A Micropuncture Study of Potassium Excretion by the Remnant Kidney

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ABSTRACT In order to study the mechanism of enhanced potassium excretion by the remaining nephrons of the remnant kidney, micropuncture and clearance experiments were carried out in rats after surgical ablation of 3/4 of the total renal mass. The potassium intake in all animals was approximately 5 meq/day. Animals were studied 24 h and 10–14 days after 3/4 nephrectomy. Balance measurements in the chronic animals before micropuncture study indicated that 24 h K^+ excretion by the remnant kidney was equal to that of the two kidneys before ablation of renal mass. Measurements of distal tubular inulin and potassium concentrations revealed progressive reabsorption of potassium in this segment of the nephron in both the 24-h and chronic 3/4-nephrectomized rats, as well as in normal control rats. A large increase in tubular fluid potassium content occurred between the end of the distal tubule and the final urine in the 3/4-nephrectomized rats, but not in the normal controls. These observations suggest that the segment of the nephron responsible for enhanced potassium excretion by remaining nephrons was the collecting duct.

In additional experiments, potassium was completely eliminated from the diet of chronic 3/4-nephrectomized rats before micropuncture study. In these animals, no addition of K^+ occurred beyond the distal tubules. Normal rats infused with 0.15 M KCl to acutely elevate serum K^+ concentration, demonstrated reabsorption of K^+ in the distal tubule and a large addition of K^+ to the urine beyond the distal tubule.

We conclude that the collecting duct is the major site of regulation of urinary potassium excretion in normal rats and is responsible for the adaptation to nephron loss by the remnant kidney.

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INTRODUCTION

It is well known that patients with advanced renal disease often are able to maintain their serum potassium concentration within the normal range until renal failure progresses to the stage of oliguria (1). Since this balance occurs even without severe restriction of potassium intake, it seems clear that the diseased kidney is able to adjust to the decrease in its functioning mass by increasing the rate of potassium excretion by the surviving nephrons. It is this increased excretion rate per nephron which allows the patient to remain in potassium balance and to avoid hyperkalemia throughout what may be many years of advancing renal failure.

The mechanism of the alteration in potassium excretion by the diseased kidney is unknown. Recently, Schultze and co-workers (2) studied this problem in dogs by reducing the nephron mass experimentally. They found that within 18 h after removal of an intact kidney, the contralateral kidney with a reduced functioning nephron population (due to ligation of branches of the renal artery) increased its rate of potassium excretion markedly to a range almost equal to that of the two kidneys before the nephrectomy. This response was found to be independent of mineralocorticoid activity and sodium intake, and it persisted after acute reduction of glomerular filtration rate (GFR) of the surviving kidney.

The present micropuncture experiments in rats were undertaken to determine the site along the nephron responsible for increased potassium excretion after reduction of renal mass, and to attempt to elucidate the mechanism. Distal tubular sodium, potassium, and inulin concentrations were measured in normal control rats and in animals 24 h or 10–14 days (chronic animals) after excision of 3/4 of the total renal mass (hemi-nephrectomy and contralateral nephrectomy). Balance studies in the chronic animals demonstrated that daily

urinary K^+ excretion by the remnant kidney was equal to that of the two normal kidneys before 3/4 nephrectomy. In all groups of rats, progressive reabsorption of K^+ was observed along the length of the distal convoluted tubule. In the normal control rats, no further reabsorption or secretion of K^+ was found beyond the distal tubule. In the experimental rats, studied both 24 h and 10–14 days after 3/4 nephrectomy, a large amount of K^+ was added to the urine beyond the end of the distal tubule, presumably due to secretion by the collecting ducts. The collecting duct secretory mechanism appeared to be markedly sensitive to changes in potassium intake and/or serum K^+ concentration in chronic 3/4-nephrectomized rats, but also could be activated acutely in normal rats by i.v. KCl infusion.

METHODS

White male Sprague-Dawley rats weighing 225–350 g were maintained in individual metabolic cages. All animals except those specified below were fed a "potassium-free" diet (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) and were given 50 ml daily of a 100 meq/liter KCl, 5% sucrose drinking solution. The diet contained 0.18 meq/g of Na^+ and 0.003 meq/g of K^+. The drinking solution was provided in a graduated J-shaped drinking tube. Since the animals usually drank most or all of the 50 ml ration, they ingested approximately 5 meq KCl daily.

