COUNTERPOINT: DEFENDING PORE THEORY

The paradigm of a charge-selective glomerular filtration barrier (GFB) was established in the 1970s when Brenner and colleagues published their seminal data on glomerular sieving coefficients (θ ; primary urine-to-plasma concentration ratios) of differently charged dextran species across the rat GFB (1). Negatively charged, sulphated dextran molecules exhibited much lower θ than neutral and cationic dextran species (Figure 1B). However, recently, several authors have questioned these results (2,3). This is due to the fact that some fractions of sulphated dextran can bind to plasma proteins and to glomerular cells, leading to an underestimation of their θ (3,4). In addition, it has been shown that polysaccharides (cf. dextran) exhibit a flexible molecular conformation, making them hyperpermeable compared to more rigid solutes, such as proteins (5,6). Ficoll, a co-polymer of sucrose and epichlorohydrine, apparently shows glomerular sieving characteristics somewhere in between those of dextran and proteins, at least for molecular radii (a_e) approaching the pore radius (r_p) (5). However, for certain ranges of molecular radii ($a_e/r_p < 0.65$), Ficoll and proteins have actually been found to have similar glomerular permeation characteristics (7,8). High molecular

weight Ficoll, passing through “large pores” (see below; $a_e/r_p < 0.65$), like albumin (7), has therefore been frequently used for assessments of glomerular permeability (9–12).

ELECTROSTATIC EFFECTS OF GLOMERULAR CHARGE

A) SOLUTE RESTRICTION

Testing carboxymethylated (CM), negatively charged but conformationally intact Ficoll (a-Ficoll) vs neutral Ficoll (n-Ficoll), Fissell and colleagues demonstrated a charge-dependent restriction for a-Ficoll in negatively charged (thin) artificial membranes (13). Using the same preparation of a-Ficoll (a gift from William Fissell), we demonstrated a reduced transport of a-Ficoll relative to n-Ficoll across the rat GFB (14). Based on these results, we recently quantified the electrostatic properties of the GFB in terms of surface charge density, modeling the glomerular barrier as a negatively charged fiber-matrix according to the model in (15). We estimated the fiber surface charge to be similar to that of known surface charge densities for many proteins in the

FIGURE 1 — “New” (A) and “old” (B) “Brenner curves” (1). Panel A shows recent data on glomerular sieving coefficients measured for anionic and neutral Ficoll and simulated for cationic Ficoll (14). The negatively charged Ficoll molecules (hatched line) were calculated to have a surface charge of similar magnitude to albumin (-0.02 C/m^2) (16). The surface charge of the positively charged Ficoll was set at 0.02 C/m^2 . B represents the “classical” Brenner curves measured for negatively charged, sulfated dextran vs neutral and cationic (DEAE) dextran. The sulfated dextran measurements (hatched line) especially have been found to be in error and to markedly exaggerate the impact of charge on the properties of the glomerular filtration barrier.

body, namely $0.005 - 0.02 \text{ C/m}^2$, which is less than previously thought (Figure 1) (16). This surface charge will exert an electrostatic effect on the permeation of negatively charged macromolecules across the GFB. The magnitude of the electrostatic restriction effect can be simulated by adding a fraction of the so-called “electrical double layer” (Debye length, which is $\sim 8 \text{ \AA}$ at physiologic ionic strength) to the permeable structures (fibers or pores) and by adding a similar length constant to the radius of the permeating charged molecule. For a surface charge of 0.02 C/m^2 about 20% of the Debye length, or $1.5 \text{ \AA}$, should be the length constant in this context (16). In a simple pore model (see below), the effect of charge is thus to increase the restriction of the permeating charged solute across the charged pores (or between the charged fibers) in a fashion that can be mimicked by adding $1.5 \text{ \AA}$ to the solute radius and subtracting $1.5 \text{ \AA}$ from the pore radius. Even though such a perturbation is small, due to the rather moderate electrical charge in the GFB, it still is critical in preventing albumin (radius $35.5 \text{ \AA}$) from permeating the “small pore” pathways, having a radius of $37.5 \text{ \AA}$. Thus, $37.5 \text{ \AA} - 1.5 \text{ \AA} = 36 \text{ \AA}$ (apparent charged small pore radius) and $35.5 \text{ \AA} + 1.5 \text{ \AA} = 37 \text{ \AA}$ (apparent “charged” albumin radius), with the consequence that the passage of albumin will normally occur only through a “large pore” pathway (radius $\sim 110 - 120 \text{ \AA}$) in the GFB (6,7,17).

B) DONNAN POTENTIAL

Another electrical phenomenon in the GFB is the so-called “Donnan potential,” which is created by the difference in protein concentration between the plasma and the primary urine. The absence of negatively charged albumin in the primary urine will cause an uneven partitioning of anions and cations, mainly sodium and chloride, across the GFB, which, in turn, creates a positive Donnan potential in the Bowman’s space with a magnitude of $\sim 1 \text{ mV}$ (16).

