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Research Article

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Determinants of Glomerular Filtration in Experimental Glomerulonephritis in the Rat

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ABSTRACT Pressures and flows were measured in surface glomerular capillaries, efferent arterioles, and proximal tubules of 22 Wistar rats in the early autologous phase of nephrotoxic serum nephritis (NSN). Linear deposits of rabbit and rat IgG and C3 component of complement were demonstrated in glomerular capillary walls by immunofluorescence microscopy. Light microscopy revealed diffuse proliferative glomerulonephritis, and proteinuria was present. Although whole kidney and single nephron glomerular filtration rate (GFR) in NSN (0.8±0.04 [SE] ml/min and 27±2 nl/min, respectively) remained unchanged from values in 16 weight-matched normal hydropenic control rats (0.8±0.08 and 28±2), important alterations in glomerular dynamics were noted. Mean transcapillary hydraulic pressure difference ($\overline{\Delta P}$) averaged 41±1 mm Hg in NSN versus 32±1 in controls ($P < 0.005$). Oncotic pressures at the afferent (π_A) end of the glomerular capillary were similar in both groups (~16 mm Hg) but increased much less by the efferent end (π_E) in NSN (to 29±1 mm Hg) than in controls (33±1, $P < 0.025$). Hence, equality between ΔP and π_E, denoting filtration pressure equilibrium, obtained in control but not in NSN rats. While glomerular plasma flow rate was slightly higher in NSN (88±8 nl/min) than in controls (76±6, $P > 0.2$), the failure to achieve filtration equilibrium in NSN rats was primarily the consequence of a marked fall in the glomerular capillary ultrafiltration coefficient, K_f, to a mean value of ~0.03 nl/(s·mm Hg), considerably lower than that found recently for the normal rat, 0.08 nl/(s·mm Hg). Thus, despite extensive glomerular injury, evidenced morphologically and by the low K_f, GFR remained normal. This maintenance of GFR resulted primarily from increases in $\overline{\Delta P}$, which tended to increase the net driving force for filtration, and thereby compensate for the reduction in K_f.

INTRODUCTION

Despite impressive advances in the understanding of the immunologic and morphologic aspects of glomerulonephritis, relatively little is known of the forces governing glomerular ultrafiltration in this disorder. This shortcoming is due in large part to the fact that glomeruli are rarely encountered as surface structures in the mammalian kidney and are therefore inaccessible to direct study *in vivo*. This restriction has been overcome, however, in that a unique strain of Wistar rats with surface glomeruli has recently been discovered, allowing direct assessment of the determinants of ultrafiltration (1–5). Accordingly, the present study was undertaken in an effort to examine, by using previously described micropuncture techniques (1–6), the initial changes in glomerular function in this disorder.

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GLOSSARY OF SYMBOLS

$\overline{AP}$	Mean femoral arterial pressure, mm Hg.
A/G	Albumin/globulin ratio.
C	Protein concentration, g/100 ml.
EABF	Efferent arteriolar blood flow, nl/min.
GBF, GPF	Glomerular blood flow and plasma flow, respectively, nl/min.
GBM	Glomerular capillary basement membrane.
GFR	Glomerular filtration rate (whole kidney).
Hct_A	Blood hematocrit in femoral artery or afferent arteriole.
H&E	Hematoxylin and eosin.
K_f	Ultrafiltration coefficient, nl/(s·mm Hg) or nl/(min·mm Hg).
KFAb	Kidney fixing antibody.
NSN	Nephrotoxic serum nephritis.
P	Hydraulic pressure, mm Hg.
PAS	Periodic acid-Schiff
P_{UF}	Net ultrafiltration pressure, mm Hg.
ΔP	Transmembrane hydraulic pressure difference, $P_{GC} - P_T$, mm Hg.
π	Colloid osmotic pressure, mm Hg.
$\Delta\pi$	Transmembrane osmotic pressure difference, $\pi_{GC} - \pi_T$, mm Hg.
R	Resistance to blood flow, dyn·s·cm^{-5}.
R_{TA}	Total arteriolar resistance, $R_A + R_E$, dyn·s·cm^{-5}.
SNFF	Single nephron filtration fraction.
SNGFR	Single nephron glomerular filtration rate, nl/min.
$(TF/P)_{In}$	Tubule fluid to plasma inulin concentration ratio.
V_{TF}	Tubule fluid flow rate, nl/min.

Superscript

—	Mean value.

Subscripts

A	Afferent arteriole.
C	Peritubular capillary.
E	Efferent arteriole.
GC	Glomerular capillary
T	Proximal tubule.

METHODS

Induction of experimental glomerulonephritis. A nearly pure suspension of glomeruli was prepared from frozen rat kidneys (Pel-Freez Biologicals, Inc., Rogers, Ark.) by a method modified from Spiro (7). After sonication glomerular basement membranes (GBM) were separated from cellular debris by washing and repeated centrifugation and were then lyophylized. Approximately 0.5 mg (dry weight) of the resultant GBM, mixed with incomplete Freund's adjuvant, was injected once each week for a period of 6 wk into each of six adult male New Zealand white rabbits. Rabbits were then repeatedly bled and given further booster injections over a several week period. Harvested sera were tested qualitatively for anti-GBM antibody potency by intravenous injection of 1-ml aliquots into adult Wistar rats; those sera causing immediate proteinuria were pooled. The antisera were inactivated at 56°C for 30 min before use. Immune rabbit gamma globulin (IgG) was separated from an aliquot of the pool by precipitation with 50% saturated ammonium sulfate and chromatographed on DEAE-cellulose. The kidney fixing antibody content (KFAb) of this IgG was assayed by using a paired label technique (8); similarly isolated normal rabbit IgG served as control. The average IgG concentration of the pooled rabbit sera was 9.5 mg/ml; the KFAb content of this IgG was found to be 1.7%. By indirect immunofluorescence microscopy, this antiserum produced 4+ linear deposits on both glomerular and tubular basement membranes of frozen sections of normal rat kidneys, up to a dilution of 1:400. No staining of tubule epithelial cell cytoplasm occurred.

Preliminary experiments showed that 33–35 days after intravenous injection of 0.5–1.0 ml of antiserum into Munich-Wistar rats, kidneys were found to be pale and swollen with scattered collapsed tubules, and to have glomeruli that were so edematous and bloodless that micropuncture of capillaries was not possible. Accordingly, the dose of antiserum was reduced to one-fourth of that which regularly produced immediate proteinuria. After control 24-h urine collections for protein determination, adult Munich-Wistar rats weighing 107–300 g were preimmunized by footpad injection with 0.5–1 mg of partially aggregated rabbit IgG in complete Freund's adjuvant. 48 h later a small dose of rabbit anti-rat GBM antiserum (41 μg of KFAb in 0.25 ml) was injected into the tail vein and a 24-h urine collection was begun. Subsequently 24-h urine collections were usually collected on day 3 or 4 and at various times thereafter. 5–16 days after injection of nephrotoxic serum the determinants of glomerular ultrafiltration were measured by appropriate micropuncture techniques.

After micropuncture measurements, a midcoronal section of each kidney was snap-frozen in isopentane in a dry ice-acetone bath for subsequent examination by immunofluorescence microscopy. An adjacent section of tissue was fixed in Bouin's solution for light microscopy. In addition, 1-mm^3 sections of cortex from four rat kidneys were fixed in 2.5% gluteraldehyde and subsequently prepared for electron microscopy. Fluorescence antisera (Cappel Laboratories, Inc., Downington, Pa.) against rabbit IgG, rat IgG, rat C3 and rat fibrinogen were used for immunofluorescence studies. Cryostat sections of tissue (6 μm in thickness) fixed in acetone were examined by fluorescence microscopy. Normal rat kidneys served as control. Sections (4 μm) stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) were examined by light microscopy.

