The Mammalian Urine Concentrating Mechanism: Hypotheses and Uncertainties

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The urine concentrating mechanism of the mammalian kidney, which can produce a urine that is substantially more concentrated than blood plasma during periods of water deprivation, is one of the enduring mysteries in traditional physiology. Owing to the complex lateral and axial relationships of tubules and vessels, in both the outer and inner medulla, the urine concentrating mechanism may only be fully understood in terms of the kidney’s three-dimensional functional architecture and its implications for preferential interactions among tubules and vessels.

During periods of water deprivation, the urine concentrating mechanism of mammals stabilizes the osmolality of blood plasma by producing a urine that has an osmolality substantially exceeding that of blood plasma. Urine is concentrated in the final stages of its production: water is absorbed, in excess of solute, from the collecting ducts and into the vasculature of the medulla, thus increasing the osmolality of the collecting duct fluid and thus the osmolality of the urine that emerges from the collecting ducts.

When a mammal is producing concentrated urine, an increasing osmolality gradient is maintained in all tubules and vessels along the cortico-medullary axis of the outer medulla by means of active NaCl transport from specialized renal tubules (viz., thick ascending limbs). However, the thin ascending limbs found in the inner medulla have no significant active transepithelial transport of NaCl or any other solute (7, 8, 29, 30). Thus active solute transport coupled with countercurrent flow does not explain the concentrating process in the inner medulla, where the steepest osmotic gradient is generated.

The most influential theory for the generation of the inner medullary osmolality gradient is the “passive mechanism” hypothesis, proposed independently in 1972 by Kokko and Rector (15) and by Stephenson (41). The passive mechanism depends on the assumption that the interstitium has a much higher urea concentration than NaCl concentration and that fluid in the descending limbs has a much higher NaCl concentration than NaCl concentration and that fluid in the thin ascending limbs has a sufficiently high permeability to NaCl and a sufficiently low permeability to urea, then much NaCl will diffuse (passively) from the ascending thin limb lumen into the interstitium, whereas simultaneously little urea will diffuse from the interstitium into the thin limb lumen. If these transepithelial concentration differences are sustained, the interstitial fluid will be concentrated while the luminal fluid is being diluted. The passive mechanism hypothesis assumes that the concentrations are sustained by continuous diffusion of urea from the collecting duct lumens and by continuous delivery of tubular fluid having a high NaCl concentration to the ascending thin limbs, this delivery depends on the descending thin limb having sufficiently low NaCl and urea permeabilities that transepithelial concentration gradients are not dissipated along the course of the descending thin limbs. Thus the passive mechanism is critically dependent on specific loop-of-Henle permeabilities to NaCl and urea.

However, mathematical models using measured values of urea permeability have generally been unable to predict a significant axial osmolality gradient (38). The inconsistency between measured urea osmolarities and the predictions of mathematical models has motivated the formulation of a number of alternative hypotheses, including the potential roles of anatomical complexity (47–49), of accumulation of an external osmolyte (6, 10, 44, 43), of muscular contractions of the pelvic wall (13, 46), and of solute secretion into the loops of Henle (24). The attempts to reconcile mathematical models with the formation of highly concentrated urine have been extensively reviewed (13, 25, 38).

In this review, we summarize new findings on the three-dimensional functional architecture of the renal medulla of the rat kidney, and we consider the significance and implications of these findings for the urine concentrating mechanism.

The Concentrating Mechanism of the Outer Medulla: Does it Depend on Countercurrent Multiplication?

In 1942, Kuhn and Ryffel (22a) proposed the generally accepted paradigm for the mechanism of the outer-medullary concentrating effect: urine is concentrated by means of the multiplication of a single effect (“Verteilungsfaltung des Einzelwirkstoffes”). More precisely, a small osmotic pressure difference (the single effect) between oppositely directed flows in parallel renal tubules is multiplied, by means of the countercurrent principle (“Gegenstromprinzip”), resulting in a large...
increase in osmotic pressure along the cortico-medullary axis, as shown in FIGURE 1A. The loop of Henle was subsequently identified as the likely principal counterflow unit (5); an axial gradient was identified along the renal medulla (51); experiments indicated an osmolality difference across the thick ascending limb epithelium (50); and an early mathematical model employed active transport of NaCl from thick ascending limb lumen to the interstitium as the single effect (22).

