Anomalous decrease in dextran sulfate clearance in the diabetic rat kidney

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Anomalous decrease in dextran sulfate clearance in the diabetic rat kidney. Am. J. Physiol. 274 (Renal Physiol. 43): F700–F708, 1998.—The anomalous increase in charge selectivity as previously observed with reduced dextran sulfate clearances in diabetic rats (L. D. Michels, M. Davidman, and W. F. Keane. Kidney Int. 21: 699–705, 1982) was confirmed in 4-wk streptozotocin (STZ) diabetic Sprague-Dawley rats using the isolated perfused kidney technique. The apparent charge selectivity in both control and diabetic rats could be abolished by increasing the dextran sulfate concentration to 200 µg/ml in the perfusate. This was demonstrated by a high rate of processing of dextran sulfate (−1,700 ng·min⁻¹·kidney⁻¹) by glomeruli in both control and diabetic kidneys and by the fact that charge interaction could not explain the concentration dependence. The amount of urinary desulfation of dextran sulfate was also found to be significantly less in the diabetic kidney as was glomerular sulfate activity compared with controls. Dextran sulfate glomerular processing is therefore altered in the STZ diabetic rat kidney but could be rationalized in terms of previous models of endothelial cell receptor-mediated uptake of dextran sulfate. The results are consistent with recent work demonstrating that there is little or no electrostatic charge interaction operating on dextran sulfate or other negatively charged molecules at the glomerular capillary wall.

dextran sulfate; isolated perfused kidney; glomerular cell uptake; fractional clearance; sulfatase; streptozotocin diabetes; charge selectivity

IN TYPE 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic patients, the development of diabetic nephropathy is characterized by the presence of proteinuria (11). Proteinuria has been associated with morphological and functional alterations of the glomerular filter, and these alterations are presently believed to arise from a loss in charge selectivity early in the disease and later a loss in size selectivity (16). It has been generally thought that the fixed negative charge provided by heparan sulfate proteoglycan in the glomerular basement membrane (GBM) electrostatically repels the negative charge on albumin and, by doing so, provides a highly restrictive barrier to albumin transport (charge selectivity). The decline in the fixed anionic charge observed in diabetic renal disease has been attributed to the loss of heparan sulfate proteoglycan (5, 7, 12–14, 19, 22, 28, 33, 34).

Studies that have investigated charge selectivity directly have employed the use of dextran sulfate as a test transport probe. This is because dextran sulfate is highly negatively charged and is not reabsorbed by postglomerular tubules, so its clearance reflects interactions occurring at the glomerular level. Recently, however, there has been some concern as to the nonoptimal value of dextran and dextran sulfate as glomerular transport probes. This has been suggested to be, in part, due to the possible role of conformation in governing transport, although recent studies (24) have confirmed earlier work (2) that these effects are not major. Other effects including osmotic-induced conformational change by high concentrations of albumin on dextran (24) and dextran sulfate binding to albumin (4, 30) have also been shown to be negligible.

Charge selectivity studies with dextran sulfate have demonstrated that for a given hydrodynamic size, negatively charged dextran sulfate exhibits a lower clearance compared with its uncharged counterpart, dextran (6). In disease, the loss of charge selectivity would therefore predict similar clearances for both dextran sulfate and dextran, and this has been reported in experimental glomerulonephritis (1). Clearances of dextran sulfate in insulin-treated and untreated alloxan-induced diabetes have shown fractional clearances that are further restricted in diabetic rats relative to dextran sulfate clearances in controls (15). These results clearly represent anomalous behavior in terms of the general understanding of charge selectivity and proteinuria. The need to reexamine these results is even more prevalent, as recent studies have demonstrated that charge selectivity is not as significant as originally thought (8). It has been shown in the case of dextran sulfate that its glomerular processing does not involve electrostatic interaction with the fixed charges of the GBM, but rather it undergoes endothelial cell receptor-mediated uptake, desulfation but not depolymerization, and then exocytosis (4, 10, 27, 30, 31).

