Architecture of the human renal inner medulla and functional implications

Guojun Wei,1 Seymour Rosen,2 William H. Dantzler,1 and Thomas L. Pannabecker1
1Department of Physiology, University of Arizona Health Sciences Center, Tucson, Arizona; and 2Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

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Wei G, Rosen S, Dantzler WH, Pannabecker TL. Architecture of the human renal inner medulla and functional implications. Am J Physiol Renal Physiol 309: F627–F637, 2015. —The architecture of the inner stripe of the outer medulla of the human kidney has long been known to exhibit distinctive configurations; however, inner medullary architecture remains poorly defined. Using immunohistochemistry with segment-specific antibodies for membrane fluid and solute transporters and other proteins, we identified a number of distinctive functional features of human inner medulla. In the outer inner medulla, aquaporin-1 (AQP1)-positive long-loop descending thin limbs (DTLs) lie alongside descending and ascending vasa recta (DVR, AVR) within vascular bundles. These vascular bundles are continuations of outer medullary vascular bundles. Bundles containing DTLs and vasa recta lie at the margins of coalescing collecting duct (CD) clusters, thereby forming two regions, the vascular bundle region and the CD cluster region. Although AQP1 and urea transporter UT-B are abundantly expressed in long-loop DTLs and DVR, respectively, their expression declines with depth below the outer medulla. Transcellular water and urea fluxes likely decline in these segments at progressively deeper levels. Smooth muscle myosin heavy chain protein is also expressed in DVR of the inner stripe and the upper inner medulla, but is sparsely expressed at deeper inner medullary levels. In rodent inner medulla, fenestrated capillaries abut CDs along their entire length, paralleling ascending thin limbs (ATLs), forming distinct compartments (interstitial nodal spaces; INSs); however, in humans this architecture rarely occurs. Thus INSs are relatively infrequent in the human inner medulla, unlike in the rodent where they are abundant. UT-B is expressed within the papillary epithelium of the lower inner medulla, indicating a transcellular pathway for urea across this epithelium.

MEDULLARY LOOPS OF HENLE AND blood vessels of the human kidney are organized together with collecting ducts (CDs) in distinctive patterns that differ in the medullary rays, the outer medulla, and the inner medulla (Fig. 1). The human kidney has a minimal outer stripe of the outer medulla in contrast to the very large outer stripe in rodents (19, 41). In both rodent and human, the inner stripe of the outer medulla consists of two lateral regions, the vascular bundle and interbundle regions. The vascular bundles consist of descending and ascending vasa recta (DVR and AVR) and include short-loop descending thin limbs (DTLs) in the inner stripe of most rodents, but not in humans (2, 19). The interbundle region consists of a diffuse distribution of long-loop DTLs, thick ascending limbs of loops of Henle, CDs, and interconnecting capillaries (22). The inner stripe therefore consists of many vascular bundles running parallel to the corticomedullary axis, with each bundle carrying countercurrent flows. In both human and rat, the DVR feed into complex capillary networks that perfuse the interbundle region, with many capillaries feeding, in turn, to AVR (32). These DVR are lined by pericytes, which have a smooth muscle component whose contractile properties have the potential for regulating blood flow in both the inner stripe and the inner medulla (32).

Recent studies have shown that in the rat inner medulla there is a significantly different, yet distinct arrangement of tubular and vascular structures compared with that of the outer medulla (18, 37–39, 42, 48–50). A similar detailed analysis of human inner medullary architecture has not been carried out. However, the same segment-specific antibodies that have been used for investigating rodent medullary architecture bind specifically to homologous proteins in the human medulla and can therefore be used to characterize human medullary architecture.

The rat kidney is characterized by a steep corticomedullary osmolality gradient that, in the outer medulla, reaches a peak osmolality about twice that of systemic plasma (about 600 mosmol/kgH2O), and in the deep papilla reaches an osmolality about 10-fold that of plasma. The osmolality in the deep papilla of the human rises to less than fivefold that of plasma, but in both species the steepest increase occurs within the inner medulla. Just how this osmolality gradient is formed remains unexplained (11). The corticomedullary osmolality gradient is paralleled by a steep corticomedullary PO2 gradient (29). Formation of both gradients reflects, in part, the tubulovascular architecture. The thin limbs of Henle, CDs, and blood vessels partition NaCl, urea, oxygen, water, and other molecules into and out of the multiple medullary compartments in a manner dependent upon their transepithelial or transendothelial permeability characteristics and gradients in addition to overall architecture (5, 20, 40). Consequently, the tubulovascular architecture can promote or constrain medullary countercurrent systems and other intercompartmental fluid, solute, and oxygen flows. In this study, our aim was to determine the structural relationships that exist between the inner medullary tubules, CDs, and vasa recta of the human kidney, thereby providing insight into the potential contributions of functional architecture to the inner medullary urinary concentrating mechanism, oxygen distribution patterns, and overall fundamental renal physiology.

