Transepithelial water and urea permeabilities of isolated perfused Munich-Wistar rat inner medullary thin limbs of Henle’s loop

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Nawata CM, Evans KK, Dantzler WH, Pannabecker TL. Transepithelial water and urea permeabilities of isolated perfused Munich-Wistar rat inner medullary thin limbs of Henle’s loop. Am J Physiol Renal Physiol 306: F123–F129, 2014. First published November 6, 2013; doi:10.1152/ajprenal.00491.2013.—To better understand the role that water and urea fluxes play in the urine concentrating mechanism, we determined transepithelial osmotic water permeability (Pw) and urea permeability (Pfu) in isolated perfused Munich-Wistar rat long-loop descending thin limbs (DTLs) and ascending thin limbs (ATLs). Thin limbs were isolated either from 0.5 to 2.5 mm below the outer medulla (upper inner medulla) or from the terminal 2.5 mm of the inner medulla. Segment types were characterized on the basis of structural features and gene expression levels of the water channel aquaporin 1, which was high in the upper DTL (DTLupper), absent in the lower DTL (DTLlower), and absent in ATLs, and the Cl− channel CICK1, which was absent in DTLs and high in ATLs. DTLupper Pw was high (3,204.5 ± 450.3 μm/s), whereas DTLlower showed very little or no osmotic Pr (207.8 ± 241.3 μm/s). Munich-Wistar rat ATLs have previously been shown to exhibit no Pr, DTLupper Pfu was 40.0 ± 7.3 × 10−5 cm/s and much higher in DTLlower (203.8 ± 30.3 × 10−5 cm/s), upper ATL (203.8 ± 35.7 × 10−5 cm/s), and lower ATL (265.1 ± 49.8 × 10−5 cm/s). Phloretin (0.25 mM) did not reduce DTLupper Pfu, suggesting that Pfu is not due to urea transporter UT-A2, which is expressed in short portions of long-loop DTLs forming bends within approximately the first millimeter below the OM (32, 34, 42). These DTL segments are considered to be lower DTLs (DTLlower), and their upper DTL (DTLupper) segments lie within the OM and do express AQPI (T. L. Pannabecker, unpublished observations) (Fig. 1). DTLs of the rat that extend deeper than the first millimeter below the OM express detectable AQ1 protein only for the first 40% of their inner medullary length (DTLupper). Thus, variable lengths of DTLupper and DTLlower coexist at most levels throughout the IM, with DTLlower and prebends as the sole descending segments near the papilla tip (Fig. 1). Urea transporter UT-A2 protein is expressed in short portions of some rat inner medullary DTLs, but only in segments close to the OM (19, 25). No other known AQPs or urea transporters are expressed in inner medullary DTLs. ATLs and prebend segments consist of type 4 epithelia, express the Cl− channel CICK1, and express no known AQPs or urea transporters.

In this study, we further characterized the structure of Munich-Wistar rat inner medullary thin limbs of Henle’s loops. We also confirmed the identity of single isolated segment types by correlating AQ1 and CICK1 mRNA expression levels with structural characteristics determined using light microscopy. We then determined water and urea transepithelial flux rates and permeabilities of structurally distinct, isolated perfused segments. The magnitudes of transepithelial fluid and solute fluxes in the thin limbs of Henle’s loops are essential to understanding the role that thin limbs play in generating the high medullary osmolality that supports the urine concentrating mechanism in the mammalian kidney (9, 31).

We found, on the basis of water permeability (Pw) or urea permeability (Pfu), that the DTL consists of two functionally distinct segments, whereas the ATL consists of a single functionally distinct segment. We propose that, as with chinchilla (7), these segments correspond to type 2 (DTLupper), type 3 (DTLlower), and type 4 (upper ATL (ATLupper) and lower ATL (ATLlower)) epithelia. The variable functionality along the DTL and very high Pfu along the ATL are not consistent with the passive mechanism as originally proposed (20, 39) and raise further questions about solute movements across the inner medullary thin limbs.

