Three-Dimensional Reconstruction of Glomeruli by Electron Microscopy Reveals a Distinct Restrictive Urinary Subpodocyte Space

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For more than 150 years, the only urinary space that has been recognized in the glomerulus conducting primary filtrate to the proximal convoluted tubule has been Bowman’s space (BS) (1). Here it is shown that ultrastructural reconstructions of the podocyte and the glomerulus reveal BS to be formed from three distinct urinary spaces through which filtrate must pass before reaching the proximal convoluted tubule. The most restricted region, the subpodocyte space (SPS; first described by Gautier in 1950), was found to cover 58 to 65% of the glomerular filtration barrier. It is morphologically distinct from the rest of BS and has a highly significant restriction to flow based on morphometric measurements. This SPS was altered during increased renal perfusion pressure, consistent with the podocyte dynamically reacting to the increase in filtration. A second anastomosing branching region draining the glomerular center, which has been termed the interpodocyte space, has fewer restrictions to flow into the final region—the shell-like peripheral urinary space. The physiologic role of the restrictive SPS is yet to be determined but likely possibilities include regulation of glomerular filtration and cleaning of the glomerular filtration barrier.

Urine is formed by modification of primary filtrate in the postglomerular nephron. This primary filtrate is formed by ultrafiltration across the glomerular filtration barrier, which consists of fenestrated endothelial cells, the glomerular basement membrane (GBM), and the slit diaphragms between podocyte foot processes (PFP).

Bowman’s space (BS) was originally defined as the microscopic space seen between the edge of the capillary tuft and the Bowman’s capsule of the renal corpuscle in the renal cortex (1). The size of the originally defined BS is not easy to demonstrate with light microscopic techniques because the glomerulus shrinks and swells with various fixation and tissue-processing methods (2,3). With the advent of the electron microscope, BS was described, from single ultrathin sections, to be continuous from the parietal cells lining the Bowman’s capsule down to the ultrafiltration barrier and the slit diaphragms between the foot processes of the podocytes (4,5). It is generally assumed that no further significant resistance to fluid movement occurs between the slit diaphragms of the glomerular ultrafiltration barrier and the exit into the proximal convoluted tubule. Deen et al. (6) in 2001 reviewed the structural representations used in modeling glomerular permeability, and for the glomerular ultrastructure, a paper published nine years earlier was referred to as sufficiently modern with no further morphologic advances having been made in the interim (7).

While considering the ultrastructural interactions between the GBM and the podocyte, it seemed to us that the processes that anchor podocytes to the GBM, directly attaching the podocyte cell body to the foot processes, were more common than had been assumed. Foot processes that arise directly from and anchor cell bodies to GBM are not described in recent structural reviews and papers on podocytes. Kanwar (7), Nagata and Kriz (8), and Mundel et al. (9) all asserted that normal podocyte cell bodies do not directly attach by foot processes to capillaries but attach by foot processes that have branched off primary processes that emerge from the cell body. These primary processes are described from scanning electron micrographs and are described according to their appearance from a plan view of the podocyte. However, transmission electron micrographs in all three papers show normal podocytes attached to the GBM by single long foot processes arising directly from the podocyte cell body or from primary processes but are covered by the cell body/primary process and are invisible to the scanning electron microscope. It is clear from the descriptions that these are not considered to be primary processes, as they are covered by the cell body. Admittedly, the proportion of foot processes that attach to the GBM directly from the cell body would be small compared with those from primary processes; nevertheless, they are present and are mentioned in earlier reviews. Elias (10) stated that the cell body contact with the basement membrane is substantially through the little feet or pedicels.
To avoid confusion with the foot processes arising from primary processes, we have called these single elongated foot processes anchoring processes. These anchoring processes that arise directly from either the podocyte cell body or occasionally from overlying primary processes form the boundaries and central elongated pillars of the subpodocyte spaces (SPS). The question arose as to whether anchoring processes and podocyte coverage could impede outflow of primary urine from the filtration slits to the proximal convoluted tubule.

To estimate whether this added resistance would be significant, the length and width of these enclosed channels plus the dimensions of openings that connect with the rest of BS needed to be assessed. However, there are no quantitative reports on the extent of the ultrastructural disposition of podocyte attachment to the underlying GBM. All of the transmission electron microscopy studies to date have relied on evidence from single sections or short runs of sections. To determine the contribution of these attachments to impedance of fluid flow, a three-dimensional (3D) view is required of the interior of the capillary tuft. To fully understand the size, shape, and physiologic significance of these attachments and the space that they enclose, we reconstructed the podocyte and its anchoring processes from micrographs of ultrathin serial sections. From these reconstructions, we have come to the startling conclusion that these podocyte attachments are so extensive that they form a distinct, restricted set of spaces underneath the podocyte that are continuous with the remainder of BS. As a result of our reconstruction, we have also defined a podocyte-lined tributary system that drains the restrictive urinary spaces, from the center to the glomerular edge, from which the filtrate then disengores into the shell-like peripheral urinary space between the edge of the glomerulus and Bowman’s capsule. We have determined some basic physical attributes of the SPS and the interpodocyte space (IPS) and the pores that lead from these urinary spaces.

A brief and simple analysis of these parameters shows that the restriction to flow is great enough from these SPS into the other urinary spaces that it is impossible to conceive that filtration into this space is of equal significance to the rest of the glomerular filtration barrier (GFB).

**Materials and Methods**

**Immersion Fixation**

Kidney fixation procedures were adapted and modified from Hayat (11). Wistar rats (n = 3; 300 g male) were killed by cervical dislocation (schedule 1 procedure according to UK Home Office regulations), and one kidney from each rat was rapidly excised and sliced in a pool of glutaraldehyde fixative (1% tannic acid and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3), approximately 10°C). Cubes (0.5 to 1 mm diameter) or slices <1 mm thick of kidney cortex were selected and further fixed at 4°C with glutaraldehyde fixative. After a minimum of 3 h of fixation, the tissues were left in fresh fixative overnight, then washed in 3× 10 min changes of 0.1 M cacodylate buffer (pH 7.3, 4°C). The tissues were postfixed for 1 h in either 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.3, 4°C) or 3% methylene tungstate (pH 7.3, 25°C) (12). Tissues were washed again in either cacodylate buffer and then distilled water (3×15 min changes, osmium fixed) or distilled water (4×15 min changes, tungstate fixed) before dehydration with ethanol, infiltration and embedding in Araldite resin (Agar Scientific, Stansted, UK).

