mode, the concentrating effect is generated, in part, by urea secretion into the LDL3, which, owing to the decoupling of the osmolality of the water-impermeable LDL3 from that of adjacent tubules, raises the osmolality of LDL3 fluid. A high ATL urea permeability, coupled with high Na⁺ permeability, results in a rapid osmotic equilibration of ATL tubular fluid with the CC (ATL fluid was hyperosmotic relative to the CC at the loop end). Thus lower LDL3 or ATL urea permeabilities resulted in less concentrated DTL tubular fluid at the loop bend, or in a more concentrated ATL tubular fluid, both of which reduced concentrating capability.

In the SS mode, when both LDL3 and ATL urea permeabilities were reduced simultaneously, osmolality showed an initial decline (to 765 mosmol/kgH₂O for urea permeabilities of 20 × 10⁻⁵ cm/s), but further reductions in these permeabilities resulted in increased urine osmolality (to 1,398 mosmol/kgH₂O for urea permeabilities of 1 × 10⁻⁵ cm/s), as the model configuration closely approximated pipe mode permeabilities. Indeed, for urea permeabilities of 1 × 10⁻⁵ cm/s, urine...
osmolality (and the corresponding FWA) was higher than the base-case values for both modes, because of the more favorable boundary conditions used in the SS mode (see Table 2).

**LDL3 length.** For the base case, based on our tubular reconstructions, we assumed that the LDL3 and prebend segments made up the lower 60% of those DTLs reaching 1 mm or more into the IM. To determine the effect of this configuration on model results, we varied the normalized LDL3 length from 0 to 1. At normalized length 0, the LDL2 was lengthened to replace the LDL3 and join the prebend segment; at normalized LDL3 length 1, the LDL3 was assumed to make up the whole IM portion of those DTLs (except for prebend segments) reaching at least 1 mm into the IM.

The effect of varying normalized LDL3 length is shown in Fig. 8, Aa–Ac. As normalized LDL3 length was increased from 0 to 1, concentrating capability increased in both modes. In the pipe mode, urine osmolality increased from 752 mosmol/kg H2O to the base-case of 1,265 mosmol/kg H2O at normalized length 0.6, and then to 1,527 mosmol/kg H2O, i.e., from a relative 87% decrease in concentrating capability to a relative 44% increase. However, as normalized LDL3 length increased from 0 to 1, urine flow decreased from 0.143 to 0.0615 nl·min⁻¹·nephron⁻¹. Combined with the effect of increasing osmolality, this resulted in an initial increase in FWA to a relatively unchanging value nearly equal to the base-case FWA, 0.240 nl·min⁻¹·nephron⁻¹. In the SS mode, as normalized LDL3 length increased from 0 to 1, urine osmolality increased from 698 to 1,248 mosmol/kg H2O, urine flow decreased from 0.109 to 0.0734 nl·min⁻¹·nephron⁻¹, and FWA increased from 0.136 to 0.222 nl·min⁻¹·nephron⁻¹.

These results indicate that the LDL3 plays an important role in both modes. In the pipe mode, urea permeability is higher in LDL2 (13 × 10⁻⁵ cm/s) than in LDL3 (1 × 10⁻⁵ cm/s). Thus a longer LDL2 (i.e., a shorter LDL3) promotes more urea secretion into the DTL, which results in a near-osmotic equilibration with the CC by both water absorption and substantial urea secretion. As a consequence, the DTL Na⁺ concentration increases less than it would if osmotic equilibration were mostly by water absorption, and urea is removed from the CC and sequestered in the ATL, which has a low urea permeability. Ultimately, the gradient favoring Na⁺ absorption is re-
duced, and the urea retained in the ATL tends to be highly dissipative.

In the base case for the SS mode, osmolality of the LDL2 fluid is close to that of the interstitial fluid (see Fig. 5A) because of the high water permeability of the LDL2, whereas osmolality of the LDL3 fluid increases significantly above the adjacent CC, because the LDL3 is water impermeable but highly urea permeable. A shorter LDL3 resulted in the DTL carrying relatively less concentrated fluid toward the loop bend and in reduced urea entry. These effects tend to reduce urea entry in the DTL and delay equilibration with the CC urea concentration. When the LDL3 is completely absent, urea entry will occur only into the loop bend and the ATL; such entry is highly dissipative because it tends to raise the osmolality of ATL flow.

**Maximum length of pure AQP1-null DTLs.** In both modes, we have assumed that the DTL of a loop of Henle that turns within the first millimeter of the IM is entirely AQP1 null and that its DTL, before the prebend (i.e., the LDL2S), is impermeable to Na⁺ and has either low (pipe mode) or moderate (SS mode) urea permeability. We investigated the effect of these pure AQP1-null DTLs by varying this maximum length (or “cutoff” length) of the AQP1-null DTLs from 0 (no pure AQP1-null DTLs) to 5 mm (all DTLs are AQP1-null). The results are shown in Fig. 8, Ba–Bc.

For the pipe mode, the percentage range of relative variation in urine osmolality (based on the measure given by Eq. 5), as the cutoff length of the AQP1-null DTLs was varied from 0 to 5 mm, was 23%; the ranges of the variations in urine flow rate and FWA were 25 and 12%, respectively. These changes were nonmonotonic and too complex to easily interpret. As the cutoff length increased from 0, more loops had low urea permeability along their entire length [LDL2S urea permeability is low (1 × 10⁻⁵ cm/s) compared with LDL2 urea permeability (13 × 10⁻⁵ cm/s)]. With less urea entering the DTLs, a high interstitial urea concentration (and a corresponding low interstitial electrolyte concentration) was sustained. However, the water-impermeable LDL2S allows no water absorption that would raise the Na⁺ concentration before tubular fluid reaches the prebend segment. These two competing factors resulted in

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Fig. 8. Parameter studies for LDL3 segment length (column A), maximum length of DTLs having LDL2S segments (column B), and prebend length (column C). Solid curve, pipe mode; dashed curve, SS mode; ●, base-case value for pipe mode; ○, base-case values for SS mode. Negative prebend length indicates that the portion of the ATL that follows the bend has the physical characteristics of the LDL3 segment.
maximum urine osmolality (and minimum urine flow rate) at a cutoff length of \( \sim 3.75 \text{ mm} \); a further increase in the cutoff length resulted in decreased urine osmolality, but despite this decrease, urine flow rate increased sufficiently to result in increased FWA.

