

Functional Profile of the Isolated Uremic Nephron

EVIDENCE OF PROXIMAL TUBULAR "MEMORY" IN EXPERIMENTAL RENAL DISEASE

WALTER TRIZNA, NORIMOTO YANAGAWA, YAACOV BAR-KHAYIM,
BARBARA HOUSTON, and LEON G. FINE, *Division of Nephrology,
Department of Medicine, University of California, Los Angeles, California 90024*

ABSTRACT In experimental models of glomerular and nonglomerular renal disease, single nephron filtration rate and proximal tubular reabsorption of fluid decrease or increase in parallel in the same nephron. To assess whether intrinsic adaptations in proximal tubular function, i.e., changes that are independent of the peritubular or humoral milieu, contribute to this phenomenon, segments of rabbit late superficial proximal convoluted tubules (PCT) were studied by in vitro perfusion. PCT were obtained from normal kidneys, from remnant kidneys, and from kidneys embolized with microspheres. Single nephron filtration rates are increased in the remnant and decreased in the embolized kidneys. Whereas the embolized-kidney rabbits were nonazotemic (the contralateral kidney was left *in situ*), the remnant-kidney animals were uremic. In order to study a nonazotemic model of increased single nephron filtration rate, PCT were also obtained from uninephrectomized rabbits.

Significant compensatory hypertrophy occurred in the PCT of the remnant kidney. Net fluid reabsorption (J_v) per unit length was increased by ~60%; J_v per unit luminal surface area was the same as in the normal PCT. Transepithelial potential difference (PD) was significantly greater than normal. This was associated with a reversal of the normal permselective properties ($P_{Cl} > P_{Na}$) of the late superficial PCT so that P_{Na} exceeded P_{Cl} . The changes could not be ascribed to some undetermined effect of the uremic state in vivo, since increases in tubule size, J_v per unit length, and PD also occurred in PCT from nonazotemic uninephrectomized rabbits.

In contrast, J_v per unit length or per unit luminal surface area, was decreased by ~50% in PCT from embolized kidneys and PD was also reduced. In these tubules, the normal permselective properties were also

reversed. Tubule size, however, was not significantly different from normal.

The increases or decreases in J_v that occurred in the different disease models were not dependent on tubular fluid flow rate or the uremic milieu in vitro.

These studies indicate that intrinsic proximal tubular function is modified by the disease state in vivo and that the "memory" of this adaptation is expressed in the in vitro situation. The changes in J_v observed in vitro parallel the increases or decreases in single nephron filtration rates that occur in vivo. Compensatory hypertrophy, with an attendant increase in luminal surface area, could explain the increased J_v per millimeter in the remnant kidneys, but the adaptation observed in the embolized kidneys cannot be ascribed to changes in tubule size.

INTRODUCTION

In chronic experimental glomerulonephritis, the glomerular filtration rates of the individual nephrons of the disease kidney differ widely and range from below to above normal values (1-3). Despite this marked heterogeneity of function within the same organ, the relationship between the single nephron glomerular filtration rate (SNGFR)¹ and the absolute rate of fluid reabsorption by the proximal tubule remains constant, i.e., glomerulotubular balance is maintained. A similar situation obtains in the nephrons of the kidneys with a reduced nephron population in which SNGFR and fluid reabsorption by the proximal tubule increase concomitantly (4-7). The mechanism underlying this remarkable parallelism between glomerular

Received for publication 14 July 1980 and in revised form 20 April 1981.

¹Abbreviations used in this paper: BUN, blood urea nitrogen; J_v , net fluid reabsorption; PCT, proximal convoluted tubules; PD, potential differences; SNGFR, single nephron glomerular filtration rate.

and proximal tubular function remains obscure, but has recently received renewed attention (8).

The present studies were designed to investigate the nature of this relationship by examining *in vitro* fluid reabsorption by proximal tubules obtained from animals with experimental renal disease in which SNGFR was either elevated or lowered. Since we have previously shown that fluid reabsorption in the rabbit proximal straight tubule correlates with the degree of compensatory hypertrophy (9), we wished to evaluate further whether changes in intrinsic tubular epithelial cell function, i.e., changes that are independent of humoral or peritubular factors, contribute to the pattern of proximal tubular function observed in chronic renal disease.

METHODS

Experimental groups

Superficial proximal convoluted tubules from three groups of 2–3-kg white New Zealand rabbits were studied.

Normal SNGFR. Tubules were obtained from the left kidneys of nine normal rabbits.

Elevated SNGFR. Tubules were obtained from the solitary remnant kidneys of nine uremic rabbits. Remnant kidneys, in which approximately one-sixth of the original single kidney mass was left intact, were prepared as described previously (9–11) and studied after 3 wk. By this time, significant compensatory hypertrophy has occurred (9) and the GFR of the remaining nephrons approximately doubles (see below).

Reduced SNGFR. Tubules were obtained from seven kidneys in which glomerular filtration was reduced by embolization of the glomeruli by microspheres injected directly into the left renal artery. The contralateral kidney was left *in situ* to minimize the stimulus for compensatory hypertrophy.

Remnant kidneys were studied after 3 wk and embolized kidneys after 7–10 d.