**Perfusion Fixation**

Wistar rats (n = 5; 300 g male) were terminally anesthetized intramuscularly with a mixture of Hypnovel (Roche Products Ltd., Welwyn Garden City, UK) and Hypnorm (Jansen Animal Health, Ripley, UK), which comprised Hypnovel:water:Hypnorm in the ratio 1:2:1 (mixed in that order). Shortly after death, the abdominal aorta distal to the renal arteries was perfused at 100 mmHg. Upstream ligatures were used to prevent blood mixing with the perfusate, and the vena cava was cut to allow egress of perfusate and blood during fixation. Kidneys were perfused first with a flush of mammalian Ringer (at 37°C) that contained 5 IU/ml Heparin (Monoparin; CP Pharmaceuticals Ltd., Wrexham, UK) until the kidneys rapidly changed color with the clear perfusate, then the initial flush solution was changed to 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.35) at 37°C for 2 to 5 min followed by the same fixative for 5 to 10 min at 0°C. Small pieces (0.5 to 1 mm-diameter cubes) of kidney cortex were excised and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.35) at 4°C; after fixation of 2 to 4 h, tissue pieces were left in fresh fixative overnight. Kidney pieces were washed in 0.1 M cacodylate buffer washes (4×15 min), postfixed in osmium tetroxide for 1 h (1% OsO4 in 0.1 M sodium cacodylate buffer [pH 7.35], 4°C), and washed in distilled water (3×15 min). Dehydration was with ethanol, and tissues were infiltrated and embedded in Araldite resin (Agar Scientific).

**Sectioning and Reconstruction**

Survey sections (500 nm thick) were cut from each kidney and stained with Toluidine Blue (1% in 1% [aqueous] borax) for light microscopy. Glomeruli were identified before the cut surface was trimmed to include one to three glomeruli (clustered) in a smaller block face suitable for ultrathin serial sectioning. Serial section runs of 20 to 250 sections long and of 100-nm thickness were cut and laid on conductive carbon Formvar slot grids and stained with 3% (aqueous) uranyl acetate and Reynolds’ lead citrate solution (11,13).

Digital micrographs were taken on a Phillips 100CS microscope at ×2600 to show the disposition of a few podocytes in each field of view from section to section. The sectioned shape of capillaries, mesangial matrix, and red cells were used as positional cues to line up podocyte profiles from section to section. Micrographs of the same region on consecutive sections were used to reconstruct areas underneath the podocyte, the podocytic anchoring foot processes, the underlying GBM and the pores connecting the area underneath the podocyte (SPS) to the rest of the urinary space (exit pores). Adobe Photoshop software (Adobe Systems Inc., San Jose, CA) was used to highlight and reconstruct the SPS from these micrographs. To simplify reconstruction, potential SPS that were cut in cross-section (at right angles to the GBM) were reconstructed rather than SPS that were cut in a more difficult reconstructive plane at an oblique angle or in a plane parallel to the GBM.

**Analysis of SPS Characteristics**

Measurements were made on the SPS reconstructions to determine the restriction to fluid flow out of the SPS. The resistance to flow of fluid from the SPS to the rest of the urinary space will be determined by the integrated cross-sectional area of the outflow from the SPS, the relative viscosity of the fluid in the SPS, and the distance that fluid has to travel from the filtration barrier to the SPS exit pore (SEP; according to Poiseuille’s law). Because the resistance is determined by the fourth power of the radius (but by the first power of the length), the exit pore width and path lengths were measured. The width of SEP was measured at the narrowest point directly on the micrographs; a rule was adopted that the first narrowing that occurred between the other uni-
nary spaces upon entering the SPS was the SEP width in that section. For all of the sections (micrographs) encompassing a pore, the maximum width measured on a micrograph was taken as the “micrograph” diameter (2rM). In addition, the pore length in the sectioning direction was estimated by counting the number of sections that encompassed the whole pore and multiplying by the section thickness (0.1 μm) to give the sectioning diameter in μm (2rS). Although the exit pores are not perfectly elliptical, they do approximate an ellipse; consequently, the areas of these ellipsoidal SEP (ASEP) were calculated from the equation for the area of an ellipse (ASEP = π × rM × rS). The area of the GBM underlying an SPS where the exit pores were situated (AGBM) was estimated by summing all of the measured lengths of GBM underlying SPS from each section and multiplying by section thickness (0.1 μm). An area fraction was calculated to express the fraction of the GBM filter area represented by the SEP area (AF = ASEP/AGBM). AF represents the fractional reduction in area from the enclosed filtration area to the SEP (all fluid filtered through 1 unit of GBM area must pass through AF units of SEP area, assuming negligible fluid losses through the podocyte cytoplasm). The width of the space encapsulated by the SPS and through which fluid must pass to get to the SEP at the periphery of the podocyte was estimated at points selected using a random-number generator by measuring the separation between the podocyte cell body or covering membrane and the GBM or cell process lying over the GBM. These SPS height measurements also included zero height when anchoring processes coincided with the randomly chosen locations. The path length for fluid from the filtration membrane through the SPS to BS was estimated by randomly selecting 20 points on the GBM of SPS micrographs and tracing the least distance for each point to an SEP from the 3D reconstruction.

Statistical Analyses

Measurements are presented as mean ± SEM unless otherwise stated. Significant differences were detected by parametric statistics (paired or unpaired t test as required). For determining the power of the statistics, G-Power was used to determine effect size (d) and power (1 − β) on the basis of a probability cut off of 5% (P < 0.05) being significant. This analysis showed that for all statistics, power was >90%, and for peripheral versus central capillaries, power was >95%.

Results

The SPS

A typical example of an electron micrograph of a capillary cross-section from a rat glomerulus is shown in Figure 1A. The capillary lumena (L) are bounded by fenestrated endothelial cells (FEC), which are supported on a GBM. PFP lie on top of the GBM, and fluid passes through the slit diaphragms between these PFP into the space outside the basement membrane but bounded by the podocyte cell body (PCB). In this section, the filtration area across the GBM of L1, L2, and L3 drains into an area immediately under a podocyte (SPS). These spaces interconnect in other sections but are shown here as four main areas (Figure 1, B and C, highlighted in yellow) and have only a single direct connection with peripheral BS in this particular section, through the SEP (highlighted in red). The basement membrane is highlighted in green and represents the filtration barrier draining into this space from capillary L1, L2, and L3. Figure 1C shows the highlighted regions extracted from the electron micrograph, which were used for 3D reconstruction.

