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Research Article

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Hemodynamic Basis for Glomerular Injury in Rats with Desoxycorticosterone-Salt Hypertension

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Abstract. Micropuncture and/or morphologic studies were performed in seven groups of uninephrectomized (UNX) adult male Munich-Wistar rats. Control groups 1, 3, and 6 received standard (24% protein) chow and tap water. Groups 2, 4, and 5 received weekly injections of desoxycorticosterone pivilate (DOC) and 1% saline for drinking, groups 2 and 4 were fed standard chow, and Group 5 a diet containing 6% protein. Group 7 received DOC, salt, and standard chow for 3 wk followed by withdrawal of DOC and salt for an additional 6 wk. 10–14 d after UNX, groups 1 and 2 exhibited similar single nephron glomerular filtration rates (SNGFR) and initial glomerular plasma flow rates (Q_A). Group 2 had higher mean arterial pressure ($\overline{AP}$) and glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) than group 1. 3–4 wk after UNX, group 4 exhibited further elevations in $\overline{AP}$ and $\bar{P}_{GC}$ as compared with groups 2 and 3. SNGFR and Q_A were similar in groups 3 and 4, but these average values were greater than typical for normal rats. Group 4 also demonstrated increased urinary protein excretion. Morphologic evaluation of glomeruli in groups 2 and 4 revealed mesangial expansion and focal intraglomerular hemorrhage whereas glomeruli of groups 1 and 3 were essentially normal. Values for $\overline{AP}$ and $\bar{P}_{GC}$ in group 5 were not different than group 3 but significantly lower than group 4. Q_A and SNGFR were lower in group 5 (low protein) than in groups 3 and 4. Furthermore, proteinuria and glomerular structural lesions were abolished in group 5. Morphologic studies performed in groups 6 and 7 showed that early DOC-SALT lesions progress to focal glomerular sclerosis. These studies suggest that continued elevations in glomerular capillary flows and pressures predispose to glomerular injury in this model of systemic arterial hypertension.

Introduction

Fifty years have passed since the initial observation that progressive azotemia and glomerular sclerosis follow extensive ablation of renal mass (1, 2). More recent studies have revealed that 1 wk after ablation, remnant kidneys demonstrate increased glomerular capillary hydraulic pressures and plasma flow rates (3). It has been suggested that these increased pressures and flows may cause glomerular damage, eventuating in mesangial sclerosis and proteinuria. Micropuncture studies in hypertensive, salt-fed Holtzman rats (4) and 2-kidney, one clip Goldblatt hypertension (5) have demonstrated an augmented pattern of glomerular capillary perfusion similar to that observed after reduction in renal mass. Furthermore, in both salt-fed Holtzman rats (4) and 2-kidney, one clip Goldblatt hypertension (6), glomerular sclerosis eventually develops. These observations suggest that increased glomerular capillary pressures and flows may play a pathogenic role in the glomerular injury which occurs in experimental hypertension.

Hypertension has also been produced in the rat by uninephrectomy and administration of desoxycorticosterone and a high salt intake (7). This model of hypertension is characterized by increased plasma volume, which is most marked during the

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initial 6 wk of DOC-SALT treatment (8). As both plasma volume expansion (9) and uninephrectomy (10) induce renal vasodilation, it seemed likely that glomerular capillary perfusion would be significantly increased in rats with DOC-SALT hypertension. Therefore, to specifically examine the hypothesis that glomerular damage results from sustained elevation in glomerular perfusion, we correlated the structural and hemodynamic alterations that occur in the glomeruli of rats with DOC-SALT hypertension.

Glossary

$\overline{AP}$	mean femoral arterial pressure, mmHg
C	protein concentration, g/100 ml
DOC	desoxycorticosterone pivilate
GFR	glomerular filtration rate (whole kidney), ml/min
Hct	blood hematocrit in femoral artery, vol/100 ml
K_f	glomerular capillary ultrafiltration coefficient, nl/(s · mmHg)
ΔP	glomerular transcapillary hydraulic pressure difference, $\bar{P}_{GC} - P_T$, mmHg
π	colloid osmotic pressure, mmHg
$\Delta\pi$	glomerular transcapillary oncotic pressure difference, $\pi_{GC} - \pi_T$, mmHg
P	hydraulic pressure, mmHg
Q	plasma flow rate
R	resistance to blood flow, dyne · s · cm^{-5}
SNFF	single nephron filtration fraction
SNGFR	single nephron glomerular filtration rate, nl/min
UNX	unilateral nephrectomy
V_G	glomerular volume

Subscripts

A	afferent arteriole
E	efferent arteriole
GC	glomerular capillary
T	proximal tubule

Methods

Sixty adult male Munich-Wistar rats weighing 200–300 g were employed in these experiments. One group of normal rats and seven groups of uninephrectomized rats were studied. As summarized in Table I, group 0 rats (n = 3) were normal controls and group 1 (n = 9) rats were fed standard chow consisting of 24% protein by weight (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) and water. Group 2 rats (n = 11) were fed standard chow, but received weekly subcutaneous injections of 10 mg of desoxycorticosterone pivilate (CIBA Pharmaceutical Co., Summit, NJ) in sesame oil and were given 1% saline for drinking. Micropuncture and morphologic studies were performed on groups 1 and 2 rats at 10 to 14 d after uninephrectomy. Group 3 (n = 12) and group 4 (n = 10) rats were treated identically to group 1 and group 2 rats, respectively, but were studied at 3 to 4 wk after uninephrectomy. Group 5 (n = 7) rats also received DOC and saline, but were fed a diet containing only 6% protein by weight that was replete with electrolytes, trace minerals, and vitamins. Rats in group 5 were also studied at 3–4 wk after uninephrectomy. Rats in groups 6 (n = 3) and 7 (n = 5) were studied 9 wk after uninephrectomy. Group 6 rats ingested standard chow and water and served as controls. Group 7 rats were treated with DOC, saline, and standard chow for 3 wk, but thereafter received only standard chow and water.

Table I. Experimental Protocols

	UNX	DOC	Salt	Protein diet	Time of study
Group 0 (n = 3)	−	−	−	24%	3–4 wk
Group 1 (n = 9)	+	−	−	24%	10–14 d
Group 2 (n = 11)	+	+	+	24%	10–14 d
Group 3 (n = 12)	+	−	−	24%	3–4 wk
Group 4 (n = 10)	+	+	+	24%	3–4 wk
Group 5 (n = 7)	+	+	+	6%	3–4 wk
Group 6 (n = 3)	+	−	−	24%	9 wk
Group 7* (n = 5)	+	+	+	24%	9 wk

* DOC and saline administered only during initial 3 wk.

Animals in groups 1–5 underwent both micropuncture and morphologic study. Morphologic studies alone were performed on rats in groups 0, 6, and 7. On the day prior to study, rats were placed in metabolic cages and 24-h urine collections were made for determination of protein and electrolyte excretion rates. On the morning of micropuncture study, rats were anesthetized with Inactin (100 mg/kg body weight intraperitoneal) and prepared in the standard fashion for micropuncture (11). To compensate for the loss of plasma associated with anesthesia and surgery, all rats received intravenous infusions of a volume of heparinized isoncotic rat plasma equal to 10 ml/kg body weight over 30 min, followed by sustaining infusions of plasma at ~0.5 ml/h, adjusted to maintain hematocrit stable at base-line values. All rats also received a 0.5 ml intravenous bolus of inulin (7 g/100 ml) in normal saline followed by a sustaining infusion of inulin at a rate of 1.2 ml/h.