The aim of this study was to examine the glomerular processing of dextran sulfate in STZ diabetic rats in relation to its apparent charge selectivity and the findings of Michels et al. (15). The use of the isolated perfused kidney (IPK) technique to estimate dextran sulfate fractional clearance has been validated in terms of in vivo clearance in a number of studies (3, 4, 27, 30). The IPK technique was therefore used to determine dextran sulfate fractional clearance and the kinetics of glomerular uptake, binding, and intracellular trafficking of dextran sulfate. In addition, ion-exchange chromatography was used to measure the degree of desulfation of dextran sulfate in the urine and sulfatase activity in glomerular extracts.
MATERIALS AND METHODS

Materials. Male Sprague-Dawley rats (200 to 250 g) were obtained from the Monash University Central Animal House. Streptozotocin (STZ) was purchased from ICN Biomedicals (Aurora, OH). Nembutal (60 mg/ml) was from Cera Chemicals (Hornsby, NSW, Australia). Synthamin 13 (a source of amino acids) was from Travenol Laboratories. Additional amino acids (asparagine, aspartate, cysteine, glutamate, glutamine, lysine, serine, and tyrosine) along with 3-[3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), EDTA, albumin [insolubilized on cross-linked 4% dextran sulfate (MW 48,000) and dextran sulfate (MW 42,000) in 1.5 ml of 5% BSA] were purchased from Pharmacia (Aurora, OH). Nembutal (60 mg/ml) was from Cera Chemicals (Mannheim, Germany). Papain was a gift from Dr. H. C. Robinson, Department of Biochemistry and Molecular Biology, Monash University. Sodium boro-[^3H]hydride (132 mCi/mg) was from Amersham International (Buckinghamshire, UK). [^3H]Inulin (2.1 mCi/g) was purchased from Du Pont (Detroit, MI).

Dextran sulfate (mol wt (Mw) = 48,000, and number average mol wt (Mn) = 40,654 (DB 001–0, 16% sulfur, degree of substitution = 1.7)) was from TdB Consultancy (Upsalla, Sweden). Dextran T-500 and dextran sulfate (Mw = 42,000; lot no. 321525), together with Sephadex G-25 in PD-10 columns, and Q-Sepharose were purchased from Pharmacia Fine Chemicals (Upsalla, Sweden). Dextran sulfate mixture (Mx1-1) containing 25% each of dextran sulfate with molecular weights of 10,000, 25,100, 39,700, and 500,000 (4) was used for albumin affinity column studies.

Laboratory sieves (aperture sizes 106, 125, and 180 µm) were used for the collection of glomeruli were purchased from Endecotts (London, UK). The metabolic cage used for the collection of urine and feces was obtained from the Central Animal House, Monash University.

Methods. Dextran, dextran sulfate, and dextran sulfate Mix-1 were labeled with tritium, using a reductive technique with sodium boro-[^3H]hydride as described by Van Damme et al. (29). The labeled preparations were applied to a Sephadex G-25 PD-10 column to separate them from free label and then dialyzed extensively against distilled water. The dialyzed preparation was applied to a PD-10 column immediately before use. Specific activities (dpm/mg) of the preparations were Dextran T-500 = 1.7870 × 10^6, dextran sulfate (Mw = 48,000) = 4.318 × 10^5, and dextran sulfate (Mw = 42,000) = 4.265 × 10^5.

Male Sprague-Dawley rats (200–250 g), starved overnight, were injected via the tail vein with STZ, dissolved in citrate buffer (pH 4.5) at a concentration of 50 mg/kg body mass, and age-related control rats were injected with citrate buffer alone. The diabetic rats received 4 U insulin subcutaneously 3 days a week. Rats were placed into metabolic cages 24 h prior to death, where urine and feces were collected. The blood glucose was measured using a strip of Ames Glucostix, and levels were determined in a standardized Glucometer II detection device (Ames Division; Miles Australia, Mulgrave, Victoria, Australia). Urinary protein levels were determined using a radioimmunoassay (25). The presence of diabetes was confirmed by measurement of blood glucose (>15 mmol/l) at 4 wk. Urinary albumin (mg/day), urinary volume (ml/day), and body weight (g) were also measured.

The IPK technique has recently been described in detail (17, 27). Briefly, kidneys from male Sprague-Dawley rats were perfused with 5% BSA in modified Krebs-Henseleit buffer that contained amino acids. The perfusion system was maintained at 37°C, and the perfusate was continually gassed with 95% oxygen and 5% carbon dioxide. Measurement of glomerular filtration rate was made with [^3H]Inulin. Fractional clearance was defined as the product of the ratio of dpm of a labeled urinary test molecule of a particular hydrodynamic radius (as determined by elution on Sephadex G-100) to dpm of a labeled perfusate test molecule with the same hydrodynamic radius times the ratio of dpm of perfusate inulin to dpm of urinary inulin.

