Role for Intrarenal Mechanisms in the Impaired Salt Excretion of Experimental Nephrotic Syndrome

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ABSTRACT A unilateral model of puromycin aminonucleoside (PAN)-induced albuminuria was produced in Munich-Wistar rats to examine the mechanisms responsible for renal salt retention. 2 wk after selective perfusion of left kidneys with PAN ($n = 8$ rats) or isotonic saline (control, $n = 7$ rats), increases in albumin excretion and decreases in sodium excretion were demonstrated in PAN-perfused but not in nonperfused kidneys of PAN-treated rats although systemic plasma protein concentration remained at control level. Total kidney glomerular filtration rate (GFR) and superficial single nephron (SN) GFR were also reduced selectively in PAN-perfused kidneys, on average by ~30%, due primarily to a marked decline in the glomerular capillary ultrafiltration coefficient (K_f), which was also confined to PAN-perfused kidneys. Values for absolute proximal reabsorption (APR) were also selectively depressed in PAN-perfused kidneys, in keeping with a similarly selective decline in peritubular capillary oncotic pressure measured in these kidneys, the latter also a consequence of the fall in K_f. In a separate group of seven PAN-treated rats, however, no differences were detected between PAN-perfused and nonperfused kidneys in the absolute amount of sodium reaching the early (0.77 ± 0.09 neq/min vs. 0.74 ± 0.08, $P > 0.40$) and late portions of superficial distal tubules (0.31 ± 0.02 neq/min vs. 0.32 ± 0.05, $P > 0.50$), despite the lesser filtered load of sodium in PAN-perfused kidneys. Suppressed sodium reabsorption in both proximal convoluted tubules and short loops of Henle of PAN-perfused kidneys contributed to this equilization of sodium delivery rates to the late distal tubule, as did comparable reabsorption along distal convolutions.

In two additional groups of PAN-treated rats, infusion of saralasin (0.3 mg/kg per h, i.v.) led to substantial increases in total kidney GFR and SNGFR in PAN-perfused but not in nonperfused kidneys. Despite these increases in total and SNGFR, urinary sodium excretion by PAN-perfused kidneys remained at a level far below that for nonperfused kidneys, again indicating that the antinatriuresis characterizing the PAN-perfused kidney is due to alterations in sodium handling by the tubules rather than changes in GFR. These results therefore indicate (*a*) that reductions in K_f and depressed sodium reabsorption by proximal tubules and Henle's loop segments in this model are brought about by intrarenal rather than circulating or systemic factors, and (*b*) assuming that superficial nephrons are representative of the entire nephron population, renal salt retention in this model is due primarily to intrarenal factor(s) acting beyond the distal convolution.

INTRODUCTION

Nephrotic syndrome is a disorder of volume regulation associated with avid salt retention by the kidney. Despite the efforts of investigators for many years, it is still uncertain whether the salt retention in this syndrome is due primarily to an intrinsic inability of the nephrotic kidney to excrete salt and water normally, or whether salt retention represents a normal renal

response to a stimulus extrinsic to the kidney, presumably geared to volume regulation (1–3). In view of the wide spectrum of renal diseases leading to the nephrotic syndrome, more than a single mechanism may be responsible for the renal salt retention observed in these diverse conditions.

Puromycin aminonucleoside (PAN) induces heavy proteinuria, avid renal salt retention, and structural changes in the glomerulus and renal tubules characteristic of minimal change glomerulopathy (4–15), namely, effacement of glomerular epithelial cell foot processes and protein reabsorption droplets in proximal tubule epithelia. To distinguish between the intrarenal vs. extrarenal mechanisms in mediating the observed salt retention, we studied a unilateral form of PAN glomerulopathy induced in the Munich-Wistar rat by selective in vivo perfusion of a single kidney with PAN. This enabled us to make micropuncture measurements of salt and water reabsorption at various sites along accessible superficial nephrons in perfused and nonperfused kidneys of the same rat.

GLOSSARY

$\overline{AP}$	mean arterial pressure
APR	absolute proximal fluid reabsorption rate, nl/min
C_A	protein concentration of femoral arterial plasma, g/dl
C_E	protein concentration of surface efferent arteriolar plasma, g/dl
GFR	total kidney glomerular filtration rate, ml/min
Hct	hematocrit, vol $\%$
K_f	glomerular capillary ultrafiltration coefficient, $nl/(s \cdot mm\ Hg)$
Π_A	afferent arteriolar oncotic pressure, $mm\ Hg$
PAN	puromycin aminonucleoside
P_{C^1}, Π_{C^1}	time-averaged hydraulic and oncotic pressures in distal-most surface branches of peritubular capillaries, $mmHg$
P_E, Π_E	time-averaged hydraulic and oncotic pressures in efferent arterioles, $mmHg$
$\bar{P}_{GC}$	mean glomerular capillary hydraulic pressure, $mmHg$
P_T	proximal tubule hydraulic pressure, $mmHg$
Q_A	glomerular capillary plasma flow rate, nl/min
R_A, R_E	resistance to blood flow along afferent and efferent arterioles, $dyn \cdot s \cdot cm^{-5}$
SNFF	single nephron filtration fraction
SNGFR	single nephron GFR, nl/min
$(TF/P)_{In}$	tubule fluid-to-plasma inulin concentration ratio
$(TF/P)_{Na}$	tubule fluid-to-plasma sodium concentration ratio
$U_{Alb}V$	total kidney albumin excretion rate, $mg/24\ h$ per one kidney
$U_{Na}V$	total kidney sodium excretion rate, $\mu eq/3\ h$ per one kidney

METHODS

General procedures

Studies were performed in adult male Munich-Wistar rats weighing 247–324 g. Unilateral proteinuria was induced in

29 rats by unilateral renal arterial infusion of PAN (6-dimethylamino-9[3'-amino-3'-deoxyribosyl] purine) (10 mg in 2 ml of 0.9% NaCl solution, Sigma Chemical Co., St. Louis, MO) 2 wk before study under pentobarbital anesthesia (Nembutal, 50 mg/kg body wt, i.p; Abbott Diagnostics, Diagnostic Products, North Chicago, IL). The technique used essentially duplicated that originally described by Bricker et al. (13) for dogs and subsequently modified for rats by Hoyer et al. (14). This approach involves selective intrarenal arterial perfusion of the left kidney with PAN while simultaneously venting the renal venous effluent from the perfused kidney. Puromycin was thus prevented from entering the systemic circulation or reaching the contralateral right kidney. A separate group of seven rats (saline group) was prepared in an identical fashion, also 2 wk before study, except that isotonic saline was substituted for PAN in the perfusion solution. All animals were allowed free access to a regular pellet diet and water both before and after these preparatory procedures.

