Permselectivity of the Glomerular Capillary Wall

STUDIES OF EXPERIMENTAL GLOMERULONEPHRITIS IN THE RAT USING DEXTRAN SULFATE

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ABSTRACT To determine whether the increased filtration of serum proteins after glomerular injury is the consequence of altered electrostatic properties of the glomerular capillary wall, we measured fractional clearances of the anionic polymer, dextran sulfate, in nine Munich-Wistar rats in the early autologous phase of nephrotoxic serum nephritis (NSN). In agreement with previous studies from this laboratory, whole kidney and single nephron glomerular filtration rates were normal in NSN rats despite histological evidence of glomerular injury, and despite a marked reduction in the glomerular capillary ultrafiltration coefficient to approximately one-third of normal. In the companion study (9), it was shown that in NSN rats the mean fractional clearances of neutral dextrans over the range of effective molecular radii from 18 to 42 Å were reduced, compared to normal. In contrast, in the present study the mean fractional clearances for dextran sulfate over the same range of molecular radii were significantly greater than those found previously for normal Munich-Wistar rats. The fractional clearance of dextran sulfate molecules of the same molecular radius as serum albumin (~36 Å) was increased markedly, from 0.015±0.005 (SEM) in non-nephritic controls to 0.24±0.03 in NSN ($P < 0.001$). The sialoprotein content of glomeruli, estimated by the colloidal iron reaction, was reduced in NSN rats as compared to normal controls. It is concluded that the abnormal filtration of anionic serum proteins, such as albumin, seen in glomerulopathies is, at least in part, the consequence of loss of fixed negative charges from the glomerular capillary wall.

INTRODUCTION

From experimental and theoretical studies it is now clear that at least three factors may influence the passage of macromolecules through the glomerular capillary wall. Two of these, molecular size and the hemodynamic determinants of glomerular filtration rate (GFR) [1] have been recognized for some time (1–7). The third factor, molecular electrical charge, has only recently been firmly established (8). Consequently, there are at least three possible explanations for the enhanced transglomerular passage of albumin, hence the proteinuria, which commonly accompanies glomerular injury: (*a*)

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[1] *Abbreviations used in this paper:* (BS/P), ratio of Bowman's space to plasma; D, dextran; DS, dextran sulfate; GFR, glomerular filtration rate; NSN, nephrotoxic serum nephritis; (U/P), ratio of urine to plasma.

an increase in the effective "pore" size and (or) number of pores, (*b*) disease-induced alterations in glomerular pressures and flows, and (*c*) changes in the electrostatic properties of the glomerular capillary wall. In the companion study, carried out in Munich-Wistar rats in the early autologous phase of nephrotoxic serum nephritis (NSN), fractional clearances of neutral dextrans (D) were found to be decreased, rather than increased, relative to values for D in normal control rats (9). Hence, the changes that must have occurred in the size of and(or) number of pores would have been expected to decrease, not increase, the transmural passage of macromolecules such as albumin. This conclusion is also consistent with the results obtained recently by Robson et al. (10) in studies of fractional polyvinylpyrrolidone clearances in children with untreated, idiopathic, nephrotic syndrome. Moreover, despite significant changes in several of the determinants of GFR, the findings in the companion study in rats (9) demonstrate that the net effect of these changes likewise would have tended to diminish, rather than enhance, the transglomerular passage of macromolecules.

It recently has been shown that for a given effective molecular radius, the anionic polymer, dextran sulfate (DS), exhibits a lower fractional clearance than does neutral D (8), suggesting that fixed negative charges on the glomerular capillary wall influence the transglomerular passage of circulating polyanions. The present study, therefore, was undertaken to test the third possible explanation for enhanced transglomerular passage of albumin, namely that of a disease-induced alteration in the electrostatic properties of the glomerular capillary wall. The results indicate that, as with albumin, and in contrast to neutral D, the fractional clearances of anionic DS molecules are increased by glomerular injury.

