Countercurrent exchange in the renal medulla

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Pallone, Thomas L., Malcolm R. Turner, Aurélie Edwards, and Rex L. Jamison. Countercurrent exchange in the renal medulla. Am J Physiol Regul Integr Comp Physiol 284: R1153–R1175, 2003; 10.1152/ajpregu.00657.2002.—The microcirculation of the renal medulla traps NaCl and urea deposited to the interstitium by the loops of Henle and collecting ducts. Theories have predicted that countercurrent exchanger efficiency is favored by high permeability to solute. In contrast to the conceptualization of vasa recta as simple “U-tube” diffusive exchangers, many findings have revealed surprising complexity. Tubular-vascular relationships in the outer and inner medulla differ markedly. The wall structure and transport properties of descending vasa recta (DVR) and ascending vasa recta (AVR) are very different. The recent discoveries of aquaporin-1 (AQP1) water channels and the facilitated urea carrier UTB in DVR endothelia show that transcellular as well as paracellular pathways are involved in equilibration of DVR plasma with the interstitium. Efflux of water across AQP1 excludes NaCl and urea, leading to the conclusion that both water abstraction and diffusion contribute to transmural equilibration. Recent theory predicts that loss of water from DVR to the interstitium favors optimization of urinary concentration by shunting water to AVR, secondarily lowering blood flow to the inner medulla. Finally, DVR are vasoactive, arteriolar microvessels that are anatomically positioned to regulate total and regional blood flow to the outer and inner medulla. In this review, we provide historical perspective, describe the current state of knowledge, and suggest areas that are in need of further exploration.

vasa recta; microperfusion; microcirculation; water channel; urinary concentration; permeability

SINCE THE EXPERIMENTAL FINDINGS of Wirz et al. (147) led to the countercurrent theory of the urinary concentrating mechanism, as described by Hargitay and Kuhn (31), most subsequent research has focused on the countercurrent multiplier function of the loops of Henle. According to the theory, a small difference in osmotic pressure (the single effect) is multiplied by countercurrent flow in adjacent channels of the limbs of Henle’s loop to produce a large axial difference in osmotic pressure between the renal cortex and the tip of the renal papilla; that is, the multiplier generates a hypertonic renal medulla. Less attention has been paid to countercurrent exchange, which is thought to preserve medullary hypertonicity rather than create it. It is generally accepted that the microcirculation of the renal medulla functions as a countercurrent exchanger that traps NaCl and urea deposited to the interstitium by the loops of Henle and collecting ducts, respectively. Early hypothetical descriptions of this process envisioned a system in which descending vasa recta (DVR) and ascending vasa recta (AVR) are parallel tubes that equilibrate by diffusion. According to that notion, blood flowing from the corticomedullary junction toward the papillary tip in DVR is concentrated by diffusive influx of NaCl and urea, and, conversely, blood flowing away from the papillary tip toward the corticomedullary junction in AVR is diluted by diffusive efflux. That theory predicts that solute is trapped due to recycling between DVR and AVR and that net rate at which solute is removed from the medulla is primarily dependent on the AVR-DVR concentration difference at the corticomedullary junction (13, 39, 40, 91, 145).
Micropuncture studies demonstrated that AVR plasma is more concentrated than adjacent DVR plasma, providing key evidence that vasa recta are countercurrent exchangers (38, 113, 114). In contrast, fluid obtained from descending thin limbs of Henle is more concentrated than that from ascending thin limbs, supporting the conclusion that the loops of Henle function as the countercurrent multiplier responsible for generating corticomedullary osmotic gradients (38–41). Subsequent studies unraveled an unexpected degree of complexity. DVR and AVR wall structures were found to be distinct and to have characteristics that vary as function of corticomedullary axis (39, 59, 91, 121). In vivo measurements of plasma protein concentrations in DVR unexpectedly revealed efflux of water from the DVR lumen to the papillary interstitium, a finding that presented two paradoxes. First, the physiological benefit derived from depositing water from DVR to medullary interstitium was enigmatic. Second, measurements of Starling forces (hydrostatic and oncotic pressure) failed to predict the observed direction of DVR transmural water movement (113, 114).

Recent physiological investigations continued to show unexpected complexity while shedding some light on the paradoxes associated with DVR equilibration. Specifically, physiological and immunochemical measurements verified that aquaporin-1 (AQP1) water channels and the facilitated urea carrier (UTB) are significant transport pathways in DVR endothelia. Unusual intracellular signaling pathways have been found in DVR endothelia (94, 99, 106). A few recent measurements of AVR properties have been obtained that show striking differences from DVR (81–83, 90, 95–98, 133). Overall, AVR remain poorly characterized because they cannot be isolated for in vitro studies. Taken together, these studies of microanatomy, tubular-vascular relationships, and transport properties demonstrate complexity and lead to the conclusion that the depiction of vasa recta as simple diffusive “U-tube” exchangers leads to conceptual errors. In this review, we will summarize the pertinent literature and, to the extent possible, give functional perspective to these observations.

In view of his numerous contributions to the urinary concentrating mechanism, among which were the introduction of the countercurrent multiplier theory of Werner Kuhn and the companion countercurrent exchange theory of the medullary circulation to American readers and the experiments by him and his coworkers testing those theories, we wish to dedicate this review to Robert W. Berliner, who died February 6, 2002.

TUBULAR-VASCULAR RELATIONSHIPS AND MICROANATOMY

Outer medulla. Blood flow to the renal medulla is largely derived from the efferent arterioles of juxtamedullary glomeruli (9, 10, 59, 73, 91, 93, 99), in addition to which a portion of the flow may traverse periglomerular “shunt” pathways (16) (Fig. 1). Afferent arterioles that supply juxtamedullary glomeruli arise from the cortical interlobular arteries at a steep recurrent angle. Those afferent arterioles are composed of one to three layers of smooth muscle cells surrounding the media and endothelial layers. Efferent arterioles of juxtamedullary glomeruli are larger in wall thickness, diameter, and length than efferent arterioles of superficial glomeruli (9, 10, 39, 91). As efferent arterioles penetrate across the corticomedullary junction to the outer stripe of the outer medulla, the muscular layer decreases and is replaced by smooth muscle remnants known as “pericytes” (Fig. 1). In an arrangement compared with a “horse’s tail,” juxtamedullary efferent arterioles then give rise to as many as 30 DVR (73). The diameter of rat DVR is generally one-half that of the parent efferent arteriole (~12 μm ID), but some larger vessels continue beyond the outer medulla to perfuse the deep inner medulla (51). When efferent arterioles become DVR, smooth muscle is replaced by pericytes and the medial layer interposed between smooth muscle and endothelium disappears. As DVR
continue into the inner medulla, the pericytes become sparse but are present (101). The transition from arteriole to capillary is most gradual in the DVR that penetrate furthest into the inner medulla (39, 91, 121).

The architectural arrangement of the outer medulla is characterized by a striking division into vascular bundles and the interbundle region (Figs. 1 and 2). The "simple" vascular bundle (rabbit, guinea pig, dog, cat, monkey, human) is comprised of DVR and AVR in close apposition with a minimum of surrounding interstitium (6, 51). AVR that lie within vascular bundles are largely those that originate within the inner medulla. AVR formed within the outer medulla from the capillary plexus of the interbundle region ascend directly to the cortex without rejoining a vascular bundle. The capillary plexus of the interbundle region arises from DVR that peel off from the periphery of the vascular bundles as they pass through the inner stripe (42, 43, 59, 73, 91). From this arrangement we infer that countercurrent exchange in the outer medulla occurs between AVR draining the inner medulla and DVR supplying both outer and inner medulla. The "complex" vascular bundle (rat, mouse, Meriones, Psammomys) differs from simple bundles due to the variable incorporation of thin descending limbs of short looped nephrons (6, 7, 52, 59, 91). In the rat, the thin descend-
ing limb is situated on the periphery of the bundles, whereas in the mouse and *Psammomys*, short looped thin descending limbs are distributed throughout the vascular bundle (7, 42, 51, 53). In some species, complex vascular bundles coalesce in the outer stripe to form “giant” bundles that traverse the inner stripe (Fig. 2A). The latter is most prominent in *Psammomys* and is to be contrasted with the vascular bundle architecture in the outer medulla of other mammalian species (Fig. 2, B-D) (42). Thus vascular bundles that characterize the inner stripe provide for countercurrent exchange between DVR and AVR returning from the inner medulla, and to a variable degree that is species dependent between vasa recta and thin descending limbs of short looped nephrons. In *Psammomys*, an additional striking feature exists. The vascular bundle periphery is brought into proximity with pelvic urine due to invaginations of the pelvic epithelium. The possibility that vascular bundles exchange solute with the pelvic urine must therefore also be considered.