Microsphere-embolized kidneys were prepared as follows. The left renal artery was exposed via a retroperitoneal flank incision and dilated by local application of 2% lidocaine. The artery was cannulated with a 30-gauge needle connected to PE 10 tubing. Approximately 4–6 million microspheres of mean (\pm SD) diameter $14.6 \pm 1.1 \mu\text{m}$ (3M Company, St. Paul, Minn.), suspended in 5 ml of physiological saline containing $50 \mu\text{l}$ of 5% Tween 80, 50 U of heparin, and 0.025 mg of 2% lidocaine, were infused directly into the renal artery over a 30-min period. The syringe was rotated constantly during the infusion to prevent sludging of the microspheres. It was found by trial and error that a smaller number of microspheres fails to reduce GFR, whereas a larger amount leads to obstruction of arterioles and the development of microinfarcts. Histological sections of all embolized kidneys used for the study revealed that the microspheres were confined exclusively to the glomeruli and were uniformly distributed throughout the cortex. This model of reduced SNGFR was chosen since homogeneous involvement of all nephrons is required to avoid sampling bias when only one nephron segment per kidney is being studied. This could not be achieved, for instance, using a model of nephrotoxic serum nephritis in rabbits in which a rapidly progressive, heterogeneous lesion results.²

The rabbits with remnant kidneys differed from those in the other two groups not only because the GFR of the remaining nephrons was elevated, but also because the animals were chronically uremic (azotemic). Consequently, an additional group of four uninephrectomized rabbits was studied. These animals were not azotemic at the time of study due to an adequate compensatory increase in the GFR of the remaining kidney.

Clearance measurements

Inulin clearances were performed on six to eight lightly anesthetized rabbits from each experimental group using a modification of methods described previously (10). A priming dose of 400 mg of inulin, in 4 ml of 0.9% NaCl, was followed by a sustaining infusion (4 mg/ml) administered at a rate of 150 mg/h. In normal animals, urine was collected via a bladder catheter and single kidney GFR calculated at 50% of total GFR. In rabbits with solitary remnant kidneys, urine was also obtained via a bladder catheter, whereas the embolized kidneys were studied by catheterizing the left ureter with PE 90 tubing through a small flank incision. Inulin concentrations in serum and urine were measured by autoanalyzer.

In vitro perfusion of superficial proximal convoluted tubules

This was performed according to methods described previously (9–12). Rabbits were killed by cervical dislocation. A 1–2-mm tangential slice of the outermost renal cortex was removed and immersed in chilled normal rabbit serum. A segment of the late superficial proximal convoluted tubules (PCT) was dissected free and transferred to a bath of normal or uremic rabbit serum (see below), maintained at 37°C and bubbled continuously with 95% O₂–5% CO₂. The “late” PCT includes the second and third millimeter of the superficial PCT. (The first millimeter was identified either by its attachment to a glomerulus or by identifying the narrow post-glomerular “neck region.” These segments were used only for the permselectivity studies described below.)

Both ends of the tubule were insulated with Sylgard 184 (Dow-Corning Corp., Midland, Mich.). [³H]methoxyinulin, dialyzed to remove small molecular weight constituents, was added to the perfusate (50 $\mu\text{Ci}/\text{ml}$) as an impermeable volume marker. Perfusion rate was adjusted to ~ 15 – $20 \text{ nl}/\text{min}$ which is the reported SNGFR of the normal rabbit (13). In two to three experiments in each group, a perfusion rate of $\sim 35 \text{ nl}/\text{min}$ was used to simulate the increased single nephron filtration rates of remnant kidneys and uninephrectomized animals. The perfusate was an isosmolal ultrafiltrate of normal rabbit serum prepared by ultrafiltering the serum through a Diaflo XM50 membrane (Amicon Corp., Lexington, Mass.).

In each experiment, three sets of observations were made sequentially by changing the type of serum bathing the tubule. The two different sequences randomly used were (a) normal serum, uremic serum, normal serum, or (b) uremic serum, normal serum, uremic serum. The mean of the results from the first and third periods was compared with the middle period to eliminate bias as a result of deterioration in the preparation as a function of time. In four additional experiments on normal PCT, uremic serum was used as the bath and a uremic serum ultrafiltrate as the perfusate. The normal and uremic sera were obtained from normal and uremic rabbits, as described previously (9), and were adjusted to the same osmolality as the perfusate immediately before the experiment.

² Knutsen, D., and L. Fine. Unpublished observations.

In each of the experimental periods, a 15-min period of equilibration was allowed before sample collections were begun. Five to six consecutive, timed collections of tubular fluid were made under mineral oil into a constriction pipette that was calibrated at the termination of the experiment.

Samples of tubular fluid were pipetted directly into Aquasol liquid scintillation fluid (New England Nuclear, Boston, Mass.) and total counts per minute were measured with a Packard Tricarb Liquid Scintillation Counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Perfusion rate (V_0) was calculated as $V_0 = S_L/(S_0)t$, where S_L is the total amount of isotope collected, (S_0) is the concentration of isotope in the perfusate, and t is the time. Net fluid reabsorption, J_v , expressed in nanoliters per millimeter tubule per minute, is thus $(V_0 - V_L)/L$, where V_L is the collection rate and L is the length of the tubule. Tubule length and internal and external diameters were measured during perfusion with a calibrated reticle in the ocular of the microscope. Tubule diameters were measured at three points along the tubule segment and the mean of the three measurements was recorded. J_v was expressed per unit tubule length and per unit luminal surface area assuming the tubule to be a perfect cylinder.