METHODS

Two human kidneys, rejected for transplant, were obtained from the International Institute for the Advancement of Medicine (IIAM.org; Edison, NJ). These whole organs experienced virtually no warm ischemia (0–60 min) and minimal cold ischemia (12–24 h) and were shipped in preservation solution directly from the operating room recovery suite, received within 24 h, and immediately dissected and fixed as described below. Anatomic studies with kidneys sourced from IIAM include Beeman et al. (4). Observations from our initial
studies with kidneys from IIAM were fully replicated in tissue obtained from the Beth Israel Medical Center. The latter specimens were complete nephrectomies (removed for tumors) that were immediately refrigerated and processed within 1–2 h of removal as described below. All patients had normal creatinine and an unremarkable urinalysis. The protocol was approved by the ethical committee of The University of Arizona, and all patients signed an informed consent form before surgery allowing the use of anonymous data from our experiments for scientific purposes, including publication.

Tissue preparation and immunohistochemistry. The renal medulla was isolated in PBS, trimmed to a size of 3 × 3 mm² and variable lengths, and immersion-fixed with periodate-lysine-paraformaldehyde (PLP; 0.01 M, 0.075 M, 2%) in PBS (pH 7.4) for 3 h at 4°C, or in 10% neutral buffered formalin overnight at 4°C, washed in PBS, and dehydrated through an ethanol series (47). Tissue pieces were dissected from near the outer medullary-inner medullary border (upper inner medulla) and from the terminal 50% of the inner medulla (lower inner medulla). Tissue was then immersed in a solution of Spurr epoxy resin (Ted Pella) and ethanol (1:1) for 16 h (room temperature), then in 100% Spurr for 48 h (4°C), and finally embedded in 100% Spurr (12 h at 60°C). Some tissue was also embedded in paraffin following ethanol dehydration and included the cortex as well. Serial transverse sections of the inner medulla were cut, beginning either at the outer medullary-inner medullary border or the papilla tip. Tissue embedded in Spurr resin was cut into 1-μm-thick sections with a Leica EM UC6 or Leica Ultracut UCT ultramicrotome. Tissue em-
bedded in paraffin was cut into 4-μm-thick sections with a Microm HM 355 S microtome.

Immunofluorescence histochemistry was performed as previously described (37–39). Before antibody application, Spurr resin was etched by applying to each slide 300 μl of a solution of 5 g NaOH, 5 ml 100% ethanol, and 5 ml propylene oxide for 3 min (24), followed by extensive washing, first with ethanol and then with distilled water. Paraffin sections were deparaffinized with three washes of xylene. Sections were then treated with 0.2% Triton X-100 (Sigma) in PBS (PBS/Triton) for 2 min and 1% SDS in PBS for 5 min. They then underwent three 5-min PBS/Triton washes. After this, they were treated for 10 min with a blocking solution consisting of 5% BSA, 1% normal donkey serum (Jackson ImmunoResearch), and 0.2% Triton X-100 diluted into PBS. Primary antibodies diluted into the blocking solution were then applied simultaneously for 2 h at room temperature followed by three 5-min PBS/Triton washes.