*Inner medulla.* Shortly after passing the inner-outer medullary junction, the vascular bundle architecture disappears and vasa recta become more evenly dispersed among nephrons and collecting ducts (39, 73, 91). In the outer medulla, interstitial space between adjacent vessels is minimal (Fig. 2B). From the inner-outer medullary junction to the papillary tip, the fraction of medullary tissue that is interstitium increases from 5 to ~30% (49). Numerous inner medullary interstitial cells, arranged horizontally like rungs of a ladder, are tethered between vessels and nephrons so that they might inhibit axial diffusion and retard dissipation of corticomedullary gradients (58). As in the outer medulla, AVR outnumber DVR. Their ratio has been reported as 1.7 to 1 in hamsters and 2.3 to 1 in the rat (35, 67, 155). DVR terminate at various levels in a sparse capillary plexus that coalesces to form AVR. DVR have a continuous, nonfenestrated endothelium and zona occludens (72, 121). Toward the termination of the DVR, fenestrations appear that characterize the wall of the subsequent capillary plexus and AVR. In the inner medulla, the fraction of the AVR wall covered by fenestrations is ~50%. That fraction decreases toward the outer medulla to ~15–30% (39, 91). On electron micrographs, fenestrations have diameters of 530 to 1,000 Å and are bridged by a 40-Å-thick diaphragm. The diaphragm has one or two concentric rings interconnected by radiating fibers and a central density (68).

**COUNTERCURRENT EXCHANGE—GENERAL CONCEPTS AND EVOLUTION OF UNDERSTANDING**

Countercurrent exchange in nature. The idea of countercurrent exchange can be traced back to Claude Bernard (14), who observed “wherever a peripheral artery flows alongside a vein there is likely to be a heat gradient between them and a transfer of heat from artery to vein. . . . [This] . . . thermal short circuit . . . carries some of the arterial heat back into the body before it reaches the periphery” [cited by Scholander and Krog (119)]. As pointed out by Scholander and Krog (119), this simple arteriovenous arrangement can become much more complex with the artery dividing into arterioles or capillaries and the vein dividing into venules or capillaries to provide an enormous area for heat exchange between arterial and venous blood, the rete mirabile (Fig. 3). The sloth is a slow-moving arboreal animal that inhabits treetops in tropical jungles. Scholander and Krog (119) dissected the brachial rete of a sloth, a 1-cm-thick bundle of parallel arteries and veins with 20–30 arteries and fewer veins. A 4-cm portion of a rete was freed, and a thermocouple probe was threaded along its length. The temperature decline in a direction away from the body was 1°C per centimeter of length, 30 times steeper than that along the human brachial artery. When the venous blood flow returning through the rete was slowed by constricting a ligature, the gradient was greatly reduced.

Cold extremities constitute an important adaptation to heat economy in many Artic mammals, such as the whale with its greatly expanded and well-vascularized fluke and fins, and the artic wading bird, which has a rete at the junction of its body and stiltlike legs. For the sloth, the ambient temperature at the treetop level can fall 15°C at night (118, 119). Given the sloth’s slowness of movement, heat conservation is at a premium (118). Besides conservation of heat, countercurrent exchange has a wide variety of applications in biology (Table 1). Countercurrent flow between blood and seawater in fish gills maximizes extraction of oxygen. The most dramatic example is found in the swim bladder of deep sea fish to regulate their buoyancy at depths of thousands of meters (Fig. 3). The pressure of gas (primarily oxygen) inside the bladder lumen is enormous, ~200 atmospheres, equaling the weight of the surrounding water. Located in the bladder neck, the rete traps oxygen to prevent its escape from the bladder. The “. . . outgoing veins, highly charged with oxygen, give it up to adjacent incoming arteries” (119). For example, the rete from a common eel weighs ~65 mg and has 100,000 arterial capillaries and about the same number of venous capillaries. The capillaries are ~4-mm long, meaning the cumulative total length of each kind of capillary is 400 m. Because the capillary diameter is 7–10 μm, the total wall endothelial surface area exceeds 20 cm² (54). Scholander (118) calculated that the oxygen pressure across a rete 1 cm long is reduced by a factor of 3,000, which means that oxygen would be completely extracted from the venous blood flowing out through the bladder neck and returned to the inflowing arterial blood.

Countercurrent exchange in the renal medulla. The idea that the countercflow arrangement of vasa recta enables efficient exchange of solutes and water originated with Kuhn and his colleagues (31, 55) and Wirz and colleagues (145–147). But for many, the introduction to countercurrent multiplication and countercurrent exchange was, at least in the United States, provided by Berliner et al. (13). The countercurrent multiplier explained the exponential rise in osmotic pressure of the renal medullary tissue from the corticomedullary junction to the papillary tip. But this
Fig. 2. Vascular bundles. A: photograph of the microvasculature of the desert rodent *Psammomys obesus* obtained by arterial injection of the kidney with Microfil. The distinct pattern of the cortex, outer, and inner medulla is apparent. In this species, the separation of the outer medulla into vascular bundles and the dense capillary plexus of the interbundle region (*) is striking because vasa recta coalesce into giant vascular bundles. OM, outer medulla; IM, inner medulla. Designations on the original figure are c, cortex; TR, transitional region (outer stripe of the OM); IS, inner stripe of the OM; IZ, inner zone (IM). B and C: injection study of vascular bundles in the OM of the rat. In contrast to *Psammomys*, individual vascular bundles do not coalesce into giant bundles. They are more evenly dispersed throughout the inner stripe of the OM. This pattern is most typical of mammalian species including the rat, mouse, and human. D: transmission electron micrograph showing AVR and DVR in the vascular bundles of the rat. AVR are fenestrated and DVR have a continuous endothelium. The interstitial space separating the DVR and AVR is small. [From Bankir et al. (7), Moffat and Fourman (73), Pallone et al. (97).]
implied that the osmotic pressure of blood entering the medulla also rises concomitantly, which posed the dilemma that even if only 5% of renal blood flow entered the medulla, the concentrating mechanism would have to concentrate a very large volume of blood to concentrate a much smaller volume of urine, severely limiting the mechanism’s efficiency. The explanation that the medullary circulation functions as a countercurrent exchanger resolved the dilemma. Wirz (146) wrote, “all the blood irrigating the medulla enters and leaves the medulla at the corticomedullary boundary, i.e., in an essentially isotonic [to systemic blood] region. In between it may adapt itself to the osmotic pressure of the surroundings by a passive uptake (on its way down) and release (on its way up) of osmotically active solutes.” Berliner et al. (13) described countercurrent exchange using the analogy of a heat exchanger, depicted in Fig. 4. In Fig. 4A, water flowing at 10 ml/min passes a heat source that supplies heat at a rate of 100 calorie/min. Accordingly, the temperature of the stream increases from 30 to 40°C. Figure 4B shows a hypothetical idealized counterflow heat exchanger created by opposing the limbs upstream and downstream from the heat source. In that case, consistent with the

Table 1. Application of countercurrent exchange (examples)

<table>
<thead>
<tr>
<th>Biology</th>
<th>Example</th>
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<tr>
<td>Conservation of body heat (rete at junction of torso with limb or appendage)</td>
<td>Sloth (118)</td>
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<td>Manatee (sea cow) (118)</td>
<td>Capybara (118)</td>
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<td>Lemur (118)</td>
<td>Anteater (118)</td>
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<td>Artic gull (119)</td>
<td>Seal (119)</td>
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<td>Whale (118)</td>
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<td>Conservation of high gas pressure (swim bladder) for buoyancy (trapping and secretion of oxygen) (rete at bladder neck)</td>
<td>Deep-sea eel (118,119)</td>
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<td>Wreckfish (119)</td>
<td>Butterfly fish (117)</td>
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<td>Extraction of oxygen from water (fish gills)</td>
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<td>Kidney medulla (mammals and birds) (13,118,119)</td>
<td>Placenta of animals (119)</td>
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<td>Man-made devices</td>
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requirement of energy conservation, water exits the exchanger at a temperature 10°C higher than the inflow, but an axial temperature gradient is created by the warming of water before its arrival at the heat source by the outflowing heated water. This large axial temperature gradient is established and maintained by thermal diffusion between the two limbs and by heat trapping due to countercurrent flow. The graph inset compares the temperature along flow tubes in each system. In Fig. 4C, countercurrent flow principle is applied to an idealized capillary loop in the medulla representing a descending vas rectum and ascending vas rectum. Note that the exchange occurs between capillary and interstitium of the medulla rather than between two adjacent capillary loops. Solute diffuses from the medullary interstitium to the blood flowing down the descending vas rectum. As blood returns in the ascending vas rectum, the solute concentration difference is reversed and solute diffuses into the interstitium. This effectively “traps” solute in the interstitium by recycling between ascending and descending capillary. In this way, blood circulates through the renal medulla without “washing out” its hypertonicity. The greater the solute permeability of the capillary, the more complete the exchange. In the absence of a high solute permeability, at any given level, osmotic equilibrium may not be completely achieved; consequently, the contents of the blood at the cortical medullary junction may be slightly hypertonic to the isotonic interstitium.

This model was consistent with the experimental findings of Wirz (145), Ullrich and Pehling (137), and Gottschalk and Mylle (30) that all structures in the rodent renal papilla are equally hypertonic. Later Jamison et al. (38), using a preparation (109) that exposed a greater portion of the rat renal papilla, found evidence that blood flowing through DVR lags in attaining osmotic equilibrium with the hypertonic surroundings.

