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Renal water reabsorption: A physiologic retrospective in a molecular era

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Renal water reabsorption: A physiologic retrospective in a molecular era. The cloning and sequencing of the aquaporin water channels has been an enormous advance in the biomedical sciences, as recognized by the award of the Nobel Prize to Peter Agre last year. Among many other examples, expression of aquaporin proteins in *Xenopus* oocytes and other heterologous expression systems has confirmed two important models of renal function: the increase in the water permeability of the collecting duct by antidiuretic hormone (ADH), and the mechanism of near isosmotic volume reabsorption by the proximal tubule. These mechanisms were the subjects of intensive investigation by numerous investigators, including Thomas E. Andreoli, who is being honored by this symposium, and who developed many of the key concepts in these areas. His early work with artificial lipid bilayer membranes and the pore-forming antibiotic amphotericin provided the rigorous foundation in experimental and conceptual modeling techniques that he later applied to physiologic and pathophysiologic mechanisms in the kidney, which are summarized in this retrospective. Dr. Andreoli and his colleagues proposed a water channel mechanism for the action of ADH, which has been confirmed by the cloning and heterologous expression of aquaporin-2. They also proposed that volume reabsorption by the proximal tubule depended on a very high hydraulic conductivity and the development of luminal hypotonicity produced by active solute reabsorption. This model has also been confirmed in mice in which aquaporin-1 expression is knocked out, resulting in a low proximal tubule water permeability that exaggerates the development of luminal hypotonicity.

The award of the Nobel Prize to Peter Agre of Johns Hopkins University last year represented the culmination of a decades-long search for the pathways for selective water movement across biologic membranes. We now know the pathways to be members of a family of water channels, or aquaporins, as Agre et al [1] named them. Prior to the landmark cloning and sequencing of aquaporin-1 (AQP1) [2], however, a large body of physiologic and biophysical information about the characteristics of these water channels had been collected, and it provided the essential background that linked a gene product from red blood cells, where AQP1 is heavily expressed, with its physiologic function.

In the 1960s and 1970s physiologists were embracing biophysical methods and computational models for understanding membrane transport processes with the same fervor that they have more recently embraced the methodologies of cell and molecular biology. It was in this era that Dr. Thomas E. Andreoli, who is being honored by this symposium, began his investigations of water channels. His work in this area formed one of the cornerstones of knowledge that led to the functional and molecular identification of the water channels. In this retrospective, I focus on these early observations in the context of what we now know to be the members of aquaporin water channel family.

MEMBRANE PORES

By the end of the 19th century, it had been clearly established that the permeabilities of solutes across the plasma membrane were determined primarily by their lipid solubility, and in the 1930s and 1940s, it became evident that the molecular radius of a solute was also an important determinant of its permeability. Furthermore, the permeabilities of water and solutes of low molecular weight, such as urea, thiourea, methanol, and acetamide were anomalously higher than expected from their lipid solubility, even when their small size was taken into account. These and similar observations led to the "mosaic membrane hypothesis," which envisioned the plasma membrane as expanses of lipid penetrated by aqueous pores that served as a facilitating pathway for solutes with molecular radii smaller than that of the pore aperture [3].

Membrane pores could explain not only the markedly higher permeabilities of small solutes, but also the characteristics of water movement across biologic membranes. The water permeability of a membrane can be measured in two ways. First, isotopes of water (D_2O or THO) can be used as tracers to measure its unidirectional flux. This measurement permits one to calculate a *diffusional water permeability*, $P_{d,w}$. Alternatively, one can impose a

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hydrostatic or osmotic pressure difference across a membrane and measure the bulk flow of water, which permits one to calculate a water flow permeability, P_f. If the mechanisms by which water permeates a membrane are the same for both measurements, $P_{d,w}$ and P_f should be the same, and this is what is found when these permeabilities are measured in unmodified lipid bilayer membranes [4, 5]. In such membranes, irrespective of the external driving force, the movement of water within the membrane involves a solubility-diffusion process, in which the driving force for the water movement is a gradient of water activity. However, in all biologic membranes P_f exceeds $P_{d,w}$, implying that the mechanism of water movement produced by a transmembrane osmotic or hydrostatic pressure difference is not diffusional.