Quantitative analysis of histology. An attempt was made to quantitate the extent and distribution of the proliferative response engendered by nephrotoxic serum administration. H&E-stained midcoronal plane sections, 4 μm in thickness, were viewed under 430× magnification, and glomerular cell counts (excluding inflammatory cells) were made randomly on ten glomeruli, five deep and five superficial, by using a special grid (Scientific Products, Charlotte, N. C., M2493-8, 10 mm, interval = 100). Deep glomeruli were defined as glomeruli situated more than one-half the distance from the capsule to the corticomedullary junction. Only glomeruli exceeding seven grid squares in diameter were counted to avoid the bias introduced by counting glomeruli sectioned at some distance from the equatorial plane. The total cross-sectional area was calculated from the average of two diameters, assuming a circular shape. The number of cells per mm^2 was then calculated. Ten experimental animals and two control animals were examined. Statistical evaluation of mean differences was performed by two-tail t test and variances were compared by the F statistic (9).

Clearance and micropuncture studies. Experiments were performed in 38 adult mutant Wistar rats weighing 134–314 g and allowed free access to food and water before study. Rats were anesthetized with Inactin (100 mg/kg) and prepared for micropuncture as described previously (10–12). 60 min before micropuncture rats received an intravenous infusion of isotonic NaCl containing 10% inulin at the rate of 0.02 ml/min. Mean femoral arterial pressure ($\overline{AP}$) was monitored by means of an electronic transducer (model P23Db, Statham Instru-

ments, Inc., Oxnard, Calif.) connected to a direct-writing recorder (model 7702B, Hewlett-Packard Co., Palo Alto, Calif.). After this 60-min equilibration period, exactly timed (1–2 min) samples of fluid were collected from each experimental tubule for determination of flow rate and inulin concentration and calculation of SNGFR. By using the collection technique of controlled suction recently validated for this laboratory (13), minimal changes in tubule diameter or the position of the distal oil block were produced. Coincident with these tubule fluid collections, femoral arterial blood samples, 100 μl in volume, were obtained for determinations of hematocrit and plasma inulin concentration.

Hydraulic pressures were measured in single glomerular capillaries within surface glomeruli by using a continuous recording, servo-nulling micropipette transducer (12, 14, 15). Micropipettes with outer tip diameters of 2–3 μm and containing 2.0 M NaCl were used. Penetration of Bowman's capsule and entry into capillaries was performed under stereomicroscopic control. Hydraulic output from the servo-system was channeled via an electronic transducer (model P23Db, Statham Instruments, Inc.) to a second channel of the recorder. Accuracy, frequency response, and stability features of this servo-system have been reported previously (12). Direct measurements of hydraulic pressure in single glomerular capillaries (P_{GC}),[1] proximal tubules (P_T), efferent arterioles (P_E), and second- or third-order peritubular capillaries (P_C) were recorded in each rat. In several NSN and control rats, values of $\bar{P}_{GC}$ were not obtained because of the absence of surface glomeruli.

To obtain estimates of colloid osmotic pressure (π) of plasma entering and leaving glomerular capillaries, protein concentrations (C) in femoral arterial and efferent arteriolar blood plasma were measured as described previously (1–6). C measured in femoral arterial plasma was taken as representative of C for the afferent arteriole. Colloid osmotic pressures for control rats were calculated from these measured values of C with the equation:

$$\pi = 1.63C + 0.294C^2 \quad (1)$$

where $4 \leq C \leq 10$ g/100 ml. This equation has been shown by us (16) to agree within 1% of the more commonly employed empirical equation derived by Landis and Pappenheimer (17). Eq. 1 assumes an albumin/globulin (A/G) ratio of 1.0, the ratio found in normal hydropenic rats in this laboratory. To test the validity of Eq. 1 for NSN rats, A/G ratios were measured in 12 of these animals. The relationship between π and C for NSN rats with differing A/G ratios was then determined by using a PM-30 membrane (Amicon Corp., Lexington, Mass.) in a fast-response membrane osmometer (16). For NSN rats with an A/G ratio of 0.8, Eq. 1 was found to be valid. In nine of the 12 rats examined, values for the A/G ratio were 0.8–1.0; hence Eq. 1 was employed. For plasma with an A/G ratio of <0.8, values of π were found to be lower than values calculated with Eq. 1. The best-fit relationship between π and C for plasma with an A/G ratio of 0.4 (the lowest A/G ratio observed in any of the NSN rats) is described by the equation:

$$\pi = 2.24C + 0.180C^2 \quad (2)$$

For plasma with A/G ratios between 0.4 and 0.8, the coefficients were determined by linear interpolation of the coefficients in Eqs. 1 and 2.

These estimates of pre- and postglomerular protein concentration permit calculation of single nephron filtration fraction (SNFF) and glomerular plasma flow (GPF) (see equations below). From direct measurements of the decline in pressure along single afferent and efferent arterioles, and estimates of blood flow through these vessels, vascular resistances to blood flow through these individual vessels were calculated (see Eqs. 8–10).

Analytical. The volume of tubule fluid collected from individual nephrons was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of inulin in tubule fluid was measured, usually in duplicate, by the microfluorescence method of Vurek and Pegram (18). Inulin concentration in plasma was determined by the macroanthrone method of Führ, Kaczmarczyk, and Krüttgen (19). Protein concentrations in efferent arteriolar and femoral arterial blood plasmas were determined, usually in duplicate, with an ultramicrocolorimeter[2] using a recently described (10) microadaptation of the method of Lowry, Rosebrough, Farr, and Randall (20).

Calculations. Single nephron glomerular filtration rate:

$$SNGFR = (TF/P)_{In} \cdot V_{TF} \quad (3)$$

where $(TF/P)_{In}$ and V_{TF} refer to transtubular inulin concentration ratio and tubule fluid flow rate, respectively.

Single nephron filtration fraction:

$$SNFF = 1 - \frac{C_A}{C_E} \quad (4)$$

where C_A and C_E denote afferent and efferent arteriolar protein concentrations, respectively.

Initial glomerular plasma flow rate:

$$GPF = \frac{SNGFR}{SNFF} \quad (5)$$

Blood flow rate per single afferent arteriole or glomerulus:

$$GBF = \frac{GPF}{1 - Hct_A} \quad (6)$$

where Hct_A, the hematocrit of afferent arteriolar blood, is assumed to equal femoral arterial hematocrit.

