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Research Article

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ABSTRACT To investigate the mechanism(s) of increased filtration of serum proteins after glomerular injury, polydisperse samples of uncharged [^{3}H]dextran (D) or anionic [^{3}H]dextran sulfate (DS) were infused into 14 control and 16 puromycin aminonucleoside- (PAN) treated Munich-Wistar rats. Fractional clearances of D or DS ranging in radius from 18 to 42Å were determined in these rats, together with direct measurements of the forces governing the glomerular filtration rate of water. Whole kidney and single nephron glomerular filtration rates were ~40% lower in PAN-treated rats, relative to controls, due mainly to a marked reduction in the glomerular capillary ultrafiltration coefficient and, to a lesser extent, to a small reduction in glomerular plasma flow rate as well. In PAN-treated rats, as in normal controls, inulin was found to permeate the glomerular capillary wall without measurable restriction, and both D and DS were shown to be neither secreted nor reabsorbed. Fractional clearances of uncharged D were reduced after PAN administration, falling significantly for effective D radii from 22 to 38Å. Utilizing a theory based on macromolecular transport through pores, these results indicate that in PAN-treated rats, effective pore radius is the same as in controls, ~44Å. In PAN nephrosis, however, the ratio of total pore surface area/pore length, a measure of pore density, is reduced to approximately one-third that of control, due very likely to a reduction in filtration surface area. In contrast to the results with uncharged D, fractional clearances of DS were found to increase after PAN administration for all DS radii studied. These results with D and DS suggest that proteinuria in PAN nephrosis is due, not to an increase in effective pore radius or number of pores, but rather to a diminution of the electrostatic barrier function of the glomerular capillary wall, thereby allowing increased passage of polyanions such as DS and albumin.

INTRODUCTION

Puromycin aminonucleoside (PAN) has been used widely to produce experimental animal models of lipoid nephrosis, a disorder characterized by enhanced urinary protein excretion (1–9). Morphologically, PAN regularly leads to loss of organization of the normally slender and interdigitating glomerular epithelial cell foot processes, or pedicels. These pedicels are replaced instead, in some areas, by more or less continuous sheets of epithelial cytoplasm, while in other areas the urinary aspect of the glomerular capillary basement membrane (GBM) appears devoid of any epithelial cell covering (9–17). In addition, biochemical and ultrastructural alterations in the GBM (3, 11, 16) as well as in mesangium have been noted (4, 8, 16).

While many morphological explanations have been put forth to account for the proteinuria, there have been remarkably few attempts to define the functional alterations in glomerular permselectivity associated with PAN administration. Such functional studies would seem to be of paramount importance in that much experimental and theoretical evidence exists to indicate that the passage of macromolecules across the glomerular capillary wall is influenced by a variety of factors (18–31). These not only include macromolecular size but also net macromolecular charge, and the hemodynamic determinants of the glomerular filtration rate of water (GFR) as well.

Accordingly, the present study was undertaken in PAN-treated and control Munich-Wistar rats to com-

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pare the determinants of the glomerular filtration of water together with an assessment of glomerular permselectivity to polydisperse uncharged and polyanionic dextrans in these two groups of animals.

GLOSSARY OF SYMBOLS

A/G	Albumin to globulin ratio.
$\overline{AP}$	Mean arterial pressure.
BS	Bowman's space.
$(BS/P)_D/(BS/P)_{IN}$	Fractional dextran clearance for a single glomerulus.
$(BS/P)_{DS}/(BS/P)_{IN}$	Fractional dextran sulfate clearance for a single glomerulus.
C	Total protein concentration.
C_A	Afferent arteriolar plasma protein concentration.
C_E	Efferent arteriolar plasma protein concentration.
D	Dextran.
DS	Dextran sulfate.
GBM	Glomerular capillary basement membrane.
GFR	Glomerular filtration rate of water.
K_f	Ultrafiltration coefficient.
PAN	Puromycin aminonucleoside.
P_C	Hydraulic pressure in third-order peritubular capillaries.
P_E	Hydraulic pressure in surface efferent arterioles.
$\bar{P}_{GC}$	Mean hydraulic pressure in surface glomerular capillaries.
P_T	Hydraulic pressure in the proximal tubule.
$\overline{\Delta P}$	Mean glomerular transcapillary hydraulic pressure difference.
Π	Colloid osmotic pressure.
Π_A	Afferent colloid osmotic pressure.
Π_E	Efferent colloid osmotic pressure.
Q_A	Glomerular capillary plasma flow rate.
r_0	Effective pore radius.
S'/l	Ratio of pore area to pore length.
SNFF	Single nephron filtration fraction.
SNGFR	Single nephron glomerular filtration rate.
$U_{Prot} \cdot V$	Urinary total protein excretion.
$(U/P)_D/(U/P)_{IN}$	Fractional dextran clearance for the kidney as a whole.
$(U/P)_{DS}/(U/P)_{IN}$	Fractional dextran sulfate clearance for the kidney as a whole.

