Glomerular permeability: a never-ending saga

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The paper by Tanner et al. (27) revisits the critical question of how the intricate glomerular capillary membrane provides such a highly restrictive barrier to macromolecules while still allowing the movement of vast volumes of protein-free fluid (1, 7, 13, 14, 20). In recent years, this topic has emerged from dormancy because of exciting new technology and because several recent studies presenting provocative data have challenged well-established concepts regarding glomerular permselectivity (5, 6, 20, 24). In particular, it has been proposed that vast quantities of protein are normally filtered and that tubular reabsorption of intact proteins is primarily responsible for preventing loss of protein from the body (6, 24). The complex nature of the glomerular capillary wall with three distinct components has given rise to many questions regarding the specific roles of each of the major barriers, with arguments going from one extreme to the other (2, 6–9, 16, 20). Furthermore, the role of charge on the glomerular endothelium and basement membrane has been challenged anew (21, 26), even though it was once thought to be comfortably settled. In essence, almost every aspect of glomerular permselectivity is under new scrutiny, and we can only hope that the new studies will bring an enlightened understanding rather than further confusion.

The utilization of fluorescently labeled probes of varying molecular size and charge coupled with applications of modern two-photon confocal microscopy has provided a new approach to answer these unresolved questions. Although numerous studies have examined the permeability properties of the glomerular membrane, the authors emphasize that the high resolution of the new technology coupled with the extraordinary width of the glomerular basement membrane (GBM) in kidneys from Necturus maculosus (salamander or commonly known as the “mud puppy”) allows visualization and quantitation of the fluorescent molecules in the GBM as well as in Bowman’s space. This allows for a more detailed consideration of the specific barrier functions of the individual components of the capillary wall. Volumes have been written about barrier functions of capillary systems throughout the body (17, 20, 28), but it is recognized that the glomerular capillaries are among the most complex and challenging to understand.

Tanner and colleagues (27) make several important points. Certainly, one of the most important conclusions is that the overall glomerular permeability to large macromolecules, in particular albumin, is extremely low with a sieving coefficient in the range of <0.005. These data support many of the “classic findings” (1, 7, 12, 14) and are at variance with suggestions by Comper and associates (5, 6, 23, 24) that much greater amounts of protein are filtered by even normal glomeruli. Another conclusion is that charge selectivity could not be detected in the glomerular membrane of Necturus kidneys (4, 15, 18, 23, 26, 29). However, the data indicating that anionic charges do not seem to have an important role is a difficult one to generalize (7, 15, 16, 20). This issue is physiologically relevant as charge has often been used to explain the fact that serum albumin has a much lower glomerular sieving coefficient than would be expected from just its molecular size and/or effective molecular radius. So, if the charge on albumin does not restrict passage, then what does? Until this is answered, the statement that “charge has no detectable effect on filterability of these macromolecules” must remain in question. We should not forget the numerous studies that contributed to the conceptual framework regarding the importance of the electrostatic barrier (1, 7, 13, 15, 16, 18). For the same equivalent radius, the fractional clearances of albumin (3.6 nm) and negatively charged dextran sulfate are considerably lower than the clearances of uncharged molecules (4, 18). Thus differences in the transport of electrically charged macromolecules have been thought to be due to the membrane-bound polyanionic glycoproteins that are rich in sialic acid and heparan sulfate residues, which set up a negative electrostatic field that repels polyanions (9, 16). These are associated with the glycoprotein coat that covers the endothelial fenestrations, the basement membrane, and the epithelial cells. Partial loss of these anionic sites can lead to albuminuria in the absence of any gross structural abnormalities and in cases of mild glomerulonephritis (11, 22). Such a loss has been induced experimentally by neutralization of the electrostatic barrier with the polycation protamine. In more severe glomerular injury-associated proteinuria, a larger fraction of the filtrate appears to pass through a population of large-diameter, nonselective pores (7, 8, 11, 12). Importantly, the authors point out that the Necturus kidney has a much lower glycosaminoglycan concentration in the GBM than exists in the rat. Nevertheless, the sieving coefficient for albumin was still very low.

In addition to size and charge, molecular configuration influences the sieving coefficient (3, 19). Rigid or globular molecules such as horseradish peroxidase or Ficoll have lower glomerular sieving coefficients for any given molecular size than neutral dextran polymers with highly deformable linear structures (29). Because shape, flexibility, and deformability contribute to the quantitative relationship between molecular size and transglomerular solute flux, it has been challenging to establish the true dimensions of the extracellular channels. Data currently available indicate that the effective radius of the channels in the glomerular membrane is in the range of 4.5 to 6 nm (7, 8, 12, 14, 29). The data provided by Tanner et al. (27) provide fresh support to the conclusions based on older more conventional techniques.

