Glomerular size and charge selectivity in the rat as revealed by FITC-Ficoll and albumin

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Ohlson, Maria, Jenny Sörensson, and Börje Haraldsson. Glomerular size and charge selectivity in the rat as revealed by FITC-Ficoll and albumin. Am J Physiol Renal Physiol 279: F84–F91, 2000.—The fractional clearances (θ) for FITC-Ficoll and albumin were estimated in isolated perfused rat kidneys in which the tubular activity was inhibited by low temperature (8°C) and/or 10 mM NH₄Cl. The Ficoll data were analyzed according to a two-pore model giving small and large pore radii of 46 Å and 80–87 Å, respectively. The estimated negative charge density was 35–45 meq/l at 8°C. Perfusion with erythrocyte-free solutions of kidneys at 37°C reduced glomerular size and charge permselectivity. Thus the large pore fraction of the glomerular filtrate (f_L) was 1.64% at 37°C compared with 0.94% at 8°C. The θ for albumin was four times higher at 37°C than at 8°C (0.86% vs. 0.19%, respectively). NH₄Cl caused further irreversible damage to the glomerular barrier. We conclude that there are no deleterious effects on the glomerular barrier of a reduction in temperature from 37°C to 8°C. Therefore our data seem to disprove the hypothesis of low glomerular permeselectivity and transtubular uptake of intact albumin and support the classic concept of a highly selective glomerular barrier.

The maintenance of an intact glomerular barrier to molecules with Stokes-Einstein radius (SE) is always used to describe molecular size, since it is derived from the free diffusion constant. Also, the passage of anionic molecules is restricted, whereas that of cationic ones seems to be enhanced (5).

Recently, an alternative hypothesis of glomerular permselectivity was presented (29). Isolated kidneys, removed by en bloc dissection, were perfused with recirculated filtered 5% BSA in a Krebs-Henseleit buffer at 37°C. The tubular reabsorption was inhibited by the use of various drugs, i.e., NH₄Cl. Dextrans with a_SE of 26–50 Å were used to determine whether the glomerular permselectivity was unaffected by the drugs. The θ for albumin in the control situation was 0.75–0.9%, increasing to ~7% when the drugs were used. To explain these high θ values, the authors suggested that a new transtubular cell pathway must be responsible for the return of intact albumin from the tubular lumen to the blood (29). Also, the glomerular charge selectivity was suggested as being insignificant.

However, the use of dextran as a transport probe for the ideal neutral sphere has been questioned. Rennke et al. (30) used the protein horseradish peroxidase (HRP) and found that the θ values for its cationic, neutral, and anionic forms were less than those for dextran with similar size and charge. Dextran forms random-coiled spheres in free solution and are vulnerable to deforming forces. It was suggested that dextran is subjected to unfolding during convective transport across the glomerulus. Dextran would thus behave as if it was of smaller dimensions than assumed. Oliver et al. (27) found that Ficolls of various radii had a lower θ than dextrans of equal a_SE. This implies that Ficoll might be a better probe for the measurement of the equivalent small and large pore radii. Also, Blouch et al. (3) found that over a molecular radius interval of 20–70 Å, θ for a given Ficoll was uniformly lower than the corresponding θ for a dextran of equal molecular radius, both in healthy and nephrotic humans. Solute shape is in fact highly important for its glomerular passage and may actually outweigh size and charge (22).

One way of inhibiting the tubular activity is low temperature. Reduced temperature inhibits tubular function as well as energy consumption and myogenic tone (8, 12), and it reduces protease activity without detectable changes on capillary permeability (33).
Thus at present there are two dramatically different views of glomerular permeability: the classic highly selective barrier, and a new hypothesis of “leaky” glomerular capillaries with massive tubular uptake. To understand the reason for the disparate results, we decided to test some of the experimental conditions used by Osicka et al. (29). We used the isolated perfused kidneys (IPK) to estimate the clearances for albumin and Ficoll with or without inhibited tubular activity, obtained by low temperature (8°C) and/or NH₄Cl.