Chronic 3/4-nephrectomy rats. In six rats, after they had been ingesting the diet and drinking solution for at least 1 wk, 24 h K^+ intake and urinary excretion were measured on 3 consecutive days. 3/4 nephrectomy was then carried out on these six rats as follows: Under light ether and i.p. Inactin (Promonta, Hamburg, West Germany) anesthesia, the right kidney was excised and the cephalad half of the left kidney ablated by a silk ligature. This latter procedure left much of the ventral surface of the experimental kidney undisturbed, to permit micropuncture collections from the surface nephrons. The abdominal wound was sutured and the animal returned to the metabolic cage where the same diet and drinking solution were provided. 7–10 days later, 24 h K^+ intake and urinary excretion were again measured for 3 consecutive days. The day after the last balance study, micropuncture, and renal clearance measurements were obtained from the remnant kidney, as described below.

In three additional rats, 3/4 nephrectomy was performed as above and the animals maintained on the same diet and drinking solution for 4–8 days after surgery. However, tap water was substituted for the KCl drinking solution 24 h before micropuncture study. Three other rats were fed a regular rat pellet diet ($Na = 0.15$ meq/g, $K = 0.27$ meq/g) before and after 3/4 nephrectomy. On the day of micropuncture study, 5–7 days after renal ablation, these three rats were infused i.v. with 0.15 M KCl at 0.05 ml/min continuously throughout the experiment in order to elevate serum K^+ concentration.

Animals studied 1 day after 3/4 nephrectomy. Micropuncture and clearance studies were carried out in five rats 24 h after 3/4 nephrectomy. These animals had been maintained on the same K^+-free diet and KCl drinking solution for at least 3 days (usually 5–7 days) before renal ablation. The surgical procedure of 3/4 nephrectomy was the same as in the chronic animals. After surgery, the animals were returned to their metabolic cages where the diet and drinking solution were provided for the next 24 h. At the end of this period, micropuncture and clearance measurements were obtained from the remnant kidney, as described below.

Control rats. Control studies were performed in 10 normal rats. In seven of these, the potassium-free diet and KCl drinking solution were the same as above, and were provided at least 3 days (usually 5–7 days) before micropuncture study. The other three normal rats were maintained on a regular rat pellet diet and tap water. In these three rats, 0.15 M KCl was infused i.v. at 0.05 ml/min throughout the micropuncture experiment, in order to acutely elevate serum K^+ concentration.

In all animals, on the day of micropuncture study, anesthesia was produced with i.p. Inactin, 10 mg/100 g. A jugular vein was cannulated with PE 50 polyethylene tubing for administration of i.v. fluids and a second PE 50 tubing was inserted into the same vein for injection of FD and C green no. 3 dye (Keystone Aniline & Chemical Co., Chicago, Ill.). A carotid artery was cannulated with heparinized PE 50 tubing connected to a Statham strain gauge (model P 23 Dc, Statham Instruments, Inc., Oxnard, Calif.) for continuous recording of blood pressure by a Grass polygraph (model 5D, Grass Instrument Co., Quincy, Mass.). A volume of Ringer's lactate solution equal to 1% of body weight was given i.v. over a 2–3 min period. In all animals except those given 0.15 M KCl infusion (see above), a constant i.v. infusion of Ringer's lactate was administered at 0.05 ml/min. [*Carboxyl*-^{14}C]Inulin, and in most experiments, [*glycyl*-2-^{3}H]*p*-aminohippuric acid (PAH) (New England Nuclear, Boston, Mass.), were added to the infusion. The concentrations of these compounds were estimated to yield dpm at least three times that of the background in the smallest samples measured. *d*-Aldosterone, 20 μg/kg per h (Ciba Pharmaceutical Co., Summit, N. J.), was added to the infusion in all experiments.