GLOSSARY OF SYMBOLS

K_f	Ultrafiltration coefficient.
$\overline{P}_{GC}$	Length-averaged value of the glomerular capillary hydraulic pressure.
$\overline{\Delta P}$	Length-averaged value of the glomerular transcapillary hydraulic pressure difference, $\overline{P}_{GC} - P_T$.
π_E	Efferent arteriolar colloid osmotic pressure.

METHODS

Animals studies

Induction of experimental glomerulonephritis. NSN was induced in nine adult Munich-Wistar rats of both sexes in the manner described in the companion report (9). 6–21 days after intravenous injection of nephrotoxic serum, the fractional clearances of DS and the determinants of glomerular ultrafiltration were measured by appropriate clearance and micropuncture techniques (7–9).

After these measurements, sections of each kidney were prepared for histological examination utilizing light and immunofluorescence techniques as described previously (11, 12). In addition, representative sections were stained for acidic glycoproteins (glomerular sialoprotein) by the colloidal iron technique (13). The severity of glomerular injury as observed by light microscopy was semiquantitatively assessed on a 0–3+ scale, based on degree of hypercellularity, occlusion of capillary loops, and polymorphonuclear infiltration. The intensity of colloidal iron staining along the epithelial side of glomerular capillary walls was similarly assessed on a scale of 0 (no staining) to 3+ (intense Prussian blue deposits). Representative sections of snap-frozen renal tissue were studied by direct immunofluorescence microscopy for deposits of rabbit IgG, rat IgG, and rat C_3 by methods described previously (11).

Studies with DS molecules of narrow size distribution. For this strain of rats under normal hydropenic conditions, it has been shown previously that fractional DS clearances obtained for the kidney as a whole (estimated from comparison of the urinary clearance of various sized DS molecules to that of inulin) can be equated with clearances of these substances across single accessible surface glomeruli (8). It also has been shown (9) that, in NSN rats, inulin appears in Bowman's space in the same concentration as in plasma water, demonstrating that inulin provides an exact measure of the GFR of water in this model of renal disease. To test the validity of equating fractional DS clearances for a single glomerulus with those for the kidney as a whole in NSN, tritiated DS molecules of narrow size distribution, prepared in the manner reported previously (8), and characterized with respect to average Stokes-Einstein radius, were used as test solutes in three rats. A 0.4-ml priming infusion, containing nonisotopic inulin (6 g/100 ml) and tritiated DS (< 300 mg/100 ml, activity $\simeq$ 0.5 mCi/ml), was injected into the left jugular vein 30 min before micropuncture, followed immediately by continuous infusion of the same solution at the rate of 1.2 ml/h. This infusion was continued throughout the duration of each experiment. During this hydropenic period, two or three 15-min urine samples were collected from a catheter in the left ureter for measurement of urine flow rate and inulin and DS concentrations. At the midpoint of each urine collection period, fluid was collected from accessible Bowman's capsules (30–60 nl/collection) and 100 μl of blood was withdrawn from the femoral artery for determination of DS and inulin concentrations.

Studies with DS molecules of wide molecular size distribution. Having established that inulin permeates the glomerular capillary wall without measurable restriction in NSN rats (9) and that fractional urinary DS clearances are the same as fractional DS clearances measured for single accessible glomeruli in the same kidney (i.e., DS molecules are neither secreted nor reabsorbed), justification is provided for relying on urinary clearances to assess the permselectivity characteristics for all glomeruli in a single kidney, now using an homologous series of DS molecules of widely varying molecular size. The methods for preparation of this homologous series have been given elsewhere (8). These experiments were performed in nine hydropenic NSN rats (ranging in body weight from 240 to 439 g), in which 0.4 ml of a solution of nonisotopic inulin in isotonic saline (10 g/100 ml) was infused intravenously 45 min before micropuncture, followed immediately by a constant infusion of the same solution at the rate of 1.2 ml/h. Just before micropuncture, 0.4 ml of an isotonic saline solution containing tritiated DS of broad molecular size distribution (DS concentration < 300 mg/100 ml, activity $\simeq$ 0.3 mCi/ml [8]) was infused intravenously, followed immediately by a

constant infusion of the same solution at the rate of 1.2 ml/h. Details of the experimental procedure for collecting, processing, and analyzing blood, urine, and tubule fluid are exactly as described in the companion report (9).