When water moves through aqueous pores that penetrate the bimolecular lipid leaflet of a membrane, as envisioned for the mosaic membrane, hydrostatic or osmotic pressure gradients across the membrane produce a convective or "bulk" flow in which assemblies of water molecules move in concert, rather than by independent Brownian movement, which underlies the diffusional movement of water or its tracer analogues. For this reason, P_f exceeds $P_{d,w}$ for a porous membrane. When the pores in the membrane are homogenous and have a right circular cylindrical bore of radius r, the $P_f/P_{d,w}$ ratio is proportional to r^2 [3].

Because the differences between P_f and $P_{d,w}$ typically measured in biologic membranes were rather large, the predicted pore radius indicated that small solutes should also permeate the pore. Not only would this account for the anonymously high permeabilities for these solutes, but, because these solutes could interact with water molecules within the pore, the osmotic pressure they produced would be less than that predicted by the van't Hoff equation. Solutes that traverse a membrane by a pore can be characterized by a reflection coefficient (σ), which is defined as the ratio of the observed to the theoretical osmotic pressure produced by that solute. Solutes that are too large to enter a pore have a reflection coefficient of 1.0 (i.e., they do not interact with water within the membrane and they exert the full osmotic pressure predicted by the van't Hoff relationship). Conversely, solutes that enter the pore have reflection coefficients that are less than 1.0 and vary in proportion to the molecular size.

HOLES IN BLACK LIPID MEMBRANES

One approach to understanding the molecular characteristics of pores and other permeation pathways in biologic membranes was to isolate transporters and study their function in artificial membranes. When mixtures of various lipids are applied to a small aperture, they form a thin planar bimolecular membrane like a plasma membrane. Because the thickness of these membranes is less than 100 Å, they appear black, and are often referred to as black lipid membranes. These planar lipid bilayer membranes, as well as artificial membrane vesicles, were widely used in the 1970s and early 1980s for functional studies of transporter proteins that had been isolated by biochemical methods. However, when the lipid bilayer method was first developed, the only molecules with transport function that had been isolated were antibiotics such as valinomycin, amphotericin, and gramacidin, which acted by altering the normal permeability properties of target microbes. It was with these techniques and model transporters that Tom Andreoli began his studies of water channels at Duke University in 1967.

After collaborating with Dan Tosteson on studies of ion transport in lipid bilayers that had been treated with valinomycin, Tom began studies on amphotericin B. In contrast to valinomycin, which operates as a carrier for K^+, amphotericin B (or nystatin, a nearly identical polyene antibiotic) was later shown (see below) to form aqueous pores through cholesterol-containing membranes. When amphotericin or nystatin was introduced into a lipid bilayer, they increased the ionic conductance of the membrane by more than six orders of magnitude [6, 7]. These antibiotics also increased $P_{d,w}$ (measured by THO) and the permeabilities solutes with molecular radii less than ~5.5 Å (approximately that of glucose) in inverse proportion to their size. Based on experimental studies and biochemical considerations, the laboratories of Andreoli [6, 7], Finkelstein [8], and de Kruijff [9] independently proposed a structure for the amphotericin pore in which amphotericin and cholesterol molecules were aligned like alternating staves in barrel, surrounding an aqueous core with a radius of 5 to 6 Å.

The measurement of solute and tracer water permeabilities was relatively straightforward from a technical point of view. For this purpose, Tom Andreoli designed the apparatus shown in Figure 1. A polyethylene sheet with an aperture in the center was clamped between two half-chambers, which were then filled with symmetrical aqueous buffer solutions. A solution of phospholipid and cholesterol in decane was then painted over the aperture and allowed to thin until it became a black membrane. Amphotericin or nystatin was added to both aqueous solutions in an ethanol stock solution, and it incorporated spontaneously into the membrane. In order to measure $P_{d,w}$ or a solute permeability, THO or the tracer-labeled solute, respectively, were added to the back chamber as shown in panel 1 of Figure 1. The flux of the tracer across the membrane was then measured from its rate of appearance in the front chamber by intermittent sampling.