Research in the past 40 years has borne out these theoretical proposals and experimental findings, although with substantial supplementation and refinement (9, 38).

The finding that, in principle, a countercurrent system can use a small transtubular osmolality difference by continuing NaCl concentration through this delivery system having sufficient tubular dilution that transepithelial dissipated NaCl and urea concentrations measured values have been unable to conform to the standard interpretation of the countercurrent multiplication. The findings on the concentrating mechanism in the outer medulla (OM), which does not conform to the standard interpretation of the countercurrent multiplication. C: the concentrating mechanism in the inner medulla (IM), based on the “solute-separation, solute-mixing” mechanism. In all panels, thick lines indicate water-impermeable portions of tubules; darkening colors represent increasing osmolality.

FIGURE 1. The original formulation of counter-current multiplication and the concentrating mechanisms in the outer and inner medulla. A: at left the original formulation of counter-current multiplication is shown, where the descending and ascending tubes are in direct contact, and NaCl is transported from the descending tube to the ascending tube. At right, a revised view of counter-current multiplication applied to a loop of Henle is shown, where the descending and ascending limbs are separated by the interstitium, and the descending limb fluid is concentrated by water absorption. B: the concentrating mechanism in the outer medulla (OM), which does not conform to the standard interpretation of the countercurrent multiplication. C: the concentrating mechanism in the inner medulla (IM), based on the “solute-separation, solute-mixing” mechanism. In all panels, thick lines indicate water-impermeable portions of tubules; darkening colors represent increasing osmolality.
Three-Dimensional Lateral and Vertical Relationships of Tubules and Vessels in the Outer Medulla and Their Functional Implications

We believe that the concentrating mechanism of the outer medulla should be reconsidered in light of the radial and axial inhomogeneity revealed in anatomical studies. Kritz and colleagues have reported that the organization of tubules and vessels is highly structured in the outer medulla of a number of mammalian species, including rats and mice (16, 17, 19, 21). A schematic diagram of the organization in the inner stripe of the rat outer medulla is shown in **FIGURE 2A**. Tubules are organized around tightly packed vascular bundles, with the collecting ducts and thick ascending limbs found distant from vascular bundles and descending limbs positioned nearer the bundles. The structural organization is believed to result in preferential interactions among tubules and vascular segments, which appear to be represented in schematic diagrams and descriptive phrasing in reviews and texts (3, 9, 27, 31, 45). These considerations suggest that the outer-medullary concentrating mechanism does not conform to a standard interpretation of countercurrent multiplication but rather is more like the mechanism that is illustrated in **FIGURE 1B**.

The Inner Medulla: Implications of Three-Dimensional Architecture

If one assumes that the mechanism that produces the osmotic gradient along the thick ascending limbs involves the interactions of all renal tubular and vascular structures, then that mechanism can be fully understood only in terms of the kidney’s three-dimensional functional architecture. To fully understand this functional architecture, a computer-assisted process was used to reconstruct the vascular and tubular structures of the inner medulla of the rat kidney from serial transverse sections (33–37).

However, immunohistochemical localization studies, in both mice (52) and rats (32, 46), have revealed a segment along the short descending limb in the inner stripe that does not express the water channel aquaporin-1 (AQP-1). Thus not only are the descending limbs of short loops distant from their contiguous ascending limbs, but also tubular fluid osmolalities along substantial inner stripe portions of short descending limbs may well be lower than osmolalities in other tubules and vessels at the same level. If these inner stripe descending limb portions do not present a load of fluid to be concentrated, then the vigorous active transport of NaCl along the thick ascending limbs may generate very substantial osmolality gradients across the thick limb epithelium. Indeed, a mathematical model of the outer-medullary urine concentrating mechanism of the rat (23) predicts such gradients, which appear to be represented in schematic diagrams and descriptive phrasing in reviews and texts (3, 9, 27, 31, 45). These considerations suggest that the outer-medullary concentrating mechanism does not conform to a standard interpretation of countercurrent multiplication but rather is more like the mechanism that is illustrated in **FIGURE 1B**.