The perfusion pipette which served as a luminal electrode was connected to a calomel half-cell through a 0.16-M NaCl-4% agar bridge. The half-cell was connected to the input of an impedance converter and the signal displayed on a Grass model 7 Polygraph with a model 7P1A Low Level DC Preamplifier (Grass Instrument Co., Quincy, Mass.). The circuit was completed through a 0.16-M NaCl-4% agar bridge connecting the bath to a reference calomel half-cell connected to a ground through a precision millivolt reference source (W-P Instruments, Inc., New Haven, Conn.). Any small potential difference due to asymmetry of electrodes was nulled before the experiment by means of a variable potentiometer in the impedance converter. Transepithelial potential difference (PD) was monitored continuously throughout the experiment.

Measurement of permselectivity to sodium and chloride

It has been demonstrated that juxtamedullary PCT have higher potentials and J_v than superficial PCT (13, 14). In the first millimeter of superficial PCT and in all segments of the juxtamedullary PCT, P_{Na} is greater than P_{Cl} , whereas the reverse is true of the late (second mm and third mm) superficial PCT. Since PCT from remnant kidneys were observed to have higher PD and J_v than normal PCT (see Results), the permselectivity to Na and Cl of both superficial and juxtamedullary PCT from five kidneys from each experimental group was examined to determine whether an association between J_v , PD, and the $P_{Na}:P_{Cl}$ ratio could be found. In addition, five segments of the first millimeters of the superficial PCT were studied in each experimental group.

Superficial PCT were dissected from a 1-mm thick slice of tissue obtained from the immediate subcapsular cortex. Localization of the origin of the segment is described above. A 1-mm thick slice underlying the superficial slice was discarded and a second 1-mm thick slice was obtained from the inner cortex for dissection of juxtamedullary PCT. PCT were perfused using an artificial bath and perfusate containing (in millimoles per liter): NaCl 105, KCl 5, $NaHCO_3$ 25, $CaCl_2$ 1.8, $MgSO_4$ 1, NaH_2PO_4 4, Na acetate 10, glucose 3.3, and alanine 5. The bath additionally contained 5% vol/vol normal rabbit serum. PD was recorded for 10 min. The bath was then switched to one containing the same con-

stituents except that NaCl concentration was 55 and 92 mM sucrose was added to obtain isosmolality. Using this bath solution, a lumen-to-bath NaCl gradient of 50 mM was thus imposed. PD was recorded for an additional 10 min. This PD was corrected for a liquid junction potential by +3.5 mV, which is the observed and calculated junction potential for these solutions (13).

Measurement of tubule hypertrophy

Two methods were used to measure tubular size: (a) Protein content per unit length was measured on duplicate pooled samples of PCT (total length 10–15 mm) according to methods described previously (11). (b) Total cell volume per unit length was measured in each perfused tubule and was calculated from the internal and external diameters assuming the tubule to be a perfect cylinder (11).

Calculations

Data are expressed as the mean \pm SE. Perfusion experiments were regarded as being technically inadequate and were excluded from the study only if they met the following preset criteria: (a) tubule length < 0.7 mm; (b) absence of a transtubular PD; and (c) $J_v < 0.1$ nl/mm per min or apparent net "secretion" of fluid. All other experiments are included in the study.

Statistical methods

Comparisons between different groups of tubules were made using an unpaired t test. Where two observations were made on the same tubule, these were compared using a paired t test.

RESULTS

Single kidney glomerular filtration rate. Inulin clearances of normal, remnant, and embolized kidneys are shown in Table I together with blood urea nitrogen (BUN) levels in the three groups of animals. Evidence that SNGFR was approximately doubled in the remnant kidneys is provided by the fact that, although the mass of the remnant kidney was reduced to approximately one-sixth of normal, the mean GFR of these kidneys was approximately one-third of normal by three weeks ($P < 0.001$ vs. normal). In addition, mean BUN, which was 114 ± 15.3 on the third post-

TABLE I
Single Kidney Inulin Clearances and BUN Concentrations of Rabbits with Normal, Remnant, and Embolized Kidneys

	Normal	Remnant	Embolized
Inulin clearance, ml/mm	3.1 ± 0.2	1.1 ± 0.1	2.0 ± 0.2
BUN, mg/dl	13.6 ± 1.3	69.1 ± 13.1	13.6 ± 1.8
<i>n</i>	(8)	(7)	(6)

Values are expressed as mean \pm SE.

operative day, was reduced to 69.1 ± 13.1 at the time of study. The GFR of the embolized kidneys was reduced to approximately two-thirds of normal ($P < 0.05$). Since all glomeruli were embolized and no areas of infarction were present, it can be assumed that SNGFR was uniformly decreased. In these animals, an increase in the GFR of the contralateral intact kidney prevented the development of azotemia.