The left kidney was exposed through a small lateral abdominal incision, the kidney dissected free of perirenal adipose tissue, and placed in a leucite cup attached to the animal table. The kidney was immobilized by silicone grease (Dow Corning Corp., Midland, Mich.) or cotton packed between it and the kidney cup. Mineral oil warmed to 38°C flowed continuously over the kidney. A Fiber optic light system was used to illuminate the kidney surface. A PE 50 polyethylene catheter was inserted into the left renal pelvis for collection of timed urine specimens. In all animals, body temperature was monitored continuously via a rectal thermistor and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) and was maintained between 37–38°C by adjusting the heat of the animal table. Arterial blood samples were collected from the carotid artery catheter at the beginning and end of the experiment for measurement of pH, total CO_2, sodium, potassium, and urea. The same determinations were carried out on most urine samples. Arteriolized blood was collected from the cut end of the tail in heparinized capillary tubes at approximately 30-min intervals throughout the experiment for analysis of radioactivity.

Tubular fluid was collected from surface distal convoluted tubules only. The distal convoluted tubules were identified by i.v. injection of 0.05 ml of 10% FD and C green dye, or by the appearance of their thin, glistening epithelium. A column of castor oil colored with Sudan black was injected into the distal tubular lumen after the direction of tubular fluid flow had been determined by injection of a few small droplets of oil. The oil column introduced was at least

TABLE I

*Plasma Acid-Base and Electrolyte Data in Normal and ¾-Nephrectomized Rats Studied 1 Day after Renal Ablation**

	pH	P_{CO_2}	HCO_3^-	Na^+	K^+	BUN
		mm Hg	*mmol/liter*	*meq/liter*	*meq/liter*	*mg/100 ml*
Normal rats						
Mean	7.41	34.5	20.7	147.5	3.8	17.8
±SE	0.01	0.7	0.6	1.1	0.06	1.3
(7)‡						
24-h ¾-nephrectomized rats						
Mean	7.37	28.5	16.0	142.4	5.5	77.6
±SE	0.02	1.2	0.5	1.9	0.3	7.4
(5)‡						
P	NS	<0.01	<0.01	<0.02	<0.01	<0.001

* Data obtained during micropuncture study. Infusion was Ringer's lactate at 0.05 ml/min.

‡ Number in parenthesis = number of animals.

10-tubule diameters in length, in order to prevent contamination of the collection by retrograde flow of fluid. The collection was timed with a stopwatch, and usually lasted between 5 and 10 min. The collection was made at a rate which maintained the oil block in place, by adjusting the level of a mercury bulb connected to the micropipette. At the end of the collection, the pipette was withdrawn from the lumen and its tip sealed by drawing in mineral oil from the surface of the kidney. The tubule was then injected with a colored latex compound (General Biological, Inc., Chicago, Ill.) for subsequent microdissection. Portions of 3 nl were taken from the tubular fluid samples for duplicate measurements of potassium and sodium with a Helium Glow photometer (American Instrument Co., Inc., Travenol Laboratories, Inc., Silver Springs, Md.). In preliminary tests with solutions containing sodium and potassium in varying concentrations, it was found that determinations for each of these electrolytes were accurate within ±5%. No interference was found between Na^+ and K^+ over a wide range of concentrations and results were highly reproducible from day to day. The remainder of the tubular fluid sample was transferred to a 0.1 mm ID constant-bore capillary tube (Corning Glass Works, Science Products, Div., Corning, N. Y.) for measurement of volume (3). The measured volume was corrected for the amount of fluid removed for the sodium and potassium determinations. The tubular fluid was then washed out of the capillary tube into liquid scintillation counting vials for measurement of ^{14}C and ^{3}H dpm (4). Radioactive counts were measured in a Nuclear-Chicago liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.), Unilux II A, and dpm calculated by the