**FIGURE 2** Schematic diagrams of tubular organization in the rat renal medulla.

A cross section through the inner stripe of outer medulla, where tubules appear to be organized around a vascular bundle. B cross section through the upper inner medulla, where tubules and vessels are organized around a collecting duct cluster. Inset: schematic configuration of a collecting duct, ascending vasa recta (AVR), an ascending thin limb, and a nodal space.
Anatomical findings distinguish three subpopulations of Henle’s loops in the inner medulla; sample loops are shown in FIGURE 3. **Subpopulation 1** corresponds to loops of Henle that reach no more than ~1 mm into the inner medulla (outer zone 1; FIGURE 4), that do not label for AQP1 in their descending limbs, and that presumably have little or no permeability to water. **Subpopulation 2** corresponds to loops that reach more than 1 mm and no more than ~3–3.5 mm into the inner medulla (outer zone 2; FIGURE 4), and that label for AQP1 in the upper ~40% of the inner-medullary portions of their descending limbs but do not express AQP1 in the remaining 60% of their lengths. These loops usually enter and then ascend within the primary collecting duct clusters for variable distances. **Subpopulation 3** corresponds to loops that reach into the final 1–2 mm of the inner medulla (inner zones 1 and 2; FIGURE 4) and that also label for AQP1 in the upper ~40% of the inner-medullary portion of their descending limbs. It is noteworthy that no detectable AQP1 is expressed in any thin descending limb segments in the final 2.0–2.5 mm of the inner medulla. In all loops, expression of the chloride channel CIC-K1 begins abruptly in the AQP1-negative portion of the descending thin limb (~150–200 μm above the hairpin bend and then continues uniformly along the entire length of the ascending thin limb within the inner medulla. About half the loops reaching into the terminal 500 μm of the inner medulla (inner zone 2; FIGURE 4), instead of having narrow hairpin bends with only a small transverse segment, are bent so that they...
have a broad transverse segment that includes part of the CIC-K1-positive prebend region of the descending thin limb and part of the ascending thick limbs (36). These broad bends tend to be close to, and curved laterally around, the very large terminal collecting ducts in this region (FIGURE 4).

The Implications of Anatomy for Function

We have proposed a "solute-separation, solute-mixing" concentrating mechanism (24) that may be regarded as a refinement of the passive mechanism (15, 41) and is shown in FIGURE 1C. However, the characterization of that mechanism as "passive" is misleading. Transport work is performed in the outer medulla, mostly in thick ascending limbs, to separate NaCl from urea ("solute-separation"). Additional transport work is required in the collecting ducts to actively transport NaCl to the interstitium and thus promote osmotic water absorption from the collecting ducts and raise the urea concentration inside collecting ducts so that the urea will diffuse into the interstitium.

In "solute-separation, solute-mixing," the "mixing" refers to the intermingling, within the interstitium and vasculature, of NaCl from loops of Henle and urea from collecting ducts. The tubular-vascular patterns and relationships that emerge from our findings suggest a concentrating mechanism that may be comprised of three countercurrent systems, as described below.