Samples of proximal tubule fluid were obtained by micropuncture for determination of flow rate and inulin concentration, the latter by the method of Vurek and Pegram (12). Efferent arteriolar blood samples were obtained for measurement of total protein concentration (13). Hydraulic pressure measurements were made in cortical tubules and vessels by using the servo-null micropipette technique (11). The details of the calculations employed have been given previously (11). Urinary protein concentration was measured by precipitation with 3% sulfosalicylic acid. Turbidity was then determined by measuring absorbance at a wavelength of 595 mμ using a Coleman Junior II spectrophotometer. Urinary and plasma sodium and potassium concentrations were determined by standard flame photometry. Urinary phosphate concentration was determined by the Fiske-Subbarow method (14). Statistical analysis was performed by Student's t test for comparisons between the means of two groups (1 vs. 2) or by one-way analysis of variance (groups 3, 4, and 5) followed by computation of modified t values and multiple pairwise comparisons according to the method of Bonferroni (15). Statistical significance was defined as $P < 0.05$.

Table II. Summary of Renal Cortical Microcirculation Studies

	Body wt	Hct	$\overline{AP}$	$\bar{P}_{GC}$	P_T	P_E	$\overline{\Delta P}$	C_A	C_E	π_A
	g	*vol/100 ml*	*mmHg*	*mmHg*	*mmHg*	*mmHg*	*mmHg*	*g/100 ml*	*g/100 ml*	*mmHg*
Group 1										
$n = 9$	256±6	44±0.3	112±3	51±1	16±1	19±1	35±1	6.3±0.2	8.9±0.4	22±0.2
Group 2										
$n = 11$	251±7	45±1	132±6	55±1	15±1	19±1	40±2	6.0±0.1	8.8±0.3	20±1
Group 3										
$n = 12$	265±6	45±1	115±3	53±1	15±1	17±1	38±1	6.0±0.1	8.2±0.3	20±1
Group 4										
$n = 10$	262±8	46±1	155±6	59±2	16±1	22±1	44±1	5.9±0.6	8.5±0.3	20±3
Group 5										
$n = 7$	201±3	40±1	124±6	50±1	14±1	16±1	37±1	5.2±0.1	7.3±0.2	17±1
P 1 vs. 2	NS	NS	<0.02	<0.0005	NS	NS	<0.025	NS	NS	NS
P 3 vs. 4	NS	NS	<0.05	<0.05	NS	<0.05	<0.05	NS	NS	NS
P 3 vs. 5	<0.05	<0.05	NS	NS	NS	NS	NS	<0.05	<0.05	<0.05
P 4 vs. 5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Mean±SEM

Following micropuncture studies, the kidneys of six animals each from groups 1–5 were fixed by perfusion for 5 min at the measured arterial pressure with 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Additional morphologic data was obtained from three intact animals (group 0), three uninephrectomy rats followed for 9 wk (group 6), and five uninephrectomy animals which received DOC and saline as drinking water for the first 3 wk and then were allowed to recover on normal diet and drinking water for an additional 6 wk (group 7). Following perfusion-fixation, one or two 3–4 mm thick coronal sections through the mid portion of the kidney were postfixed in 4 g/100 ml buffered formaldehyde solution and processed routinely for light microscopy through paraffin embedding. Sections 3 μm in thickness were stained with hematoxylin/eosin and by the periodic-acid Schiff technique. The glomerular tuft volume (V_G) for each animal was determined following the procedure described by Weibel (16). For this purpose, the mean glomerular random cross-sectional area ($\bar{A}_G$) was determined on 100 systematically sampled glomerular tuft profiles by point counting at a final magnification of 200 X utilizing a 361-point ocular grid covering a 369664 μm^2 microscopic field. The $\bar{V}_G$ was then calculated as: $V_G = \frac{\beta}{\kappa}(\bar{A}_G)^{3/2}$, where $\beta = 1.38$ is the shape coefficient for spheres (the idealized shape of glomeruli) and $\kappa = 1.1$ is a size distribution coefficient (16, 17). The mean value for each group ($\bar{V}_G$) and standard deviation were calculated. Significance of differences between all groups was determined by one-way analysis of variance; twelve predetermined pairwise comparisons were assessed for significance by modified *t* statistics; and critical values were calculated by the methods of Bonferroni, Tukey, and Scheffe (15). Statistical significance was defined as $P < 0.05$. The frequency of focal and segmental glomerular lesions was determined for each group of animals by examining all glomerular profiles contained in one coronal section from each animal. The number of damaged glomeruli and the total number of examined glomeruli in each group as a whole were recorded and expressed as a frequency per 10,000 glomeruli; an average of 223 glomeruli/animal (range, 164 to 274) were examined. Other glomerular changes, such as expansion of the mesangial areas and abnormalities of arteries and arterioles, were assessed nonquantitatively by light microscopy. Small, randomly selected fragments of cortex were also processed for electron microscopy by osmium postfixation and epoxy-resin embedding. Ultrathin sections of selected glomeruli from each group stained with lead citrate and uranyl acetate on the grid were then examined for ultrastructural abnormalities in a Philips 201 electron microscope at 60 kV. Two to three glomeruli from at least three animals in each group (groups 1–5) were examined by electron microscopy. Nonquantitative evaluation of the light microscopic appearance of cortical sections taken from rats in groups 0–7 were performed in a blinded fashion by a single observer. Quantitative morphologic measurements were performed by the same investigator in a standard fashion as described above but were not blinded.

Results

Micropuncture studies. Mean values for body weight; Hct; plasma sodium and potassium concentration; whole kidney GFR; $\overline{AP}$; SNGFR; and the pressures, flows, and resistances governing glomerular ultrafiltration for animals in groups 1–5 are summarized in Table II. After uninephrectomy and 10–14 d of DOC-SALT treatment, mean arterial pressure ($\overline{AP}$) averaged 132±6 (SE) mmHg in group 2 rats, significantly greater ($P < 0.02$) than the mean value of 112±3 mmHg measured in group 1 rats. Mean glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) and the mean glomerular transcapillary hydraulic pressure difference ($\overline{\Delta P}$) were also increased in group 2 rats, averaging 55±1

π_E	SNFF	SNGFR	GFR	Q_A	$R_A \times 10^{10}$	$R_E \times 10^{10}$	$R_T \times 10^{10}$	Plasma [Na]	Plasma [K]
mmHg		*nl/min*	*ml/min*	*nl/min*	*dyne·s·cm^{-5}*	*dyne·s·cm^{-5}*	*dyne·s·cm^{-5}*	*meq/liter*	*meq/liter*
38±3	0.29±0.02	57.8±4.5	1.70±0.08	208±31	1.52±0.22	0.97±0.15	2.49±0.36	143±1	4.1±0.2
37±2	0.32±0.02	62.1±3.0	1.60±0.08	206±19	1.86±0.24	1.04±0.11	2.90±0.34	148±2	3.6±0.3
33±2	0.27±0.02	65.3±2.7	1.54±0.13	236±16	1.21±0.19	0.81±0.05	2.02±0.20	142±2	4.3±0.1
35±2	0.30±0.04	68.0±4.6	1.55±0.17	220±18	1.97±0.17	0.92±0.07	2.86±0.21	143±3	3.4±0.3
27±1	0.29±0.02	39.8±3.8	1.04±0.08	142±26	3.03±0.60	1.68±0.29	4.72±0.89	143±1	3.6±0.2
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.05
<0.05	NS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	NS	=0.05
<0.05	NS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	NS	NS

and 40±2 mmHg, respectively; both values were significantly greater than the corresponding average values of 51±1 and 35±1 mmHg measured in group 1 rats. A selective increase in $\overline{\Delta P}$ would be predicted to increase SNGFR; however, values for whole kidney GFR and SNGFR were similar in groups 1 and 2, averaging 1.70±0.08 ml/min and 57.8±4.5 nl/min, respectively, for group 1 and 1.60±0.08 ml/min and 62.1±3.0 nl/min for group 2. Average values for initial glomerular plasma flow rate (Q_A) and systemic plasma protein concentration (C_A) were also not significantly different between groups 1 and 2. The only remaining explanation for the similarity of SNGFR in groups 1 and 2, despite the increase in $\overline{\Delta P}$ observed in group 2, is that the glomerular capillary ultrafiltration coefficient (K_f) must have been lower in group 2 rats. Filtration pressure equilibrium (where $\pi_E = \overline{\Delta P}$) obtained in five of eight rats in group 1. The mean of the minimum K_f values calculated for animals at equilibrium taken together with the unique K_f values calculated for animals at disequilibrium was 0.122±0.021 nl/(s·mmHg) in this group. In group 2, all rats except one were at filtration pressure disequilibrium. The unique values of K_f averaged 0.082±0.011 nl/(s·mmHg) among these rats.[1] As shown in Table II, average values for afferent (R_A), efferent (R_E), and total (R_T) arteriolar resistances were not statistically different in group 1 and 2 rats.

