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Research Article

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Glomerular Hypertrophy Aggravates Epithelial Cell Injury in Nephrotic Rats

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Abstract

Glomerular function and structure were assessed after reduction of nephron number and restriction of protein intake in rats with adriamycin nephrosis. Rats received an injection of adriamycin and were divided into three groups with similar values for albuminuria after 4 wk. Group 1 rats then served as controls, group 2 rats were subjected to four-fifths renal ablation, and group 3 rats were placed on a low protein diet (8% protein) while group 1 and group 2 rats remained on a standard diet (24% protein). Micropuncture and morphometric studies were performed 10 d later. Estimated single-nephron albuminuria (SN_{alb}) was increased by renal ablation in group 2 and decreased by protein restriction in group 3 (group 1, 20±2 μg/d; group 2, 68±7 μg/d; group 3, 12±1 μg/d, $P < 0.05$ groups 2 and 3 vs. 1). Increased SN_{alb} was associated with increased glomerular volume in group 2 and reduced SN_{alb} was associated with reduced glomerular volume in group 3. (group 1, $1.44\pm0.04 \times 10^6$ μm³; group 2, $1.66\pm0.08 \times 10^6$ μm³; group 3, $1.26\pm0.03 \times 10^6$ μm³, $P < 0.05$ groups 2 and 3 vs. 1). Increased SN_{alb} in group 2 was not associated with an increase in glomerular transcapillary hydraulic pressure. The area of epithelial cell detachment from the peripheral capillary wall was markedly increased in group 2 but not perceptibly altered in group 3 (group 1, $16\pm5 \times 10^2$ μm²; group 2, $65\pm17 \times 10^2$ μm²; group 3, $18\pm5 \times 10^2$ μm²; $P < 0.05$ group 2 vs. 1). These studies show that glomerular hypertrophy is associated with increased epithelial cell detachment from the peripheral capillary wall and with increased remnant nephron albuminuria after reduction of nephron number in rats with established nephrosis. (*J. Clin. Invest.* 1990. 85:1119–1126.) adriamycin • epithelial cell • glomerulus • hypertrophy • nephrosis

Introduction

Reduction in functioning nephron number leads to increases in remnant glomerular filtration rate and remnant glomerular volume. It has recently been emphasized that these changes in remnant glomerular function and structure can cause glomerular injury after renal ablation in rats with initially normal kidneys and accelerate glomerular injury in rats with preexisting renal disease (1–4). A number of studies have related remnant glomerular injury to increased glomerular capillary pressure after renal ablation (5). Further, recent studies suggest that glomerular hypertrophy promotes remnant glomerular injury by a mechanism which is independent of capillary hypertension (4, 6–8). The mechanisms by which glomerular hypertrophy promotes glomerular injury remain to be identified. In a previous study, we found that glomerular hypertrophy after reduction of nephron number in nephrotic rats was accompanied by a dramatic increase in remnant nephron proteinuria (4, 6). This finding suggests that glomerular hypertrophy can aggravate injury to glomerular epithelial cells. In the current study, morphometric techniques were used to identify changes in epithelial cell structure that accompany glomerular hypertrophy after renal ablation in rats with adriamycin nephrosis. Further studies sought to identify changes in epithelial cell structure in nephrotic rats subjected to dietary protein restriction, a maneuver which has been shown to reduce glomerular volume and proteinuria in these animals (9).

Methods

Male Munich Wistar rats weighing 235–275 g were given a single injection of adriamycin (doxarubicin HCl), 3.3 mg/kg, via tail vein under brevital anesthesia (50 mg/kg, i.p.). All rats were maintained on standard laboratory chow until 3 wk after injection of adriamycin. They were then placed on a synthetic laboratory diet with protein content matched to the standard laboratory chow (24% protein by weight, TD 85424, Teklad, Inc., Madison, WI). Baseline measurements were obtained at 4 wk after injection of adriamycin and rats were divided into three groups with similar albumin excretion rate, serum albumin concentration, and body weight. A first group of nephrotic rats, group 1 ($n = 8$), served as controls and continued to receive the synthetic laboratory diet for 10 d. A second group of rats, group 2 ($n = 9$), was subjected to 4/5 ablation of renal mass by surgical right nephrectomy and ligation of two to three branches of the left renal artery and then maintained on the same 24% protein diet for 10 d. A third group of rats, group 3 ($n = 8$), received a synthetic diet with protein content reduced to 8% by weight (TD 87126, Teklad, Inc.) for 10 d. The mineral content of the two synthetic diets matched the mineral content of the standard laboratory chow. All animals had unrestricted access to food and drinking water. Repeat measurements of albumin excretion rate and serum albumin were made at 10 d. After these measurements micropuncture studies were performed and kidneys were prepared for morphometric analysis.