Since the remnant-kidney animals differed from the other two groups in being chronically azotemic, a nonazotemic model of increased SNGFR was studied. In four uninephrectomized rabbits, a compensatory increase in GFR maintained BUN levels at 14.8 ± 1.4 mg/dl.

Indices of compensatory hypertrophy. Tubule size, expressed in protein per millimeter length or total cellular volume per millimeter length, is listed in Table II. Significant hypertrophy occurred in the PCT obtained from remnant kidneys ($P < 0.001$ vs. normal), whereas no difference in tubule size was observed between the normal and the embolized kidneys. The internal and external diameters of the remnant kidney PCT were significantly greater than in the normal PCT ($P < 0.01$). These diameters were not significantly different from normal in the embolized kidneys (Table III). In the uninephrectomized animals, protein content and cellular volume per millimeter were 0.45 ± 0.20 μ g and $15.63 \pm 2.11 \times 10^{-4}$ cm^3 , respectively. These values are comparable to those observed in the remnant kidney PCT.

Net water flux and potential difference of PCT perfused in vitro. Transtubular PD and net water fluxes (Jv) in the three groups of animals are listed in Table III. Each tubule was perfused in a bath of both normal and uremic rabbit serum. In none of the three groups were differences in PD or Jv observed between the two baths indicating that differences in transport rates were intrinsic to the tubular epithelium and independent of the peritubular environment.

In normal rabbit serum, mean net water flux was 1.00 ± 0.09 nl/min per mm or $1.6 \pm 0.12 \times 10^4$ nl/min per cm^2 in normal PCT. Jv per millimeter tubule length increased to 1.66 ± 0.16 in the hypertrophied PCT of the remnant kidneys ($P < 0.005$) (Fig. 1);

TABLE II
Indices of Compensatory Hypertrophy in PCT Obtained from Rabbits with Normal, Remnant, and Embolized Kidneys

	n	Protein content	Cellular volume
		$\mu\text{g/mm}$	$\times 10^{-4} \text{ mm}^3/\text{mm}$
Normal	(9)	0.21 ± 0.04	9.58 ± 0.51
Remnant	(9)	0.49 ± 0.15	16.47 ± 1.43
Embolized	(7)	0.23 ± 0.02	10.90 ± 1.22

Mean \pm SE.

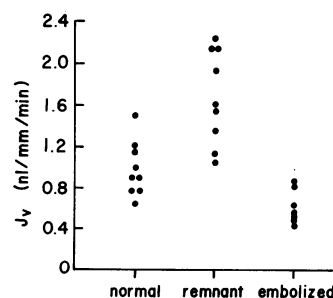


FIGURE 1 Net water flux per millimeter tubule length (Jv) in superficial proximal convoluted tubules from normal, remnant, and embolized kidneys perfused in a bath of normal rabbit serum.

Jv per square centimeter luminal surface area was not different from normal. PD was also increased significantly in this group of tubules ($P < 0.005$) (Fig. 2). Jv, in four normal PCT bathed in uremic serum and perfused with a uremic ultrafiltrate, was 1.08 ± 0.18 nl/min per mm. Since the increased rate of fluid transport per unit length observed in the remnant PCT could be related either to the chronic increase in SNGFR (or some concomitant thereof), or to an undetermined effect of exposure to a "uremic" milieu, additional studies were performed on hypertrophied PCT obtained from the four nonazotemic uninephrectomized rabbits. Mean PD was -6.8 ± 0.8 mV and Jv was 1.38 ± 0.08 nl/min per mm, values which are also increased above those observed in the normal kidney ($P < 0.01$).

In contrast to the above, both Jv [expressed per unit length ($P < 0.01$) or per unit luminal surface ($P < 0.005$)] and PD ($P < 0.01$) (Table III; Figs. 1 and 2) were decreased in the PCT obtained from the embolized kidneys. In this group, mean Jv was ~50% of the normal value.

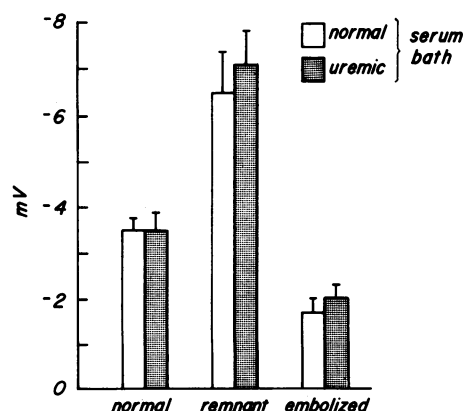


FIGURE 2 Transepithelial PD, lumen-negative, of superficial proximal convoluted tubules from normal, remnant, and embolized kidneys. Tubules were perfused in a bath of normal and uremic rabbit serum.