For the SS mode, the percentage range of relative variation in urine osmolality (based on the measure given by Eq. 5) was 37%; the ranges of variations in urine flow rate and FWA were 10 and 11%, respectively. As the cutoff length increased from 0, more DTLs had moderate permeability (13 \( \times \) \( 10^{-3} \text{ cm/s} \)) before their prebend segments, which resulted in lower urine concentrations at the loop bends compared with base case. Thus urea continued to enter along the ATLs, and this entry produced a dissipative effect that decreased urine-concentrating capability.

We conclude from these results that our model study does not indicate, in the context of UCM function, a clear rationale for those loops of Henle having water-permeable segments in the DTL.

**Prebend segment length.** Prebend segments having lengths of 166.7 \( \mu \text{m} \) are represented in both modes, and in both modes base-case Na\(^+\) permeability was assumed to abruptly increase from 0 to \( 80 \times 10^{-5} \text{ cm/s} \) at the beginning of the prebend segment and to remain at that value throughout the prebend segment and the ATL. The impact of varying prebend segment length is shown in Fig. 8, Ca–Cc. Negative length indicates that a corresponding portion of the terminal ATL was assigned the transport characteristics and diameter-decrease pattern of the LDL3. The base-case prebend length corresponds to approximately the optimal concentrating effect: nearly maximum urine osmolality and FWA (but nearly minimum urine flow) were obtained. Doubling the prebend length resulted in only small changes: in the pipe mode, relative urine osmolality was increased by 0.17% to 1,266 mosmol/kgH\(_2\)O, whereas in the SS mode relative urine osmolality was decreased by 2.2% to 1,023 mosmol/kgH\(_2\)O; changes in other variables were also small. However, when the prebend was replaced by a “postbend” (i.e., when the prebend length was set to negative values), a more significant reduction in concentrating effect was obtained. With a postbend length of 166.7 \( \mu \text{m} \) (which is equal to the base-case prebend length), relative urine osmolality decreased by 40% to 1,028 mosmol/kgH\(_2\)O in the pipe mode and by 41% to 883 mosmol/kgH\(_2\)O in the SS mode. It is noteworthy that these effects are generated by relatively small structural changes: the base-case prebend length is only \( \sim 3.3\% \) of the IM length.

**Loop of Henle population distribution.** To determine the effect of the loop of Henle population distribution, we conducted simulations using alternative fractional distributions of the form

\[
\begin{align*}
\psi(x) &= \left[ 1 - 0.95a^{1/6} \left( \frac{x}{L} \right)^{2} e^{-2.8a^{1/6}(x/L)} \right],
\end{align*}
\]

where 0 \( \leq a \leq 2.5 \). The case of \( a = 1.0 \) corresponds to the base-case distribution (cf. Eq. 1), while \( a = 2.5 \) corresponds to a case where many more loops turn back near the OM-IM boundary, and \( a = 0 \) corresponds to the case where all loops reach to the papillary tip. Representative distributions are illustrated in Fig. 9Aa, and our assessment measures, as a function of variable distribution parameter \( a \), are shown in Fig. 9, Ab–Ad. In both modes, as \( a \) was increased from 0 to \( \sim 1 \) (i.e., as fewer loops reached into the deep IM), urine osmolality and FWA increased, and urine flow rate decreased. We interpret these results to mean that if \( a \) is near 0, so that a large fraction of the loops reach far into the IM, then the tubular fluid in the lengthened loops puts an additional load on the concentrating mechanism, the cascade effect of overlapping loop bends diminishes, and the ratio of urea-to-Na\(^+\) concentration in the CC is reduced by urea secretion into the lengthened loops (the reduced ratio diminishes the driving force for Na\(^+\) absorption from loop bends and ATLs). However, as \( a \) was increased to 1.0, the impact of the lengthened loops was reduced, the cascade was restored, and a configuration with adequately high CC urea concentration (and sufficiently low CC Na\(^+\) concentration) for the model to concentrate effectively was reached.

As \( a \) increased from 1.0 to 2.5, urine osmolality decreased and urine flow rate increased; FWA also increased, although slowly. We interpret these results to mean that sufficient Na\(^+\) must be delivered (via the loops) to the deep IM for the modes to be effective in concentrating urine. The FWA was sustained at high values for this range of \( a \) because increased urine flow more than compensated for reduced urine osmolality.

An overall implication of these results, as \( a \) ranges vary its full range, is that at each level of the IM, a balance between Na\(^+\) delivery from loops and urea delivery from the CDs must be maintained to ensure a vigorous concentrating effect.

**CD urea permeability profile.** In the model, CD urea permeability is low (\( P_{0} = 1 \times 10^{-5} \text{ cm/s} \) (22) in the first half of the IM but increases exponentially to \( P_{1} = 110 \times 10^{-5} \text{ cm/s} \) (59) in the second half of IM. Thus CD urea permeability \( P_{CD,\text{urea}} \) is given by

\[
\begin{align*}
P_{CD,\text{urea}}(x) &= \begin{cases} 
P_{0} & x \leq bL, \\
(P_{0} + (P_{1} - P_{0})e^{2ab/(1-b) - 1}/(e^{2ab/(1-b) - 1} - 1)) & x > bL, 
\end{cases}
\end{align*}
\]

where \( \alpha = 7 \) and in the base case, \( b = 0.5 \) (cf. Eq. 3). Differing permeability profiles, corresponding to \( b = 0.1, 0.25, 0.5, 0.75, \) and 0.95, are illustrated in Fig. 9Ba. For larger values of \( b \), CD urea permeability remains low for a longer portion of the IM. The impact of differing permeability profiles, as a function of \( b \), for \( b \) ranging from 0.1 to 0.95, is shown in Fig. 9, Bb–Bd. For sufficiently low values of \( b \), much urea is absorbed near the OM-IM boundary, which reduces the urea available to drive Na\(^+\) absorption from the loops of Henle that reach deep into the IM. However, as \( b \) increased to \( \sim 0.35 \), maximum urine osmolalities were obtained, \( \sim 1,420 \) and \( \sim 1,121 \) mosmol/kgH\(_2\)O, for the pipe and SS modes, respectively. As \( b \) increased further, urine flow rate increased whereas urine osmolality decreased; these two competing effects resulted in increased FWA. The osmolality decrease is due to decreased urea efflux from the CD, which arises from the increased high-permeability area that is available for efflux. Sufficient urea efflux from a sufficiently long portion of the terminal CD, and a resulting sufficiently high CC urea concentration, promotes the effective operation of the two modes.