Perhaps the most challenging issue addressed by Tanner et al. regards the barrier function of the main components of the glomerular capillary wall. As mentioned, the greater width of the GBM and the capabilities of the new technology allowed...
evaluation of the concentrations of the fluorescently labeled macromolecules within the GBM itself. The authors noted that the GBM/plasma concentration ratios for many of the macromolecules did not differ significantly from that of inulin and suggest further that these high concentrations indicate that GBM does not discriminate among these molecules. Because of these “surprisingly high” concentrations of macromolecules within the GBM, the authors suggest that the GBM only weakly impedes passage of large molecules. They are clearly justified in concluding that “the sharp drop-off in concentration that occurs between GBM and filtrate in Bowman’s capsule suggests that the podocyte layer is the major barrier restricting the passage of macromolecules.” However, they may have been too hasty in assigning the GBM such a minor role. Indeed, accumulation of macromolecules in GBM has been noted before in rats and was shown to be affected by blood flow (25). These data led Ryan et al. (25) to conclude that “the structural pores in the epithelial layer—more specifically, in the slit diaphragm region—are smaller than those in the GBM.”

Most studies involving quantitative consideration of macromolecular passage through capillary membranes have relied on the thermodynamic approach developed by Kedem and Katchalsky (10). Derivations for solute flux ($J_s$) across a constraining membrane include a convection term, which is the solute flux that occurs as a consequence of the bulk volume flow ($v$), and a diffusion flux, which is a function of the concentration gradient of the solute. Thus, solute flux due to both factors is defined as

$$J_s = J_C(1 - \sigma) + PS(\Delta C),$$

where $J_C$ is the volume flow (in this case the GFR), $C_C$ is the average concentration across the membrane, and $\sigma$ is the reflection coefficient. $\Delta C$ is the concentration difference across the capillary wall, and $PS$ is the diffusional, permeability surface-area product coefficient. With small uncharged molecules, such as glucose, $\sigma$ approaches 0 and thus glucose flux is simply defined by the product of GFR and the plasma glucose concentration. For very large molecules that are restricted with almost complete efficiency, $\sigma$ approaches 1 and thus solute flux due to convection is negligible. The most relevant example is for plasma albumin. Using a value of 1 mg/dl for albumin concentration in early tubular fluid and a systemic plasma albumin concentration of 3,600 mg/dl, $\sigma$ is >0.999. Furthermore, the $PS$ coefficient is so low (0.001 ml/min) that solute flux due to diffusion also approaches 0. These quantitative considerations also highlight the difficulty in attempting to evaluate mechanisms of proteinuria. Theoretically, an increase in protein passage across the glomerular membrane of 100-fold could be accounted for by a change in $\sigma$ from 0.999 to 0.90. Similarly, relatively small changes in $\sigma$ caused by the anionic residues could easily lead to a perceptible difference in the glomerular sieving coefficient (10, 14, 28).

However, what about the GBM? Does the accumulation of macromolecules in the GBM mean that it has a very low $\sigma$? I would contend that it does not. The accumulation of the solutes in the Necturus GBM only means that $\sigma$ of the GBM is less than $\sigma$ of the podocyte layer. However, the GBM could still have a $\sigma$ of 0.9 or even higher, which would contribute greatly to the exclusion of macromolecular passage and yet, with time, there would still be a slow accumulation of the solutes in the GBM. The consideration of permeability properties of barriers in series is quite complex and requires detailed quantitative analysis. Qualitatively, however, it can be concluded that if the second barrier has a higher $\sigma$ than the first barrier, then solutes will accumulate within the first barrier over time even if $\sigma$ of the first barrier is still relatively high. Thus some entry of these large molecules would still occur over time, and the concentration would continue to approach the plasma concentration because of the greater restriction to passage by the podocyte layer. Interestingly, the authors indicate in the legend to Fig. 6 in their article (27) that dextran fluorescence in the GBM appeared to increase with time and was greater at 5 h. This finding suggests that the GBM does restrict entry of the macromolecules. In essence, unless the rate of accumulation of the macromolecules in the GBM is carefully assessed, it can only be concluded that $\sigma$ is greater for the podocyte layer than for the GBM, but it is not justifiable to conclude that the GBM is a minor player in restricting passage of macromolecules. Perhaps the authors will consider a study evaluating the time-dependent changes in GBM macromolecular concentrations using fluorescent probes with different effective pore radii to obtain a better estimate of the actual restrictive properties of the GBM in the Necturus kidney. These data, coupled with a mathematical model evaluating the barrier functions of the three glomerular barriers in series, could provide an improved understanding of the relative roles of each component.

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REFERENCES


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