TABLE III
Transtubular PD and Net Jv of Superficial PCT Obtained from Normal, Remnant, and Embolized Rabbit Kidneys

	Length	i.d.	o.d.	Lumen surface area	Perfusion rate	Transtubular PD		Jv		Jv	
						NS	US	NS	US	NS	US
	mm	μm	μm	$\times 10^{-4} \text{ cm}^2$	nl/min	mV	mV	nl/min/mm	nl/min/mm	$\times 10^4 \text{ nl/min/cm}^2$	$\times 10^4 \text{ nl/min/cm}^2$
Normal kidneys	0.71	20.5	38.9	4.57	15.35	-3.9	-5.0	1.48	1.35	2.29	2.09
	0.70	19.4	38.9	4.26	22.04	-3.5	-3.5	1.13	1.37	1.85	2.25
	0.81	21.0	39.2	5.34	21.21	-4.0	-4.1	0.91	1.18	1.38	1.74
	1.64	20.8	45.0	10.71	14.74	-4.1	-4.0	1.06	1.08	1.62	1.65
	0.87	18.4	36.8	5.03	13.96	-2.1	-2.0	0.63	0.83	1.09	1.43
	1.09	16.3	37.8	5.60	14.59	-3.0	-3.0	0.77	0.76	1.49	1.47
	1.38	18.4	37.4	7.97	38.76*	-4.3	-2.8	0.77	0.63	1.33	1.09
	1.15	22.4	34.9	8.09	35.65*	-4.5	-4.0	1.05	1.03	1.49	1.46
	1.57	20.5	41.0	10.11	41.20*	-3.0	-3.9	1.20	0.98	1.86	1.86
Mean	1.10	19.7	39.0	6.85	—	-3.6	-3.6	1.00	1.20	1.60	1.67
\pm SEM	0.12	0.6	7.1	0.81	—	0.2	0.3	0.09	0.08	0.12	0.12
Remnant kidneys	0.89	31.5	54.0	8.81	17.02	-6.2	-7.4	2.24	2.00	2.26	2.02
	0.78	18.5	40.5	4.53	25.02	-9.5	-12.5	1.37	1.12	2.35	1.92
	0.72	37.5	54.0	8.48	23.89	-8.0	-6.5	2.16	3.68	1.83	3.12
	1.48	27.0	54.0	12.55	26.89	-8.0	-7.0	1.94	1.89	2.28	2.22
	0.74	23.2	53.0	5.39	14.75	-9.6	-1.1	1.60	1.75	2.19	2.40
	0.76	40.5	67.5	9.67	12.98	-2.4	-3.0	2.15	2.08	1.68	1.63
	0.85	20.5	48.4	5.47	36.72*	-6.7	-7.2	1.14	1.36	1.77	2.11
	1.21	22.5	54.2	8.55	32.80*	-3.5	-4.0	0.96	—	1.35	—
	1.03	31.5	45.5	10.19	36.42	-5.4	-6.4	1.34	—	1.35	—
Mean	0.94	28.1	52.3	8.18	—	-6.6	-7.2	1.66	1.98	1.89	2.20
\pm SEM	0.09	2.5	2.5	0.87	—	0.8	0.5	0.16	0.29	0.13	0.18
Embolized kidneys	1.14	22.2	39.6	7.95	23.16	-1.4	-1.6	0.65	0.51	0.93	0.73
	1.11	28.9	39.9	10.07	12.54	-2.6	-4.0	0.82	0.61	0.90	0.67
	0.76	20.2	44.4	4.82	21.95	-2.5	-3.0	0.48	0.76	0.75	1.19
	0.93	20.2	44.4	5.90	23.99	-1.0	-1.0	0.51	0.50	0.80	0.79
	0.67	26.2	48.4	5.51	21.85	-1.0	-1.0	0.44	1.15	0.56	1.39
	0.71	19.0	36.0	4.24	33.00*	-1.5	-1.5	0.84	0.47	1.41	0.78
	0.89	14.2	36.4	3.97	34.76*	-2.2	-2.0	—	0.53	—	1.18
Mean	0.89	21.5	41.3	6.06	—	-1.7	2.0	0.62	0.57	0.89	0.96
\pm SEM	0.07	1.8	1.7	0.83	—	0.3	0.4	0.07	0.13	0.12	0.11

* Tubules perfused at rapid perfusion rates.

NS, normal rabbit serum.

US, uremic rabbit serum.

Net water flux, Jv, is expressed per unit tubule length (nanoliters per minute per millimeter) and per unit lumen surface area (nanoliters per minute per square centimeter).

Permselectivity to sodium and chloride. By imposing a 50-mM NaCl gradient from lumen-to-bath, a change in steady-state PD reflects the relative permeability of the PCT to sodium and chloride. An increase in lumen negativity indicates $P_{\text{Na}} > P_{\text{Cl}}$, whereas a decrease indicates $P_{\text{Cl}} > P_{\text{Na}}$.

As shown in Table IV, the first millimeter of the normal PCT was Na selective (lumen negativity increased), whereas the late segment was chloride selective (lumen negativity decreased). In both the

remnant and the embolized kidneys, there was reversal of normal permselectivity in the late segments so that P_{Na} was observed to be greater than P_{Cl} . (Since the mobility of Cl is ~ 1.5 times that of Na, the apparently equal Na and Cl permeabilities in the embolized group, in which PD did not change significantly upon imposing a NaCl gradient, is indicative of Na permselectivity.) In both of these groups, the first millimeter of the superficial PCT was also Na selective.