Determination of the glomerular residence time for the dextran sulfate was made through cold-chase experiments. These experiments involved the perfusion of [^3H]dextran sulfate for 30 min where a steady state in the uptake of glomerular dextran sulfate is known to occur (27). The perfused kidney was then disconnected from the perfusion circuit containing the labeled dextran sulfate and immediately connected to a second perfusion circuit that contained either no labeled dextran sulfate or cold dextran sulfate. The perfusion was then continued for 5 min. At the end of the perfusion, the glomeruli were isolated and the amount of remaining labeled dextran sulfate was determined.

The procedure for isolation of glomeruli was based on the method described by Spiro (26). Glomeruli isolated in this way are essentially free of the outer Bowman’s capsule and collecting tubules and are ~95% pure. The glomeruli were suspended in ~6 ml of 0.85% NaCl and kept on ice. A 20-µl aliquot was taken by a microcapillary tube for counting under a phase-contrast microscope. The glomeruli were then papain digested, whereby samples (1 ml) were incubated with 0.15 ml of papain buffer (1.0 M sodium acetate, pH 5.5, and 0.05 M EDTA) containing 10 mg/ml cysteine hydrochloride and 10 µl of papain suspension (16 mg/ml) for 24 h at 60°C. Glomeruli were isolated postperfusion to determine the amount of dextran sulfate binding during the perfusion and to conduct binding experiments with dextran sulfate.

Binding of [^3H]dextran sulfate to isolated glomeruli preparations was conducted following the isolation of glomeruli from normal and diabetic kidneys. Binding studies were carried out at various incubation times of 5, 15, 30, 60, and 120 min. Isolated glomeruli (~2,000) were incubated with 15 µg[^3H]dextran sulfate (Mw = 42,000) in 1.5 ml of 5% BSA in Krebs-Henseleit buffer incubation medium at 37°C with gentle tumbling. Following incubation, the samples were centrifuged at 3,000 rpm in a KS-5200C Kubota centrifuge and washed twice with chilled 0.85% NaCl. The supernatant and washes were stored in preweighed vials. The glomeruli were then papain digested and counted for radioactivity. In addition, the washes were also counted for radioactivity to determine the recovery of tritium.

The amount of urinary desulfation was calculated by subjecting urine and perfusate samples to ion-exchange chromatography using a 19 × 1-cm column of Q-Sepharose. The sample was applied in 6 M urea, 0.05 M Tris, 0.05% CHAPS, pH 7.0, and eluted with a linear gradient of 0.15-2.5 M NaCl in the same buffer. The recoveries using this technique were in the range 95–100%.

Sulfatase activity was determined by isolating glomeruli and incubating with ~6 ml of PBS, pH 7.4, containing 136 mM NaCl. 2.68 mM KCl. 81 mM NaH2PO4, and 1.5 mM K2HPO4 and placed on ice for 1 h. The Sonicator Ultrasonic Processor (Heat Systems, Farmingdale, NY) was applied for 15 s, using a 0.3 s on and 0.7 s off cycle using a microtip probe. The output setting was set at 4.5. The resultant suspension
was centrifuged at 3,500 rpm for 20 min at 4°C using an MSE Centaur 2 centrifuge. The resultant pellet was incubated with 5% BSA in Krebs-Henseleit buffer with 15 µg/ml dextran sulfate (M_w = 48,000) for 24 h at 37°C. Following the incubation, the pellet was spun down for 10 min at 3,000 rpm in a K-5200C Kubota centrifuge. The supernatant was then subjected to ion-exchange chromatography.

Net glomerular anion charge was measured by a ^22Na and ^36Cl exchange technique, which has been previously described in detail (9). Radioactivity was determined by the use of 1:3 ratio of aqueous sample to scintillation mixture and measured on a Wallac 1410 scintillation counter.

All quantitative data expressed as means ± SD, where n represents the number of determinations, except for protein excretion, which is expressed as geometric mean ×/* tolerance factor. Significance of difference between means were analyzed by unpaired Student's t-test. The criterion for statistical significance was taken to be *P < 0.05.