RESULTS

Histopathology. Renal tissue was available for light microscopic study from eight rats. Using a semiquantitative scoring system of 0–3+, the average score was 1.5 (range 1.0–3.0). The lesions consisted of glomerular hypercellularity with occlusion of some capillary loops by proliferating mesangial cells, infiltration with polymorphonuclear leukocytes, and occasional mitotic figures (Figs. 1A and 1B). Tubulointerstitial lesions were infrequent and blood vessels were normal. Heavy linear deposition of rabbit IgG and weaker deposition of rat IgG and C_3 in a similar distribution was seen in each instance. These observations are similar to those previously described for this model (11). The intensity of glomerular colloidal iron staining was markedly decreased in the NSN rats. The average score was 0.4 ($n = 8$, range: 0–0.5) vs. 2.0 ($n = 5$, range: 1.0–3.0) for a group of non-nephritic normal Munich-Wistar rats stained at the same time and sectioned at the same thickness (Figs. 1C and 1D).

Studies with DS molecules of narrow size distribution. Fig. 2 presents data comparing $(BS/P)_{DS}/(BS/P)_{IN}$ ratios with simultaneously measured values of $(U/P)_{DS}/(U/P)_{IN}$. As shown, fractional DS clearances obtained for six separate surface glomeruli from three NSN rats were essentially the same as ratios measured for the kidney as a whole. These data were obtained for DS molecules ranging in effective radius from 15.5 to 19.5 Å. These findings demonstrate that in Munich-Wistar rats with NSN, DS molecules, like neutral D (9), are neither secreted nor reabsorbed by the renal tubules. The data further suggest that fractional clearances of DS are homogeneous from glomerulus to glomerulus within a single kidney in this form of glomerulonephritis, as in non-nephritic rats (8).

Studies with DS molecules of broad size distribution. The relationship between the fractional clearance of DS, given by the ratio $(U/P)_{DS}/(U/P)_{IN}$, and effective DS radius for nine NSN rats during hydropenia is summarized in Fig. 3. The points represent mean values ±1 SE. Table I contains the individual values from all nine rats. For comparison, the fractional clearance profile for DS recently obtained by us (8) in seven normal hydropenic Munich-Wistar rats is also shown in Fig. 3, and the mean values are given in Table I. In NSN rats, mean fractional DS clearances were substantially greater, at any given molecular size, than those found in normal rats. These findings are to be contrasted with the findings in the companion study (9), in which we found the fractional clearances of neutral D in NSN rats to be reduced below values found in normal rats.

