Illuminating the Glomerular Filtration Barrier, Two Photons at a Time

William H. Fissell
Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio


The mechanisms by which the kidney retains albumin and larger proteins in the circulation while excreting smaller solutes remain enigmatic despite intense investigation. Although the controversy largely plays out in academic journals, the problem is anything but academic, because the health of millions of adults hinges on effective treatment of proteinuric CKD. Proteinuria is associated with cardiovascular and all-cause mortality. Indeed, albuminuria has emerged as the single best predictor of progression of CKD, and control of proteinuria is clearly associated with preservation of GFR.

To illustrate the public health burden of CKD, the work by Coresh et al. used National Health and Nutrition Examination Surveys data to estimate that nearly 8% of the US population had moderate or severe reductions in GFR and slightly more than 8% had microalbuminuria. Almost all of the increased prevalence of albuminuria over the prior decade could be explained by increased prevalence of hypertension, diabetes, and obesity, suggesting that increased prosperity in the so-called developing world will bring an increased burden of proteinuric CKD.

In JASN, the work by Sandoval et al. presents a detailed description of factors influencing the accuracy of a recently developed technique (two-photon intravital microscopy) that has unexpectedly lent support to a controversial model of glomerular filtration and tubular reclamation. The importance of this paper is highlighted by background of the debate.

Although albumin circulates in blood at a concentration of 40 g/L, micropuncture studies have repeatedly reported albumin concentrations in Bowman’s capsule of 10 mg/L or less. Thus, physiologic models of renal protein handling have been based on the generally shared tenet that the glomerular capillary wall forms a stringent barrier to albumin passage. Indeed, clinical syndromes with proteinuria are characterized by ultrastructural changes to the glomerular capillary wall on electron microscopic examination. Conversely, tubulointerstitial disorders are typically associated with low-level proteinuria. Finally, the link between congenital nephropathy of the Finnish type, nephrin, and nephrin’s expression at the slit diaphragm codified the role of the glomerular filtration barrier in the language of molecular biology.

Albumin seems to enjoy a special status in measurements of glomerular integrity. Similar-sized polysaccharide molecules, such as ficoll, are hindered by the glomerular capillary wall to a lesser extent than albumin. However, measurements of macromolecular transport through well-defined artificial membranes or agarose gels have not shown similar discrepancies, and the differential in sieving coefficient between albumin and ficoll is much less pronounced in other capillary beds.

Insight into albumin’s special status in the kidney can be derived from studies of albumin metabolism by proximal tubule cells and reports of albumin fragments found in urine and the renal vein. Antibody-based assays seem to underestimate urinary albumin excretion compared with studies that use radiolabeled albumin. Thus, the work by Osicka et al. suggested that the low fractional excretion of albumin by the kidney might be attributable to tubular transport and metabolism rather than the unique properties of the glomerular capillary wall. However, because clinicopathologic and genetic data were most consistent with a glomerular barrier, a significant role for tubular albumin transport was difficult to integrate with prior models of renal physiology.

In an attempt to gain insight into the ongoing controversy by using a new technique, the work by Russo et al. employed the recently developed toolkit of two-photon intravitral microscopy to determine the concentration of albumin in Bowman’s capsule and measured a glomerular sieving coefficient for albumin (GSCa) of about 0.034 (or 50- to 100-fold higher than the coefficient determined from micropuncture studies). Furthermore, rats treated with puromycin aminonucleoside display reduced proximal tubule brush border albumin uptake and little change in GSCa. The appearance of this study in 2007 stimulated animated discussion about the two-photon technique and the significance of the results, and the reader may follow the controversy in the original literature. There have been technical criticisms of the 2007 paper. Others using the two-photon technique have obtained more conventional values for the filtration barrier. The high value for GSCa may be an artifact of low signal to noise ratio, inflating the measured values in Bowman’s capsule. Signal saturation or interference of passing red blood cells in the capillary artifactually may lower the values in the capillary loop, and a transmission electron micrograph interpreted as showing fusion of a vesicle laden with albumin with the basolateral cell membrane may have been either a fixation artifact or a chance occurrence.

The work by Sandoval et al. marks a significant reinforcement of the application of the two-photon technique to
glomerular physiology, because it highlights control of sources of artifact and addresses apparent discrepancies between their data and data from others. Amid the details of photodetector geometry and polymer polydispersion, two features emerge that deserve attention beyond discussions of technique. First, the two-photon technique reported in the work by Sandoval et al.\textsuperscript{10} measures a GSCA\textsubscript{L} in fasted Frömter strain Munich–Wistar rats that is much closer (GSCA\textsubscript{L} \textasciitilde 0.007) to the GSCA obtained from micropuncture studies than the 0.034 measurements (in a different strain) published in the 2007 paper.\textsuperscript{11} These values for GSCA may overlap values obtained by the work of Ohlson et al.\textsuperscript{19} using a chilled isolated perfused kidney from Wistar rats. This finding opens the possibility that the differences in measured values for GSCA between techniques might originate in differences between strains and physiologic conditions rather than the experimental error of which Russo et al.\textsuperscript{11} have been accused. Second, the work by Sandoval et al.\textsuperscript{10} presents multiple images of fluorescent intracellular bodies appearing to approach or fuse with the basolateral membrane, suggesting that the prior observation was not just a freak occurrence but a phenomenon that merits more quantitative examination.

The data still require caution in interpretation, but hopefully, they will redirect discourse to the mechanisms by which the apparent GSCA might have much greater spatial and temporal variation than previously imagined. Perhaps not all glomeruli are created equal, and by its nature, the two-photon technique only probes the behavior of glomeruli exactly at the surface of the kidney just like micropuncture studies.\textsuperscript{39} The roles of trauma, fasted/fed status, and other short-term physiology in altering glomerular permeability deserve increased mechanistic attention.\textsuperscript{40} Other studies support the hypothesis that albumin is taken up by proximal tubule cells and peptides from albumin proteolysis are observed on apical and basolateral sides of the epithelium; however, fluorescence microscopy cannot distinguish whether the fluorophore was bound to an intact albumin molecule or an albumin-derived peptide fragment.\textsuperscript{41} The data and the debate regarding the filtration barrier continue to accumulate while a record 113,636 patients started dialysis in the United States in 2009. Patients and practitioners alike are eager for mechanism-based treatments that will delay disease progression. The relative contributions of endothelium, basement membrane, podocyte, and tubule to renal protein handling in health and disease could be better understood just as we as physicians and researchers might better understand each other’s results.

DISCLOSURES
None.

REFERENCES
25. Greive KA, Balazs NDH, Comper WD: Protein fragments in urine have been considerably underestimated by various protein assays. Clin Chem 47: 1717–1719, 2001