The measurement of water flow through the lipid bilayer posed a more formidable challenge. The delicate lipid bilayers could not withstand any mechanical pressure, thus an osmotic pressure difference was required to drive water flow, but even so, the bilayers proved to

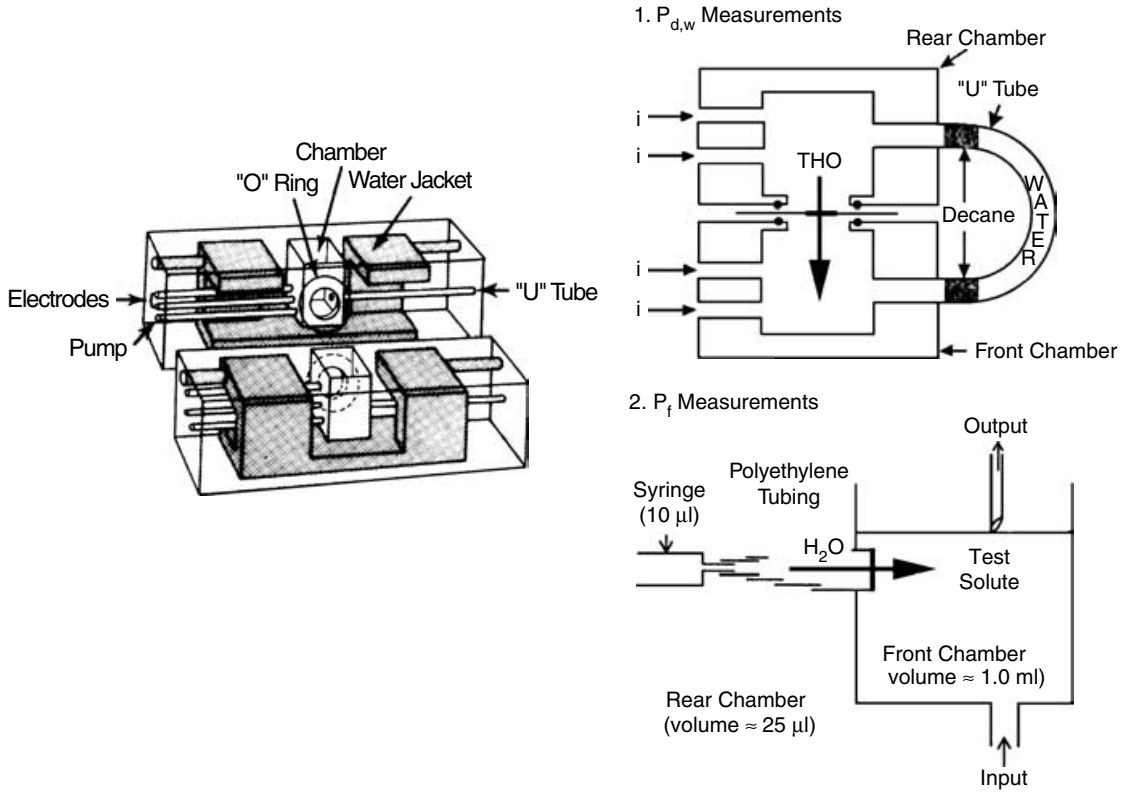


Fig. 1. Chamber for measurement of water permeabilities in lipid bilayer membranes. As shown on the left, the chamber consisted of two halves that were typically separated by a thin polyethylene septum with a 1 to 3 mm aperture over which the lipid membrane was formed. The temperature of the aqueous solutions was controlled by water circulating through the water jackets (from [11]). Panel 1: Radioactive tracer flux measurements. The two half-chambers contained identical solutions and THO, or a labeled solute was added to the back chamber. $P_{d,w}$ was calculated from the rate of THO diffusion from the back to the front chamber. Panel 2: Water flow measurements. The lipid membrane was formed on the end of a polyethylene tube that was introduced into a single half-chamber via a sealed port. The polyethylene tube was connected to a 10-µL syringe mounted in a micrometer for the precise adjustment of the volume in the polyethylene tubing. When the osmolality of the half chamber was raised by the addition of a test solute, water flowed from the closed chamber formed by the polyethylene tube. The experimenter adjusted the syringe micrometer so as to keep the lipid membrane, which was observed continuously through a dissecting microscope, flat. The rate of water flow out of the polyethylene tubing, and subsequently, P_f were calculated from rate of change of the micrometer readings. Drawings in panels 1 and 2 are adaptations of similar illustrations by Andreoli et al [10].