Efferent arteriolar blood flow rate:

$$EABF = GBF - SNGFR \quad (7)$$

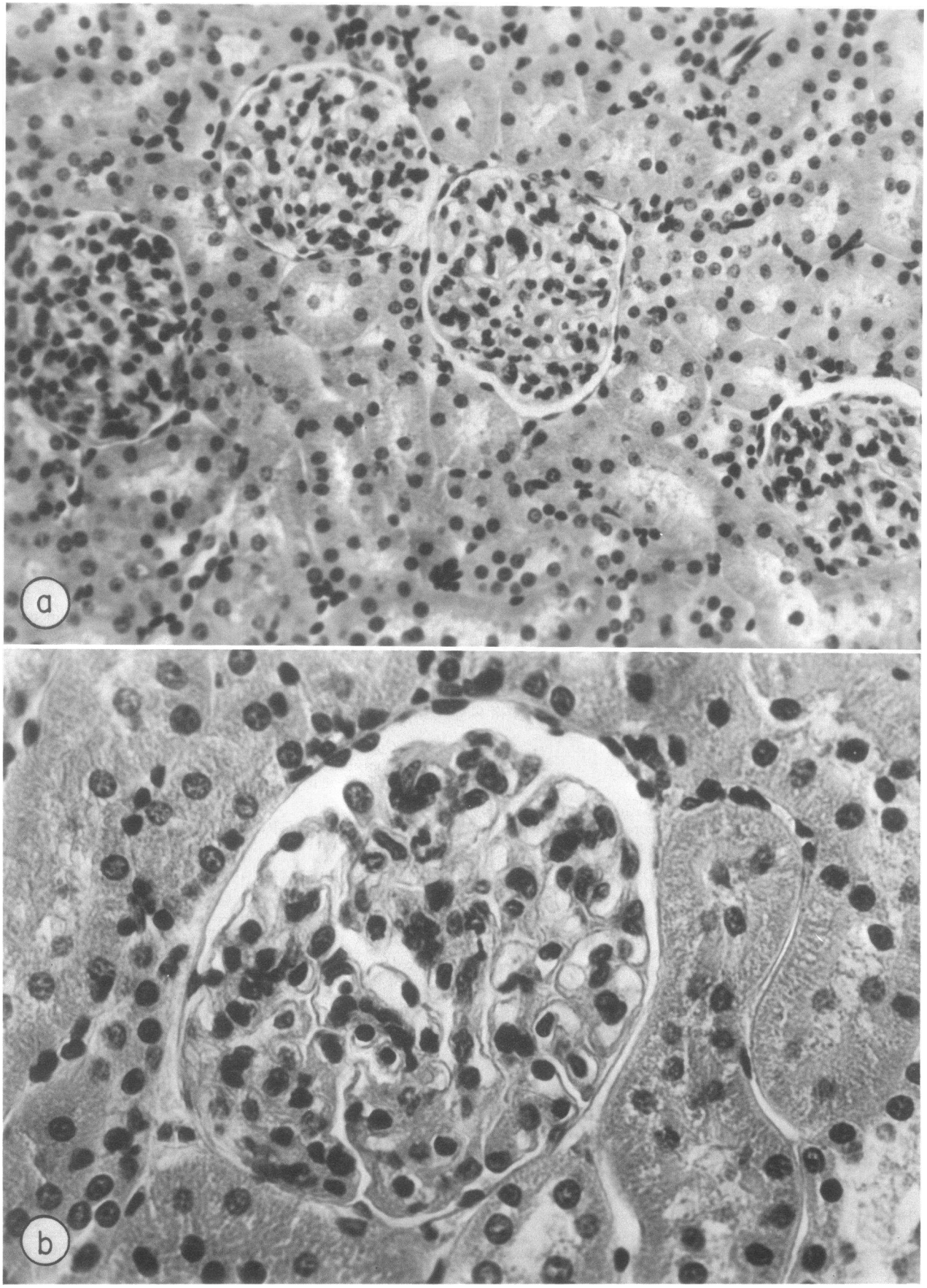
Resistance per single afferent arteriole:

$$R_A = \frac{\overline{AP} - \bar{P}_{GC}}{GBF} \times (7.962 \times 10^{10}) \quad (8)$$

where the factor 7.962×10^{10} is used to give resistance in units of dyn·s·cm^{-5} when $\overline{AP}$ and $\bar{P}_{GC}$ are expressed in mm Hg and GBF in nanoliters per minute.

[1] Values for P_{GC} as given in the present study represent time averages. As reported previously (1), peak-to-valley amplitudes of single glomerular capillary pressure pulses average approximately 8 mm Hg and generally bracket these time-averaged values equally during systole and diastole. The term "$\bar{P}_{GC}$" represents P_{GC} averaged over the length of the glomerular capillary, the justification for which has been discussed previously (2).

[2] Designed and constructed by Dr. Gerald Vurek, Laboratory of Technical Development, National Heart and Lung Institute, Bethesda, Md.


a
b

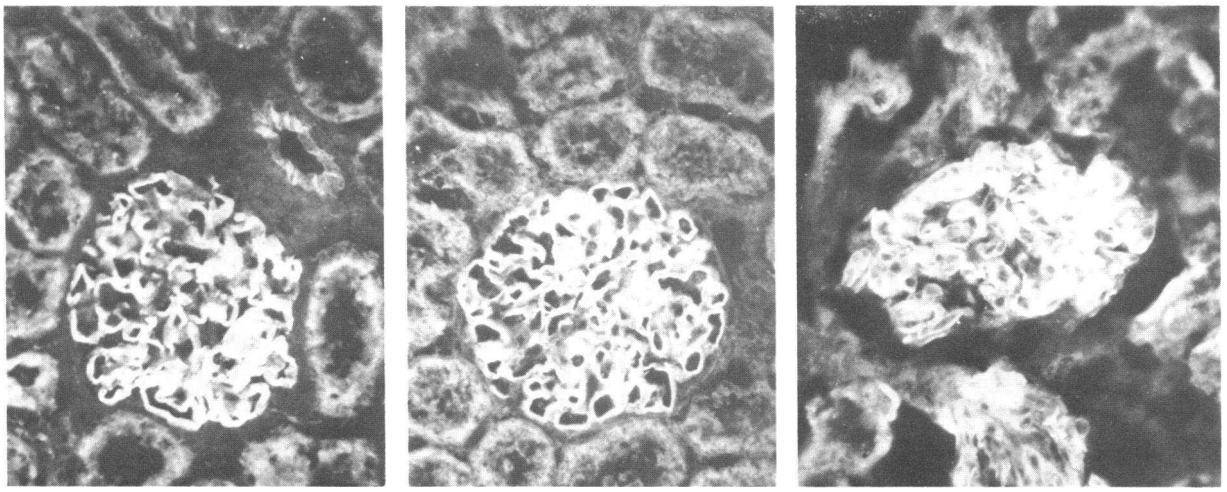


FIGURE 2 Immunofluorescence microscopy of renal cortex from a rat with nephrotoxic serum nephritis, revealing the presence of rabbit and rat gamma globulin and rat C3 component of complement in a linear distribution in glomerular capillary walls.

Resistance per single efferent arteriole:

$$R_E = \frac{\bar{P}_{GC} - P_C}{EABF} \times (7.962 \times 10^{10}) \quad (9)$$

Total arteriolar resistance for a single pre- to postglomerular vascular unit:

$$R_{TA} = R_A + R_E \quad (10)$$

An estimate of the net ultrafiltration pressure at the afferent end of the glomerular capillary (PUF_A) is given by the expression:

$$PUF_A = P_{GC} - P_T - \pi_A \quad (11)$$

An estimate of the net ultrafiltration pressure at the efferent end of the glomerular capillary (PUF_E) is given by the equation:

$$PUF_E = P_{GC} - P_T - \pi_E \quad (12)$$

Eqs. 11 and 12 contain the assumptions that glomerular capillary hydraulic pressure is relatively constant along the glomerular capillary network (2) and that the colloid osmotic pressure of fluid in Bowman's space (π_T) is negligible. This latter assumption has been validated by the finding in two NSN rats (with heavy proteinuria) that the protein concentration of fluid in Bowman's space is less than 200 mg/100 ml. Accordingly, π_T is well below 1 mm Hg.