TABLE II

*Urinary Electrolyte and Renal Hemodynamic Data in Normal Rats**

Rat no.	Sample no.	V	pH	HCO_3^-	$U_{Na}V$	U_KV	GFR	C_{PAH}	EF_{Na}	EF_K
		μl/min per kg		*mmol/liter*	*μeq/min per kg*	*μeq/min per kg*	*ml/min per kg*	*ml/min per kg*	%	%
1	U_1	47.0	6.72	4.40	6.16	3.03	8.04	8.80	0.49	10.48
	U_2	58.5	6.76	4.40	7.43	3.66	8.16	18.01	0.59	12.45
2	U_1	128.8	6.13	0.36	2.45	5.35	7.18	15.38	0.23	19.60
	U_2	139.0	6.16	0.37	2.71	5.21	9.15	17.55	0.20	15.00
3	U_1	35.1	6.98	9.54	3.22	2.28	10.98		0.21	5.81
	U_2	16.1			1.55	1.74	8.00		0.13	6.02
4	U_1	28.8	6.60	3.34	0.59	0.43	9.64		0.04	1.13
5	U_1	16.0	6.03	0.67	1.44	3.24	11.34		0.05	4.99
	U_2	30.6	5.87	0.70	0.64	1.24	7.36		0.06	4.30
6	U_1	59.2	5.95	0.29	0.30	0.26	4.92	6.53	0.04	1.42
	U_2	96.0	5.68	0.19	0.46	0.66	6.78	9.81	0.04	2.65
	U_3	44.8	5.93	0.36	0.31	0.71	4.26	15.46	0.05	4.54
7	U_1	38.6	5.57	0.20	0.20	3.09	13.08	36.44	0.04	6.43
	U_2	32.1	5.54	0.35	0.35	2.87	7.00	21.82	0.03	11.00
	U_3	37.7	5.52	0.53	0.53	6.52	8.92	22.34	0.04	19.36
Mean		53.9	6.10	1.84	1.89	2.69	8.32	17.21	0.15	8.35
±SE		9.8	0.13	0.72	0.57	0.50	0.60	2.72	0.04	1.56

V, urine flow rate; $U_{Na}V$, rate of sodium excretion; U_KV, rate of potassium excretion; GFR, glomerular filtration rate (inulin clearance); C_{PAH}, clearance of *p*-aminohippurate; EF_{Na}, excreted fraction of filtered sodium; EF_K, excreted fraction of filtered potassium.

* The data were obtained from the left (experimental) kidney. Values for excretion rates have been multipled × 2 to approximate rates for both kidneys.

TABLE III

*Urinary Electrolyte and Renal Hemodynamic Data in ¾-Nephrectomized Rats Studied 24 h after Renal Ablation**

Rat no.	Sample no.	V	pH	HCO_3^-	$U_{Na}V$	U_KV	GFR	C_{PAH}	EF_{Na}	EF_K
		μl/min per kg		*mmol/liter*	*μeq/min per kg*	*μeq/min per kg*	*ml/min per kg*	*ml/min per kg*	%	%
8	U_1	43.6	5.76	0.60	0.61	3.94	0.85	3.61	0.51	84.62
	U_2	52.6	5.68	0.14	1.08	5.26	0.90	4.92	0.85	106.70
9	U_1	32.8	6.45	1.59	0.43	2.49	0.89		0.37	42.77
	U_2	38.5	6.46	1.37	0.50	2.71	0.84		0.46	48.68
	U_3	33.3	6.34	1.78	0.33	2.83	0.76		0.33	53.77
10	U_1	31.7	6.61	4.28	1.08	1.94	0.91	3.93	0.81	44.97
	U_2	39.1	6.67	4.57	1.78	2.58	0.67	3.04	1.83	79.08
	U_3	33.7	6.45	3.04	1.26	2.81	0.79	3.57	1.10	70.13
	U_4	14.5			0.64	1.16	0.41	2.16	1.08	54.36
11	U_1	28.8	6.70	3.76	1.97	1.44	1.13	3.15	1.13	31.88
	U_2	35.7	6.31	2.29	2.05	2.14	1.83	4.59	0.75	28.74
	U_3	23.9	6.14	1.20	0.86	1.85	1.38	3.88	0.43	32.24
12	U_1	33.1	6.01	0.54	0.22	2.55	0.83	4.08	0.18	49.73
	U_2	39.6	5.99	0.70	0.28	4.08	0.85	3.98	0.23	74.66
	U_3	47.1	5.86	0.44	0.33	5.75	1.01	4.65	0.23	85.82
Mean		35.2	6.25	1.88	0.89	2.90	0.94	3.80	0.69	59.21
±SE		2.4	0.09	0.40	0.16	0.34	0.08	0.22	0.12	5.97