**CD maximum Na\(^+\) active transport rate.** Active Na\(^+\) absorption from the initial portion of the model CD facilitates the modes by raising the urea concentration in CD tubular fluid and
by reducing the load presented to the concentrating mechanism in the deep portion of the model IM. Because a wide range of values have been reported for Na$^+$ active transport rates in CD (75), we determined the sensitivity of model results to this parameter by scaling the base-case $V_{\text{max}, \text{Na}}$ profile by a factor $c$, where $c$ ranged from 0.5 to 1.5. The base-case profile and the profiles corresponding to $c = 0.5$ and $c = 1.5$ are shown in Fig. 9C.

As the scaling parameter $c$ increased from 0.5 to 1.5, urine osmolality in the pipe mode increased from 875 to the base-case 1,265 at $c = 1$ to 1,813 mosmol/kg H$_2$O, i.e., from a relative decrease in concentrating capability of 66% to a

---

**Fig. 9.** Parameter studies for loop of Henle distribution (column A), CD urea permeability profile (column B), and maximum CD Na$^+$ active transport rate ($V_{\text{max}, \text{Na}}$; column C). Aa: sample loop distributions, as a function of scaling parameter $a$ in Eq. 6. Ba: sample CD urea permeability profiles, as a function of scaling parameter $b$ in Eq. 7. Ca: sample CD $V_{\text{max}, \text{Na}}$ profiles, as a function of proportional scaling factor $c$. Solid curve, pipe mode; dashed curve, SS mode (rows b, c, and d); •, base-case value for pipe mode; ○, base-case values for SS mode.
relative increase of 93%; in the SS mode, urine osmolality increased from 819 to the base case of 1,031 to 1,148 mosmol/kgH₂O, i.e., from a relative decrease of 59% to a relative increase of 33% (see Fig. 9Cb). The osmolality increases were accompanied by decreasing urine flows (to minimums of 0.032 and 0.062 nl·min⁻¹·nephron⁻¹ in the pipe and SS modes, respectively for cd = 1.5; see Fig. 9Cc). These two competing effects resulted in a decrease in FWA (see Fig. 9Cd), although the rate of decrease was reduced for cd > 1.0. These results suggest that a sufficiently high Vmax,Na is necessary to raise CD urea concentration and sustain effective modes. Moreover, with a Vmax,Na profile that is higher than base case, a higher urine osmolality may be obtained, although urine flow is reduced.

We also computed the model’s concentrating capacity when the base-case Vmax,Na profile was replaced with a spatially uniform constant-rate Vmax,Na, and that constant rate was varied from 5 to 7 nmol·cm⁻²·s⁻¹ (results not shown). We obtained the same general patterns for osmolality, urine flow, and FWA as those shown in Fig. 9, Cb–C d. To make a comparison with results using the base-case Vmax,Na, we identified constant rates of Vmax,Na that would produce the same osmolalities as produced by our base-case profiles; those rates are 5.91 and 5.37 nmol·cm⁻²·s⁻¹ for the pipe and SS modes, respectively. However, the corresponding urine flow rates for constant Vmax,Na are 0.0686 and 0.0782 nl·min⁻¹·nephron⁻¹, whereas those for the base case are 0.0780 and 0.0889 nl·min⁻¹·nephron⁻¹. Thus at equal urine osmolalities, the nonuniform base-case Vmax,Na produces ~11% more urine, for both the pipe and SS modes, than in the case of constant Vmax,Na, and because the FWAs are proportional to the urine flows when osmolalities are fixed, the corresponding FWAs will also be ~11% greater for the base case. These findings suggest that the modes are not critically dependent on the spatial pattern of Vmax,Na. However, these findings also suggest that higher Na⁺ absorption rates from the CD nearer the base of the IM (relative to rates in the papilla) tend to produce larger urine flows for given urine osmolalities than will uniformly distributed absorption capacity.

CD inflow rate. CD flow rate at the OM-IM boundary is difficult to ascertain by experimental means, and it almost certainly varies substantially with the state of the animal (1). Therefore, we investigated the effect of the CD inflow rate on the modes; results for rates ranging from 1.5 to 4.0 nl·min⁻¹·nephron⁻¹ are shown in Fig. 10, A–C (base-case values for the pipe mode and SS mode are 2.4 and 2.2 nl·min⁻¹·nephron⁻¹, respectively).

In both modes, as CD inflow rate increased from its base-case value, urine flow rate and, consequently, FWA increased. However, urine osmolality decreased to 811 and 749 mosmol/kgH₂O (relative decreases of 77 and 79%) for the pipe and SS modes, respectively, for an inflow rate of 4.0 nl·min⁻¹·nephron⁻¹. This reduction in urine osmolality results principally from the increase in load arising from increased CD flow. When the CD inflow rate was decreased from the base-case values, urine osmolality initially increased, owing to a reduced load, to maximums of ~1,539 and 1,101 mosmol/kgH₂O, respectively (relative increases of 46 and 20%) for the pipe and SS modes, respectively, at an inflow rate of ~1.8 nl·min⁻¹·nephron⁻¹. However, a further reduction in inflow rate resulted in decreased urine osmolality. This decrease resulted from reduced urea flow into the IM, a urea flow that was no longer sufficient to support the transepithelial Na⁺ concentration gradient that promotes Na⁺ absorption from loops of Henle.

Nonideal countercurrent exchange in the CC. Our CC formulation does not take into account potential gradient-diminishing effects arising from nonideal countercurrent exchange by the VR. Indeed, our formulation assumes that the transendothelial permeabilities of the VR to solutes and water are essentially infinite, a configuration that corresponds to maximally effective countercurrent exchange and that may produce optimal concentrating effects (24). Nonideal vascular countercurrent exchange may be represented in a CC model by including a term in the CC solute conservation equation that represents diffusion along the corticomedullary axis of the CC (63) (see Eq. A3 and accompanying text in the APPENDIX). However, the appropriate magnitude of the diffusion coefficient for that term depends on several factors, and these factors, involving flow rates and effective permeabilities (63), are spatially inhomogeneous and difficult to characterize. Stephenson et al. (66) used diffusion coefficients for NaCl and urea that are about an order of magnitude larger than corresponding values for solute self-diffusion in dilute aqueous solution; the
self-diffusion coefficients for Na$^+$ and urea are $\sim 1.5$ and $\sim 1.38 \times 10^{-5}$ cm$^2$/s, respectively (42, 74).