2 wk later, rats were anesthetized with Inactin (100 mg/ kg, i.p.) and placed on a temperature-regulated micropuncture table. Immediately after the induction of anesthesia, the left femoral artery was catheterized with a PE-50 polyethylene tube, and a base-line collection of 70 μl of arterial blood was made. This arterial catheter was used for subsequent periodic blood sampling and estimation of mean arterial pressure (AP). AP was monitored with an electronic transducer (model P23Db, Gould Inc., Medical Products Div., Oxnard, CA) connected to a direct-writing recorder (model 775A, Hewlett-Packard Co., Palo Alto, CA). Polyethylene catheters were also inserted into the left and right jugular veins for infusion of inulin and saralasin and into the left femoral vein for infusion of isooncotic rat plasma. Intravenous infusion of 7% inulin solution in 0.9% NaCl (1.2 ml/h) were then started. To achieve eurolemic condition homologous rat plasma was also infused, using the protocol described in detail previously (16). Following tracheostomy, the left (or right) kidney was exposed by a subcostal incision and separated from the adrenal gland and the surrounding perirenal fat. The kidney was suspended on a Lucite holder, and its surface illuminated with a fiberoptic light source and bathed with 0.9% NaCl heated to 35°–37°C.

Micropuncture assessment of base-line single nephron function

In 15 PAN-treated and 7 saline-treated animals, micropuncture measurements were started 60–80 min after onset of plasma infusion and carried out as described below. In addition to performing a series of micropuncture measurements on the kidneys (left) previously perfused either with PAN or saline, micropuncture measurements were also carried out on contralateral (right) kidneys. In these PAN- and saline-treated rats, the micropuncture of ipsilateral and contralateral kidneys was carried out in random manner. Timed (1–2 min) samples of fluid were collected from late surface proximal convolutions from each of two or three nephrons for determination of flow rate and inulin concentration and calculation of single nephron glomerular filtration (SNGFR) and absolute proximal fluid reabsorption rates (APR). End-proximal convolutions were identified by observing the passage of the Lissamine green injected into surface proximal convolutions with small tip perfusion pipettes. Coincident with these tubule fluid collections, two or three samples of femoral arterial blood were obtained periodically for determination of hematocrit (Hct) and plasma concentrations of

protein, inulin, and sodium. In addition, two or three samples of urine from each kidney were collected for determination of flow rate, inulin, sodium and albumin concentrations, and calculations of total kidney glomerular filtration rate (GFR) and total kidney sodium ($U_{Na}V$) and albumin ($U_{Alb}V$) excretion rates. For these urine collections, indwelling ureteral polyethylene catheters (PE-10) were used.

Measurements of glomerular and peritubular capillary hemodynamics. In eight PAN- and seven saline-treated rats, time-averaged pressures were also measured in surface glomerular capillaries ($\bar{P}_{GC}$), proximal tubules (P_T), efferent arterioles (P_E) and distal-most surface branches of peritubular capillaries (P_{Cl}) with a continuous-recording, servo-null micropipette transducer system (model 3, Instrumentation for Physiology and Medicine, San Diego, CA). Micropipettes with outer tip diameters of ~2 μm and containing 2.0 M NaCl were used. Hydraulic output from the servo system was coupled electronically to a second channel of the Hewlett-Packard recorder by means of a pressure transducer.

To estimate the colloid osmotic pressure of plasma entering and leaving glomerular capillaries, protein concentrations (C) of femoral arterial (C_A) and surface efferent arteriolar (C_E) plasma were measured as described previously (16). Colloid osmotic pressure (Π) was calculated according to the equation derived by Deen et al. (17). Values for C_A, and thus Π_A, for femoral arterial plasma are taken as representative of values of C and Π for the afferent end of the glomerular capillary network. These estimates of pre- and postglomerular plasma protein concentration permit calculation of single nephron filtration fraction (SNFF), initial glomerular capillary plasma flow rate (Q_A) and glomerular capillary ultrafiltration coefficient (K_f) using the equations described in detail elsewhere (17).

Segmental analysis of tubule sodium reabsorption. In a separate group of seven PAN-treated rats, rates of sodium delivery into and reabsorption from sequential segments of superficial nephrons were determined in PAN-perfused and nonperfused kidneys in each rat. In these rats, therefore, not only end-proximal convolutions but also early and late distal tubules were identified by observing the appearance and disappearance of Lissamine green, as described by Wright (18). Thus, in addition to proximal tubule fluid collections, samples of tubule fluid were also collected from both early and late distal convolutions for the determination of flow rate and sodium concentration. For late distal tubule fluid collections, longer collection times (3-5 min) were required.

Effects of saralasin

Measurements of whole kidney function. A third group of PAN-treated rats ($n = 6$) were used only for whole kidney clearance studies. In these rats, saralasin (Sarenin, Eaton Laboratories, Norwich, NY) was infused intravenously at a rate of 0.3 mg/kg per h beginning immediately after completion of the pertinent base-line measurements and collections in both kidneys. At 30-60 min after the onset of saralasin, measurements were repeated for both kidneys.

Measurements of glomerular hemodynamics. Since saralasin infusion was found to raise GFR in the third group of PAN-treated rats, the mechanism responsible for this rise in GFR was explored in a fourth group of PAN-treated rats ($n = 8$). In this latter group, left (PAN-perfused) kidneys were subjected to micropuncture study for the determination of glomerular hemodynamics while whole kidney inulin clearance was simultaneously measured in contralateral kid-

neys. After initial measurements and collections, saralasin was infused at a rate of 0.3 mg/kg per h, and measurements were repeated at 30-60 min after the onset of this infusion.

Analytical methods

The volume of fluid collected from individual proximal tubules 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 (19). Inulin concentrations in plasma and urine were determined by the macroanthrone method of Führ et al. (20). C_E and C_A were determined, usually in duplicate, with an ultramicrocolorimeter using the method of Viets et al. (21). Albumin concentrations in urine samples were determined by the immunodiffusion method (22). Sodium concentrations of the distal tubule fluid samples were assayed by electron probe microanalysis (23). Sodium concentration in proximal tubule fluid was estimated from the sodium concentration in systemic plasma as assessed by standard flame photometry. Urine sodium concentration was also measured by flame photometry.

Electron microscopy

To test the validity of the procedure used for inducing unilateral proteinuria, perfused and nonperfused kidneys were examined by electron microscopy in seven PAN-treated animals that had also been subjected to micropuncture study. Thin sections of cortex from these kidneys perfusion-fixed with glutaraldehyde (1.25 g/dl in 0.1 cacodylate buffer, pH 7.4) were processed for electron microscopy (24).