METHODS

Kidney Perfusion Technique

Fifteen male rats weighing between 250 and 310 g (Wistar strain; Møllegaard, Stensved, Denmark) were used. The rats were kept on standard chow and had free access to water prior to the experiments. The local ethics committee approved the experiments.

We used a modification of the isolated perfused rat kidney preparation described by Johnsson and Haraldsson in 1992 (18) that has been described in detail previously (37). Anesthesia was induced by an intraperitoneal injection of pentobarbital (50 mg/kg; Apoteksbolaget, Umeå, Sweden), and a thermostatically controlled heating pad maintained the body temperature of the rat at 37°C. The tail artery was cannulated to establish a route for subsequent administration of drugs and for recording of the arterial pressure (Pₐ). The experiments were performed at either 8°C or 37°C.

Both kidneys from six rats were used in the cold experimental setup. The rat was eviscerated, and the intestines were removed. Cannulation of both ureters (PE-25 cannulas) was facilitated by enhanced diuresis after injection of furosemide (2 mg/kg; Benzon Pharma, Copenhagen, Denmark) and saline (0.4 ml). The rat was heparinized (1,000 IU; Lövens Läkemedel, Malmö, Sweden), and the aorta was thereafter cannulated in a retrograde direction, thus allowing for artificial perfusion of the kidneys by use of a peristaltic pump (model IPC-04 V1.32; Ismatech, Zurich, Switzerland). A second cannula was inserted into the thoracic aorta with the tip close to the right renal artery. The two kidneys were perfused with completely separate perfusion lines by ligating the aorta between the renal arteries.

Nine kidneys from nine rats were used in the warm experiments. The first part of the preparation did not differ from the cold setup, except that only the left ureter was cannulated. The aorta was ligated distal to the renal arteries and cannulated in a retrograde direction, providing the kidneys with both perfusate and their own circulation. Following a ligature on the aorta proximal to the left renal artery, the caval vein was cut open.

Care was taken not to touch the kidneys during the preparation, and the kidneys were fully perfused with either blood or perfusate during the entire preparation.

Near the aortic inlets, T-tubes connected to pressure transducers (PVB Medizintechnik, Kirchensee, Germany) recorded mean Pₐ values. The urine was collected in small vials and continuously weighed for calculation of urine flow. A computer (PC 586), using Labview computer software, monitored Pₐ and urine weight changes as well as urine flow and pump speed.

Perfusates

Three different perfusates were used, all based on a modified Tyrode solution containing human albumin (18 g/l; Pharmacia & Upjohn, Uppsala, Sweden). The standard perfusate contained the following: 113 mM NaCl, 4.3 mM KCl, 2.5 mM CaCl₂, 0.8 mM MgCl₂, 25.5 mM NaHCO₃, 0.5 mM NaH₂PO₄, 5.6 mM glucose, nitroprusside (0.9 mM; Merck, Darmstadt, Germany), and furosemide (10 mg/l, Benzon Pharma). For the second perfusate, 2 g/l of FITC-labeled Ficoll (Ficoll-70; Bioflow, Uppsala, Sweden) was added to the standard solution. The third perfusate was obtained by adding 2 g/l of FITC-Ficoll and 10 mmol/l NH₄Cl. The perfusates were protected from light and bubbled with 5% CO₂ in O₂. All solutions were made with fresh distilled water (MilliPore) with a resistivity of 18.2 MΩ/cm. The pH was 7.4 and kept stable during the experiments. Essential amino acids (Vamin, 14 g/l; Pharmacia & Upjohn, Uppsala, Sweden) were added to the perfusates used at 37°C. The concentrations of the individual amino acids were as follows (in mmol/l): 1.6 glycine, 0.4 aspartate, 0.6 glutamate, 2.7 alanine, 1.0 arginine, 0.07 cysteine, 0.7 histidine, 0.7 isoleucine, 1.0 leucine, 0.9 lysine, 0.6 methionine, 0.7 phenylalanine, 0.9 proline, 0.6 serine, 0.7 threonine, 0.1 tryptophan, 0.02 tyrosine, and 0.9 valine. We also added 2–3 mmol/l mannitol to the perfusates used in the warm experiments. Mannitol is an oxygen radical scavenger and an osmotic diuretic often used in, for example, treatment of acute renal failure. The perfusates used at 37°C also contained ⁵¹Cr-labeled EDTA (0.37 MBq/l; Amersham Pharmacia Biotech, Buckinghamshire, UK) and ¹₂⁵I-labeled albumin (2 MBq/l; Isopharma, Kjeller, Norway). The tracer was eluted on an equilibrated desalting column (Sephadex G-25 PD-10; Amersham Pharmacia Biotech, Uppsala, Sweden) to reduce the free iodide content and then added to the perfusates.