METHODS

Animal studies. PAN nephrosis was induced in 16 adult female and male Munich-Wistar rats, ranging in body weight from 170 to 302 g. 10 mg of PAN (Sigma Chemical Co., St. Louis, Mo.) dissolved in 1 ml of 0.9% NaCl was administered subcutaneously at a dose of 1.67 mg/100 g body wt/day for 6–7 days. On the day after the last injection of PAN, rats were anesthetized with Inactin (100 mg/kg) (Promonta, Hamburg, W. Germany) and prepared for micropuncture as described previously (32). The fractional clearances of dextran (D) or anionic dextran sulfate (DS) were also determined.

In all rats studied, 24-h urine collections were obtained both before administration of PAN and on the 6–7th day of PAN injection (i.e., the day before study). The total protein content of the urine was determined by the Biuret method.

Studies with D and DS molecules of narrow size distribution. It has been shown previously that in the normal hydropenic Munich-Wistar rat fractional D or DS clearances obtained for the kidney as a whole, $(U/P)_D$ or $_{DS}/(U/P)_{IN}$, respectively, (estimated from comparison of the urinary clearance of various sized D and DS molecules to that of inulin) can be equated with clearances of these substances across single accessible surface glomeruli (23, 25, 29–31). It also has been shown previously that inulin appears in Bowman's space (BS) in the same concentration as that measured simultaneously in plasma water, under a variety of experimental conditions, demonstrating that inulin provides an exact measure of the glomerular filtration rate of water (23, 25, 31). Likewise it was necessary in the present study to test the validity of (*a*) equating fractional D or DS clearances for a single glomerulus $[(BS/P)_D$ or $_{DS}/(BS/P)_{IN}]$ with those for the kidney as a whole $[(U/P)_D$ or $_{DS}/(U/P)_{IN}]$ in PAN-treated rats, and (*b*) the assumption that inulin provides a valid marker for the transglomerular passage of water. These tests were accomplished as follows: tritiated D and DS molecules of narrow size distribution, prepared as reported previously (25) and characterized with respect to average Stokes-Einstein radius, were used as test solutes in four hydropenic, PAN-treated rats. A 0.4-ml priming infusion, containing nonisotopic inulin (7 g/100 ml) and tritiated D or DS (<300 mg/100 ml, sp act ≈ 50 μCi/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 for the duration of each experiment. During this infusion 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 D or 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 D or DS and inulin concentrations.

Studies with D and DS molecules of wide molecular size distribution. As will be discussed below, fractional urinary D and DS clearances in PAN rats are the same as fractional D and DS clearances measured for single accessible glomeruli in the same kidney (i.e., D and DS molecules are neither secreted nor reabsorbed). It is therefore justifiable to rely on urinary clearances to assess the permselective characteristics of all glomeruli in a single kidney, with a homologous series of D or DS molecules of widely varying molecular size. The methods for preparation of these polydisperse D and DS molecules have been given elsewhere (23, 25). Experiments with D were performed in seven hydropenic PAN-treated rats and with DS in nine hydropenic PAN-treated rats. In 13 of the PAN-treated rats studied with D or DS, micropuncture measurements of all determinants of single nephron glomerular filtration rate (SNGFR) were also made.

Initially in all experiments 0.4 ml of a solution of nonisotopic inulin in isotonic saline (7 g/100 ml) was infused intravenously followed immediately by a constant infusion of the same solution at the rate of 1.2 ml/h, for a period of 30–45 min before micropuncture. Approximately 45 min later, two or three exactly timed (1–2-min) samples of proximal tubule fluid were collected from surface proximal convolutions for determination of flow rate and inulin concentration and calculation of SNGFR. In addition samples of efferent arteriolar blood were collected from two or three superficial "star vessels" for determination of total protein concentration. The total protein concentration (C) measured in femoral arterial plasma is considered to be representative of the protein concentration in afferent arteriolar plasma. These estimates of afferent (C_A) and efferent (C_E) arteriolar plasma protein concentration permit calculation of afferent (Π_A) and efferent (Π_E) colloid osmotic pressure, single nephron filtra-

TABLE I

Comparison of Fractional D and DS Clearances for Single Glomeruli and the Kidney as a Whole in PAN-Treated Rats

Rat no.	Glomerulus no.	$\frac{(BS/P)_D}{(BS/P)_{IN}}$	$\frac{(U/P)_D}{(U/P)_{IN}}$
1	1	0.64	0.58
	2	0.89	0.78
2	1	0.79	0.83
		$\frac{(BS/P)_{DS}}{(BS/P)_{IN}}$	$\frac{(U/P)_{DS}}{(U/P)_{IN}}$
3	1	0.26	0.31
4	1	0.36	0.36

tion fraction (SNFF), and initial glomerular capillary plasma flow rate (Q_A). Samples of femoral arterial plasma were obtained from several PAN-treated rats and the albumin to globulin (A/G) ratio was determined with standard quantitative electrophoresis techniques. Mean arterial pressure ($\bar{AP}$) and hydraulic pressures in surface glomerular capillaries ($\bar{P}_{GC}$), proximal tubules (P_T), surface efferent arterioles (P_E), and third-order peritubular capillaries (P_C) were measured with continuous-recording, Servo-Null micropipette transducer techniques (Instruments for Physiology and Medicine, San Diego, Calif.) described previously (33).