METHODS

Animals. Male Munich-Wistar rats (average age: ~90 days) were purchased from Simonsen Laboratories (Gilroy, CA) or Harlan (Indianapolis, IN) or were reared in the University Animal Care facility at the University of Arizona (Tucson, AZ) and were provided with rat chow (Teklad 7001) and water ad libitum. Animals were euthanized with CO2. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and were approved by the Institutional Animal Care and Use Committee of the University of Arizona.
Tubule dissection and identification of tubule segments on the basis of gene expression. Tubule segments were also characterized by quantifying expression levels of mRNA coding for AQP1 and CIC-K. Expression of cyclophilin A served as an indicator that cDNA was successfully synthesized and amplified from each tubule. Single inner medullary thick limb segments were isolated in sucrose-HEPES buffer and selected for quantitative PCR analysis by the same structural criteria used for obtaining segments for perfusion and the study of osmotic $P_f$ and $P_w$ described above. Each thin limb segment was transferred with $5–10 \mu$l buffer into a 0.5-ml microcentrifuge tube. After removal of the buffer, the tubule was lysed in 10 mM L-arginine and 1 % Triton X-100 (pH 2.5, Sigma Aldrich, St. Louis, MO). cDNA was synthesized from 12.5 $\mu$g of lysate with Maxima HMinus Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA). Two microliters of cDNA were included in each 10-$\mu$l real-time PCR using SYBR Select Master Mix for CFX (Life Technologies, Austin, TX) on a Bio-Rad CFX96 Real-Time system (Bio-Rad Laboratories, Hercules, CA). Reactions were amplified for 40 cycles. AQP1 (Accession No. BC090068) was amplified with 5'-CCGAGACTTAGGTGGCTCAG-3' (sense) and 5'-TCATGCGGTCTGTAAAGTCG-3' (antisense), CIC-K (Accession No. BC081761) was amplified with 5'-TATCCCTTGGTGGAGAC-3' (sense) and 5'-GTCACGAAACACGGACTGAAG-3' (antisense), and cyclophilin A (Accession No. NM1017101) was amplified with 5'-CGAGCTGTTTGCAGACAAAG-3' (sense) and 5'-GCATA-

**Solutions.** Kidneys were bathed and dissected on ice in a solution consisting of 280 mM sucrose and 10 mM HEPES adjusted to pH 7.4 with Tris base and gassed with 100% $O_2$ (sucrose-HEPES buffer). The solution used for perfusing and bathing the isolated tubules consisted of (in mmol/l) 118 NaCl, 25 NaHCO$_3$, 2.5 K$_2$HPO$_4$, 2 CaCl$_2$, 1.2 MgSO$_4$, 5.5 glucose, and 5 urea adjusted to pH 7.4 and gassed with 95% $O_2$-5% $CO_2$ (bicarbonate buffer). Solution osmolalities were about 290 mosmol/kg H$_2$O.

**Tubule dissection and identification of tubule segments on the basis of structural features.** Tubule segments for perfusion and the study of osmotic $P_f$ and $P_w$ were teased from the isolated IM in sucrose-HEPES buffer without the aid of enzymatic agents under a stereomicroscope using reflected light below the dissection dish. First, DTL$_{upper}$ segments were teased from 0.5 to 2.5 mm below the OM (Fig. 1). We took care to select segments that did not include the first 0.5 mm below the OM-IM border to avoid any possibility of including UT-A2. We also chose only DTL segments from loops that extended well beyond the first 1 mm of the IM because DTLs of loops that turn within the first 1 mm lack detectable AQP1 protein and because they would have been too short to perfuse if the first 0.5 mm was not included. These selected segments would all have some AQP1 expression but could also include portions that lack AQP1 (Fig. 1). Second, DTL$_{lower}$ segments were teased from the terminal 2.5 mm of the IM. DTLs from this region have no detectable AQP1 protein. We were also careful to avoid the prebend region (a DTL$_{lower}$ segment that exhibits structural and functional characteristics of the ATL) as determined by its diameter (Fig. 2). Third, ATL$_{upper}$ segments were teased from 0.5 to 2.5 mm below the OM and were not from loops that turned within the first 1 mm. Fourth, DTL$_{lower}$ segments were teased from the lower 2.5 mm of the IM. Finally, DTLs are distinguished from ATLs by their smaller diameter and by their cell type. DTLs have nuclei that show protrusions into the lumen, whereas ATLs have round, flat nuclei (3, 33). These differences are shown most clearly when the tubule segments are viewed under a compound microscope with differential interference contrast optics (Fig. 2), but they can also be recognized under a stereomicroscope.