For fluorescence immunohistochemistry, proteins were labeled using affinity-purified polyclonal or monoclonal antibodies that recognize homologous proteins in rats (38) and humans: water channel aquaporin-1 (AQP1) mouse [ab9566, Abcam, (25)]; goat, sc-9878, Santa Cruz Biotechnology; or chicken provided by John Regan and Dan Stamer, The University of Arizona to label DTLs and DVR; kidney-specific chloride channel (ClC-K1), rabbit [AB5392, Chemicon (8)] to label ascending thin limbs of Henle’s loop (ATLs); water channel aquaporin-2 (AQP2) [goat, 9882, Santa Cruz Biotechnology (28)] or urea transporter (UT-A) rabbit [AB3070, Chemicon (1, 31)] to label DTLs and CDs; urea transporter B (UT-B) provided by Jeff Sands and Janet Klein, Emory University (46) to label DVR; or mouse CD34 [M7165, Dako (23)] to label all vasa recta and capillaries. All tubules and blood vessels were labeled nonselectively with fluorescein-conjugated wheat germ agglutinin (FL-1021, Vector Laboratories) or with α-crystallin (mouse, SPA-222, Stressgen). Secondary antibodies conjugated to fluorescent probes (Invitrogen/Molecular Probes or Jackson ImmunoResearch) were applied as described previously (47). Sections were mounted with Dako fluorescent mounting medium (Carpinteria, CA) and were viewed with epifluorescence microscopy (Applied Precision, DeltaVision).

For immunoperoxidase staining using the streptavidin/biotin technique (Life Sciences), sections were incubated with a primary antibody that recognizes pan-keratin in all thin limbs of Henle’s loops and CDs (Clone AE1/AE3; CAM 5.2 mouse, GA053, Dako) or smooth muscle myosin heavy chain (SMMS-1 mouse, ISO6630-2, Dako) followed by incubation with biotinylated anti-mouse immunoglobulins, which was then followed by incubation with horseradish peroxidase-streptavidin conjugate. Immunoreactive proteins were revealed by reaction with the substrate chromogen (0.05% 3,3’-diaminobenzidine; Kirkegaard and Perry Laboratories) and 0.01% peroxide. Sections were counterstained with Gill’s number 2 hematoxylin (Fisher Scientific).

Electron microscopy. Renal medullas were prepared for electron microscopy by trimming to a size of ~0.5 mm³ and were immersion-fixed with Karnovsky’s fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M PIPES buffer, pH 7.4, for 1 h at 4°C. Tissue was then immersed in a solution of 0.1 M glycine and 0.1 M PIPES (1:1 by volume) for 15 min, rinsed in PIPES, postfixed with 1% osmium tetroxide in 0.1 M PIPES, pH 7.4, for 1 h at 4°C. After dehydration through a graded series of ethanol solutions, and embedded in Spurr epoxy resin. Thin sections were cut on a Leica Ultracut microtome (Leica, Deerfield, IL) and were stained with 3% lead citrate for 3 min. Sections were imaged using a Tecnai Spirit Biotwin electron microscope and photographed with an AMT sidemount 4 MP camera, and images were saved as eight-bit tiffs.

RESULTS

Two lateral regions of the inner medulla. In transverse and longitudinal tissue sections, groups of coalescing CDs can be viewed as forming the organizing motif with nephrons and vascular segments arranged around them in a regular pattern, similar to that shown for three-dimensional reconstructions of the rat inner medulla (38, 39). Thus the inner medulla consists of two lateral regions: 1) the region dominated by CDs that...
coalesce as they descend toward the tip of the papilla; this region also includes capillaries and thin limbs of Henlé’s loops, and 2) the vascular bundle region, which is occupied chiefly by vasa recta and AQP1-positive DTLs. Outer and inner medullary CDs are labeled with antibodies for the proteins AQP2 and UT-A (Figs. 2 and 3). In transverse sections, inner medullary CDs are grouped together in clusters of about 6–12 segments (Figs. 4 and 5). CDs coalesce as they descend through the inner medulla, forming fewer and larger diameter CD segments. The terminal CDs then form the ducts of Bellini at the tip of the papilla.

DVR, which lie in vascular bundles of the outer and inner medulla of the human kidney, express the urea transporter UT-B (Fig. 2) (46). These vascular bundles are particularly well defined in the outer medulla and in the outer ~50%, by length, of the inner medulla. The inner medullary vascular bundles are continuations from outer medullary vascular bundles. The number of UT-B-positive DVR that continue into the innermost 50% of the inner medulla is noticeably reduced from the numbers present in the outermost 50%, judging from the reduced immunofluorescence levels in the deeper zone (Fig. 2).

This reduction reflects, in part, the absence of UT-B in the DVR that extend into the deeper inner medulla, leading to a significant number of UT-B-null DVR, as has been shown for rats (49). The reduction also reflects the decline in the total number of DVR as the DVR join the capillary plexus throughout their descent, again as has been shown for rats (49).