Immediately after the micropuncture measurements had been completed 0.4 ml of an isotonic saline solution containing either tritiated D or DS of broad molecular size distribution (D or DS concentration <300 mg/100 ml, sp act $\simeq$ 25 μCi/ml) 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 procedures for collection, processing and analyzing blood, urine and tubule fluid have been given previously (23, 25, 29, 31, 34).

Calculations. Details of the equations employed for calculation of SNGFR, Π_A and Π_E, SNFF, Q_A, and the ultrafiltration coefficient (K_f) have been reported previously (34, 35). With a theory based on macromolecular transport through an isoporous membrane, the effective pore radius and the ratio of pore surface area to pore length were calculated as discussed in detail elsewhere (24).

RESULTS

Before injection of PAN, 24-h urinary total protein excretion ($U_{Prot} \cdot V$) was found to average 10±2 mg(SE) in 13 rats and increased in each rat, on average to 67±25 mg ($P < 0.05$) after 6–7 days of PAN administration.[1] Although the mean increase in $U_{Prot} \cdot V$ with PAN treatment was nearly sevenfold, urinary protein excretion increased only two- to threefold in most rats studied. In three rats, however, greater degrees of proteinuria were observed, thereby accounting for the large average increase.

[1] The dose of PAN employed in this study was chosen since it has been shown to result in characteristic glomerular morphological abnormalities by 6–7 days (16).

Studies with D or DS molecules of narrow size distribution. Table I presents data comparing $(BS/P)_D$ or $_{DS}/(BS/P)_{IN}$ ratios with simultaneously measured values of $(U/P)_D$ or $_{DS}/(U/P)_{IN}$. It is evident that fractional D or DS clearances obtained for five single superficial glomeruli from four PAN-treated rats were essentially the same as fractional clearances measured for the kidney as a whole. These data were obtained for dextrans ranging in molecular radii from 22 to 24Å. For all paired measurements the ratio of $(BS/P)_D$ or $_{DS}/(BS/P)_{IN}$ vs. $(U/P)_D$ or $_{DS}/(U/P)_{IN}$ averaged 1.01±0.05. These findings demonstrate that in these Munich-Wistar rats treated with PAN, D and DS molecules appear to be neither secreted nor reabsorbed by the renal tubules, in accord with our previous findings in this same strain of rats during normal hydropenia (23, 25), after induction of nephrotoxic serum nephritis (29, 31), and in response to angiotensin II infusion (30). The results further suggest that fractional clearances of D and DS are homogeneous from glomerulus to glomerulus within a single kidney under the conditions of the present experiments. Furthermore, the mean value for the ratio of inulin concentration in BS to plasma water was found to equal 1.06±0.05 ($n = 5$), a value not significantly different from unity ($P > 0.2$), indicating that PAN treatment as employed in the present study does not lead to measurable restriction to the transglomerular passage of inulin.

Studies with D or DS molecules of broad size distribution. In view of the evidence (Table I) that fractional D or DS clearances for the whole kidney provide a reliable measure of D or DS permeation across capillaries of a single superficial glomerulus in PAN-treated rats, it was possible to characterize the glomerular transport of a wide range of molecular sizes of both D and DS in these rats. The methods employed in the present study allowed collection of sufficient quantities of blood and urine to permit chromatographic separation of D or DS of wide molecular size distribution into constituent narrow molecular size fractions. Fractional D or DS clearance profiles were therefore constructed for each rat based on simultaneous clearances for dextrans ranging in Stokes-Einstein molecular radii from 18 to 42Å. The relationship between the fractional clearances of D or DS, given by the ratio $(U/P)_D$ or $_{DS}/(U/P)_{IN}$, and effective D or DS radius for PAN-treated hydropenic rats is summarized in Tables II and III. For comparison, mean values for fractional D or DS clearances obtained in two separate and concurrently studied groups of normal control hydropenic rats are also given. As can be seen for normal control rats in Table II, there was no measurable restriction to the transport of uncharged D until the effective molecular radius exceeded 20Å. Fractional clearances of D decreased progressively with further increases in molecular size,

TABLE II

Comparison of Fractional Clearances of D in PAN-Treated Rats Vs. Normal Hydropenic Rats