![Fig. 1. Four structurally distinct segments of rat medullary thin limbs of Henle’s loops (designated as types 1–4). Descending thick limbs (DTLs) that descend less than about 1 mm below the outer medulla (OM) express detectable aquaporin 1 (AQP1) in their OM segments and little or no AQP1 in their inner medullary (IM) segments. DTLs that descend more than about 1 mm below the OM express detectable AQP1 protein only along the initial 40% of their IM length; the terminal 60% expresses no detectable AQP1 protein (32, 34). The ascending thin limb (ATL) and prebend segment express the Cl channel CIC-K along their entire lengths (18, 32, 40). Urea transporter UT-A2 is abundant in DTL upper (3, 33); however, as shown in this image, luminal projections in DTLs are also clearly seen with a stereomicroscope.](http://ajprenal.physiology.org/)

![Fig. 2. Differential interference contrast image of the DTL$_{lower}$ (DTL) and ATL$_{lower}$ (ATL) of a Munich-Wistar rat. Segments are recognized by their position relative to the loop bend and on the basis of structural characteristics (see METHODS). We have previously shown that luminal projections are abundant in DTL$_{upper}$ (3, 33); however, as shown in this image, luminal projections are sparse in DTL$_{lower}$ (arrows). The diameter of the DTL$_{lower}$ is less than that of the ATL and the prebend segment (PB). Diameter differences and luminal projections in DTLs are also clearly seen with a stereomicroscope.](http://ajprenal.physiology.org/)
CAGTCCTGGCATCT-3' (antisense). Standard curves were used to calculate mRNA levels (in arbitrary units), and final values are expressed per millimeter of tubule length.

Perfusion of isolated tubule segments. Tubule segments were perfused in vitro by a modification of the technique previously described by Burg et al. (4). Tubules were placed into a temperature-controlled Lucite or brass chamber with a glass bottom and observed with a stereomicroscope and inverted compound microscope. The upstream portion of the tubule was drawn into a holding pipette, which contained two concentric pipettes: a perfusion pipet, which was inserted into the tubule lumen, and an exchange pipet, for exchanging the luminal perfusate. The downstream end of the tubule was drawn into a holding pipet that had a tip of appropriate diameter to form a complete seal between perfusate and bath solution. The perfusion rate was generally between 15 and 30 nl/min. The bath temperature was held at 37°C during the collection period. Tubule dimensions were determined with an ocular micrometer.

Osmotic P1 measurements. Osmotic P1 was calculated from net transepithelial fluid flux (Jv) determined in the presence of an imposed 100 mosmol/kg H2O osmolality gradient. The peritubular bath consisted of bicarbonate buffer with 100 mM sucrose added to create a 100 mosmol/kg H2O bath-to-lumen osmolality gradient. The DTL has been shown to exhibit no significant transepithelial permeability to sucrose, as shown in the hamster (14). Measurements were conducted by collecting the perfusate, which contained [14C]dextran (~70 kDa) as the impermeant volume marker in bicarbonate buffer. The protocol for determining Jv for DTLupper, DTLlower, and ATL is shown in three representative experiments in Fig. 3. Each experiment was conducted over the course of about 60–80 min. For each tubule, the perfusion fluid in the downstream pipet was initially collected for four 5-min intervals in the absence of the osmolality gradient. The bath was then changed to bicarbonate buffer with 100 mM sucrose added, and perfusion fluid was collected for four additional 5-min intervals. Finally, the perfusion fluid was collected for another three or four 5-min intervals, again in the absence of the osmolality gradient. Jv (in nl/min·mm tubule length⁻¹) was determined using the following equation:

\[ J_v = \frac{V_v}{t} \left( \frac{X_l}{X_o} - 1 \right) \]

where \( V_v \) is the fluid collection rate per millimeter of tubule length, \( X_l \) is the [14C]dextran concentration of collected fluid, and \( X_o \) is the [14C]dextran concentration in the perfusate (in pcts). Jv was calculated from the following equation:

\[ J_v = \frac{P_i}{A} \left( J_{\text{urea}} - J_{\text{permeate}} \right) \]

where \( P_i \) is the paracellular water permeability, \( A \) is the tubule luminal surface area [which was calculated as \( \pi DL \) from the tubule inner diameter (D) and the tubule length (L)], \( V_w \) is the partial molar volume of water (18 ml/mol), and \( \delta C_{\text{osmol}} \) is the log-mean transepithelial osmolality gradient along the length of the perfused tubule [calculated as \( \delta C_1 - \delta C_2/\ln(\delta C_1/\delta C_2) \), where \( \delta C_1 \) and \( \delta C_2 \) are the transepithelial osmolality differences at the perfusion and collection ends, respectively]. Perfusate and bath osmolalities were measured with a vapor pressure osmometer (Wescor, Logan, UT). The collected fluid osmolality was estimated as follows: \( O_p(X_l/X_o) \), where \( O_p \) is perfusate osmolality.

P urea measurements. P urea was determined by measuring the unidirectional [14C]urea flux resulting from an ~5 or ~0.1 mM lumen-to-bath or bath-to-lumen urea concentration gradient. Because the unidirectional fluxes were nearly the same in either direction, the data were combined. The perfusate and peritubular bath consisted of bicarbonate buffer. In some experiments, 5 mM urea in either the lumen or bath was replaced with 5 mM raffinose (see RESULTS). The unidirectional flux of [14C]urea from the lumen to the bath (J urea; in pmol·min⁻¹·mm tubule length⁻¹) was calculated using the following equation:

\[ J_{\text{urea}} = \frac{C_o V_o - C_l V_l}{t} \]

where \( C_o \) and \( C_l \) are the urea concentrations in the perfusate and collectate, respectively, \( V_o \) is the perfusion rate per unit tubule length, and \( V_l \) is the collection rate per unit tubule length. The bath-to-lumen urea flux simplifies to

\[ J_{\text{urea}} = -C_l V_l \]

since the perfusate urea concentration was 0 mM.

P urea (in cm/s) was calculated for lumen-to-bath fluxes from the following equation:

\[ P_{\text{urea}} = \frac{J_{\text{urea}}}{(\pi D^2 C)} \]

where \( D \) is the tubule diameter and \( C = \delta C_1 - \delta C_2/\ln(\delta C_1/\delta C_2) \) (where \( \delta C_1 \) is the urea

concentration gradient at the perfusion end of the tubule and \( \delta C_2 \) is the urea concentration gradient at the collection end of the tubule). (5). Net volume flow is zero in the absence of a transepithelial osmolality gradient (see RESULTS), and tracer backflux was negligible as short tubule segments were used and tracer concentration in the trans compartment was ~10% of that in the cis compartment.