Rats studied 3–4 wk after uninephrectomy and administration of DOC and salt (group 4) demonstrated even greater hemodynamic alterations as compared with UNX controls. $\overline{AP}$ averaged 155±6 mmHg in group 4 rats, a value markedly greater ($P < 0.05$) than that observed in control group 3 animals (Table II). As was the case in group 2 rats, this increase in systemic blood pressure was transmitted to the glomerular circulation, as average values for $\bar{P}_{GC}$ and $\overline{\Delta P}$ were also increased in group 4 rats, to 59±2 and 44±1 mmHg, respectively, vs. a mean value for $\bar{P}_{GC}$ of 53±1 mmHg and $\overline{\Delta P}$ of 38±1 mmHg in group 3 rats. As shown in Table II, average values for GFR, SNGFR, Q_A, C_A, and P_T were similar in these two groups. Once again, SNGFR failed to rise with the increase in $\overline{\Delta P}$ seen in group 4 rats because K_f tended to be lower in these animals. All but two rats in group 3 were at filtration pressure disequilibrium. The average of the minimum and unique K_f values calculated for all rats in group 3 was ≥0.119±0.014 nl/(s·mmHg). Group 4 contained two rats at filtration pressure equilibrium for which only minimum values for K_f could be calculated. However, among the seven group 4 rats for which unique values for K_f were calculated, K_f averaged 0.071±0.014 nl/(s·mmHg), a value significantly less than that seen in group 3 rats.[2] Although numerically greater in group 4 rats, the average

1. With the inclusion of the minimum value of K_f calculated for the one rat in group 2 at filtration pressure equilibrium, K_f averages 0.087±0.008 nl/(s·mmHg) in this group.

2. When the minimum K_f values calculated for the two rats in group 4 in which filtration pressure equilibrium obtained are included in the determination of the mean, K_f averages 0.078±0.011 nl/(s·mmHg), still significantly less ($P < 0.05$) than the corresponding value observed in group 3.

values of the afferent, efferent, and total arteriolar resistances were not significantly different in group 3 and 4 rats.

Despite identical treatment with DOC and salt, group 5 rats maintained on a low protein diet exhibited strikingly different glomerular hemodynamic patterns from those fed standard chow (group 4). The mean values for GFR (1.04±0.08 ml/min) and SNGFR (39.8±3.8 nl/min) observed in group 5 rats were significantly lower ($P < 0.05$) than those found in both groups 3 and 4. This lower SNGFR resulted, in part, from lower average values of $\overline{AP}$ (124±6 mmHg), and therefore, $\bar{P}_{GC}$ (50±1 mmHg) and $\overline{\Delta P}$ (37±1 mmHg) in group 5 rats compared with those observed in group 4. In fact, these values for $\overline{AP}$, $\bar{P}_{GC}$, and $\overline{\Delta P}$ in group 5 were not significantly different from corresponding values measured in UNX rats not receiving DOC and salt (group 3). In addition, the average values of Q_A (142±26 nl/min) and C_A (5.2±0.1 g/100 ml) were significantly lower in group 5 rats than in groups 3 and 4. All animals in group 5 were at filtration pressure disequilibrium and, on average, K_f was reduced in these rats to 0.051±0.011 nl/(s·mmHg), a value significantly lower ($P < 0.05$) than in group 3. Thus, low protein feeding markedly altered the hemodynamic pattern in DOC-SALT rats, causing reductions in the mean values of $\overline{AP}$, $\bar{P}_{GC}$, $\overline{\Delta P}$, GFR, SNGFR, Q_A, and K_f.

As shown in Table III, rats drinking water (group 3) excreted an average of 0.95±0.19 meq of sodium/24 h. DOC-SALT rats drinking 1% saline (groups 4 and 5) excreted 7.35±1.78 and 8.28±0.75 meq of sodium/24 h, respectively; these values are significantly greater ($P < 0.05$) than that observed in group 3. Potassium excretion rates were similar in all three groups of rats measured 3 wk after uninephrectomy, averaging 2.34±1.36 in group 3, 1.76±0.78 in group 4, and 2.20±0.50 meq/24 h in group 5. Mean phosphate excretion rates were also similar in group 3 (0.46±0.12 mM/24 h) and group 4 (0.56±0.15); however, rats on the low protein diet (group 5) excreted nearly twice as much phosphate (0.93±0.13) as rats in groups 3 and 4. Phosphate content was similar in the standard (0.99%) and low protein diets (0.70%), and rats appeared to ingest approximately equal amounts of the two feeds. It is possible that increased phosphate excretion in group 5 resulted from increased gastrointestinal absorption of phosphate; this is a consequence of the fact that, in the low protein diet, most phosphate was supplied as inorganic sodium and potassium salts. Alternatively, urinary phosphate excretion may have been increased in group 5 in the absence of differences in phosphate absorption. If so, then serum phosphate levels might have been lower in group 5 rats. As serum phosphate levels were not determined in these studies, we are unable to distinguish between these two hypotheses. Nevertheless, it is apparent that the different hemodynamic and morphologic (see below) patterns observed in DOC-SALT rats fed a 6% (group 5) vs. a 24% (group 4) protein diet did not result from reductions in the amounts of sodium, potassium, or phosphate ingested by low protein rats.

Table III. Urinary Sodium, Potassium, and Phosphate Excretion

	$U_{Na}V$	U_KV	$U_{Phos}V$
	meq/24 h	*meq/24 h*	*mM/24 h*
Group 3			
$n = 10$	0.95±0.19	2.34±1.36	0.46±0.12
Group 4			
$n = 7$	7.35±1.78*	1.76±0.78	0.56±0.15
Group 5			
$n = 8$	8.28±0.75*	2.20±0.50	0.93±0.13*

Values are given as mean±SEM.
* $P < 0.05$ vs. group 3.

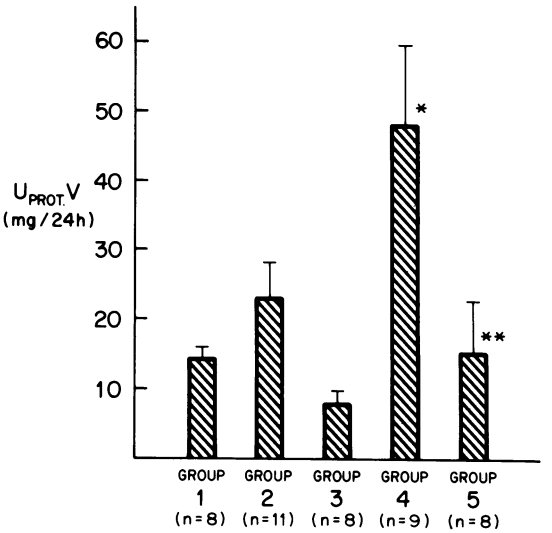


Figure 1. 24-h urinary protein excretion rates measured on the day prior to sacrifice in rats in groups 1–5. Bars represent means±SEM. Groups are defined in Table I. *, $P < 0.05$ vs. group 3; **, $P < 0.05$ vs. group 4.