* Data obtained from single remaining remnant kidney.

channels ratio method, using ^{133}Ba as an external standard. From the labeled inulin concentrations in plasma (P) [1] (corrected for a water content of 94%) and tubular fluid (TF), single nephron glomerular filtration rates (SNGFR) were calculated using the expression:

$$SNGFR = TF/P_{In} \times TFR$$

where TF/P_{In} is the ratio of inulin in tubular fluid/plasma, and TFR is the tubular fluid flow rate (TFR). The SNGFR values were used as a check on the technique of collection of each tubular fluid sample. If fluid was inadvertently collected distal to the oil block, due to too short a column or incorrect determination of the direction of tubular fluid flow, unreasonably high values for SNGFR were obtained in comparison with other values from the same kidney. Such samples were discarded.

At the end of each experiment, the experimental kidney was removed, macerated in 50% HCl at 37°C for 2 h, and the latex casts of the distal tubules dissected out for determination of the puncture site.

Arterial blood and urine pH was measured at 37°C with a Metrohm pH meter (model E 322, Brinkmann Instruments, Inc., Westbury, N. Y.). Sodium and potassium concentrations in urine and blood were measured with a model 143 flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Total CO_2 in plasma and urine was measured with a Natelson microgasometer (Scientific Industries, Inc., Springfield, Mass.) and urea concentration by the method of Kaplan (5).

All calculations and statistical analyses were carried out with an Olivetti Programma 101 (Olivetti Corp. of America, New York), using appropriate computer programs.

RESULTS

Studies 1 day after 3/4 nephrectomy. The data on arterial blood acid-base parameters, sodium, potassium, and urea for the five 3/4-nephrectomized animals studied 1 day after renal ablation and seven normal control rats are shown in Table I. As can be seen, a slight metabolic acidosis was present in the 3/4-nephrectomized animals, partially compensated for by a reduction in P_{CO_2}. Serum K^+ was significantly elevated to 5.5 from a control value of 3.8 meq/liter. The blood urea nitrogen (BUN) had increased from a control level of 17.8 to 77.6 mg/100 ml.

The results of the renal hemodynamic and clearance studies in these two groups of animals are presented in Tables II and III. The excretion rates for the normal rats, shown in Table II, were obtained from the left kidney but have been multiplied times 2 in order to approximate total excretion for the two kidneys. Since excretion rates for the left (micropuncture) kidney might have been slightly lower than for the right, because of the surgical manipulation of the left kidney,

[1] *Abbreviations used in this paper:* SNGFR, single nephron glomerular filtration rate; TF/P_{In}, ratio of inulin in tubular fluid plasma; TFR, tubular fluid flow rate.

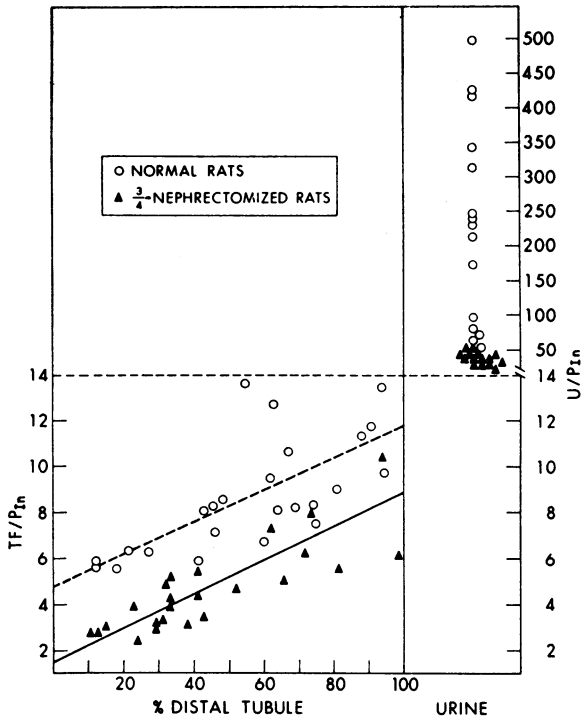

FIGURE 1 Distal TF/P_{In} ratios in normal and 3/4-nephrectomized rats studied 24 h after renal ablation. The interrupted and solid lines represent the calculated linear regression lines for the control and experimental groups respectively. U/P_{In} ratios are shown to the right.