Experimental Protocol

The perfusion started with the standard solution for 20 min. Then the perfusate with FITC-Ficoll was used for about 15 min, after which the perfusate containing both FITC-Ficoll and NH₄Cl was used for another 15 min. Finally, the kidneys were perfused with the standard solution with FITC-Ficoll. Total perfusion time was approximately 1 h. During each perfusion period, three to five urine samples were collected for determination of glomerular filtration rate (GFR) and values for Ficoll and albumin. Albumin concentrations were measured by radioimmunoassay (Pharmacia Upjohn Diagnostics, Uppsala, Sweden) in the cold series. For the experiments performed at 37°C, ¹₂⁵I-albumin concentrations were measured in a gamma counter (Cobra, Auto-Gamma Counting systems; Packard Instrument, Meriden, CT). Corrections were made for background activity and ⁵¹Cr-EDTA spillover. For calculation of GFR in the experiments performed at 8°C, a urine-to-plasma (U/P) ratio for ⁵¹Cr-EDTA of 1.15 was used in accordance with previous measurements at 8°C (15, 18–20, 22).

Analysis of Ficoll Concentrations

The perfusates and the urine samples were subjected to gel filtration on a Superose 12 PC 3.2/30 column (SMART HPLC; Amersham Pharmacia Biotech, Uppsala, Sweden) for calculation of the sieving coefficients for FITC-Ficoll. The total bed volume of the column was 2.4 ml, and the void volume was 0.97 ml. A 0.05 M phosphate buffer with 0.15 M NaCl with pH 7.0 was used as an eluent. A 20-µl sample was analyzed at 490 nm with a flow of 40 µl/min. The pressure was ~1.2
MPa and the temperature was kept at 8°C during the analysis. The distribution of FITC-Ficoll in the perfusate and in the urine, from a representative experiment at 37°C (control situation), is shown in Fig. 1. The estimation of θ for Ficoll is determined with a high degree of precision. Thus the single-wavelength noise for the µPeak Monitor (SMART HPLC) is <1x10^-4, i.e., the error is less than 10% if the absorbance is 0.001 absorbance units (AU). Absorbance levels less than 0.001 AU were all outside the chosen molecular range and were not included in the analysis. We estimated the error in U/P ratios for Ficoll to be <1% for most molecular sizes, increasing toward 10% for the smallest and the largest Ficolls.

**Calibration Curve**

Six monodisperse samples of Ficoll with known molecular radii, generously provided by Dr. Torvald Andersson (Amersham Pharmacia Biotech, Uppsala, Sweden); blue dextran 2000, vitamin B12, thyroglobulin, ferritin, albumin, ovalbumin, aldolase, chymotrypsinogen A, and RNase A, were used to obtain a calibration curve on the Superose 12 PC 3.2/30 column (SMART HPLC). The relationship for FITC-Ficoll is well described by y = 1.53 - 0.114Ln(x), with a correlation coefficient (r) of 0.999. The protein curve is given by y = 1.32 - 0.0893Ln(x), with r = 0.990.