Vascular bundles become more loosely organized at deeper levels where they are distributed between the CD clusters as seen in transverse sections. Bundles are particularly distinct in sections from near the outer medullary-inner medullary border (Fig. 4), thereby forming the two lateral regions in the inner medulla as noted above. This arrangement is comparable to that seen in the outer medulla, although the two regions consist of different tubulovascular compositions. These spatially distinct regions are arrayed across the entire inner medulla in the transverse dimension in a relatively uniform fashion (Fig. 4).

DVR, AVR, and capillaries are labeled with an antibody raised against the protein CD34, which is a glycosylated transmembrane sialomucin (3) (Fig. 5). AVR are large-diameter, UT-B-negative vessels and are juxtaposed with UT-B-positive DVR within vascular bundles. Capillaries that are...
UT-B negative are diffusely distributed among the CD clusters. The DVR and AVR appear as vessels with wide-open lumens in transverse sections because they run perpendicular to the plane of the section. This morphology is in sharp contrast to that of the capillaries, which appear to have somewhat elongated lumens, an appearance that arises from their smaller diameter and because many of them tend to run in the transverse dimension parallel to the plane of the section (Fig. 5).
sections from deeper levels of the inner medulla (Fig. 5), DVR become smaller in diameter and tend to collapse more readily compared with DVR in sections nearer to the OM (Fig. 4).

The contractile protein smooth muscle myosin heavy chain is expressed in outer medullary DVR, and expression continues into inner medullary DVR, which lie in vascular bundles (Fig. 6). Smooth muscle myosin heavy chain protein expression becomes increasingly sparse with depth below the outer medullary-inner medullary border.

Epithelial cells of the inner medullary thin limbs of Henle’s loops and CDs are labeled by the cocktail of antibodies AE1/AE3; CAM 5.2, a pan-cytokeratin immunostain. Thin limbs of Henle’s loops appear to be distributed fairly uniformly within regions occupied by CD clusters (Fig. 7). However, this is actually a heterogeneous population of DTL and ATL segments mixed among the CDs. AQP1 is strongly expressed in long-loop DTLs of the human kidney throughout the outer medulla and much of the inner medulla (Fig. 3) and is weakly expressed in DVR (Fig. 8) (30). The AQP1-positive DTLs lie predominantly within the vascular bundles alongside the UT-B-positive DVR (Fig. 9), in the vascular bundle regions that are spatially separate from regions occupied by CDs, in an ar-

Fig. 6. Longitudinal section showing expression of smooth muscle myosin heavy chain (brown) in DVR of the inner stripe of the outer medulla and inner medulla. Boxed areas in A are enlarged in B and C. Tissue is paraffin embedded. Scale bars = 500 μm (A) and 50 μm (B and C).

Fig. 7. Transverse section of human inner medulla. All thin limb and CD segments (brown) are labeled with the epithelial cell cytokeratin marker AE1/AE3 CAM 5.2. Several vascular bundle regions (outlined in red) and intervening CD clusters are shown. Section is from the outer 50% of the inner medulla. Tissue is paraffin embedded. Scale bar = 100 μm.
Fig. 8. Transverse section of human inner medulla. Single section from the outer 50% of the inner medulla. A: AQP1 strongly labels DTLs (large-diameter red tubules) and weakly labels DVR (small-diameter red vessels; arrows). The number of CIC-K1-positive tubules is greater than the number of AQP1-positive tubules, indicating that there are DTL segments that do not express detectable levels of AQP1. B: same as A with expression of αB-crystallin, which strongly labels thin limbs of Henle’s loops that express neither AQP1 nor CIC-K1. These are the AQP1-negative DTLs (lower DTLs). Tissue is embedded in Spurr epoxy resin. Scale bars = 50 μm.