Figure 2 shows the area of basement membrane that drains into the yellow SPS (arrows) and that has a direct connection (red SEP) with the peripheral BS shown in Figure 1. This connection is tortuous from many points, such as point A, where fluid must filter through a narrow (100 to 200 nm), long (10 μm) channel, to reach the peripheral BS. Moreover, the areas of basement membrane highlighted with arrowheads drain into areas that have no direct connection in this section with BS. Figure 3 shows 30 consecutive serial sections used in the reconstruction of this SPS. The GBM is outlined in green, and only the SPS (not the cell cytoplasm or membrane) is shown in yellow. Over the entire drainage area of these 30 sections, the only connection between this space under the podocyte and the rest of the urinary space is the small pore highlighted in red, measuring 0.65 μm wide at its widest point and running for 10 sections, from section s98 to s107 (inset, 1.0 μm long). Sections farther than s107 and before s98 show that the exit is now closed. In the space underneath the podocyte at points where there is no lateral connection, connection to the rest of the SPS occurs out of the plane of the sectioning. There is, therefore, a urinary space underneath the podocyte that has intermittent connections with urinary space outside. The extent of the connections will determine whether this space forms a restriction to fluid flow and whether this is a functionally different component of the urinary filtration pathway or simply an anomaly of sectioning.

The SPS therefore is defined as a space on the urinary side of the GBM that is bound by the GBM and the foot processes on one side and the underside of the podocyte cell body, process, or membrane on the other. It forms an ultrastructural restriction between the filtration barrier and the freely continuous space between the parietal and visceral epithelial cells (podocytes). The restriction is due partly to the low height of the podocyte above the filtration barrier and the anchoring foot processes, which would baffle fluid movements within the SPS. Therefore, it is possible to determine whether a space is anatomically subpodocyte (or just in close association with a podocyte) only by serial section reconstruction. Figure 4 shows just such a reconstruction. In individual sections, the space that is considered to be an SPS is highlighted in yellow, the connections between that space and BS are colored red, and the GBM is in green. The next section is aligned by eye with the previous one, and this process repeated for the next 121 sections. Gaps have been left in the figure to facilitate clarity, particularly where some sections (nine in total) were lost. This reconstruction shows almost an entire SPS. It can be seen that for the entire reconstructed podocyte space, 121 sections or 12.1 μm deep by approximately 20 μm wide, there are only a few tiny exits into BS. All of the filtration fluid draining into this space across the GBM highlighted in green will need to pass through these pores before being able to reach the proximal tubule. The largest exit pore has been reconstructed two dimensionally to show an x–z section of the pore. It splits into two arms and has dimensions of 0.9 by 3.3 μm and a pore area of 2.3 μm². In this entire reconstruction, there were 22 pores, averaging <0.4 μm² each (8.6 μm² total) despite draining an area of 210 μm² of basement membrane. Fluid that crosses the filtration membrane therefore needs to pass through an SEP area that is 4% of the filtration area to get into BS.
To determine the distance that fluid has to travel through the SPS, we measured the mean path length from randomly selected points on the GBM to the rest of BS using 3D reconstruction. Figure 5 shows an example of how path length was measured. The distance from two points on the GBM were

Figure 1. (A) Electron micrograph of glomerular capillaries (lumen L₁, L₂, and L₃), associated podocyte cell bodies (PCB), and mesangial matrix (M). Bowman’s space (BS) is the space between the parietal epithelial cells and the podocyte foot processes (PFP). Fluid is generally believed to filter through the fenestrations of the endothelial cells (FEC), the basement membrane (BM), and the slit diaphragms of the PFP. Once the fluid has crossed the slit diaphragms, it is considered to be in free communication with all of BS and can easily bypass anchoring processes (AP). W, white cell attached to capillary luminal wall. (B) The filtration barriers of L₁ and parts of L₂ and L₃ shown in green drain into the highlighted yellow portion of BS, which is a subpodocyte space (SPS). The uncolored SPS2 shows no connection with the yellow SPS in all sections taken. Direct communication that determines the resistance to flow (narrowest part) into the rest of BS is highlighted in red. (C) The electron micrograph has been subtracted to show the SPS (yellow), the filtration barrier represented by the BM (green), and the connection between this space and BS (SPS exit pore [SEP]) shown in red. Scale bars = 5 μm.

Figure 2. The SPS acts to restrict the flow of primary urine. In this section (enlarged from Figure 1C), the only connection between the SPS and the peripheral urinary space (PUS) was through a single SEP. Any fluid filtering across the BM at the area marked by the arrows (e.g., at point A) would need to drain through this SEP to reach the rest of BS (B) or move out of the plane of section to a closer SEP. Fluid filtering across the BM through areas labeled with arrowheads must move out of the plane of this section to drain into BS elsewhere. Scale bar = 5 μm.

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measured as shown. For point “a,” the nearest exit pore was in a plane two sections away, so fluid would have to travel 0.2 μm out of the plane of the section and 0.5 μm along the plane of the section to give a total path length of 0.7 μm. For point “b,” the nearest exit pore is in the same section and can be reached within 2.1 μm. In six SPS, the mean path length from random points along the GBM to the nearest exit from the SPS was 4.7 ± 1.3 μm. To put this distance in context, fluid has to travel through the equivalent of a capillary diameter in length and one tenth in height.

A study of 41 capillaries that were chosen at random from sections of glomeruli from three immersion-fixed kidneys showed that 61 ± 2% of the filterable area of the GBM (excluding mesangium) filtered into an SPS and the other 39% filtered directly into the rest of BS. The filtration surface coverage by SPS in Table 1 has been split into surface capillaries and central capillaries. The peripheral capillaries were those adjacent to the urinary space next to Bowman’s capsule; the central capillaries were all other glomerular capillaries (i.e., inside the tuft). The results in Table 1 show that SPS coverage of the GBM is independent of position (central versus peripheral, 65.5 ± 4 versus 59.5 ± 3%; P > 0.05 unpaired t test).