RESULTS

Characterization of the diabetic rat model. The diabetic state was established based on changes in physical characteristics and metabolic functioning. STZ diabetic rats had significant increases in blood glucose levels (*P < 0.001), and their body weight was markedly lower than that of control rats (Table 1). Urinary protein levels and volume were found to be significantly lower than that of control rats (Table 1). Urinary protein excretion, which is expressed as geometric mean ×/* tolerance factor. Significance of difference between means were analyzed by unpaired Student's t-test. The criterion for statistical significance was taken to be *P < 0.05.

IPDK perfusion parameters. Table 2 compares the IPK perfusion parameters to the isolated perfused diabetic kidney (IPDK) perfusion parameters. Perfusion flow rate was found to be decreased in the IPDK, and this decreased perfusion flow also appeared to be a factor relating to the decreased renal perfuse flow resistance. The glomerular filtration rate was also found to be slightly lower, but no significant difference was found between diabetic and control rats (*P < 0.05). Urine flow rates for diabetic and control rats were also not significantly different.

Reduced fractional clearance of dextran sulfate in the IPDK. Table 3 shows the fractional clearance of dextran and dextran sulfate in the IPDK compared with the normal control rats.

### Table 1. Criteria for establishing STZ diabetes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>368.0 ± 12.0</td>
<td>296.0 ± 20.0†</td>
</tr>
<tr>
<td>Feces excretion, g/day</td>
<td>9.0 ± 1.0</td>
<td>18.1 ± 2.2</td>
</tr>
<tr>
<td>Urinary protein, mg/day</td>
<td>0.36 × 1.15</td>
<td>2.63 × 1.19†</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>1.6 ± 0.08</td>
<td>2.0 ± 0.14†</td>
</tr>
<tr>
<td>Urine volume, ml/day</td>
<td>11.8 ± 1.3</td>
<td>88.7 ± 32.3†</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>7.4 ± 1.1</td>
<td>191.2 ± 2.7†</td>
</tr>
<tr>
<td>Kidney-to-body weight ratio, g/g</td>
<td>4.45 × 10⁻³</td>
<td>6.72 × 10⁻³</td>
</tr>
<tr>
<td>Glomerular charge, μeq/g</td>
<td>3.27 ± 0.18</td>
<td>2.48 ± 0.9†</td>
</tr>
</tbody>
</table>

Values represent determinations for n = 6, except those for glomerular charge where n = 16; see Methods for complete description of data expression. STZ, streptozotocin. Values for the diabetic rats were determined at 4 wks following induction and compared to nondiabetic control rats. *P < 0.05. †P < 0.01. ‡P < 0.001.

### Table 2. IPDK perfusion parameters compared with IPK control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perfusion Time, min</th>
<th>IPK Control (n = 4)</th>
<th>IPDK (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFR, ml/min</td>
<td>30</td>
<td>43.5 ± 5.1</td>
<td>30.4 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>45.5 ± 2.1</td>
<td>32.4 ± 4.9</td>
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<tr>
<td></td>
<td>120</td>
<td>46.8 ± 3.9</td>
<td>31.5 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.93 ± 0.69</td>
<td>2.09 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.24 ± 0.46</td>
<td>1.96 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>2.84 ± 0.48</td>
<td>1.93 ± 0.18</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>30</td>
<td>0.83 ± 0.15</td>
<td>0.76 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.77 ± 0.11</td>
<td>0.74 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.61 ± 0.10</td>
<td>0.45 ± 0.19</td>
</tr>
<tr>
<td>Urine flow rate, ml/min</td>
<td>30</td>
<td>0.17 ± 0.10</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.22 ± 0.08</td>
<td>0.25 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>0.19 ± 0.05</td>
<td>0.21 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of determinations. IPDK, isolated perfused diabetic kidney; IPK, isolated perfused kidney; PFR, perfusion flow rate; RPFR, renal perfuse flow resistance; GFR, glomerular filtration rate.

IPK control. The fractional clearance of dextran was not significantly different in diabetic and control IPK; however, dextran sulfate fractional clearance in the IPDK was significantly lower than that of the IPK control (*P < 0.05). When the perfusate concentration of dextran sulfate was increased from 15 to 200 µg/ml, a concentration dependence was observed with an increase in fractional clearance of dextran sulfate in both control and diabetic kidneys (Table 3); however, the differences in fractional clearance between the dextran sulfate clearances were abolished. (Note that the close numerical correspondence between dextran clearance and dextran sulfate clearance at high concentration is coincidental, as the two preparations have different molecular weight distributions.) The fractional clearance results for control dextran sulfate are comparable to previous reports (27, 30), and the fractional clearances observed for diabetic rats in this study are similar to in vivo experiments by Michels et al. (15) for insulin-treated and untreated alloxan-diabetic rats.