	$(U/P)_D/(U/P)_{IN}$												
	18Å	20Å	22Å	24Å	26Å	28Å	30Å	32Å	34Å	36Å	38Å	40Å	42Å
	Effective dextran radii												
Puromycin rat no.													
1	0.76	0.62	0.48	0.34	0.25	0.17	0.11	0.08	0.04	0.025	0.015	0.009	0.007
2	1.04	0.92	0.78	0.62	0.48	0.34	0.23	0.15	0.09	0.044	0.030	0.018	0.014
3	0.88	0.81	0.63	0.51	0.38	0.27	0.19	0.11	0.08	0.038	0.018	0.012	0.008
4	0.64	0.56	0.49	0.40	0.32	0.25	0.19	0.14	0.10	0.060	0.035	0.018	0.010
5	1.00	0.99	0.84	0.65	0.52	0.38	0.25	0.16	0.08	0.055	0.021	0.012	0.010
6	1.02	0.93	0.80	0.64	0.49	0.36	0.25	0.17	0.10	0.057	0.030	0.017	0.015
7	0.96	0.91	0.84	0.73	0.56	0.36	0.23	0.14	0.08	0.044	0.020	0.011	0.010
Mean	0.88	0.82	0.69	0.56	0.43	0.30	0.21	0.14	0.07	0.046	0.024	0.014	0.011
±1 SE (n = 7)	0.04	0.06	0.06	0.05	0.04	0.03	0.02	0.01	0.01	0.005	0.003	0.001	0.001
Normal hydropenic rats													
Mean	1.00	0.97	0.87	0.73	0.60	0.45	0.32	0.22	0.15	0.090	0.045	0.022	0.008
±1 SE (n = 7)	0.02	0.03	0.04	0.04	0.03	0.03	0.02	0.02	0.01	0.007	0.007	0.005	0.001
P value*	NS	NS	<0.05	<0.025	<0.01	<0.005	<0.005	<0.001	<0.001	<0.001	<0.025	NS	NS

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

approaching zero at 42Å. These results are similar to those reported previously (25, 31). After treatment with PAN, fractional clearances of D decreased substantially relative to the values obtained in normal control rats, the mean decrease being statistically significant for D over a range of effective radii between 22–38Å (Table II). As shown in Table III, fractional clearances of polyanionic DS in control rats were substantially lower than those of uncharged D (Table II), reaffirming our previous conclusion that the normal glomerular capillary wall restricts the passage of polyanions to an extent greater than that of uncharged polymers (23, 29). The fractional clearances of polyanionic DS, however, were higher in PAN treated rats than in controls (Table III), the increase being statistically significant for effective DS radii of 30–42Å.

Measurements of glomerular dynamics. Table IV and Fig. 1 summarize individual and mean values for several indices of single nephron function in 13 of the PAN-treated rats studied with D or DS, together with

TABLE III

Comparison of Fractional Clearances of DS in PAN-Treated Rats Vs. Normal Hydropenic Rats

	$(U/P)_{DS}/(U/P)_{IN}$												
	18Å	20Å	22Å	24Å	26Å	28Å	30Å	32Å	34Å	36Å	38Å	40Å	42Å
	Effective dextran sulfate radii												
Puromycin rat no.													
8	0.52	0.33	0.19	0.10	0.05	0.03	0.019	0.010	0.004	0.002	0.001	0.0005	0.0002
9	0.37	0.20	0.10	0.05	0.03	0.02	0.016	0.011	0.006	0.003	0.001	0.0007	0.0006
10	0.52	0.31	0.17	0.09	0.05	0.04	0.031	0.024	0.018	0.011	0.006	0.004	0.002
11	0.78	0.63	0.43	0.26	0.14	0.08	0.047	0.029	0.017	0.008	0.002	0.0005	0.0003
12	0.66	0.49	0.32	0.20	0.14	0.10	0.073	0.050	0.031	0.019	0.010	0.004	0.002
13	0.56	0.38	0.24	0.15	0.10	0.07	0.060	0.045	0.029	0.017	0.009	0.003	0.001
14	0.56	0.42	0.26	0.14	0.07	0.04	0.020	0.014	0.009	0.003	0.002	0.001	0.0005
15	0.49	0.31	0.19	0.12	0.07	0.05	0.041	0.029	0.018	0.010	0.003	0.0015	0.0006
16	0.67	0.51	0.37	0.27	0.19	0.14	0.107	0.079	0.050	0.025	0.011	0.003	0.0005
Mean	0.57	0.40	0.25	0.15	0.09	0.06	0.046	0.032	0.020	0.011	0.005	0.002	0.0009
±1 SE (n = 9)	0.04	0.04	0.04	0.03	0.02	0.01	0.010	0.007	0.005	0.003	0.001	0.0005	0.0002
Normal hydropenic rats													
Mean	0.56	0.35	0.19	0.11	0.06	0.032	0.020	0.013	0.007	0.003	0.0009	0.0004	0.0002
±1 SE (n = 7)	0.05	0.03	0.02	0.02	0.01	0.007	0.004	0.003	0.001	0.0003	0.0001	0.0001	0.0001
P value*	NS	NS	NS	NS	NS	NS	<0.05	<0.05	<0.05	<0.025	<0.025	<0.025	<0.025

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

TABLE IV

Summary of Several Measures of Single Nephron and Microvascular Function in PAN-Treated Rats Vs. Normal Control Rats