The mean height of the SPS was measured from random sampling of 280 sections. The height followed a multimodal

Figure 4. (A) Reconstruction of a near-complete SPS. A single SPS was selected in a section, and sections on either side were examined to reconstruct the entire SPS. The space extended 12.1 μm, from section 36 to section 157. In nine sections, the preparation procedures resulted in damage, and no image could be taken. The SPS drained five luminal cross-sections (i.e., capillaries) and contained 22 exit pores (some are obscured by the viewing aspect). The mean size of the exit pores was 1.3 × 0.3 μm—a mean area of 0.4 μm² (8.6 μm² in total)—and this drained a BM area of 210 μm². One region of SPS (shown in orange) was connected to the rest of the SPS only through a narrow connection in section Y and so was initially colored differently. Scale bar = 5 μm. (B) Two-dimensional representation of pore shape shown in X above. The total length of the pore was 3.3 μm (running from sections 124 to 156), and maximum width was 0.9 μm
distribution with modes at 0.5 and 0.90 μm but 23.5% of the SPS heights being above 1.0 μm (Figure 10, Table 1). Within this SPS height distribution, some of the values were zero because anchoring processes attached to the GBM were at the randomly selected measurement site. The filterable area of the GBM under the SPS covered up by podocyte anchoring foot processes was measured as 11.3 ± 3.0% in 12 SPS. The sizes of the pores between the SPS and BS (the SEP) were measured from eight SPS from three glomeruli. The mean width or micrograph diameter (2rM) of the SEP was just 0.33 ± 0.043 μm. The mean length or sectioning diameter (2rL) of the SEP was 1.10 ± 0.25 μm (or 11 sections). This gives a mean area of the SEP of 0.285 μm².

To assess the restriction provided by the SPS, we estimated the ratio between the area of the SEP and the GBM area filtering into SPS. If there were no restriction of flow (i.e., no SPS coverage) then As would approach 1.0. The mean area fraction (or the area of the SEP relative to the GBM) was 0.044 ± 0.0018 (or 4.4%), indicating that the SPS area was <5% of the GBM area. This varied for each SPS from 0.25 to 8.7%.

The IPS

While making the serial section reconstructions, we noticed that the area through which fluid drained from the SEP was narrow and tortuous and also often narrowed before opening up into the peripheral part of BS between the edge of the glomerular tuft and the Bowman’s capsule. Figure 6 shows an example of a single section where clear channels run along the cell body of the podocyte, on the other side from the SPS. This is highlighted in orange. Filtered fluid needs to drain along these channels and tributaries in at least one plane, from the center of the tuft to enter the proximal convoluted tubule through the periphery of the glomerulus. No matter what the plane of sectioning, we noticed that this space was bound on both sides by podocytes—usually different cells. These channels drain into the peripheral remainder of BS through apertures that are sometimes smaller than the width of the IPS channels. We have termed this part of BS the IPS. The remaining peripheral part of BS we therefore have termed the peripheral urinary space (PUS) to distinguish it from the IPS. The IPS offers fluid a relatively lower resistance pathway than the SPS into the PUS and often drains capillaries directly (e.g., blue arrows, Figure 6). Figure 7 shows a larger scale section where the spaces have been defined by 3D reconstruction. There are three clear urinary spaces: the SPS, IPS, and PUS. In central capillaries of the glomerular tuft, 34.5 ± 4% of the basement membrane area drained directly into the IPS and 65.5 ± 4% drained into the SPS, whereas for capillaries on the periphery of the glomerular tuft, 20 ± 1.5% drained into the IPS, 20.5 ± 2% drained into the PUS, and 59.5 ± 2.5% drained into the SPS. Figure 8 shows the distribution of the filterable area into SPS, IPS, and PUS viewed schematically.

Effect of Perfusion Fixation

To investigate a possible role of the SPS in regulation of fluid filtration, five kidneys were fixed during perfusion at physiologic flow rates and pressures (100 mmHg arterial pressure). Eleven SPS were reconstructed, and measurements were taken as above. A few striking changes were seen upon reconstruction of SPS and measurements of the IPS. First, the mean area fraction of the SPS coverage did not change (Table 1). This suggests that the SPS is not disrupted by high flows, as has been suggested by Elias et al. (14). Second, the PUS enlarges dramatically during perfusion fixation (Figure 7), whereas it is closely opposed to the capsule in immersion-fixed kidneys (Figure 6). The change in parameters of the SPS is more subtle. The distribution of heights in the SPS was altered substantially by perfusion. The lower heights (closer to the SEP) show the podocyte getting closer to the GBM with both lower modes moving closer to zero (0.3 and 0.7 μm), indicating clamping down of the podocyte on the GBM (Figure 10, Table 1). The highest heights (in more cavernous areas), however, became more frequent, with 30% of all values being above 1 μm in height (Figure 10, Table 1), as opposed to 23.5% in immersion-fixed glomeruli, indicating ballooning of selective areas of the SPS. Moreover, the SEP width is 50% wider in immersion-fixed (0.330 ± 0.043 μm) than perfusion-fixed glomeruli (0.208 ± 0.028 μm; P < 0.05, unpaired t test; Table 1). There is therefore

Figure 5. To determine the mean path length from BM to the nearest SEP, measurements were made on reconstructed SPS (yellow). Five highlighted regions derived from micrographs of serial sections are shown from a larger series. Points were randomly selected along the glomerular BM (GBM; green) in the larger series, and distance was measured from that point to the nearest SEP (red). Two examples are given. Point “a” is a point on the BM that drains into a SPS that has no SEP in that section. The nearest SEP is 200 nm away (two sections) out of the plane of section. The distance therefore is measured from point “a” to the section that contains the SEP (blue arrow) line and then to the SEP (black arrows to red pore). Another point, “b,” is in a section that does have a SEP, and the shortest path length is shown (black arrows to red pore). Scale bar = 2 μm.
Table 1. Data collated from measurements made on micrographs or reconstructions from micrographs of serial sections

<table>
<thead>
<tr>
<th></th>
<th>Immersion Fixation</th>
<th>Perfusion Fixation</th>
<th>Unpaired t Test Immersion and Perfusion Comparison Unless Indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of kidneys</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>No. of SPS reconstructed</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>$A_F$ (%)</td>
<td>$4.4 \pm 1.8$ ($n = 6$; range 0.25 to 8.7)</td>
<td>$3.7 \pm 1.3$ ($n = 11$; range 0 to 14.8)</td>
<td>NS $P &gt; 0.05$</td>
</tr>
<tr>
<td>SPS height ($\mu m$)</td>
<td>multimodal: modes at 0.5 and 0.9 $\mu m$</td>
<td>multimodal: modes at 0.3, 0.7, and 1.3 $\mu m$</td>
<td>Multimodal distribution (see Figure 10)</td>
</tr>
<tr>
<td>Mean SEP maximum width$^a$ ($\mu m$)</td>
<td>$0.33 \pm 0.043$ ($n = 6$)</td>
<td>$0.208 \pm 0.028$ ($n = 5$)</td>
<td>Sign.diff. $P &lt; 0.05$</td>
</tr>
<tr>
<td>Mean SEP length$^d$ ($\mu m$)</td>
<td>$1.10 \pm 0.25$ ($n = 6$)</td>
<td>$1.06 \pm 0.33$ ($n = 5$)</td>
<td>NS $P &gt; 0.05$</td>
</tr>
<tr>
<td>Mean SEP area$^e$ ($\mu m^2$)</td>
<td>$0.291 \pm 0.070$ ($n = 6$)</td>
<td>$0.176 \pm 0.066$ ($n = 5$)</td>
<td>NS $P &gt; 0.05$</td>
</tr>
<tr>
<td>Anchorage area$^f$ (%)</td>
<td>$11.3 \pm 3.0%$ ($n = 12$)</td>
<td>$26.0 \pm 1.0$ ($n = 17$)</td>
<td>Sign.diff. $P &lt; 0.0001$</td>
</tr>
</tbody>
</table>