Micropuncture methods

Rats were anesthetized with Inactin, 70 mg/kg, i.p., and placed on a temperature regulated micropuncture table. A PE-50 tubing catheter was inserted in the left femoral artery and used for subsequent blood sampling and estimation of mean arterial pressure ($\overline{AP}$).[1] $\overline{AP}$ was continuously monitored with an electronic transducer connected to a di-

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1. *Abbreviations used in this paper:* $\overline{AP}$, mean arterial pressure; HPEM, high-power electron micrograph; K_f, ultrafiltration coefficient; SN, single nephron; see footnotes to Tables I–IV for other abbreviations and symbols.

rect writing recorder. After tracheostomy PE-50 catheters were inserted in the right and left internal jugular veins for infusion of rat plasma, saline, and radiolabeled inulin. Plasma was obtained from retired breeder rats made nephrotic by injection of adriamycin (3.5 mg/kg). Plasma was infused in an amount equal to 1% body weight for 40–45 min, followed by a reduction of the infusion rate to 0.5 ml/h for the duration of the study. Saline was infused in an amount equal to 4% body weight for 80–100 min, followed by a reduction of the infusion rate to 2.4 ml/h for the remainder of the study. After 100 min, tritiated methoxy-inulin was added to the saline to achieve an infusion rate of 40–120 μCi/h after a loading dose of $\sim$ 40 μCi.

Micropuncture measurements were then carried out over two or three 30- to 40-min clearance periods. In each period, a 200-μl arterial blood sample was obtained for determination of hematocrit and plasma inulin and protein concentrations. A renal vein blood sample was obtained with each arterial blood sample for determination of filtration fraction by renal vein inulin extraction. Time averaged hydraulic pressures were measured in surface glomerular capillaries (one to four determinations)[2], proximal tubules (three to nine determinations), and efferent arterioles (two to five determinations) with a continuous recording servo-null micropipette transducer system (model V, Instrumentation for Physiology and Medicine, San Diego, CA). Hydraulic output from the servo system was coupled to a second channel of the direct writing recorder by means of a pressure transducer. Timed (3.5–4 min) samples of tubule fluid were collected from surface proximal convolutions of three to five nephrons for determination of single-nephron glomerular filtration rate (SNGFR).

Morphometric methods

After micropuncture study kidneys were fixed by retrograde aortic perfusion with 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). A 2–3-mm transverse section of kidney tissue was postfixed in 4% formaldehyde solution, methacrylate-embedded, and stained with hematoxylin-eosin for light microscopy and measurement of mean glomerular volume. Additional small blocks of outer cortical tissue were postfixed in osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Thick sections were cut from these blocks and examined by light microscopy. When whole glomerular profiles were observed, thin sections were mounted on slotted grids and counterstained with uranyl acetate and lead citrate for electron microscopy. Electron micrographs were thus made from sections of outer cortical glomeruli selected without regard for severity of epithelial cell injury. The precise magnification of these electron micrographs was determined from micrographs of a calibration grid.

Mean glomerular volume. The mean glomerular tuft volume ($\bar{V}_G$) for each animal was determined from mean glomerular cross-sectional area ($\bar{A}_G$) as assessed by light microscopy. $\bar{A}_G$ was determined on 100 consecutive glomerular profiles on each of two sections from each animal using a computer assisted morphometric unit (Bioquant II software). $\bar{V}_G$ was then calculated as:

$$\bar{V}_G = \beta/k \cdot (\bar{A}_G)^{3/2}, \tag{1}$$

where $\beta = 1.38$ is the shape coefficient for spheres (the idealized shape of glomeruli) and $k = 1.1$ is a size distribution coefficient (10, 11).

Surface area. Three surface areas were measured in the present study: S_{PCW}, epithelial–peripheral capillary wall interface; S_{Mes}, epithelial–mesangial interface; S_{PCWd}, peripheral capillary wall without overlying epithelium. These surface areas were assessed by determining the respective surface densities $S_{V(PCW)}$, $S_{V(Mes)}$, and $S_{V(PCWd)}$ on low-magnification electron micrographs as described by Østerby and Gundersen (12). In each animal, three whole glomerular profiles were photographed at $\approx$ ×750 and printed on 11 × 14 paper to yield a final magnification of $\approx$ ×1,750. On each print a string polygon was drawn around the glomerulus and the print covered with a point and line lattice in which each point was one end of a 20-mm line segment. The number of points falling within the polygon and the intercepts of the line segments with the relevant surfaces was counted and the surface densities calculated as:

$$S_{V(PCW)} = \frac{2 \cdot \Sigma I_{(PCW)}}{k^2 \cdot \Sigma P_{(polygon)}}; \tag{2}$$

$$S_{V(Mes)} = \frac{2 \cdot \Sigma I_{(Mes)}}{k^2 \cdot \Sigma P_{(polygon)}}; \tag{3}$$

$$S_{V(PCWd)} = \frac{2 \cdot \Sigma I_{(PCWd)}}{k^2 \cdot \Sigma P_{(polygon)}}, \tag{4}$$

where $\Sigma I_{(PCW)}$ = number of intercepts of epithelial-PCW interface with lines, $\Sigma I_{(Mes)}$ = number of intercepts of epithelial-Mes interface with lines, $\Sigma I_{(PCWd)}$ = number of intercepts of PCW without overlying epithelium with lines, $\Sigma P_{(polygon)}$ = number of points falling within the polygon, and k = real length of the lattice line segments = 20 mm per print magnification. The average surface areas S_{PCW}, S_{Mes}, and S_{PCWd} expressed in square micrometers per glomerulus were then calculated as the products of the respective surface densities and the mean glomerular volume for each animal determined by light microscopy as described above.