Water uptake by the medullary microcirculation. The foregoing models neglect the exchange of water in the medulla. In reality, water is supplied to the medullary interstitium by reabsorption from the pars recta of the proximal tubule in the outer medulla, the descending thin limb of Henle’s loop in the inner medulla, and the collecting duct. Since the epithelium of the ascending thin and thick limb of Henle’s loop is impermeable to water and there are no lymphatics in the inner medulla and few in the outer medulla, any water added to the interstitium must be removed by AVR. Ullrich et al. (136) derived a model for the countercurrent exchanger similar to Berliner’s except that solute is added along the length of the exchanger instead of being added only at the tip of the loop. The
differential equations are the same (126). In rodents, measurements of hydrostatic pressure in the DVR averaged 17 mmHg (136, 137). Ullrich posulated that this would drive water from DVR, short-circuiting water along with permeable solutes to AVR and concentrating plasma protein in the DVR. In accord with this hypothesis, the ratio of vasa recta plasma to systemic plasma protein was found to be >1, ranging from 1.08 to 1.40. (However, as the authors noted, an alternative explanation for the high plasma protein is ultrafiltration upstream in juxtamedullary glomeruli.) The authors predicted that addition of water in the AVR would reduce plasma protein to its preglomerular capillary value.

Micropuncture of the vasa recta in antidiuretic rats (41, 113, 114) and hamsters (30) near the papillary tip confirmed the findings of Ullrich et al. (136) that the plasma protein concentration at the end of the DVR is elevated above that of the blood entering the medulla. Sanjana and colleagues (113, 114) analyzed the driving forces and transmembrane volume movement. In young rats with a systemic plasma protein concentration of 4.1 g/dl, micropuncture of DVR and AVR at the base of the exposed papilla revealed mean plasma protein concentrations of 7.1 and 5.6 g/dl, respectively. Assuming the vasa recta are impermeable to protein, this finding implies dilution of plasma proteins by fluid uptake between the DVR and AVR, a finding corroborated by Zimmerhackl et al. (155). In contrast, micropuncture of the DVR at the base and tip of the exposed papilla revealed protein concentrations of 5.6 and 6.4 g/dl, respectively, indicating net fluid loss from the DVR. Water uptake in the AVR exceeded water removal from the DVR. The difference accounted for the water added to the medulla from the descending limb of Henle and collecting duct and confirmed mass balance for fluid volume movement in the inner medulla.

After the blood begins to ascend in AVR toward the cortex, the solute concentration in the plasma lags in equilibration with the continuously decreasing axial concentration of interstitial solute. At some point the direction of the transendothelial small solute concentration difference will reverse and the luminal solute concentration will exceed that in the interstitium and, if anything, will augment the Starling forces, favoring water uptake by the capillary. The analysis is complicated, however, by anatomic and structural differences between AVR and DVR. First, AVR outnumber DVR between 2 and 2.5 to 1 (35, 155). The transit time of plasma flow through AVR is thereby increased, which allows more time for equilibration of small solutes between plasma and interstitium. Second, the endothelium of AVR is fenestrated so that reflection coefficients for small solutes are likely to be lower than in DVR, which has a continuous endothelium.

Comparisons of oncotic pressure exerted by plasma protein with hydraulic pressure revealed that volume efflux from the DVR occurred despite an oncotic pressure that exceeds hydraulic pressure. This was in apparent conflict with conventional wisdom with regard to transcapillary forces that determine water movement across the capillary endothelium, as derived by Starling (124)

\[ J_v = L_p[(P_v - P_l) - (\pi_v - \pi_l)] = L_p\Delta P - \Delta\pi \]  

where \( J_v \) is the transmembrane volume flux per unit of membrane surface area, \( L_p \) is the hydraulic conductivity, \( P \) is the hydraulic pressure, \( \pi \) is the oncotic pressure, and the subscripts c and i refer to values in the capillary lumen and interstitium, respectively. As illustrated in the model exchanger (Fig. 4C) and confirmed by Jamison et al. (38), plasma concentrations of “small” (nonprotein) molecules such as NaCl lag in osmotic equilibration with the surrounding interstitium, creating a transendothelial difference in concentration. Sanjana et al. (114) hypothesized that such a gradient of small solutes might provide the additional osmotic driving force required for volume efflux from the DVR. According to nonequilibrium thermodynamics, volume flux across a membrane is defined by the following equations (44)

\[ J_v = L_p(\Delta P - \sum \sigma_i\Delta\pi_i) \]  

where \( \Delta P \) is the transmembrane hydraulic pressure difference, \( \Delta\pi_i \) is the transmembrane oncotic pressure difference due to the \( i \)th solute, and \( \sigma_i \) is the reflection coefficient of the membrane to the \( i \)th solute. The equation states that volume flux occurs in response to a transmembrane hydraulic pressure difference and the sum of the transmembrane oncotic pressures exerted by all solutes that are osmotically active across the membrane. Applying this to the DVR

\[ J_v = L_p(\Delta P - \sigma_{pr}\Delta\pi_{pr} - \sigma_{ss}\Delta\pi_{ss}) \]  

where \( \sigma_{pr} \) and \( \sigma_{ss} \) are the reflection coefficients of the capillary membrane to proteins and small solute, respectively, and \( \Delta\pi_{pr} \) and \( \Delta\pi_{ss} \) are the transmembrane oncotic pressure due to protein and small solutes, respectively. From Van’t Hoff’s law

\[ \Delta\pi_{ss} = RT\Delta C_{ss} \]
abolished. Accessible DVR near the surface of the papilla were perfused with buffers differing in osmolality from the interstitium. Perfusion with solutions made hyperosmotic or hyposmotic to the interstitium, by addition or removal of NaCl, was accompanied by water uptake into the capillary and efflux from the capillary, respectively (85).

Equation 3 describes transport of water across the DVR wall as a whole, simulating it as though it occurs through a single pathway, the hydraulic conductivity of which is \( L_p \). More recently the discovery of water channel proteins, the aquaporins, has provided a long sought after biophysical explanation for selective water permeability of biological membranes (2, 3, 76–78, 103, 108, 115). It is now understood that AQP1 is expressed by DVR endothelia and is the transport pathway across which small hydrophilic solutes such as NaCl and urea drive water flux. As will be discussed in subsequent sections, transport of water across the DVR wall is more rigorously described by simulating parallel pathways. One pathway is the highly selective AQP1 molecule (\( \sigma_{ss} = 1.0 \)) and a parallel pathway that conducts both water movement as well as convective and diffusive flux of small solutes (\( \sigma_{ss} \approx 0 \)) (77, 87, 89, 95, 135). Expression of AQP1 in DVR has been hypothesized to play an important role in the optimization of renal medullary countercurrent exchanger function (87).

The finding that urea transport across the collecting duct in the presence of AVP is much greater than can be explained by diffusion and is reduced by phloretin and urea analogs led to the discovery of a transporter that facilitates urea movement. In the last decade, two families of urea transporters have been identified (5, 17), UTA and UTB. UTA isoforms are present in the collecting duct and descending limb of Henle’s loop (133), and UTB is found in erythrocytes and DVR (8, 80, 97). In subsequent sections, the role of UTB (Fig. 5) and AQP1 (Fig. 6) in the optimization of urinary concentrating ability will also be considered.

**TRANSPORT PROPERTIES—GENERAL DEFINITIONS**

To understand the physiology of microvascular exchange in the renal medulla and review the associated literature, one must grapple with a few of the fundamentals of membrane transport theory. As described above in association with Equations 1–4, DVR equilibrates with the medullary interstitium by passive transport of solutes and water through a variety of pathways. In this section, we provide definitions of key parameters that define the properties of those pathways. Measurement of those parameters has been the goal of many studies (Tables 1 and 2). Some description of the essentials is provided in the Appendix and the reader is directed to authoritative sources (4, 19, 26, 44, 69, 71, 102, 120, 144).

Water and solutes permeate the walls of microvessels, including DVR, by passive convection and diffusion, driven by gradients of potential energy provided by transmural differences in hydrostatic and osmotic pressure (19, 69, 71). Quantitative analysis of passive transport is based on nonequilibrium thermodynamics, which states that fluxes through a membrane are proportional to driving forces if they are small enough and if the system is not too far from equilibrium (19, 44, 120). Permeability coefficients quantify relationships between transmembrane fluxes and forces or between different fluxes. Hydraulic permeability (\( L_p \)) relates the total flux of solvent (water) plus solute through a membrane (volume flux, \( J_w \)) to the difference in hydrostatic pressure between the two sides of the membrane (\( \Delta P \)). \( L_p \) equals \( J_w/\Delta P \), when the transmembrane dif-
and $P_f$ are related; $P_f = (L_p \cdot V_w)/(RT)$, where $V_w$ is the partial molal volume of water.

Diffusional permeability to a solute ($P_s$) relates the net molar flux of solute through a membrane ($J_s$) to the transmural concentration difference, $\Delta C$, when transmural volume flux ($J_v$) and therefore convective solvent drag is zero. Under these conditions, $P_s$ simply equals $(J_s/\Delta C)$ and can be viewed as the "resistance" of the membrane to diffusion of the solute. Transport of solute across a membrane can have both diffusional and convective components the directions of which need not be the same. Equations that describe this more complex scenario are provided in the APPENDIX.