be extremely labile, especially when treated with amphotericin. Also, because the water flows produced were quite small, their accurate measurement posed a considerable technical challenge. The experimental chamber was reconfigured, as shown schematically in panel 2 of Figure 1, to address at least the latter challenge. The lipid bilayer was formed on the end of a segment of polyethylene tubing inserted into a single half-chamber. The polyethylene tubing formed the compartment on one side of the bilayer, and its volume was controlled by injecting or withdrawing aqueous solution using a precision syringe, the plunger of which was coupled to a micrometer for measuring the volume injected or withdrawn [10, 11]. When a test solute was added to the larger half-chamber, it created an osmolality difference that resulted in a bulk flow of water from the tubing to the hyperosmotic half-chamber.

This approach had the advantage that the reflection coefficients of different solutes could also be measured by comparing the water flow produced by one test solute with that produced by an impermeable solute, such as raffinose, whose reflection coefficient could reasonably be assumed to be 1.0. On the other hand, the measurements were tedious and often frustrating. The lipid bilayer had to be constantly observed through a dissecting microscope and maintained planar by adjusting the micrometer controlling the volume of solution in the polyethylene tubing. Frequently, the membrane broke during the course of the measurement, which was abruptly signaled by the rasping sound of the strip chart recorder, which was continuously recording the high resistance of the lipid bilayer, "pinning" to zero, and a few uncensored "expletives" from the experimenter. The value of P_f in a lipid bilayer was a hard-won parameter.

The results from these experiments confirmed that the $P_f/P_{d,w}$ ratio in "native" bilayers (unmodified by antibiotics) was ~1.0 [4, 5] after the appropriate corrections were made for the unavoidable inadequacy of convective mixing of the solutions in the chambers (i.e., "unstirred layers"). I joined Tom Andreoli's laboratory in the

Department of Physiology at Duke University in 1969, at a time when the complications introduced by unstirred layers for diffusional permeability measurements in lipid bilayers were just beginning to be appreciated. In my first publication with Tom, I assisted with the mathematical modeling of the unstirred layer effects on the experimental measurements of solute reflection coefficients in amphotericin B–treated bilayers [12]. After corrections were made for unstirred layers, analysis of the relative permeabilities of small solutes, their reflection coefficients, and the $P_f/P_{d,w}$ ratio indicated that the pores formed by amphotericin and cholesterol had a radius of in the range of 4 to 5.6 Å [5, 13]. Pores of this size permitted facilitated diffusion of water, ions, and nonelectrolytes smaller than glucose, as well as the bulk flow of water.

WATER AND UREA PERMEABILITY OF THE CORTICAL COLLECTING TUBULE

As a nephrologist, Tom Andreoli was interested in applying approaches similar to those he was using in lipid bilayers to the study of antidiuretic hormone (ADH, or vasopressin) action in the cortical collecting duct. For many years, the frog skin and toad bladder served as model systems for studying the action of ADH. In 1939, Krogh [14] examined the effect of ADH on water permeability in the frog skin using D_2O, which was one of the first uses of isotopes that were being produced by the then emerging particle-accelerator technology. He showed that P_f was greater than $P_{d,w}$, and that, although ADH increased both permeabilities, it increased the $P_f/P_{d,w}$ ratio, suggesting that the hormone might increase the water permeability of the epithelium by increasing the radii of pores through which the water moved. Despite the usefulness of these studies in anuran epithelia in establishing the fundamental basis of ADH action, such as the localization of the permeability change to the apical membrane, and the second messenger action of cAMP (e.g., see [15]), they posed limitations to more detailed functional studies. It was also important to confirm that mechanisms established in the anuran epithelia were also operative in the cortical collecting tubule (CCT), which is the primary site of ADH-dependent water reabsorption in the nephron, but which is inaccessible to in vivo micropuncture.

The methods for perfusing isolated segments of the nephron, which had been developed by Moe Burg et al [16, 17] at the National Institutes of Health during the same time period that Tom was studying lipid bilayers, provided the means to examine this segment of the nephron, and to manipulate solution composition and other experimental parameters in vitro. By the time I reached Duke in 1969, the machine shop in Duke's Department of Physiology had already constructed the pipet holders and advance mechanisms for isolated tubule perfusion, using blueprints that had been graciously provided by Moe Burg. We then began to assemble the other equipment needed for the experiments, while learning to construct the different micropipets on the microforge and to dissect the nephron segments, all with the encouragement and advice of Dr. Burg and others in his laboratory.