To demonstrate that our modes can produce significant concentrating effects for nonideal countercurrent exchange, we conducted simulations using CC diffusion coefficients that are factors of $j = 1, 10, 100$ of the self-diffusion coefficients for Na$^+$ and urea. Resulting values of key assessment variables for model urine and for CC solute concentrations at $x = L$ are given in Table 3. (The nonreabsorbable solute NR plays no explicit role in this sensitivity study, because NR is confined to the model CD lumen.) In the pipe mode, urine osmolality was reduced from the base-case value of 1,265 to 1,255, 1,181, and 1,039 mosmol/kgH$_2$O for $j = 1, 10$, and 100, respectively; these reductions correspond to decreases of 1.69, 14.2, and 38.2% of the base-case osmolality increase along the IM. These reductions mostly arise from reductions in CC urea concentration more than Na$^+$ concentration because the change in urea concentration along the corticomedullary axis (a 636% increase relative to the value at the OM-IM boundary) is much larger than the change in Na$^+$ concentration. Osmolality is expressed as mosmol/kgH$_2$O; Na$^+$ and urea concentrations are expressed in mM; and urine flow and free water absorption (FWA) are expressed in nL/min−1·nephron−1, based on 38,000 nephrons.

Table 3. Sensitivity to diffusion in CC

<table>
<thead>
<tr>
<th></th>
<th>Pipe Mode</th>
<th></th>
<th>SS Mode</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$j=1$</td>
<td>$j=10$</td>
<td>$j=100$</td>
<td>$j=1$</td>
</tr>
<tr>
<td>Osmolality</td>
<td>1,255</td>
<td>1,181</td>
<td>1,039</td>
<td>1,025</td>
</tr>
<tr>
<td>Na$^+$ concentration</td>
<td>252</td>
<td>241</td>
<td>235</td>
<td>140</td>
</tr>
<tr>
<td>Urea concentration</td>
<td>593</td>
<td>551</td>
<td>453</td>
<td>610</td>
</tr>
<tr>
<td>NR concentration</td>
<td>117</td>
<td>110</td>
<td>91</td>
<td>95</td>
</tr>
<tr>
<td>Flow</td>
<td>0.0790</td>
<td>0.0836</td>
<td>0.0896</td>
<td>0.0892</td>
</tr>
<tr>
<td>FWA</td>
<td>0.241</td>
<td>0.235</td>
<td>0.211</td>
<td>0.206</td>
</tr>
<tr>
<td>CC Osmolality</td>
<td>1,267</td>
<td>1,195</td>
<td>1,050</td>
<td>1,037</td>
</tr>
<tr>
<td>Na$^+$ concentration</td>
<td>380</td>
<td>363</td>
<td>336</td>
<td>246</td>
</tr>
<tr>
<td>Urea concentration</td>
<td>585</td>
<td>544</td>
<td>444</td>
<td>602</td>
</tr>
</tbody>
</table>

Central core (CC) diffusion coefficients are $j$ times solute diffusion coefficients. Osmolality is expressed as mosmol/kgH$_2$O; Na$^+$, urea, and NR concentrations are expressed in mM; and urine flow and free water absorption (FWA) are expressed in nL/min−1·nephron−1, based on 38,000 nephrons.

results demonstrate that nonideal countercurrent exchange can affect base-case results, but a more comprehensive model will be required to better assess the impact of VR properties on our hypothesized modes.

DISCUSSION

Summary

We have proposed two modes for IM urine concentrating function that were suggested to us by new studies in rats that utilize methods of immunohistochemical localization and computerized three-dimensional tubular reconstruction. The configurations for the modes differ only in their descending limb water permeabilities, their loop of Henle urea permeabilities, and their CD inflow conditions. Our mathematical models suggest that both modes can generate significant osmolality gradients along the corticomedullary axis of the IM, as shown in the base-case results (Figs. 4 and 5). To varying degrees, however, both modes are sensitive to parameter choices, as shown in the parameter studies.

In the SS mode, simulated urine osmolality decreases as osmotic water permeability in LDL3 increases (Fig. 6, $Aa$–$Ac$), because combined LDL3 urea and NaCl concentrations cannot as effectively attain osmolalities exceeding interstitial osmolality. Both modes are sensitive to increased LDL3 Na$^+$ permeability (Fig. 6, $Ba$–$Bc$), which tends to diminish NaCl absorption from loop bends; and both modes require sufficiently high ATL Na$^+$ permeability for effective operation (Fig. 6, $Ca$–$Cc$) to promote significant NaCl absorption near the loop bend and to ensure that ATL tubular fluid NaCl concentration nearly equilibrates with that of adjacent interstitial fluid. In the case of insufficient equilibration, the relatively concentrated tubular fluid carried toward the OM by ATLs tends to dissipate the axial gradient.

The pipe mode concentrates more effectively when loop of Henle urea permeabilities (in units of $10^{-5}$ cm/s) are sufficiently small ($\sim 1–5$), whereas the SS mode concentrates more effectively when the loop urea permeabilities are sufficiently large (more than or equal to $\sim 100$); intermediate values (e.g., $\sim 10–50$) significantly decrease concentrating capability in both modes (Fig. 7, $Aa$–$Ac$). Because perfused tubule studies have indicated that chinchilla loops are highly permeable to urea (6, 39), whereas our preliminary data suggest that rat loops are nearly impermeable to urea (see HYPOTHESIS: TWO CONCENTRATING MODES), both the low and high urea permeability limits (i.e., both the pipe and SS mode) could be exploited in vivo, depending on species. On the other hand, definitive evidence that the loops are only moderately urea permeable when animals are in an antidiuretic state would cast doubt on both modes.

