Measurement of Albumin Reflection Coefficient With Isolated Rat Glomeruli

Virginia J. Savin, Ram Sharma, Helen B. Lovell, and Dan J. Welling

ABSTRACT

Macromolecular permeability of the glomerular capillary has been inferred from the clearance of endogenous protein or infused macromolecules. Permeability is increased after treatment with polycations as well as after renal injury. It has previously been shown that the capillaries of glomeruli isolated from normal mammals expand or collapse in response to transcapillary albumin gradients and that the magnitude of the changes in capillary volume and in total glomerular volume are directly proportional to the applied oncotic gradients. In the experiments presented here, the volume responses of control glomeruli and of glomeruli treated with protamine (100 to 600 g/mL for up to 60 min) were used to calculate the albumin reflection coefficient, \( \sigma_{albumin} \), and the convectional permeability, \( P_{convectional albumin} = (1 - \sigma_{albumin}) \), of the capillary wall. \( \sigma_{albumin} \) for normal glomeruli was about 1 (\( P_{convectional albumin} = 0 \)); \( \sigma_{albumin} \) fell to a minimum of 0.2 ± 0.1 (\( P_{convectional albumin} = 0.8 \pm 0.1 \)) after incubation with protamine sulfate (600 g/mL) for 30 min. Retraction and fusion of podocyte foot processes and denudation of the underlying matrix was seen on scanning electron micrographs of protamine-treated glomeruli. These results confirm that it is possible to study macromolecular permeability of the glomerular capillary in vitro and to calculate \( \sigma_{albumin} \) independent of hemodynamic and systemic humoral influences. This method will permit the assessment of the effects of individual mediators of glomerular injury and the study of glomeruli from kidneys affected by experimentally induced or naturally occurring renal diseases.

Key Words: Glomerular capillary, albumin permeability, polycation, protamine, proteinuria

The glomerular capillary has a unique role in maintaining fluid balance. It is sufficiently permeable to water and small solutes to produce the initial filtrate of plasma and yet sufficiently impermeable to macromolecules to permit conservation of plasma proteins. The characteristics of the filtration pathway depend on a series of barriers that are both size and charge selective (1-4). Proteinuria is a function of the forces that act on the capillary wall as well as of the characteristics of the capillary barrier itself. The relative concentrations of the proteins that appear in the urine compared with their concentrations in the plasma fractional clearance have been used as the basis for modeling the character of the changes in the barrier and for speculation about the mechanisms of injury. Fractional clearance of midsize molecules is increased not only when there is a change in the permeability of the capillary wall but also when the glomerular perfusion rate is diminished as is observed after the infusion of angiotensin II (5). Thus, the relative contributions of hemodynamic factors and alterations in the glomerular capillary itself may be difficult to distinguish. Because of the complex dependence of molecular sieving on pressure and flow in individual capillaries, it is desirable to develop an in vitro model in which the effects of individual mediators of injury can be studied individually in the absence of perfusion and during blockade of many of the secondary cellular responses.

We have previously shown that glomerular volume varies as capillaries expand or collapse in response to transcapillary oncotic gradients. The magnitude of the change in glomerular volume is directly proportional to the oncotic gradient used (6). Capillary distension in hypoprotic medium is readily visible by light microscopy. An example of a capillary in medium with oncotic pressure of 17 mm Hg that was transferred to medium of 2 mm Hg is shown in Figure 1.