10–14 d after uninephrectomy, DOC-SALT rats in group 2 excreted, on average, 23.2±5.2 mg of protein in 24 h, a value numerically but not significantly greater than that measured in group 1 rats (14.4±1.5). However, as shown in Fig. 1, by 3–4 wk after uninephrectomy, DOC-SALT rats (group 4) had substantial proteinuria, excreting an average of 48.1±12.7 vs. 8.0±1.8 mg of protein/24 h excreted by normo-tensive group 3 rats ($P < 0.05$). This increase in urinary protein in DOC-SALT rats was essentially reversed by low protein feeding, as rats in group 5 excreted on average only 15.2±7.5 mg/24 h; a value significantly less ($P < 0.05$) than that excreted by group 4 animals, but not different that that observed in rats in group 3. Interestingly, rats allowed to recover from DOC-SALT treatment for 6 wk (group 7) still had proteinuria, excreting 23.0±4.7 mg of protein/24 h vs. 11.9±2.0 in uninephrectomized controls.

Morphologic studies. All nephrectomized animals showed an increase in mean glomerular capillary tuft volume (Table IV) when compared with the two-kidney control group

Table IV. Quantitative Morphological Data

Group	n	V_G* $\times 10^{-6}$	Segmental lesions‡	Segmental lesions/10,000 glomeruli
		μm^3		
0	3	0.690±0.024	0/670	0
1	6	0.730±0.121	0/1,381	0
2	6	1.025±0.112	12/1,484	81
3	6	1.018±0.046	1/1,258	7.9
4	6	1.625±0.139	54/1,303	414
5	6	0.783±0.123	1/1,299	7.7
6	3	1.204±0.194	1/644	15.5
7	5	1.414±0.251	35/1,090	321.1

* Mean±SD.
‡ Numerator equals damaged glomeruli; denominator equals total no. of glomeruli examined.

(0.690×10^{-6} μm^3, group 0); the difference attained statistical significance (Table V) in all but groups 1 and 5. The rate of compensatory volume change in nephrectomized animals appears minimal during the first 10–14 d (from 0.690×10^{-6} to 0.730×10^{-6} μm^3), is maximal between days 10 and 21 (from 0.730×10^{-6} to 1.018×10^{-6} μm^3), and levels off by 9 wk (1.204×10^{-6} μm^3), which is the last observation made in this series of experiments. As a result of DOC-SALT administration, there was a significant increase in glomerular volume in nephrectomized animals both at 10–14 d (1.025×10^{-6} in group 2 vs. 0.730×10^{-6} μm^3 in group 1) as well as 3–4 wk (1.625×10^{-6} in group 4 vs. 1.018×10^{-6} μm^3 in group 3). Low protein diet in group 5 animals receiving DOC-SALT was effective in reducing the glomerular volume to 0.783×10^{-6} μm^3, which is a value significantly lower when compared with that observed in animals given DOC-SALT only for 3 wk (group 4, 1.625×10^{-6} μm^3) and intermediate between the figures calculated for nephrectomy animals at 3 wk (group 3, 1.018×10^{-6} μm^3) and the intact animals (group 0, 0.690×10^{-6} μm^3), but not significantly different from either. The effect of DOC-SALT treatment for 3 wk on mean glomerular volume is partially reversed when animals are allowed to recover for an additional 6 wk on standard diet (group 7) resulting in a mean glomerular volume of 1.414×10^{-6} μm^3, a value numerically higher but not statistically different from that calculated for animals at 9 wk postnephrectomy (group 6, 1.204×10^{-6} μm^3).

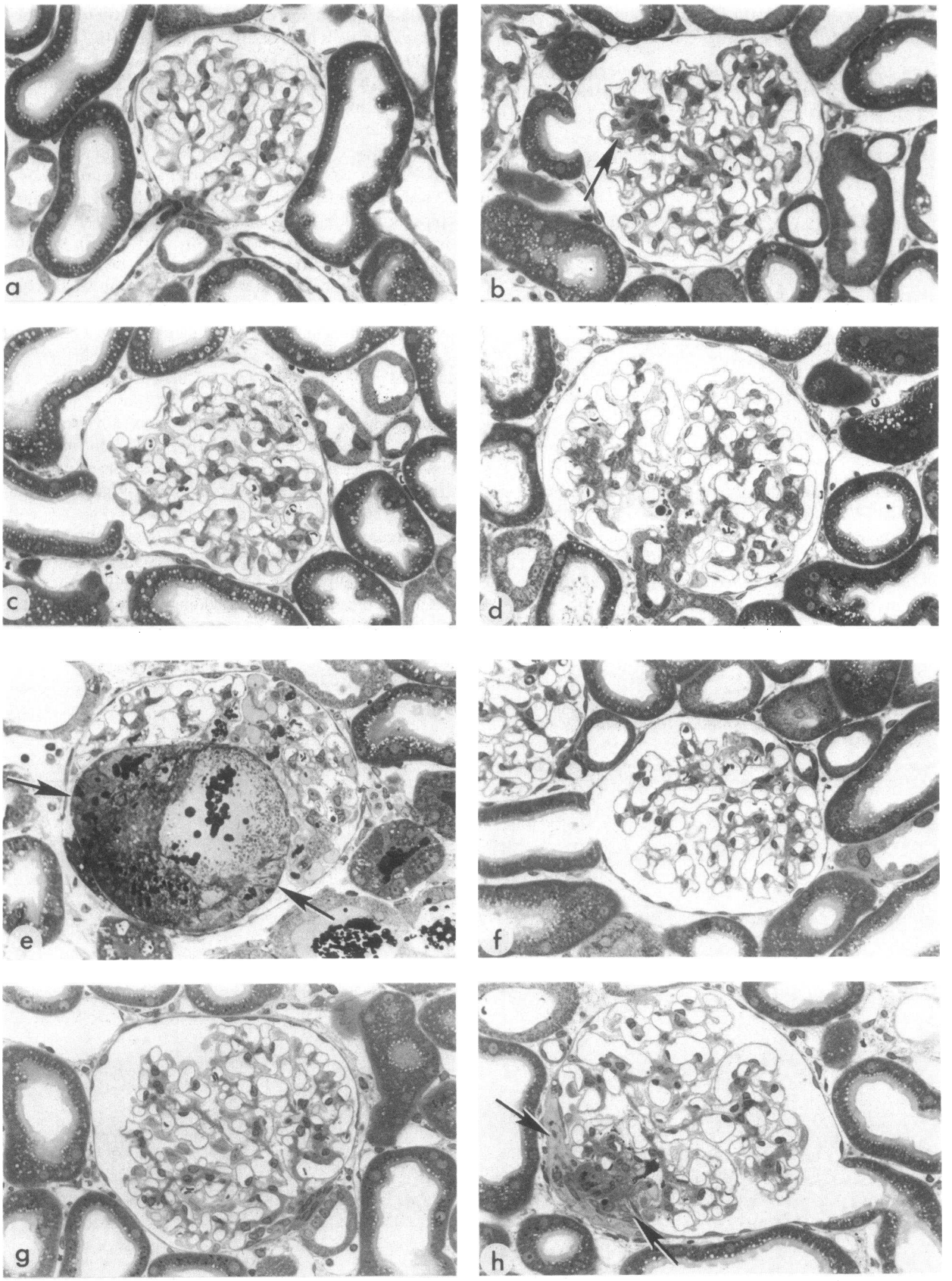
Nephrectomized animals (group 1) showed virtually no morphologic changes at days 10–14 (Fig. 2 *a*) with only very occasional areas of segmental glomerular sclerosis (1 of 1,258 glomeruli in group 3 and 1 of 644 glomeruli in group 6) and minimal increase in mesangial areas at 3–4 wk (Fig. 2 *c*) and 9 wk postsurgery (Fig. 2 *g*). The frequency of segmental lesions increased markedly when DOC-SALT was administered (Table IV). Animals in groups 2 and 4 developed partially thrombosed segmental microaneurysms of the glomerular tuft, segmental sclerosis, focal microthrombi in glomerular capillaries, and expansion of the mesangium (Figs. 2 *b, d,* and *e*). The frequency