RESULTS

Animal and whole kidney data

Mean values for body weight, $\overline{AP}$, Hct, and C_A obtained in PAN- and saline-treated animals are summarized in Table I. These two groups were similar with respect to body and kidney weight and Hct. Values for C_A were also similar, averaging 5.7±0.1 g/dl for both groups, documenting the absence of systemic hypoproteinemia in PAN-treated rats.[1] Table I also contains pertinent whole kidney data from perfused and nonperfused kidneys obtained in PAN-treated rats, and are compared with values measured in perfused and nonperfused kidneys in saline-treated rats. Marked albuminuria was uniformly detected in PAN-perfused kidneys, with excretion rates averaging 101±30 mg/24 h (range: 35-274). However, albumin excretion in the nonperfused contralateral kidneys of these PAN-treated rats [3±1 mg/24 h (range: 0-10)] remained at the same low level as was measured in

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[1] Albumin-globulin ratio determined by immunodiffusion method in five PAN-treated rats averaged 0.96±0.13.

TABLE I
Summary of Animal and Whole Kidney Data Obtained in Experimental Animals

Condition	Body weight	$\overline{AP}$	Hct	C_A	$U_{Alb}V$	GFR	$U_{Na}V$
	g	mm Hg	vol %	g/dl	mg/24 h	ml/min	μeq/3 h
PAN (n = 15 rats)	290	118	43.9	5.7			
	±13	3	0.7	0.1			
Perfused kidney					101	0.82	23
					30	0.13	7
Nonperfused kidney					3	1.35	76
					1	0.14	9
Saline (n = 7 rats)	294	115	44.6	5.7			
	15	3	0.6	0.1			
Perfused kidney					3	1.30	74
					0	0.10	29
Nonperfused kidney					2	1.30	67
					1	0.07	27
P (PAN vs. saline)	>0.50	>0.50	>0.40	>0.50			
P*					<0.001	<0.001	<0.01
P‡					<0.001	<0.025	>0.10
P§					>0.50	>0.50	>0.50
P‖					>0.50	>0.40	>0.50

Values are expressed as means±1SE.

* Perfused vs. nonperfused kidneys in PAN group.

‡ PAN-perfused vs. saline-perfused kidneys.

§ Perfused vs. nonperfused kidneys in saline group.

‖ Nonperfused kidneys of PAN group vs. nonperfused kidneys of saline group.

perfused [3±0 mg/24 h (range: 0–3)] and nonperfused kidneys [2±1 mg/24 h (range: 0–4)] of rats given saline. While GFR remained essentially unaffected in saline-perfused kidneys when compared with their contralateral partners (1.30±0.10 ml/min vs. 1.30±0.07), GFR was significantly lower in PAN-perfused kidneys than in nonperfused contralateral kidneys (0.82±0.13 ml/min vs. 1.35±0.14, P < 0.001). $U_{Na}V$ in the saline-perfused kidneys remained essentially at the same level measured in the contralateral kidneys (74±29 μeq/3 h vs. 67±27). However, $U_{Na}V$ was significantly lower in PAN-perfused kidneys than in nonperfused contralateral kidneys of the same animals (averaging 23±7 μeq/3 h vs. 76±9, P < 0.01).

Micropuncture assessment of base-line function

Glomerular hemodynamics. As with GFR, SNGFR was found to be reduced in PAN-perfused kidneys. Thus, as shown in Table II, the average value for SNGFR was substantially lower in PAN-perfused kidneys (31.8±2.8 nl/min) than in nonperfused contralateral kidneys of the same animals (48.6±2.0,

P < 0.001), a difference of 35% and essentially identical to that noted from GFR. By contrast, the mean SNGFR value in these contralateral kidneys was not different from that measured in perfused (46.9±1.3 nl/min) and nonperfused (49.0±1.3) kidneys of the saline-treated group.

Table II also contains the average values for the hemodynamic determinants of SNGFR. In contrast to the considerable difference in SNGFR values between PAN-perfused and nonperfused kidneys, values for Q_A were only slightly lower in the former than in the latter (129±12 nl/min vs. 142±8, P > 0.20), indicating a substantially lower SNFF on the PAN-perfused side (0.25±0.01 vs. 0.35±0.01, P ≤ 0.001). Moreover, values for $\overline{P}_{GC}$ and P_T, and hence $\overline{\Delta P}$, also were very nearly the same in perfused and nonperfused kidneys of PAN-treated animals. Since Π_A of the plasma entering perfused and nonperfused kidneys must have been the same, and was measured to be at normal levels, the low values for SNGFR and SNFF in PAN-perfused kidneys proved to be due to the substantially reduced average value for K_f. Indeed, as shown in Table II, the average value for K_f in PAN-perfused kidneys was at least 50% lower than the mean value measured in non-

TABLE II
Mean Values for SNGFR and Its Determinants in PAN- and Saline-perfused Rats

Condition	SNGFR	Q_A	SNFF	Π_A	$\bar{P}_{GC}$	P_T	$\overline{\Delta P}$	$\overline{\Pi_E/\Delta P}$	K_f
	nl/min			mm Hg	mm Hg	mm Hg	mm Hg		nl/(s·mm Hg)
PAN (n = 8 rats)									
Perfused kidney	31.8	129	0.25	18.5	51.1	15.1	35.4	0.81	0.047
	±2.8	12	0.01	0.2	0.8	0.5	0.8	0.02	0.005
Nonperfused kidney	48.6	142	0.35	18.6	50.4	14.9	35.5	1.02	≥0.102*
	2.0	8	0.01	0.2	0.5	0.4	0.7	0.02	0.008
Saline (n = 7)									
Perfused kidney	46.9	147	0.33	19.0	49.5	15.1	34.4	1.03	≥0.108*
	1.3	11	0.02	0.6	0.7	0.7	0.9	0.03	0.006
Nonperfused kidney	49.0	155	0.32	19.0	49.3	15.2	34.1	1.03	≥0.122*
	1.3	9	0.02	0.6	0.7	0.6	1.0	0.04	0.017
P ‡	<0.001	>0.20	<0.001	>0.50	>0.20	>0.50	>0.50	<0.001	<0.001
P §	<0.001	>0.20	<0.001	>0.50	>0.10	>0.50	>0.40	<0.001	<0.001
P ‖	>0.20	>0.10	>0.40	>0.50	>0.50	>0.50	>0.20	>0.50	>0.20
P ¶	>0.50	>0.20	>0.20	>0.50	>0.20	>0.50	>0.20	>0.50	>0.20

Values are expressed as means±1SE.

* Average of minimum K_f values.

‡ Perfused vs. nonperfused kidneys in PAN group.

§ PAN-perfused vs. saline-perfused kidneys.

‖ Perfused vs. nonperfused kidneys in saline group.