Table II summarizes individual and mean values for

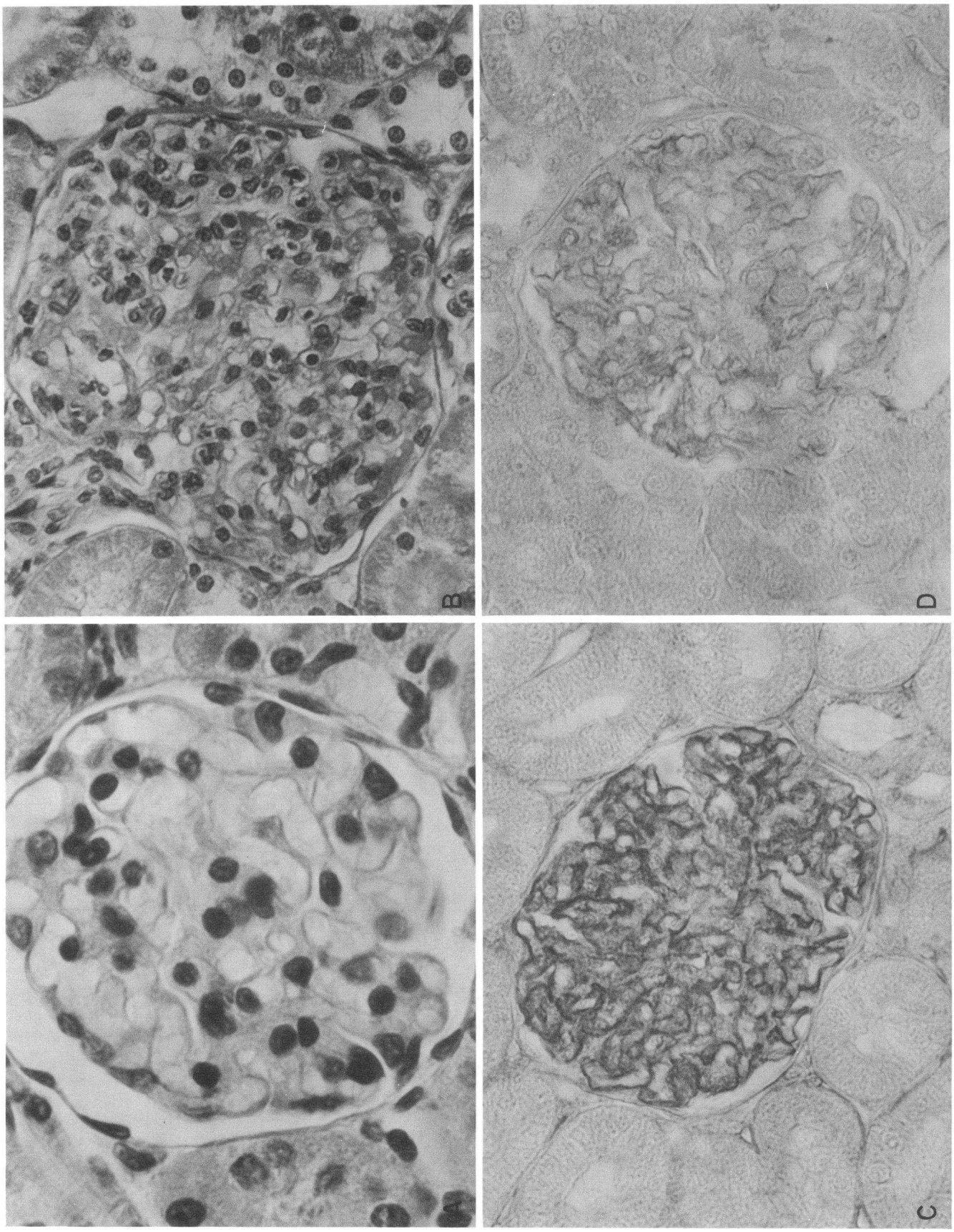
TABLE I

Comparison of Fractional Clearances of DS in NSN Rats vs. Normal Hydropenic Rats

	$(U/P)_{DS}/(U/P)_{IN}$												
	18 Å	20 Å	22 Å	24 Å	26 Å	28 Å	30 Å	32 Å	34 Å	36 Å	38 Å	40 Å	42 Å
NSN rats													
No.													
9	0.70	0.68	0.65	0.60	0.53	0.43	0.32	0.26	0.19	0.14	0.10	0.05	0.015
10	0.65	0.62	0.58	0.54	0.49	0.43	0.35	0.28	0.21	0.14	0.09	0.05	0.02
11	0.88	0.82	0.76	0.69	0.63	0.55	0.48	0.40	0.32	0.24	0.17	0.09	0.04
12	0.76	0.66	0.58	0.51	0.46	0.42	0.36	0.30	0.23	0.16	0.10	0.05	0.015
13	0.97	0.91	0.85	0.78	0.71	0.64	0.55	0.48	0.38	0.29	0.21	0.13	0.07
14	1.05	1.02	0.97	0.93	0.83	0.71	0.60	0.52	0.43	0.35	0.26	0.15	0.07
15	0.97	0.93	0.88	0.80	0.68	0.55	0.43	0.36	0.30	0.24	0.18	0.12	0.06
16	1.05	1.03	1.00	0.90	0.78	0.67	0.55	0.48	0.41	0.35	0.29	0.20	0.10
17	1.06	1.05	1.03	1.00	0.82	0.66	0.51	0.43	0.35	0.29	0.22	0.17	0.07
Mean	0.90	0.86	0.81	0.75	0.66	0.56	0.46	0.39	0.31	0.24	0.18	0.11	0.05