Osmotic reflection coefficient ($\sigma_d$) is a property of a membranous pathway that describes the selectivity of the pathway for solvent vs. solute. $\sigma_d$ Can take on values between zero and one. $\sigma_d$ Is one for a semipermeable membrane that sieves or "reflects" all solute from solution, but zero for a nonelective membrane that does not distinguish between solute and solvent. An ultrafiltration coefficient ($\sigma_i$) is the ratio of the convective solute flux reflected at a membrane to that carried through the membrane, given by $[1 - (J_s/J_v \cdot C_1)]$, where $C_1$ is the solute concentration at the upstream surface of the membrane and $\Delta C$ is zero. For practical purposes in physiological dilute solutions, $\sigma_d$ and $\sigma_i$ are equal. The equality is, however, only approximate for nonideal solutes, such as albumin (19, 69). As illustrated by EQUATIONS 2 and 3, when the reflection coefficient to the $i^{th}$ solute ($\sigma_i$) < 1.0, a transmural gradient of the solute will exert less than its total ideal osmotic driving force for water movement. When $\sigma_i$ = 1, solvent traversing the membrane will be rendered solute free at the downstream membrane surface (complete sieving). Conversely, when $\sigma_i$ = 0, movement of water across the membrane carries solute freely, without restriction.

**TRANSPORT OF WATER THROUGH THE DVR WALL**

Water moves through the walls of DVR via pathways of at least two kinds (77, 89, 90, 135). Analysis of the permeabilities of DVR indicates that a "shared" transmural pathway for water and hydrophilic solutes exists in parallel with a "water only" pathway ($\sigma \approx 1.0$) that excludes hydrophilic solutes.

**Shared pathway.** Evidence for a shared pathway conducting diffusion of hydrophilic solutes through the walls of DVR comes from measurements of the correlations between the diffusional permeabilities of these microvessels to $^{22}$Na ($P_{Na}$) and to tritiated water ($P_D$), $^{36}$Cl, $[3H]$rafﬁnose ($P_{raf}$), $[14]$C]urea, and $[14]$C]inulin (90). The simplest interpretation of correlated variations in diffusional permeability to hydrophilic solutes is that they arise from variations in a shared aqueous (porous) pathway. Simultaneous measurement of permeability to two solutes was obtained by perfusing DVR in vitro with pairs of radioactive tracers and calculating both permeabilities (e.g., $P_{Na}$ and $P_{raf}$) from lumen-to-bath efflux using dual isotope detection methods in the perfusate and collectate (Figs. 5–8, 9A, B, C).

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**Fig. 6. Osmotic water permeability** ($P_f$) of OMDVR. A: $P_f$ was measured in glutaraldehyde fixed rat OMDVR by measuring transmural water flux generated by imposing a bath-to-lumen gradient of NaCl. Sequential measurements in controls revealed stability, whereas exposure to p-chloromercuribenzene sulfonate (pCMBS, 2 mM) reduced $P_f$ to nearly zero. Glutaraldehyde fixation was necessary to prevent deterioration of the vessel caused by either the large osmotic gradient or prolonged pCMBS exposure. B: $P_f$ was measured in AQP1 null (−/−) or replete (+/+) murine OMDVR by imposing bath-to-lumen gradients of NaCl, urea, glucose, or rafﬁnose. Deletion of AQP1 reduced DVR $P_f$, measured by driving water flux with NaCl, from a control value of ~1,100 μm/s to nearly zero. Water flux driven by rafﬁnose (molecular wt 564) was markedly reduced in the AQP1(−/−) vessels, compared with AQP1(+/+) vessels, but remained unexpectedly high in the former. Similarly, glucose (molecular wt 180) and urea (molecular wt 60) gradients drove measurable water flux across AQP1(−/−) DVR. See Water uptake by the medullary microcirculation for further discussion. [From Pallone et al. (87).]
From Fick's first law (see APPENDIX), the diffusional permeability of a porous membrane to a solute ($P_s$) is given by

$$P_s = D_p \phi (A_p/\Delta x)$$

where $D_p$ represents the diffusion coefficient of the solute inside the membrane pores, the partition coefficient ($\phi$) is the equilibrium ratio of solute concentration in a pore to that in bulk solution, and $A_p$ is the fraction of the membrane area occupied by pores and $\Delta x$ is pore length (19, 66). Using Equation 5, it can be predicted that, for diffusion of small hydrophilic solutes in large pores, the ratio of the permeabilities to those solutes should be equal to the ratio of their diffusion coefficients in bulk solution. This was verified in DVR for several pairs of tracers (Table 2) (90). It was experimentally possible to demonstrate the correlations in rat DVR because the permeability of individual vessels varies. The variation in $P_s$ between DVR is probably attributable to variations in $A_p/\Delta x$, because the shared pathway in these microvessels does not significantly sieve small hydrophilic solutes (see below).

In contrast to AQP1-mediated water transport (Fig. 6), the shared pathway also conducts most of the transmural convection ($J_v$) driven by oncotic pressure differences across the walls of DVR (135). Evidence for this comes from paired estimates of $J_v$ and $P_{raf}$ in isolated DVR perfused with a high molecular weight fluorescent volume marker plus $[^3 \text{H}]$raffinose and intermittently exposed to high concentrations of albumin (135). The product of hydraulic conductivity and reflection coefficient to albumin ($L_p \phi_{alb}$) was calculated from volume flux ($J_v$) driven by the known transmural osmotic pressure difference provided by albumin ($\Delta \pi_{alb}$) when $\Delta P$ was negligible. $L_p \phi_{alb}$ correlates with $P_{raf}$ in DVR, with an intercept close to zero (Fig. 9), indicating that most $J_v$ driven by $\Delta \pi_{alb}$ goes through the shared pathway. The shared pathway is insensitive to mercurial compounds, unlike the exclusive water pathways described below, because $p$-chloromercuribenzenesulfonate (pCMBS; see Fig. 6) does not change $L_p \phi_{alb}$ in

![Fig. 7. Perfusion rate dependence of permeability measurements. $[^3 \text{H}]$raffinose permeability (A) and $[^{14} \text{C}]$inulin permeability (B) are shown as a function of perfusion rate of in vitro isolated, perfused DVR from outer medullary vascular bundles of the rat. Change in permeability is rapidly reversible upon lowering the perfusion rate (data not shown). Heavy line connects mean ± SE of individual points.](http://ajpregu.physiology.org/)

![Fig. 8. Correlations of solute permeability in OMDVR. A: dual isotope perfusions of DVR were performed with $^{22} \text{Na}$ and either $^{36} \text{Cl}$ or $[^3 \text{H}]$raffinose to measure simultaneous permeability. Permeability to $^{36} \text{Cl}$ or $[^3 \text{H}]$raffinose (ordinate) is highly correlated with that to $^{22} \text{Na}$ (abscissa). Strong correlation and zero intercept are consistent with permeation of these solutes via a shared pathway. B: dual isotope perfusions of DVR were performed with $^2 \text{H}_2 \text{O}$ and $^{22} \text{Na}$. Permeability to these isotopes is correlated but the intercept is nonzero. The latter finding is consistent with the interpretation that $^2 \text{H}_2 \text{O}$ permeates the DVR wall through a pathway shared with $^{22} \text{Na}$ and through an additional independent pathway, likely AQP1.](http://ajpregu.physiology.org/)
across in vitro perfused OMDVR with hyperoncotic albumin. Diffu-

table 2. Permeability of vasa recta to
hydrophilic solutes

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<tr>
<th>Permeability × 10−6 cm/s</th>
<th>Species</th>
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<th>IMDVR</th>
<th>IMAVR</th>
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Permeability Ratio

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<th>IMDVR</th>
<th>IMAVR</th>
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Table 3. Hydraulic conductivity, osmotic water permeability, and reflection coefficients

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<th>OMDVR</th>
<th>IMDVR</th>
<th>IMAVR</th>
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<td>Hydraulic pressure</td>
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<td>64,85</td>
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</table>

Glutaraldehyde-fixed DVR (89). Fixation of DVR by brief exposure to glutaraldehyde prevents damage by mercurials or hyperosmolar solutions and does not change permeabilities (77, 87, 89).

Flow appears to increase the hydraulic and diffusional permeabilities of the shared pathway in DVR by a mechanism that is unknown in these microvessels (Fig. 7). Lp, αNa, Praf, and PNa correlate with perfusion rate in isolated DVR, but the ultrafiltration coefficient for albumin does not (89, 90, 135). Mean Lp values reported for DVR are >1.4×10−6 cm·s−1·mmHg−1 in vivo (96) and 1.56×10−6 cm·s−1·mmHg−1 in vitro (if αNa is 1) (135) (Table 3).

Molecular sieving by the shared pathway in DVR appears to be slight for small hydrophilic solutes but has not been systematically investigated. The shared pathway in rat DVR apparently offers little restriction to diffusion or entry of hydrophilic solutes up to the size of inulin, because diffusional permeability ratios for pairs of these solutes resemble corresponding ratios of free diffusion coefficients (D) (90). If solutes enter the shared pathway with little restriction (φ close to 1, Equation 5), then this implies that their solutions undergo little ultrafiltration and exert only small fractions of their total osmotic pressures across this pathway. Mathematical modeling indicates that osmotic reflection and ultrafiltration coefficients are ~1−φ2 for porous membranes or for fibrous networks (such as the glyocalyx lining microvessels) and therefore are small if φ is close to one (19, 29, 69, 71). Osmotic reflection coefficients for NaCl solutions at the walls of rat DVR are indeed small, estimates being 0.032 in vitro (135) and <0.05 in vivo (85). These values are calculated from the relative abilities of NaCl and albumin solutions to drive transmural volume flux in unfixed DVR, assuming that αNa is one. They are probably overestimates of the osmotic reflection coefficient of NaCl (αNa) at the shared pathway, because they describe molecular sieving by whole DVR, which occurs at highly selective exclusive water pathways as well as at the shared pathway (see below and Appendix). In fact, transport of water across the DVR wall may be better...
described by simulating parallel transport through the shared pathway \((\sigma_s \approx 0)\) and water channels \((\sigma_s \approx 1.0)\).