In this article, we further define the relationship between glomerular volume increment and effective
METHODS

Glomerular Isolation and Measurement of Capillary Volume Response to Oncotic Gradients

Glomeruli were isolated from the renal cortex of adult Sprague-Dawley rats by standard sieving techniques. Glomeruli selected for study were free of Bowman’s capsule and of visible arteriolar fragments (6). Isolation medium was isotonic to plasma and contained, in millimoles per liter: sodium chloride, 115; potassium chloride, 5; sodium acetate, 10; di-basic sodium phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 1.2; calcium chloride, 1.0; and glucose, 5.5. The oncotic content of the medium was varied by the addition of BSA at concentrations of 4, 6, or 8 g/dL or high-molecular-mass neutral dextran (252 kd) at concentrations of 4 or 6 g/dL. BSA was chosen because it may have physical characteristics similar to human albumin, a molecule of interest in relation to glomerular injury that should provide a sensitive probe for changes in the permeability barrier of the glomerular capillary. Neutral dextran of high molecular weight was chosen because data from sieving experiments indicate that the glomerular capillary remains impermeable to molecules with molecular radius >60 Å (dextran 0) even after the most severe injury (1). The oncotic pressure of the media was measured with a membrane oncometer (Model 4100; Wescor, Inc., Logan, UT). The function of this instrument is based on the transudation of water through a semipermeable membrane. The membrane used had a molecular mass cut-off of 30 kd (Amicon PM 30; Amicon Division, W.R. Grace and Co., Beverly, MA). The relationship between concentrations of solute and oncotic pressure may not be strictly linear because dimers and higher aggregates may be present. The values for the oncotic pressure of the media are shown in Table 1. The pH of the medium was adjusted to 7.4 by bubbling with 95% O2/5% CO2 before use.

The volume response of the glomeruli was examined in the following manner. An individual glomerulus was selected, held by gentle suction on a micropipette, and observed by videomicroscopy. Glomerular volume, which included both capillary and extracapillary extracellular spaces as well as intracellular spaces of the glomerular cells, was calculated

Table 1. Oncotic pressure of media used for volumetric studies

<table>
<thead>
<tr>
<th>Oncotic agent</th>
<th>Concentration (g/dL)</th>
<th>Oncotic Pressure (mm Hg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>1</td>
<td>3.2 ± 0.2 (4)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>16.2 ± 0.8 (5)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>39.4 ± 0.8 (2)</td>
</tr>
<tr>
<td>Albumin</td>
<td>1</td>
<td>2.3 ± 1.3 (4)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>17.5 ± 2.2 (8)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>24.8 ± 1.6 (4)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>37.7 ± 4.4 (4)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses are number of measurements of different solutions.
from the average diameter measured from the video monitor. After an initial period of observation, the medium was replaced by fresh medium of different oncotic composition. Volume changes consequent to this change in medium composition occurred within 5 s and were maintained for at least several minutes in both control glomeruli and in glomeruli that had been incubated with protamine to alter the permeability barrier (vide infra). \( \sigma_{\text{albumin}} \) was calculated by the glomerular volume before and 30 s after medium exchange.

The calculations of \( \sigma_{\text{albumin}} \) detailed in the following protocols are valid only if one assumes that the capillary contents are conserved during the period of observation. To determine whether this assumption is warranted, we observed glomeruli for up to 20 min after replacing albumin medium with albumin-free dextran medium. Glomerular volume was measured at 1-min intervals for 5 min and then at 5-min intervals. Medium was replaced with fresh medium every 5 min to assure that evaporative concentration did not play an important role in the volume observations. Because no significant hydraulic gradient is present across the capillary wall of isolated glomeruli with open arteriolar fragments (6), we interpreted any decrease in capillary volume as evidence of diffusional loss of intracapillary albumin across the capillary wall. This interpretation is valid even though diffusion of both albumin and dextran occurred at the remnants of the arterioles because there was no barrier to diffusion of solute at these sites and no driving force for net fluid flux that would result in a change in capillary volume.

Estimation of Albumin Reflection coefficient, \( \sigma_{\text{albumin}} \)

We used the following equation for the calculation of reflection coefficients:

\[
J_r = L_p \left( \sum \sigma_{\text{solute}} \Delta \pi_{\text{solute}} \right) \quad \text{Equation 1}
\]

This equation is valid when the arteriolar fragments are patent because there is no significant transcapillary hydrostatic pressure gradient under these conditions. In this case, equilibrium, defined as \( J_r = 0 \), occurs when there is no significant oncotic gradient, \( \Delta \pi \) (6).

