Much remains to be investigated, but evidence to support an electrostatic hypothesis would be forthcoming if the pD and its orientation is confirmed *in vivo* in mammals; the macro-molecular flux, in Peti-Peterdi's *in vivo* model changes in response to polycation protamine or anionic heparin—that is, using multi-photon technology, can real-time macromolecular flux be controlled purely by altering the charge on the barrier? This would be strong evidence to support the electrostatic hypothesis. However, it may be very difficult to define clearly that this effect is related purely to a change in physics and not to cellular or other electrostatically mediated responses within the GFB. Podocytes, for example, exposed to protamine *in vitro* are known to undergo architectural and phenotypic change in the absence of other cells or flow.[15]

Notwithstanding all of this, we are sure this intriguing study will prompt some brisk and animated debate, and we await with expectation the next installment in the saga reassured that the new technologies[4] now available lend themselves to asking these challenging questions and perhaps continuing to unsettle our place in the comfort zone—a place that after all is rarely all that comfortable.

DISCLOSURES

None.

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See related article, "Electrical Forces Determine Glomerular Permeability," on pages 2053–2058.

A New Role for Charge of the Glomerular Capillary Membrane

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Things move when acted on by forces. In body fluids and at tissue-fluid interfaces, Coulomb forces hold oppositely charged molecules in close proximity to one another. When one half of a charge couple is more susceptible than the other to some external driving force, imposing that driving force will cause some degree of charge separation, which creates an electric dipole. The electric field associated with aligned dipoles balances the effect of the original outside force on the more mobile species, thereby maintaining bulk charge neutrality over measurable distance. Summing the electric fields contrib-

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uted by dipoles within a porous membrane yields the transmembrane voltage.

The most well-appreciated example of this in medicine and physiology is the negative cell membrane voltage, which results from diffusion of potassium out of cells, and the Donnan voltage, which arises after permeable ions diffuse to electrochemical equilibrium while a charged macromolecule is confined to one side of a semipermeable membrane. In both of these cases, the outside force is provided by a concentration difference and the mobile species moves by diffusion. When the mobile species moves by solvent drag rather than diffusion, the resulting charge separation gives rise to a so-called *streaming potential*.

Streaming potentials are used by engineers to identify the amount and nature of fixed surface charges on porous or fibrous membranes where an electrical double layer is formed by the membrane charges and loosely associated free ions in solution. The earliest theoretical description of electrical double layers is traceable to Helmholtz in the 1850s, and the idea of surface charge on the glomerular filtration barrier (GFB) dates back nearly half a century. Whereas kidney physiologists have invoked the electrical double layer to explain the charge selectivity of the GFB *vis à vis* effective pore radius, no one has considered the streaming potential in glomerular physiology, until now.

In this issue of *JASN*, Hausmann *et al.*[1] report what seems to be a streaming potential across the GFB.[2] However, the direction of the voltage drop (negative in Bowman's space) is opposite what would be explained by streaming across the filter bearing a fixed negative surface charge layered with mobile cations. So although reliable physical theory predicts that a streaming potential exists, the finding of Hausmann *et al.*[1] suggests a major gap in our understanding of the construction of the capillary wall. This observation is supported by the finding that administration of a smaller cationic polymer, protamine, eliminates the streaming potential. However, this finding does not explain why the elimination of a negative charge in the urinary space is possible given the negative charge of the glomerular membrane.

The magnitude of the streaming potential (0.045 mV per 10 cmH_2O filtration pressure) is small relative to the expected Donnan potential (2.3 mV positive in Bowman's space for impermeant plasma protein, accounting for 12 mEq/L anion gap when plasma sodium concentration is 140 mEq/L). Nonetheless, the authors suggest that the streaming potential is a critical part of the filtration barrier to albumin sieving that prevents the barrier from fouling by driving albumin back into the plasma.

In support of this hypothesis, they cite several instances in which increased albumin sieving might be tied to loss of streaming potential. Electrical properties of the glomerular barrier have now been postulated to retard albumin filtration by two mechanisms. Earlier mechanisms involved restricted access of albumin to the filtration barrier by steric hindrance. Now it is imagined that the streaming potential affects albumin by electrophoresis. The notion of steric hindrance by the glomerular basement membrane ran into trouble when it was learned that the actual surface charge density was too small to yield the imagined effect.[2] Hausmann *et al.*[1] present a model for electrophoresis that may or may not encounter similar difficulties down the road.

Is it possible for such a small voltage as this streaming potential to exert a major retrograde force on albumin? Recall that the Coulomb force exerted on albumin at any point is determined by the electric field at that point. The membrane voltage divided by membrane thickness gives the average electric field within the membrane. If the electric field that results from streaming is inhomogeneous and concentrated over a short distance within the membrane, then it could exert a large force on albumin in that region while making a meager contribution to transmembrane voltage.