Mean glomerular transcapillary hydraulic pressure difference:

$$\overline{\Delta P} = \bar{P}_{GC} - P_T \quad (13)$$

The ultrafiltration coefficient (K_f) is calculated with a differential equation which gives the rate of change of protein concentration with distance along an idealized glomerular capillary. This equation, together with its derivation and the method for its solution, is given in detail elsewhere (21).

RESULTS

General. Rats with nephrotoxic serum nephritis (NSN) appeared to be in good health throughout the period of observation (up to 16 days) and ate and drank normally. Edema formation was not detected. Grossly, the kidneys were of normal size and color. Microscopic examination of the cortical surface at the time of micropuncture revealed remarkable homogeneity of tubule and glomerular size and color. No dilated, pale, blood-

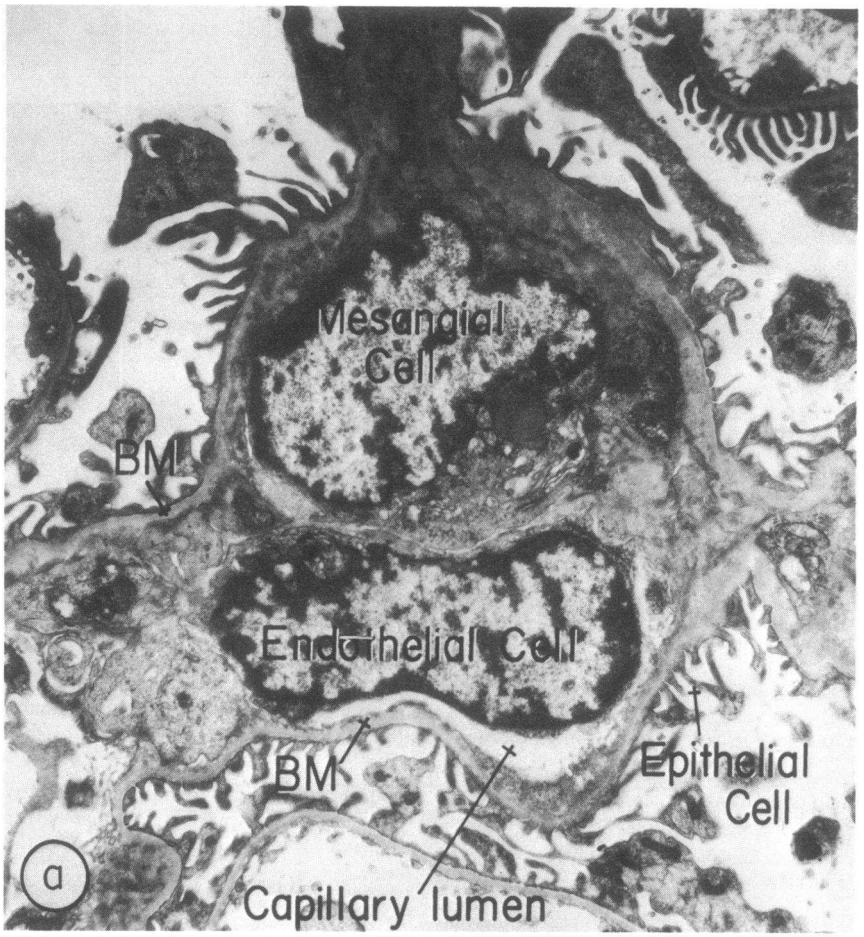
TABLE I

Quantitative Analysis of Histology in Control and Experimental Rats

Condition	Area/glomerulus	Cells/area
	mm^2	$no./mm^2$
Glomerulonephritis		
Superficial (50)	1.47±0.03	60.5±1.3
Deep (50)	1.48±0.03	62.2±1.3
Control		
Superficial (20)	1.34±0.04	51.7±1.9
Deep (20)	1.26±0.05	57.3±1.3

Numbers in parentheses refer to number of glomeruli. Results are expressed as means±1 SE.

FIGURE 1 Representative section of cortex from a rat with nephrotoxic serum nephritis. (*a*) Demonstrates the diffuse nature of the glomerular lesion. ×150. (*b*) Reveals the segmental nature of the proliferative response in an individual glomerulus (hematoxylin and eosin). ×300.


Mesangial Cell
BM
Endothelial Cell
BM
Epithelial Cell
a
Capillary lumen

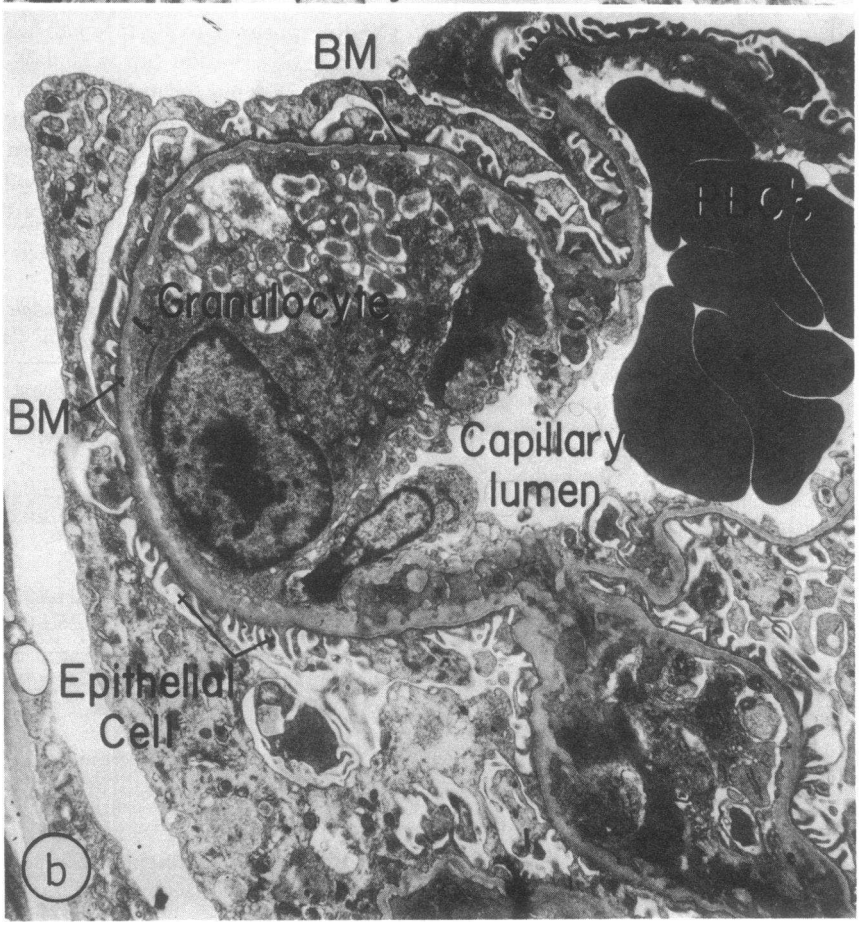

BM
RBC's
Granulocyte
BM
Capillary lumen
Epithelial Cell
b

TABLE II

Whole Kidney Function in Control and Experimental Rats

Condition	$\overline{AP}$	Hct	GFR	$\frac{V}{GFR} \times 100\%$	$\frac{(U/P)_{Na}}{(U/P)_{In}} \times 100\%$	$\frac{(U/P)_{K}}{(U/P)_{In}} \times 100\%$	$U_{Na}V$	$U_{K}V$	C_A
	mm Hg		*ml/min*				*μeq/min*	*μeq/min*	*g/100 ml*
Control	113	0.50	0.85	0.27	0.05	20.4	0.07	1.07	5.2
	±2	±0.01	±0.06	±0.02	±0.006	±3.2	±0.02	±0.23	±0.1
	(16)	(16)	(11)	(11)	(6)	(5)	(5)	(5)	(16)
Glomerulonephritis	114	0.49	0.77	0.33	0.09	17.7	0.10	0.73	5.1
	±3	±0.01	±0.04	±0.02	±0.02	±2.5	±0.02	±0.11	±0.1
	(22)	(22)	(19)	(19)	(14)	(14)	(14)	(14)	(18)
P value*	>0.5	>0.5	>0.2	>0.1	>0.1	>0.5	>0.4	>0.1	>0.4

Values are shown as means±1 SE. Numbers in parentheses refer to number of rats.