**Fractional clearance for albumin and Ficoll.** The renal clearance (Cl) of a solute, x, can be estimated from the amount excreted in the urine (Cu) during a certain time period over the plasma concentration (Cp), i.e., Cl = (Cu/ Cp) · Q. The θ of a solute is given by its Cl over GFR. The two-pore model. The exchange can be described using the following parameters: small pore radius, rs, large pore radius, rl, the large pore fraction of the glomerular filtrate, fL, and finally the unrestricted pore area over diffusion distance, A0/Δx. The net fluxes of fluid and solutes are calculated for each pore pathway separately using nonlinear flux equations (32). In the analysis, A0/Δx was assumed to be equal to or larger than 10,000 cm. The viscosity of water, unique for every molecular radius, was included in the model. The temperature will influence charge interactions as evident from the equations for Debye length (see Ref. 37). The effect is, however, small (5%) and was therefore not included in the analysis.

**Charge selectivity.** The effects of molecular charge were estimated using the Donnan concept of charge-charge interactions as described in a previous report (37). The crucial parameter to determine is the concentration of fixed negative charges within the gel, ω.

**Statistics**

Results are presented as means ± SE, and differences were tested using the Wilcoxon signed rank sum test or Student’s t-test where appropriate.

**RESULTS**

**General**

Mean values ± SE for P0, pump flow (Q), vascular resistance (PRU100), GFR, and θ for albumin during the different perfusion periods at both 8°C and 37°C are presented in Tables 1 and 2, respectively. The θ for albumin was measured before the perfusate with FITC-Ficoll was introduced, and there was no significant difference of FITC per se compared with the control period. However, there was a time-dependent increase in θ for albumin of 1.4% per hour at 37°C, described as θalbumin = 0.0144t + 0.0032, where t is time in hours (Fig. 3). At 8°C, the increase in θalbumin
during control situation, during perfusion with NH4Cl, respectively. At 8°C, Q, ml/min 7.8 ± 0.3, PRU100, mmHg 61.7, GFR, ml min⁻¹ (g ww)⁻¹ 0.211 ± 0.016, and θalbumin 0.0017 ± 0.0007 were 0.021 ± 0.008, 0.132 ± 0.012, 0.0028 ± 0.0011, and 0.040 ± 0.0015, respectively (Table 1).

Values are means ± SE for recorded parameters at 8°C during the four perfusion periods; n = 8. PA, arterial pressure; Q, pump flow; GFR, glomerular filtration rate; PRU 100, vascular resistance; ww, wet weight; θalbumin, fractional clearance of albumin.

### Table 2. Summary of recorded parameters at 37°C

<table>
<thead>
<tr>
<th></th>
<th>Perfusion Before FITC-Ficoll</th>
<th>Control</th>
<th>Perfusion with NH4Cl</th>
<th>After NH4Cl Perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pₐ, mmHg</td>
<td>61.7 ± 3.4</td>
<td>74.3 ± 2.9</td>
<td>80.5 ± 2.6</td>
<td>82.8 ± 2.7</td>
</tr>
<tr>
<td>Q, ml/min</td>
<td>14.9 ± 0.59</td>
<td>14.9 ± 0.60</td>
<td>13.1 ± 0.79</td>
<td>12.2 ± 0.96</td>
</tr>
<tr>
<td>PRU₁₀₀ mmHg·min⁻¹ (100 g ww)·ml⁻¹</td>
<td>0.054 ± 0.004</td>
<td>0.062 ± 0.005</td>
<td>0.081 ± 0.008</td>
<td>0.094 ± 0.010</td>
</tr>
<tr>
<td>GFR, ml min⁻¹ (g ww)⁻¹</td>
<td>0.265 ± 0.019</td>
<td>0.254 ± 0.021</td>
<td>0.200 ± 0.019</td>
<td>0.193 ± 0.017</td>
</tr>
<tr>
<td>θalbumin</td>
<td>0.0043 ± 0.0006</td>
<td>0.0086 ± 0.0012</td>
<td>0.0187 ± 0.0038</td>
<td>0.0136 ± 0.0018</td>
</tr>
</tbody>
</table>

Values are means ± SE for recorded parameters at 37°C during the four perfusion periods (n = 8).

was slightly less than the 0.30% per hour previously determined (θalbumin = 0.0030 ± 0.0023) (15).