Does the streaming potential explain filtration properties that cannot be explained otherwise? The authors mention postural proteinuria and imagine low glomerular capillary pressure leads to low hydraulic water flux when patients are upright, but glomerular filtration and hydraulic water flux are synonymous, and the only way to reduce the streaming potential without a proportionate decline in GFR is to reduce the shear stress and charge separation within the filtration barrier by increasing the surface area for filtration while lowering the driving pressure. However, increasing filtration surface will sustain GFR only when there is filtration disequilibrium, which will not occur when capillary pressure is low. Furthermore, it is generally observed that proteinuria and glomerular capillary hypertension go hand-in-hand.

Finally, Hausmann *et al.*[1] invoke the *streaming potential* as a novel mechanism to explain why the albumin-sieving coefficient is high during low GFR. The usual explanation for this is that, when GFR is low, each element of filtrate spends more time in Bowman's space, allowing more time for albumin to gain access to the urine by diffusion. Intuitively, the sieving coefficient will approach unity as GFR approaches zero with or without an associated loss of streaming potential.

To summarize, demonstrating a streaming potential across the GFB essentially validates the laws of physics *in vivo*, which is a technical feat, but a streaming potential that is negative toward Bowman's space is more noteworthy because it invalidates the prevailing view of the glomerular capillary as a filtration barrier covered by fixed negative surface charge. The relative contribution of the streaming potential to the reflection of albumin in various clinical circumstances invites additional quantitative reasoning.

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DISCLOSURES

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Yet Another Advance in Understanding Albuminuria?

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Although the clinical relevance of proteinuria, especially albuminuria, has been well documented in chronic kidney disease,[1] the quantitative mechanistic significance of different barrier components to albuminuria remains an area of considerable excitement and debate. The glomerular filtration barrier (GFB), formed by fenestrated endothelia with their glycocalyx, podocytes with their interdigitated foot processes and slit diaphragms, the subpodocyte space, and the glomerular basement membrane (GBM), has been long believed to be the major determinant of albuminuria with podocyte foot process fusion being central.[2] Multiple genetic, molecular, and morphologic lines of evidence implicate the podocyte and its slit diaphragm as playing a central role in the GFB. However, changing concepts regarding the mechanistic relevance and clinical importance of each of these individual components of the GFB have been advancing at a rapid rate.

The glycocalyx of glomerular endothelial cells has been shown by enzymatic degradation,[3] use of vascular endothelial growth factor antibodies to induce glomerular endothelial injury,[4,5] and mutations of the laminin β3 gene[6] to result in macroalbuminuria without morphologic podocyte injury, although controversy remains.[2] Basement membrane charge properties and their molecular constituents have also been reexamined and deleted, respectively, in attempts to understand further their importance. Clearly, laminin β2 mutants and null mice for either laminin β2 or the α3 chain of type IV collagen demonstrate the potential role of the GBM in albuminuria.[7–10] In addition, the glomerular endothelial cell and GBM may be able to compensate for changes in the podocyte that lead to foot process fusion and loss of the heparin sulfate glycosaminoglycan and anionic charge.[11] The recent discovery of a subpodocyte space has added another dimension to the GFB, although its role in protein filtration remains unknown.[12,13]

On this background, is it little wonder that a talented investigative team, with considerable experience in the area, used a new technological advancement in scanning electron microscopy in this issue of *JASN* to produce data that bring into question long-held beliefs regarding the structure and function of the podocyte slit diaphragm.[14] They describe the existence of heterogeneous ellipsoidal and circular pores, located in the central region of the slit diaphragm, log normally distributed with a mean diameter far greater than envisioned previously. This was accomplished with careful attention to potential fixation, imaging, and quantitative analysis artifacts, although high-pressure freezing would have been the preferred tissue preparation.

Like many scientific advances challenging previously held beliefs, it is likely to be initially refuted vigorously, which is a healthy and required part of the scientific evaluation process. Pore sizes were quantified and compared between Munich Wistar Fromter (MWF) rats, known to develop albuminuria with age and develop focal glomerular sclerosis, and Wistar rats that do not develop albuminuria. The mean pore sizes of both strains were similar, but MWF rats had a small increase in the very largest pores, which the authors propose to be the mechanism of albuminuria. However, in both rat strains, unique images with high resolution and state-of-the-art quantitative morphometric analysis revealed pore sizes that beg the question, "Is albumin filtered across the glomerulus under normal physiologic conditions in levels previously deemed unrealistic by many investigators in the field?"[15] This is especially true given the accumulating evidence from a number of studies using genetic, biochemical, imaging, and molecular approaches and indicating an important role for proximal tubule epithelial cells in albumin reabsorption and reclamation.[16–24]

The present data could easily be interpreted to substantiate further the role of the proximal tubule under physiologic and pathologic conditions in minimizing albuminuria. The authors invoke endothelial and GBM barriers in

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