Table V. Analysis of Variance Data for Mean Glomerular Volume

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F ratio
Between groups	7	4.14×10^{12}	5.92×10^{11} (TSS)	30.19
Within groups	33	6.47×10^{11}	1.96×10^{10} (ESS)	($F_{0.01}(7, 33) = 3.23$)

Results of Relevant Pairwise Comparisons

Compared groups	Modified t value	Bonferroni m = 12 critical t* = 3.12	Tukey critical value = 3.24	Scheffe critical value = 4.02
0–1	0.41	NS	NS	NS
0–2	3.38	*	*	NS
0–3	3.31	*	*	NS
0–4	9.45	*	*	*
0–5	0.94	NS	NS	NS
0–6	4.49	*	*	*
0–7	7.08	*	*	*
1–2	3.65	*	*	NS
3–4	7.51	*	*	*
4–5	−10.42	*	*	*
3–7	−2.91	NS	NS	NS
6–7	2.06	NS	NS	NS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of segmental lesions (microaneurysms and segmental sclerosis) increased in DOC-SALT animals from 0.8% of glomeruli at day 10 to 14 to >4% at the end of 3–4 wk of mineralocorticoid and salt treatment (Table IV). When animals were maintained on a 6% protein diet (group 5), the effects of DOC-SALT treatment on glomerular morphology were completely prevented (Fig. 2 *f*, Tables IV and V).

Animals allowed to recover on a standard chow for 6 wk following a 3-wk treatment with DOC-SALT (group 7) only showed areas of segmental glomerular sclerosis (Fig. 2 *h*) with a frequency (3.2% of glomeruli) similar to that observed for microaneurysms at the end of the active treatment phase in group 4 (4.1%). Nephrectomized animals not treated with DOC-SALT and followed for 9 wk (group 6) showed a 20-fold reduction in the rate of segmental glomerular lesions (Table IV).

Small arteries and arterioles in hypertensive animals receiving DOC-SALT for 3–4 wk revealed occasional segmental thickening of the vessel wall, perivascular edema, and infiltration of the vessel wall by amorphous, eosinophilic material ("fibrinoid necrosis"). The above described fibrinoid necrosis was only very rarely observed in arteries and arterioles in the vicinity of glomeruli with microaneurysmal lesions. Therefore, a direct association of both glomerular and vascular lesions was not evident from our observations.

At the ultrastructural level, animals in group 4 showed additional alterations of the glomerular capillary wall (Fig. 3). Epithelial cells showed focal obliteration of foot processes, attenuations of the cytoplasm with bleb formation, and increases in the number of cytoplasmic vacuoles and lysosomes (Fig. 3 *b*). The endothelial cell layer showed thickened expanses of cytoplasm devoid of the characteristic fenestrae and focal detachment from the underlying basement membrane (Fig. 3 *b*). Occasional capillaries contained platelets, fibrin, and inflammatory cells (Fig. 3 *b*). Glomeruli from animals in group 2 showed similar changes, although lower in frequency. In contrast, ultrastructural analysis of kidneys from groups 1, 3, and 4 failed to disclose changes as described above.

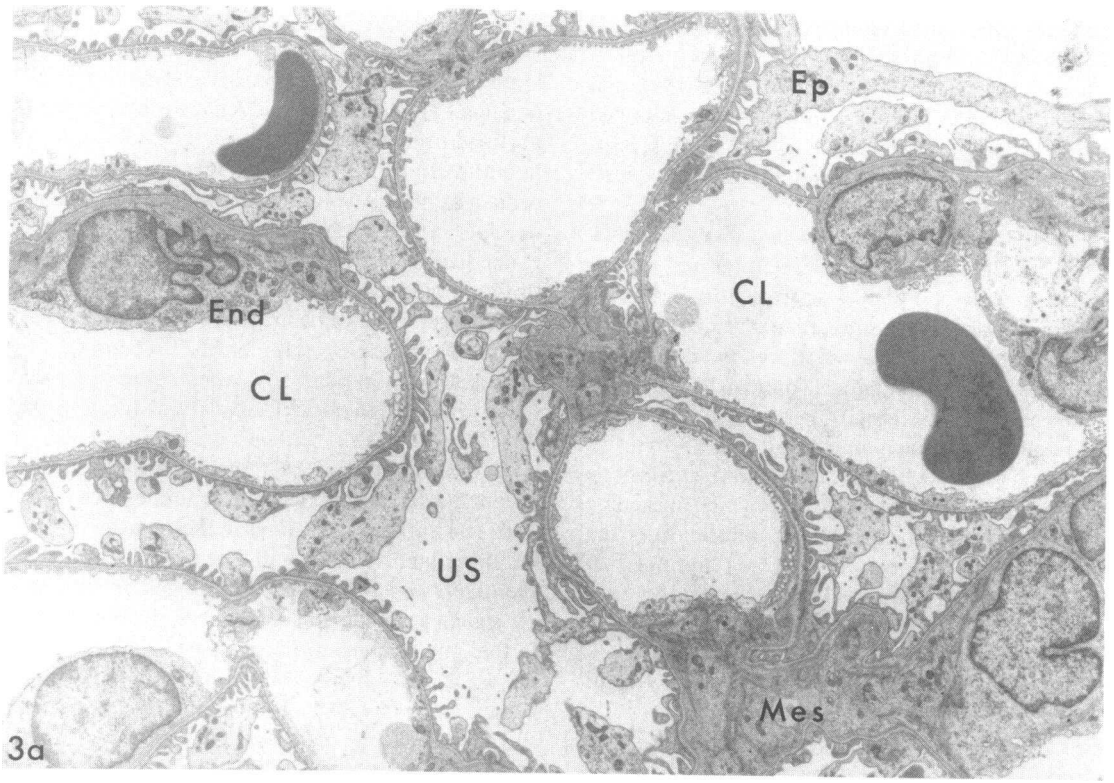
Discussion

Deen et al. (10) assessed glomerular dynamics in rats 3 wk after unilateral nephrectomy. The renal cortical micropuncture data obtained in that study is not exactly comparable with our data in that Deen et al. (10) studied rats under conditions of hydropenia and plasma volume expansion whereas our experiments were performed under euvolemic conditions. Nevertheless, the glomerular hemodynamic patterns observed in UNX rats were similarly altered in both studies. Accordingly, although the uninephrectomized rats in groups 1 and 3 served as controls in our study, the mean values for SNGFR and Q_A observed were increased by more than 50% when compared with values previously reported in normal, euvolemic Munich-Wistar rats from this laboratory (18).

Administration of DOC and a high salt intake produces systemic arterial hypertension in uninephrectomized rats and leads to additional alterations in the determinants of glomerular ultrafiltration. After only 10 d of DOC-SALT treatment, mean systemic arterial and glomerular capillary hydraulic pressure are significantly increased. By 3 wk, these pressures have risen further and significant morphologic evidence of glomerular injury is already apparent. Muller-Süur et al. (19) measured whole kidney GFR in DOC-SALT rats 4 wk after uninephrectomy and found it to be increased to a value comparable with that reported here. The same authors also estimated mean glomerular capillary hydraulic pressure using stop-flow techniques and, in contrast to the present study, found it to be normal. However, the validity of the stop-flow method of estimating $\bar{P}_{GC}$ has recently been questioned (20, 21). Therefore, technical artifacts may account for the differences between that study and ours, where $\bar{P}_{GC}$ was found to be clearly elevated when measured directly.