Glomerular volume response to oncotic gradients was used to assess \( \sigma_{\text{albumin}} \) by three protocols.

Response to Isoosmotic Medium Change

Glomerular volume was first measured in BSA medium identical to that used for isolation. The plasma contained within the capillary lumens had had sufficient time to equilibrate with the medium in which BSA was the only oncotic agent, so that \( \Sigma \sigma_{\text{solute}} \Delta \pi_{\text{solute}} = 0 \). The bathing medium was then replaced with an albumin-free solution of neutral dextran of the same osmolality so that \( \pi_{\text{dextran}} = \pi_{\text{albumin \ initial \ medium}} \). After this exchange, equilibrium was rapidly reestablished by movement of fluid across the glomerular capillaries. The new glomerular volume was stable within 5 s and remained unchanged for several minutes. If very little plasma protein was lost, it follows that

\[
\sigma_{\text{albumin}} = \frac{V_{\text{capillary \ final}}}{V_{\text{capillary \ initial}}} \quad \text{Equation 2}
\]

Because \( \sigma_{\text{dextran}} = 1 \) and media concentrations were chosen such that \( \pi_{\text{dextran}} = \pi_{\text{albumin \ initial \ medium}} \), glomerular volume either remained the same or decreased. If the exchange of BSA medium for neutral dextran medium of the same oncotic pressure did not result in any net fluid flux or change in capillary volume, \( \sigma_{\text{albumin}} \) was 1. In contrast, if capillary volume decreased and equilibrium was achieved at a diminished capillary and glomerular volume, \( \sigma_{\text{albumin}} \) was less than 1. In these experiments, we are not able to precisely measure initial and final capillary volumes but have used changes in the total glomerular volume as a qualitative measure of capillary volume changes. Thus, exchange of BSA medium for iso-osmotic neutral dextran medium permitted a qualitative assessment of the reflection coefficient.

Determination of Isotonic Conditions

Equilibrium conditions were used to derive a unique value for \( \sigma_{\text{albumin}} \). We used successive approximations to establish isotonic conditions, i.e., those in which replacement of BSA medium by neutral dextran medium resulted in no change in glomerular volume. In this case, Equation 1 may again be used and \( \sigma_{\text{albumin}} \) may be rigorously defined by the ratio of the oncotic pressure of dextran and albumin that produces no change in capillary volume, \( V_{\text{capillary \ initial}} = V_{\text{capillary \ final}} \). Under these conditions:

\[
\sigma_{\text{albumin}} = \frac{\pi_{\text{dextran}}}{\pi_{\text{albumin \ initial \ medium}}} \quad \text{Equation 3}
\]

Volume Responses to Sequential Medium Changes

The glomerulus consists of several distinct spaces, including both intracellular and extracellular components. The capillary space constitutes about 23% of the total volume in medium with 4 g/dL of BSA (7). It is primarily this space that responds to changes in the oncotic concentration of the bathing medium. The increase in glomerular volume after changes in bathing media is a complex function that depends on the relative exchangeable volume of the glomerulus, the spatial arrangement of the capillaries, and the compliance and elasticity of the capillary wall, as well as on the permeability characteristics of the
filtration barrier. Nonetheless, we have previously shown that there is a direct relationship between the increase in glomerular volume and the oncotic gradient applied across the capillary wall (8). We used this principle to calculate \( \sigma_{\text{albumin}} \) by comparing the volume responses with albumin and neutral dextran.