the total excretion rates might be underestimated to some extent. When the data for the two groups of animals are compared, it can be seen that urine flow rate in the remnant kidney was about 65% of that for the two kidneys of the control animals. There was no significant difference in urine pH or HCO_3^- concentration. The mean sodium excretion rate was lower for the remnant kidney than for the two kidneys of the control rats, but the mean potassium excretion was almost identical (2.69±0.50 vs. 2.90±0.34 μeq/min per kg). GFR and clearance of PAH (C_{PAH}) were approximately 11 and 22% respectively of the total values in the control animals. The excreted fraction of filtered sodium (EF_{Na}) was slightly but significantly increased from 0.15 to 0.69 in the 3/4-nephrectomized rats ($P < 0.001$). Potassium excretion, expressed as a percent of the amount in the glomerular filtrate (EF_K), increased from 8.35% in the control animals to 59.21% in the experimental animals.

The micropuncture data obtained from distal convoluted tubules in these two groups of animals are presented in Figs. 1–3. In Fig. 1 are shown the TF/P_{In} ratios related to the site of puncture, and the corresponding linear regression lines for the control and 3/4-nephrectomized animals. As can be seen, TF/P_{In} was significantly lower along the length of the distal tubule in the 3/4-nephrectomized rats, as compared with the control animals, but the slopes of the two lines were almost identical. The data indicate that a larger fraction of the glomerular filtrate entered the beginning of the distal tubule and the collecting ducts in these 3/4-nephrectomized rats. A striking difference in fractional water reabsorption was also evident between the end of the distal tubule and the final urine, as indicated by the large difference in U/P_{In} between the two groups of animals.

The handling of sodium and potassium by the distal convoluted tubule of the control and 3/4 nephrectomized animals is shown in Figs. 2 and 3. As can be seen in

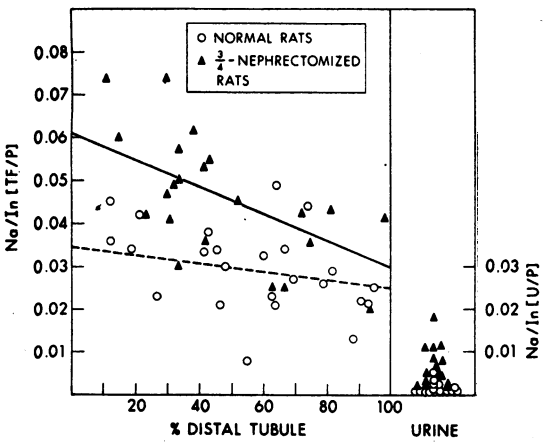

FIGURE 2 Fraction of filtered sodium remaining in distal tubule and final urine of control and 24 h 3/4-nephrectomized rats. The interrupted and solid lines are the linear regression lines for the control and experimental groups respectively.

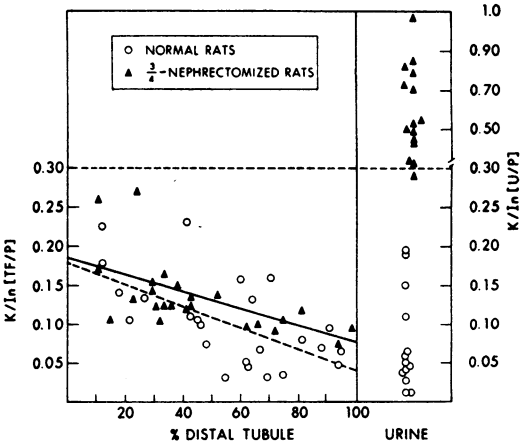

FIGURE 3 Fraction of filtered potassium remaining in distal tubule and final urine of control and 24 h 3/4-nephrectomized rats. The interrupted and solid lines are the linear regression lines for the control and experimental groups respectively.

Fig. 2, a somewhat larger fraction of filtered sodium entered the beginning of the distal tubule in the 3/4-nephrectomized rats, but by the end of the distal tubule, only 2–3% of the filtered sodium remained in the lumen in both groups of rats. The fraction of filtered sodium in the final urine was slightly higher in the experimental animals than in the controls.