¶ Nonperfused kidneys of PAN group vs. nonperfused kidneys of saline group.

perfused kidneys of the same animals (0.047±0.005 nl/(s·mm Hg) vs. 0.102±0.008, $P < 0.001$).[2] Because of this low level of K_f, filtration pressure equilibrium was not achieved in PAN-perfused kidneys, as indicated by the value of $\Pi_E/\Delta P$, significantly <1 (Table II), contrasted with the values of nearly unity in the other three groups. Of further note, average values for Q_A, ΔP, and K_f for contralateral kidneys in PAN-treated rats remained at levels measured in perfused and nonperfused kidneys of saline-treated control animals, as also shown in Table II.

Proximal tubule fluid reabsorption and peritubular capillary dynamics. Table III summarizes the behavior of the proximal tubule in perfused and nonperfused kidneys of PAN- and saline-treated animals. Despite the tendency for overall salt retention in PAN-perfused kidneys (Table I), APR was substantially lower in PAN-perfused kidneys (11.9±2.1 nl/min) than in nonperfused kidneys of these same PAN-treated rats

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[2] Whereas filtration pressure equilibrium, i.e., $\Pi_E/\Delta P \simeq 1.0$, was achieved in six of eight nonperfused kidneys, this condition was not obtained in any of the PAN-perfused kidneys. Nevertheless, the unique value for K_f calculated for the PAN-perfused kidney was uniformly lower than the minimum value obtained in the contralateral kidney in each PAN-animal studied.

(21.1±2.2, $P < 0.005$) or in saline controls (20.8±1.5 nl/min, $P < 0.005$ for the perfused and 22.1±1.5, $P < 0.005$ for nonperfused kidneys). As indicated by the similarity in the values for tubule fluid-to-plasma inulin concentration ratios, $(TF/P)_{In}$, calculated for end-proximal tubules in perfused and nonperfused kidneys of both PAN- and saline-treated animals (Table III), proximal fractional reabsorption was nearly the same in all groups, ranging from 0.37±0.03 to 0.45±0.02.

To explore the mechanism(s) responsible for the low level of APR in PAN-perfused kidneys, two of the forces governing peritubular capillary uptake of APR, namely, peritubular capillary oncotic and hydraulic pressure prevailing at both proximal- and distal-most ends of the peritubular capillary network were determined for each kidney in both PAN- and saline-treated animals. Thus, as shown in Table III, values for P_E are essentially identical among all of the groups of kidneys studied, averaging 18.5±0.5, mm Hg, 18.5±0.8, 18.8±0.4, and 18.9±0.7 in perfused and nonperfused kidneys of PAN- and saline-treated rats, respectively. Likewise, P_{C^i} values were nearly equal among these kidneys, averaging 10.3±0.5, 9.7±0.6, 9.7±0.6, and 10.0±0.4 mm Hg. By contrast, values for Π_E were significantly lower in PAN-perfused kidneys (28.7±0.6

TABLE III

Superficial Proximal Tubule Function and Some of the Determinants of Peritubular Capillary Uptake
of Absolute Proximal Fluid Reabsorption

Condition	APR	$(TF/P)_{in}$	P_E	$P_{C'}$	Π_E	$\Pi_{C'}$
	nl/min		mm Hg	mm Hg	mm Hg	mm Hg
PAN (n = 8 rats)						
Perfused kidney	11.9	1.62	18.5	10.3	28.7	23.9
	±2.1	0.09	0.5	0.5	0.6	0.5
Nonperfused kidney	21.1	1.77	18.5	9.7	36.1	26.1
	2.2	0.08	0.8	0.6	1.0	0.7
Saline (n = 7 rats)						
Perfused kidney	20.8	1.80	18.8	9.7	35.2	25.7
	1.5	0.08	0.4	0.6	1.4	1.0
Nonperfused kidney	22.1	1.85	18.9	10.0	35.1	25.5
	1.5	0.07	0.7	0.4	1.7	1.0
P^*	<0.005	>0.10	>0.50	>0.20	<0.001	<0.025
$P\ddagger$	<0.005	>0.10	>0.50	>0.40	<0.005	>0.10
$P\S$	>0.20	>0.20	>0.50	>0.20	>0.50	>0.50
$P^\parallel$	>0.50	>0.40	>0.50	>0.50	>0.50	>0.50

Values are expressed as means±1 SE.
* Perfused vs. nonperfused kidneys in PAN group.
‡ PAN-perfused vs. saline-perfused kidneys.
§ Perfused vs. nonperfused kidneys in saline group.
‖ Nonperfused kidneys of PAN vs. nonperfused kidneys of saline group.

mm Hg) than in contralateral (36.1±1.0, $P < 0.001$) or saline-treated ipsilateral (35.2±1.4, $P < 0.005$) or contralateral kidneys (35.1±1.7, $P < 0.01$). While values for $\Pi_{C'}$ were also significantly lower in PAN-perfused kidneys (23.9±0.5 mm Hg) than in the contralateral kidneys (26.1±0.7, $P < 0.025$), the former was only numerically but not significantly lower than mean values measured in perfused (25.7±1.0 mm Hg, $P > 0.10$) and contralateral nonperfused (25.5±1.0 mm Hg, $P > 0.10$) kidneys of the saline group.

Segmental analysis of tubule sodium reabsorption. Fig. 1 provides a schematic comparison of the amounts of sodium delivered to and reabsorbed from the various segments of surface nephrons in PAN-perfused and nonperfused kidneys. Because of the reduction in SNGFR measured only in PAN-perfused kidneys, the filtered load of sodium was significantly lower in PAN-perfused (5.6±0.7 neq/min) than in nonperfused kidneys (8.2±0.6 neq/min, $P < 0.001$). Despite the concomitant reductions of APR observed in perfused kidneys (2.3±0.3 neq/min vs. 3.8±0.3, $P < 0.001$), the amount of sodium arriving at the end-proximal convolution was still lower on the PAN-perfused side (3.3±0.4 neq/min vs. 4.4±0.5, $P < 0.001$).

Fig. 1 also describes the behavior of the so-called short loops of Henle, that is, the segments located be-tween the last accessible portion of the proximal convoluted tubule and the earliest accessible portion of the distal convoluted tubule of superficial nephrons. It is evident from this figure that the amount of sodium reaching the early distal tubule in PAN-perfused (0.78±0.09 neq/min) and nonperfused kidneys (0.74±0.08, $P > 0.4$) was the same, indicating that the amount of sodium reabsorbed by these short loops was significantly less in PAN-perfused kidneys (2.5±0.4 neq/min vs. 3.7±0.5 in contralateral kidneys, $P < 0.01$).

As also shown in Fig. 1, the average amount of sodium reaching the end-distal convolution was essentially the same between the two kidneys (0.31±0.02 neq/min for perfused, 0.32±0.05 for nonperfused kidney), just as was the case for the early distal tubule. Thus, the absolute amount of sodium reabsorbed along the distal convoluted tubule per se was equivalent in both kidneys in the PAN-treated group.