Volumes of glomerular components. Volumes of glomerular components were measured on five high-power electron micrographs (HPEM) from each of four glomerular profiles per animal. These micrographs were printed on 8 × 10 paper at a final magnification of $\approx$ ×13,000 and covered with a 7 × 9 point grid. The fractional volumes of the following components were assessed: Epi, epithelial cells including podocytes; End, endothelial cells; Cap, capillary lumen; GBM, glomerular basement membrane; Mes, mesangium (cells and matrix). Fractional volumes for each component (x) were calculated as:

$$V_{V(x)} = \frac{\Sigma P_{(x)}}{\Sigma P_{(Epi+End+Cap+GBM+Mes)}}, \tag{5}$$

where $\Sigma P_{(x)}$ = number of points falling on component (x), and where the denominator $\Sigma P_{(Epi+End+Cap+GBM+Mes)}$, is the number of points falling on the structures constituting the glomerular tuft, excluding the urinary space. The absolute volumes of individual components expressed as cubic micrometers per glomerulus were then calculated as:

$$V_{(x)} = V_{V(x)} \cdot \bar{V}_G \cdot V_{V(tuft)}, \tag{6}$$

where $\bar{V}_G$ is the mean glomerular volume determined by light microscopy, and where $V_{V(tuft)}$ is the fraction of this volume occupied by tuft as determined by point counting on the low power electron micrographs with

$$V_{V(tuft)} = \frac{\Sigma P_{(tuft)}}{\Sigma P_{(polygon)}}, \tag{7}$$

where $\Sigma P_{(tuft)}$ = number of points within the string polygon falling on the tuft (excluding urinary space), and $\Sigma P_{(polygon)}$ = total number of points falling within the polygon.

Slit frequency. Average slit frequency was determined by counting the number of slits overlying the peripheral capillary basement membrane on HPEM. Peripheral capillary basement membrane was distinguished from mesangial basement membrane by the loss of parallelism between epithelial and endothelial cells as described by Mauer et al. (13). Peripheral capillary basement membrane length was measured on HPEM using the computer assisted morphometric unit. Slit frequency was calculated as:

$$\text{Slit Freq} = \frac{\Sigma \text{ slits}}{\Sigma \text{ BML}}, \tag{8}$$

where Σ slits = the number of slits counted on the HPEM from four glomeruli from each animal, and Σ BML = the total peripheral capillary basement membrane length on the HPEM for that animal.

2. A single value for glomerular capillary pressure was obtained in only one rat.

Analytical

Albumin excretion rate was determined by averaging two consecutive 24-h urine collections. Plasma protein concentration was measured by the modified Lowry technique to avoid interference by plasma lipids (14). Plasma and urine albumin concentrations were measured by rate nephelometry (Beckman Immunochemistry Analyzer II, Beckman Instruments, Inc., Palo Alto, CA), using anti-rat albumin antibody (Cooper Biomedical, Inc., Malvern, PA). Radioinulin content of plasma, urine, and tubule fluid was assessed by liquid-phase scintillation counting.

Calculations and statistics

Values for glomerular hemodynamic and morphometric parameters for each animal were recorded as the mean of values obtained in that animal. Group mean values were then calculated as the mean of values for individual animals. Efferent arteriolar plasma protein concentration (C_E) was calculated from the relation: $C_E = C_A/(1 - FF)$. A recently derived set of equations relating oncotic pressure to plasma protein concentration in nephrotic rats was used to calculate plasma oncotic pressure (15). A standard mathematical model was used to derive the glomerular capillary ultrafiltration coefficient (K_f) from these values for oncotic pressure and from measured parameters (16). The statistical significance of differences among values for individual parameters in the three experimental groups was evaluated by the analysis of variance. Values for SN_{alb} were log-transformed for statistical analysis. The Newman-Keuls multiple range test was used to assess the significance of differences between individual group means (three comparisons) with significance defined as $P < 0.05$. Comparison of values for blood pressure and albumin excretion rate at different time points within each group was made by the paired t test. Results are expressed as means±SE throughout.

Results

Albumin excretion rate and body growth. By design, baseline albumin excretion rate at 4 wk after injection of adriamycin was similar in the three groups (Table I), averaging 838±51 mg/d in group 1, 820±69 mg/d in group 2, and 874±56 mg/d in group 3. Albumin excretion rate rose slightly to 923±63 mg/d in group 1 animals at 10 d. Despite four-fifths reduction in nephron mass, albumin excretion rate was reduced only slightly to 646±27 mg/d in group 2 rats at 10 d. Dietary protein restriction reduced albumin excretion rate to 578±27 mg/d in group 3 rats at 10 d. Baseline values for serum albumin concentration were similar in the three groups and were not altered significantly at ten days. Baseline values for body weight were also similar in the three groups. Body weight was slightly but significantly reduced in group 2 and group 3 rats at 10 d, falling from 291±7 g to 264±5 g in group 2 rats and from 280±5 g to 264±4 g in group 3 animals.