Both modes require that the LDL3 make up a sufficient fraction of the DTL (Fig. 8, $Aa$–$Ac$); otherwise, the LDL2, which is highly water permeable and somewhat permeable to urea, tends to dissipate the transepithelial loop-to-interstitium osmolality gradients on which the modes depend, and the system will operate much like previous, and unsuccessful, model formulations of the passive mechanism (e.g., 36, 39, 46, 66, 77). Indeed, LDL3 is essential to both modes; in the pipe mode it prevents significant urea entry, whereas in the SS mode it decouples DTL tubular fluid osmolality from that of adjacent interstitial osmolality.
The pipe mode is not particularly sensitive to assigning to all loops transport properties like those in loops that reach into the first millimeter of the IM and are AQP1 null (i.e., the case where maximum AQP1-null DTL length is set to 5 mm in Fig. 8, Ba–Bg), but SS mode urine osmolality and FWA are diminished significantly, because urea entry is reduced by the assumption of lower urea permeability of the LDL2s, relative to LDL3. Although the model prebend segment has a length of 167 μm, a length that is a small fraction of the length of most loops, calculations nonetheless predict that prebend length can significantly affect concentrating capability and that the length found in vivo is nearly the optimal length for both modes, with respect to obtaining a high urine osmolality (Fig. 8, Ca–Cc).

Loop distributions that differ significantly from the base case yield substantially decreased concentrating capability (Fig. 9, Aa–Ad). If additional loops reach deeper into the IM, urea absorption from CDs is insufficient to maintain the favorable gradients that drive passive NaCl absorption in the deep IM; when too few reach deep into the IM, NaCl absorption from loops is insufficient to result in a significant concentrating effect.

The alternative CD permeability profiles represented in our parameter studies indicate that sufficient urea must be absorbed at sufficient IM depth (Fig. 9, Ba–Bd). Studies in which the maximum CD Na⁺ absorption rate was multiplied by a scalar predict that NaCl absorption must be sufficiently large to ensure sufficiently high urea concentrations in the deep IM and that increasing NaCl absorption tends to increase urine osmolality while decreasing urine flow and FWA (Fig. 9, Ca–Cd).

The rate of inflow from the OMCD to the IMCD has a significant impact on model results (Fig. 10, A–C). When flow is reduced below ~1.75 nl·min⁻¹·nephron⁻¹, too little urea is delivered to the IM for effective concentrating mechanism function; as flow increases above ~2.0 nl·min⁻¹·nephron⁻¹, the load presented to the papilla exceeds the capacity of the concentrating mechanism.

Among the most noteworthy of these parameter study findings, in the context of the new experimental results on which they are based, is that LDL3 length plays an important role in both modes: its low permeability to urea is indispensable for an effective pipe mode, and its low permeability to water is indispensable for an effective SS mode. It is also noteworthy that the prebend segment appears to contribute a small, although significant, increment to total concentrating capability.

Significance and Comparisons

The most significant finding of this study is that mechanisms that are similar to those envisioned by the passive hypothesis and that show substantive consistency with emerging data may be capable of generating significant osmolality gradients along the IM. The passive hypothesis proposed by Stephenson (63) and by Kokko and Rector (31) depended on particular NaCl, urea, and water transport properties for the IM portions of long loops of Henle: the passive hypothesis assumed high water permeability for DTLs, and it depended on sufficiently low urea and NaCl permeabilities for DTLs and sufficiently low urea permeability, relative to NaCl permeability, for ATLS. However, as has been noted in a number of reviews (e.g., 16, 27, 57), perfused tubule experiments provided evidence for loop of Henle urea and NaCl permeabilities that were incompatible with the passive hypothesis, and that incompatibility has been demonstrated in a number of modeling studies (including, e.g., 36, 39, 46, 66, 77) that were based on the experimental permeabilities but that failed to generate significant axon osmolality gradients. Consequently, many researchers have abandoned the passive hypothesis and have proposed alternatives.

Notable among these alternatives are hypotheses that depend on three-dimensional structure, on external osmolytes, or on the peristalsis of the renal papilla. The role of three-dimensional structure was investigated in the highly influential “WKM” model proposed by Wexler et al. (78). Although this model, in its original formulation, predicted a significant IM gradient that was localized at the base of the IM and away from the papilla, the model drew criticisms for some of its structural and transport assumptions (12, 20, 64, 65). Subsequent studies sought to address the criticisms yet ultimately acknowledged that the preferential interactions that depended on the accepted three-dimensional structure were not sufficient to explain the IM concentrating effect in the papilla (69, 72, 73). The theoretical feasibility of external osmolytes contributing to a concentrating effect appears well established (13, 19, 68, 69), although experimental evidence is inconclusive. The concentrating potential of peristalsis (27, 35, 61) is more speculative: no convincing model calculations for its effective operation have been published, to our knowledge.

Because no generally accepted alternatives to the passive hypothesis have emerged, the hypothesis has remained popular, despite an absence of convincing support from mathematical models. The principle of using the potential energy contained in separated streams of fluid, one having high urea concentration, relative to NaCl concentration (as in CD), and the other having high NaCl concentration, relative to urea concentration (as in DTL), has remained appealing, because such energy may be sufficient to provide an adequate thermodynamic explanation for high urine osmolalities (63).

How do our concentrating modes differ from previous model studies? And how are significant axial osmolality gradients generated by our two modes?

Unlike most other recent model studies (36, 39, 46, 66, 77), we have not considered our loop of Henle urea permeability choices to be bound by average values reported in perfused tubule experiments (e.g., 14, 15, 47); rather, in conformity with our own recent experiments (49) and unpublished preliminary observations (see hypothesis: two concentrating modes), we have assumed low loop of Henle urea permeabilities in AQP1-null loop segments, or, alternatively, we have assumed high urea permeabilities in those segments, similar to values reported in chinchilla (6). Urea permeabilities obtained in perfused tubule measurements in the rat, hamster, and rabbit have exhibited a large range of variability (14, 15, 47), and the measurements were obtained under experimental circumstances that may not have been representative of normal function in antidiuretic animals.