Table IV provides a segmental analysis of fractional sodium and water reabsorption along the nephron in perfused and nonperfused kidneys of PAN-treated rats. As can be seen, the value of $(TF/P)_{in}$ was slightly (although not significantly) depressed at end-proximal (1.74±0.13 vs. 1.94±0.11, $P > 0.05$) and early distal (2.63±0.38 vs. 3.44±0.32, $P > 0.05$) puncture sites in
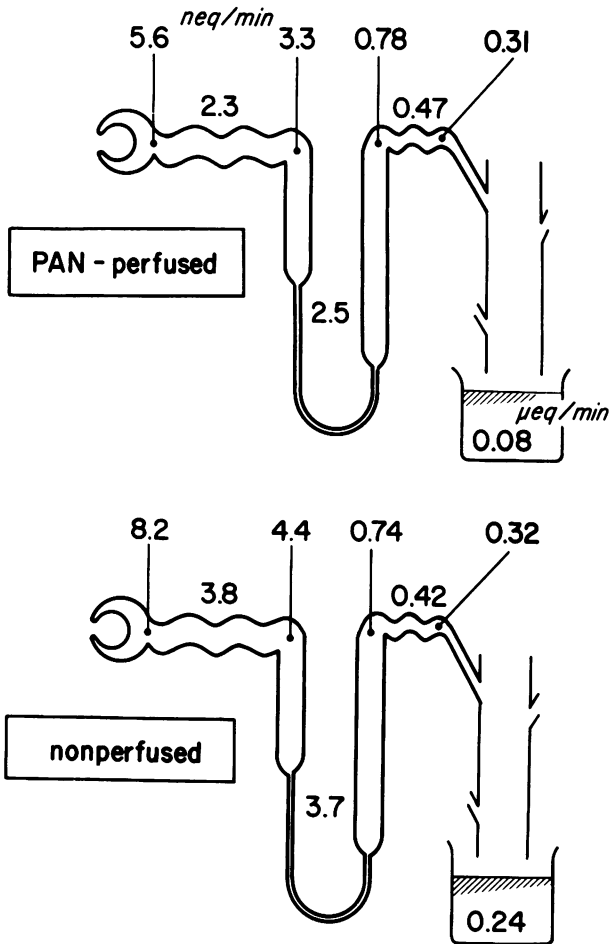
FIGURE 1 Schematic representation of sodium delivery and reabsorption along superficial nephron segments in PAN-perfused and nonperfused kidneys. Only absolute mean values are given.
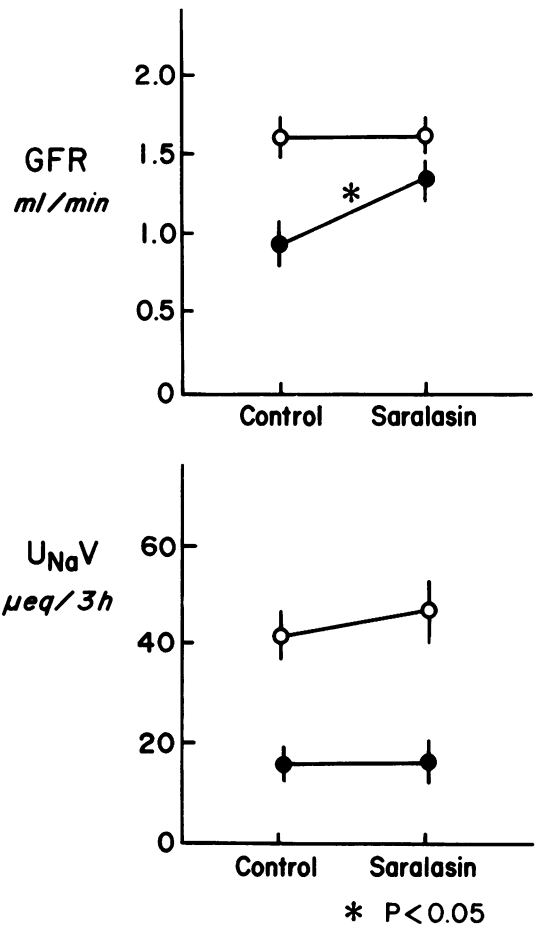

FIGURE 2 Summary of GFR and $U_{Na}V$ data obtained in PAN-perfused (●) and nonperfused (○) kidneys given saralasin ($n = 6$ rats). Values are expressed as means±1 SE.

perfused kidneys, relative to nonperfused kidneys. By the accessible end of superficial distal tubules however, $(TF/P)_{In}$ values (5.90±0.89 vs. 11.0±3.3, $P < 0.01$), differed markedly and significantly. As also indicated in Table IV, mean values for $(TF/P)_{Na}$ were comparable between perfused and nonperfused kidneys in PAN-treated rats at all sites examined. The fraction of filtered sodium remaining in the lumen was higher in perfused than in nonperfused kidneys at each site, whereas values for fractional reabsorption of sodium for each of the three segments examined, namely, proximal convoluted tubule, short loops of Henle, and distal convoluted tubule did not differ significantly between perfused and nonperfused kidneys.

Effects of saralasin

Measurements of whole kidney function. Fig. 2 summarizes whole kidney clearance data from a third

group of six PAN-treated rats given saralasin. As shown in the top panel, intravenous infusion of this angiotensin II antagonist, in a dose of 0.3 mg/kg per h, failed to alter GFR in nonperfused kidneys (1.7±0.2 ml/min before vs. 1.7±0.2 during saralasin). By contrast, GFR increased significantly in PAN-perfused kidneys during saralasin infusion (from 0.9±0.3 ml/min to 1.3±0.3, $P < 0.01$). Despite this substantial increase in GFR, urinary sodium excretion (lower panel) remained unchanged in PAN-perfused kidneys (from 18±5 μeq/3 h to 18±5) and far-below values measured in nonperfused kidneys (41±11 μeq/3 h before vs. 47±14 during saralasin).

Measurements of glomerular hemodynamics. For the additional group of eight rats, whereas inulin clearance from nonperfused kidneys was again unaffected (1.22±0.09 ml/min before vs. 1.19±0.07 during saralasin infusion), saralasin infusion induced a significant increase in SNGFR in proteinuric kidneys (Table V). However, on average, the magnitude of this change

TABLE IV
Segmental Analysis of Fractional Sodium and Water Reabsorption Along
Superficial Nephrons of PAN-treated Rats

	Perfused kidney			Nonperfused kidney		
	End-proximal	Early distal	End-distal	End-proximal	Early distal	End-distal
$(TF/P)_{In}$	1.74	2.63	5.90	1.94‡	3.44‡	11.0*
	±0.13	0.38	0.89	0.11	0.32	3.3
$(TF/P)_{Na}$	1.00§	0.35	0.35	1.00§	0.30‡	0.33
		0.02	0.06		0.02	0.06
$(TF/P)_{Na}/(TF/P)_{In}$‖	0.59	0.15	0.057	0.52‡	0.090*	0.037*
	0.04	0.02	0.007	0.03	0.006	0.007
$\dot{V}, nl/min$¶	20.4	15.2	7.6	29.7*	16.7	7.4
	1.7	1.7	2.1	3.5	1.7	1.8
	Proximal	Short loops	Distal	Proximal	Short loops	Distal
Fraction of filtered Na	0.41	0.44	0.094	0.48‡	0.43	0.054‡
reabsorbed by each segment	0.04	0.03	0.023	0.03	0.03	0.011

Values are expressed as means±1SE.
* $P < 0.05$.
‡ $0.05 < P < 0.10$.
§ Assumed but not measured.
‖ Fraction of filtered sodium remaining to point of puncture.
¶ Tubule fluid flow rate.