Rat no.*	$\overline{\Delta P}$	$\hat{P}_{GC}$	P_T	C_A	C_E	Π_A	Π_E	$\Pi_E/\overline{\Delta P}$	SNGFR	Q_A	SNFF	K_f
		mm Hg		*g/100 ml*		*mm Hg*			*nl/min*			*nl/s·mm Hg*
									13.7			
					5.4				12.5			
					5.6				19.2			
1	125	41	12	3.8	5.5	10.8	17.9	0.62	15.1	48.7	0.31	0.017
									18.2			
					7.1				25.0			
					7.1				16.7			
2	120	46	11	5.1	7.1	16.1	26.2	0.75	20.0	71.4	0.28	0.024
									20.5			
					7.2				18.2			
					6.8				21.6			
3	110	46	12	4.7	7.0	14.3	25.6	0.75	20.1	60.9	0.33	0.023
									13.2			
					5.3				12.5			
					5.5				17.3			
4	110	41	12	3.6	5.4	10.1	17.4	0.60	14.3	43.3	0.33	0.015
									29.8			
					7.7				30.7			
					8.7				28.8			
5	120	46	12	4.8	8.2	14.8	32.9	0.97	29.8	54.2	0.55	0.046
									21.3			
					7.6				19.2			
					7.8				23.8			
6	105	42	10	4.6	7.7	13.9	29.8	0.93	21.4	53.5	0.40	0.039
					6.5							
					6.1							
7	115	45	12	4.4	6.3	13.1	21.8	0.66	12.2	30.5	0.40	0.010
					6.2				30.1			
					6.7				23.3			
					6.1				26.2			
8	115	48	11	5.1	6.3	16.1	21.8	0.59	26.5	139.5	0.19	0.024
					6.1				24.7			
					6.5				18.5			
					5.6				25.2			
9	105	48	12	4.5	6.1	13.5	20.8	0.58	22.8	87.7	0.26	0.020
					7.7				14.5			
					5.5				16.3			
10	110	44	11	3.8	6.7	10.8	23.4	0.71	15.4	36.7	0.42	0.016
									15.4			
									12.1			
11	120	44	11	4.3	7.8	12.7	30.4	0.92	13.8	30.7	0.45	0.021
									14.7			
									15.4			
13	120	45	11	5.1	7.5	16.1	28.5	0.84	15.1	47.2	0.32	0.022

TABLE IV (*Continued*)

Rat no.*	$\overline{\Delta P}$	$\bar{P}_{GC}$	P_T	C_A	C_E	Π_A	Π_E	$\Pi_E/\overline{\Delta P}$	SNGFR	Q_A	SNFF	K_f
	mm Hg			*g/100 ml*		*mm Hg*			*nl/min*			*nl/s·mm Hg*
									21.1 23.9			
14	105	47	12	4.6	7.9	13.9	31.0	0.89	22.5	53.6	0.42	0.030
Overall												
mean	114	45	11	4.5	6.9	13.6	25.2	0.75	19.2	58.3	0.36	0.024
±1 SE	2	1	0.2	0.1	0.3	0.6	1.4	0.04	1.5	8.1	0.03	0.003
(n = 13)												
Normal hydropenic rats												
Mean	112	45	11	5.1	8.3	16.1	33.8	1.00	30.8	79.4	0.39	0.060‡
±1 SE	2	0.3	0.3	0.1	0.1	0.3	0.6	0.02	1.5	4.1	0.01	0.005
(n = 14)												
P value§	NS	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	NS	<0.001

* Fractional clearances of DS were determined in nine rats in this group whereas indices of single nephron and microvascular function were determined in six of the rats, identified as rat nos. 8–11, 13, 14. Rat nos. 1–7 were studied with neutral D.
‡ Value represents the mean±1 SE of the individual minimum K_f values calculated in each rat in this group.
§ P values calculated from unpaired data with Student's t test.

mean values for these same indices obtained in 14 control normal hydropenic rats. As shown, mean values for SNGFR in these PAN-treated rats were significantly lower, on average by ~40%, than in normal controls, in accord with the reductions in whole kidney GFR which averaged 0.56±0.04 ml/min with PAN in comparison to the normal control mean value of 0.95±0.06 ml/min ($P < 0.001$). A lesser but statistically significant reduction in initial glomerular plasma flow rate, Q_A, averaging about 20%, was also observed with PAN treatment. This greater reduction in SNGFR than Q_A accounts for the slight fall in SNFF. Since arterial hematocrit remained unaffected by PAN (50.9±1 ml/100 ml vs. control mean of 50.5±1 ml/100 ml $P > 0.5$) the reduction in glomerular blood flow was proportional to that in Q_A. $\overline{\Delta P}$, $\bar{P}_{GC}$, and P_T were not significantly affected by PAN treatment; the mean glomerular transcapillary hydraulic pressure difference ($\overline{\Delta P}$) was therefore similarly unaffected by PAN administration, averaging 33±1 mm Hg compared to the normal control value of 34±1 mm Hg ($P > 0.5$) (Fig. 1). Although not given in Table IV, values for P_C and P_E averaged 9±0.4 and 15±0.7 mm Hg, respectively, in PAN-treated rats, compared to control values of 9±0.3 and 13±0.5 mm Hg. In spite of the nearly identical mean values for $\overline{\Delta P}$ observed in control and PAN-treated rats, equality between $\overline{\Delta P}$ and Π_E was obtained only in control but not in the PAN-treated group. The condition of filtration pressure disequilibrium, denoted by ratios of $\Pi_E/\overline{\Delta P}$ significantly less than unity, which was obtained in 11 of the 13 PAN-treated rats studied, was due to marked falls in the value of C_E, hence Π_E. Values for C_A and thus Π_A, were also reduced significantly in PAN-treated rats, presumably due to the associated proteinuria. Although not shown, the A/G of systemic plasma obtained in PAN-treated rats was found to be close to unity, thus validating the use of the Landis-Pappenheimer equation (36) for calculation of oncotic pressure (Π) from measured values of C. As previously discussed in detail (35), the condition of filtration pressure disequilibrium permits calculation