In the present study, SNGFR and GFR were comparable in uninephrectomized control and DOC-SALT hypertensive rats despite the fact that $\overline{\Delta P}$ was significantly greater in the hypertensive animals; hence, K_f tended to be lower in the DOC-

Figure 2. Light micrographs of renal cortex. Representative light micrographs of renal cortex from different experimental animals after perfusion fixation. 1 μm thick epoxy-resin sections were stained with 1% toluidine blue (260 X). (*a*) Group 1: 10 d postnephrectomy on regular diet. The glomerulus shows a delicate mesangial matrix with few cellular elements. (*b*) Group 2: 10 d postnephrectomy on DOC-SALT regimen. The glomerulus is noticeably enlarged showing segmental areas of mesangial expansion and hypercellularity (arrow). (*c*) Group 3: 3–4 wk postnephrectomy. The glomerulus is moderately enlarged, segmental lesions are not evident. (*d*) Group 4: 3–4 wk postnephrectomy on DOC-SALT regimen. The glomerular tuft is markedly enlarged. Notice prominent mesangial elements and occasional dense reabsorption droplets in visceral epithelial cells (center, lower half of the capillary tuft). (*e*) This micrograph illustrates a typical segmental lesion observed in animals from group 4, 3–4 wk postnephrectomy on DOC-SALT treatment. The lower part of the capillary tuft is occupied by a massively dilated capillary loop (microaneurysm) showing partial occlusion by fibrin, platelets, and other cellular elements (arrows). Notice also an increase in reabsorption droplets in glomerular epithelial cells and erythrocyte fragments in the lumen of a distal convoluted tubule (lower right corner). (*f*) Group 5: 3–4 wk postnephrectomy on DOC-SALT and low protein diet. The volume of glomeruli from animals in this group is statistically less than that measured in group 3 (Fig. *c*), but not different from that obtained in the 2-kidney controls (group 0, not illustrated). Notice also the lack of segmental lesions and narrow mesangial areas. (*g*) Group 6: 9 wk postnephrectomy. The glomerulus illustrated shows an increased diameter and prominent mesangium. (*h*) Group 7: 9 wk postnephrectomy and after an initial 3 wk on DOC-SALT regimen. The enlarged glomerular tuft shows an area of segmental sclerosis with adhesion of the tuft to Bowman's capsule (arrows).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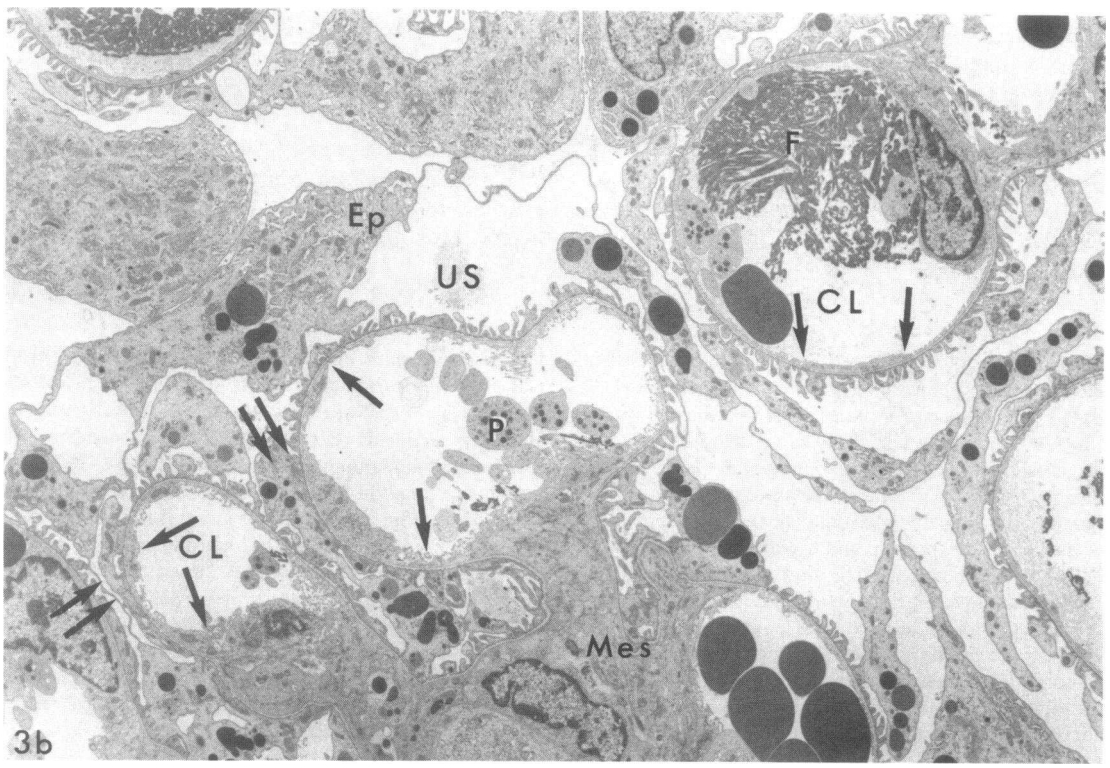
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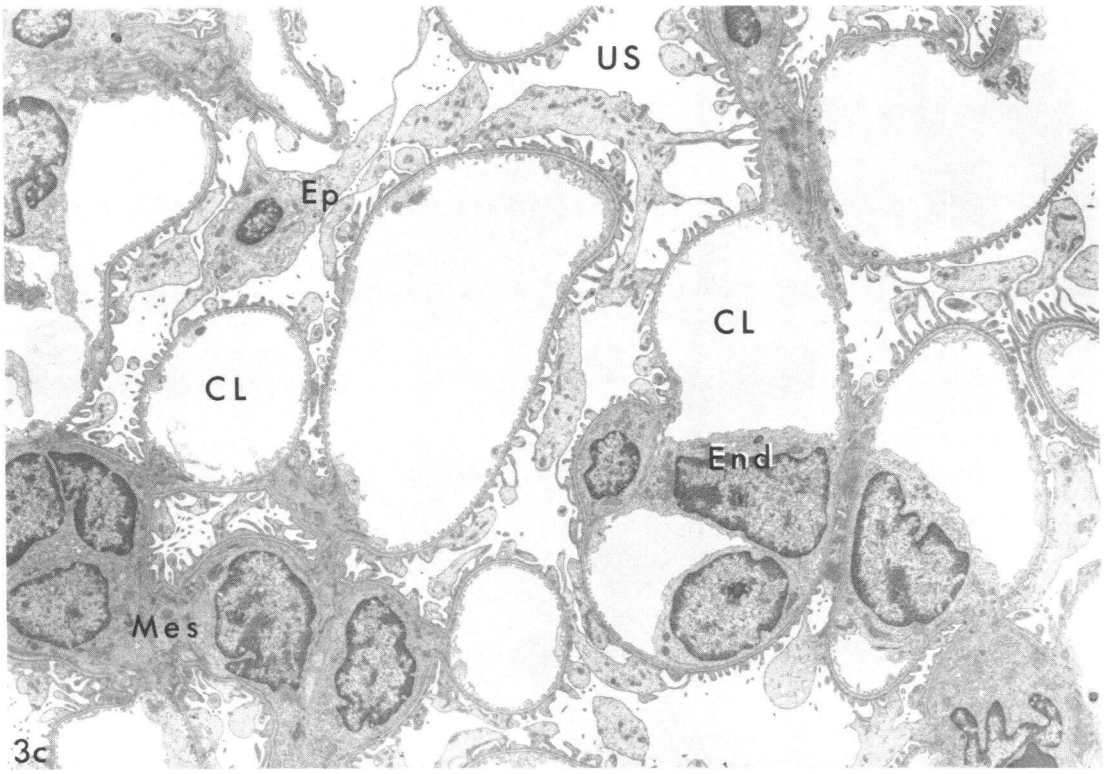

Figure 3. Electron micrographs of glomerular capillaries following perfusion fixation (*EP,* visceral epithelial cells; *End,* endothelium; *Mes,* mesangium; *US,* urinary space; *CL,* capillary lumen; *F,* fibrin; *P,* platelet). (*a*) Group 3. The capillary lumina are patent; the endothelial cells show regularly spaced fenestrae; and the epithelial cell reveals normal foot process architecture (4,000 X). (*b*) Group 4. The capillaries are partly occluded by fibrin (*F*) and platelets (*P*). The endothelium shows extensive areas devoid of normal fenestrae (single arrows) and the epithelium shows focal obliteration of foot processes (double arrows), attenuation of cytoplasm and increase in lyzosomes (reabsorption droplets) (3,600 X). (*c*) Group 5. Most of the changes observed in group 4 animals could be prevented by low protein feeding, as illustrated by this segment of the glomerular tuft taken from a nephrectomized animal on low protein diet treated for 3 wk with DOC-SALT (3,450 X).