| No. of IPS measured      | 29                 | 12                 |                                                                    |
| Mean IPS width$^g$ ($\mu m$) | $0.74 \pm 0.06^i$ ($n = 29$) | $1.38 \pm 0.19^k$ ($n = 12$) | Sign.diff. $P < 0.001$ |
| Mean IPS exit pore width$^h$ ($\mu m$) | $0.61 \pm 0.08^i$ ($n = 29$) | $0.77 \pm 0.15^k$ ($n = 12$) | NS $P > 0.05^i$ |
| Central capillary coverage by SPS ($n = 3$) (34.5 $\pm$ 4% IPS$^l$) | $65.5 \pm 4$ % SPS$^l$ | $58 \pm 6.5%$ SPS$^m$ | NS $P > 0.05^l$ |
| Peripheral capillary coverage by SPS ($n = 3$) (40.5 $\pm$ 3% IPS, PUS) | $59.5 \pm 3$ % SPS$^l$ | $53 \pm 7%$ SPS$^m$ | NS $P > 0.05^m$ |

$^a$All data are means ± SEM unless otherwise specified. $A_F$ indicates area fraction; SPS, subpodocyte space; SEP, SPS exit pore; IPS, interpodocyte space; GBM, glomerular basement membrane; PUS, peripheral urinary space.

$^b$The fraction of the GBM filterable area into an SPS represented by the SEP area (expressed here as a percentage). Area fraction % = (SEP area/GBM filtration area) $\times$ 100.

$^c2r_M$ from micrograph. In all of the sections encompassing an individual SEP, the width was measured across the entrance to the SPS in the micrographs. For individual SEP, the largest value was taken as the SEP maximum width. The mean SEP maximum width for each SPS was used to calculate the tabulated values.

$^d2r_s$ (in sectioning direction). Number of sections that contain an open SEP on the micrograph $\times 0.1 \mu m$. The mean SEP length for each SPS was used to calculate the tabulated values.

$^e$The areas of SEP were calculated assuming them to be elliptical (area = $\pi \times r_S \times r_M$). SEP area = $\pi \times$ (SEP maximum width/2)(SEP length/2). Mean SEP area for each SPS was used to calculate tabulated values.

$^f$The fraction of the GBM area of the SPS occluded on the urinary side by the attachment of podocytic anchoring processes, expressed as a percentage. Anchorage area % = (anchor area/SPS GBM filtration area) $\times$ 100.

$^g$The width of the IPS channels (including tributaries) was measured every 4 $\mu m$ from the IPS pore at the narrowest point of the IPS channel (IPS pores leading into the rest of BS (IPS and PUS)). The single long foot processes that attach onto the GBM directly from podocytes, which we refer to as anchoring processes, seem to have reacted to the perfusion pressure by widening their attachment to the GBM. In contrast, the foot processes derived from the primary processes, which provide the bulk of the filtration surface, seem relatively unaffected.

$^h$The IPS also changes with perfusion; however, unlike the SPS, the mean IPS width distends from 0.74 ± 0.06 to 1.38 ± 0.08 $\mu m$ multimodal: modes 0.05 to 1.3 $\mu m$.
0.19 μm (P < 0.001, unpaired t test) (Table 1). However, the size of the exits from the IPS to the PUS does not significantly change (from 0.61 ± 0.08 to 0.77 ± 0.15 μm; P > 0.05, unpaired t test; Table 1), suggesting that the IPS exit pore is more tightly regulated than the rest of the IPS with the pore width constrained even during high flow. The majority of the SPS changes are consistent with a reactive response to the increase in flow or pressure, whereas the changes of the IPS are consistent with an increased flow forcing open most of the IPS but not the exit to the PUS.

Discussion

We describe here, for the first time, 3D reconstruction of the urinary spaces of the renal glomerulus. There are three distinct spaces that together form BS. Although the three dimensional reconstructions are entirely new and this technique has not previously been used to demonstrate these spaces clearly, the existence of one of them has been previously postulated. A series of papers in the 1950s and 1960s discussed the concept that the anchoring processes that tether the podocyte to the GBM could form a space beneath the podocyte. The concept of subpodocytic lacunae, or “lacunaire peri-capillaire,” was first proposed by Gautier et al. (15) in 1950, who claimed that these spaces were not identical with the lumen of Bowman’s capsule. Elias et al. (10,14) reported that the podocytes seemed to cover the foot processes either by their cell bodies or by extending membranes over the urinary side of the filtration membrane. These podocytic membranes had also been described by Pak Foy (16) in 1957. In 1965, Elias asserted that after filtering through the GBF, primary urine pooled in the SPS before being taken up in podocytic vacuoles. Intracellular “treatment” was followed by discharge into BS; Elias argued that unimpeded filtration across the filtration barrier might take place only if the podocytic membranes “actually burst in life” (14). This view of vacuolar uptake was subsequently discarded (probably correctly), unfortunately the concept of the SPS’s being a restrictive space was discarded with it.

The problem with early electron microscopic studies like those of Gautier and Elias was that all of the inferences arose from observations of single or isolated sections and, rarely, short runs of serial sections. These then were extrapolated to a 3D view, supported occasionally by stereology. Technical advances have made serial sectioning of tissues over many tens of micrometers more feasible than before. Computer reconstruction of the digitally acquired micrographs enables rapid assembly of the 3D view.