In late juxtamedullary segments, the PD recorded

TABLE IV
Transtubular PD in the Absence and Presence of a Lumen-to-Bath Sodium Chloride Gradient in Late and Early Superficial PCT of Normal, Remnant, and Embolized Kidneys

	Late proximal		Early proximal	
	No gradient	50 mM gradient*	No gradient	50 mM gradient
	mV	mV	mV	mV
Normal	-4.0±0.5	-1.3±1.5	-4.0±0.1	-5.8±0.8
<i>P</i>	<0.5		<0.5	
Remnant	-5.8±0.9	-7.3±1.4	-6.0±1.0	-7.8±0.9
<i>P</i>	<0.025		<0.01	
Embolized	-2.6±0.4	-1.1±0.6	-2.1±0.3	-5.6±1.1
<i>P</i>	NS		<0.02	

* PD is corrected for a liquid junction by +3.5 mV. Early proximal refers to the postglomerular segment (1st mm); late proximal refers to segments that include the 2nd and 3rd mm of the PCT.

in the absence and presence of a NaCl gradient respectively were: normal -4.9 ± 0.5 mV and -6.7 ± 0.6 mV ($P < 0.001$); remnant -5.6 ± 0.9 mV and -6.8 ± 0.8 mV ($P < 0.02$); embolized -2.4 ± 0.4 mV and -3.8 ± 0.8 mV ($P < 0.5$). Thus, $P_{Na} > P_{Cl}$ in all three groups.

DISCUSSION

The adjustments in proximal tubular function that occur in various forms of experimental renal disease appear to parallel changes in the filtration rates of the glomeruli that delivered filtrate into these segments of the nephron. In models of reduced nephron mass, for instance, SNGFR is uniformly increased (4–7) and this is accompanied by an increase in absolute fluid reabsorption by the proximal tubule (4–7). In models of experimental glomerulonephritis, marked heterogeneity of SNGFR is evident within the same kidney and, yet, proximal glomerulotubular balance is the same from nephron to nephron (1–3, 8). Ichikawa et al. (8) have recently provided preliminary evidence that this close coupling of glomerular and tubular function can be explained, in part, by differences in efferent arteriolar oncotic pressure and initial peritubular capillary flow rate.

The present study was designed to examine whether intrinsic changes in proximal tubular function accompany chronic changes in SNGFR independently of the peritubular and humoral factors that influence reabsorptive function. Stated simply, if SNGFR is increased or decreased, is intrinsic proximal tubular function programmed to parallel such a change and, if so, can this be demonstrated in an in vitro situation in which the peritubular environment of the tubule is controlled? Precedent for this approach is provided by previous studies from this laboratory in which a relationship between fluid reabsorption and compen-

satory hypertrophy was observed in the rabbit proximal straight tubule studied in vitro (9).

Two experimental settings were selected in which a uniform increase and decrease in SNGFR were experimentally induced in the rabbit kidney. Superficial proximal convoluted tubules were removed from these kidneys and studied in vitro. Each tubule was exposed to both a normal and a "uremic" milieu³ provided by serum obtained from normal and subtotal nephrectomized rabbits. Both experimental models, i.e., the remnant kidney and the embolized kidney, are characterized by uniform involvement of all nephrons so that the single proximal tubule studied was regarded as being representative of the whole kidney.

The values for transtubular PD and J_v obtained in normal PCT in this study are comparable to those reported by other workers (15, 16). Characteristically, a reasonably wide variation in these normal values was found (16). Perfusion rate was ~ 20 nl/min in most experiments. In order to simulate the high flow rates observed in the remnant kidney in vitro, perfusion rate was increased to ~ 35 nl/min in certain experiments in each group. This had no effect on J_v in confirming the fact that the reabsorptive rate of the PCT in vitro is not simply a function of perfusion rate (15).

In the remnant kidneys, the mean transtubular PD of ~ -7 mV was significantly greater than the normal value observed in the present and other studies

³ We have recently found that uremic rabbits, maintained on an ad lib. diet, do not develop secondary hyperparathyroidism (unpublished observations). Hyperparathyroidism only occurs if dietary phosphorus intake is augmented. The uremic sera used in this study are from animals on an ad lib. intake and thus do not have increased parathyroid hormone concentrations.

(13–18). Such values have not been observed in the superficial PCT under any other circumstances, but have been described in juxtamedullary PCT (13).

Differences in the permselectivity to Na and Cl (a property of the paracellular pathway) have been observed between superficial and juxtamedullary PCT; the late superficial PCT is chloride selective, whereas the late juxtamedullary PCT is sodium selective (13). It was therefore important to establish whether the increased PD of the superficial PCT of the remnant kidney was associated with a reversed permselectivity similar to that observed in the late juxtamedullary PCT. The present studies confirm previous observations on the normal PCT, although it should be noted that the changes in PD induced by a lumen-to-bath NaCl gradient are smaller than those described by Jacobson and Kokko (13). In contrast, P_{Na} is greater than P_{Cl} in the late superficial PCT of the remnant kidneys. In the embolized kidneys, P_{Na} was also greater than P_{Cl} . Sodium selectivity was observed in the first millimeter of the superficial PCT and in the late juxtamedullary PCT of all three groups.