Table I. Body Weight and Albuminuria

		BW	$U_{alb}V$	S_{alb}	C_{alb}
		g	*mg/d*	*g/dl*	*ml/min*
Group 1	0d	289±6	838±51	1.06±0.12	0.060±0.008
(n = 8)	10d	292±7	923±63	0.94±0.14	0.077±0.009‡
Group 2	0d	291±7	820±69	0.99±0.10	0.063±0.009
(n = 9)	10d	264±5*	646±27*‡	1.11±0.09	0.043±0.004*‡
Group 3	0d	280±5	874±56	1.05±0.12	0.066±0.010
(n = 8)	10d	264±4*	578±27*‡	1.15±0.09	0.037±0.003*‡

Values are mean±SEM.
Abbreviations: BW, body weight; $U_{alb}V$, albuminuria; S_{alb}, serum albumin concentration; C_{alb}, albumin clearance; 0d, baseline value at 4 wk after administration of adriamycin; 10d, final value at 10 d after baseline value.
* $P < 0.05$ group 2 and 3 vs. group 1.
‡ $P < 0.05$ 10d value vs. 0d value.

Glomerular hemodynamic function. Mean values for $\overline{AP}$ under anesthesia, hematocrit, GFR, SNGFR, and the pressures and flows governing glomerular ultrafiltration for the three groups of rats are summarized in Table II. $\overline{AP}$ was increased after renal ablation in nephrotic rats, averaging 150±6 mm Hg in group 2 as compared with 118±2 mm Hg in group 1 and 116±3 mm Hg in group 3. Hematocrit and plasma protein concentration was not different in the three groups.

As expected, ablation of a major portion of the renal mass led to a decline in total GFR in group 2. GFR was reduced to 0.56±0.05 ml/min in group 2 as compared to 1.68±0.12 ml/min in group 1. As it does in normal rats, reduction in renal mass evoked remnant nephron hyperfiltration, with SNGFR increasing to 56±3 nl/min in group 2 as compared to 36±2 nl/min in group 1. In accord with our previous results, however, the hemodynamic changes responsible for the increase in remnant nephron GFR in nephrotic rats were different than those identified in normal rats (2, 6). Thus the increase in remnant SNGFR in group 2 rats was largely due to increases in glomerular plasma flow rate (Q_A) and K_f while no significant increase in ΔP over the value observed in group 1 rats was observed. Dietary protein restriction did not significantly alter glomerular hemodynamic function in group 3. Values for total GFR, SNGFR, Q_A, and ΔP in group 3 were slightly reduced as compared to the values observed in group 1, but the differences were not statistically significant.

The ratio of total GFR to SNGFR was used to estimate the functioning nephron number in each animal. Mean values of 47±3 × 10^3 and 48±4 × 10^3, respectively, were obtained in group 1 and group 3 rats with intact kidneys. Renal ablation reduced estimated nephron number in group 2 rats to 10±1 × 10^3. Estimates of nephron number in each animal were in turn used to calculate values for single nephron albumin excretion rate as summarized in Table III. A mean value of 20±2 μg/d was obtained in group 1. The calculated value for single nephron albumin excretion rate was increased threefold to 68±7 μg/d after renal ablation in group 2 and was reduced to 12±1 μg/d by dietary protein restriction in group 3.

Glomerular structure. Mean values for glomerular volume and for the surface areas and volumes of different glomerular components are summarized in Table IV. Glomerular volume in group 1 nephrotic rats averaged 1.44±0.04 × 10^6 μm^3, a value no different than that obtained in normal male Munich-Wistar rats with similar body weight in our laboratory. Increased remnant nephron albuminuria at 10 d after renal ablation was associated with hypertrophy of remnant glomeruli in group 2. At this stage of glomerular hypertrophy, no increase in the surface area of the peripheral capillary wall, S_{PCW}, or the epithelial–mesangial interface, S_{MES}, was apparent. There was, however, a marked increase in the area of epithelial cell detachment from the basement membrane of the peripheral capillary wall, S_{PCWd}. Epithelial cell detachment from the peripheral capillary wall, as illustrated in Fig. 1, has regularly been observed in rats with toxic epithelial cell injury induced by puromycin aminonucleoside and by adriamycin (17, 18). Application of quantitative morphometric techniques revealed