### Sieving Coefficients for Ficoll

The sieving coefficients for FITC-Ficoll were not significantly different between 8°C and 37°C for the various perfusion periods used. Figure 4 shows the sieving coefficients for FITC-Ficoll after perfusion with 10 mM NH₄Cl. The θ values for Ficoll with aₜrans of 36 Å at 8°C were 0.021 ± 0.003, 0.037 ± 0.006, and 0.035 ± 0.004, during control situation, during perfusion with NH₄Cl, and after perfusion with NH₄Cl, respectively. At 37°C, the θ values for Ficoll with aₜrans of 54 Å were 0.0011 ± 0.0002 during control, 0.0019 ± 0.0003 with NH₄Cl, and 0.0017 ± 0.0003 after NH₄Cl. At 37°C, the θ values for Ficoll with aₜrans of 36 Å were 0.029 ± 0.002 during control situation, 0.037 ± 0.002 during perfusion with NH₄Cl, and 0.035 ± 0.003 after perfusion with NH₄Cl. The θ values for Ficoll with aₜrans of 54 Å at 37°C were 0.0017 ± 0.0003, 0.0025 ± 0.0005, and 0.0021 ± 0.0005 during control, with NH₄Cl, and after NH₄Cl, respectively.

### Two-Pore Model Analysis

The Ficoll data were analyzed according to a two-pore model (32). The results from that analysis are presented in Table 3. The small pore radius was rather precise at both 8°C and 37°C, being 45.5 ± 0.36 and 46.8 ± 0.25 Å, respectively. Moreover, the small pore radius did not differ between the different perfusion periods (Fig. 5). The large pore radius varied between 80 and 87 Å, at the different temperatures and for the different perfusion periods that were used. The large pore fraction of the glomerular filtrate (f₁) was 1.64 ± 0.12% at 37°C compared with 0.94 ± 0.13% at 8°C (P < 0.05, n = 8) (Fig. 6). Perfusion with NH₄Cl increased f₁ even further to 2.42 ± 0.15% at 37°C (P < 0.05, n = 8) and 1.72 ± 0.10% at 8°C (P < 0.01, n = 8) (Fig. 6). The increase in f₁ was not completely reversible when returning to the perfusate without NH₄Cl. During control, both at 8°C and 37°C, the pore area over diffusion distance (Aₓ/Δx) reached the lower limit of 10,000 (Table 3). Perfusion with NH₄Cl induced recruitment of pore area, giving Aₓ/Δx of 196,000 ± 24,000 cm at 8°C (P < 0.001, n = 8) and 53,200 ± 9,120 cm at 37°C (P < 0.01, n = 8) (Table 3). The recruited pore area did not return to control when returning to the perfusate without NH₄Cl (Table 3).

### Sieving Coefficients for Albumin

During control, the θ for albumin was higher at 37°C than at 8°C, 0.0086 ± 0.0012 and 0.0019 ± 0.0006, respectively (Fig. 7) (P = 0.002, n = 8). Moreover, NH₄Cl increased the θ for albumin to 0.019 ± 0.0038 at 37°C (P < 0.01, n = 8) and to 0.0028 ± 0.0011 at 8°C (not significant (NS), n = 8) (Fig. 7).
and analysis for the parameters can also be tested by performing the pore experiments in each group. The stability of the mean pore analysis are shown in Table 3. They are based on the standard perfusate (Fig. 8). Infusion of 10 mM of NH₄Cl caused a fall in the geometric mean of the ratios was 4.2 with an SE of 1.4 and 11 (n = 8). Infusion of 10 mM of NH₄Cl caused a fall in the ratio to 2.9 (NS), where it remained when returning to the standard perfusate (θ ratio to 2.8). The average charge densities at 37°C were 24.2, 19.5, and 19.0 meq/l, respectively, with the latter two being significantly less than those at 8°C (P < 0.05, n = 8) (Fig. 8).