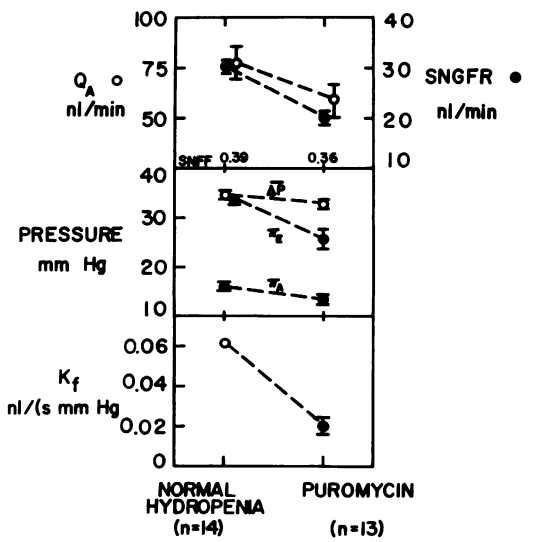

FIGURE 1 Summary of effects of 6–7 days of PAN treatment on the determinants of SNGFR. Mean data±1SE obtained from 14 normal control Munich-Wistar rats (left) and 13 PAN-treated rats (right) are shown.

of unique values for the ultrafiltration coefficient, K_f. In PAN-treated rats, the value of K_f was found to average 0.024±0.003 nl/(s·mm Hg) compared to a minimum mean value in normal hydropenia of 0.060 nl/(s·mm Hg) ($P < 0.001$).

Membrane parameters derived from pore theory. As discussed in detail elsewhere (24) it is possible, using pore theory, to calculate both effective pore radius and the ratio of pore surface area/pore length from measurements of K_f, $\overline{\Delta P}$, Q_A, C_A, and the mean values of $(U/P)_D/(U/P)_{IN}$ ratios for each effective D radius. Values of pore radius computed in this manner for D of varying molecular size are shown in Fig. 2. Values are shown for the PAN-treated rats (open circles) and for the hydropenic control rats (solid circles). Over the range of effective dextran radii studied, effective pore radius was found to be relatively independent of dextran size, averaging approximately 44Å both in PAN-treated rats and control rats. In contrast, the ratio of pore surface area to pore length, a measure of pore density, in PAN-treated rats was approximately one third of that in control rats (Fig. 2), an effect attributable to the marked fall in K_f with PAN treatment, in the absence of a substantial change in pore radius.[2]

DISCUSSION

In rats treated with PAN for 6–7 days in the dose employed in this study, the kidneys in situ appeared normal by stereomicroscopy. Although micropuncture data gave evidence of substantial reduction in SNGFR (to ~60% of control), values for SNGFR in any one kidney, estimated from fluid collections in several different surface proximal tubules, were remarkably similar (Table IV). These findings indicate that this PAN-induced lesion was generally uniform with regard to glomerular function. Further evidence for homogeneity of function in PAN-treated rats is provided by the close correspondence of fractional D and DS clearances obtained for single glomeruli and the kidney as a whole (Table I).

Values for SNGFR and whole kidney GFR were about 40% lower in PAN treated rats than in controls. Oken and co-workers, using a single injection method for inducing PAN nephrosis, reported equivalent reductions in both SNGFR and whole kidney GFR (2, 5). It has been suggested, on the basis of indirect evidence, that in certain glomerulopathies, including PAN-induced nephrosis, inulin clearance may not reflect the true value of GFR due to a decrease in glomerular permeability to molecules as small as inulin (37, 38). This possibility was effectively excluded in the present study by the finding that inulin concentrations in fluid from BS failed to differ significantly from concentrations in plasma water. Similar lack of restricted transglomerular passage of inulin was demonstrated by us in rats with nephrotoxic serum nephritis as well (31). In addition to its unrestricted passage across the glomerular capillary wall, inulin has been shown in tubular microinjection studies in PAN-treated rats to be recovered completely in final urine, thereby excluding the possibility of a significant transtubular leak of inulin (38, 39). Thus, inulin clearance is taken to provide an accurate measure of GFR in the present study.