* Significance of differences in means between control and experimental rats determined from unpaired data by Student's *t* test. $(U/P)_{Na}$, $(U/P)_{In}$, $(U/P)_{K}$, urine to plasma sodium, inulin, and potassium concentration ratios, respectively; $U_{Na}V$, $U_{K}V$, absolute sodium and potassium excretion rates, respectively.

filled, or atrophic tubules were noted. Kidney weight in NSN rats averaged 0.88±0.05 (SE) g compared to a mean value of 0.90±0.08 g in controls ($P > 0.5$).

Histopathology. Evidence of glomerular injury was found by light microscopy to be relatively uniform throughout the cortex. The lesions consisted principally of segmental proliferation of mesangial and/or endothelial cells. Although some variation in the extent of alterations was noted among glomeruli, all showed a proliferative response. In most glomeruli there was obliteration of some but not all of the capillary lumina by proliferating mesangial cells. Occasional glomeruli revealed segmental necrosis. Epithelial cell crescents were seen only rarely. No significant vascular changes other than those observed in glomerular capillaries were found. In general, the capillary wall was of normal thickness as estimated by light microscopy, and only slight to moderate increase in mesangial matrix was seen by PAS stain. Interstitial changes were infrequent and inconstant, consisting mainly of focal collections of mononuclear cells. No significant alterations in tubule morphology were noted. A low- and high-power view of a typical cortical section of kidney from an NSN rat stained with H&E is shown in Fig. 1.

By immunofluorescence microscopy there was uniform linear distribution of rabbit IgG in the capillary walls of all glomeruli examined (Fig. 2, left). Since the rats had been preimmunized with rabbit IgG, there was also uniform linear distribution of rat IgG in all glomeruli (Fig. 2, middle), even in the rats sacrificed as early as 6 days. The presence of rat C3 component of complement was detected in a linear distribution in all glomeruli (Fig. 2, right). Renal tubule cells and basement membranes and nonglomerular vascular structures did not stain with any of these immunofluorescence reagents, except for scattered, interrupted linear deposits of C3 in the basement membranes of tubules, seen also in kidneys of control rats. Occasional glomeruli revealed intense segmental deposits of fibrin.

Quantitative analysis of histology. The results of the quantitative analysis of the proliferative response is shown in Table I. The mean cross-sectional area of glomeruli in NSN rats was slightly but significantly greater than in controls ($P < 0.002$), but no difference was noted between superficial and deep glomeruli ($P > 0.2$). Glomerular cell counts per mm^2 were increased significantly above normal in nephritic glomeruli ($P < 0.001$); again there was no difference between superficial and deep glomeruli ($P > 0.1$). Most importantly, the variance of the cell counts was no different between superficial and deep glomeruli ($P > 0.5$) nor between NSN rats and controls ($P > 0.5$), indicating that the proliferative lesions were relatively uniform among the constituent glomeruli.

Electron microscopy. Electron micrographs of sections from two rats revealed nearly identical patterns. The glomerular basement membrane appeared normal in most capillary loops (Fig. 3*b*). There was proliferation of cellular elements, most notably of the mesangium. In some areas, glomerular capillary loops were nearly or completely obliterated, and capillary walls were thickened and distorted by interposition of proliferating mesangial cells and matrix (Fig. 3*a*) and circulating leukocytes (Fig. 3*b*). In other areas, capillary walls were uninvolved or only thickened focally. Endothelial cells appeared normal, although in focal areas the endothelium was absent and replaced by polymorphonuclear leukocytes (Fig. 3). Epithelial cells

FIGURE 3 Electron microscopy of portions of glomeruli from a rat with nephrotoxic serum nephritis revealing the focal areas of a proliferative lesion. ×6,500. BM, glomerular capillary basement membrane. See text for details.

TABLE III

Summary of the Measured Determinants of Glomerular

Animal no.	Body wt	Kidney wt	GFR	$\overline{AP}$	$\overline{P}_{GC}$	P_T	P_C	C_A	C_E
	g		*ml/min*	*mm Hg*		*mm Hg*		*g/100 ml*	
1	173	0.64	—	98	42	9	9	5.4	8.4
2	210	—	—	132	47	13	9	5.4	8.7
3	192	—	—	115	44	13	13	5.6	8.4
4	214	—	—	117	43	13	10	5.6	8.3
5	173	—	0.68	112	43	15	13	5.2	8.4
6	201	—	0.66	118	46	14	10	5.0	7.9
7	244	—	0.72	120	43	11	8	5.0	8.4
8	274	0.64	0.82	128	46	12	9	5.6	8.6
9	163	0.56	—	108	46	11	12	5.8	8.8
10	215	0.76	0.47	112	37	10	8	4.8	7.6
11	269	0.98	1.02	114	46	10	8	5.6	8.5
12	259	0.92	1.12	97	42	11	8	4.8	8.0
13	260	1.06	1.18	106	—	12	12	4.8	7.9
14	314	1.26	1.26	104	—	12	10	5.2	7.5
15	264	0.99	0.63	120	—	12	10	4.4	7.5
16	254	1.22	0.77	111	43	11	12	5.6	—
Mean	230±11 (16)	0.90±0.08 (10)	0.85±0.08 (11)	113±2 (16)	44±0.7 (13)	12±0.4 (16)	10±0.5 (16)	5.2±0.1 (16)	8.2±0.1 (15)

Values are summarized as means ±1 SE. Numbers in parentheses refer to number of rats.

appeared nearly normal except for focal areas of swelling and effacement of foot processes. No subepithelial deposits were observed.

Proteinuria. In NSN rats mean 24-h urine protein excretion before injection of nephrotoxic serum was 12±9 mg, similar to the value in control rats of 10±9 mg. Both were slightly higher than the value in a commercial strain of Wistar rats, 3±3 mg. In the 24-h period following injection of nephrotoxic serum, protein excretion averaged 8±6 mg, a value not significantly different from control. At 4 days, many but not all rats exhibited significant proteinuria, averaging 58±73 mg (range 4.0–203 mg). 24-h urine collections performed after 6–16 days revealed that most, but not all, rats eventually developed proteinuria. At the time of micropuncture, NSN rats were found to have markedly elevated protein concentrations in bladder urine, averaging 11.5±5 mg/ml as compared to <1 mg/ml in controls. No rats developed gross hematuria, and microscopic examination of urine sediment obtained by bladder aspiration in six NSN rats revealed no abnormal cells or casts.