If, for a given experimental condition, the relative exchangeable capillary volume and the elastic and distensibility characteristics of the glomerulus are constant, if \( \Delta V \) is proportional to \( (\sigma_{\text{solute}} \Delta x_{\text{solute}}) \), and if \( \sigma_{\text{dextran}} = 1 \), then

\[
\sigma_{\text{albumin}} = (\Delta V_{\text{albumin}}/\Delta x_{\text{albumin}})/(\Delta V_{\text{dextran}}/\Delta x_{\text{dextran}})
\]

Equation 4

where

\[
\Delta V = V_{\text{final}} - V_{\text{initial}} \quad \text{and} \quad \Delta x = x_{\text{initial}} - x_{\text{final}}
\]

Glomeruli for these studies were isolated in media with albumin concentrations up to 8 g/dL (38 mm Hg) or high-molecular-weight neutral dextran concentration of up to 6 g/dL (39 mm Hg). Medium was replaced in a stepwise fashion by solutions with lower concentrations of the same solute. Volume response was measured after 30 s.

We also calculated \( \sigma_{\text{albumin}} \) using the relationship between \( \Delta V_{\text{albumin}} \) and \( \Delta x_{\text{albumin}} \) of control and experimental glomeruli. In this case, we first determined that the volume response of control glomeruli to BSA and neutral dextran gradients did not differ, that is, \( \sigma_{\text{albumin control}} = 1.0 \), and that the responses of control and experimental glomeruli to neutral dextran were also the same, that is, \( \sigma_{\text{dextran experimental}} = 1.0 \). If these conditions are fulfilled, then

\[
(\sigma_{\text{albumin}})_{\text{experimental}} = (\Delta V_{\text{albumin}}/\Delta x_{\text{albumin}})_{\text{experimental}}/(\Delta V_{\text{albumin}}/\Delta x_{\text{albumin}})_{\text{control}}
\]

Equation 5

**Convectional Permeability**

Convectional permeability was defined as \( P_{\text{convectional}} = (1 - \sigma_{\text{albumin}}) \) to describe the movement of albumin consequent to water flow. This parameter is distinct from diffusional permeability, which is independent of solvent movement. When the reflection coefficient, \( \sigma_{\text{albumin}} \), is zero, albumin moves at the same rate as water and \( P_{\text{convectional}} \) is 1.0. When \( \sigma_{\text{albumin}} \) is one, albumin cannot cross the membrane with water and \( P_{\text{convectional}} \) is zero.

**Treatment of Glomeruli to Decrease \( \sigma_{\text{albumin}} \) and Increase Permeability**

Glomeruli were isolated as above and incubated in medium containing BSA and protamine (100 to 600 µg/mL) for 5 to 60 min. After treatment, glomeruli were observed during incubation in albumin-free dextran medium to assess diffusional losses of intracapillary proteins. Media exchanges were made to establish the volume response to iso-oncotic neutral dextran, to determine isotonic concentrations of BSA and dextran, and to define the volume response to albumin and dextran gradients.

**Morphologic Assessment of Glomeruli**

Samples of glomeruli were fixed in 2% glutaraldehyde after control or protamine incubation and examined by scanning electron microscopy with a JEOL JSM-35 (JEOL, Limited, Tokyo, Japan) scanning transmission electron microscope at magnifications of x1,000 to x10,000.

**Statistical Analyses**

Volume responses of control and experimental glomeruli were compared by nonpaired t tests. Values given in the text are mean ± SE. P values of <0.05 were considered significant. Values in text and figures are mean ± SE.

**RESULTS**

**Normal Glomeruli**

Volume of control glomeruli remained stable for up to 20 min after albumin medium was replaced by dextran medium of the same oncotic pressure (Figure 2). This finding was consistent with a very slow diffusional loss of albumin in the absence of filtration. As noted above, the calculation of \( \sigma_{\text{albumin}} \) in nonperfused glomeruli requires the use of the assumption that intracapillary volume and solute content are constant within the first 30 s after medium change. The fact that capillary volume was maintained for many minutes after the substitution of high-molecular-weight dextran for albumin in the bathing medium supports this assumption.