Table II. Summary of Renal Cortical Microcirculation Studies

	$\overline{AP}$	Hct	C_A	GFR	SNGFR	FF	Q_A	P_{GC}	P_T	P_E	ΔP	π_A	π_E	K_f
	mmHg	*%*	*g/dl*	*ml/min*	*nl/min*	*%*	*nl/min*			*mm Hg*				*nl/(s × mm Hg)*
Group 1														
(n = 8)	118±2	39±1	5.03±0.07	1.68±0.12	36±2	0.24±0.02	154±17	62±1	15±1	15±1	46±1	11±1	19±1	0.019±0.002
Group 2														
(n = 9)	150±6*	37±1	4.97±0.05	0.56±0.05*	56±3*	0.25±0.01	228±15*	63±2	15±1	16±1	49±2	11±1	18±1	0.028±0.002*
Group 3														
(n = 8)	116±3‡	39±1	4.85±0.05	1.44±0.08‡	33±2‡	0.26±0.02	126±13‡	56±1*‡	13±1	14±1	43±1‡	11±1	18±1	0.019±0.002‡

Mean values±SEM. Abbreviations: $\overline{AP}$, mean arterial pressure; Hct, hematocrit; C_A, afferent arteriolar plasma protein concentration; GFR, glomerular filtration rate (obtained by doubling measured left kidney GFR in groups 1 and 3); SNGFR, single nephron glomerular filtration rate; FF, filtration fraction; Q_A, glomerular plasma flow rate; P_{GC}, mean glomerular capillary hydraulic pressure; P_T, proximal tubule hydraulic pressure; P_E, efferent arteriolar hydraulic pressure; ΔP, mean glomerular transcapillary hydraulic pressure difference; π_A and π_E, afferent and efferent arteriolar colloid osmotic pressure; K_f, glomerular capillary ultrafiltration coefficient. * $P < 0.05$ groups 2 and 3 vs. group 1. ‡ $P < 0.05$ group 3 vs. group 2.

Table III. Estimated Single Nephron Albuminuria at 10 d

	Group 1	Group 2	Group 3
$U_{alb}V$ (*mg/d*)	923±63	646±27*	578±27*
Nephron (n, $\times 10^3$)	47±3	10±1*	48±4‡
SN_{alb} (*μg/d*)	20±2	68±7*	12±1*‡

Values are mean±SE.
Abbreviations: $U_{alb}V$, albuminuria at 10 d; SN_{alb}, single nephron albumin excretion rate calculated from $U_{alb}V$ and nephron number.
* $P < 0.05$ groups 2 and 3 vs. group 1.
‡ $P < 0.05$ group 3 vs. group 2.

that the area of epithelial cell detachment from the peripheral capillary wall was 0.016±0.005 × 10^5 μm^2, in group 1 nephrotic rats in the present study. The area of epithelial cell detachment increased to 0.065±0.017 × 10^5 μm^2 in association with glomerular hypertrophy in group 2. This change represents an increase in the area of epithelial detachment from 1.0±0.3% to 4.3±1.2% of the total peripheral capillary wall, as illustrated in Fig. 2.

Glomerular hypertrophy was associated with significant increases in epithelial cell volume, endothelial cell volume, and glomerular basement membrane volume in group 2 rats. Epithelial slit frequency was determined to assess whether the extent of epithelial foot process retraction was altered in the course of glomerular and epithelial cell hypertrophy in nephrotic rats. As expected, slit frequency in group 1 nephrotic rats, 0.42±0.04 slits/μm, was reduced below the value obtained in normal rats of similar body weight in our laboratory, as illustrated in Fig. 2. No further reduction in slit frequency was associated with the threefold increase in remnant nephron albuminuria which accompanied glomerular hypertrophy after renal ablation in group 2 rats.

In contrast to the increase in remnant nephron albuminuria associated with glomerular hypertrophy after renal ablation in group 2, reduction in albuminuria was associated with reduction of glomerular volume after dietary protein restriction in group 3. The magnitude of the reduction in albuminuria in group 3, however, was less than the magnitude of the increase in remnant nephron albuminuria in group 2. Reduction in glomerular volume in group 3 was not accompanied by a perceptible change in the area of epithelial cell detachment from the basement membrane or in epithelial cell slit frequency, as illustrated in Fig. 2.

Discussion

Recent studies have emphasized that progressive glomerular injury follows reduction of nephron number in the rat. Numerous studies have documented early development of glomerular injury following ablation of renal mass in rats with initially normal kidneys (1, 2). Further studies have shown that ablation of renal mass accelerates the progression of experimental renal disease (3, 4).

The potential analogy of renal disease progression in rats subjected to renal ablation and humans with renal insufficiency has prompted investigation of the mechanisms responsible for glomerular injury following reduction in nephron number. A variety of mechanisms of injury have been identi-

fied (19). One line of evidence suggests that changes in glomerular function and structure which serve to elevate remnant nephron glomerular filtration rate cause remnant glomerular injury (20). Thus elevated capillary pressure has been shown to accelerate remnant glomerular injury in several disease models (5, 21). Recent studies suggest that glomerular hypertrophy also promotes glomerular injury independent of glomerular capillary hypertension (4, 6–8). In rats with established nephrosis, glomerular hypertrophy following renal ablation is accompanied by a large increase in remnant nephron proteinuria (4, 6). This increase in remnant nephron proteinuria is followed by rapid development of glomerular sclerosis (4). The main objective of the present study was to identify a structural basis for increased remnant nephron proteinuria associated with glomerular hypertrophy in nephrotic rats.