Whole kidney and single nephron function in nephrotoxic nephritis vs. control rats. Table II summarizes the mean values for a number of indices of whole kidney function during hydropenia in control and NSN rats. Despite extensive glomerular injury, as evidenced morphologically and by the abnormal protein excretion rates, whole kidney GFR, on average, was not significantly reduced in NSN rats relative to values in controls. Mean values for arterial pressure ($\overline{AP}$), arterial hematocrit, and systemic total protein concentration were similar in the two groups, and there were no significant alterations in absolute or fractional sodium or potassium excretion rates or fractional volume excretion. The values in control rats agree closely with results in normal hydropenic rats reported by us previously (1–3, 5, 22).

The measured determinants of glomerular ultrafiltration in control and NSN rats are summarized in Tables III and IV, respectively. The two groups were similar with respect to body weight and kidney weight. As with whole kidney GFR, mean values for SNGFR were not significantly different between groups, averaging 27.2 nl/min in NSN rats and 28.0 nl/min in control. A measure of the homogeneity of function among individual nephrons in NSN rats is provided by the finding that the coefficient of variation[3] in SNGFR was very nearly the same as in the control group, averaging 20±3 vs. 14±2% ($P > 0.1$), respectively. Average values for SNFF were lower in NSN rats (0.32±0.02) than in the control group (0.37±0.01, $P > 0.05$). Values of GPF averaged 88.4±7.8 and 75.7±5.7 nl/min

[3] Coefficients of variation were calculated as the ratio of the standard deviation to the mean value of SNGFR, expressed as a percentage. This calculation was performed in all rats in which three or more values of SNGFR were obtained.

π_A	π_E	P_{UF_A}	P_{UF_E}	$\frac{\pi_E}{\overline{\Delta P}}$	SNGFR	SNFF	GPF	R_A	R_E
mm Hg		*mm Hg*			*nl/min*		*nl/min*	$\times 10^{10}$ *dyn·s·cm*$^{-5}$	
17	34	16	−2	1.03	19.5	0.36	54.2	3.8	2.7
17	36	18	−2	1.06	25.2	0.38	66.3	5.4	3.1
18	34	13	−3	1.10	29.2	0.33	88.5	3.3	1.8
18	34	11	−4	1.13	22.3	0.33	67.6	4.9	2.7
16	34	12	−6	1.21	24.6	0.38	64.7	4.4	2.4
16	31	16	1	0.97	28.6	0.37	77.3	3.8	2.3
16	34	17	−2	1.06	26.1	0.44	59.3	5.2	2.9
18	36	16	−2	1.06	19.6	0.35	56.0	5.0	2.6
19	37	15	−2	1.06	22.2	0.34	65.3	3.6	2.4
15	29	12	−3	1.07	20.6	0.37	55.7	5.6	2.7
18	35	18	1	0.97	41.2	0.34	121.2	2.1	1.4
15	32	17	0	1.03	41.7	0.40	104.2	2.1	1.6
15	31	—	—	—	31.4	0.39	80.5	—	—
16	28	—	—	—	36.0	0.31	116.1	—	—
13	28	—	—	—	23.9	0.41	58.3	—	—
18	—	14	—	—	35.3	—	—	—	—
17±0.4 (16)	33±0.7 (15)	15±0.7 (13)	−2±0.6 (12)	1.06±0.02 (12)	28.0±1.8 (16)	0.37±0.01 (15)	75.7±5.7 (15)	4.1±0.3 (12)	2.4±0.2 (12)

($P > 0.2$) in NSN and control rats, respectively. Glomerular capillary hydraulic pressures ($\bar{P}_{GC}$) were significantly higher in NSN rats than in controls, averaging 52 mm Hg and 44 mm Hg, respectively ($P < 0.001$). Values for P_T measured at random sites along surface proximal convolutions were not different between groups. A second measure of the homogeneity of function in NSN rats is given by the finding that the coefficient of variation in P_T among different nephrons in individual rats averaged 7±1%, compared to 7±1% in controls ($P > 0.5$). No differences were noted for values of P_C or P_E (15±0.5 mm Hg in control vs. 14±0.5 in NSN, $P > 0.5$). The mean values of $\bar{P}_{GC}$, P_T, P_C, and P_E for the controls were essentially the same as those reported by us previously for normal hydropenic rats (1–3, 5). The mean transcapillary hydraulic pressure difference, $\overline{\Delta P}$, was significantly higher in NSN than in control rats, averaging 41±1.3 mm Hg in the former and 32±0.7 in the latter. The hydraulic pressure drop along surface afferent arterioles, $\overline{\Delta P} - \bar{P}_{GC}$, averaged 70.3 mm Hg in control and was lower, averaging 64.4 mm Hg, in NSN rats, although this difference was not statistically significant. These values were uniformly and significantly higher ($P < 0.001$) than the pressure drop along surface efferent arterioles ($\bar{P}_{GC} - P_C$), which averaged 33.8 mm Hg for controls and 41.5 mm Hg for NSN rats. As shown in Tables III and IV, calculations of absolute resistance to blood flow through single afferent arterioles (R_A) in NSN rats yielded values that were, on average, approximately 20% lower than in controls, although this difference was not significant statistically. Resistance to flow in efferent arterioles (R_E) was similar, on average, between groups.

Measurements of protein concentration in preglomerular (C_A) and efferent arteriolar (C_E) plasma in control rats (Table III) yielded values typical of those usually found for normal hydropenic conditions (1–3, 5, 22). Although normal values of C_A were observed in NSN rats, on average C_E was significantly less than in controls (Table IV). π_A and π_E calculated from these values of C_A and C_E are also shown in Tables III and IV.[4] As given in Eqs. 11 and 12, the measurements in the present study allow determination of the magnitude of the glomerular transcapillary pressure difference favoring ultrafiltration at afferent (P_{UF_A}) and efferent (P_{UF_E}) ends of the capillary network in each rat. In

[4] In rats 17 and 31 in Table IV, A/G ratios were not determined. In calculating π_A and π_E from measured values of C_A and C_E in these rats, we assumed an A/G ratio of 0.8, or equal to the mean value found for the NSN group. For rat 17, even if an A/G ratio of 0.4 were assumed (the lowest value found in NSN rats), this would have no appreciable effect on π. This is so because C_A and C_E were low, and differences in values of π obtained by using Eqs. 1 and 2 become appreciable only when values of C are about 8 g/100 ml or greater. In rat 31, even if an A/G ratio of 0.4 were assumed, π_E would be increased by only 2 mm Hg and would still be far less than $\overline{\Delta P}$.