An intracortical countercurrent system

The countercurrent system in the central region of the collecting duct cluster appears to function specifically to raise the osmolality of collecting duct tubular fluid by facilitating the targeted delivery of NaCl via loop-descending segments and by mostly confining absorbed NaCl, urea, and water within the collecting duct clusters. The microdomains (or nodal spaces, FIGURE 2B, INSET) formed by the collecting ducts, ascending vasa recta, and ascending thin limbs appear ideally configured to mix NaCl from loops with absorbate from the collecting ducts (which consists mostly of water, urea, and NaCl) to produce isolated sites of high osmolality that will promote water withdrawal from adjoining collecting ducts. The ascending vasa recta alongside the collecting ducts provide, at each medullary level, a low-resistance sink for absorbed solutes and water. The absorbate is carried via these ascending vasa recta to higher levels within the collecting duct cluster, where the local absorbate will likely have lower solute concentrations and lower osmolality, and where the ascension in ascending vasa recta fluid that is concentrated relative to local fluid would help elevate the osmolality of the collecting duct tubular fluid and contribute to an optimization of concentrating efficiency.

An intercluster countercurrent system

In the outer 3-3.5 mm of the inner medulla (outer zone; FIGURE 4), the tubules and vessels of the peripheral regions of the collecting duct clusters appear to form a second countercurrent system that is separated from the countercurrent system within the central regions of the collecting duct clusters. In the peripheral regions, fluid in descending limbs and vasa recta flows toward the tip of the papilla, whereas fluid in ascending limbs and vasa recta flows toward the outer medulla. It seems plausible that the principal role of this system is to remove water from the water-permeable portions of descending thin limbs (the upper 40% of these limbs) and thus to raise the tubular NaCl concentration within descending thin limbs before they enter the central regions of the collecting duct clusters and come in contact with the nodal spaces. The efflux of NaCl and urea from the intercluster portions of ascending thin limbs and the upward flow of relatively concentrated ascending vasa recta fluid may promote this water withdrawal, and the ascending vasa recta may serve to carry the absorbate to the outer medulla. Moreover, the separation of the loop-of-Herde portions in the peripheral regions of the collecting duct clusters may serve to separate urea concentration maxima from the intercluster areas, where NaCl and urea are all mixed within the fluid, as the fluid has a solute concentration that is impaired throughout.

A papillary countercurrent system

A third countercurrent system appears in the final 1.5-2 mm of the collecting duct cluster (FIGURE 4), where the collecting ducts are no longer distinguished from the vasa recta, and all vasa recta are fenestrated. These broad bends continue to be readily distinguishable from this portion of the medulla, and the vasa recta are all fenestrated and separated by fenestrated capillaries. These capillaries are highly permeable, and they form a broad transverse segment that includes part of the CIC-K1-positive prebend region of the descending thin limb and part of the ascending thick limbs (36). These broad bends tend to be close to, and curved laterally around, the very large terminal collecting ducts in this region (FIGURE 4).
the “mixing” interstitium and urea from the descending limbs suggest a process desmosome, as indicated by the interloop pattern below.

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A region of the medulla specifically to deliver NaCl to the descending vasa recta, located at the interface of the outer and inner medulla, may be considered the principal site of solute mixing (Fig. 2E). Importantly, the interloop interstitium may also play an important role in facilitating solute mixing and thereby support a rapid efflux of NaCl at the bend. In particular, the chloride transporter ClC-K1 is likely to be the principal sites of solute mixing.

**Summary**

The mammalian kidney is an intricate, highly specialized organ with a complex structure that allows for the efficient production of concentrated urine. This organ, known as the kidney, is responsible for regulating the body’s fluid and electrolyte balance by filtering blood, reabsorbing essential substances, and excreting waste products and excess fluids. The kidney is composed of two main parts: the renal cortex and the renal medulla. The renal medulla is further divided into the outer medulla and the inner medulla, with the inner medulla being the site of urine concentration.

Key features of the mammalian kidney include:

- **Countercurrent Multiplication (CCM):** A mechanism where blood enters the outer medulla and is diluted by urine, while urine returns to the outer medulla, effectively concentrating solutes.
- **Tubular Counterflow:** The countercurrent flow of urine and blood through the renal medulla helps in concentrating solutes efficiently.
- **Solute Mixing:** The mixing of solutes within the tubules and interstitial spaces facilitates the concentration of solutes.

**References**