SALT rats. The mechanism of this reduction in K_f is uncertain, but several possibilities should be considered. The ultrafiltration coefficient, K_f, is equal to the product of the hydraulic conductivity of the glomerular capillary wall (k), and the total capillary surface area available for filtration (S). Therefore, reductions in the value of K_f can be brought about by declines in k or S. In the present study, alterations in k might have resulted from the glomerular capillary wall damage which was evident in groups 2 and 4. However, although focal abnormalities of the glomerular capillary wall were noted in hypertensive rats by electron microscopy, most of the available filtration surface appeared normal. Furthermore, neither permselective nor ultrastructural alterations of the glomerular capillary wall have been detected by us in several other experimental settings associated with reductions in K_f (22, 23). Therefore, selective alterations in k may not fully account for the low K_f seen in DOC-SALT rats.

Alternatively, K_f might have been reduced because declines occurred in the value of S. Segmental glomerular lesions and focal microthrombosis as noted above may have contributed to a reduction of the value of S in group 4 animals. Recently, considerable evidence has been amassed to suggest that a variety of vasoactive agents may induce reductions in S by virtue of their actions to promote mesangial cell contraction (24). Mesangial cells in culture exhibit contractile responses to angiotensin II, antidiuretic hormone, and norepinephrine at concentrations well within the physiologic range (25). Furthermore, both circulating antidiuretic hormone (26) and catecholamine (27) levels are elevated in rats with DOC-SALT hypertension. Therefore, it is attractive to speculate that the low K_f observed in DOC-SALT rats might have resulted from hormonally induced, mesangial cell contraction tending to reduce glomerular capillary surface area in vivo.

Associated with increased glomerular capillary pressures and flows, glomeruli in DOC-SALT hypertensive rats revealed morphologic evidence of injury by 10 d after uninephrectomy. By 3 wk, a diffuse increase in mesangial matrix and cells as well as focal areas of intraglomerular thrombosis and hemorrhage

were noted. Electron microscopic examination revealed that in individual glomerular capillary loops, endothelial cells had become focally detached from the basement membrane. These morphologic alterations are similar to those described previously (28, 29, 30) in kidneys of rats with this form of steroid-induced hypertension.

To test the possibility that focal intraglomerular hemorrhage might result in permanent structural damage, we performed morphologic studies on another group of uninephrectomized rats in which DOC and salt administration were discontinued after 3 wk (group 7). Interestingly, proteinuria persisted in these animals despite cessation of DOC-SALT treatment. At the time of sacrifice, light microscopic examination of the kidneys of these rats demonstrated a similar frequency of segmental lesions as was seen at the end of the 3–4 wk DOC-SALT treatment. However, the lesions had evolved from thrombosed microaneurysms, as seen in group 4 animals, to "healed" segmental sclerosis with hyalinosis.

Hostetter and co-workers (3) have recently suggested that, after reduction in renal mass, alterations in glomerular hemodynamics lead to eventual glomerular injury. The possibility that a similar mechanism might be responsible for renal injury which results from sustained elevations in systemic arterial pressure has also been proposed (4, 29). In the present study, structural alterations and substantial proteinuria, both indicative of widespread glomerular injury, were associated with increased glomerular capillary pressures and flows in DOC-SALT hypertensive rats. To test whether these changes in glomerular dynamics per se might have been responsible for the observed glomerular injury, a group of uninephrectomized DOC-SALT rats were fed a low protein diet. This maneuver has been shown to markedly influence intrarenal hemodynamics, and to blunt the increase in SNGFR which develops after surgical reduction in renal mass (3) or as a consequence of normal maturation (31).

As predicted, protein restriction markedly affected both systemic and intrarenal hemodynamics. Interestingly, protein restricted DOC-SALT rats exhibited significantly lower values for $\overline{\Delta P}$ than rats fed standard chow. Moreland et al. (32) have also found blood pressure to be lowered in DOC-SALT rats fed a 5% protein diet for 4 wk. The mechanism of the reduction in systemic arterial pressure induced by protein restriction is uncertain. However, Moreland et al. (32) observed that vascular strips taken from protein restricted DOC-SALT animals were less sensitive to norepinephrine than those taken from DOC-SALT rats fed standard chow (22.5% protein). This decreased reactivity was associated with decreased membrane stores of calcium so that less calcium was available for activation by norepinephrine in these rats.

At the level of the renal circulation, whole kidney and single nephron GFR were lower in group 5 rats as compared with groups 3 and 4. These lower filtration rates were, in turn, the result of reductions in both Q_A and $\overline{\Delta P}$. Furthermore, the increase in $\bar{V}_G$, segmental lesions, and proteinuria seen in DOC-SALT rats fed a standard diet was essentially abolished in rats ingesting the low protein diet. Therefore, in our study, a maneuver which blunted the hemodynamic consequences of DOC-SALT hypertension also prevented structural glomerular injury. In addition, glomerular hemodynamic alterations were present in DOC-SALT rats 10 d after uninephrectomy, prior to the appearance of marked proteinuria and widespread structural abnormalities. These findings are consistent with the hypothesis that sustained hemodynamic changes observed in hypertensive rats were themselves responsible for the glomerular injury.

It should be noted that, during the course of micropuncture study, blood pressure tended to decline in hypertensive rats. This fall occurred despite constant plasma infusion designed to maintain a stable intravascular volume. Therefore, it is possible that the glomerular capillary pressures and flows we observed in anesthetized, hypertensive rats were not truly representative of those which prevailed in the awake state. It seems likely, however, that, as blood pressure fell only in the hypertensive groups, the values of $\overline{\Delta P}$ and Q_A we observed in DOC-SALT animals probably represent lower limits for the true values existing in these rats before micropuncture. Accordingly, it seems valid to make correlations between the patterns of glomerular perfusion we observed in anesthetized rats and the histologic alterations that developed in these same animals over a 2–3-wk period.

Other studies have also demonstrated an effect of varying dietary protein content on the progression of renal disease. For example, Blatherwick et al. (33) and later Lalich et al. (34) observed accelerated glomerular sclerosis when uninephrectomized rats were fed a high protein diet. In other studies (35, 36), graded reductions in dietary protein content led to stepwise increases in the lifespan of rats undergoing extensive renal ablation. More recently, in studies of rats after 90% ablation of renal mass, maintenance of glomerular hemodynamics at nearly normal levels by protein restriction was associated with preservation of glomerular architecture and absence of proteinuria (3). In subsequent experiments, Meyer et al. (37) compared the effects of a 6% vs. a 40% protein diet on the incidence of glomerular sclerosis and proteinuria in rats after a 50% or 67% reduction in renal mass. 8 mo after surgical ablation, graded loss of renal mass led to increases in proteinuria and glomerular sclerosis. However, low protein feeding was associated with lower inulin clearance, reduced protein excretion, and less sclerosis after either 50 or 67% ablation. These findings are also consistent with the hypothesis that the reduced GFR which accompanies protein restriction may limit progressive glomerular damage following loss of renal mass.