TABLE IV

Summary of the Measured Determinants of Glomerular

Animal no.	Body wt	Kidney wt	GFR	$\overline{\Delta P}$	$\bar{P}_{GC}$	P_T	P_C	C_A	C_E
	g		*ml/min*	*mm Hg*		*mm Hg*		*g/100 ml*	
17	174	0.86	0.66	100	56	10	13	4.9	6.2
18	157	—	0.78	102	—	10	7	4.6	8.2
19	153	0.74	0.53	105	49	11	10	5.0	7.7
20	—	—	0.80	105	—	11	9	5.1	8.6
21	169	0.90	0.73	101	—	10	8	—	—
22	134	0.81	—	95	52	12	12	—	—
23	145	0.75	—	102	—	11	8	—	—
24	210	0.79	0.51	125	—	12	8	5.1	6.8
25	176	0.69	0.57	116	—	10	7	5.1	8.0
26	189	0.68	0.56	114	51	12	10	4.9	—
27	189	0.69	0.70	108	—	11	11	5.9	8.6
28	170	0.56	0.84	132	48	10	8	4.9	8.0
29	308	1.26	1.20	113	—	12	10	5.1	8.2
30	198	0.70	0.65	123	—	13	13	—	—
31	291	1.10	—	100	58	11	11	5.0	8.0
32	276	1.23	1.03	115	—	10	10	5.4	7.7
33	260	1.26	0.94	142	51	12	10	5.3	6.8
34	264	1.02	0.88	112	55	12	10	5.1	6.4
35	220	0.91	0.86	127	58	11	10	4.1	6.8
36	211	1.02	1.00	135	44	12	13	5.4	7.8
37	188	0.80	0.66	118	55	11	10	5.7	7.3
38	184	0.77	0.71	118	48	11	10	5.3	8.8
Mean	203±11 (21)	0.88±0.05 (20)	0.77±0.04 (19)	114±3 (22)	52±1 (12)	11±0.2 (22)	10±0.4 (22)	5.1±0.1 (18)	7.6±0.2 (17)
P value*	>0.1	>0.5	>0.2	>0.5	<0.001	>0.05	>0.5	>0.4	<0.025

Values are summarized as means±1 SE. Numbers in parentheses refer to number of rats.
* As defined for Table II.

control rats, P_{UF_A} averaged 15±0.7 mm Hg. By the efferent end of the glomerular capillary network, the imbalance of pressures favoring ultrafiltration essentially disappeared, P_{UF_E} averaging −2±0.6 mm Hg. In NSN rats, P_{UF_A} was significantly higher ($P < 0.001$) than in controls, averaging 25±1.7 mm Hg. In contrast to control rats, by the efferent end of the glomerular capillaries there was still a significant force for ultrafiltration, P_{UF_E} averaging 13±2.7 mm Hg, thereby indicating that filtration pressure equilibrium did not obtain in these animals.

A unique value of the glomerular capillary ultrafiltration coefficient, K_f, can be determined only under conditions in which filtration pressure equilibrium is not achieved, i.e., when $\pi_E/\overline{\Delta P} \neq 1$. In nine of the ten NSN rats in which all of the determinants of glomerular ultrafiltration were measured, equilibrium was not achieved. Individual values for K_f are given in Fig. 4 (right) and averaged 0.026±0.005 nl/(s·mm Hg).[5] Control values in Fig. 4 (left) were taken from a previous study from our laboratory (4); K_f in the normal rat was found to average 0.078±0.003 nl/(s·mm Hg), or three times that obtained in the present study in NSN animals.

The mean driving pressure for ultrafiltration, $\bar{P}_{UF}$, was calculated by using the equation:

$$\bar{P}_{UF} = \frac{SNGFR}{K_f} \qquad (14)$$

Accordingly, $\bar{P}_{UF}$ averaged 17.4 mm Hg in NSN rats compared with a mean value of 5.6 mm Hg estimated for the control group. It is important to recognize that under conditions of filtration pressure equilibrium, such

[5] The equations used to compute K_f in this study (21) assume a pressure drop of zero from afferent to efferent ends of the glomerular capillaries, which has been found to be a good approximation in normal rats (2). We have calculated, using an equation which permits inclusion of a nonzero axial pressure drop (21), that even if the axial pressure drop in NSN rats was as high as 20 mm Hg, which seems unlikely, the value of K_f determined for NSN rats would underestimate the true K_f by not more than 10%.

π_A	π_E	P_{UF_A}	P_{UF_E}	$\frac{\pi_E}{\Delta P}$	SNGFR	SNFF	GPF	R_A	R_E
mm Hg		*mm Hg*			*nl/min*		*nl/min*	$\times 10^{10}$ *dyn·s·cm*$^{-5}$	
15	21	31	24	0.46	13.9	0.21	66.2	2.8	3.1
—	—	—	—	—	41.2	0.44	93.6	—	—
16	30	22	8	0.79	25.7	0.35	73.4	2.8	2.4
16	36	—	—	—	34.4	0.41	83.9	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	20.6	—	—	—	—
—	—	—	—	—	16.0	0.25	64.0	—	—
—	—	—	—	—	18.4	0.36	51.1	—	—
15	—	24	—	—	29.0	—	—	—	—
20	32	—	—	—	26.8	0.31	86.5	—	—
15	29	23	9	0.76	36.4	0.38	95.8	3.9	2.4
16	33	—	—	—	28.9	0.38	76.1	—	—
—	—	—	—	—	26.5	—	—	—	—
16	31	32	16	0.66	37.0	0.37	100.0	1.6	2.2
17	30	—	—	—	37.0	0.30	123.3	—	—
17	25	22	14	0.64	36.6	0.22	166.4	2.2	1.1
16	23	28	21	0.53	25.8	0.21	129.0	1.6	1.4
12	23	34	23	0.49	16.8	0.40	42.0	5.8	4.9
17	30	15	2	0.94	22.2	0.31	71.6	5.0	2.0
19	27	26	17	0.61	26.9	0.22	122.3	2.0	1.6
17	37	20	0	1.00	23.1	0.40	57.8	5.2	3.6
16±0.5	29±1.3	25±1.7	13±2.7	0.69±0.06	27.2±1.8	0.32±0.02	88.4±7.8	3.3±0.5	2.5±0.4
(15)	(14)	(11)	(10)	(10)	(20)	(17)	(17)	(10)	(10)
>0.5	<0.025	<0.001	<0.001	<0.001	>0.4	>0.05	>0.2	>0.2	>0.5

as obtained in control rats, only a minimum value of K_f can be calculated (4, 21). Hence the value of $\bar{P}_{UF}$ in control rats of 5.6 mm Hg represents the maximum value consistent with experimental observations. Any lower value for $\bar{P}_{UF}$ (corresponding to achieving filtration equilibrium at a more afferent point along the capillary) will only serve to accentuate the differences in K_f between control and NSN groups.

DISCUSSION

The method of induction of glomerulonephritis in the present study resulted in a remarkably uniform glomerular lesion. The dose of nephrotoxic serum was insufficient to produce immediate proteinuria (heterologous phase) (23), but in the presence of an optimized antibody response to rabbit IgG, accomplished by preimmunization, an autologous phase developed in all rats studied (23). This was confirmed by the finding of rat IgG and C3 component of complement in all glomeruli examined by immunofluorescence microscopy. Despite the morphologic evidence of widespread glomerular injury, rats developed few of the features that characterize human acute glomerulonephritis. Other than mild to moderate proteinuria and variable hypoalbuminemia, we found no evidence of arterial hypertension, edema, sodium retention, hematuria, or cylindruria. Moreover, at the time of micropuncture, the kidneys appeared normal, and glomerular filtration rates (single nephron and whole kidney) and glomerular plasma flow rates were not different, on average, from values in control rats.