Statistical analysis. Data combined from three or more samples are reported as means ± SE; n is the number of replicates. The statistical significance of differences between means was determined with Student’s paired t-test or one-way ANOVA and Duncan’s post hoc test (P < 0.05).
**RESULTS**

**Isolation of structurally distinct thin limb segments.** DTL_upper and ATLs from the rat IM are distinguishable from each other on the basis of structural criteria, as we have previously reported (3, 33) (also see METHODS). Notably, the DTL_upper has numerous cells with nuclei protruding into the tubule lumen, whereas ATLs have round, flat nuclei. In the present experiments, the DTL_lower was initially identified by dissecting the descending segment along with its bend and following it to its upper descending segment. Segments from the lower IM are shown in Fig. 2 as observed with differential interference contrast optics. These segments are also apparent using a stereomicroscope with subillumination. There is also commonly a distinct thickening in diameter several hundred micrometers above the bend on the descending side of the loop (Fig. 2). This short thickened segment is similar in diameter to that of ATL_lower. We generally relied on making a visual comparison of DTL_lower and ATL_lower tubule diameters in neighboring segments from each papilla to further confirm the identification of ATL_lower segments (3, 33).

**Quantitative PCR.** We isolated single segments of DTL_upper, DTL_lower, and ATLs on the basis of the structural characteristics described above and measured expression levels of mRNA encoding AQP1 and ClCK1 in each segment. Relative to other segments, DTL_upper expresses high mRNA levels of AQP1 and low mRNA levels of ClCK1, DTL_lower expresses essentially no AQP1 mRNA and low levels of ClCK1 mRNA, and ATL expresses essentially no AQP1 mRNA and high levels of ClCK1 mRNA (Fig. 4). These data parallel the known expression levels of AQP1 and ClCK1 protein in these segments as determined by immunohistochemistry (29, 32, 34, 41).

**Water permeabilities of isolated perfused thin limb segments.** In the absence of a transepithelial osmolarity gradient, J_w was essentially zero for DTL_upper, DTL_lower, and ATL isolated perfused segments, as shown in representative experiments (Fig. 3). With the application of a 100 mosmol/kg H_2O bath-to-lumen osmolarity gradient (100 mM sucrose added to the bath), a substantial volume flux was observed in DTL_upper but not in DTL_lower and ATL (Fig. 3). The volume flux rapidly diminished in DTL_upper upon removal of the osmolarity gradient. The effects of imposing a 100 mosmol/kg H_2O bath-to-lumen osmolarity gradient on J_v and P_f values for all three segments are shown in Table 1. Under this condition, J_v and P_f values for DTL_upper were substantially higher than values for DTL_lower and for ATL, with the latter previously published by us in an earlier report (44) (Table 1). J_v and P_f values for DTL_lower and ATL were not significantly different from zero.

**Urea permeabilities of isolated perfused thin limb segments.** P_urea was determined by imposing a transepithelial urea gradient of about 0.1 mM for DTL_upper, DTL_lower, ATL_upper, and ATL_lower. The gradient was applied in a bath-to-lumen orientation or in a lumen-to-bath orientation. Values were similar for each orientation and were combined. P_urea for DTL_upper, although high, was only about one-fourth of P_urea for DTL_lower and both ATL segments (Table 2). P_urea values for DTL_lower and both ATL segments were not significantly different from each other. P_urea was also determined for DTL upper and ATL_upper by imposing a transepithelial urea gradient of −5 mM. Experiments conducted with an −5 mM urea gradient resulted in a P_urea value that was similar to that seen with a urea gradient of 0.1 mM.

**Urea permeability of the isolated perfused DTL_upper in the presence of phloretin.** Phloretin inhibits urea transport mediated by members of the solute carrier 14 (SLC14) family of UTs, including UT-A2. Because UT-A2 protein is expressed in inner medullary DTL segments lying near the OM-IM boundary (19, 25, 43), we tested effects of phloretin on urea transport in DTL_upper to test if urea flux occurs by way of a member of