Fractional reabsorption of potassium by the distal tubule, shown in Fig. 3, was closely comparable in the control and 3/4-nephrectomized rats. Under these experimental conditions, there was no evidence of potassium secretion along the distal tubule. Rather, as can be seen, progressive reabsorption of potassium was observed. In spite of the fact that serum K^+ was significantly higher in the 3/4-nephrectomized rats than in the normal controls, the fraction of filtered potassium remaining in the lumen at the end of the distal tubule was approximately the same in the two groups of rats (0.07 vs. 0.04). In striking contrast to the distal tubule findings, a large addition of potassium took place between the end of the distal tubule and the final urine in the 3/4-nephrectomized rats, whereas in the control animals, no definite reabsorption or secretion occurred beyond the distal tubule.

Studies 1–2 wk after 3/4 nephrectomy. The data on potassium intake and urinary excretion, measured in six rats before and after 3/4 nephrectomy, are shown in Table IV. Intake and output were measured for 3 consecutive days in each rat before 3/4 nephrectomy, and for a second 3-day period starting 7–10 days after the 3/4 nephrectomy. Potassium intake could be measured precisely, since virtually all of the K^+ ingested was via the drinking solution. As can be seen, before renal ablation, all rats consumed the entire daily 5 meq potassium ration and excreted 4.81 meq/day in their urine. Approximately 1 wk after 3/4 nephrectomy, their fluid consumption was slightly but not significantly lower, and their 24 h urinary K^+ excretion was almost identical with what it had been before 3/4 nephrectomy. These observations imply that the remnant kidney had adapted so that each of the remaining nephrons was excreting roughly four times as much potassium as before 3/4 nephrectomy.

The plasma acid-base and electrolyte data obtained from the same six chronic 3/4-nephrectomy rats at the time of micropuncture study are shown in Table V. Except for the BUN, all values were within the normal range, and the data are not significantly different from the normal control rats. In the chronic 3/4-nephrectomy rats, the BUN was moderately elevated to 25.6 mg/100 ml, as compared with 17.8 mg/100 ml in the control animals.

The urinary electrolyte and renal hemodynamic data obtained from the six chronic 3/4 nephrectomy rats are presented in Table VI. As can be seen, the GFR and C_{PAH} of the remnant kidney was considerably greater than in animals studied 24 h after 3/4 nephrectomy, implying that hypertrophy had occurred. Urinary pH and HCO_3^- concentration were not significantly different from the other groups of animals. The EF_K averaged 30.62%, a value which is significantly higher than in the normal control rats, but lower than in the 3/4-nephrectomized rats studied 24 h after surgery. The higher GFR in the chronic 3/4-nephrectomy animals accounts entirely for the lower EF_K, since U_KV was higher in these animals than in those studied 24 h after 3/4 nephrectomy.

The micropuncture data for the chronic 3/4-nephrectomy rats are shown in Figs. 4–7. Fig. 4 shows plotted TF/P_{In} ratios along the distal tubule for the six chronic animals maintained on a constant K^+ diet and infused with Ringer's lactate during micropuncture study. The regression lines for the normal control and 24 h 3/4-nephrectomy rats have been included for comparison. As can be seen, fractional reabsorption of water in the chronic 3/4-nephrectomized rats was closely comparable with that in the normal control animals, and was higher than in the 24 h 3/4-nephrectomized rats. The U/P_{In} ratios, shown to the right of the figure, were intermedi-

TABLE IV

*24 h K^+ Intake and Urinary Excretion (meq)**

	Control period	¾ Nephrectomy	*P*
Intake			
Mean	5.00	4.75	
±SD	0.87	0.63	>0.1
Output			
Mean	4.81	4.36	>0.5
±SD	2.17	1.06	

* Data obtained from six rats. Intake and output were measured for 3 consecutive days in the control period and 3 consecutive days starting 7–10 days after ¾ nephrectomy.