Molecular sieving by a shared pathway in DVR is poorly defined even in microvessels from AQP1 knockout mice, which retain only minimal exclusive water pathways (87). Osmosis drives volume efflux from these DVR through a mercurial-insensitive pathway, which appears to show increased sieving of progressively larger hydrophilic solutes. Hyperosmolar solutions of NaCl are ineffective, but urea, glucose, and raffinose are increasingly able to drive volume efflux from unfixed and fixed AQP1 knockout DVR (Fig. 6B). PCMBS does not inhibit raffinose-driven volume efflux from these microvessels. Similarly, AQP1 knockout DVR apparently do not ultrafilter luminal \(^{22}\)Na, but do retain some \(^3\)H-raffinose and \(^{14}\)C-inulin during volume efflux driven by hyperosmolar raffinose. Unfortunately, mathematical simulations of ultrafiltration and transmural diffusion along mouse DVR do not yield reliable estimates of osmotic reflection or ultrafiltration coefficients for small hydrophilic solutes because of the high diffusional permeabilities of mouse DVR to these tracers (87). Hence, the appearance of size-dependent molecular sieving by AQP1 knockout DVR, although attributable to a shared pathway, is equally consistent with complete sieving at remaining exclusive water pathways, combined with slower transmural diffusion of larger solutes.

In contrast with small hydrophilic solutes, the macromolecule albumin undergoes considerable sieving at the shared pathway in DVR. The mean ultrafiltration coefficient of albumin solutions at whole DVR is 0.89 (and not significantly different from 1), according to retention of fluorescently labeled perfusate albumin during volume efflux driven by unlabeled albumin (135). This probably is close to the ultrafiltration coefficient of albumin solutions at the shared pathway, because this pathway dominates the \(L_p\) of DVR.

**Exclusive water pathways.** Water apparently diffuses through the walls of DVR via pathways that exclude hydrophilic solutes, as well as via pathways, because the permeability to tritiated water (\(P_D\)) of these microvessels is always high, even when \(P_{Na}\) is low (90) (Fig. 8B).

Exclusive water pathways also conduct most of the convection driven through the walls of DVR by transmural osmotic pressure gradients due to small hydrophilic solutes (85, 89). Evidence for this is that mathematical simulations indicate that small hydrophilic solutes are mainly driven through the walls of DVR during volume efflux driven by hyperosmolar NaCl, most accurately predict the observed retention of \(^{22}\)Na and \(^3\)H-raffinose when ultrafiltration and osmotic reflection coefficients are assumed to be one at the convective pathway (135) (Fig. 10).

The walls of DVR therefore seem to contain exclusive water pathways in parallel with a shared pathway that sieves small hydrophilic solutes poorly and macromolecules well. For parallel pathways (19), the volume flux driven through the walls of DVR \((J_v)\) by transmural differences in concentration of small hydrophilic solutes \((\Delta c_{os})\) can be described by

\[
J_v = P_{fa} \sigma_{fa} V_w \Delta C_{fa} = (P_{fa} A_w \sigma_{fa,w} + P_{fw} A_w \sigma_{fw,w}) V_w \Delta C_{fa} \quad (6)
\]

where \(V_w\) is the partial molar volume of water, \(\sigma_{fa}\) is the osmotic reflection coefficient for a small hydrophilic solute, \(A\) is the fractional area of a pathway, and the subscripts \(a, w,\) and \(p\) denote values for the whole microvessel for exclusive water and for shared pathways, respectively, so that

\[
P_{fa} = P_{fw} A_w + P_{fw} A_w \quad (7)
\]

Mean osmotic permeabilities of exclusive water pathways \((P_{fw} A_w)\) in unfixed and fixed DVR from rats are between 900 and 1,300 \(\mu\)m/s (89, 135). These esti-
mutes of $P_{f,w}A_w$ ("apparent $P_f"$) of DVR are calculated from $J_v$ driven by $\Delta C_{ss}$ due to NaCl, because of the evidence that osmotic reflection coefficient of NaCl is one at the exclusive water pathway ($\sigma_{ss,w}$) but low at the shared pathway ($\sigma_{ss,p}$). This evidence implies that NaCl drives volume flux mainly through the exclusive water pathway, because it exerts little effective osmotic pressure across the shared pathway. From Equation 6, $J_v \approx P_{f,w}A_w \Delta C_{ss}$ if $\sigma_{ss,p} \approx 0$. Flow apparently does not modulate $P_{f,w}A_w$ in glutaraldehyde-fixed DVR (89), as it does $P_{f,a}$ in the unfixed microvessels (135) or $P_{f,a}$ in unfixed (90) or fixed (89) DVR.

The osmotic permeability of exclusive water pathways in DVR ($P_{f,w}A_w$) is one order of magnitude lower than that of the shared pathway ($P_{f,a}A_p$) (Table 3). This follows because an osmotic permeability for whole DVR ($P_f$) of 16,700 $\mu$m/s can be calculated from the mean $L_p$ of $1.56 \times 10^{-6}$ cm $\cdot$ s $^{-1}$ $\cdot$ mmHg $^{-1}$ for microvessels in vitro (89, 135). These permeabilities are of the shared plus exclusive water pathways in DVR, because this $L_p$ is calculated from $J_v$ driven by albumin solutions, which apparently have osmotic reflection coefficients close to one at both pathways.

The low osmotic reflection coefficient of NaCl solutions at whole DVR (85, 135) is consistent with the osmotic permeabilities and molecular sieving properties attributed to these shared and exclusive water pathways. For parallel pathways (19), from Equations 6 and 7

$$\sigma_{ss,a} = (P_{f,a}A_p \sigma_{ss,p} + P_{f,w}A_w \sigma_{ss,w})/P_{f,a} \quad (8)$$

This predicts that $\sigma_{ss,a} \approx 0.06$, if $\sigma_{ss,p}$ is zero, $\sigma_{ss,w}$ is one, $P_{f,w}A_w$ is 1,000 $\mu$m/s, and $P_{f,a}$ is 16,700 $\mu$m/s, which agrees reasonably well with experimental values for $\sigma_{ss,a} < 0.05$ for NaCl solutions (Table 3) (85, 135). Note that $\sigma_{ss,a}$ (Equation 8) is the same as $\sigma_{ss}$ in Equation 3 as originally applied to the DVR wall by Sanjana et al. (114).

Identification of exclusive water pathways in DVR begins with the observation that they are mercurial sensitive, unlike the shared pathway. pCMBS strongly inhibits volume efflux driven by hyperosmolar NaCl from fixed DVR from rats (89) (Fig. 6A) and significantly reduces $P_D$ without changing $P_{f,a}$ (77). Similarly, pCMBS abolishes volume efflux driven by NaCl from wild-type mouse DVR and reduces that driven by raffinose (87). This suggests that aquaporins form a transcellular exclusive water pathway in DVR, although mercurials do not block all (2) or only (80) aquaporins.

AQ1 is highly selective for water, mercurial sensitive (2, 3), and expressed by DVR (77, 78) in sufficient quantity to account for exclusive water pathways (89). Polyclonal antibodies to AQ1 label the plasma membranes (including caveolae) of the continuous endothelium of DVR in the inner medulla of rat kidney, but not the surrounding pericytes or the fenestrated endothelium of AVR (77). The AQ1 content of rat DVR, measured by enzyme-linked immunosorbent assay, predicts an osmotic permeability of 1,344 $\mu$m/s, if AQ1 is equally distributed between luminal and abluminal endothelial plasma membranes in series (89). This is close to measured osmotic permeabilities for exclusive water pathways ($P_{f,w}A_w$) in rat DVR of between 900 and 1,300 $\mu$m/s (89, 135). Deletion of AQ1 in knockout mice eliminates nearly all volume efflux driven by hyperosmolar NaCl from fixed DVR, reducing $P_{f,w}A_w$ by $\sim$50-fold in homozygous animals and by about twofold in heterozygous animals (Fig. 6B) (87).

Urea transporters in the DVR wall. The distribution and permeabilities of UTB urea transporters indicate that these proteins may conduct urea through the walls of outer and perhaps inner medullary DVR. Outer medullary DVR express urea transporters, because apparent $P_{urea}$ is high, even in isolated microvessels with low $P_{f,a}$ and therefore a poorly permeable shared pathway (90, 97) (Fig. 5A). Also, thiourea, methylurea, phloretin, or pCMBS inhibits the apparent $P_{urea}$ of outer medullary DVR in vitro (Fig. 5B, Table 2) (80, 90, 97). Thiourea at maximal inhibitory concentration (80) reduces the $P_{urea}$ of outer medullary DVR to reveal a strong correlation with $P_{f,a}$ attributable to the shared aqueous transmural pathway (80, 97). Inner medullary DVR may lack functional urea transporters, because they show a close correlation between estimated $P_{urea}$ and $P_{f,a}$ in vivo, unaffected by thiourea or phloretin, with an intercept near to zero (97). This evidence is not conclusive, because unstirred layers in the renal interstitium might obscure the $P_{urea}$ and $P_{f,a}$ of inner medullary DVR in vivo, but this seems unlikely (85, 90, 97). Antibodies to UTB label the continuous endothelium of rat DVR, most strongly in the outer medulla, but not pericytes or the fenestrated endothelium of AVR (47, 131, 148).