±1 SE	0.05	0.06	0.06	0.06	0.05	0.04	0.03	0.03	0.03	0.03	0.02	0.02	0.01
($n = 9$)													
Normal hydropenic rats*													
Mean	0.74	0.58	0.42	0.29	0.19	0.13	0.08	0.05	0.03	0.015	0.01	0.00	0.00
±1 SE	0.04	0.04	0.04	0.03	0.02	0.01	0.01	0.01	0.005	0.005	0.00	0.00	0.00
($n = 7$)													
P value‡	<0.05	<0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* Reported in detail elsewhere (8).

‡ *P* values calculated from unpaired data using Student's *t* test.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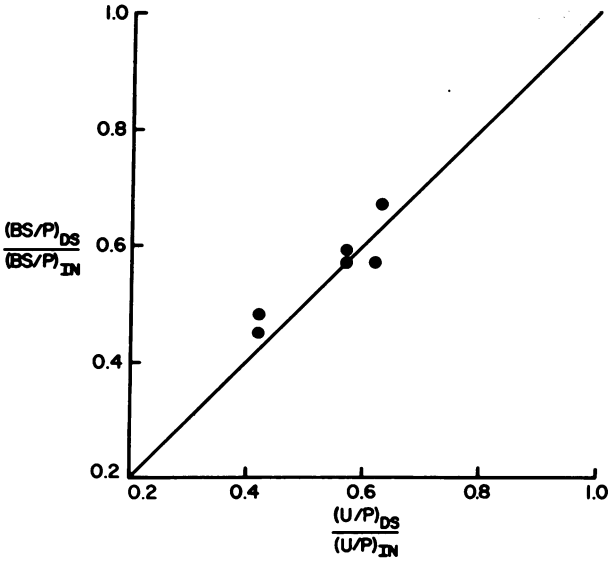

FIGURE 2 Comparison of $(BS/P)_{DS}/(BS/P)_{IN}$ ratios with $(U/P)_{DS}/(U/P)_{IN}$ ratios obtained simultaneously in three NSN rats. The line of identity is indicated.

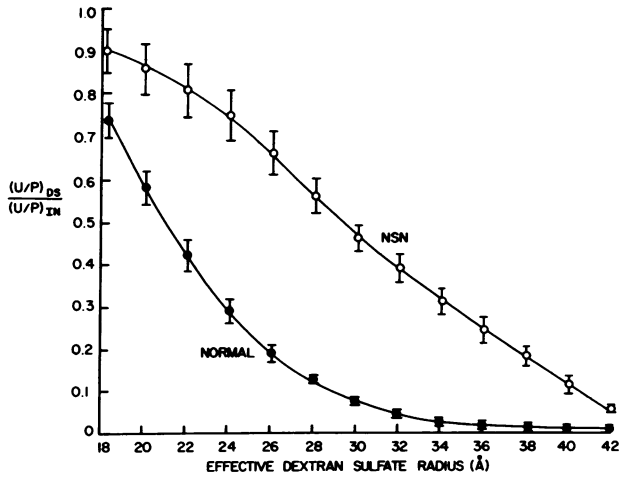

FIGURE 3 Comparison of fractional DS clearances plotted as a function of effective DS radius for NSN rats (○) and for a group of non-nephritic normal hydropenic control rats (●) reported previously (8). Values are expressed as means±1 SE.