Our pipe mode has much in common with the passive hypothesis (31, 63), in which the DTLs were assumed to have high osmotic water permeability so that DTL tubular fluid would be in near-osmotic equilibrium with the interstitium, whereas NaCl absorption was conceptualized to occur along the water-impermeable ATLS. NaCl absorption was hypothesized to be driven by a gradient rendered favorable for NaCl uptake.
diffusion by urea absorption from the CD system. However, concentrating capability from this absorption has to be utilized to raise the osmolality of DTL fluid, which presents a load to the concentrating mechanism. In the pipe mode, this load is offset by increased Na\(^+\) absorption around the loop bend. In the SS mode, the LDL3 is considered water impermeable, and thus the osmolality of LDL3 tubular fluid is decoupled from the adjacent interstitial osmolality, and tubular fluid can exceed interstitial osmolality. In both modes, DTLs function essentially as conduits for NaCl delivery to the loop bend, where the rate of NaCl absorption is highest, owing to the high NaCl permeability at the beginning of the prebend segment. The loop bend is at the deepest portion of the medulla reached by a particular loop, and at that deepest point, it is in contact with the highest interstitial urea concentration that is encountered by that loop. Both of the modes depend on DTL NaCl concentrations that are mostly generated by water absorption as the limbs pass through the OM; if the interstitial urea concentrations of the IM are large enough, the DTL NaCl concentrations attained in the OM provide a sufficient gradient for vigorous NaCl absorption from bends of long loops.

In both our modes, the long loops of Henle act to target NaCl delivery to the loop bends, to promote maximal NaCl absorption there, and along the first portion of the ATL (thought of in the direction of tubular flow) additional NaCl is absorbed as the NaCl concentration equilibrates with the interstitium. In essence, the ATLS act as NaCl-equilibrating segments, and overlapping loops of Henle act to preserve a composite ATL fluid that is osmotically dilute relative to other IM fluids at each level.

In the SS mode, owing to urea entry into DTLs and to the decoupling of DTL osmolality from that of the interstitium, loop fluid osmolality in the deepest loop portions exceeds that of the adjacent interstitium. Thus at the loop bend, and along the ATL, both NaCl and urea are absorbed and contribute to the axial osmolality gradient. This aspect of SS mode function is similar to a case described by Knepper and others (25, 27, 28): the medulla may concentrate not only by means of relatively dilute ascending segments but also by means of relatively concentrated descending segments.

Both the pipe mode and SS mode depend on the sufficient delivery of urea to IMCD and on the conveyance of much of that urea to the papilla. Moreover, the urea concentration in the CD, along the papilla, must be sufficiently large, relative to CD NaCl concentration, to ensure that solute absorbed from the CD will result in an interstitial fluid with a sufficiently high urea concentration relative to NaCl concentration. Such a configuration is consistent with experimental studies showing high urea concentration relative to NaCl concentration in urine (see below). To ensure sufficiently high CD urea concentration in our model studies, we adjusted CD urea permeabilities to promote urea absorption mostly in the papilla. This absorption pattern is consistent with measurements of urea permeability (57) and evidence that suggests an absence of urea transporters in the initial portion of the IMCD (71).

To ensure that the urea concentration in the CD remains sufficiently high, relative to NaCl concentration, along the course of the CD, we have assumed active NaCl absorption along the IMCD, absorption that is especially vigorous near the IM base. This absorption also promotes osmotic water absorption from the CD, and thus it helps regulate the load that CD contents present to papilla. Active NaCl absorption from the IMCD has been represented in some previous model studies (e.g., 39, 66, 72). However, our maximum transport rate \( V_{\text{max,Na}} \) (9 nmol cm\(^{-2}\) s\(^{-1}\) near the IM base) is higher than rates previously used in models of the UCM, although it is compatible with experimental studies (75). (We acknowledge, however, that the spatially inhomogeneous NaCl transport used in our model is not based on experimental findings but is formulated to regulate CD flow and urea content along the model papilla.)

Because of our use of substantial NaCl active transport rates along the IMCD, we may not be justified in characterizing our modes as passive, because they depend on active NaCl transport from the IMCD (especially in the IM base) to continue the separation of urea from NaCl that was begun in the thick ascending limbs of the OM and sustained and augmented by the intervening cortical segments and the OMCD. However, like the passive hypothesis, our modes depend on the separation of urea from NaCl and on the subsequent mixing of that urea and NaCl in the interstitium and vasculature of the IM. Perhaps these modes, and the passive hypothesis itself, could more accurately be called "solute-separation, solute-mixing" hypotheses.

We have previously demonstrated, in principle, that solute absorption localized near the bends of the loops of Henle (37) can increase concentrating capability, and modeling studies for Gambel’s quail (38, 43) have indicated that active NaCl absorption around the ends of looped avian nephrons may play a role in the efficiency of the avian UCM. These studies suggest the general principle that it is desirable for a loop of Henle to deliver solute, free of accompanying water, as deep in the medulla as practicable and as plentifully as possible at the deep site (37). This general principle is exploited by both of our modes, as can be seen in the significant decrease in Na\(^+\) concentration around loop bends in Figs. 4C and 5C. In the longest loop, the average rates of Na\(^+\) absorption along the prebend segment are 47.2 and 53.0 nmol cm\(^{-2}\) s\(^{-1}\) for the pipe and SS modes, respectively. The corresponding rates along the postbend length corresponding to the prebend length are 24.4 and 35.8 nmol cm\(^{-2}\) s\(^{-1}\). Thus in each mode, Na\(^+\) absorption rates, from the overlapping effect of the bend of a single loop of Henle, are two to three times greater than the Na\(^+\) absorption rate that can be estimated (7) for the thick ascending limb in the inner stripe of the OM. The principle of near-bend absorption is coupled with a distribution of loops that is approximately exponential and that tracks the exponential coalescences of the CDs. This suggests that solute absorption from loops of Henle, at each medullary level, will be balanced with the CD flow that must be concentrated at that level.

A rationale that was advanced for the efficacy of solute absorption from near loop bends was that solute so absorbed does not have to concentrate the shorter DTLs that have already turned back (37). This rationale may apply to the pipe mode, but it does not appear to be generally applicable to loops of Henle that have long terminal regions that are water impermeable, as in the SS mode. However, it may apply near the base of the IM, where sufficiently long loops have a water-permeable descending segment (here called LDL2). The rationale may also apply to the load presented by VR flow, which
decreases with medullary depth. Another reason for efficacy of near-bend absorption may be that solute absorbed from loop bends will have the lowest likelihood of entering ATLs of other loops of Henle.