Whereas the membranes described by Elias occurred throughout the capillary tuft, subsequent scanning electron microscopic views of the surface of capillary loops on the surface of the capillary tuft (17) clearly showed exits from spaces covered by podocyte cytoplasm. The authors suggested that the podocytic membranes developed from flattened primary processes that failed to cover up fully the filtration mem-
brane and therefore did not present a significant barrier in glomerular filtration, a view that was reiterated by many, including Nagata and Kriz (8), who viewed the space as “usually communicating freely with the common urinary space of Bowman’s capsule.” However, scanning electron micrographs, which investigate only the surface of tissue or a fractured plane (least line of resistance), could not determine the location or the structure of the spaces that were covered by podocyte primary processes or membranes. To view underneath the podocyte, transmission electron microscopy and 3D reconstruction is necessary.

The 3D reconstructions carried out here demonstrate that this SPS forms an ultrastructurally (and possibly functionally) distinct space within the glomerulus. From the reconstructions, there seems to be a highly significant resistance between the SPS and the adjacent IPS or PUS and a less significant restriction between the IPS and the PUS. From the SEP dimensions and the area of filtration into the SPS, the resistance to the movement of fluid from the filtration barrier to the proximal convoluted tubule can be estimated.

Poiseuille’s law can be used to determine the relative increase in resistance on the basis of measurements of SEP radii and length, and known lengths and areas of the GBM. Poiseuille’s law states that the resistance (R) to a fluid flowing through a cross-sectional area of radius (r), length (l), and viscosity (η) is

\[ R = \frac{8\eta l}{\pi r^4} \]  

(1)

The relative resistance of the GFB to the SEP (R_{GFB}/R_{SEP}) therefore can be calculated for the simplest assumption of the

Figure 8. Parameters of the urinary spaces. (A) Capillaries in the center of the glomerular tuft have no direct connection with PUS. Of the capillary surface, 65.5% drains into an SPS and 34.5% drains into an IPS channel. (B) For capillaries at the periphery of the glomerular tuft, 20% of the surface area drains directly into the PUS, 20% drains into the IPS, and the remainder (60%) drains into the SPS. (C) Dimensions of the SPS from micrographs. The green bar shows the mean GFB drainage per section, the red bar shows the mean SEP width that drains that area of the SPS, and the height of the red bar above the green is proportionally the distance that fluid must travel on average to reach the SEP.

Figure 9. The SPS alters in response to an increase in perfusion pressure. (Ai) Electron micrograph showing a representative SPS from an immersion-fixed glomerulus. (Aii) The SPS (yellow) has a low height (h), a few narrow anchoring processes (ap), and exit pores (red) defined through serial section reconstruction. (Bi) Electron micrograph showing an SPS from a perfusion-fixed glomerulus. (Bii) Podocyte attachment to the GBM surface is through more extensive anchoring processes. The SEP is narrower than in the immersion-fixed glomerulus, and the SPS height is reduced in some areas and increased (not shown) in others. Scale bars = 5 μm.

Figure 10. Comparison of the frequency distribution of SPS height in immersion (○) and perfusion-fixed (▲) kidneys. The bin midpoints (bin width 0.2 μm) plotted show a multimodal distribution in SPS height. SPS height in the two lower modes decreases from the immersion-fixed values of 0.5 and 0.9 μm to 0.3 and 0.7 μm after perfusion fixation, which can be interpreted as the podocyte clamping down on the GBM. However, the proportion of measurements that were >1 μm increased from 23.5% of the total in the immersion-fixed glomeruli to 30% of the total in perfusion-fixed glomeruli, indicating ballooning of other parts of the SPS.
shape of the two areas in series (i.e., two circles), assuming that the fluid inside the SPS has the same viscosity as that that crosses the GFB:

\[ \frac{R_{\text{GFB}}}{R_{\text{SEP}}} = \left( \frac{r_{\text{SEP}}}{r_{\text{GBM}}} \right)^2 \left( \frac{l_{\text{GBM}}}{l_{\text{SEP}}} \right) \]  

(2)

The relative area of the GBM (same as GFB) to the SEP (\(A_{r} = 0.04\)) is equivalent to the square of the relative radii (\(r_{\text{SEP}}^2 / r_{\text{GBM}}^2\)). Therefore, the relative radius is 0.2 (\(r_{\text{SEP}} / r_{\text{GBM}}\)). The mean length from the GBM to the SEP is 4.65 \(\mu m\), and the length of the exit pore is 0.3 \(\mu m\) (0.3 and 4.65 \(\mu m\) assumed to be \(l_{\text{SEP}}\) minimum and maximum), whereas the length from the endothelial fenestrations to the slit diaphragm (start of the SPS) is approximately 0.2 \(\mu m\) (\(k_{\text{GFB}}\)). From these measurements, the relative resistance can be calculated from equation 2 to approximate between 1.1 \(\times 10^{-3}\) and 6.8 \(\times 10^{-3}\) or a 900- to 14,500-fold increase in resistance to flow between GFB and SEP, depending on the relative contributions of the SPS and the SEP. This is a very simplistic assumption, because it assumes that the permeability of the GFB and the SEP are the same, which is clearly unlikely, and it assumes that the free area for filtration equates to that of the basement membrane rather than the slit diaphragms. However, it does show that the SPS is a distinct restrictive space within the glomerulus. Considerably more work is required on the 3D structure of the SPS to determine the extent of this resistance. Moreover, there are other good reasons for why even this remarkably high figure might be a significant underestimation of the resistance to flow.

It is known that the podocytes, like many other cells, have a covering of glycosaminoglycans or a glyocalyx; this layer is arguably 30 nm thick with extensions up to 50 nm or more (18–20). Nearly one half (60 to 100 nm) of the 208-nm-wide SEP (perfusion fixed) will be clogged with glyocalyx. Many smaller exit pores would be completely blocked with flow-restrictive glyocalyx if one accepts the glyocalyx thickness reported by Rostgaard and Qvortrup (21), who managed to stain a glomerular endothelial glycocalyx three to five times thicker than the values above by perfusing with oxygen-carrier solutions.

