

Fluid Transport Across Leaky Epithelia: Central Role of the Tight Junction and Supporting Role of Aquaporins

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Fischbarg J. Fluid Transport Across Leaky Epithelia: Central Role of the Tight Junction and Supporting Role of Aquaporins. *Physiol Rev* 90: 1271–1290, 2010; doi:10.1152/physrev.00025.2009.—The mechanism of epithelial fluid transport remains unsolved, which is partly due to inherent experimental difficulties. However, a preparation with which our laboratory works, the corneal endothelium, is a simple leaky secretory epithelium in which we have made some experimental and theoretical headway. As we have reported, transendothelial fluid movements can be generated by electrical currents as long as there is tight junction integrity. The direction of the fluid movement can be reversed by current reversal or by changing junctional electrical charges by polylysine. Residual endothelial fluid transport persists even when no anions (hence no salt) are being transported by the tissue and is only eliminated when all local recirculating electrical currents are. Aquaporin (AQP) 1 is the only AQP present in these cells, and its deletion in AQP1 null mice significantly affects cell osmotic permeability (by 40%) but fluid transport much less (20%), which militates against the presence of sizable water movements across the cell. In contrast, AQP1 null mice cells have reduced regulatory volume decrease (only 60% of control), which suggests a possible involvement of AQP1 in either the function or the expression of volume-sensitive membrane channels/transporters. A mathematical model of corneal endothelium we have developed correctly predicts experimental results only when paracellular electro-osmosis is assumed rather than transcellular local osmosis. Our evidence therefore suggests that the fluid is transported across this layer via the paracellular route by a mechanism that we attribute to electro-osmotic coupling at the junctions. From our findings we have developed a novel paradigm for this preparation that includes 1) paracellular fluid flow; 2) a crucial role for the junctions; 3) hypotonicity of the primary secretion; and 4) an AQP role in regulation rather than as a significant water pathway. These elements are remarkably similar to those proposed by the laboratory of Adrian Hill for fluid transport across other leaky epithelia.

I. INTRODUCTION

The mechanism of epithelial fluid transport constitutes arguably the last major problem of epithelial function still unsolved. In recent times, much evidence for the paracellular route for fluid flow across leaky epithelia has been dismissed in favor of explanations based on trans-

cellular flow across aquaporins. In contrast, we discuss here the clear-cut evidence for paracellular flow in the corneal endothelium. In this light, we discuss and put in perspective past evidence and interpretations. From our conclusions, the matter is ripe for a pendular swing towards paracellular flow in leaky epithelia, with transcellular flows playing only a compensatory role.

ing in the junctions. We may have taken this central matter a step further. These questions remain obvious targets for further investigation. But nothing this far has disproved that electro-osmosis is the missing link.

The preceding remarks apply to leaky epithelia. It appears, however, that fluid-transporting tight epithelia belong instead in a separate track and that for that group transcellular local osmosis is the leading explanation. The case in point is the recent work of Erik Hviid Larsen and colleagues on toad skin (56, 76).

III. FLUID TRANSPORT ACROSS EPITHELIA: THE REVIEW IN DETAIL

A. A Historical Perspective

The idea of transcellular fluid transport started somewhat off-key. An early proposal for it was pinocytosis; alas, in 1960 Adrian Hogben demolished it, calling it "the last refuge of the intellectually bankrupt" (45). In truth, there is no evidence for substantial fluid movements by it (88, 89).

In looking for more logical explanations, one has to consider the two different pathways water can travel across an epithelium: transcellular and paracellular. That there are in some epithelia paracellular pathways with high conductance for water became increasingly clear as the electrical resistance of epithelial layers was studied in the early 1970s. By about that time, epithelia were categorized as tight, intermediate, and leaky, mainly through the work of Frömter and Diamond (27). Subsequently, Whittombury and Reuss (119) pointed out that several epithelia that transport fluid isotonicly were electrically leaky, viz. kidney proximal tubule, gallbladder, intestine, and corneal endothelium. There are, however, other fluid-transporting epithelia such as retinal pigment epithelium, choroid plexus, and ciliary epithelium for which the geometry has so far precluded definitive measurements of its electrical resistance. Hence, it seems prudent to restrict our current arguments to proven leaky epithelia.