Desulfation of urinary dextran sulfate in the IPDK. Figures 1 and 2 show the elution of labeled dextran sulfate from urine from IPK and IPDK with 15 µg/ml of dextran sulfate, respectively. The ion-exchange columns were eluted with a linear salt gradient of 0.15–2.5 M NaCl. Dextran sulfate in perfuse normally elutes with a sharp peak at ~1.9 M NaCl. Material that elutes at lower NaCl concentrations therefore will have a lower sulfate content. The IPK control shows that

### Table 3. Fractional clearance of dextran and dextran sulfate in STZ diabetic rat kidney compared with normal control rats

<table>
<thead>
<tr>
<th>Fractional Clearance</th>
<th>Diabetic (n = 6)</th>
<th>Control (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Dextran</td>
<td></td>
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<tr>
<td>15 µg/ml</td>
<td>0.138 ± 0.024</td>
<td>0.125 ± 0.015</td>
</tr>
<tr>
<td>200 µg/ml</td>
<td>0.0236 ± 0.0092</td>
<td>0.0520 ± 0.0230†</td>
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</table>

Values are means ± SD. *P < 0.05.
89 ± 5% (n = 4) of the dextran sulfate in the urine has been desulfated to some extent. This value has been calculated from the change in the area under the curve of the urine versus perfusate profiles. These results are in agreement with past studies (10, 30). In the IPDK, however, only 15 ± 8% (n = 4) desulfation occurred.

Sulfatase activity in glomerular extracts. The pattern of desulfation of dextran sulfate was confirmed by analysis of glomerular sulfatase activity in both diabetic and control glomerular extracts as shown in Table 4. It can be seen that the sulfatase activity in the diabetic rat kidney was significantly lower (9 ± 3%) than that of the control (74 ± 9%), thus indicating that sulfatase activity is partially inhibited (P < 0.05).

Depolymerization of dextran sulfate in the IPDK. To examine whether desulfation of dextran sulfate was accompanied by depolymerization, perfusate and urine samples were analyzed by size-exclusion chromatography to determine whether all the urine material could be accounted for as originating from the perfusate. An example of the fractionation of urine and perfusate is shown in Fig. 3. Analysis of the urine profiles confirms that all the material was derived from the perfusate and that there was no degradation or depolymerization occurring during renal passage. Using these profiles, it is also possible to determine fractional clearance as a function of molecular radius as shown in Fig. 4. These profiles exemplify the trends expected from the fractional clearance of the unfractonated preparations given in Table 3.

Dextran sulfate binding to albumin. The possibility that dextran sulfate binding to albumin influences its fractional clearance would appear to be negligible (4, 30). We have established that normal apparent charge selectivity occurs with dextran sulfate (15 µg/ml) in the presence of 50 mg/ml albumin (Table 3), which is a molar ratio of albumin to dextran sulfate of 2,300:1.

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Desulfation, %</th>
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<tbody>
<tr>
<td>STZ diabetic</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Normal</td>
<td>74 ± 9*</td>
</tr>
</tbody>
</table>

Values are means ± SD for glomeruli following a 24-h incubation with dextran sulfate (M_w = 48,000) at 15 µg/ml; n = 4. *P < 0.05.
Charge selectivity can be destroyed by decreasing this ratio to 173:1 using 200 µg/ml of dextran sulfate (MW 48,000; Table 3) or 40 µg/ml of dextran sulfate (MW 10,000) (30). There is no physical basis to suggest that apparent charge selectivity may be influenced by binding interactions that may be modulated by changing the molar ratios from 173 to 2,300. These numbers are out of the range of the maximum number of nearest albumin molecules to make close contact with a dextran sulfate molecule at any one time (calculated to be 3–10 molecules of albumin depending on the conformation of dextran sulfate). Further evidence is that, at the ratio of albumin to dextran sulfate of 173:1 or lower at 34:1 (1,000 µg/ml dextran sulfate M_w = 48,000), the dextran sulfate is ultrafiltered in the same manner as dextran over the range of radii of 18 to 44 Å (30). Figure 3 also demonstrates that no aggregates of dextran sulfate and albumin are forming in the perfusate as the elution profiles of the perfusate samples (Fig. 3, A and B; mixture of dextran sulfate and albumin at the molar ratio of 1:2,300) are identical to the elution profile of dextran sulfate alone (Fig. 3C). Other evidence for the lack of complexes is demonstrated by the fact that perfusate dextran sulfate is not eluted on ion-exchange resins at lower ionic strengths, which it would be if involved in electrostatic complexes with albumin (Fig. 2).