several indices of single nephron function in these nine NSN rats. Values for these quantities are in close agreement with those found in the companion investigation (9) as well as in previous studies of rats with NSN (11, 12). Mean values of single nephron GFR in these NSN rats (30±2 nl/min) were similar to those obtained by us in non-nephritic Munich-Wistar rats (11, 12). Likewise, values for whole kidney GFR (mean 0.93 ml/min) agree closely with values in non-nephritic rats of the same strain. Glomerular capillary hydraulic pressure, $\overline{P}_{GC}$ (Table II), and the mean transcapillary hydraulic pressure difference, $\overline{\Delta P}$, were considerably higher in NSN rat than values typically found in non-nephritic Munich-Wistar rats (11, 12). As in the companion study (9), the ratio $\pi_E/\overline{\Delta P}$ (which averaged 0.73±0.03) deviated significantly from unity, indicating that filtration pressure equilibrium was not achieved. When filtration pressure equilibrium is not achieved, a unique value of the glomerular capillary ultrafiltration coefficient (K_f) can be calculated (14). For the eight NSN rats in which all of the determinants of glomerular ultrafiltration were measured, K_f averaged 0.029±0.002 nl/s·mm Hg, a value similar to that reported previously for NSN rats (9, 11, 12) and approximately one-third that found in normal Munich-Wistar rats (15, 16).

DISCUSSION

To a large extent, current views of the mechanisms governing both normal and altered permselectivity of the glomerular capillary wall to macromolecules derive from differential solute clearance studies. In such studies the urinary excretion of some test macromolecule (such as D or polyvinylpyrrolidone) is compared to that of a reference solute (such as inulin) which appears in Bowman's space in the same concentration as in plasma water. If both test and reference solutes are neither secreted nor reabsorbed, fractional clearance determined in this way is equivalent to the ratio of the concentration of the test macromolecule in Bowman's space to that in plasma water.

Studies from this laboratory have employed this approach to measure the transport of uncharged as well as polyanionic, tritiated D across glomerular capillaries in the normal Munich-Wistar rat and in rats with NSN. As shown in Table III, the fractional clearance of a neutral D molecule of the same effective radius as albumin, ~36 Å, greatly exceeds that of albumin in normal

FIGURE 1 (A) Representative glomerulus from a normal Munich-Wistar rat. Note open capillaries and normal cellularity. (hematoxylin-eosin stain, original magnification × 500). (B) Representative section of a glomerulus from a rat with nephrotoxic serum nephritis. Note occlusion of capillary lumina, cellular proliferation, and polymorphonuclear leukocytic infiltration. (hematotylin-eosin stain, original magnification × 312). (C) Representative glomerulus from a normal rat stained for sialoprotein. Note dark (Prussian blue) stain along epithelial side of basement membrane. (Colloidal iron, original magnification × 312). (D) Representative glomerulus from a rat with NSN stained for sialoprotein. Note scanty staining of most capillary walls. (Colloidal iron, original magnification × 312).

TABLE II

Summary of Several Measures of Single Nephron and Microvascular Function in NSN Rats during Hydropenia*

