Glomerular Filtration Barrier and Molecular Segregation: Guilty as Charged?

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The normal phenotype of the glomerular filtration barrier (GFB) and the mechanisms that underlie it have intrigued physiologists, microvascular biologists, and renal physicians for decades, especially because, in health, the multilayered barrier differentially restricts the passage of molecules of various sizes, shapes, and charges. Although poorly permeable to large lipid–insoluble or anionic molecules, the GFB is highly permeable to water and small water–soluble moieties. The individual or combined roles of the layers of the semipermeable GFB, including the glycocalyx-covered fenestrated glomerular endothelium, the glomerular basement membrane (GBM), the slit diaphragm between interdigitating podocyte foot processes, and a restrictive subpodocyte space (SPS) remain controversial. In contrast, most would agree that this strict segregation of glomerular filtrate is impaired or lost in glomerular disease, and we are spurred on to understand the underlying mechanisms of the selectivity of the barrier because we often deal with the consequences of glomerular disease.

Our initial understanding of differential sieving stems from our use of everyday items to separate material of different sizes in the kitchen, garden, or laboratory, hence the sieve, the riddle, or the filter—that is, tools that separate on the basis of size alone using the force of gravity or occasionally pressure if the agent to be retained is excessively small. Although poorly permeable to large lipid–insoluble or anionic molecules, the GFB is highly permeable to water and small water–soluble moieties. The individual or combined roles of the layers of the semipermeable GFB, including the glycocalyx-covered fenestrated glomerular endothelium, the glomerular basement membrane (GBM), the slit diaphragm between interdigitating podocyte foot processes, and a restrictive subpodocyte space (SPS) remain controversial. In contrast, most would agree that this strict segregation of glomerular filtrate is impaired or lost in glomerular disease, and we are spurred on to understand the underlying mechanisms of the selectivity of the barrier because we often deal with the consequences of glomerular disease.

In this issue of *JASN*, Hausmann et al. add to the complex concepts of physical structure and glomerular cell function by highlighting the potential role of electrostatic forces within the GFB. Through in vivo cannulation experiments in *Necturus maculosus* (an aquatic amphibian), they show a measurable and modifiable potential difference (pD) across the GFB and propose it is this pD, generated in situ by the differential flow of charged ions, that excludes albumin from being filtered. The pD is proposed to be analogous to that of a streaming potential, a concept unfamiliar to most molecular biologists and perhaps nearly all clinicians.

In a streaming potential model of the GFB, a negatively charged porous glomerular barrier divides two spaces where the assumed charge is fixed and distributed homogeneously. If one adds an ionized solution such as dilute NaCl, then positive ions will be attracted to the fixed negative charge on the barrier in a uniform distribution. Although there may be minor charge gradients locally, there is no net charge gradient (pD) across the GFB. If one introduces unidirectional flow into the system such that fluid flows under pressure across the barrier, then many positive ions will travel through the barrier and beyond its influence, but some will not escape capture by the fixed negative charge and will accumulate on the nonplasma side of the barrier, producing an uneven distribution of positive charge. Likewise, although some negative ions will traverse the barrier because of convective flux—less so than the positive ions—some negative ions will be reflected back by the fixed negative charge of the barrier, producing a corresponding negative charge on the plasma.

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surface of the barrier, hence the potential that occurs in a stream.

The claim that it is this streaming potential that underpins the GFB’s high oncotic reflection coefficient will, we are sure, disturb the comfort zone of many investigators because, by implication, it suggests albumin exclusion primarily depends on physical chemistry with cell biology as secondary to this effect—an intriguing concept to be sure, but further work will be required to reconcile some objections that may be swift in coming.