Our results show that glomerular hypertrophy in nephrotic rats is associated with increased epithelial cell detachment from the peripheral capillary basement membrane. Glomerular hypertrophy at 10 d after renal ablation in group 2 rats was accompanied by a fourfold increase in the mean area of epithelial detachment from the peripheral capillary basement membrane. This structural change was associated with a threefold increase in remnant nephron albuminuria. Epithelial cell detachment from the basement membrane has previously been associated with proteinuria in rats with adriamycin and puromycin nephrosis and in rats with initially normal kidneys subjected to renal ablation (17, 18, 22). Epithelial cell detachment from the basement membrane has also been observed in several human glomerular diseases characterized by heavy proteinuria (23, 24). In rats with puromycin nephrosis the onset of proteinuria coincides with the appearance of epithelial detachment from the basement membrane and the degree of proteinuria is correlated with the extent of this lesion (18, 24, 25). These observations have prompted the suggestion that increased protein passage through capillary wall areas denuded of epithelial cells causes proteinuria after toxic epithelial cell injury. The present results are consistent with this hypothesis, and show further that epithelial cell detachment from the peripheral capillary wall increases when injured glomeruli undergo compensatory glomerular hypertrophy.

The extent of epithelial cell injury was assessed not only by measuring epithelial cell denudation from the basement membrane but also by estimating the degree of broadening of epithelial cell foot processes as reflected by epithelial slit frequency. Broadening or "fusion" of epithelial cell foot processes is regularly associated with heavy proteinuria in experimental and in human renal disease. Studies employing scanning electron microscopy have established that this alteration in epithelial cell structure does not reflect actual fusion of adjacent foot processes but rather retraction of foot processes and simplification of epithelial cell structure (26). As expected, group 1 nephrotic rats in the current study exhibited broadening of epithelial cell foot processes reflected by reduction in epithelial slit frequency below values observed in normal rats. Of note, however, no further broadening of epithelial cell foot processes accompanied the threefold increase in remnant nephron albuminuria associated with nephron loss and compensatory glomerular hypertrophy in group 2 rats. The average slit frequency of 0.52±0.05 slits/μm observed in group 2 was not different than the average slit frequency of 0.42±0.04 slits/μm observed in group 1. This finding is consistent with the recent demonstration by Fries et al. (27) that protein ex-

Table IV. Summary of Morphometric Studies

	$\bar{V}_G$	$V_{V_{Tuft}}$	S_{PCW}	S_{PCW_d}	S_{Mes}	S Freq	V_{Epi}	V_{End}	V_{Mes}	V_{Cap}	V_{GBM}
	$10^6 \mu m^3$			$10^5 \mu m^2$		slits/μm			$10^6 \mu m^3$		
Group 1 (n = 8)	1.44±0.04	0.76±0.01	1.63±0.08	0.016±0.005	0.39±0.04	0.42±0.04	0.29±0.02	0.09±0.01	0.13±0.01	0.54±0.02	0.05±0.01
Group 2 (n = 9)	1.66±0.08*	0.75±0.01	1.59±0.08	0.065±0.017*	0.45±0.02	0.52±0.05	0.35±0.01*	0.12±0.01*	0.14±0.01	0.55±0.03	0.08±0.01*
Group 3 (n = 8)	1.26±0.03*‡	0.78±0.01	1.63±0.10	0.018±0.005‡	0.39±0.03	0.52±0.06	0.27±0.02‡	0.09±0.01‡	0.11±0.01	0.47±0.02*‡	0.05±0.01‡

Mean values±SEM. Abbreviations: $\bar{V}_G$, mean glomerular volume; $V_{V_{Tuft}}$, fractional tuft volume; S_{PCW}, surface area of peripheral capillary wall; S_{PCW_d}, surface area of peripheral capillary wall denuded of epithelial cells; S_{Mes}, surface area of mesangial–epithelial interface; S Freq, average slit frequency; V_{Epi}, epithelial cell volume; V_{End}, endothelial cell volume; V_{Mes}, mesangial volume (cells plus matrix); V_{Cap}, capillary luminal volume; V_{GBM}, glomerular basement membrane volume. * $P < 0.05$ groups 2 and 3 vs. group 1. ‡ $P < 0.05$ group 3 vs. group 2.