	Permeability (μm/sec)	
	–ADH	+ADH
Water, $P_{d,w}$	3.8 – 4.7	9.7 – 14.2
Water, P_f	6 – 10	157 – 186
Urea	0.03 – 0.10	0.02 – 0.12

Fig. 2. Urea and water permeabilities in isolated perfused rabbit cortical collecting tubule in the absence and presence of antidiuretic hormone (ADH). Values represent the range of mean values reported in the studies of Grantham and Burg and their collaborators [17], and Schafer and Andreoli [18, 19]. The diffusional permeabilities of water ($P_{d,w}$) and urea were measured using, respectively, THO and ^{14}C-labeled urea with isotonic solutions on both sides of the epithelium. The water flow permeability (P_f) was measured from the volumetric flow of water produced by a hypotonic perfusate and an isotonic basolateral bathing solution. Measurements were made in the absence (−ADH) or presence (+ADH) of 250 μU/mL of ADH in the bathing solution.

In the first study of the CCT, Grantham and Burg [17] confirmed several important predictions from anuran epithelia. They demonstrated that either ADH, by a selective action at the basolateral surface, or cAMP, increased the net water flow produced by a transepithelial osmolality difference (hypotonic luminal perfusate and isotonic basolateral bathing solution) and $P_{d,w}$, as measured by THO in the absence of an osmolality difference. The surprising finding in their studies was that, despite an almost three-fold increase in $P_{d,w}$ with ADH, there was no change in the urea permeability, which remained extremely low.

Our first studies with the CCT confirmed that ADH had no effect on the urea permeability, but increased P_f and $P_{d,w}$, and they also showed, as expected from studies in anuran epithelia, that the apical membrane was rate-limiting to water movement in the absence of ADH [18, 19]. Our primary objective was, however, to reconcile the disparate effects of ADH on water and urea permeability. Figure 2 presents a comparison of water and urea permeabilities in the CCT in the absence and presence of ADH. In the absence of ADH, the $P_f/P_{d,w}$ ratio was not significantly different from 1.0, and could be explained by limited water permeation through the unmodified lipid phase of the limiting apical membrane. ADH increased

P_f to a greater extent than $P_{d,w}$, giving a $P_f/P_{d,w}$ ratio of ~13. As discussed above, these results could be explained if ADH acted by producing pores in the apical membrane, but the large $P_f/P_{d,w}$ ratio would require that these pores have a radius of ~12 Å [19]. Pores of this size were totally incompatible with the very low permeability of the CCT to urea, whose molecular radius is only 1.8 Å.

As shown in Figure 2, even in the presence of vasopressin (AVP), the urea permeability of the CCT was not significantly different from 0, and in any case was more than an order of magnitude less than that of THO [17]. Our experiments also showed that urea and other small solutes had reflection coefficients of 1.0 [18]. The dilemma was, therefore, how a to explain the high $P_f/P_{d,w}$ ratio in terms of a pore in the face of evidence that urea, a solute whose size approached that of the water molecule, was excluded. As shown in lipid bilayers, unstirred layers in the aqueous solutions result in artifactually low values of $P_{d,w}$ [5, 12]; however, we were able to demonstrate that unstirred layers external to the epithelium were insignificant in the isolated perfused CCT preparation [18, 19]. The development of unstirred layers was prevented by the axial perfusion of the tubule lumen, which has a radius of only ~10 μm, the effective mixing of the basolateral solution, and the simplicity of the CCT, which consisted only of the single cell layer of the epithelium and its supporting basement membrane. Therefore, we considered the possibility that the cytoplasm of the epithelial cells provided an abnormally high resistance to diffusion, which we referred to as cellular "constraints to diffusion" [19], as had been demonstrated in the toad bladder by Parisi and Piccinni [20].