In particular, because the streaming pD (and putative albumin exclusion) is proportional to flow rate, the electrostatic hypothesis is consistent with the data from Rippe’s group demonstrating that the sieving coefficient of Ficoll and globular proteins decreases as GFR increases; however, clinically, it might therefore be predicted that agents that reduce intraglomerular pressure and single-nephron GFR, such as calcineurin or angiotensin-converting enzyme inhibitors, might increase proteinuria rather than reduce it. Although mathematical modeling may suggest the pD is sufficient to prevent the passage of albumin into the primary filtrate, mathematical models usually depend on simplifications and assumptions, requiring further testing by functional evidence. For instance, if the GFB charge is removed in a way that guarantees no change in cellular phenotype, then does protein flux increase in mammalian systems in vivo? Such studies are awaited, but indirect evidence in the literature seems confusing: Altering charge with exogenous agents such as protamine or ionic perfusate of variable composition may derange GFB function; in contrast, transgenic manipulation of the endogenous constitutive anionic properties of the GBM seems to have little effect.

The proposal by Hausmann et al. is supported by the hypothesis that trans-GFB filtration is homogeneous but the forces that drive filtration change throughout the glomerulus, thereby almost certainly introducing variability to filtration per unit area. The glomerular intracapillary hydrostatic pressure is pulsatile, and its mean value drops from one end to the other while oncotic pressure rises. Cellular and acellular (fenestral and filtration slit) coverage of the GFB results in heterogeneous velocity profiles across the GFB. Migrating podocytes and the influence of the SPS, which cover only part of the GFB, also contribute to heterogeneity.

Ultrafiltration is also much more likely to be unhindered at the edge of the glomerulus than at its center, and the only way the filtration per unit area across the GFB could be the same in the different lobules of the glomerulus (in parallel with each other) is for the forces driving filtration (hydrostatic and oncotic) to be identical and for hydraulic pressure that would require the resistance afforded by segmental glomerular capillary structure to intraluminal flow, particularly length, width, and tortuosity, also to be identical. Furthermore, if the primary force that retains albumin is electrophoretic, then the mechanisms of proteinuria in genetic defects in GBM and podocytes also need to be reconciled.

Most intriguing, why did Hausmann et al. find the measured pD the wrong way around from the conventional view? They discuss possible reasons, such as postulated double layers of counter ions around the barrier, which would explain the seeming reversal of the fixed charge of the barrier. However, such a phenomenon should be multiple, not just double layered, and positively charged protamine should not be able to reduce the amplitude of the filtration-dependent potential. This result would logically lead to the hypothesis that this would be the likely property of hyperperfusion with heparin or albumin. With respect to the orientation of the pD, perhaps this is because we are considering the GFB as exactly what we know it is not—an inanimate, single-layered barrier—when in fact it is a living, dynamic entity with many layers adapting to forces and physiochemical signaling.

Because the pD is measured across the entire barrier and the movement of ions that produce it occurs through all layers, all of which have anionic glycocalyx (podocytes and endothelial cells) or anionic GBM heparan sulfate proteoglycan charges, it begs the question as to whether each layer has a streaming potential of its own. Is there one at all on the parietal surface of the podocytes and their processes because primary filtrate exits around them not through them? In effect, the charge on the barrier may be functionally one sided, affecting the orientation of a streaming potential.

There may also be a more mundane reason for the unanticipated reorientation of the pD that underlines why these experiments may soon be done in mammals. First, renal tubular transepithelial resistances show marked differences between mammals and amphibians, at least in terms of magnitude (eightfold). Also, the same study showed that the orientation of streaming potentials changes from one end of the proximal tubule to the other as a result of claimed differences in electrogenic transport mechanisms by pumps and channels; in addition, streaming potentials can be obscured by overlying diffusion potentials—potentials produced by the differential permeability of a barrier to Na+ in one direction rather than another. Furthermore, these authors found that this is particularly a problem when hypotonic solutions are present; specifically, they found that an intraluminal positive pD, as in the report by Hausmann et al., was produced with the use of low Na+ solutions. The physiologic Na+ for many amphibians is 100 to 120 mmol/L and is 100 to 110 mmol/L for this species. Also, the perfusate in experiments by Hausmann et al. contained 90 mmol/L Na+. In mammals, therefore, the pD may in fact be in the predicted orientation.

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 macromolecular 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 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.

REFERENCES


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