**Limitations of Model Modes**

Our model modes attain urine osmolalities consistent with a moderate antidiuresis, and our parameter studies suggest reasonable parameter and boundary condition changes that could produce significantly higher osmolalities than those found in our base cases. However, it seems unlikely that osmolalities consistent with the highest measured values for the rat (nearly 3,000 mosmol/kgH₂O) can be attained in our current model modes while also maintaining realistic urine flows. This discrepancy may be attributable in part to long-term adaptation in water-deprived animals. Trinh-Trang-Tan et al. (70) have shown that the thickness of the inner stripe of OM can be substantially increased in Brattleboro rats having diabetes insipidus by chronic administration of antidiuretic hormone, or in Wistar rats by restricting water intake. A marked increase in concentrating capability that accompanies the inner stripe hypertrophy may be due to altered boundary conditions at the OM-IM junction, including higher osmolality in descending tubular flows, higher NaCl concentration in DTLs, and lower flow rates, which would correspond to a reduced load for the IM concentrating mechanism.

Aspects of renal structure and function that are not represented in our model may promote one or both of the concentrating modes that we have described. These aspects include the VR, which may help to sequester urea in the IM; the three-dimensional structure, which may facilitate preferential interaction among tubules and vessels; renal peristalsis, which may reduce osmotic washout by reducing IM blood flow, or which may have a more active role by mediating a temporal cycle that contributes to, or supplements, our concentrating modes (35); solutes not currently represented in our model, such as potassium, which might contribute to favorable trans-epithelial gradients for NaCl absorption from loops of Henle; or external osmolyte accumulation. Thus although we have proposed potential passive modes that appear consistent with emerging data and that produce significant IM osmolality gradients in our mathematical models, we acknowledge that these modes, if they do indeed play a role in the IM concentrating mechanism, are likely to be supported by, or may work in parallel with, other mechanisms.

**Comparison with Experimental Results**

In Wistar rats in various states of diuresis, Atherton et al. (2) examined the ionic composition and osmolality of the final urine and of slices of kidney taken at six intervals from inner cortex to papillary tip. These studies, or course, simply averaged the composition of fluid in all structures within a given slice. In moderately antidiuretic rats, the average osmolality in the IM base, (~700 mosmol/kgH₂O) was similar to our model osmolalities at the OM-IM boundary (Figs. 4 and 5), and the average urine volume flow rate (~3 µl·min⁻¹·kidney⁻¹) and average osmolality at the tip of the papilla (~1,200–1,300 mosmol/kgH₂O) are in reasonable agreement with base-case values predicted by both model modes (Table 4).

More detailed comparisons can be made between the base-case model predictions and the results of in vivo micropuncture studies that measured the composition of fluid from loops of Henle and CD tips and/or final urine. Because the thin limb reconstructions that stimulated development of our model modes involved the rat kidney, the most directly applicable study appears to be that of Pennell et al. (52), which examined the composition of these fluids in normal moderately antidiuretic Sprague-Dawley rats. The significant comparisons with model results for osmolality, Na⁺ concentration, urea concentration, and flow rates are shown in Table 4. In the experiments by Pennell and co-workers, the urine flow rate was ~2.4 µl·min⁻¹·kidney⁻¹, which is somewhat lower than our base-case rates of 3.0 and 3.4 µl·min⁻¹·kidney⁻¹ predicted by the pipe mode and SS mode, respectively. Nonetheless, the model rates are appropriate for animals in a moderate antidiuresis, and the model rates could be reduced with even higher simulated urine osmolality with reduced boundary inflow into the IMCD (Fig. 10A). The urine osmolalities predicted by the two modes (~1,000–1,300 mosmol/kgH₂O) and those observed in the rats (~1,200 mosmol/kgH₂O) are comparable. Moreover, both modes predict a urine urea concentration substantially higher than the urine Na⁺ concentration, in accord with the experimental results. A similar relationship between urine urea and Na⁺ concentrations is also observed in hamsters with comparable urine osmolalities (8). However, the pipe mode predicts substantially higher urine urea and Na⁺ concentrations than observed in the rat study despite a higher predicted urine flow rate. Nevertheless, the predictions of both modes for urine osmolality, urea concentration, and Na⁺ concentration (Table 4) are in generally good agreement with corresponding measurements in both rats (52) and hamsters (8) that are in moderate antidiuresis.

The osmolalities predicted by the model for tubular fluid in the bends of the longest loops are similar to osmolalities...
measured in antidiuretic rats (Table 4), and the model osmolalities are also similar to measured osmolalities in the final urine (or in the fluid at the end of the CDs) (52). An osmolality of the fluid at the bend of the long loops nearly identical to that in the fluid at the terminal end of the CDs is also observed in hamsters (8, 9). The osmolality predicted by both modes for the terminal end of the CC (1,277 and 1,043 mosmol/kg H2 O for the pipe and SS modes, respectively) is similar to that predicted for urine (or fluid in the terminal end of the CDs). Moreover, the osmolality measured in VR near the papillary tip (4 mm above the papillary tip, 150 mosmol/kg H2 O) observed previously (38) is comparable to the average concentration in tubular fluid at the terminal end of the CDs. Therefore, the composite CD used in the model need not contain factors that represent the sums of the circumferences of all CDs to accurately represent the osmolality to the sum of the cross sections of all CDs to compute accurate steady-state solute concentration profiles. However, some differences between physiological data and model predictions appear in the urea and Na\(^+\) concentrations at the bend of Henle’s loop (Table 4). Studies in moderately antidiuretic rats (52) and hamsters (8, 9, 44, 45) failed to bend to equal the observed or predicted for the fluid at the bend of the loops or the CDs at the same level of the papilla (9, 21, 44, 45, 53).

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In an early micropuncture study in rats with a part of the kidney cut away to reveal more of the papilla, Jamison et al. (17) found that, in the region 2–4 mm above the papillary tip, fluid in the ATL was significantly hypotonic to fluid in the DTL at the same level. This difference between the DTLs and ATLs observed in the osmolality profiles of the DTLs and ATLs predicted by both of our modes (Figs. 4B and 5B). Moreover, an estimate of the average difference in osmolality between the two limbs (~100–150 mosmol/kg H2 O) observed in this micropuncture study (17) is comparable to the average difference predicted by both of our modes (Figs. 4 and 5).