**Response to Iso-oncotic Gradients, Definition of Isotonic Conditions**

Replacement of albumin medium (\( \sigma_{\text{albumin}} = 17.5 \) mm Hg) by approximately iso-osmotic neutral dextran medium (\( \sigma_{\text{dextran}} = 18.2 \) mm Hg; \( \sigma_{\text{dextran}}/\sigma_{\text{albumin}} = 1.04 \)) resulted in no significant change in glomerular volume (\( \Delta V = -1.3 \pm 0.7\% \); \( N = 10 \)). This finding is consistent with \( \sigma_{\text{albumin}} \) not different from 1.0.

**Response to Sequential Medium Changes**

The volume response of control glomeruli isolated in dextran medium of 39.4 mm Hg and transferred to medium of sequentially lower dextran concentrations is illustrated in Figure 3. In this figure, we have
Glomerular Volume Increment, %

![Glomerular Volume Increment Graph](image)

Figure 2. Time course of decrease in glomerular volume after replacement of albumin-containing medium by isotonic albumin-free dextran medium. Medium was changed at time 0. No significant volume change occurred at the time of medium change. Volume of control glomeruli (circles) remained constant for up to 20 min. We have interpreted this as evidence that there was no significant diffusional loss of intracapillary solutes during incubation. Volume of protamine-treated glomeruli (crosses) diminished gradually during the observation period. We have interpreted this finding as evidence of increased diffusional permeability in protamine-treated glomeruli. Despite this gradual decrease, volume during the first several minutes was not significantly different from the initial volume. We have used these data to support our assumption that intracapillary solute is conserved during the first 30 to 60 s after medium exchange.

### Protamine-Treated Glomeruli

Glomeruli were isolated in 4 g/dL of BSA medium, incubated in medium with protamine (600 μg/mL) for 30 min, and observed during incubation with 1% dextran medium. As noted below, the initial change to 1% dextran medium did not result in a significant change in glomerular volume. Glomeruli were observed for 20 min to determine whether the assumption that albumin loss was very slow was valid even for protamine-treated glomeruli. Glomerular volume decreased by less than 0.1% during the first minute, by 3% at 5 min, and by a total of 7% at 20 min (Figure 2). The gradual volume loss indicated concurrent loss of solute (plasma proteins) and water from the capillaries of protamine-treated glomeruli. This loss was more rapid in protamine-treated glomeruli than in control glomeruli but was still slow enough that measurements of volume responses in the first 30 s after media change could be used to estimate δ_{albumin}. As noted above, diffusion of plasma proteins from the openings of arteriolar fragments was balanced by inward diffusion of dextran and did not result in net water movement.

### Response to Iso-oncotic Medium

When BSA medium (17.5 mm Hg) was replaced by neutral dextran medium (18.2 mm Hg) the volume of protamine-treated glomeruli diminished in every case. The average volume increment was -4.6 ± 0.8% (N = 11). Because the diffusion of albumin out of the capillary was very slow even in these glomeruli, as shown in Figure 2, we have interpreted this result as evidence that δ_{albumin} was less than 1 after incubation with protamine (Equation 2).

### Definition of Isotonic Conditions

The conditions for which BSA and dextran media were isotonic, i.e., did not result in a significant volume change, were established by trial and error. The replacement of BSA medium of 18.2 mm Hg by dextran medium of 2.3 mm Hg oncotic pressure resulted in a volume increment of 0.3 ± 0.2% (N = 14); this value was not different from 0. The reflection coefficient calculated from the ratio of dextran and albumin concentrations (Equation 3) was 0.13.