In order to explain the disparity between P_f and $P_{d,w}$ in the presence of ADH (Fig. 2), an unstirred layer of a solution resembling extracellular fluid would have to be over 150 μm thick, whereas the epithelial cells of the CCT were only 6 to 7 μm high. In other words, if the diffusion constraints imposed by the cell layer accounted totally for the disparity between $P_{d,w}$ and P_f, they would have to provide ~25 more resistance to THO diffusion than a solution layer of the same height. Such an amplification of diffusion resistance could be explained if the cross-sectional area for diffusion within the cytoplasm was restricted, if the pathway were tortuous, or if the viscosity of the cytoplasm was high [19]. In order to assess the diffusion resistance of the cytoplasm, we used a series of lipophilic solutes whose diffusion through lipid bilayers we could show was limited far more by unstirred solution layers than by the bilayer. These solutes served as probes of the diffusion resistance of the cytoplasm, and measurement of their permeabilities showed that the cytoplasm offered more than 10 times the resistance predicted for an aqueous solution of the same thickness. Our subsequent experiments, coupled with mathematic modeling in collaboration with Clifford Patlak at the NIH, suggested that the high diffusional resistance of the cytoplasm was due to a restricted area for diffusion [21], most likely due to cytoskeletal matrix.

Based on these findings, we initially speculated that, after correction for the cellular constraints to diffusion, $P_{d,w}$ might be equal to P_f, implying that pores need not be involved to explain water permeation in the CCT, and that ADH acted by increasing the diffusional solubility of water in the apical membrane (e.g., by increasing the fluidity of the lipid bilayer) [19, 21, 22]. The primary failing of this "solubility-diffusion mechanism" was the inability to explain the fact that the permeabilities of small hydrophilic solutes such as urea did not increase in parallel with the permeabilities to water and lipophilic solutes, as they do in artificial lipid bilayer membranes of increasing fluidity [18, 23].

These shortcomings of the solubility diffusion mechanism for ADH action brought us back to the same dilemma that was posed by the pore mechanism (i.e., how could ADH increase the water permeability of the apical membrane without increasing the urea permeability). However, at about the same time that we were reconsidering the solubility-diffusion mechanism, Levitt [24] showed that single-file channels could also be responsible for the discrepancy between P_f and $P_{d,w}$. A single-file channel is essentially a pore with a bore so narrow that water molecules cannot pass each other within it. In this case, the $P_f/P_{d,w}$ ratio is predicted to be proportional to the number of water molecules that can reside simultaneously in the channel [24]. Hebert and Andreoli [25] measured the activation energies for water and a series of moderately lipophilic solutes in the CCT. Their analysis of that data was consistent with a model in which, in the absence of ADH, cellular constraints to diffusion explained the discrepancy between P_f and $P_{d,w}$, whereas, in the presence of ADH, single-file channels that held ~6 water molecules contributed to the discrepancy [25]. At about the same time, similar conclusions were reached about the action of ADH in the toad bladder [26].

In the mid 1980s, the only model for a pore that would require single-file water movement and exclude urea was the channel-forming antibiotic gramicidin; however, this agent also markedly increased the ionic conductance of lipid bilayer membranes [3]. Thus, final validation of the single-file channel model for ADH action in the CCT and toad bladder had to wait for the cloning and sequencing of the aquaporin water channels more than a decade later. In 1993, Peter Agre et al [2] cloned the first of these aquaporins, AQP1, which is found in red blood cells, the proximal tubule, descending limb of the loop of Henle, and vasa recta. This landmark paper, for which Agre received a Nobel Prize, was followed in the same year by that of Fushimi et al [27], which described the expression cloning from the rabbit kidney of AQP2—the member of the aquaporin family that is localized to the apical

membrane of the CCT and connecting tubule [28]. Not only does AQP2 provide the necessary pathway for water permeation that is regulated by ADH-dependent trafficking to the apical membrane [29], but it also has an immeasurably low permeability to urea and ions even as small as H_3O^+ or H^+ [28]. The latter characteristics are of particular importance in maintaining a high luminal urea concentration and a typically low pH in the CCT even in antidiuresis.