Stability of the Two-Pore Parameters

The standard errors of the four parameters from the pore analysis are shown in Table 3. They are based on the mean θ values for Ficoll and albumin of similar size (36 Å). From these ratios, the charge densities (ω) in the glomerular membrane were determined (37) and found to be unevenly distributed. Geometric means were therefore calculated, and at 8°C the θ ratio was 13.4 with a standard error in the mean (SE) of +6.4 and −4.3 (n = 7). During and after infusion of 10 mM of NH₄Cl the θ ratios were 14.4 and 12.1, respectively. These values correspond to average charge densities of 38.6, 39.5, and 37.3 meq/l (Fig. 8). At 37°C the geometric mean of the θ ratios was 4.2 with an SE of +1.4 and −1.1 (n = 8). Infusion of 10 mM of NH₄Cl caused a fall in the θ ratio to 2.9 (NS), where it remained when returning to the standard perfusate (θ ratio to 2.8). The average charge densities at 37°C were 24.2, 19.5, and 19.0 meq/l, respectively, with the latter two being significantly less than those at 8°C (P < 0.05, n = 8) (Fig. 8).

Glomerular Charge Density

Fractional clearance ratios were calculated from the θ values for Ficoll and albumin of similar size (36 Å). This is from these ratios, the charge densities (ω) in the glomerular membrane were determined (37) and found to be unevenly distributed. Geometric means were therefore calculated, and at 8°C the θ ratio was 13.4 with a standard error in the mean (SE) of +6.4 and −4.3 (n = 7). During and after infusion of 10 mM of NH₄Cl the θ ratios were 14.4 and 12.1, respectively. These values correspond to average charge densities of 38.6, 39.5, and 37.3 meq/l (Fig. 8). At 37°C the geometric mean of the θ ratios was 4.2 with an SE of +1.4 and −1.1 (n = 8). Infusion of 10 mM of NH₄Cl caused a fall in the θ ratio to 2.9 (NS), where it remained when returning to the standard perfusate (θ ratio to 2.8). The average charge densities at 37°C were 24.2, 19.5, and 19.0 meq/l, respectively, with the latter two being significantly less than those at 8°C (P < 0.05, n = 8) (Fig. 8).

Stability of the Two-Pore Parameters

The standard errors of the four parameters from the pore analysis are shown in Table 3. They are based on the mean θ values for each molecular radius from all the experiments in each group. The stability of the parameters can also be tested by performing the pore analysis for the θ distribution x − SE and x + SE. Thus, for x − SE at 8°C and control situation, the rₛ = 45.0 ± 0.03 Å, rₓ = 84.4 ± 1.7 Å, fₓ = 0.98 ± 0.13%, and Aₓ/Δx = 10,000 cm. For x + SE at 8°C and control situation, the rₛ = 46.5 ± 0.38 Å, rₓ = 88.8 ± 2.5 Å, fₓ = 0.99 ± 0.15%, and Aₓ/Δx = 35,500 cm. Thus all pore parameters were stable and rather precise.

DISCUSSION

This study supports and extends the classic view of the highly selective glomerular barrier. First, our data seem to disprove the recently launched “albumin retrieval” hypothesis (29), which suggests a low glomerular permeability together with massive tubular uptake of intact albumin. The low θalbumin at 8°C, a temperature expected to strongly inhibit endocytotic uptake of filtered proteins (25, 26), provides strong evidence in favor of the classic view of high glomerular permselectivity.

The present θalbumin of 0.19% is far less than that reported from Osicka et al. (29), where the tubular uptake of proteins was inhibited by various toxins, i.e., 150 mM lysine, 10 mM NH₄Cl, 0.1 mM chloroquine, and 0.02 mM cytochalasin B. Under such conditions, θalbumin reached 7%, which led the authors to postulate the presence of a transtubular cell pathway transporting intact albumin from the tubular lumen to the blood. The present study offers limited scope for such mechanisms.