---

**Definition of Glomerular Reflection Coefficient**

The glomerular reflection coefficient, δ, is a measure of the relative concentration of albumin and dextran in the glomerular capillary lumen. It is defined as:

\[ δ = \frac{C_\text{dextran}}{C_\text{albumin}} \]

where \( C_\text{dextran} \) is the concentration of dextran and \( C_\text{albumin} \) is the concentration of albumin in the glomerular capillary lumen. This coefficient is typically used to study the permeability of the glomerular capillary wall and the exchange of solutes between the plasma and Bowman's space.
Volume Responses to Albumin and Dextran Gradients

The volume response of protamine-treated glomeruli to neutral dextran gradient exchange is illustrated in Figure 4. The relationship is similar to that for control glomeruli. These observations are consistent with the interpretation that net contributions of the relative exchangeable capillary volume and of the physical characteristics of capillary compliance are not markedly altered by neutralization of polyanions.

The volume response of glomeruli incubated with protamine (600 μg/mL) for 30 min after sequential changes in albumin concentration is also shown in Figure 4. The finding that the volume response to albumin gradients was markedly diminished compared with the response of the same glomeruli to dextran gradients or of control glomeruli to albumin or dextran gradients coupled with the observation that the response to dextran was not changed confirms that \( \sigma_{\text{albumin}} \) is decreased after protamine treatment.

The volume response of additional glomeruli isolated in 4 g/dL of albumin medium, treated with protamine (600 μg/mL for 30 min), and washed with 1 g/dL of BSA medium averaged only 1.6 ± 0.2% (N = 38). This response was much less than that of glomeruli that had not been treated with protamine (6.0 ± 0.4%; N = 27). \( \sigma_{\text{albumin}} \), calculated from the ratio of the responses of individual protamine-treated glomeruli and the average response of control glomeruli after 4- to 1-g/dL albumin washes (Equation 5), was 0.27 ± 0.04 (N = 38).

The volume response of glomeruli isolated in 4 g/dL of dextran, treated with protamine, and transferred to 1 g/dL of dextran was 5.0 ± 0.5% (N = 11). \( \sigma_{\text{albumin}} \), calculated with the ratio of responses of individual protamine-treated glomeruli to an albumin gradient and the average response of protamine-treated glomeruli to a comparable dextran gradient (Equation 4), was 0.32 ± 0.04 (N = 38).

The effects of 30 min of incubation with several concentrations of protamine were tested. Volume response was significantly diminished after incubation with 100 μg/mL of protamine. Incubation with 200 μg/mL produced an additional decrement, whereas the responses after incubation with 400 or 600 μg/mL were similar. \( \sigma_{\text{albumin}} \), calculated from Equation 5, is shown in Table 2.

Additional experiments were performed to determine the duration of incubation with protamine (600 μg/mL) required to achieve a minimum \( \sigma_{\text{albumin}} \). Volume response to albumin gradients was slightly diminished after 5 min and decreased progressively after incubations of 15 and 30 min. The volume response remained at about the same low level after incubation of up to 60 min. \( \sigma_{\text{albumin}} \) is shown in Table 3.

Morphology of Glomeruli

Isolated glomeruli used in these experiments were free of Bowman's capsule and appeared intact by

| Table 2. \( \sigma_{\text{albumin}} \) after incubation of isolated glomeruli for 30 min with protamine in varying concentrations\(^a\) |
|-------------------|-------------------|
| Protamine Concentration (μg/mL) | \( \sigma_{\text{albumin}} \) |
| 100               | 0.7 ± 0.2 (13)    |
| 200               | 0.5 ± 0.2 (12)    |
| 400               | 0.2 ± 0.1 (14)    |
| 600               | 0.2 ± 0.1 (21)    |

\( a \) Values indicate mean ± SE. Numbers in parentheses are number of glomeruli studied.

| Table 3. \( \sigma_{\text{albumin}} \) after incubation of isolated glomeruli with protamine (600 μg/mL) for varying durations\(^a\) |
|-------------------|-------------------|
| Duration of Incubation (min) | \( \sigma_{\text{albumin}} \) |
| 5                 | 0.9 ± 0.1 (9)     |
| 15                | 0.5 ± 0.1 (9)     |
| 30                | 0.2 ± 0.1 (21)    |
| 40 to 60          | 0.2 ± 0.1 (13)    |