Fig. 9. Transverse section of human inner medulla. Regions exhibiting no labeled tubules or vessels (marked with X) are occupied by groups of unlabeled CDs, which can be identified by their diameter and thick epithelial wall (not shown). AQP1-positive DTLs and UT-B-positive DVR lie in regions that are spatially separate from regions occupied by CDs. DTLs tend to lie at the periphery of vascular bundles. Spatial separation of DTLs from CDs is unlike the arrangement in the outer medulla, where they both lie in the interbundle region along with thick ascending limbs. Few DVR label with AQP1 antibody. Tissue is embedded in Spurr epoxy resin. Scale bar = 500 μm.
rangement similar to that in rats (40). Thus the close association of long-loop DTLs with CDs in the outer medulla undergoes an anatomic transition as DTLs descend from the outer medulla into and through the inner medulla, where they tend to lie distant from CDs. As with UT-B expression in DVR, AQP1 expression in DTLs declines with depth below the outer medulla and there are progressively fewer and fewer AQP1-positive DTLs in the deeper inner medulla (Fig. 3). This reflects both the absence of AQP1 expression in each DTL, leading to a significant number of AQP1-null DTL segments (see below) and also reflects the fact that the total number of DTLs declines at an exponential rate with depth below the outer medulla as the DTLs make a 180° turn at all depths to form the ATLs.

The inclusion of DTLs and DVR within vascular bundles clearly occurs in the upper 50% of the inner medulla, but other segment-specific markers or electron microscopy studies will have to be employed to determine the extent to which this architecture continues into the deeper inner medulla where AQP1 and UT-B protein expression are markedly reduced (Figs. 2 and 3). The chloride channel ClC-K1 is expressed in the inner medullary ATL and in a short prebend segment of the terminal DTL (16, 17) (Fig. 8). The number of ClC-K1-positive segments (ATLs) in transverse sections is higher than the number of AQP1-positive DTLs in the inner medulla (Fig. 8) because AQP1 is not expressed along the entire length of the DTL, as noted above, whereas ClC-K1 is expressed along the entire length of the ATL as well as in the prebend segment of the DTL. Because AQP1-positive DTLs lie predominantly within the vascular bundle region, ATLs and likely AQP1-null DTLs make up the population of thin limbs that lie within the CD clusters.

CD cluster region. Interconnecting capillaries run vertically and horizontally to form a plexus of vessels that surround the CD clusters (Fig. 10). These vessels are distributed in a relatively sparse pattern throughout a large interstitial space and abut a relatively small proportion of the CD surface area. This is seen even in the outer inner medulla, whereas in rodent kidneys the interstitial volume tends to be relatively small. In electron micrographs of transverse sections, the peritubular capillaries are seen to lie relatively distant from the CD surface membrane (~10 μm or more) (Fig. 11). This is in sharp contrast to the fenestrated capillaries that closely abut CDs in the rodent outer inner medulla, which lie within 0.5–1.0 μm away from CDs, and along with ATLs form what have been referred to as interstitial nodal spaces (15, 38).
**UT-B expression in the papillary epithelium.** UT-B is strongly expressed along the papillary epithelium, especially near the papilla tip (Fig. 2), suggesting that the papillary epithelium may have significant transcellular urea permeability.

**DISCUSSION**

We have shown that distinct structural relationships exist between the inner medullary thin limbs of Henle’s loops, CDs, vasa recta, and peritubular capillaries in the human kidney. Just as there are two distinct lateral regions in the inner stripe of the outer medulla (the vascular bundle and the interbundle regions), there are two distinct lateral regions in the inner medulla of humans and rodents: the vascular bundle region (also referred to as the intercluster region) and the CD cluster region (summarized in Fig. 12) (39, 40). The vascular bundle regions of the inner stripe and the inner medulla in the kidney of humans, rats, and kangaroo rats are dominated by DVR and AVR (15, 49). Whereas the vascular bundle region of the rodent inner stripe includes short-loop DTLs, the vascular bundle region of the human inner stripe includes no DTLs (19). On the other hand, the vascular bundle region of both the human and rodent inner medulla includes AQP1-positive long-loop DTLs. The CD cluster region of the inner medulla includes CDs, ATLs, probably AQP1-negative DTLs, and a peritubular capillary bed.

As with the outer medulla, especially in rodents, the inner medulla exhibits significant zonation along the corticomedullary axis. The most obvious changes in zonation of the inner medulla that we observed in both humans and rodents (39, 48) include 1) the loss of detectable AQP1 in the lower 50% of the inner medulla, 2) the loss of detectable urea transporter UT-B in the DVR in the lower 50% of the inner medulla, and 3) the disappearance of smooth muscle myosin heavy chain protein expression in the lower 50% of the inner medulla.