The reversal of the normal permselective properties in the late PCT of the remnant and embolized kidneys could not provide a unifying explanation for the observed changes in J_v . It has been argued that neutral organic solute-sodium cotransport depolarizes the luminal cell membrane and generates a lumen-negative potential difference. The sodium ion, having gained access to the peritubular surface, could either diffuse back into the lumen or chloride could diffuse out of the lumen through the paracellular pathway. If the tubule is more permeable to chloride than it is to sodium, the majority of current flow will be in the form of chloride ions moving out of the lumen. If the permselectivity is reversed, the majority of sodium ions will be recycled back into the lumen (19). Thus, in chloride-selective tubules, the reabsorption of organic solutes leads to significant NaCl reabsorption, whereas in sodium-selective tubules, little NaCl is reabsorbed secondary to organic solute reabsorption.

In the present study, the chloride selectivity of the late proximal tubule was converted to sodium selectivity in both disease models. In the remnant model, J_v was normal (or increased), whereas in the embolized model, it was decreased. Thus, the permselectivity alone cannot explain the differences in J_v between the two models.

Berry et al. (20) have also pointed out that anion concentration gradients could drive an important diffusive flux of sodium chloride through the paracellular pathway, but the length of tubules perfused, the composition of the perfusate, and the rate of perfusion would not have led to the generation of anion gradients in these studies.

The altered transtubular PD in the disease models

could be explained by one or more of a number of changes in the epithelium. The increased PD of the remnant PCT, for instance, could be due to an increase in electrogenic sodium transport across the peritubular membrane, to depolarization of the luminal membrane, or to a decrease in shunting through the paracellular shunt pathway. Since the resistance of the shunt pathway is largely determined by the cross-sectional area of the junctional complexes (21), it is likely that, in the disease models, changes in tubular geometry alone could significantly alter the PD and permeability of the tubule.

Among the mechanisms underlying the observed increases in reabsorptive rate and PD could be (a) the *in vivo* chronic uremic state, (b) compensatory hypertrophy of the tubule, and (c) an undetermined “programming” of tubular function by a chronic change in SNGFR or some concomitant thereof. In order to evaluate the role of chronic uremia *per se*, uninephrectomized animals were studied. These animals had normal BUN levels 3 wk after the nephrectomy indicating that the SNGFR of the nephrons of the remaining kidney had increased significantly. The proximal tubules of these kidneys had hypertrophied in a manner similar to that observed in the remnant kidneys. Despite the existence of a nonazotemic state, both J_v and PD increased in these uninephrectomy PCT effectively eliminating the uremic state from consideration as an important underlying cause for the changes.

The hypertrophy observed in the PCT of the remnant and uninephrectomy kidneys appears to offer an explanation for the increased J_v per unit length in these models, because J_v increased proportionally to the increase in luminal surface area. It should be pointed out, however, that measurement of the internal tubular diameter and calculation of the apparent reabsorptive surface area in no way takes into account the fact that the true reabsorptive surface of the proximal tubule is the brush border of the apical membrane. It may, therefore, be overly simplistic to suppose that there is a direct relationship between the true and the apparent luminal surface area.

In the embolized-kidney PCT, both PD and J_v were significantly lower than normal. Once again, the animals were nonuremic. However, in contrast to the association between J_v and tubule size observed in the hypertrophied PCT, tubule size was not different from normal. The decreased rate of fluid reabsorption could thus be ascribed neither to a decrease in the size of the tubule nor to chronic uremia.

From the above, it appears as if an alteration in intrinsic proximal tubular function is induced by a chronic change in the filtration rate of the glomerulus, which feeds into it, or to a peritubular event associated with such a change. Whether the change is in-

duced by the chronic alteration in "filtered load" per se or by some consequence of the altered filtration rate such as peritubular capillary blood flow, peritubular oncotic pressure, etc., is not clear. It is evident, however, that the PCT demonstrates a memory of its in vivo pattern of function and continues to operate accordingly in an in vitro situation.

Preliminary evidence supportive of the concept that the function of the proximal tubule can be modified by altering its in vivo environment has recently been presented by Knepper and Burg (22), who found that fluid reabsorption by proximal straight tubules is enhanced by deoxycorticosterone acetate pretreatment of the rabbits only if they are maintained on a normal salt intake. Salt deprivation abolished this effect and no relationship between endogenous aldosterone levels and fluid reabsorption could be discerned. It was concluded that some consequence of deoxycorticosterone administration such as changes in filtration rate, rather than a direct effect of the mineralocorticoid, leads to this alteration in intrinsic tubular function.

The link between changes in filtration rate and intrinsic tubular reabsorption remains obscure. While compensatory hypertrophy per se could be an important factor in enhancing fluid reabsorption, tubular size alone does not explain the reduction observed in the model of decreased GFR. Similarly, neither the presence of chronic uremia nor the composition of the peritubular milieu can account for the adaptations observed. Somehow, proximal tubular function is programmed to adapt to the increased or decreased filtered load imposed by the disease state and the memory of this function persists in vitro.