\( a \) Values indicate mean ± SE. Numbers in parentheses are number of glomeruli studied.
light microscopy. The capillary lumens at the periphery were visible by standard illumination, and epithelial cell bodies could be distinguished over some loops. Capillary lumens in 4 g/dL of BSA medium were open, had slightly oval cross-sections, and were neither maximally distended nor collapsed. Replacement of incubation medium with hyperoncotic medium resulted in capillary collapse, whereas hyponcotic medium resulted in capillary distension. Scanning electron microscopy of isolated glomeruli revealed podocyte bodies that were closely adherent to the capillary and normal foot process morphology (Figure 5).

During protamine incubation in 4 g/dL of BSA medium, glomerular capillary configuration and glomerular diameter did not change. However, differential interference videomicroscopy (magnification, X400) showed that the epithelial cell bodies became more prominent during incubation. The replacement of incubation medium with BSA medium of more or less oncotic pressure resulted in little change in capillary volume compared with the changes observed in control glomeruli.

The impression of altered epithelial cell morphology after incubation with protamine was confirmed by scanning electron microscopy as shown in Figure 6. After 60 min of incubation in 600 µg/mL of protamine, epithelial cell bodies were enlarged and their attachment to the capillary wall was interrupted, leaving only tenuous attachments. The foot processes of many podocytes had retracted and fused.

DISCUSSION

The permselectivity of the capillary wall is determined by a combination of size and charge barriers (1-4). Charged substances in the glomerular capillary wall include the anionic glycosaminoglycans, heparan sulfate (9,10) and chondroitin sulfate, and sialoproteins, including podalyxin of the glycoprotein...
crease charge density has been noted in both naturally occurring and experimental renal diseases. Dextran sieving studies in proteinuric rats and in humans with diverse renal diseases show both size- and charge-selective defects. A dynamic charge barrier, maintained by filtration, is also important in preventing the filtration of anionic molecules.

The epithelial cells also contribute significantly to the permeability barrier, as evidenced by the fact that agents that acutely injure cells may cause proteinuria. Podocyte morphology and structure of the intracellular slit-pore junctions are altered after loss of fixed charges through neutralization by polycations, through enzymatic digestion of extracellular matrix components, or after injury to glomerular epithelial cells. In some states, proteinuria may be the result of detachment of epithelial cells from the basement membrane.

Infusion of protamine was shown to cause foot process "fusion" by Seller et al. in 1975. Systemic or intrarenal infusions of protamine or other polycations have been used to mimic nephrotic proteinuria and enhanced filtration of other macromolecules such as ferritin. The magnitude of albumin excretion is directly related to the dose of polycation, and proteinuria may be prevented or reversed by infusion of heparin. Anionic sites may not be immediately accessible to neutralization because about 1 h was required for maximum binding of cationic dextran to isolated glomeruli.

In these experiments, normal glomeruli showed $\sigma_{\text{albumin}} = 1.0$ and diffusional permeability is nearly 0. Perturbation of the system by neutralization of anionic charge resulted in a marked decrease in $\sigma_{\text{albumin}}$ and a smaller increase in diffusion. $\sigma_{\text{albumin}}$ was diminished after as little as 15 min of incubation with 600 $\mu$g/mL of protamine and fell to a minimum of 0.2 after 30 min. The decrease in $\sigma_{\text{albumin}}$ was also dose dependent. Dose and time dependence are analogous to those after intrarenal perfusion and are consistent with the hypothesis that protein leak is related to neutralization of charges to which protein has only limited accessibility. Alternatively, a cellular mechanism such as swelling or retraction of foot processes may be required to produce the protein leak. Our observations of gradually increasing prominence of epithelial cell bodies during protamine incubation and of apparent retraction of foot processes observed by scanning electron microscopy are consistent with an important role for epithelial cell attachment and structure in modulating protein permeability.