**SPS Hydrostatic Pressure**

With such a small size of the SEP relative to the GBM area (\(A_{r} = 4\%\)), the size of the exit pore should dictate fluid movements in the SPS. The flow of fluid might be expected to inflate the space-inducing mechanical stress in the overlying podocytes, and this inflation was apparent in parts of the perfusion-fixed glomeruli. The SPS pressure can be predicted from the ratios of the resistances of the GFB and the SEP, because under steady-state conditions the flow into the SPS must equal the flow out. Just as the pressure in the capillaries can be calculated from the precapillary and postcapillary resistance ratio and the arteriolar and venular pressures, so the SPS pressure can be calculated from the ratios of the resistances of the GFB (\(R_{\text{GFB}}\)) and the SEP (\(R_{\text{SEP}}\), the net driving pressure or ultrafiltration pressure (\(P_{\text{UF}}\)), and the pressure in the rest of BS or IPS and PUS (\(P_{1-P}\)) (equation 3). \(P_{\text{UF}}\) is the pressure that drives fluid across the GFB and is the hydrostatic pressure difference across the GFB pushing fluid out of the capillary minus the colloid osmotic pressure sucking fluid back into the capillary (i.e., \(P_{\text{UF}} = \Delta P - \kappa \Delta \pi\); see equation 5 below).

It can be seen from equation (3) that as the resistance through the SEP approaches that of the GFB, so the pressure in the SPS approaches the midway point between the pressure in the rest of the urinary space (1+P) and capillary ultrafiltration pressure. If the SEP resistance significantly exceeds the GFB resistance, then the SPS pressure approaches the capillary ultrafiltration pressure. The regulation of the resistance across the SEP therefore is going to be a critical regulator of the pressure in the SPS, as well as the flow through the SPS.

\[ P_{\text{SPS}} = \frac{\frac{R_{\text{GFB}}}{R_{\text{SEP}}} \cdot P_{1-P} + P_{\text{UF}}}{1 + \frac{R_{\text{GFB}}}{R_{\text{SEP}}}} \]  

(3)

The pressure in the SPS therefore must be higher than in the rest of the urinary space (\(P_{1-P} \sim P_{\text{BS}} = 15\) to 25 mmHg). A possible indicator of the inflation pressure in the SPS has come from recent work on cultured podocytes by Morton et al. (22), who applied a pressure of 10 to 20 cmH2O (7 to 15 mmHg) to a small area of podocyte membrane and activated a stretch-sensitive channel on the podocyte surface. The identity of the stretch-sensitive channel was unknown, but it was postulated that stretch-induced calcium entry to the podocyte would raise local calcium concentrations, suggesting that these cells respond to “filtration pressure.” With the rediscovery of the SPS, we propose that this stretch channel may be involved in regulation of SPS pressure that is 7 to 15 mmHg above that in the rest of BS.

The surrounding medium for the podocytes in vivo is the primary urine in the urinary space of the SPS and the BS. It is known that \(P_{\text{BS}}\) is 15 to 25 mmHg (average approximately 20 mmHg), depending on species and technique (23–25). If the podocyte is regulating pressure via membrane stretch, then the SPS hydrostatic pressure could be held at 27 to 35 mmHg. Such a regulatory mechanism would involve intracellular calcium fluxes proposed by Morton et al. (22) and would require podocytic contraction (contractile closure of the SEP, filtration slits, or global contraction of the podocyte all could be contenders). In mechanically stressed cultured podocytes, the podocyte processes get thinner and longer and the cell body decreases (26). No apoptosis was reported in mechanically stressed podocytes, but stress fibers that organized radially with a focus on an actin-rich center formed. Stress fibers oriented radially would support the concept of inflation of the SPS beneath the podocyte because this distribution would provide a suitable platform to alleviate the stress of inflation from beneath. It is interesting that we did see an increase in the area of GBM covered by anchoring foot processes (Table 1), suggesting that the podocyte does display rapid reorganization of cell processes in response to the increased filtration rate and presumably pressure of the perfusion fixation. F-actin reorganization in podocytes in response to mechanical stress has been shown to occur, although it is not known whether anchoring processes contain actin (26). However, cultured cells do not reveal the
whole story, because calcium influx dominates the signal in cultured cells but calcium store release is more prevalent in intact glomeruli (27).

Effect of SPS on Fluid Filtration

Below we have considered the Starling forces driving fluid movement out of glomerular capillaries and the effect when fluid filters into an SPS. According to the Starling hypothesis,

\[ L_p \Delta P = \sigma \Delta \pi \]  

(4)

\( L_p \) is the hydraulic conductivity of the GFB, \( \Delta P \) is the hydrostatic pressure difference between the glomerular capillary and the urinary spaces of the glomerulus, \( \Delta \pi \) is the oncotic pressure difference between the glomerular capillary and the urinary spaces of the glomerulus, and \( \sigma \) is the reflection coefficient that is the fraction of oncotically active molecules reflected from the GFB (not passing into the urinary spaces). The net pressure that drives fluid across the ultrafiltration barrier is often defined as the \( \Delta P \); therefore,

\[ \Delta P = \Delta \pi - \sigma \Delta \pi \]  

(5)

For most oncotic macromolecules, \( \sigma \) is close to 1 for the GFB.

\[ \Delta P = \Delta \pi \]  

(6)

The protein concentration in BS has been shown to be very low (28), and the oncotic pressure within BS has been shown to be negligible (\( \pi_{BS} \approx 0 \) (25). Because the measurement of these parameters would be from fluid sampled from the PUS, it is assumed that the oncotic pressure in the lower resistance pathways of IPS and PUS is similarly small (\( \pi_{IPS} \approx 0 \)). On the basis that the barrier to the movement of proteins seems to be the GFB, we further assume that the oncotic pressure in SPS is also small (\( \pi_{SPS} \approx 0 \)). Therefore, for filtration directly into IPS and PUS where \( \Delta P \) is the \( \Delta \pi \) into IPS and PUS:

\[ \Delta P_{I+P} = (\pi_{CAP} - \pi_{I+P}) - (\pi_{CAP} - \pi_{I+P}) \]

\[ \Delta P_{I+P} = P_{CAP} - P_{I+P} - \pi_{CAP} \]

(7)

And for filtration into SPS where \( \pi_{SFS} \) is the \( \Delta \pi \) into SPS,

\[ \Delta P_{SPS} = (\pi_{CAP} - \pi_{SPS}) - (\pi_{CAP} - \pi_{SPS}) \]

\[ \Delta P_{SPS} = P_{CAP} - P_{SPS} - \pi_{CAP} \]

(8)

Where subscripts “I+P,” “CAP,” and “SPS” refer to IPS plus PUS, capillary, and SPS. If the hydraulic conductivity is assumed to be constant along the length of the glomerular capillaries (based on the indistinguishable ultrastructures at both podocyte covered and naked sites), then changes in capillary IPS+PUS and SPS hydrostatic pressure and capillary (plasma) oncotic pressure will be the factors that affect fluid filtration rate per unit area across the glomerular ultrafiltration barrier.