**Table 1. Transepithelial osmotic water permeability of isolated perfused Munich-Wistar rat thin limb segments**

<table>
<thead>
<tr>
<th>Segment Type</th>
<th>Number of Replicates</th>
<th>Tubule Length, μm</th>
<th>Collection Rate, nl·mm⁻¹·mm⁻¹</th>
<th>Collected-to-Perfusate [¹⁴C]Dextran Concentration, mM</th>
<th>J_v, nl·mm⁻¹·mm⁻¹</th>
<th>P_f, μm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTL_upper</td>
<td>6</td>
<td>775</td>
<td>28.1 ± 1.2</td>
<td>1.31 ± 0.04</td>
<td>7.7 ± 0.9</td>
<td>3,204.5 ± 450.3</td>
</tr>
<tr>
<td>DTL_lower</td>
<td>5</td>
<td>629</td>
<td>33.1 ± 3.2</td>
<td>1.03 ± 0.03</td>
<td>0.8 ± 0.8*</td>
<td>207.8 ± 241.3*</td>
</tr>
<tr>
<td>ATL†</td>
<td>10</td>
<td>829</td>
<td>16.0 ± 3.0</td>
<td>1.01 ± 0.02</td>
<td>0.3 ± 0.3*</td>
<td>69.4 ± 72.5*</td>
</tr>
</tbody>
</table>

DTL_upper and DTL_lower, upper and lower descending thin limbs, respectively; ATL, ascending thin limbs; P_f, water permeability; J_v, net transepithelial fluid flux. *Significantly different from DTL_upper (by one-way ANOVA, P < 0.05). †Data from Ref. 44.
the SLC14 family of transporters. In the absence of phloretin, $P_{\text{urea}}$ was 83.2 ± 25.7 × 10^{-5} cm/s; in the presence of phloretin (0.25 mM), $P_{\text{urea}}$ was 81.9 ± 26.0 × 10^{-5} cm/s (means ± SE, $n = 4$, not significantly different by Student’s paired t-test, $P > 0.5$).

**DISCUSSION**

The “passive mechanism” hypothesis of urine concentration, as originally proposed by Kokko and Rector (20) and Stephenson (39), requires high transepithelial osmotic $P_i$ and low $P_{\text{urea}}$ and Na$^+$ permeability along the DTL and low $P_{\text{urea}}$ along the ATL. These $P_i$ and $P_{\text{urea}}$ to a significant degree, exist neither in the isolated perfused thin limbs of the rat, as shown here, nor in the chinchilla (5, 6). The Na$^+$ permeability of the chinchilla DTL, although high, may be sufficiently low to adhere to the Kokko and Rector and Stephenson models (6). The separation of urea from NaCl that is initiated in the outer medullary thick ascending limb and sustained and augmented by intervening cortical segments and the outer medullary collecting duct most certainly involves active transport. However, the subsequent mixing of urea and NaCl in the interstitium and vasculature of the IM could involve passive mechanisms. These mechanisms likely differ to some degree from those proposed in the Kokko and Rector and Stephenson models. Unexplained Na$^+$ and urea imbalances in the mouse Cl$^-$ channel and UT knockout models underscore our ignorance of the mechanisms that balance inner medullary interstitial and vascular NaCl and urea composition (1, 10a, 41a). A more complete understanding of thin limb water, NaCl, and urea permeabilities and transport properties is essential to understanding a “solute-separation, solute-mixing” mechanism of urine concentration (23).

The rat inner medullary DTL consists of two structurally distinct segments that can be identified with light-level microscopy. We described structural features of the rat DTL in earlier publications (3, 33), which are distinct from those of the DTL, as shown here. Nuclear protrusions, which are abundant in DTL, sparse in DTL, and absent in ATL, enable one to identify each of these three segments with a stereomicroscope. A comparison of tubule diameters for neighboring DTL and ATL from each papilla also facilitates the identification of ATL segments (3, 33).