When 240 µl (4.7 × 10^7 dpm) of 15 µg/ml of dextran sulfate Mix-1 was applied to 5 ml of the albumin affinity gel in Krebs-Henseleit buffer and washed with 20 ml of Krebs-Henseleit buffer, there was no radioactivity associated with the albumin affinity column. These studies demonstrate that dextran sulfate binding to albumin under physiological conditions is negligible.

Glomerular levels of dextran sulfate in the IPDK. Figure 5 shows the level of [3H]dextran sulfate present in glomeruli isolated postperfusion with dextran sulfate at a concentration of 15 µg/ml. It is evident that there is a significant increase in uptake of dextran sulfate for STZ diabetic rat kidney (43.21 ± 10.03 ng/1,000 glomeruli) compared with control kidneys (19.58 ± 2.18 ng/1,000 glomeruli; P < 0.05). There was also no time dependence of dextran sulfate accumulation in diabetic glomeruli, with the similar amounts of dextran sulfate present at 20 min (44.65 ng/1,000 glomeruli) and 5 min (45.2 ng/1,000 glomeruli).

**Fig. 3.** Representative profiles of the size-exclusion chromatography on Sephadex G-100 of [3H]dextran sulfate (M_w = 48,000) at 15 µg/ml in urine (○) and perfusate (●) in the IPK (A, 50,000 dpm in urine and 35,000 dpm in perfusate) and IPDK (B, 100,000 dpm in urine and 35,000 dpm in perfusate) and in the [3H]dextran sulfate (M_w = 48,000) preparation (C). V_o, void volume of the column as determined with blue Dextran T2000; V_t, total column volume as determined with 3H2O.

**Fig. 4.** Fractional clearance of [3H]dextran (●, n = 4) and [3H]dextran sulfate at 15 µg/ml in the IPK (○, n = 4) and IPDK (▲, n = 4). *P < 0.05, **P < 0.01, ***P < 0.001.
glomeruli), 40 min (55.78 ng/1,000 glomeruli), and 60 min (48.69 ng/1,000 glomeruli).

Binding of [3H]dextran sulfate to isolated glomeruli was also examined (Fig. 6). These studies show that the potential for glomeruli to bind dextran sulfate is far higher than that observed in terms of resident dextran sulfate measured in glomeruli isolated postperfusion. The dextran sulfate binding curve was also significantly greater for diabetic glomeruli compared with controls (P < 0.05).

DISCUSSION

This study has confirmed the findings by Michels et al. (15) in that the fractional clearance of dextran sulfate is reduced in the diabetic rat kidney. These results demonstrate that the kidney is displaying anomalously increased charge selectivity, particularly when it has been generally recognized that the fixed charge concentration of the glomerular capillary wall is reduced in diabetes (Table 1). We have recently shown that charge interactions governing glomerular permeability are not as great as originally thought (8) and that apparent charge selectivity is due to factors other than charge-charge interactions (4, 10, 17, 27, 30–32). The glomerular processing of dextran sulfate by normal kidneys has been successfully interpreted in terms of receptor-mediated uptake by endothelial cells (4, 10, 27, 30–32). It means therefore that dextran sulfate processing in the diabetic kidney may also be governed by a cell-mediated process as discussed below.

Multiple populations. We have demonstrated from cold-chase studies that the rate of glomerular processing of dextran sulfate $M_W = 48,000$ is $\sim 46$ ng/min (assuming 40,000 glomeruli/kidney; Ref. 21), whereas in the diabetic kidney it is $\sim 21$ ng/min. The diabetic kidney appears to have two populations of glomerular dextran sulfate. One population appears to be a dynamic cell-associated dextran sulfate that also seems to be present in normal kidneys. The only difference is that the diabetic kidney population does not undergo desulfation. A new population of relatively static dextran sulfate is identified in the diabetic kidney, which binds early during the course of the perfusion, does not accumulate, and does not undergo significant exchange.