Rat no.	Body wt	Kidney wt	$\overline{AP}$	$\bar{P}_{GC}$	P_T	P_C	C_{PA}	C_{PE}	π_A	π_E	$\frac{\overline{\pi E}}{\overline{\Delta P}}$	SNGFR	Q_A	SNFF
	g	*g*		*mm Hg*			*g/100 ml*		*mm Hg*			*nl/min*		
								9.3				34.4		
								9.7				26.1		
								—				—		
9	327	1.31	120	—	12	7	5.7	9.5	18.8	41.0	—	30.2	75.5	0.40
												36.9		
								8.3				32.5		
								8.0				30.7		
								—				—		
10	439	1.10	115	55	12	10	5.3	8.2	17.0	32.9	0.76	33.4	95.4	0.35
												32.7		
								8.5				34.9		
								8.4				23.6		
								—				—		
11	325	1.42	110	54	11	9	5.1	8.4	15.9	34.3	0.80	30.4	76.0	0.40
												40.4		
								6.9				37.2		
								6.6				35.7		
								—				—		
12	296	1.15	124	52	10	7	4.5	6.8	13.5	24.5	0.58	37.7	111.5	0.34
												39.4		
								7.8				46.4		
								7.2				43.5		
								—				—		
13	299	1.15	113	52	11	7	4.2	7.5	12.3	28.5	0.70	43.1	98.0	0.44
								8.1				27.2		
								8.0				19.5		
								7.5				17.5		
								—				—		
14	251	0.93	113	48	11	10	5.6	7.9	18.3	31.0	0.84	21.4	73.8	0.29
								6.7				30.3		
								6.8				29.3		
								6.4				23.1		
								—				—		
15	252	0.99	115	48	12	10	4.9	6.6	15.3	22.6	0.63	27.6	106.1	0.26
												27.1		
								7.5				20.8		
								7.5				19.3		
								—				—		
16	245	0.86	115	49	10	7	5.3	7.5	16.9	27.2	0.70	22.4	77.2	0.29
												19.2		
								7.5				27.3		
								7.6				25.3		
								—				—		
17	240	1.11	121	44	10	7	5.3	7.6	16.9	29.0	0.85	23.9	79.7	0.30
Overall Mean	297	1.10	116	50	11	8.2	5.1	7.8	16.1	30.1	0.73	30.0	88.1	0.34
±SE	21	0.06	1	1	0.3	0.5	0.2	0.3	0.7	1.8	0.03	2.4	4.9	0.02

Abbreviations: $\overline{AP}$, mean arterial pressure; C_{PA}, plasma protein concentration; C_{PE}, efferent arteriolar plasma protein concentration; P_C, hydraulic pressure in third-order branch peritubular capillaries; P_T, hydraulic pressure in the proximal tubule; π_A, initial glomerular capillary oncotic pressure; Q_A, initial (afferent) glomerular plasma flow rate; SNFF, single nephron filtration fraction; SNGFR, single nephron glomerular filtration rate.

* All values except for body and kidney weights represent the mean of several measurements in each rat.

hydropenic rats (7). In other words, albumin is restricted to a much greater extent than would be predicted from consideration of size alone.

Taking note of the fact that albumin is a polyanion in physiological solution, we previously examined the effect of electrical charge on glomerular permeability to macromolecules by studying the transport of DS, an anionic polymer structurally similar to neutral D (8). As can be seen in Table III, the effect of the negative charge on the DS molecule with an effective radius of

TABLE III

Fractional Clearance of Albumin Compared to that of Dextrans of Similar Molecular Size

Macromolecule (M)	Molecular radius	Condition	$(U/P)_M/(U/P)_{IN}$	Reference no.
	Å			
Albumin	36	Normal hydropenia	<0.001*	29
Neutral dextran	36	Normal hydropenia	0.19±0.01 SE (n = 7)	7
		NSN hydropenia	0.14±0.01 (n = 8)	9
DS	36	Normal hydropenia	0.015±0.005 (n = 7)	8
		NSN hydropenia	0.24±0.03 (n = 9)	Present study

* This value represents the ratio $(BS/P)_{albumin}/(BS/P)_{IN}$.

36 Å was sufficient to reduce its fractional clearance in normal rats to a value approaching that of albumin. We have interpreted this difference between the transport of DS and neutral D of the same size to indicate that there is electrostatic repulsion of circulating anionic macromolecules by some fixed, negatively charged component of the glomerular capillary wall.