In PAN-treated rats initial Q_A was observed to fall, on average, to a lesser extent than did SNGFR (Fig. 1), implying that in addition to the decline in Q_A, there must also have been an alteration in one or more of the other determinants of ultrafiltration to account for the greater decline in SNGFR. One possible candidate, $\overline{\Delta P}$, was found to be similar in PAN and control rats. Although essentially normal values for $\overline{\Delta P}$ were obtained in PAN-treated rats, equality between $\overline{\Delta P}$ and Π_E did not obtain (Fig. 1), due to the failure of Π_E to rise to a value sufficient to equal and oppose $\overline{\Delta P}$. This reduction in Π_E, relative to control rats, was due entirely to the profound PAN-induced reduction in the ultrafiltration coefficient, K_f. Thus, the marked falls in SNGFR and total kidney GFR observed in the present study in response to PAN administration appear to be due primarily to the fall in K_f, and to a lesser extent, to the fall in Q_A as well.

In addition to the morphological findings of others of widespread glomerular injury after PAN treatment (9–17), there is also functional evidence to indicate that the proteinuria associated with PAN administration is primarily of glomerular origin. Thus, Oken et al. demonstrated relative equality between the amount of albumin in proximal tubule fluid and that in final urine in PAN-treated rats (5). Further, in tubule microinjection studies, Lewy and Pesce (39) found little difference between PAN and control rats in the recovery in final urine of early proximal injections of ^{125}I-albumin. To investigate the mechanism(s) whereby PAN induces an increase in the transglomerular passage of macromolecules, fractional clearances of both uncharged D and polyanionic DS of various effective radii were measured in the present study. As shown in Table II, fractional clearances of D decreased after PAN administration for all effective radii studied, the decrease being significant for radii from 22 to 38Å.

[2] The relationship between K_f, effective pore radius (r_0), and the ratio of pore area to pore length (S'/l), derived from pore theory and discussed in detail elsewhere (24) is: $K_f = (S'/l)\ (r_0^2/8\eta)$, where $\eta = 0.007$ poise is the viscosity of water at 37°C. In this calculation, uncharged macromolecular transport is assumed to occur across an isoporous membrane. The word "membrane" is used here to refer to the functional properties of the multicomposite structure of the glomerular capillary wall, and is not to be confused with any specific anatomical structure such as the GBM.

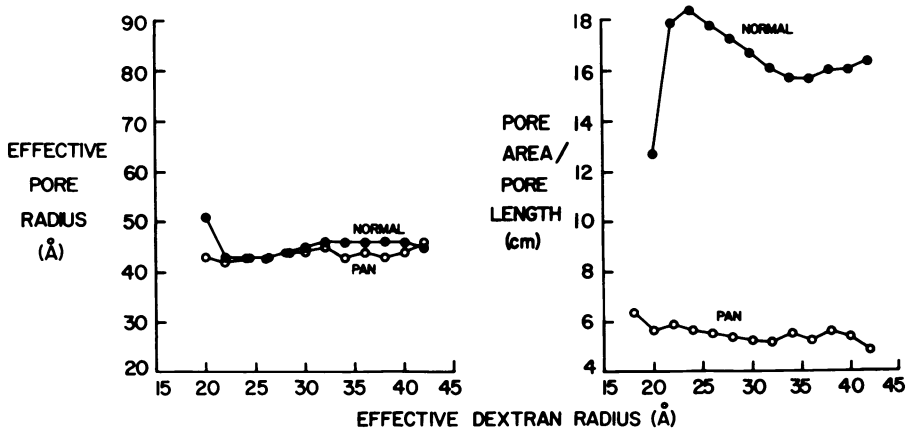

FIGURE 2 The relationship between effective pore radius and effective dextran radius for PAN-treated and normal control rats is shown on the left. The relationship between the ratio of total pore area/pore length and effective dextran radius for PAN-treated and normal control rats is shown on the right.

These decreases in fractional D clearances were found, using pore theory, to be the result of a decrease in the ratio of pore surface area/pore length (a measure of pore density). Whether the latter reflects a reduction in the available filtration surface area, increase in pore length, or both, cannot be ascertained from the present results. Nevertheless, either or both of these possibilities seem reasonable in view of the observed fall in K_f since the effective pore radius remained essentially unchanged (Fig. 2). Q_A, the other hemodynamic determinant of glomerular ultrafiltration which was altered (i.e. reduced) by PAN treatment, would on theoretical (24) and experimental grounds (30) be expected to have resulted in enhanced rather than reduced fractional clearances of macromolecules. Thus, the observed changes in Q_A fail to explain the observed decline in fractional D clearances noted in the present study. Accordingly, our findings suggest a reduction in the permeability of the nephrotic glomerular capillary wall to uncharged macromolecules and therefore fail to explain the concomitant proteinuria, since, if plasma proteins, including albumin, behaved in a manner analogous to that of uncharged dextrans, the filtration and excretion of these plasma proteins would have been expected to diminish, rather than increase, with this form of glomerular injury.