ISOSMOTIC VOLUME REABSORPTION BY THE PROXIMAL TUBULE

Although we did not realize it at the time, an aquaporin also lay at the heart of the second problem that Tom Andreoli and I puzzled over in our decade-long collaboration—the mechanism of isosmotic volume absorption in the proximal tubule. When we began our studies with isolated perfused proximal tubules, it had been well established that they had a continuously high rate of volume reabsorption that exceeded even that of the small intestine, and that this absorption occurred in the apparent absence of any transepithelial driving force. The most widely held view was the "standing gradient" hypothesis of Diamond and Bossert [30], which proposed that the lateral intercellular spaces became hyperosmotic due to active NaCl transport selectively into their apical ends. This active NaCl addition would cause the intercellular spaces to become hyperosmotic if they offered a large resistance to diffusion with the serosal solution due to their limited cross-sectional area and/or tortuosity. In other words, the model viewed the intercellular space in effect as an unstirred layer, which allowed an osmotic driving force to be developed within the epithelium itself, despite the isosmolality of the external solutions.

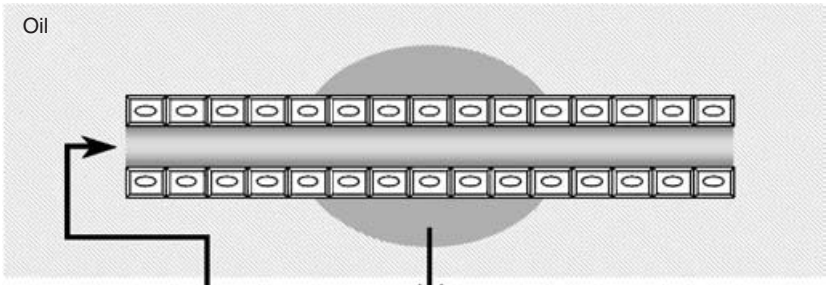
Our initial studies with proximal tubules led us to question whether the diffusion resistance of the lateral intercellular spaces was sufficient to develop a significant standing gradient of osmolality along their length. Based on our experimental data and mathematic modeling of the spaces [31–33], as well as the previously known high ionic conductance of the proximal tubule, we concluded that the osmolality at the apical ends of the spaces would be well less than 1 mmol/L hyperosmotic to the external solution even under the most extreme conditions. The lack of a significant diffusion resistance in the lateral spaces of the proximal tubule was also supported by examinations of their three-dimensional morphology. Welling et al [34] showed that the lateral membranes bounding the intercellular space, and hence, the cross-sectional area of the space, increased dramatically along its length like a ruffled skirt, with the "waist" at the junctional complexes and the ruffles adjoining the basement membrane. The apparent tortuosity of the spaces observed by two-dimensional histology was an artifact of observing their highly infolded structure in a single plane, whereas the path from the junctional complexes to the basement membrane was in fact a straight line.

We also confirmed that the hydraulic conductivities of both the convoluted and straight segments of the proximal tubule were extremely high [35, 36], which we now know is due to the high expression of AQP1 in both the luminal and basolateral membranes [1]. Because of the high hydraulic conductivity, a transepithelial osmolality difference of only 2 to 15 mOsmol/L could produce the rates of volume reabsorption measured in the proximal tubule. We proposed that volume reabsorption in the proximal tubule could be explained by luminal dilution ("absolute" hypotonicity) produced by active NaCl in combination with an "effective" luminal hypotonicity produced by the preferential reabsorption of solutes such as glucose, amino acids, and bicarbonate, which have higher reflection coefficients than the NaCl that remains in the lumen [37].

In fact, the earliest measurement of the osmolality of fluid samples obtained from the proximal tubule in mammals by Walker et al [38] suggested that the lumen might be hypotonic by 2 to 3 mOsmol/L compared with systemic plasma, but this and similar differences measured by others were ignored because of the variability of the ultrmicro osmolality determinations. More recently, Green and Giebisch [39] applied the difficult technique of simultaneously perfusing the proximal tubule lumen and the surrounding peritubular capillaries with simple isotonic NaCl solutions to avoid the complications introduced by the preferential reabsorption of solutes with high reflection coefficients. They were able to demonstrate that the luminal fluid became diluted in these segments by 1.7 to 3.9 mOsmol/L with volume reabsorption rates of 0.9 to 1.2 nL/min.

A very novel approach for demonstrating a transepithelial osmolality difference was developed by Delon Barfuss while he was working in my laboratory. He found that when isolated proximal tubule segments were perfused under oil, absorbate formed on the basolateral surface in small droplets, which could be sampled and analyzed [40–42]. A summary of his results is presented in Figure 3, which shows that significant transepithelial differences in osmolality, and the concentrations of Na^+ and glucose, could be developed by the proximal tubule.