($\sim$30%) was somewhat smaller than that of GFR seen in the previous PAN-treated group ($\sim$40%). As also shown in Table V, the pressures and flows governing glomerular ultrafiltration were largely unaltered by saralasin. Values for Q_A increased slightly in association with numerically small and statistically insignificant falls in R_A (from $4.2\pm0.8 \times 10^{10}$ dyn·s·cm^{-5} to 2.9 ± 0.4, $P > 0.10$) and R_E (from $2.2\pm0.4 \times 10^{10}$ dyn·s·cm^{-5} to 1.5 ± 0.1, $P > 0.10$). Likewise, values for $\bar{P}_{GC}$ and P_T remained essentially constant, as did $\Delta \bar{P}$ values. By contrast, values for K_f increased significantly in response to saralasin infusion, on average by 60% above

presaralasin levels. Because of this rise in K_f, SNFF increased significantly (Table V) reflecting the observed rise in SNGFR despite the near constancy in Q_A. In spite of the increased SNGFR, values for $U_{Na}V$ again remained unaltered at low levels during saralasin infusion (20 ± 7 μeq/3 h before vs. 23 ± 7 during saralasin infusion).

Electron microscopy

Both PAN-perfused and nonperfused kidneys appeared unremarkable by light microscopy: proximal

TABLE V
Summary of Effects of Saralasin on Glomerular Hemodynamics in PAN-perfused Kidneys

	SNGFR	Q_A	SNFF	Π_A	$\bar{P}_{GC}$	P_T	$\overline{\Delta P}$	$\Pi_E/\overline{\Delta P}$	K_f
	nl/min			mm Hg	mm Hg	mm Hg	mm Hg		nl/(s·mm Hg)
PAN-perfused kidneys ($n = 8$ rats)									
Before saralasin	23.1	91	0.25	18.3	48.3	15.1	33.1	0.88	0.044
	±1.3	13	0.02	0.6	0.3	0.7	1.0	0.07	0.005
During saralasin	30.3	109	0.29	17.7	47.5	16.2	31.4	0.96	0.071
	1.0	15	0.01	0.7	0.2	0.8	0.6	0.05	0.004
P	<0.05	>0.20	<0.025	>0.10	>0.40	>0.50	>0.10	>0.10	<0.01

Values are expressed as means±1SE.

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and distal convoluted tubules revealed no histologic abnormalities. Electron microscopy of PAN-perfused kidneys showed effacement of glomerular epithelial cell cytoplasm, prominent increases in podocyte dense bodies (lysosomes), and obliteration of slit pores. The basement membrane was of normal thickness. Glomeruli taken from nonperfused kidneys appeared ultrastructurally intact (Fig. 3).

DISCUSSION

PAN has been used widely to induce animal models of minimal change glomerulopathy, a disorder characterized by marked albuminuria (4–15). Using the technique of selective unilateral renal arterial perfusion with PAN, Bricker et al. (13) and Hoyer et al. (14) demonstrated that albuminuria was confined to the perfused kidney, clearly indicating that the proteinuria seen in this disease is a consequence of intrarenal mechanism(s) rather than systemic or circulating factors. It seemed to us that, by the same token, this model of unilateral heavy proteinuria might provide a means for ascertaining whether the avid salt retention known to characterize the nephrotic state is due to an intrinsic inability of the proteinuria kidney to excrete salt and water normally, or whether salt retention merely represents a normal renal response to a stimulus extrinsic to the kidney (e.g., hypoalbuminemia or hyperaldosteronism).

In this study it was possible to demonstrate that marked albuminuria and effacement of glomerular epithelial cell foot processes were confined only to PAN-perfused kidneys and these changes coincided with ipsilateral reduction in salt excretion. This observation has recently been duplicated by Chandra et al. (25). In their study, a compensatory increase in sodium excretion was observed in contralateral non-PAN-perfused kidneys. In our study, however, sodium excretion in contralateral kidneys of PAN-treated rats was, on average, at the level comparable to that measured in perfused or nonperfused contralateral kidneys in control saline animals. Since we did not carry out an evaluation of external sodium balance in these rats before micropuncture study, it remains uncertain whether the observed ipsilateral fall in salt excretion (without apparent enhancement of salt excretion in contralateral kidneys) reflects on-going chronic salt retention in conscious PAN-treated animals. Nevertheless, our findings implicate the operation of intrinsic renal mechanism(s) in the causation of both the unilateral renal salt retention and protein loss. Of note, this selective salt retention by the proteinuric kidney was observed in the absence of a reduction in systemic plasma protein concentration.

One intrinsic renal mechanism potentially responsible for the lower excretion rate of sodium in the PAN-

perfused kidney appears to be GFR, which was also significantly lower in PAN-perfused kidneys than in nonperfused contralateral kidneys or kidneys from control rats. By measuring all the factors potentially capable of accounting for this reduction in GFR, it was possible to ascribe this defect in GFR to a small reduction in Q_A and an even more pronounced decline in K_f. This pattern of glomerular dynamics essentially duplicates the pattern previously noted in rats given PAN systemically (26). Moreover, since the mean value of GFR and its determinants for nonperfused contralateral kidneys in PAN-treated rats remained at levels similar to those of kidneys in control animals, intrinsic renal mechanisms rather than circulating substances or alterations in plasma composition (such as hypoproteinemia) must again be invoked.

One such mechanism appears to be locally enhanced action of angiotensin II. Infusion of saralasin, a specific angiotensin II antagonist, led to a significant increase in GFR and SNGFR in proteinuric kidneys largely via its effect on the ultrafiltration coefficient whereas this antagonist failed to evoke changes in GFR in nonproteinuric kidneys. Nevertheless, the observation that values for GFR obtained during saralasin infusion in proteinuric kidneys still remained far below the levels measured in contralateral or sham-treated controls suggests that factors other than angiotensin II also contributed to the observed fall in GFR in the PAN-perfused kidney. Presumably, structural alterations, including narrowing of the slit diaphragm complex, remained unaffected by saralasin and account for the persistent impairment in GFR and SNGFR that was observed.