In this connection, there have been debates between proponents of the transcellular and paracellular routes for fluid transport. A review by Alan Weinstein and Erich Windhager (114) discusses these issues for kidney proximal tubule, as well as a paper by Subrata Tripathi and Emile Boulpaep (105). This last paper calls attention to the fact that, depending on the relative areas of the lateral versus the basal membranes, the transcellular route can be predominantly transbasal or translateral. The first modern models for epithelial fluid transport were those of Peter Curran and co-workers (47, 79) and Diamond and Bossert (13); in both, fluid was driven by local osmotic gradients at both the apical and basolateral cell membranes and could traverse cell membranes and intercel-

lular junctions. These models set high standards for the field and included transcellular osmosis in a feasible geometrical frame. They began to appear in textbooks as explanations for this phenomenon.

Still, objections began to appear. Adrian Hill (35) pointed out that fluid transported through cells as theorized by Diamond would be hypertonic, while epithelia transported isotonicly. Hill's objections brought the matter to a standstill. Still, if the flow could not be transcellular, it had to be paracellular, and somehow no consensus for that could be developed either. In several papers, Hill's and other laboratories showed evidence suggesting solvent drag of solute caused by paracellular, transjunctional water flow. However, there were counterarguments that a similar drag of solute would take place if fluid would travel via lateral membranes and the paracellular space.

As a result of this impasse, the local osmosis model survived in textbooks, which to this day almost invariably explain fluid transport across leaky epithelia by some version of local transcellular osmosis. The recent avalanche of evidence for the presence of water channels in fluid-transporting epithelia has of course helped this thinking. And yet, things may not be as simple, as we look at the mechanism more closely.

B. Water Channels

For water to traverse cell membranes, it has to be helped to cross the lipid bilayer. So the idea of a plasma membrane water channel emerged early on, championed by Arthur K. Solomon and colleagues (96). As the first water channel protein (AQP1) was characterized (3, 4) and molecularly identified (12, 85, 86), attention turned to its presence in epithelia. As summarized in an earlier section, findings reinforced views from review writers that fluid transport presumably traversed epithelial cell membranes (89, 98).

C. Problems for Transcellular Water Transport Surface and Resurface

If fluid transport traverses epithelial cells via AQPs, one would expect *prima facie* that absence of aquaporins would affect that transport markedly. Yet, that expectation has not been fulfilled.

In the last decade, Alan Verkman's laboratory in collaboration with several others have extended such studies greatly through the experimental use of AQP knockout mice (110, 112). These results raised similar questions as to whether AQPs are the main route of fluid transport through epithelia. A thorough analysis of the results with AQP knockout mice appears in a review by Hill's group ("What are aquaporins for?" Ref. 39). A paragraph from it

proposal is a qualitative jump that may bring the field very near a solution to these long-standing questions. Interestingly, we have also arrived at a paradigm of paracellular flow, which seems a remarkable convergence for two laboratories using different methodologies, and working independently.

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This review is in honor of the recently deceased Dr. Friedrich P. J. Diecke, a former Chairman of the Department of Pharmacology and Physiology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School. Dr. Diecke and the author had a very fruitful collaboration dating back to 1998. He was to have been a coauthor of this review, but disease prevented that. Among his many virtues, Dr. Diecke counted with a privileged mind, a unique sense of humor, a classical physiological training, and an encyclopedic knowledge of general and comparative physiology and the biological transport literature. Several ideas discussed here carry his brilliant imprint. An outstanding scientist and a gentleman, he will be missed.

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DISCLOSURES

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