Reduction in expression of these proteins along the corticomedullary axis would impact the dynamics of compartment-to-compartment flows of fluids, solutes, and oxygen at different levels of the inner medulla. Studies with mouse AQP1 gene deletions have shown that AQP1 is the principal pathway for transepithelial water flux in the long-loop DTL, and, in its absence, DTLs exhibit reduced transepithelial water permeability (10). If AQP1 is the principal pathway for transepithelial water flux in human kidneys as in rodents, then the human AQP1-null DTLs are likely to have very low transepithelial water permeability. Equilibration of tubule fluid by transcellular water efflux is therefore unlikely to occur in these segments, and if they have exceedingly high urea and NaCl permeabilities as in the rat and chinchilla, then they likely equilibrate by way of solute entry (9, 27). Urea and NaCl entry into the AQP1-null DTL segment in human and rodent kidney are essential for producing a concentrated urine, although these entry pathways remain unstudied (11, 35). The DVR in the innermost 50% of the rat and kangaroo rat inner medulla also express little or no UT-B (15, 49). However, functional studies in rats have shown that vasa recta from the deep papilla exhibit substantial urea...
permeability, apparently even in the absence of a known urea transporter. Transepithelial urea flux in these segments possibly occurs by way of the paracellular pathway (33).

Smooth muscle myosin heavy chain protein is expressed in renal arterioles (26, 44), and its expression along the upper inner medullary DVR in the human kidney suggests that segments in the upper zone of the inner medulla have contraction and relaxation properties, as has been shown for DVR of the inner stripe of the outer medulla (32). The contractile properties of segments at deeper levels of the inner medulla, where smooth muscle myosin heavy chain protein expression is distributed more sparsely or is absent altogether, likely are different. Thus regulation of blood flow from DVR into capillary beds of the outer inner medullary CD cluster region may involve contractile mechanisms that include smooth muscle myosin, whereas blood flow from DVR in the lower inner medulla does not.

Corticomedullary zonation in the rodent inner medulla is also defined by another architectural feature consisting of repeating microdomains aligned alongside CDs and formed by fenestrated capillaries and ATLs. Proposed to serve as mixing compartments for NaCl, urea, and water reabsorbate (21), these interstitial nodal spaces are abundant in the rodent outer inner medulla and decline significantly in number with increasing depth below the outer medulla (13, 36). If these compartments do play a role in rodent renal physiological processes, then the absence of this architectural feature in the human inner medulla could be related to substantive differences between human and rodent physiology.

The architecture of medullary rays and the inner stripe has long been considered an important factor in determining blood flow and oxygen delivery to metabolically active nephron segments and may also apply to the inner medulla. Medullary rays are supplied by the venous vasa recta and thus are particularly vulnerable to hypoxia (7, 19). Oxygenation in this region is preserved by the adjacent peritubular capillary network. In the outer medulla, separation of the DVR from the metabolically active thick ascending limbs and CDs underlies a significant Po2 radial gradient. In situations of inner stripe hypoxic injury, the epithelial elements in continuity with the vascular bundles are much less vulnerable than the epithelial elements contiguous to the more centrally located CDs. The inner medulla is far less vulnerable to hypoxic injury than the inner stripe, with its high oxygen utilization (6, 14). Because of the physical separation of long-loop AQPI-positive DTLs and DVR from CDs (Fig. 12), fluid and solute reabsorbate from DTLs and DVR and oxygen diffusing from DVR have limited exchange with CDs. Mathematical modeling studies have estimated that inner medullary vascular bundles of rat may generate significant radial Po2 gradients, thereby preserving oxygen delivery to the deep papilla (12). For example, models suggest that in the absence of vascular bundles and with homogeneous distribution of tubules and vessels, the fractional delivery of O2 to the inner medulla that reaches the terminal 1.5 mm drops from 3.3 to 0.8%.

The urea transporter UT-B has previously been shown to be expressed in rat urothelia (45). UT-B possibly provides a transcellular pathway for transepithelial urea flux across the human papillary epithelium. However, functional studies with rat papillary epithelium suggested that the low measured transepithelial urea permeability combined with the low transepithelial concentration gradient that exists under normal steady-state conditions is unlikely to account for a physiologically important urea flux (43).

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