Unlike the experimental animal model used in this study, moderate to marked hypoalbuminemia is characteristic of the nephrotic syndrome in humans. While such reduction in serum albumin concentration would tend to promote a higher rate of filtration, the reductions in K_f and Q_A demonstrated in the present study would act to dampen this effect.[3] Since GFR is often reduced in clinical minimal change disease (30, 31), despite hypoalbuminemia, similar reductions in Q_A and K_f may also be occurring in this disorder in man. Of importance, however, the reduction in GFR observed uniformly in our experimental animals does not appear to be a requirement for salt retention in proteinuric kidneys, in that despite a rise in GFR toward

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[3] Hypoalbuminemia induced acutely in vivo has been reported to lead to a reduction in the value of K_f (27, 28), whereas an inverse relationship between perfusate albumin concentration and K_f has recently been reported in vitro (29). The effect of chronic hypoalbuminemia on K_f, however, has not been studied and remains unknown at present.

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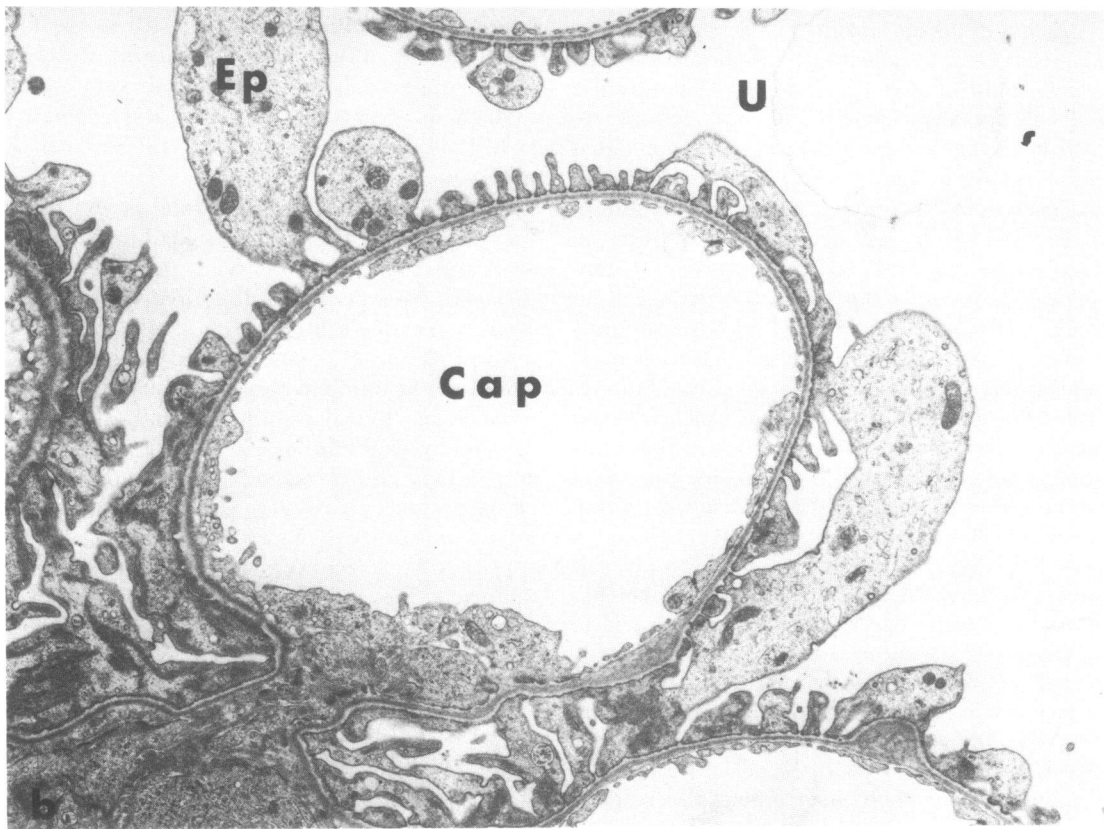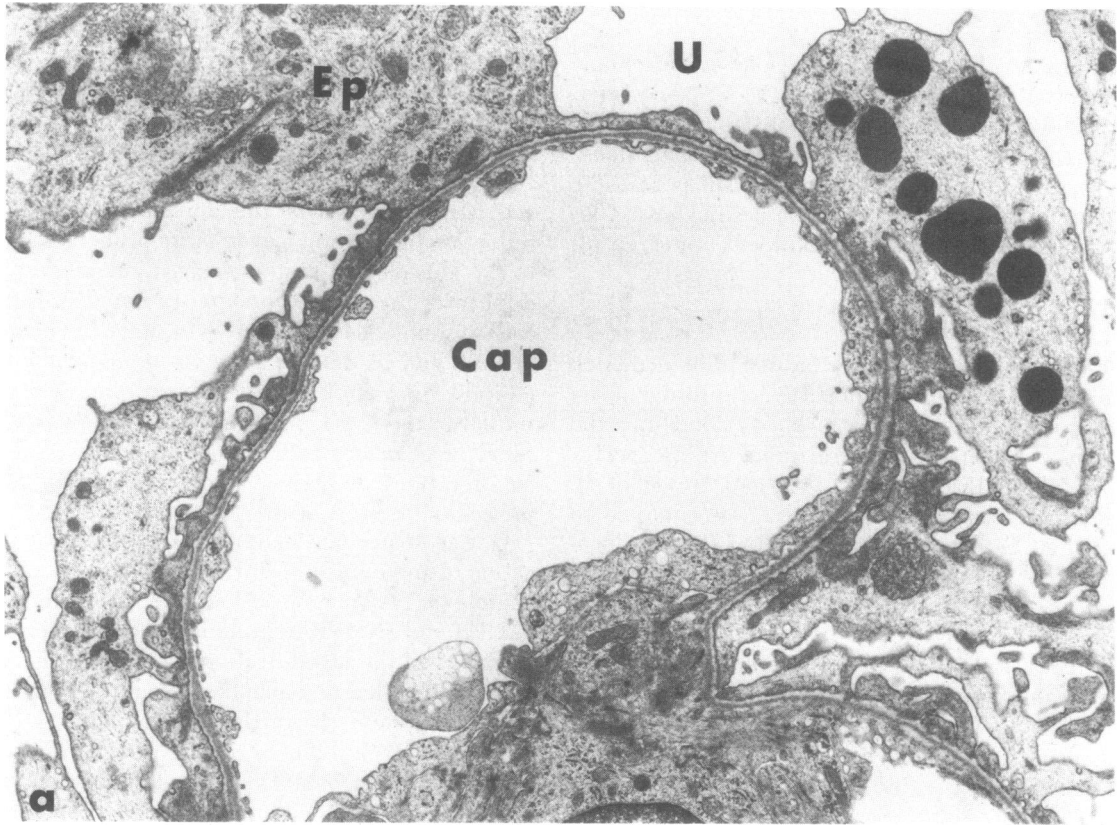
normal levels with saralasin we failed to augment sodium excretion in PAN-perfused kidneys (Fig. 2).