Fluid filtration rate per unit area of GFB into

\[ IPS + PUS = L_p(\pi_{CAP} - \pi_{I+P} - \pi_{CAP}) \]

(9)

Fluid filtration rate per unit area of GFB into

\[ SPS = L_p(\pi_{CAP} - \pi_{SPS} - \pi_{CAP}) \]

(10)

In equations 9 and 10, for constant \( L_p \), the direction of flow and the relative flow can be evaluated from the ultrafiltration pressures calculated from the Starling pressures. The Starling pressures \( P_{CAP}, P_{BS} \) and \( \pi_{CAP} \) quoted in various papers show a range of values dependent on species and technique (23,24,29). We used \( P_{BS} \) values as \( \pi_{I+P} \) values and opted for ‘mammalian’ values quoted by Navar et al. (25). We have set \( P_{SPS} \) varying from a low value of 7 mmHg greater than \( P_{BS} \) to a high value of 15 mmHg greater than \( P_{BS} \) (to represent the podocytic membrane stretch activation pressure quoted by Morton et al. [22]).

Starling Forces.

- Capillary hydrostatic pressure \( P_{CAP} \) 60 mmHg
- Capillary oncotic pressure \( \pi_{CAP} \) 30 mmHg (average)
- BS (IPS+PUS) hydrostatic pressure \( P_{BS}(P_{I+P}) \) 20 mmHg
- SPS hydrostatic pressure low \( i/P_{SPS} \) 27 mmHg. SPS hydrostatic pressure high \( i/P_{SPS} \) 35 mmHg

Resulting Ultrafiltration Pressures.

- IPS+PUS Ultrafiltration pressure \( P_{I+P} \) +10 mmHg
- SPS Ultrafiltration pressure low \( i/P_{SPS} \) +3 mmHg
- SPS Ultrafiltration pressure low \( i/P_{SPS} \) -5 mmHg

This simple illustration shows that filtration would occur in 40% of the GFB area that opens directly into IPS or PUS (\( P_{I+P} = 10 \) mmHg). However, adjacent areas of membrane opening into SPS show very little filtration or even transient resorption (or reverse filtration) (\( P_{SPS} \) range, 3 to −5 mmHg), and this would increase if \( P_{CAP} \) were decreased or \( P_{SPS} \) increased. This is possible due to the extremely high ratio of the post- to pre-SPS resistance. However, any filtration would have to be transient as the reverse filtration would quickly lead to collapse of the SPS (or equilibration of pressures) and hence reduce SPS pressure.

This constant pressure illustration can be complicated further if the variations in glomerular capillary pulse pressure are factored in. This raises the possibility of areas of GBM that undergo transient flow reversal across the GFB in time with the pulse pressure. Although further work is required to show whether this occurs in vivo, there is a very significant role for transient and intermittent flow reversal across a filtration barrier. One of the unexplained mechanisms of maintenance of the GFB is how the barrier is unclogged or cleaned. Many proposals have been made involving mesangial cells phagocytosing Ig aggregates (30), which clog the GBM in disease states, sulfated glycosaminoglycans of the GBM acting as anticlogging agents (31), or the hypothesis that the GBM is a concentrated gel that allows rapid fluid flow and slow macromolecular diffusion (32). The podocyte has also been proposed as a continuous filter cleaner becoming phagocytic in certain pathologic states (33), but details of the mechanism of how the filter is cleaned under normal conditions are not known. Our discovery of a highly significant resistance to flow through the SPS leads to the possibility that the mechanism for cleaning the filter relies on
the ability of the SPS to reverse the flow and cause transient or intermittent reverse filtration or reabsorption into the glomerular capillaries (perhaps in synchrony with the pulse pressure). Glomerular backwashing of a proportion of the GBM, linked with a dynamic podocyte (as evidenced above) and hence a movable SPS would allow cleaning of the whole filtration surface, not just the 60% covered at any one time by the podocytes.

Model Problems

This new subdivision of the urinary space in the glomerulus results in difficulties in the current concept of free movement of fluid into the tubular system once filtration has occurred. Our current calculations suggest that of the capillary area previously regarded as filtering fluid into the BS, less than half of this area (40%) is actually responsible for the vast majority of the filtration into the urinary spaces. In the SPS-covered areas, there is low or no net fluid exchange, depending on pressure regulation in the SPS. Models of glomerular filtration have not previously factored in this extra resistance. At the simplest level, modelers describe flow through the filtration barrier as occurring through repeating units, where a unit is specified as several fenestrations underlying an area of GBM and a single filtration slit opening into BS (34). If it is assumed that IPS and PUS are low-resistance pathways and therefore would fit with the old concept of filtration into a low-resistance BS, then these models now require the addition of units that open into the high-resistance SPS. The implication for estimates of GFB hydraulic conductivity with only 40% of the observable (with transmission electron microscopy) filtration surface area filtering fluid is that these original values must be 2.5 times higher than originally calculated for the same volume of fluid to filter out through the smaller surface area.

Conclusion

Here we have shown that 3D reconstruction of the glomerulus using serial section transmission electron microscopy reveals that the SPS, originally described as “lacunaire peri-capillaire” (15), forms a highly restrictive barrier to fluid flow. This current study is the first using 3D reconstruction to confirm that the SPS occurs in glomeruli and presents contorted pathways that restrict the movement of primary urine on its journey from GFB to the renal tubular system and that the podocyte can respond dynamically to changes in filtration rate. Ultrastructural reconstructions from serial sections have also defined the presence of two other separate urinary spaces (IPS and PUS), splitting our current concept of BS or urinary space into three parts: The SPS in close contact with the GFB and restrictive to the movement of filtrate, the IPS with less restriction to flow that drains the SPS or drains uncovered GFB directly, and PUS—the space originally conceived by Bowman around the edge of the capillary tuft—which drains the uncovered GFB on the edge of the capillary tuft and receives outflow from the IPS and peripheral SPS. The PUS guides the primary filtrate to the proximal convoluted tubule.

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References

Correction


In this editorial from the April 2005 issue of JASN, there was an error in listing the web site URL for the International Standard Randomized Controlled Trial Number registry.

The correct URL is www.controlled-trials.com. The publisher regrets this error.

Related Editorial


Dr. Vivette D’Agati commented on the report by Neal et al., found in the May issue of JASN, in an editorial that appears in the same issue (D’Agati V: And You Thought the Age of Anatomic Discovery Was Over. J Am Soc Nephrol 16: 1166–1168, 2005). The article by Neal et al. did not include the usual reference to this related editorial. The publisher regrets this oversight.