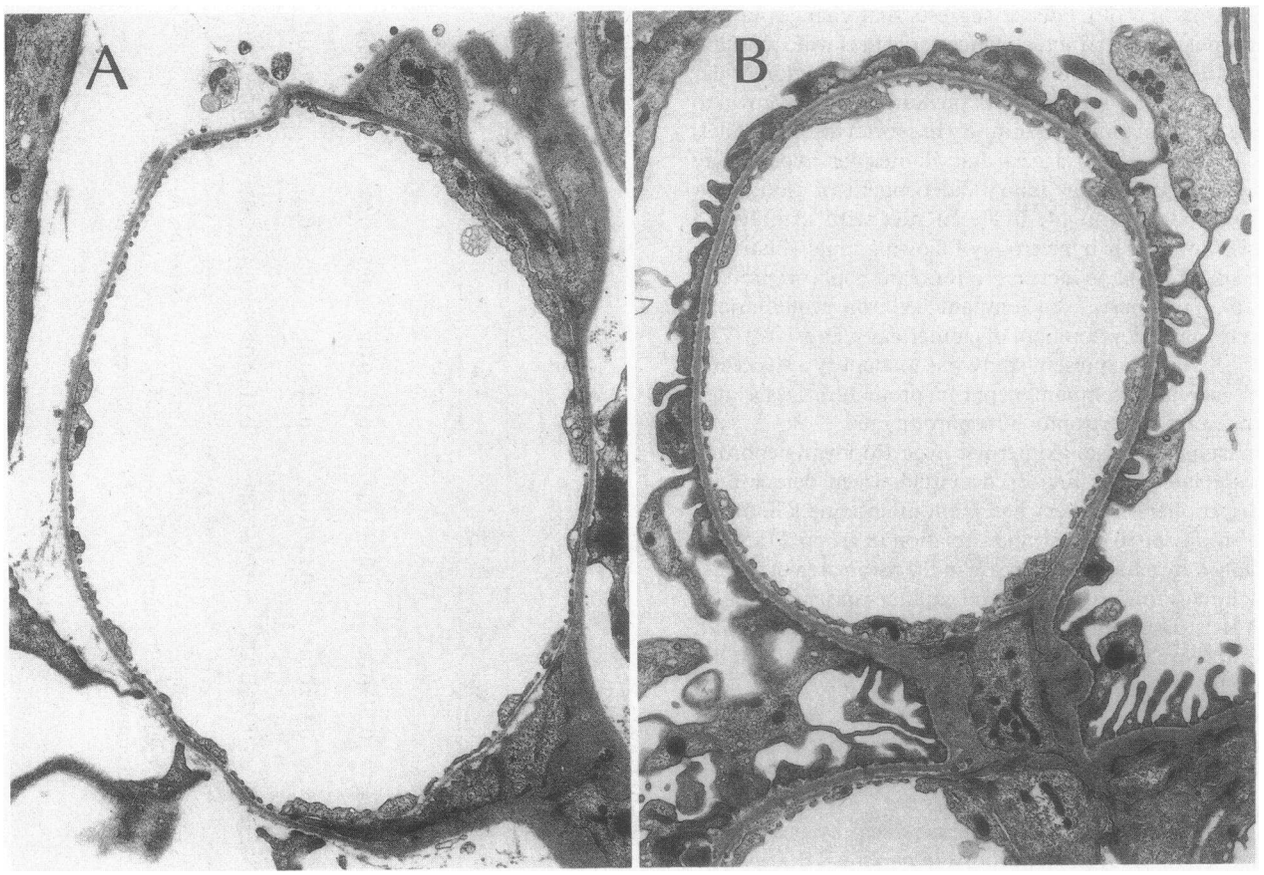

Figure 1. Epithelial cell injury after adriamycin nephrosis and renal ablation. (*A*) A capillary loop showing extensive detachment of epithelial cell foot processes. The severe injury illustrated in this micrograph was seen only focally. (*B*) Another capillary loop from the same section of the same glomerulus showing relative preservation of epithelial cell structure. ×7,500.

cretion rate is not correlated with epithelial cell foot process width in nephrotic humans. Together, our results and those of Fries et al. (27) indicate that impairment of glomerular permselectivity is not determined by the extent of foot process retraction. By themselves, these results do not exclude the possibility that foot process retraction represents a threshold response, occurring when a certain degree of proteinuria is exceeded. Previous studies have demonstrated, however, that retraction of epithelial cell foot processes precedes development of proteinuria in rats with puromycin nephrosis (18, 24). Results of these studies seem inconsistent with the hypothesis that foot process retraction is a consequence of impaired permselectivity and proteinuria.

Micropuncture studies were performed to assess the dependence of remnant nephron albuminuria and remnant glomerular structure on glomerular capillary pressure. These studies showed that increased remnant nephron albuminuria and epithelial cell detachment from the basement membrane were not caused by an increase in ΔP after renal ablation in nephrotic rats. The mean ΔP value of 49±2 mm Hg in group 2 was not significantly increased above the mean ΔP value of 46±1 mm Hg in group 1. This result is consistent with our previous finding that remnant nephron proteinuria increases after renal ablation in nephrotic rats even when a converting enzyme inhibitor is used to prevent any increase in ΔP (6). Together, these results suggest that changes in epithelial cell structure observed in group 2 rats can properly be attributed to glomerular hypertrophy rather than to glomerular capillary hypertension.