These studies can only be viewed as a qualitative assessment of the different populations of dextran sulfate associated with the kidney. The measured rates of glomerular processing are low in relation to those calculated for the dextran fractional clearances observed (see below). The decreased fractional clearance of dextran sulfate in the control or diabetic kidney means that the total amount of dextran sulfate being processed by the endothelial cell at a perfusate concentration of 15 µg/ml will be $\sim 1,700$ ng·min$^{-1}$·kidney$^{-1}$ (see below). This is far higher than that measured in...
glomeruli isolated postperfusion. This is primarily due to the fact that during the course of glomeruli isolation, cell surface dextran sulfate (30–32) appears to be removed since, in the chase experiments, there was no difference regardless of whether the exchange chase medium contained dextran sulfate. This loss alone precludes any quantitative assessment of the absolute turnover of dextran sulfate. The binding data, too, of dextran sulfate to isolated glomeruli, demonstrate the potential for high-capacity turnover, where dextran sulfate binding to glomeruli may be as high as 48,000 ng/kidney (assuming 40,000 glomeruli/kidney).

Concentration dependence. An important finding of this study is that in both normal and diabetic kidneys, the apparent charge selectivity is concentration dependent. At perfusate concentrations of 200 µg/ml, the fractional clearance of dextran sulfate is the same in both normal and diabetic kidneys. It has been established (30) that dextran and dextran sulfate clearance are identical at this perfusate concentration in normal kidneys. Yet at this perfusate concentration, the negative charge concentration of the perfusate dextran sulfate (0.6 meq/l) is considerably lower than that of the glomerular capillary wall (7.6 meq/l) (9). These conditions would provide ample opportunity to allow charge-charge interactions to take place between the fixed charge of the glomerular wall and the dextran sulfate, but these interactions are not manifested in differential clearance. The negative charge provided by the circulating dextran sulfate is also well below the negative charge concentration provided by the ambient albumin (13 meq/l), which is thought to be charge affected in its transport across the capillary wall. The concentration dependence of the dextran sulfate handling in the IPK and IPDK argues against the electrostatic model as the basis of its apparent charge selectivity.

The concentration dependence of dextran sulfate handling is consistent with previous model of a receptor-mediated cellular uptake of dextran sulfate that can be saturated at relatively high concentrations (4, 30). In fact, concentration-dependent fractional clearance values are important in describing the dynamic interactions of the system. For control kidneys the fractional clearance of dextran sulfate ($M_w = 48,000$) at 15 µg/ml is 0.052, which represents the amount excreted in a desulfated form. Therefore, glomerular sulfatase activity must be encountering dextran sulfate at a rate of 0.052 × 15,000 (perfusate concentration in ng/ml) × 0.8 (glomerular filtration rate in ml/min) = 624 ng·min$^{-1}$·kidney$^{-1}$. If glomerular processing was not occurring, then the fractional clearance would be 0.143 as demonstrated from concentration dependence studies. In this case the glomeruli would process dextran sulfate at a rate of 0.143 × 15,000 × 0.8 = 1,716 ng·min$^{-1}$·kidney$^{-1}$. This extra amount (above 624 ng·min$^{-1}$·kidney$^{-1}$) will be returned to the perfusate.

For diabetic kidneys, the fractional clearance value of 0.0236 equates to a processing rate of 283 ng·min$^{-1}$·kidney$^{-1}$. Since fractional clearance is concentration dependent, the maximum processing by the glomeruli will be close to 1,800 ng·min$^{-1}$·kidney$^{-1}$ (when fractional clearance = 0.150). The potential for increased processing of dextran sulfate is also seen in the binding curves of isolated diabetic glomeruli (Fig. 6).

The dynamic processing of dextran sulfate by the diabetic kidney would also eliminate any suggestion that dextran sulfate is clogging up the endothelial cell; in addition to this, a clogged endothelial cell would not affect dextran sulfate transport in steady state and therefore would not manifest a decreased fractional clearance.