Indeed, previous morphological studies in rat (2) and in humans (34) indicate that almost no endogenous albumin enters the urine. Also, Ryan and Karnovsky (35) showed that as long as the kidneys were perfused, no detectable amounts of albumin entered the urine (35). Moreover, albumin entering the proximal tubule seems to be degraded (24). Against this background, we suggest that the glomerular barrier may have been damaged in the studies by Osicka et al. (29), resulting from the ischemia-reperfusion, accumulation of substances in the perfusate due to recirculation, and ultimately resulting from toxic effects of the drugs used to inhibit tubular uptake.

Second, the Ficoll data show that glomerular size selectivity is well described by a two-pore model. The functional small pore radius is close to 46 Å, whereas the less frequent large pores have a functional radius of 80–90 Å. Our results are well in accordance with the previously noted clearance values for Ficoll in humans (3) and in rat (28). The large pore radius found in the present study is similar to that found when using two lactate dehydrogenase (LDH) isoforms as tracer molecules (23), being 75–85 Å. Note, however, that the functional pore radii in the classic dextran studies

Table 3. Results of the two-pore analysis

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Perfusion Period</th>
<th>rₛ, Å</th>
<th>rₓ, Å</th>
<th>fₓ, %</th>
<th>Aₓ/Δx, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>8°C</td>
<td>Control</td>
<td>45.5±0.36</td>
<td>86.8±2.07</td>
<td>0.94±0.13</td>
<td>10,000±0</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>46.9±0.22</td>
<td>81.2±0.53</td>
<td>1.72±0.10</td>
<td>196,000±24,000</td>
</tr>
<tr>
<td></td>
<td>After NH₄Cl</td>
<td>47.2±0.26</td>
<td>81.8±0.96</td>
<td>1.53±0.13</td>
<td>155,000±24,500</td>
</tr>
<tr>
<td>37°C</td>
<td>Control</td>
<td>46.3±0.25</td>
<td>80.3±0.67</td>
<td>1.64±0.12</td>
<td>10,000±0</td>
</tr>
<tr>
<td></td>
<td>NH₄Cl</td>
<td>46.8±0.26</td>
<td>80.3±0.55</td>
<td>2.42±0.15</td>
<td>53,200±9,120</td>
</tr>
<tr>
<td></td>
<td>After NH₄Cl</td>
<td>46.9±0.31</td>
<td>82.8±1.00</td>
<td>1.84±0.17</td>
<td>42,200±10,100</td>
</tr>
</tbody>
</table>

Values are means ± SE; rₛ, small pore radius; rₓ, large pore radius; fₓ, large pore fraction of the glomerular filtrate; Aₓ/Δx, pore area over diffusion distance.
were considerably higher (10). Indeed, there are few dextran studies that suggest lower pore radii (21). Third, the glomerular barrier does not seem to be significantly affected by a reduced temperature. Thus the small and large pore radii were similar in kidneys perfused either at 8°C or at 37°C (Fig. 5). Low temperature does not affect the permselective properties of the glomerular barrier, which are in accordance with data from skeletal muscle (31). Moreover, the reported results cannot be due to toxic effects of FITC on the glomerular cells, since albumin was measured before and after FITC-Ficoll administration both at 8°C and at 37°C. There was, however, a slight time-dependent increase in albumin of 0.30% per hour at 8°C and 1.4% per hour at 37°C. Indeed, the present albumin is only slightly higher than that reported by Tojo and Endou (38) from a micropuncture study. That study is particularly interesting since it was designed to correct for tubular modification of the urine composition. Thus albumin and inulin U/P ratios were determined, and the U/P for albumin was then plotted vs. U/P for inulin.

Extrapolation to U/P = 1 for inulin would represent the true glomerular filtrate θalbumin.