Variation in dietary protein content has also been shown to retard the progression of immune-mediated glomerular injury, including nephrotoxic serum nephritis in rats (38, 39) and the lupus-like nephropathy of the New Zealand Black/New Zealand White mouse (40). In man, a recent study has suggested that moderate protein restriction may also delay the progression of renal functional deterioration in patients with early renal failure

(41). While careful hemodynamic measurements were not performed in that study, it is attractive to speculate that the protective effect of low protein feeding resulted from the prevention of hyperperfusion in surviving nephrons in these patients.

Nevertheless, it is possible that the low protein diet protected DOC-SALT rats from glomerular damage by some effect other than one relating to glomerular hemodynamics. It is clear, however, that this effect was not the result of differences in sodium or potassium excretion or balance, as rats in groups 4 and 5 excreted similar amounts of these electrolytes in 24 h. Alternatively, it might be suggested that changes in dietary phosphate accounted for the different morphology of groups 4 and 5. Alfrey and co-workers (42, 43) have reported that phosphorus restriction protects rats with renal injury from developing progressive renal insufficiency. However, dietary phosphorus content was similar in the standard and low protein diets employed in this study. Furthermore, as can be seen from Table III, rats on low protein diet (group 5) actually excreted almost twice as much phosphate in 24 h as did rats on standard chow. Therefore, it seems unlikely that changes in dietary phosphate account for the difference between groups 4 and 5.

Studies in several other experimental models of hypertension are consistent with the notion that hemodynamic alterations predispose to glomerular injury in this setting. Azar et al. (4) performed micropuncture and morphologic studies in one-kidney "postsalt" hypertensive rats of the Holtzman strain. At 5 mo, obvious nephrosclerosis was present in the kidneys of all hypertensive rats. Furthermore, these morphologic abnormalities were associated with marked increases in the mean values of SNGFR, Q_A, and $\overline{\Delta P}$ in these animals. Similarly, Feld et al. (44, 45) suggested that the relative failure of juxtamedullary nephrons to autoregulate is responsible for the glomerular injury that selectively affects this population of nephrons in the spontaneously hypertensive rat. Finally, it has long been known that when hypertension is produced by constricting one renal artery in the rat, glomerular lesions are produced in the opposite kidney but not in the ipsilateral clipped kidney (6, 29). Recent micropuncture studies (5) indicate that, in rats with this form of experimental hypertension, the unclipped kidney has augmented glomerular capillary pressures and flows and single nephron filtration rates, while, in contrast, these same parameters are reduced in the clipped "protected" kidney. Thus, the glomerular hemodynamic pattern observed in the unclipped kidneys of rats with 2-kidney Goldblatt hypertension closely resembles that observed in DOC-SALT hypertensive rats. Taken together, these studies provide further evidence that altered dynamics per se contribute to glomerular injury in experimental hypertension.

It might be argued that the increase in $\overline{\Delta P}$ of 6 mmHg in group 4 vs. group 3 rats is of small magnitude and, therefore, unlikely to account for a dramatic difference in glomerular morphology. However, it should be noted that this increase in mean glomerular transcapillary hydraulic pressure difference is superimposed on glomerular capillary plasma flows which are already substantially elevated as a result of the uninephrectomy. Q_A averaged 236 nl/min in euvolemic uninephrectomized rats in group 3, a value ~50% greater than that which has been observed in normal 2-kidney Munich-Wistar rats in this laboratory (18). Of note, the glomeruli of rats in this group demonstrated a diffuse increase in glomerular mesangium that was easily distinguishable from normal. Eventually, these uninephrectomized rats go on to develop obvious glomerular sclerosis in the remaining kidneys (37). The findings in the present study suggest that when glomerular capillary hypertension is superimposed on a vasodilated kidney, the progression to glomerular sclerosis in greatly accelerated.

Consistent with this hypothesis, Raij et al. (46) have recently suggested the combination of systemic hypertension and preglomerular afferent arteriolar vasodilation may exacerbate ferritin-antiferritin immune complex glomerulonephritis. They studied hypertensive rats of the spontaneously hypertensive rat and Dahl strains because it has been demonstrated that relative afferent arteriolar vasoconstriction protects the superficial glomeruli of spontaneously hypertensive rats but not Dahl rats from increases in perfusion pressure (47). As predicted, Dahl rats developed more severe mesangial sclerosis than spontaneously hypertensive rats in response to intraperitoneal injections of ferritin. Thus, the presence of arterial hypertension amplified immune-mediated glomerular injury only in the presence of relative renal vasodilation. Similarly, Tikkanen et al. (48) examined the effects of DOC-SALT treatment on the course of autologous immune complex nephritis (Heymann nephritis) in rats. DOC-SALT nephritic rats developed hypertension and a significant increase in GFR although these parameters were unaffected by the induction of nephritis alone. Associated with these hemodynamic alterations, DOC-SALT nephritic rats demonstrated increased proteinuria and more severe renal histologic lesions than nephritic rats not receiving DOC and salt. It is attractive to speculate that, as in the study of Raij et al. (46), augmented glomerular capillary hydraulic pressures and plasma flow rates enhanced the immune-mediated glomerular injury.

The mechanism whereby augmented glomerular capillary pressure and flow alter glomerular structure is not known. One possibility is that the increased glomerular transcapillary hydraulic pressure difference, $\overline{\Delta P}$, may injure the capillary network by some mechanism analogous to the effects of hypertension on the systemic arterial vessels, possibly involving mechanical disruption of normal vascular integrity (49). This hypothesis is consistent with the observation that increased $\overline{\Delta P}$ and glomerular lesions coexisted in group 4 rats. Alternatively, increased transglomerular traffic of plasma proteins may have had an injurious effect on glomerular structure (50–52). A rise in the filtration rate of macromolecules would be expected as a result of the increase in glomerular capillary plasma flow rate and transcapillary hydraulic pressure observed in these animals (53). In addition, changes in the intrinsic permeability of the glomerular wall might have occurred and resulted in augmented movement

of macromolecules through the wall, thereby contributing to the ultimate injury of hypertensive glomeruli (51, 52).

Clinically, chronic renal insufficiency often leads to end-stage renal failure at a predictable rate (54, 55). It has also been observed that uncontrolled systemic arterial hypertension complicating chronic renal insufficiency may hasten this progression to uremia (56). Furthermore, therapy with antihypertensive agents has been reported to stabilize renal function at existing levels in some hypertensive patients with mild to moderate renal dysfunction (57). The finding in the present study that glomerular capillary hypertension accelerates glomerular injury after reduction in renal mass provides an attractive explanation for these clinical observations, and provides a rationale for the aggressive control of blood pressure in the hypertensive patient with renal disease.

In summary, rats made hypertensive by uninephrectomy and 3 wk of treatment with DOC and high salt intake exhibit striking structural abnormalities of their glomeruli and increased urinary protein excretion, both in association with augmented glomerular capillary plasma flow rates and transcapillary hydraulic pressure gradients. A low protein diet prevents these increments in glomerular capillary pressures and flows, and protects DOC-SALT rats from developing proteinuria and morphologic evidence of glomerular injury. This protective effect of low protein feeding is not explained by differences in sodium, potassium, or phosphate excretion or balance. These findings, therefore, suggest that glomerular capillary hypertension in the presence of augmented capillary perfusion predisposes to glomerular injury in this model of systemic arterial hypertension. Furthermore, they lend additional support to the view that elevations in glomerular pressures and flows constitute a general mechanism for eventual glomerular destruction in a wide variety of renal diseases.

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