ELECTROKINETIC EFFECTS AFFECTING SOLUTE TRANSPORT ACROSS CHARGED MEMBRANES

In the absence of charge, filtration (solvent flow) can be described as the sum of hydraulic flow and osmotic flow. Furthermore, solute flux is classically the sum of diffusion and convection and their mutual interactions, which can be described by the global non-linear flux equation (Patlak equation) (18–20). However, across charged membranes, the transport of charged solutes, such as albumin, is much more complicated due to the development of a variety of electrokinetic phenomena. Fluid flow through a charged barrier gives rise to an electrical (streaming) potential to prevent the flow of counterions (on the charged pore wall and on the macromolecule surface) in the direction of the volume flow. This streaming potential has essentially 2 effects on the solute transport across the barrier. First, the streaming potential will reduce the volume flow (via counter-electroosmosis) and, thus, the overall solvent flow. This apparent reduction of the hydraulic conductance (LpS) has been shown to be negligible

at a physiologic ionic strength (21,22). Second, the streaming potential will also produce an electrophoretic transport of charged solutes, increasing the clearance of solutes of the same charge as the barrier. Hence, electrophoresis will normally improve the transport of anions across an anionic barrier such as the GFB. However, the electrophoretic effect is quite small compared with diffusion and convection under most circumstances, as has been shown by several authors (21–24).

POTENTIAL FLAWS IN THE CALCULATION OF ELECTROKINETIC FORCES ACROSS THE NECTURUS GFB IN THE STUDY OF HAUSMANN *ET AL.* (25)

A) ELECTROPHORESIS IS NOT A CONVECTIVE FLUX

In order to quantify transport across the GFB, one has to make some assumptions concerning the structural basis of the hindrance exerted on solute and fluid transport. Common physical models of the GFB are, e.g. 1) the negatively charged fiber-matrix plus large pore model (15,16), 2) the log-normal distributed pore plus shunt model (12,26,27), and 3) the 2-pore model (17,18). These models predict a marked restriction in the GFB of solute transport for molecules in the radius range of $20 - 40 \text{ \AA}$, resulting in a very low θ for albumin (1×10^{-4}), and, furthermore, that proteins larger than albumin can actually to some extent also enter the primary urine. The 2-pore model sets a discrete upper limit to the size-selectivity of the normal GFB by endowing the large-pore radius with a radius similar to that of immunoglobulin M ($110 - 120 \text{ \AA}$) (17). In the calculations by Hausmann *et al.* (25), pores of radius $\sim 42 \text{ \AA}$ were used to model the size-selectivity of the GFB. Across this pore system, proteins larger than albumin (e.g. immunoglobulin G and α_2 -macroglobulin) are prevented from passing the GFB. It was further assumed that the hindrance factor for electrophoretic transport is identical to that of convective transport. However, electrophoresis is equivalent to ion migration, i.e., a diffusive phenomenon, not a convective one. Actually, it can be shown that the hindrance factor for electrophoresis can be approximated to that of diffusion under the assumption that the electric field has the same (or opposite) direction to that of solute transport (21). Thus, it is subject to much higher transport restriction than convection. The difference is ~ 130 -fold! In other words, by treating electrophoresis as a convective phenomenon, the electrophoretic transport was inflated by a factor ~ 130 in (25). This is why electrophoresis was considered to be of importance for albumin transport.

B) THE “STREAMING POTENTIAL” WAS REVERSED IN THE NECTURUS GFB

In the *Necturus*, contrary to theoretical predictions, the streaming potential polarity was opposite to that observed in simple negatively charged *in vitro* channels in which there is a flux of negatively charged solutes. Hausmann *et al.* (25) attributed this charge reversal to an “overcharging” phenomenon,

which might occur if additional solute counterions (e.g. cations) are bound to the (negatively) charged filter walls, for example by chemical interactions. It is conceivable that such a phenomenon may be ascribed to the rather atypical filtration barrier present in the *Necturus*. The *Necturus* GFB is thus 10-fold wider ($3.5\ \mu\text{m}$) than that of mammals ($0.2 - 0.3\ \mu\text{m}$). The “tissue-like” structures forming the *Necturus* GFB are lined by podocyte foot processes (having slit diaphragms) and by a thin endothelium. However, the extracellular matrix in between these 2 layers is not a classical glomerular basement membrane, but is mostly electron lucent with numerous cell processes within it, an overall rather heterogeneous structure (28). In such a “tissue-like” barrier, charge reversal might be a possibility, similar to the situation in the bovine lens basement membrane, which is an order of magnitude thicker than the *Necturus* GFB ($\sim 30\ \mu\text{m}$) (29). However, even though charge reversal might be a possibility in complex “tissue-like” membranes, the electrophoretic term, when correctly estimated, is too small to make a significant contribution to overall albumin transport (Figure 2).