A further objective of the present study was to identify glomerular structural changes responsible for decreased albuminuria after restriction of protein intake. Recent studies have shown that restriction of protein intake reduces proteinuria both in nephrotic rats and in nephrotic humans (28–31). The current study showed that reduction of albuminuria was associated with reduction of glomerular volume after dietary protein restriction in rats with established adriamycin nephrosis. Beukers et al. (9) have previously found that development of proteinuria and glomerular volume are reduced when dietary protein restriction is initiated at the time of adriamycin injection. The present study did not, however, identify any changes in epithelial cell structure associated with reduction of albuminuria and reduction of glomerular volume in protein restricted nephrotic rats. In particular, no decrease in the area of epithelial cell detachment from the peripheral capillary basement membrane was observed in protein-restricted nephrotic rats. The mean area of detachment of $0.018\pm0.005 \times 10^5$ μm^2 per glomerulus in group 3 was no different from the mean area of detachment of $0.016\pm0.005 \times 10^5$ μm^2 per glomerulus in group 1. The precision of these results, however, is not sufficient to establish that protein restriction did not affect epithelial cell detachment from the basement membrane. Marked intraglomerular variability of epithelial cell injury was observed in the current study as in previous studies of toxic epi-

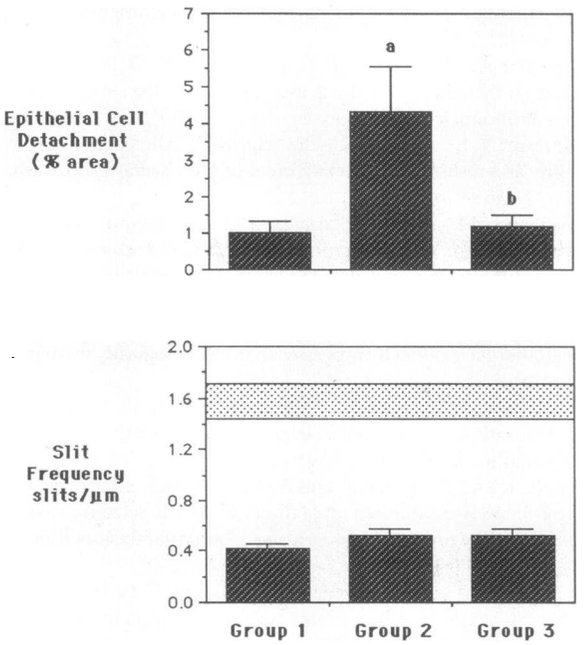

Figure 2. Area of epithelial cell denudation from the peripheral capillary wall (expressed as percent peripheral capillary wall area) and average slit frequency in nephrotic rats. Epithelial cell denudation from the peripheral capillary wall increased in group 2 rats subjected to renal ablation. Foot process retraction in each group was reflected by reduction in average slit frequency below normal values. Average slit frequency was not altered following renal ablation in group 2 or protein restriction in group 3. $^{a}P < 0.05$ group 2 vs. group 1. $^{b}P < 0.05$ group 3 vs. group 2. The shaded band depicts the normal range (mean±2 SD) for slit frequency in male Munich-Wistar rats (from reference 34).

thelial cell injury induced by puromycin (18). Because of this variability, examination of a large number of glomerular profiles would be required to determine whether the area of epithelial cell detachment changed in proportion to albumin excretion rate in group 3 rats. Surface area measurements obtained from 24 glomerular profiles in groups 1 and 3 in the current study cannot distinguish whether dietary protein restriction reduces epithelial cell detachment from the basement membrane or whether this dietary treatment reduces albuminuria in nephrotic rats by a different mechanism. The present study does suggest, however, that reduction in albuminuria cannot be attributed to reduction in ΔP in protein restricted rats with adriamycin nephrosis. ΔP was only slightly lower in group 3 than in group 1 in the current study, and this difference did not achieve statistical significance. We have previously shown that albuminuria in rats with adriamycin nephrosis is not reduced by converting enzyme inhibitor treatment which causes a larger, statistically significant reduction in ΔP (32). Similarly, Beukers et al. (9) have shown that proteinuria in rats with adriamycin nephrosis is not reduced by converting enzyme inhibitor treatment which causes a prominent reduction in systemic blood pressure. Together, these results indicate that lowering of ΔP cannot by itself account for the reduction in albuminuria in nephrotic rats maintained on a low protein diet. It remains possible, however, that reduction in ΔP acts in concert with reduction in glomerular volume to reduce the albumin excretion rate in these animals.

Previous studies have shown that normal rats subjected to renal ablation exhibit a gradual increase in proteinuria. Development of proteinuria in these animals can be largely prevented by normalization of glomerular capillary pressure (2, 33). These findings suggest that normal epithelial cells can maintain their structure and preserve glomerular permselective function as the glomerulus enlarges following reduction in nephron number. In contrast, a large increase in remnant nephron albuminuria was observed at ten days following renal ablation in nephrotic rats in the present study. This increase in remnant nephron albuminuria was associated with increased epithelial cell detachment from the peripheral capillary wall. These findings suggest that injured epithelial cells are unable to maintain their attachment to the capillary wall when the glomerulus enlarges in nephrotic animals. In a previous study, we found that increased remnant nephron albuminuria was associated with rapid development of glomerular sclerosis in nephrotic rats with reduced nephron number (4). This finding and the results of the current study lead us to speculate that glomerular hypertrophy can accelerate glomerular sclerosis by aggravating epithelial cell injury when nephron number is reduced in the setting of established nephrosis.

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