Of particular importance for the function of a solute-mixing model involving NaCl movement out of the ATL (and, in our present model, the prebend segment of the DTL) is the general requirement of a concentration gradient for NaCl between the fluid in these loop regions and the surrounding interstitium. Such a gradient is predicted by our model modes (Figs. 4 and 5). Moreover, it is supported by the micropuncture study by Johnston et al. (21) in moderately antidiuretic rats: they found that the Na\(^+\) concentration in the fluid around the loop bends of the papillary thin limbs was ~30–60 mM higher than the concentration in the neighboring VR (assumed to reflect the interstitium) (21). These values are similar to the differences between tubular fluid in the longest loop bend and the CC predicted by the base-case modes (84 and 55 mM for the pipe and SS modes, respectively). Thus these micropuncture data are compatible with our model results.

Our models of the pipe and SS modes produce a urine concentration comparable to that observed in rats in a moderate antidiuresis. Moreover, other key model predictions fit reasonably well with many available experimental data. However, to obtain further information on which, if either, of these two models is more appropriate and to determine what major changes or refinements are necessary, additional experimental data are required. First, all known urea transporters for which appropriate antibodies are available need to be included in the three-dimensional functional reconstructions to provide a better picture of the tubule regions of significant urea permeability. Second, the permeabilities of the AQP1-null segments of the DTLs to Na\(^+\), water, and urea must be measured directly, probably in isolated perfused segments. Third, the full functional three-dimensional arrangement of the structures in the IM and their distances and dimensions need to be determined to make appropriate modifications to the distributed-loop CC model that we have used for the present study.

APPENDIX

Model Equations

Model equations represent the conservation and transmural transport of solutes and water. Detailed justification for the model equations can be found in Ref. 40; the model is similar to one we have used previously (38). The DTL, ATL, CD, and CC are indexed by \(i = 1, 2, 3, \) and 4, respectively; Na\(^+\), urea, and NR are indexed by \(k = 1, 2, \) and 3, respectively.

In a distributed-loop configuration, a loop of Henle reaches to each medullary level, and two space variables are used: \(x\) denotes the level of the IM, whereas \(y\) indexes a loop by the level at which it turns; both \(x\) and \(y\) range along the IM from 0 to \(L = 5\) mm, with \(y = x\). Thus at time \(t\), in a distributed DTL or ATL (\(i = 1\) or 2) reaching to medullary level \(y\), the water flow rate at level \(x\) is denoted by \(F_{W} = F_{W}(x, y, t)\). Analogous function notation and arguments are used for other loop variables.

For a given tubule \(i\), the transmural water line flux is given by \(J_{W}\), assumed positive for transport into the tubule. With this notation, water conservation in tubule \(i\) is given by

\[
\frac{\partial}{\partial x} F_{W} = J_{W}. \tag{A1}
\]

Solute conservation in DTL, ATL, or CD (i.e., \(i = 1, 2, \) or 3) is given by

\[
\frac{\partial}{\partial t} C_k = \frac{1}{A_i} \left( - F_{W} \frac{\partial}{\partial x} C_k + J_k - C_k J_{W} \right). \tag{A2}
\]

where \(C_k\) is the concentration of solute \(k\), \(A_i\) is the cross-sectional area of the tubule, and \(J_k\) is the transmural line flux of solute \(k\), taken positive into the tubule. The three terms inside the parentheses on the right arise from axial intratubular solute advection, transmural solute transport, and transmural water transport, respectively. The steady-state solution of Eq. A2, obtained when \(\partial / \partial t C_k = 0\), does not depend on \(A_i\); therefore, the composite CD used in the model need not maintain fidelity to the sum of the cross sections of all CDs to compute accurate steady-state solute concentration profiles. However, the transport terms \(J_k\) and \(J_{W}\) on the right-hand side of Eq. A2 must contain factors that represent the sums of the circumferences of all CDs at level \(x\); i.e., the effective transport area of the CD epithelium, as a function of depth, must be accurately represented to compute accurate concentration profiles.
The finite water and solute permeabilities of the barriers separating descending and ascending VR give rise to nonideal countercurrent exchange by vasculature, the effects of which can be represented in the model by axial diffusion in the CC; thus

\[
\frac{\partial}{\partial t} C_{ik} = \frac{1}{A_k} \left[ - F_{Av} \frac{\partial}{\partial x} C_{ik} + J_{Wi} - C_{ik} J_{Wi} + D_k \left( A_k \frac{\partial}{\partial x} C_{ik} \right) \right],
\]

where \(D_k\) is the diffusion coefficient for solute \(k\).

When nonideal countercurrent exchange is represented, the diffusion coefficients are nonzero, which requires the specification of additional boundary conditions for Eq. A5. We assumed that there was no diffusive solute flow from the CC across the papillary tip; thus it was assumed that \(\partial/\partial x C_{ik}(L, t) = 0\). At the OM-IM boundary, the gradients of interstitial solute concentrations were computed by assuming that the interstitial solute concentrations are linear in the OM and are equal to blood plasma solute concentrations at the corticomedullary boundary; i.e., it was assumed that \(\partial/\partial x C_{ik}(0, t) = (C_{ik}(0, t) - C_{\text{plasma}, k})/WOM\). The length of the OM, denoted by \(WOM\), was taken to be 2 mm. Plasma Na\(^+\) and urea concentrations, denoted by \(C_{\text{plasma}, 1}\) and \(C_{\text{plasma}, 2}\), were set to 160 and 15 mM, respectively.

The transmural water line flux into a DTL, ATL, or CD \((i = 1, 2,\) or \(3)\) is given by

\[
J_{Wi} = 2\pi r_i d \sum_{k=1}^{3} \phi_k (C_{ik} - C_{iM} - C_{iA}),
\]

where \(r_i\) is the radius of the tubule, \(d\) is the product of partial molar volume of water \(V_w\) and the transmural osmotic water permeability coefficient \(P_{ri}\), and \(\phi_k\) is the osmotic coefficient of solute \(k\). The transmural solute line flux into a tubule is given by

\[
J_{si} = 2\pi r_i \left[ P_{si} (C_{ik} - C_{iA}) - \frac{V_{\text{max},ik} C_{ik}}{K_{M,ik} + C_{ik}} \right].
\]

The first term inside the parentheses on the right is transmural diffusion characterized by permeability \(P_{si}\); the second term, which represents active transport by a saturable expression having the form

\[
F_{si} = F_{si} \left( \frac{C_{ik} - C_{ir}}{J_{si}} \right),
\]

where \(D_{si}\) is the diffusion coefficient for solute \(k\).


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