As compelling as this evidence was, it was somewhat compromised by the rather nonphysiologic conditions that were necessary both in the in vivo experiments of Green and Giebisch [39], and in our in vitro experiments with the tubules under oil [40–42]. Again, the methods of molecular biology provided the ultimate proof for a model based on functional observations. Vallon et al [43] have shown that, as expected, volume reabsorption in the proximal tubule of knockout mice in which AQP1 is absent (AQP1 −/−) is significantly less than in the



	Perfusate	PCT	PST
Osmolality (mOsm/l)	291-294	307	302
Na^+ (mM)	148	152	143
Glucose (mM; PCT)	4.4	30	–
(mM; PST)	1.1	–	4.5

Fig. 3. Hypertonicity of proximal tubule absorbate. When isolated proximal tubule segments are perfused under oil, absorbate forms as droplets on the basolateral surface, as shown here diagrammatically. The osmolality and Na^+ and glucose concentrations of the absorbate and perfusate are presented for experiments using proximal convoluted (PCT) and proximal straight (PST) tubule segments. Note, the glucose concentration of the perfusate used for the experiments with the PST was lower than that used in the PCT in order to mimic that observed in vivo. Data from Barfuss and Schafer [40–42].

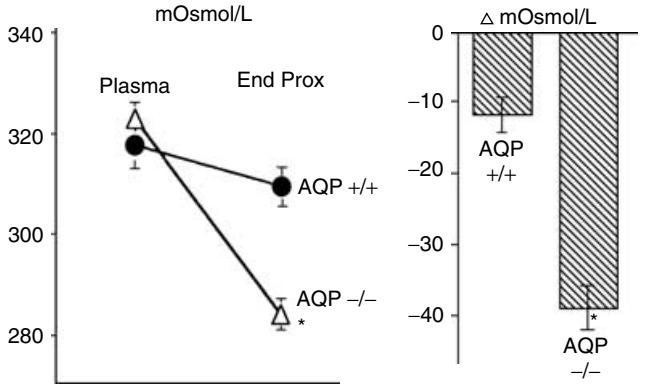


Fig. 4. Loss of aquaporin-1 (AQP1) augments luminal hypotonicity. Data are from the free-flow micropuncture experiments of Vallon et al [43] in mice with a knockout of AQP1 gene expression (AQP1 –/–) and wild-type (AQP +/+) controls. Left panel: The fall in osmolality of proximal tubule fluid along the length of the proximal tubule is significantly greater in AQP –/– than in AQP +/+ mice. Right panel: The transepithelial osmolality difference (ΔmOsmol/L, luminal minus plasma osmolality) is significantly greater in AQP –/– than in AQP +/+ mice. Adapted from Figure 1 of Vallon et al [43]. Note, AQP –/– data presented are from knockout mice that were hydrated so that the plasma osmolality was the same as in the AQP +/+ control mice [43].

proximal tubule of wild-type controls (AQP1 +/+). As shown in Figure 4, despite the lower rate of volume reabsorption, the luminal fluid became significantly more dilute in AQP1 –/– than in AQP1 +/+ proximal tubules. The explanation for this result is that the steady-state transepithelial osmolality difference that develops across the proximal tubule is directly proportional to the rate of active solute reabsorption, but inversely proportional to the hydraulic conductivity. Presuming that active transport is not affected, the decreased hydraulic conductivity in the AQP1 –/– mice results in a more dramatic fall in luminal hypotonicity, thus confirming the transepithelial driving force model.

CONCLUSION

Molecular cloning techniques have confirmed the models for two important renal mechanisms—volume reabsorption by the proximal tubule, and the action of ADH in the collecting duct—that had been developed from physiologic studies in live animals and in vitro preparations. The accuracy of these and many other models of transport when verified by gene expression in other tissues is gratifying, and, admittedly, often somewhat surprising. Of course, this burgeoning information about genes and proteins are of importance not only because they may support extant physiologic explanations of function, but also because they open a window on a new age in which genetic and pharmacologic treatment of abnormal function will be based on the structure and operation of these genes and proteins.

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