Fourth, the classic notion of a charge barrier is supported in the present study by the much lower sieving coefficient of albumin compared with Ficoll of equivalent size (36 Å). The sieving data for Ficoll and albumin were analyzed according to a simplified model of charge-charge interactions (37). The charge density, ω, in the gel (or membrane) was 43 meq/l in the experiments performed at 8°C. Similar charge density values for the glomerular wall have been found using native and charge-modified myoglobin (42), LDH isoenzymes (23), and native and charge-modified HRP (37). Interestingly, Huxley et al. (17) estimated a charge density of 34 meq/l in single mesenteric capillaries perfused with plasma. Thus peripheral and glomerular capillaries may actually have similar charge-selective properties but differ in the radii of the small and large pore pathways. Thus the charge density was probably overestimated using dextran sulfate, with ω values of 120–170 meq/l (11).
Fifth, hypoxia and/or ammonium chloride may damage the glomerular barrier. Perfusion with erythrocyte-free solution at 37°C increased the number of large pores. Thus the large pore fraction of the glomerular filtrate ($f_l$) was 1.64% at 37°C and 0.94% at 8°C. Perfusion with ammonium chloride increased the number of large pores even further, with $f_l$ values of 2.42% and 1.72% at 37°C and 8°C, respectively. These changes were not completely reversible when returning to the perfusate without NH$_4$Cl. It is well known from other organs that inflammatory reactions increase the number of large pores rather than the average pore radii (14, 31). These effects are caused by endothelial cell contraction, which has been extensively described (39). For the glomerular barrier, the exact position of the size selectivity is debated (basement membrane or podocyte slit-membrane?), making interpretations highly speculative.

Both high temperature and NH$_4$Cl reduced the charge selectivity. Thus the $\theta$ for albumin was four times higher in the warm experiments than in the cold ones (0.86% and 0.19%, respectively). Ammonium chloride increased the $\theta$ for albumin to 1.9% at 37°C and to 0.28% at 8°C. Again, these changes were not completely reversible when returning to the standard perfusate. Figure 8 shows that the isolated kidneys perfused at 37°C had significantly less fixed negative charges (29 meq/l) compared with the charge density fused at 37°C had significantly less fixed negative fusate. Figure 8 shows that the isolated kidneys completely reversible when returning to the standard perfusion at 37°C. Indeed, erythrocyte-free solution at 37°C increased the number of large pores even further, with $f_l$ values of 2.42% and 1.72% at 37°C and 8°C, respectively. Ammonium chloride seemed to reduce $\omega$ even further.

The effects of temperature on the number of large pores and on the charge density are probably due to ischemic damage in both the size and charge restrictivity of the glomerular barrier induced by artificial perfusion of the kidneys at 37°C. Indeed, erythrocyte-free perfusion at 37°C is known to cause hypoxic cell damage in the proximal tubule (1, 16, 36) as well as in the thick ascending limb cells (4). Moreover, the endothelial glycocalyx (9, 41) as well as the glomerular basement membranes (40) have been shown to be sensitive to hypoxia/reperfusion, which leads to loss of proteoglycans (40).

In summary, the present study supports the classic view of glomerular permselectivity. We found no support for the so-called “albumin retrieval hypothesis.” On the contrary, the glomerular membrane is highly permselective to macromolecules. Artificial perfusion of the kidney at 8°C does not affect glomerular permselectivity. Ammonium chloride and erythrocyte-free perfusion at 37°C increased the number of large pores and reduced the charge density. Taken together with our recent findings on the effects of ionic strength on the charge barrier (37), the present data are compatible with the following notions of the glomerular barrier: 1) The glomerular membrane is composed of two separate barriers in series, one charge selective and one size selective. 2) The size barrier is heteroporous with numerous small pores (radius 46 Å) and far less frequent large pores (80–87 Å). 3) The charge barrier behaves as a gel containing 35–45 meq/l of fixed negative charges. 4) Glomerular charge selectivity is exceeded by a structure that is more dynamic and vulnerable than the size barrier. 5) Finally, we propose that proteoglycans produced by the endothelial cells play a much more important role for glomerular charge selectivity than hitherto suggested.

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