As mentioned earlier, at least three possible mechanisms may account for the enhanced transglomerular passage of albumin seen with glomerular injury. One possibility, involving a change in permselectivity based on molecular size, may be discarded for our experimental model of glomerulonephritis in view of the results in the companion study (9). This study demonstrated that fractional clearances of neutral D over a broad range of radii were reduced in NSN, relative to values in normal hydropenia. Thus, as shown in Table III, for neutral D of a molecular size essentially equal to that of albumin, transport was diminished rather than increased. A second possible mechanism, involving disease-induced alterations in glomerular pressures and flows, also failed to provide an explanation for enhanced transglomerular passage of albumin (9). Indeed, the results of this previous study suggest that if albumin behaved like neutral D of similar size, its transport across the glomerular capillary wall would have been decreased in NSN, relative to normal hydropenia, in contrast to our regular finding of mild to moderate proteinuria in NSN (9, 11, 12).

To test the third possible mechanism for proteinuria, that of a disease-induced alteration in the fixed charge of the glomerular capillary wall, we examined the transport of DS in nine NSN rats in the present study. For NSN rats fractional clearances for DS were substantially greater, at any given molecular size, than those found in normal Munich-Wistar rats (Fig. 3 and Tables I and III). These findings indicate that in NSN there is a reduced ability of the glomerular capillary wall to selectively restrict the transport of polyanionic, but not neutral macromolecules (9).

The results of the present study suggest that loss of fixed negative charges from the glomerular capillary wall may be a major cause of increased filtration of plasma proteins after experimental or spontaneous glomerular injury. As to the identity of the substance or substances contributing these fixed negative charges, it has been known for some time that the glomerular epithelial cell and its foot processes are covered with a thin layer of an acidic glycoprotein (sialoprotein or glomerular polyanion) which is highly negatively charged (17–20). Furthermore, recent studies have shown that the slit pore membrane (the membrane lying between the channels formed by the interdigitating foot processes and adjacent to the outer aspect of the basement membrane) is also coated with sialoprotein (20). The glomerular basement membrane proper has also been shown to contain small amounts of sialic acid (21, 22). The sialoprotein layer(s) may function to restrict the passage of anionic macromolecules such as plasma proteins (17) and DS. In support of this possibility, glomerular sialoprotein content has been found to be diminished in glomerulopathies associated with proteinuria (19, 23), a finding also confirmed in the present study from histochemical measurements employing colloidal iron. Moreover, the sialic acid content of isolated glomerular basement membrane is reduced in experimental and clinical proteinuria states (21, 24, 25). As further confirmation of the loss of fixed negative charges in glomerulopathies, it has been shown that the electrophoretic mobility of glomerular basement membrane isolated from rats with NSN is reduced significantly (26).

At present, however, the interrelationships among loss of glomerular polyanion, swelling, distortion, or obliteration of foot processes, and the abnormal filtration of plasma proteins are unclear. A possible sequence

suggested by previous (17, 23) and present observations is as follows: immunological injury causes glomerular capillary wall damage leading to (*a*) a reduction in sialic acid content of glomerular basement membrane and the sialoprotein layer of the glomerular epithelial cells, and (*b*) occlusion of capillary lumina with reduction in the surface area for ultrafiltration. As a consequence of these changes, the transcapillary movement of neutral macromolecules is restricted, whereas the movement of anionic macromolecules is enhanced. At the present time, however, it is not possible to establish unequivocally a causal relationship between loss of glomerular polyanion and development of proteinuria. Proteinuria produced by infusions of albumin (protein overload proteinuria) is associated with ultrastructural alterations of the foot processes and reduction of glomerular sialoprotein content (27). Similar changes in glomeruli have been observed recently following infusion of polycations such as protamine sulfate or poly-L-lysine (28). These effects of polycation infusions were reversed by infusion of heparin or other polyanions (28).

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