The use of isolated glomeruli permits us to assess the capillary permeability barrier directly. The finding that replacement of albumin medium by dextran medium of the same oncotic pressure resulted in an immediate loss of capillary volume provides a rigorous demonstration that $\sigma_{\text{albumin}}$ was decreased after protamine incubation. The minimum value for $\sigma_{\text{albumin}}$ determined from the null point for volume change ($J_v = 0$) during exchange of albumin for dextran medium was 0.2, and comparable values for $\sigma_{\text{albumin}}$ were derived from glomerular volumetric responses. The observation that protamine-treated glomeruli had volumetric responses to dextran that were not different from those of untreated glomeruli is consistent with the interpretation that capillary volume and compliance are not markedly altered by protamine and that diminished $\sigma_{\text{albumin}}$ accounts for the differences in volume responses.

There are at least two possible explanations for the apparent differences between values for $\sigma_{\text{albumin}}$ in our experiments and during filtration in vivo. First, the injury that we produced in vitro may far exceed any that occurs in vivo. The findings that the decrease in $\sigma_{\text{albumin}}$ was maximal only after prolonged incubation with a high concentration of protamine and that there was morphologic evidence of marked alterations in podocyte morphology with extensive areas of denudation of glomerular basement membranes are consistent with this postulate.

Second, the use of nonperfused and nonfiltering glomeruli to define $\sigma_{\text{albumin}}$ may reveal a mechanism for controlling protein permeability that has not been previously recognized. The convectional movement of a molecule through a membrane depends on both the characteristics of the pores through which the solute must pass and on the driving forces, including the effective solute concentration in the pore. We have assumed that the local concentrations on the inside and outside of the nonperfused capillary are identical to the concentrations in the bathing media and that the concentration in the pore is the average of these. These assumptions are the same as those made in modeling in vivo filtration and are most likely valid in the first instants after medium exchange and in experiments in which there is no volume change. If the effective gradient in vitro was diminished because albumin within the capillaries was prevented from entering the pore, then the calculated value for $\sigma_{\text{albumin}}$ decreases. A diminished gradient might occur in vivo because of laminar flow or because of the presence of a dynamic charge barrier. A decreased effective gradient could also result from trapping of filtered albumin in the filtration pathway either within the extracellular matrix or on the urinary side of the capillary barrier; these mechanisms might be important during steady states or reverse filtration. Further experiments will be required to determine which of the postulated explanations is correct.

In the interim, diminished values of $\sigma_{\text{albumin}}$ in vitro indicate...
the presence of altered macromolecular permeability and provide us with a means to assess the degree of injury to the capillary filtration barrier.

In summary, we have developed a method for assessing the macromolecular permeability of the glomerular capillary in vitro and have used this method to document that morphologic and functional changes after polycation treatment are independent of hemodynamic factors. In this model, control glomeruli have $\sigma_{\text{albumin}}$ that is not different from that of dextran (252 kDa) and exhibit no measurable diffusion of albumin during 20 min of observation. Incubation with protamine reduced $\sigma_{\text{albumin}}$ markedly and also increased albumin diffusion.

This method offers a unique opportunity to determine a numeric value for $\sigma_{\text{albumin}}$ after a variety of experimental manipulations. Hemodynamic influences on the filtration of solutes are eliminated, and the reflection coefficient and diffusional permeability may be assessed independently. Individual mediators may be studied and blockers may be used to dissect the mechanisms of injury. The in vitro method may also be applied to human renal disease by measuring the volumetric responses of glomeruli isolated from percutaneous renal biopsies.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grant 22040 and by grants from the Kansas Affiliate of the American Heart Association, the Merit Review Board of the Veterans' Administration, and the BRSG at the University of Kansas Medical Center.

REFERENCES