Sulfatase activity. In the control kidney, we have demonstrated that, if the degree of substitution of sulfate on the sugar residue of dextran sulfate was $\geq 1.7$, then essentially all the material that is excreted, whether in IPK or in vivo, is desulfated to some extent (10, 30). This means that dextran sulfate must undergo an obligatory interaction with sulfatases of the endothelial cell prior to filtration. The importance of this interaction in governing apparent charge selectivity was previously demonstrated by the fact that material that did not undergo desulfation did not exhibit charge selectivity. This could be brought about by decreasing the degree of sulfate substitution on the dextran sulfate (30), by increasing the concentration of dextran sulfate in the perfusate of the IPK (low-molecular-weight dextran sulfate was most effective) (4, 30), or by using agents that inhibited endocytotic uptake (30). The sulfatase activity seems to be primarily intracellular, since inhibitors of endocytotic uptake or protein synthesis affect apparent charge selectivity and desulfation (27). The integral relationship between apparent charge selectivity and exposure to sulfatase activity has been rationalized (30) on the basis that dextran sulfate takes a bypass route through the endothelial cell and is ultimately released as a desulfated molecule, which cannot bind to the putative receptor again (due to requirement of degree of sulfate substitution $\geq 1.7$). Some of this desulfated material will be transported across the glomerular capillary wall, whereas some will be released into the capillary lumen. Although the desulfation process may not be exclusive to endothelial cells, in steady-state transport it is only this bypass pathway that will lower the fractional clearance.

In the diabetic state an unique situation arises when the sulfatase activity is markedly reduced as demonstrated by the degree of desulfation of the excreted material as well as significant reduction in sulfatase activity in glomerular extracts. The reduction in sulfatase activity is likely to reflect alterations synonymous with decreased activities of enzymes associated with lysosomal degradation in diabetes (20). This change in sulfatase activity is accompanied by an increase in apparent charge selectivity, opposite to that observed in normal kidneys.

How can we rationalize less desulfation of dextran sulfate in diabetes with increased apparent charge selectivity or decreased fractional clearance? A number of possibilities exist. We have demonstrated previously (30) that when the degree of sulfate substitution on dextran sulfate was lowered, charge selectivity decreased as did the degree of desulfation. We would...
interpret this as the receptor recognizing the degree of sulfate substitution. We know that dextran sulfate with a degree of substitution of 1.7 sulfates groups per monosaccharide or greater is avidly taken up by the endothelial cell and desulfated. In fact the material will not be filtered unless it is taken up by the endothelial cell and desulfated. The exocytosed desulfated dextran sulfate will not effectively bind again to the receptor, as now its degree of sulfate substitution is much lower, and it can escape into the urinary filtrate or back into the plasma. The important point is that in diabetes the sulfatase activity is reduced. The dextran sulfate that is endocytosed is now exocytosed with its sulfates intact. Therefore, a possible explanation is that, unlike the situation in the normal kidney, this dextran sulfate, which has already had a history of intracellular trafficking, has now a further opportunity to be re-endocytosed by the receptor and undergo further intracellular trafficking. This recycling could then contribute to the increased apparent charge selectivity and the increase in glomerular residence of dextran sulfate observed in diabetes. The dextran sulfate may also simply escape back into the capillary. Another possibility is that there may be simply more receptors for dextran sulfate available for the processing of dextran sulfate in the diabetic rat kidney.

Concluding remarks. There are several characteristic biochemical and physiological changes that occur in 4-wk STZ diabetic rats. These include the turn-off of glomerular sulfatase activity, the increased uptake but slower turnover of glomerular dextran sulfate, and the decreased fractional clearance of dextran sulfate. All these changes could be integrated into a model that involves a receptor-mediated uptake of dextran sulfate by glomerular endothelial cells. In both normal kidneys and diabetic kidneys the apparent charge selectivity exhibited by dextran sulfate could be abolished by increasing the concentration of the perfusate dextran sulfate. This would argue strongly against any significant electrostatic interaction governing fractional clearance.

Implications for the mechanism of albuminuria. The processing of dextran sulfate by diabetic kidneys is consistent with a body of other work which demonstrates that glomerular capillary wall restriction of polyanion transport such as dextran sulfate and albumin is not as great as originally thought (4, 8–10, 17, 23, 27, 30–32). This means that some other major mechanism must account for albumin processing. It has been suggested that albumin is simply size selected at the capillary wall, undergoes uptake by the proximal tubular cells, and is returned to the blood supply undergraded (17, 18). The high capacity of tubular uptake would make it the most likely source of albuminuria in renal disease, including diabetes.

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