Some urea transporters can increase the osmotic permeability of cells, according to swelling or shrinkage experiments (122, 150, 151), although perhaps only at unphysiologically high levels of expression (122). Nevertheless, UTB expressed in Xenopus oocytes apparently has a single channel osmotic permeability approach that of AQ1, with ultrafiltration and osmotic reflection coefficients for urea of $\sim$0.3 (151). UTB also mediates an appreciable osmotic permeability in erythrocytes, according to data from knockout mice lacking UTB and/or AQ1 (150). The osmotic permeability conferred by UTB is insensitive to mercurials, but inhibited by methylurea (151) or phloretin (150).

These reports indicate that UTB might form a shared pathway for water and some small hydrophilic solutes in DVR, but the quantitative importance of such a pathway is unclear. Whether or not water movement through other nonaquaporin membrane proteins (60) contributes to transmural fluxes in DVR is unknown.

TRANSPORT OF WATER IN OTHER CONTINUOUS MICROVESSELS

The shared and exclusive water pathways of DVR apparently resemble those already described in other microvessels with continuous endothelium, although DVR have high $L_p$ values for continuous microvessels.
(1, 19, 21, 29, 69, 71). Shared aqueous transmural pathways account for the correlation between \( L_p \) and \( P_s \) to small hydrophilic solutes seen across different microvascular beds, despite large variations in absolute permeabilities. Continuous microvessels sieve small hydrophilic solutes, showing smaller ratios of diffusional permeability to free diffusion coefficient (\( P_s/D \)) for tracers of larger molecular radius, indicating greater restriction of diffusion or entry by the shared pathway. Continuous microvessels also sieve macromolecules, displaying high and uniform ultrafiltration or osmotic reflection coefficients, despite wide variations in \( L_p \) and \( P_s \) to small solutes. The shared pathways in continuous microvessels appear to be extracellular routes through interendothelial clefts containing interrupted strands of tight junction and covered by glyocalyx, which acts principally as a macromolecular filter. The evidence for this comes from quantitative studies of electron-dense tracers, correlations between ultrastructure and permeabilities, and mathematical modeling of pathways. Flow increases diffusional permeabilities in several microvascular preparations, by a mechanism involving nitric oxide synthase in mammalian tissue (20, 71).

Exclusive water pathways seem to mediate <10\% of \( L_p \) in most continuous microvessels (21, 71). Their existence emerged from comparisons of \( P_s \) and \( P_{Na} \), and from analyses of sieving of small hydrophilic solutes. These exclusive water pathways are probably transcellular, mediated as in DVR by AQP1, which is abundant in continuous microvessels of many tissues (33, 78, 138). Nevertheless, exclusive water pathways might include routes through the tight regions of interendothelial junctions. UTB-mediated transport of urea, and therefore probably of water, seems insignificant in most microvascular beds examined (71), although UTB is widely expressed in vascular endothelium (139).

### EQUILIBRATION OF NaCl AND UREA ACROSS THE DVR WALL

As blood flows from the corticomedullary junction toward the papillary tip, rising interstitial concentrations of NaCl and urea are encountered. Those solutes equilibrate with the DVR lumen; however, the lag creates transmural gradients so that interstitial NaCl and urea concentrations exceed their respective concentrations in DVR plasma. Two processes contribute to equilibration. One is diffusion. Diffusive influx of NaCl and urea occurs through an aqueous pathway that provides minimal steric restriction to diffusion or convection of low molecular weight hydrophilic solutes. Diffusive influx of urea also occurs via a transcellular route that employs a facilitated carrier (Fig. 5) (80, 90, 97, 134). Molecular sieving by AQP1 is the second mechanism that concentrates NaCl and urea in the DVR lumen. Driven by transendothelial NaCl and urea gradients, water flows via AQP1 channels from the DVR lumen to the medullary interstitium (76–78, 85, 87, 89, 95) (Fig. 6). The conformation of AQP1 is such that water movement through its central pore excludes NaCl and urea so that transcellular water efflux concentrates NaCl and urea within the DVR lumen.

Quantification of diffusive permeabilities of the DVR wall to NaCl has been achieved by measurement of the rate of efflux of \(^{22}\text{Na}\) and \(^{14}\text{C}\)urea from microperfused vessels. Those experiments have been performed both in vivo on the surface of the exposed papilla (Fig. 5A) (67, 75, 83, 85, 96, 97) and in vitro in microperfused DVR isolated from outer medullary vascular bundles (80, 87, 89, 90, 95, 97, 135). A summary of reported permeability measurements is provided in Table 2. Some limitations of the methods employed to obtain the values should be noted. In vivo perfusion can lead to underestimation of permeabilities if the rate of diffusion of the isotopes away from the vessel in the surrounding interstitium is too low. In that case, \(^{22}\text{Na}\) or \(^{14}\text{C}\)urea concentrations on the abluminal surface accumulate, violating the assumption of zero abluminal concentration. In vitro perfusion, due to the presence of a continuously flowing bath, is less likely to yield errors from such boundary layer effects, but necessitates the trauma of isolation and exposes the vessel to artificial buffers that could alter transport properties.

As shown in Fig. 7, \(^{3}\text{H}\)raffinose and \(^{14}\text{C}\)inulin permeability of in vitro perfused outer medullary DVR is strongly dependent on perfusion rate (90). Similar behavior is observed when permeability to \(^{22}\text{Na}\) is examined. Whether this rate dependence exists in vivo has important implications for the manner in which these vessels equilibrate. For example, if the true NaCl permeability is very low, then abstraction of water across AQP1 might be the dominant mode of NaCl equilibration. As discussed below, that mode of equilibration may reduce blood flow to the deeper regions of the medulla and thereby enhance interstitial osmolality near the papillary tip (87).

Measurement of \(^{14}\text{C}\)urea efflux yields a permeability value that is the sum of transport via the transcellular, carrier-mediated route and other, e.g., pericellu- lar, pathways (80, 90, 97). When the urea carrier in the DVR wall is inhibited by phloretin, pCMBS, or structural urea analogs (thiourea, methylurea), the measured urea permeability falls (Fig. 5B, Table 2) (80, 90). Histochemical evidence and in situ hybridization have identified the DVR urea transporter as the same as that expressed by the RBC-UTB. This carrier is distinct from the vasopressin-stimulated cAMP-responsive transporter of the collecting tubule and the related isoforms found in thin descending limb of Henle (UTA1, UTA2, etc.) (5, 8, 25, 36, 47, 48, 104, 105, 110–112, 131–134, 139, 148–151).

### TRANSPORT OF SOLUTES AND WATER ACROSS THE AVR WALL

Blood ascending toward the cortex in AVR encounters decreasing NaCl and urea concentrations so that the lag in equilibration results in luminal osmolality that exceeds that in the adjacent interstitium. The transmural NaCl and urea gradients generated in vivo
are likely to be small. This is because AVR are larger and more numerous than DVR so that the combination of high permeability, large surface area and slower blood flow favors a high degree of equilibrium between AVR plasma and interstitium (35, 39, 67, 91, 155). AVR solute permeability has not been as thoroughly evaluated as that in DVR because AVR have not been isolated and perfused in vitro, owing to technical difficulties. Transport properties have been measured only by the difficult approach of in vivo microperfusion of vessels on the surface of the exposed papilla of rats and hamsters (Table 2) (67, 75, 81, 83). The values so obtained exceed DVR permeabilities but, even so, are probably underestimated. As discussed above, the underestimation is likely because the $^{22}\text{Na}$ and $[^{14}\text{C}]$urea tracers in the perfusate might cross the AVR wall and accumulate in the interstitium to significant levels.

As required for overall mass balance in the medulla, AVR must remove the water deposited to the interstitium by nephrons, collecting ducts, and DVR. Transmural oncotic pressure gradients favor water uptake across the AVR wall and AVR hydraulic conductivity is very high (Table 3) (64, 82, 98, 113, 114). In vivo, transmural gradients in the AVR generated by the osmotic lag between blood and interstitium are directed to favor water uptake (luminal concentration > interstitial concentration). For AVR water uptake to be augmented by those gradients, the AVR wall would have to have nonzero reflection coefficients to NaCl and/or urea and transmural gradients of those solutes would need to be of significant magnitude. In the AVR, rigorous measurements of NaCl and urea reflection coefficients have been technically impossible to obtain but the general hypothesis that NaCl might augment transmural volume flux has been tested. In contrast to similar experiments in DVR (85), in vivo microperfusion of AVR with buffers made hypertonic or hypotonic to the papillary interstitium yielded no measurable transport across the AVR wall. Theoretical analysis of macromolecules has been considered in several studies (64, 86, 141, 153). The variation in the data from those experiments was large so that low levels of small solute-driven AVR water transport would not have been readily detected (83).