In reconciling this apparent paradox between uncharged dextrans and plasma proteins, it is important to recognize that, in addition to macromolecular size, the passage of macromolecules across the glomerular capillary wall is influenced to an important extent by the net electrical charge of the circulating molecules (23, 28–30). The wall of the glomerular capillary has been shown to possess significant quantities of negatively charged glycosialoproteins (glomerular polyanion) (40–46), believed to constitute an electrostatic barrier to the passage of circulating polyanions such as albumin (23, 28, 29, 40). Therefore, the use of anionic probe macromolecules such as DS may represent more appropriate markers for the transglomerular passage of anionic proteins such as albumin than do uncharged polymers such as dextrans or polyvinylpyrrolidone.[3] As is apparent from Table III, the fractional clearances of polyanionic DS molecules after PAN treatment were generally greater for any given molecular radius than values observed in normal controls, the increase being statistically significant over the range of effective DS radii from 30 to 42Å. Relative to the increase in $U_{Prot} \cdot V$ observed with PAN-treated rats (which in most rats increased two- to threefold, compared to pre-PAN values), it is of interest that the absolute clearance of 36Å DS, equivalent in size to albumin, also increased approximately twofold in PAN rats. This increase in absolute DS clearance arises from the fact that the increase in fractional clearance of 36Å DS was nearly fourfold, whereas the simultaneous decline in total GFR was about 40%. Accordingly, the increments in urinary protein and 36Å DS excretions were generally comparable for most rats given PAN.[4]

[3] It is worth stressing, however, that the use of uncharged dextrans as probe molecules provides unique insights into glomerular permeability to macromolecules in terms of assessing changes in pore radius and/or pore density. In part, this is due to the fact that it is not yet possible to derive these same membrane parameters using fractional clearances of negatively charged DS molecules since neither the net valence of the DS molecule nor possible changes in the transglomerular electrical potential difference due to the presence of DS are known.

[4] There are, however, several factors which make us reluctant to place much emphasis on this general similarity between changes in $U_{Prot} \cdot V$ and those in $U_{DS} \cdot V$. One factor pertains to the Biuret method employed to measure $U_{Prot} \cdot V$.
(Footnote 4 continued on next page.)

The findings in the present study are therefore suggestive of a reduction in the electrostatic barrier function of the glomerular capillary wall in PAN nephrosis, an effect that serves to enhance the transglomerular passage of circulating negatively charged macromolecules, including albumin. Further evidence that loss of glomerular polyanion may be an important factor in the pathogenesis of the proteinuria observed in PAN nephrosis derives from several studies in which it has been shown that the binding of cationic substances to anionic sites on the glomerular capillary wall is reduced substantially in PAN-treated rats, when compared to controls (41, 46–48). Of importance, our observations with polyanionic DS are suggestive of a reduction, rather than complete loss, of glomerular polyanion after PAN treatment in that fractional clearances of DS remained significantly lower than those of uncharged D (Tables II and III), suggesting persistence of some electrostatic barrier function.

Caulfield and Farquhar (16), employing ultrastructural techniques, reported an increase in the passage of uncharged dextran molecules into the urinary space in nephrotic rats subjected to the same PAN dose regimen as was employed in the present study. Their findings are thus at a variance with those reported herein, as well as with those of Buerkert et al. who also reported decreased fractional clearances (38) of uncharged polymers in rats with PAN nephrosis. Moreover, Robson et al. (37) also found reductions in fractional clearances of uncharged polymers in children with minimal change nephrotic syndrome, as did Chang et al. in rats with experimentally induced nephrotoxic serum nephritis (31). This discrepancy between these various fractional clearance data obtained in vivo and the findings of Caulfield and Farquhar (16) is probably due to the protocol employed by the latter workers. In their study, relatively large quantities of nonisotopic dextrans were infused in both normal and PAN-treated rats in doses proportional to rat body weight, but without determination of the concentrations of dextran in blood. In view of the profound reductions in whole kidney GFR that undoubtedly occurred after PAN administration, infusion of equivalent amounts of dextran in PAN-treated and control rats would probably have led to considerably higher blood dextran levels in PAN-treated than in control rats, thereby resulting in greater absolute excretion rates for dextrans in nephrotic rats, but not necessarily greater fractional dextran clearances. A second major flaw with the study of Caulfield and Farquhar (16) derives from their failure to characterize the radius of the molecules gaining access to the urinary space, both in normal and PAN rats. Despite utilization of dextran fractions of relatively narrow size distribution, a broad range of molecular sizes is inevitable, even though most of the molecules may conform to a narrow population. It is therefore possible that the dextrans demonstrated in the urinary space of PAN rats in their study were of relatively small molecular radius. For these reasons, the conclusion of Caulfield and Farquhar (16) that PAN enhances glomerular permeability to uncharged dextrans is open to question.

Since this method is quantitatively inexact, we believe it unreasonable to assign more than qualitative importance to the measured changes in protein excretion induced by PAN. Another factor to be appreciated is that the contribution of tubular reabsorption of various filtered plasma proteins to overall urinary total protein excretion rate has not been as fully defined as has been possible for DS. Finally, since the composition of the urinary proteins excreted after PAN treatment was not assessed in this study, the magnitude of the increase in albumin excretion (radius = 36Å) that occurred is unknown, so that a rigorous quantitative comparison between $U_{DS} \cdot V$ (for DS radius = 36Å) and $U_{Prot} \cdot V$ is not possible.

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