The data shown here indicate that the Munich-Wistar rat inner medullary DTL and DTL are two functionally distinct segments. In isolated perfused tubules, the DTL exhibits high osmotic $P_i$ and moderately high $P_{\text{urea}}$, whereas the DTL exhibits low osmotic $P_i$ and very high $P_{\text{urea}}$. The ATL uniformly exhibits low osmotic $P_i$ and very high $P_{\text{urea}}$ along its entire length. $P_{\text{urea}}$ values of the rat thin limbs are substantially higher than $P_{\text{urea}}$ measured for free diffusion across the human red blood cell plasma membrane (1 × 10^{-6} cm/s) (2). The osmotic $P_i$ and $P_{\text{urea}}$ values reported here are similar, in most respects, to those reported for chinchilla DTL, DTL, and ATL (in the chinchilla studies (5–7, 24), the authors used the terms middle and lower part of the long-loop descending limb to refer to the DTLs isolated from the outer 30% of the IM and from near the papilla tip, respectively). One exception, though, is the much higher $P_{\text{urea}}$ for the Munich-Wistar rat DTL compared with chinchilla DTL (203 × 10^{-5} vs. 47.6 × 10^{-5} cm/s). The physiological impact of this difference remains to be determined. The DTL osmotic $P_i$ values that we found for the Munich-Wistar rat are comparable to those found in DTLs isolated from near the OM-IM boundary for the Wistar rat (13), hamster (14), and mouse (8), but the DTL osmotic $P_i$ values reported here are lower than those found in the Sprague-Dawley rat DTL (6) and are not significantly different from zero.

In the Munich-Wistar rat, the high or low osmotic $P_i$ of each segment correspond, respectively, to high or low levels of AQP1 protein (30, 31, 34) and mRNA expression (Fig. 4). CICK1 protein expression is high in the ATL and prebend segment with little or no expression in the DTL (18, 32, 40), corresponding to high expression of CICK1 mRNA in the ATL and its near absence in the DTL, as shown here.

The identity of the transepithelial pathway for urea in the rat DTL and ATL remains an enigma. The absence of inhibition by phloretin in the DTL indicates that UT-A2 is not the pathway in this segment, the only thin limb segment known to express any of the SLC14 class of transporters in the rat IM. Likewise, phloretin did not inhibit urea fluxes in the chinchilla DTL, DTL, or ATL (5). If urea flux is transeellular, then it must involve one or more unknown UTs. Although urea and water can flow across the tight junction and through the paracellular pathway in some systems (12), there is no strong evidence to suggest that urea, a molecule similar to but larger than water, could traverse this pathway when water could not in the thin limb segments exhibiting no osmotic $P_i$.

Luminal fluid at the bends of the loops of the moderately antidiuretic rat and hamster that lie near the tip of the papilla is in approximate osmotic equilibrium with capillary fluid (11, 16, 27, 28, 36) and is likely in equilibrium with interstitial fluid.
fluid. The high transepithelial osmotic $P_f$ values of rat, hamster, mouse and chinchilla DTL-upper thin limbs suggest that osmotic equilibration in these segments could involve relatively high water flux. This water flux may play a critical role in the urine concentrating mechanism, as mice lacking AQP1 have greatly reduced concentrating capacity (8). In contrast, because of the low $P_f$ in rat and chinchilla DTL-lower, little or no osmotic equilibration can occur via osmotic water flux. Osmotic equilibration across the DTL-lower more likely occurs primarily by solute secretion, as suggested for the rat, hamster, and Psammomys (10, 26, 35). Because the DTL-lower exists at all levels of the IM, equilibration by solute secretion may be important in the urine concentrating mechanism at all levels along the corticopapillary axis of the IM. A role for both osmotic water equilibration and solute secretion in thin limbs has been captured in recent mathematical models of the urine concentrating mechanism (22, 23); however, the maximum urine concentration obtained in this and all other models remains well below the maximum concentrating capacity of rats.

In summary, each inner medullary DTL, consisting of two segment types (DTL-upper and DTL-lower), and each ATL, consisting of a single segment type, can be distinguished on either structural grounds or on the basis of relative mRNA expression levels of AQP1 and CIC1. $P_{area}$ is only moderately high in the segment that both expresses AQP1 and has high osmotic $P_f$, whereas $P_{area}$ is very high in all thin limb segments that lack the water channel AQP1 and have no osmotic $P_f$. Additional studies are needed to understand how these characteristics contribute to producing the corticopapillary osmolarity gradient in the IM.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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