C) THE FIELD STRENGTH WAS OVERESTIMATED FOR THE *NECTURUS* GFB

To obtain the field strength generated by the streaming potential, the potential difference should be divided by $3.5\ \mu\text{m}$ (cf. Ref. 25), which is the width of the GFB in *Necturus*, not by the width of the human GFB ($0.3\ \mu\text{m}$). However, the calculations of Hausmann *et al.* seem to have yielded a more than 10-fold higher field strength than actually measured. In fact, since the electrodes in the experiments were even farther apart ($\sim 200 - 300\ \mu\text{m}$), the overestimation might be even higher. Figure 2 shows the impact of electrophoresis on the transport of proteins across the human GFB (using correct diffusive hindrance factors) and assuming either the field strength suggested by Hausmann *et al.* ($-1,600\ \text{V/m}$) or a hypothetical field strength generated by the Donnan potential ($4,800\ \text{V/m}$).

IMPLICATIONS FOR THE PERITONEAL MEMBRANE?

In the peritoneum, the effects of both electrostatic and electrokinetic phenomena are minor, as recently reviewed (30). The peritoneal membrane is a compound transport barrier with the so-called interendothelial slits of the continuous capillary walls coupled in series with the peritoneal interstitial matrix (31). The transport characteristics of this complex barrier are very different from those of the GFB, in that there is no functional charge selectivity across the peritoneum and no selection of solutes due to shape or flexibility (32). The capillary interendothelial slit is a tortuous pathway restricting solute transport across “semi-tight” junctions interrupted by “junctional breaks” close to the capillary lumen. In the wider, downstream portions of the slit (width $\sim 200\ \text{\AA}$) there are, in addition, adherens junctions. The peritoneal capillary barrier in many respects seems to resemble a gel filtration column and is thus very different from the straightforward and thin

FIGURE 2 — Glomerular sieving curves for negatively charged molecules modeled as hard spheres with a surface charge of $-0.02\ \text{C/m}^2$ for 3 different field strengths: $0\ \text{V/m}$, $-1,600\ \text{V/m}$ (25) and $4,800\ \text{V/m}$. A 2-pore model was employed, where the small pore radius (r_s) was set at $36.6\ \text{\AA}$, the large pore radius (r_l) at $98.6\ \text{\AA}$, and the fractional ultrafiltration coefficient accounted for by large pores (α_l) at 6×10^{-5} (33). Neither the streaming potential reported by Hausmann *et al.* (25), nor the hypothetical field strength generated by a ($1.5\ \text{mV}$) Donnan potential ($4,800\ \text{V/m}$) significantly affected the transport of charged solutes across the GFB. The effects are particularly low for a solute the size of albumin. Note that a (small) reversed streaming potential would be totally counteracted by the effect of the Donnan potential.

structure of the mammalian GFB, which selects solutes based on size, shape, charge, and flexibility (30).

CONCLUDING REMARKS

In summary, several flaws in the calculations of the electrophoretic term in (25) seem to have led to an overestimation of the electrokinetic contribution to albumin transport by at least 3 orders of magnitude. This overestimation derives mostly from an incorrect restriction factor for electrophoresis and an incorrect calculation of the field strength. Furthermore, the model presumes that there is a reversal of the streaming potential in the GFB, a phenomenon that is only seldom seen in thin membranes, such as the mammalian GFB. Finally, the Donnan potential, being $\sim 1\ \text{mV}$ (Bowman’s space positive), should have been recorded at baseline in the experiments. However, a Donnan potential was not recorded.

Could the measured “streaming potential” be an artifact or just reflect an anomalous behavior of the “tissue-like” GFB in the *Necturus*? Hausmann *et al.* (25) performed careful control experiments to exclude artifactual tip potentials and pressure- and flow-induced potential differences. Furthermore, they measured streaming potentials in an *in vitro* setup

under identical conditions to the experimental ones, and the theoretically predicted polarity was then obtained, being positive behind a negatively charged filter. Yet the fact that the electrodes were filled with perfusion solution (a modified Ringer's solution) with some added protein (0.5% bovine serum albumin) should have caused the formation of a local Donnan potential in the probe during glomerular filtration, i.e., whenever the protein concentration in Bowman's space fell toward 0. The sign of the net potential depends on the local potential difference of the probe vs that across the GFB. If the latter were lower than the local Donnan potential across the probe, a reversed polarity would result. Another explanation of the reversed "streaming potential" may be that the thick, "tissue-like" structures forming the *Necturus* GFB may have given rise to more complex electrokinetic phenomena than can be described by simple "streaming potentials." In support of this, Fissell and colleagues (see above) measured a reversed "streaming potential" over the thick bovine lens membrane (29). A similar phenomenon for a thin biological barrier has, to our knowledge, never been observed. In any case, the field strength measured is not sufficient to significantly affect the albumin permeation across the GFB. In conclusion, the results from the laboratory of Marcus Moeller and his colleagues are intriguing. Based on their findings they claim that the GFB, although negatively charged, actually physiologically behaves as a *positively* charged barrier! For this paradigm there is, however, little, if any, independent experimental evidence.

DISCLOSURES

The authors have no financial conflicts of interest to declare.

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