**TRANSPORT OF UREA AND WATER ACROSS THE PLASMA MEMBRANE OF RED BLOOD CELLS**

In addition to DVR and AVR, red blood cells (RBCs) participate in the medullary recycling of solute and water. The RBC membrane and DVR endothelia express the same transporters for water (AQP1) and urea (UTB) (8, 104, 105, 131–134, 139, 148–151). Thus urea returning from the inner medulla in AVR plasma and RBC cytoplasm has high conductivity transcellular pathways across which it can be returned to the medullary interstitium. Using mathematical simulations, Macey and Yousef (62) concluded that the RBC urea carrier might protect the RBC from osmolar lysis.

**TRANSPORT OF LARGE MOLECULES BY THE MEDULLARY MICROCIRCULATION**

It is generally accepted that the renal medulla is devoid of lymphatics (11, 22). As such, the interstitial space must be cleared of large molecules by some other mechanism. Logically, that mechanism must be solvent drag across the AVR wall. Water continuously deposited by loops of Henle, collecting ducts, and DVR must be taken up into AVR (63–65, 86, 141, 153). That water movement will also carry albumin and other large molecules to the AVR wall where they could potentially be carried by convection into the AVR lumen. The related transport issues are complex, because, depending on the “pore” structure of the AVR wall, molecular sieving would tend to limit such permeation and secondarily concentrate macromolecules within the interstitium. Hypothetically, the tendency toward high interstitial oncotic pressure could be offset if deposition of water to the interstitium by nephrons and DVR dilutes macromolecules and raises hydraulic pressure enough to drive water flux across the AVR wall. It has been predicted that a small net transmural driving force would suffice to accommodate mass balance because the AVR wall has a very high hydraulic conductivity (Table 3) (63–65, 70, 81, 82). Commensurate with this notion, AVR tolerate reduction of luminal hydraulic pressure without collapsing (65). With respect to transport of macromolecules, the reflection coefficient of the AVR to albumin has been measured by different methods. Values of 0.59–0.72 and an average of 0.78 were obtained by MacPhee and Michel (64) and Pallone (81), respectively (Table 3). Those nonzero values predict significant sieving of albumin by the AVR wall. Theoretical analysis of macromolecular trafficking has been considered in several studies (64, 86, 141, 153).

**INSIGHTS FROM MODELING: THE ROLE OF AQPI TO ENHANCE EXCHANGER EFFICIENCY**

Mathematical models of the urinary concentration have played an important role in the evolution of our understanding (31, 34, 56, 74, 125–130, 136, 143). The daunting complexity involved in the simulation of both nephrons and the microcirculation has often led investigators to focus on one while neglecting the other. In the case of the vasa recta, there have been many mathematical models of microvascular exchange in the renal medulla. These have been motivated by the desire to simulate countercurrent exchange and account for low oxygen tensions within the medulla (136, 152). The earliest efforts simulated the vasa recta as limbs of a countercurrent exchanger that equilibrates by diffusion either across a simple membrane or via an interposed interstitial space. Those efforts have been previously summarized and will not be reviewed here (91). Instead, we will draw attention to more recent efforts and the insights derived from them.

Most models that focus on vasa recta have specified corticomedullary solute concentration profiles as inputs; however, Wang and Michel (140, 141) recognized...
that it is more realistic to specify the rate of deposition of NaCl, urea, and water to the medullary interstitium. Mathematically this introduces generation terms into the governing differential equations that account for interstitial mass balance. Their elegant models maintained sufficient simplicity to avoid the need for numerical integration. When shunting of blood flow from DVR to AVR with medullary depth was included as a feature, an exponential increase in corticomedullary solute concentration was predicted, a result that agrees with prior electron probe measurements (50, 140, 142). A weakness of all such models is that they neglect simulation of loops of Henle and collecting ducts (23, 24, 140–142). Solute generation rates in the interstitium are assigned as inputs to the model and interstitial solute concentrations are calculated predictions. Thus variations of transport properties of the microvessels cannot affect the interstitial appearance of NaCl, urea, and water as would occur in vivo. Convincing evidence has been provided that structure and properties of nephrons can abruptly vary with medullary depth (100). The effects of such variation cannot be accounted for by models that focus solely on the microcirculation.

Many of the key parameters needed for a detailed theoretical model to describe microvascular exchange in the renal medulla (solute permeabilities, reflection coefficients, hydraulic conductivities) have been experimentally measured over the last two decades. That information has been combined with anatomic detail and recent insights concerning transcellular pathways for urea and water transport in DVR endothelia to enable increasingly realistic simulations by mathematical modeling. Recent work incorporated a description of AQP1- and UTB-mediated urea and water transport into an evaluation of microvascular exchange in the renal medulla by accounting for parallel pathways for transport of water described above. Following the lead of Wang and colleagues (140, 142), mass balance equations incorporated the rate of introduction of NaCl, urea, and water into the medullary interstitium as a function of corticomedullary axis. As a result, interstitial solute concentrations and the magnitude of corticomedullary gradients were predicted rather than specified as inputs (23, 24, 87). Commensurate with the demonstration that collecting duct urea permeability rises markedly in the inner medulla (111), the rate of generation of urea was assumed to rise exponentially toward the papillary tip. The predictions showed that AQP1 might play an important role to raise medullary interstitial osmolarity by driving water efflux from DVR to the medullary interstitium across AQP1 water channels, effectively shunting DVR plasma volume to the AVR and reducing blood flow to deeper regions of the medulla. The lowering of blood flow favors high exchanger efficiency in the inner medulla where urea is added to the interstitium from the collecting duct. It also reduces the volume of fluid to be concentrated (13, 39). The net effect is optimization of interstitial osmolarity (Fig. 11). Without AQP1, the model predicts that water influx will occur into DVR along its length, driven by Starling forces, with reduction of interstitial osmolarity throughout the medulla. Thus the effect of water channels is to reduce blood flow rate to the inner medulla to improve solute trapping and interstitial osmolarity in that region (87). Interest in this intriguing prediction is heightened by the observation that transmural water flux can be driven across the wall of AQP1 null mice by solutes other than NaCl (Fig. 6). If the non-AQP1 pathway is important in vivo, it might represent another route through which water is shunted from DVR to AVR in the medulla.

**SUMMARY AND FUTURE DIRECTIONS FOR RESEARCH**

The primary lesson from recent studies is that the tendency to conceptualize vasa recta as a diffusive U-tube countercurrent exchanger is a misleading oversimplification. In the case of purely diffusive equilibration between lumen and interstitium, countercurrent exchanger efficiency would be enhanced by high permeability, high surface area, and low flow rate. Each of those factors tends to decrease the transmural gradient generated by osmotic lag with the interstitium and therefore the difference in solute concentration between descending and ascending limbs (Fig. 4). If vasa recta were purely diffusive exchangers, the difference in concentration at the corticomedullary junction would govern the overall rate of solute removal from the medulla. Due to high rates of equilibration, it is
likely that AVR solute concentrations only slightly exceed those in DVR and that the extent to which AVR blood flow rate exceeds DVR blood flow rate actually serves as the major determinant of overall solute removal.

Expression of the UTB urea transporter results in high DVR urea permeability and minimization of transmural urea gradients. In contrast to transport of urea, the permeability of at least some DVR to NaCl is low (Fig. 5A), favoring equilibration of NaCl by AQP1-mediated water efflux. We conclude from this that a tradeoff has probably evolved to optimize urinary concentrating ability. Relatively low DVR NaCl permeability favors shunting of water from DVR to AVR via AQP1, the purpose of which may be to lower blood flow rate toward the papillary tip. When the net outcome is fully analyzed by mathematical simulation, the prediction is that AQP1-mediated shunting of water could enhance medullary interstitial osmolality (Fig. 11) (87). Stated another way, the continuous shunting of water across AQP1 in DVR, driven primarily by transmural gradients of NaCl, might serve to reduce blood flow, thereby both enhancing equilibration near the papillary tip (where the need to preserve steep corticomedullary gradients is greatest) and reducing the overall flow that needs to be concentrated.

It has been technically feasible to isolate outer medullary DVR (OMDVR) by microdissection from vascular bundles. These vessels have been extensively characterized with respect to their solute and water transport properties and vasoactive characteristics. In contrast, inner medullary DVR (IMDVR) have not been so thoroughly evaluated because they cannot be readily dissected from the rat medulla and subjected to in vitro experimentation. Solute permeabilities of IMDVR measured by in vivo microperfusion may have been underestimated (Table 2). Given the low permeabilities measured in some OMDVR (Fig. 5A) when perfused at slow rates, it seems likely that their properties vary as a function of medullary depth. This might be verified if DVR could be isolated from various medullary locations in a species other than the rat.

Countercurrent exchange in the inner medulla and in vascular bundles of the outer medulla has been best characterized through mathematical models and measurements. Exchange within the interbundle region remains the subject of speculation. There have been no studies of the properties of the interbundle capillary plexus. It stands to reason that a highly interconnected plexus (Fig. 2) could not support efficient countercurrent exchange. Lateral solute gradients extending across the inner stripe from vascular bundles to the interbundle region might exist. There have been no attempts to simulate this feature of medullary microvascular exchange, either alone or as part of a larger model of the concentrating mechanism.