ACKNOWLEDGMENTS

We acknowledge the excellent secretarial assistance of Esther Vanegas and Roberta Franklin.

This work was supported by grants 5 R01 AM 26098 and 5 R01 AM 25301 from the National Institutes of Health. Dr. Fine is the recipient of a Research Career Development Award from the National Institutes of Health.

REFERENCES

1. Rocha, A., M. Marcondes, and G. Malnic. 1978. Micropuncture study in rats with experimental glomerulonephritis. *Kidney Int.* 3: 14-23.
2. Allison, M. E. M., C. B. Wilson, and C. W. Gottshalk. 1974. Pathophysiology of experimental glomerulonephritis in rats. *J. Clin. Invest.* 53: 1402-1423.
3. Lubowitz, H., D. C. Mazumdar, J. Kawamura, J. T. Crosson, F. Weisser, D. Rolf, and N. S. Bricker. 1974. Experimental glomerulonephritis in the rat. Structural and functional observations. *Kidney Int.* 5: 356-364.
4. Schultze, R. G., F. Weisser, and N. S. Bricker. 1972. The influence of uremia on fractional sodium reabsorption by the proximal tubule of rats. *Kidney Int.* 2: 59-66.
5. Weber, H., K. Y. Lin, and N. S. Bricker. 1975. Effect of sodium intake on single nephron glomerular filtration rate and sodium reabsorption in experimental uremia. *Kidney Int.* 8: 14-20.
6. Wen, S. F., N. L. M. Wong, R. L. Evanson, E. A. Lockhart, and J. H. Dirks. 1973. Micropuncture studies of sodium transport in the remnant kidney of the dog. The effect of graded volume expansion. *J. Clin. Invest.* 52: 386-397.
7. Buerkert, J., D. Martin, J. Rasad, S. Chambless, and S. Klahr. 1979. Response of deep nephrons and the terminal collecting duct to a reduction in renal mass. *Am. J. Physiol.* 236: F454-F464.
8. Ichikawa, I., J. R. Hoyer, and B. M. Brenner. 1980. Intact nephron hypothesis in glomerulonephritis: mechanism of glomerulotubular (GT) balance. *Clin. Res.* 28: 534A. (Abstr.).
9. Fine, L. G., W. Trizna, J. J. Bourgoignie, and N. S. Bricker. 1978. Functional profile of the isolated uremic nephron. Role of compensatory hypertrophy in the control of fluid reabsorption by the proximal straight tubule. *J. Clin. Invest.* 60: 1508-1518.
10. Fine, L. G., D. Schlondorff, W. Trizna, R. M. Gilbert, and N. S. Bricker. 1978. Functional profile of the isolated uremic nephron. Impaired water permeability and adenylate cyclase responsiveness of the cortical collecting tubule to vasopressin. *J. Clin. Invest.* 60: 1519-1527.
11. Fine, L. G., N. Yanagawa, R. G. Schultze, M. Tuck, and W. Trizna. 1979. Functional profile of the isolated uremic nephron. Potassium adaptation in the rabbit cortical collecting tubule. *J. Clin. Invest.* 64: 1033-1043.
12. Chonko, A. M., R. W. Osgood, A. E. Nickel, T. F. Ferris, and J. H. Stein. 1975. The measurement of nephron filtration rate and absolute reabsorption in the proximal tubule of the rabbit. *J. Clin. Invest.* 56: 232-235.
13. Jacobson, H. R., and J. Kokko. 1976. Intrinsic differences in various segments of the proximal convoluted tubule. *J. Clin. Invest.* 57: 818-825.
14. Jacobson, H. R. 1976. Characteristics of volume reabsorption in rabbit superficial and juxtamedullary proximal convoluted tubule. *J. Clin. Invest.* 63: 410-418.
15. Burg, M. B., and J. Orloff. 1968. Control of fluid reabsorption in the renal proximal tubule. *J. Clin. Invest.* 47: 2016-2024.
16. Grantham, J. J., J. M. Irish III, and D. A. Hall. 1978. Studies of isolated renal tubules in vitro. *Am. Rev. Physiol.* 40: 249-277.
17. Burg, M. B., and J. Orloff. 1970. Electrical potential difference across proximal convoluted tubules. *Am. J. Physiol.* 219: 1714-1716.
18. Cardinal, J., M. Lutz, M. B. Burg, and J. Orloff. 1975. Lack of relationship of potential difference to fluid reabsorption in the proximal tubule. *Kidney Int.* 7: 94-102.
19. Berry, C. A., and F. C. Rector, Jr. 1980. Active and passive sodium transport in the proximal tubule. *Mineral Electrolyte Metab.* 4: 149-160.
20. Berry, C. A., D. G. Warnock, and F. C. Rector, Jr. 1978. Ion selectivity and proximal salt reabsorption. *Am. J. Physiol.* 235: F234-F245.
21. Reuss, L., and A. L. Finn. 1974. Passive electrical properties of toad urinary bladder epithelium. Inter-cellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* 64: 1-25.
22. Knepper, M. A., and M. B. Burg. 1980. Increased fluid reabsorption by proximal tubules following deoxycorticosterone acetate (DOCA) administration. *Clin. Res.* 28: 453A. (Abstr.).