In evaluating the renal tubule handling of salt and water in PAN-perfused and nonperfused contralateral kidneys, we found that the amounts of fluid and sodium reaching the end of the accessible proximal convoluted tubule in proteinuric kidneys of PAN-treated rats proved to be significantly lower than on the nonperfused side (Fig. 1). These lower values were the consequence of a reduced filtered load as well as reduced rates of reabsorption along the proximal convoluted tubule segments. Once again, therefore, the changes in proximal reabsorption point to a mechanism intrinsic to the proteinuric kidneys. One such mechanism for explaining the lower absolute rates of salt and water reabsorption in the proximal convolutions is suggested by the finding of a lower K_f and hence, lower filtration fraction in PAN-perfused glomeruli, the effect of which would be expected to selectively reduce postglomerular plasma oncotic pressure. This linkage between K_f and postglomerular plasma oncotic pressure was in fact demonstrated by direct measurement; whereas mean values for peritubular capillary hydraulic pressure did not differ among any of the groups of kidneys studied, peritubular capillary plasma protein concentrations, and therefore oncotic pressure, were significantly lower, on average, in PAN-perfused kidneys (Table III). It should be noted that this lower average value for peritubular capillary plasma protein concentration was the consequence, not of a fall in systemic plasma protein concentration but rather of the unilateral decline in K_f. Thus, this lower value for K_f creates at least one important intrinsic renal mechanism for depressing proximal fluid reabsorption. In this study reductions in APR were closely and directly correlated with concurrent declines in SNGFR. Of note is the recent evidence obtained from several isolated tubule microperfusion studies (36, 37), indicating that an alteration in proximal tubule luminal flow rate is capable of modifying transepithelial fluid transport. The possibility therefore exists that the K_f-induced reduction in SNGFR in PAN-perfused kidneys brings about a concurrent fall in APR by virtue of a luminal influence on transepithelial fluid transport.

In the typical clinical case of lipoid nephrosis, even further reduction in proximal reabsorption may be effected by the associated hypoproteinemia. Grausz et al. (31) found in patients with clinically overt nephrotic syndrome that fractional proximal reabsorption (estimated by whole kidney fractional sodium reabsorption during administration of massive doses of chlorothiazide and ethacrynic acid) was markedly reduced, compared with values measured in similar fashion in normal individuals. In their series, patients with minimal change disease and other primary glomerulopathies were included, and a reduction in proximal fluid reabsorption was felt to be a general feature of the nephrotic state.

Similar reductions in fractional and/or absolute proximal fluid reabsorption have been demonstrated by direct micropuncture measurements in several experimental forms of primary glomerulopathy. In rats in the autologous phase of nephrotoxic serum nephritis with severe albuminuria and hypoalbuminemia, Rocha et al. (32) found that absolute proximal fluid reabsorption was markedly depressed, albeit in proportion to the fall in SNGFR. Fractional as well as absolute proximal fluid reabsorption has also been demonstrated to be significantly depressed in a milder form of nephrotoxic serum nephritis in the absence of hypoproteinemia (33). Furthermore, in rats with autologous immune complex nephropathy, i.e., a form of membranous glomerulopathy, both fractional and absolute proximal sodium reabsorption was again found to be lowered significantly below control levels (2). As with minimal change glomerulopathy, these forms of glomerular injury are also associated with marked reductions in K_f (33–35). In consequence, since filtration fraction and hence, peritubular capillary colloid osmotic pressure, were either found or can be assumed to be reduced, it is attractive to speculate that this change in peritubular Starling forces plays a causal role in the reductions in proximal fluid reabsorption seen in all primary glomerulopathies, irrespective of whether hypoproteinemia is present or not.

As with proximal tubules, superficial loop segments also failed to account for the observed salt retention by the proteinuric kidneys (Table IV and Fig. 1). Indeed, the difference in the amount of sodium remaining in the lumen between PAN-perfused and nonperfused kidneys was further lessened by the time fluid reached the early distal tubule puncture site, due to a significant reduction in sodium reabsorption by the loop in PAN-perfused kidneys (Table IV and Fig. 1). Since the quantity of sodium delivered to the loop segment was lower on the PAN-perfused side, the finding that total sodium reabsorption in the loop segment

FIGURE 3 (a) Representative glomerular capillary loop from a PAN-perfused kidney. There is marked simplification of foot processes and obliteration of filtration slits. Epithelial cells show an increase in lysosomal structures (upper right corner). (b) Representative glomerular capillary loops from the nonperfused kidney in a PAN-treated rat. The epithelial cells appear unremarkable. (Ep, epithelial cell; U, urinary space; Cap, capillary lumen) ×8000.

was also lower than on the nonperfused side is consistent with prevailing notions regarding the flow-dependence of sodium reabsorption by these segments (38). Given that this quantity of sodium to the distal tubules was comparable, it is hardly surprising that the absolute reabsorption of sodium in the distal convoluted tubule was also comparable on the two sides.

Thus, neither the fall in filtered load of sodium nor the measured changes in sodium reabsorption in the various nephron segments to the end of the accessible distal tubule of superficial nephrons adequately account for the more pronounced antinatriuretic response of the proteinuric kidney. Similar conclusions were reached recently by Bernard et al. (2) in a segmental analysis of tubule sodium reabsorption in volume expanded rats with autologous immune complex nephropathy. We are therefore left with the two familiar alternatives that regularly emerge from all such micropuncture attempts at sequential analysis of tubule sodium reabsorption, namely, (a) that the difference in sodium excretion between the two kidneys is a consequence of more pronounced sodium reabsorption in the remainder of the nephron in PAN-perfused kidneys or (b) that our findings in superficial nephrons are not representative of all of the nephrons that contribute to urine formation and that differences in the handling of sodium by deep nephrons account for the finding of a reduced urinary sodium excretion by PAN-perfused kidneys. Clearly, the present study does not enable us to choose between these possibilities.

Given the evidence indicating the presence of local intrarenal mechanism(s) causing avid salt retention by proteinuric kidneys, we attempted to interfere with one of the important volume-regulating mechanisms that can operate locally, that is, the renin-angiotensin system. Whereas saralasin, an angiotensin antagonist, increased GFR and SNGFR substantially in PAN-perfused kidneys, ipsilateral urinary sodium excretion remained depressed. Thus, although the renin-angiotensin system seems to play an important role in the genesis of the decline in GFR in the PAN-perfused kidney, this system appears to be of little importance in mediating the overall salt retention of the proteinuric kidney.

ACKNOWLEDGMENTS

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