Many other issues remain unresolved. One that has received insufficient attention is the possibility that vasa recta transport properties are regulated to optimize urinary concentration. There is evidence that reduction of inner medullary blood flow accompanies hydropenia (27, 28, 91, 93, 154); however, the extent to which this is due to vasoconstriction of juxtapapillary resistance vessels or shunting of water from DVR to AVR via AQP1 is unknown. Hypothetically, a reduction of DVR permeability to NaCl in the hydropenic state would favor the latter, but this is untested. The large variation of permeability measurements obtained by in vitro microperfusion implies that subtle differences between animals maintained in different states of hydropenia or volume depletion would be difficult to resolve.

The flow rate dependence of DVR permeability measurements (Fig. 7) raises two possibilities. One is that, owing to artifacts induced by vessel isolation and perfusion, all in vivo measurements of DVR permeability to hydrophilic solutes have been overestimated (80, 87, 90, 95, 97). If DVR NaCl permeability in vivo is, in fact, generally low, then DVR in vascular bundles equilibrate largely by AQP1-mediated water abstraction (NaCl) and UTB-mediated facilitated diffusion (urea). Variation of permeability with in vitro perfusion rate might not be an artifact. Such flow rate dependence of microvessel permeability has been observed in other microvascular beds and is often nitric oxide dependent (20, 71). If flow rate dependence of DVR NaCl permeability exists in vivo, it would imply that reduction of medullary blood flow during hydropenia is accompanied by a shift of DVR NaCl equilibration from diffusion to AQP1-mediated water abstraction. Given the prediction that the latter favors enhancement of interstitial solute concentrations by reducing blood flow (Fig. 11), it is theoretically enticing that enhancement of DVR permeability with perfusion rate is a true physiological effect.

UTB null mice have a mild urinary concentrating defect, a finding that is consistent with a role for UTB to enhance urea trapping and countercurrent exchange (149). It is known that UTA urea transporter splice variants undergo both short- and long-term regulation (8, 110, 133). Evidence that UTB also undergoes long-term regulation is emerging. Upregulation of mRNA for UTB has been identified during glosis in the central nervous system (12). Within the kidney, lithium-induced diuresis is accompanied by reduced UTB expression in the medulla (48). Interestingly, UTB expression in the renal medulla is downregulated when the vasopressin V2 agonist dDAVP is supplied to vasopressin-deficient Brattleboro rats (104, 105, 132). The latter observation seems counterintuitive, if a role for UTB is to enhance countercurrent exchange and urinary concentration. Functional measurements to confirm variation of DVR urea permeability with antidiuretic state would shed light on this question.

Also, it would be of importance to know whether UTB mediates water efflux across the DVR wall in vivo. When solutes other than NaCl are used to drive water movement, the DVR of AQP1 null mice exhibit a significant osmotic water permeability (Fig. 6), implying that an additional pathway exists. It remains to be tested whether UTB could mediate such small solute driven water movement. Such a hypothesis might be
tested using UTB null mice or selective inhibitors of UTB-mediated water transport. Similarly, the reflection coefficient of the non-AQP1 pathway to small solutes should be determined. Even if that pathway conducts water movement in vivo, low reflection coefficients to small solutes would imply that it concentrates the macromolecules but not NaCl or urea in DVR plasma. The existence of an additional solute other than NaCl and urea has been postulated to accumulate in the renal medulla to provide a driving force for urinary concentration (34, 128–130). If such a solute is identified, its ability to drive water flux across the DVR wall of AQP1 null mice should be tested.

AQP1 seems to be constitutively expressed. AQP1 null mice have a severe urinary concentrating defect, reduction of glomerular filtration rate, and general failure to thrive. Those abnormalities are partially restored by gene replacement via an adenovirus (60, 76); however, the importance of AQP1 to provide a dominant pathway that mediates reabsorption of glomerular filtrate in the proximal nephron is more important and probably overshadows the effect of its deletion from DVR. Isolated deletion of AQP1 from DVR or selective replacement of AQP1 into DVR of AQP1 null mice would be needed to resolve that issue. It has been shown that ureteral obstruction is associated with a global reduction of AQP1 expression in the cortex and medulla, an effect that extends to other aquaporins (46). Similarly, gentamicin toxicity is associated with diminished urinary concentration and decreased expression of AQP1 (57). Thus instances of AQP1 downregulation in disease states have been described, but there is little evidence for short- or long-term regulation in nonpathological states.

In addition to transport-related functions, DVR are vasoactive. In vitro, on abluminal exposure to agents such as angiotensin II, endothelins, vasopressin, and norepinephrine, DVR constrict at various foci along their length (84, 92–94, 99, 123). This might modulate corticomedullary gradients in at least two ways. First, an increase in vascular resistance might reduce blood flow to the inner medulla. Second, alteration of permeability to NaCl and urea or reduction of surface area for exchange might occur. If permeability falls during vasoconstriction, exchanger efficiency would be expected to decline, but that effect might be offset because a secondary increase in the magnitude of transmural gradients that drive water efflux across the DVR wall would result. As discussed above, this would favor increased shunting of water from DVR to AVR via AQP1, an effect that has been predicted to reduce blood flow to the deep medulla and enhance interstitial osmolality (87).

Like that of plasma proteins, trafficking of intermediate molecular weight solutes is unexplored but potentially of great interest. Paracrine agents such as angiotensin II, bradykinin, endothelins, and prostaglandins are generated in the medulla. In the presence of a countercurrent exchanger, their release into the interstitium would result in trapping and establishment of a corticomedullary concentration gradient. The concentration of the paracrine hormone present at any level in the medulla is expected to be a complex function of the rate of generation, the rate of degradation, and the permeability of DVR and AVR walls to the solute in question.

Renomedullary interstitial cells are stacked like “rungs of a ladder” and tethered to inner medullary structures. That arrangement might augment concentrating ability by preventing dissipation of axial NaCl and urea gradients within the interstitium (58). Renomedullary interstitial cells are contractile (37) and the possibility that contraction and relaxation of these cells could alter transport properties or vascular resistance cannot be ruled out. Even if they do not modulate transport functions, it is possible that their stacking isolates sliletike regions of interstitium through which the parallel structures present in the inner medulla must exchange (58, 59, 74, 125–127).

Finally, there is a large literature that points to the importance of contractions of the ureter and renal pelvis in the optimization of urinary concentration (15, 18, 22a, 32, 49a, 79, 88, 107, 116, 117). When the ureter is excised to expose the papilla for micropuncture, urinary concentrating ability falls dramatically. Whether this is due to interruption of urea recycling across the pelvic epithelium, hemodynamic effects on inner medullary blood flow, or release of vasoactive agents (18, 32) has not been resolved. When one observes the papilla through the translucent ureter before ureteral excision, blood flow in individual vasa recta seems pulsatile and, on average, much slower than it appears after excision (personal observations). The latter has not been quantified but an increase in inner medullary blood flow after ureteral excision might conceivably contribute to alteration of exchanger efficiency and reduction of concentrating ability.

**APPENDIX**

In simplest form, volume flux ($J_v$) across a membrane, driven by transmural difference of hydraulic pressure and osmotic pressure of a single solute ($\Delta\pi$) is given by

$$J_v = \frac{L_v(\Delta P - \sigma_\Delta\pi)}{\sigma_d}$$

(A1)

where $\sigma_d$ is the osmotic reflection coefficient of the membrane to the solute. Note that this is a special case of Equation 3 in which only one solute exerts osmotic pressure.

When $J_v$ is zero, then solute flux ($J_s$) is given by applying Fick’s first law of diffusion to a membrane (120)

$$J_s = -D_p\phi\Delta C/\Delta x = -P_s C/\Delta x$$

(A2)

where $D_p$ is the diffusion coefficient of the solute in the membrane, $\phi$ is the partition coefficient (the equilibrium ratio of solute concentration in the membrane to that in bulk solution), and $\Delta x$ is membrane thickness.

When $J_v$ is more than zero, $J_s$ can be described by (19, 69, 102)

$$J_s = J_v(1 - \sigma_d)(C_1 - C_2e^{–\psi})/(1 - e^{–\psi})$$

(A3)

where $C_1$ and $C_2$ are solute concentrations on each side of the membrane and $Pe$ is the Péclet number, which is the ratio of the velocity for a solute imposed by convection to that by diffusion.
Equation A3 is best explored by considering its limits. When \( J_v \to 0 \) and diffusive flux dominates, it is readily shown that \( J_v \to P_v \Delta C \). Conversely, when \( P_v \) is very large, so that convective solute flux (solvent drag) dominates over diffusion, \( J_v \to \sqrt{P_v \Delta C} \).

The volume flux of water \( (J_w) \) through a porous membrane approximates \( J_w \) only in the case of dilute solutions. \( J_w \) can be described by (26)

\[
J_w = P_v V_w \left[ \Delta P/RT - \sigma \Delta C \right]
\]

where \( P_v \) is osmotic permeability and \( V_w \) is the partial molar volume of water. Assuming that \( J_w \approx J_v \), either because \( J_v \) is zero or as an approximation for dilute solutions, then \( P_v \) can be calculated from \( L_p \) (4, 26, 144)

\[
P_v = L_p RT/V_w
\]

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REFERENCES


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