Although glomerular filtration rates were normal, marked alterations in the glomerular driving forces were observed. Despite no difference in mean systemic arterial blood pressure between groups, mean glomerular capillary hydraulic pressure was significantly higher in NSN rats than in control rats, whereas proximal tubule hydraulic pressures were the same. Therefore, $\overline{\Delta P}$, the mean transcapillary hydraulic pressure difference, was significantly greater in NSN rats, averaging 41 mm Hg compared to 32 in controls. Since systemic protein concentrations were the same in both groups, values

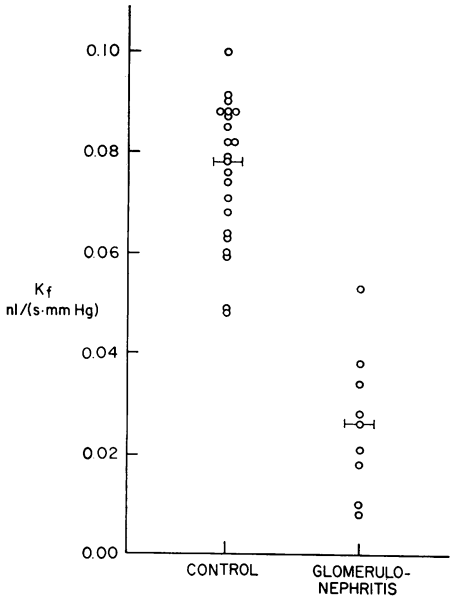


FIGURE 4 Comparison of K_f values calculated for individual rats in the NSN group with values obtained from normal Wistar rats reported previously (4).

for π_A, the oncotic pressure of plasma at the afferent end of the glomerular capillary network, were also the same. As shown in the left-hand panel of Fig. 5, in control rats the net pressure for ultrafiltration at the afferent end of the capillary, the difference between the transcapillary hydraulic pressure difference of 32 mm Hg and the oncotic pressure of 17, averaged approximately 15 mm Hg. Because of ultrafiltration, the value of the oncotic pressure is seen to increase with distance along the capillary, rising to a mean value which approximately equals $\overline{\Delta P}$, denoting achievement of filtration pressure equilibrium. The oncotic pressure profile in the left-hand panel of Fig. 5 was computed by assuming a K_f value of 0.08 nl/(s·mm Hg), a value obtained in normal Wistar rats reported previously (4). Since this K_f value yielded equilibrium very near the efferent end of the capillary, it is clear that the value of K_f in the control rats in the present study, on average, was no less than the value found previously for normal Wistar rats (4). As seen in the right-hand panel of Fig. 5, in NSN rats, the net pressure for ultrafiltration at the afferent end of the capillary was nearly twice that in controls because of the higher value of $\bar{P}_{GC}$. Despite this greater initial force for filtration, the capillary oncotic pressure failed to reach the value of $\overline{\Delta P}$, so that filtration pressure equilibrium was not achieved. The shaded areas in each panel of Fig. 5 denote the mean net ultrafiltration pressure, $\bar{P}_{UF}$. As can be seen, this mean driving force for filtration was much greater in NSN rats than in controls, averaging 17.4 mm Hg compared with a maximum estimate of 5.6 mm Hg for controls. If in control rats K_f were larger than the value assumed in calculating the oncotic pressure profile in Fig. 5 (left), equilibrium would occur nearer the afferent end of the capillary than as shown, so that $\bar{P}_{UF}$ would be less than as shown. This would only serve to magnify the differences in $\bar{P}_{UF}$ between groups.

The glomerular lesion induced in the present study resulted in a reduction in K_f to roughly one-third of normal. K_f is the product of the effective hydraulic permeability of the capillary wall and the total capillary surface area per glomerulus. It is very likely that the fall in K_f observed in NSN rats is at least partly the result of the often marked reduction in the number of patent capillaries in each glomerulus, i.e., a reduction in surface area available for ultrafiltration. This was readily evident by both light and electron microscopy, which revealed that in some areas capillary loops were nearly or completely obliterated, and capillary walls were thickened and distorted by interposition of proliferating mesangial cells and circulating leukocytes. Whether effective hydraulic permeability was also decreased cannot be ascertained from morphologic appearances, so that the quantitative contribution of this parameter to the measured decline in K_f cannot yet be determined.

The present study of experimental glomerulonephritis in the rat is unique in two respects. First, direct measurements of glomerular transcapillary hydraulic and oncotic pressures were made. Second, the lesion produced in the present study was remarkably homogeneous from nephron to nephron, both morphologically and functionally. It is this homogeneity that validates inferences regarding the functional behavior of a

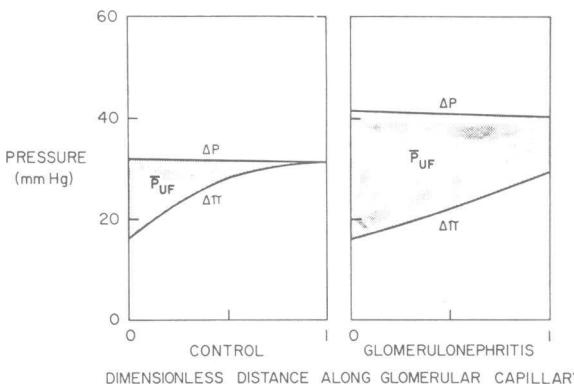


FIGURE 5 Comparison of transcapillary hydraulic (ΔP) and oncotic ($\Delta\pi$) pressure profiles along idealized glomerular capillaries in control and NSN rats, calculated in a manner described in detail elsehwere (4, 21). Input quantities consisted of average values of $\overline{\Delta P}$, π_A, GPF, and K_f for control and NSN rats. The latter, for control rats, was taken from findings reported previously (4).

representative nephron from individual measurements made in a number of separate tubules and capillaries. Previous studies by others (24–26) of experimental glomerulonephritis in the rat were characterized by much more severe glomerular injury, often studied later in the course of the disease, with marked heterogeneity of nephron function and structure. Of these, only the study of Allison, Wilson, and Gottschalk (25) sought to estimate the changes in the driving forces for ultrafiltration in glomerulonephritis. In attempting to surmount the problem of heterogeneity of nephron function, Allison et al. (25) measured stop-flow and free-flow hydraulic pressures and SNGFR in the same tubule. Rather than obtaining measurements of C_E for these same nephrons, however, these workers estimated C_E by assuming that single nephron filtration fraction (SNFF) was the same for all nephrons and equal to that measured for the kidney as a whole. Since SNFF is determined in part by $\overline{\Delta P}$ and GPF, and since these quantities varied markedly from nephron to nephron in a given animal, it seems unlikely that SNFF was uniform in all nephrons studied. In addition, these authors used the indirect stop-flow pressure technique to estimate $\bar{P}_{GC}$ but did not present data to validate this technique under the pathological conditions studied. The latter seems essential since direct and indirect measurements of $\bar{P}_{GC}$ in normal rats have not always yielded similar results (27, 28). Nevertheless, in accord with the results of the present study, the findings by Allison et al. (25) were interpreted to indicate that $\bar{P}_{GC}$ increases in experimental glomerulonephritis. If their interpretation is correct, their finding of elevations in $\bar{P}_{GC}$ in rats in advanced stages of experimental glomerulonephritis raises the possibility that elevations in $\bar{P}_{GC}$ represent an adaptation to glomerular capillary injury, prevailing in both early (present study) and late phases (25) of this disease. As was evident in the present study, in the early stage of glomerulonephritis glomerular filtration rate remained normal despite a marked reduction in K_f, because of the compensating increase in $\bar{P}_{GC}$. If $\bar{P}_{GC}$ remains elevated in chronic glomerulonephritis, as suggested by the findings of Allison et al. (25), the progressive deterioration in filtration rate characteristically noted in more advanced stages of this disorder must result from further reductions in K_f and/or declines in glomerular plasma flow rate. It should be made clear, however, that elevations in $\bar{P}_{GC}$, as estimated by indirect stop-flow techniques, have not always been observed when glomerular injury has been severe (24). For this reason, future studies should rely on direct methods, such as were employed in the present study, to determine whether $\bar{P}_{GC}$ indeed remains elevated in chronic stages of experimental glomerulonephritis.

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