TABLE V

*Plasma Acid-Base and Electrolyte Data in Chronic ¾-Nephrectomized Rats**

	pH	P_{CO_2}	HCO_3^-	Na^+	K^+	BUN
		mm Hg	*mmol/liter*	*meq/liter*	*meq/liter*	*mg/100 ml*
Chronic ¾-nephrectomized rats						
Mean	7.47	30.9	21.4	144.6	4.2	25.6
±SE	0.03	3.8	1.3	2.4	0.1	4.0

* Data obtained in six rats during micropuncture study. Infusion was Ringer's lactate at 0.05 ml/min.

TABLE VI

*Urinary Electrolyte and Renal Hemodynamic Data in Chronic ¾-Nephrectomized Rats**

Rat no.	Sample no.	V	pH	HCO_3^-	$U_{Na}V$	U_KV	GFR	C_{PAH}	EF_{Na}	EF_K
		μl/min per kg		*mmol/liter*	*μeq/min per kg*	*μeq/min per kg*	*ml/min per kg*	*ml/min per kg*	%	%
13	U_1	21.4			2.08	1.99	1.04	3.49	1.40	45.60
	U_2	17.7			1.49	0.82	1.19	4.28	0.88	16.41
14	U_1	16.4	5.83		0.72	1.52	2.69	9.59	0.18	14.08
	U_2	23.8	5.91		1.89	1.58	2.43	8.52	0.55	16.29
15	U_1	20.3	5.78	1.68	2.34	1.99	1.26	4.67	1.35	35.00
	U_2	26.2	5.86		1.99	2.08	1.85	7.09	0.78	25.58
16	U_1	28.4	6.87	5.20	2.44	2.19	2.82	9.70	0.54	22.54
	U_2	16.9	7.04		2.19	1.16	1.42	4.87	0.98	23.58
17	U_1	44.8	5.99	0.70	0.94	5.78	5.45	19.38	0.12	26.20
	U_2	97.2	5.89	0.20	10.06	12.98	5.81	17.55	1.12	55.15
	U_3	75.7	5.87	0.70	8.78	8.29	4.66	14.60	1.28	43.92
	U_4	59.5	5.85		5.86	6.90	4.78	13.12	0.83	35.65
18	U_1	34.8	6.80	4.50	1.58	6.47	4.65	12.83	0.23	27.84
	U_2	50.2	6.56	3.10	2.13	9.04	4.95	13.79	0.29	36.48
	U_3	38.2	6.82	4.40	1.55	7.37	4.22	11.94	0.25	34.94
Mean		38.1	6.24	2.56	3.07	4.68	3.28	10.36	0.72	30.62
±SE		6.15	0.14	0.70	0.73	0.96	0.44	1.27	0.12	3.05

* Data obtained from single remaining remnant kidney.

ate between the normal rats and 24 h 3/4-nephrectomy rats.

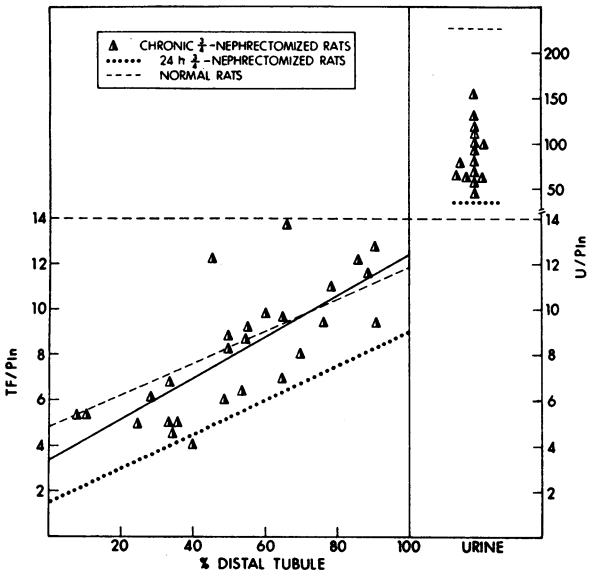

FIGURE 4 Distal TF/P_{In} ratios in chronic 3/4-nephrectomized rats studied 10–14 days after renal ablation. The solid line is the linear regression line for this group. The regression lines for the other groups are shown for comparison, as well as the mean U/P_{In} values.

Fig. 5 shows the data for fractional Na^+ excretion in the same six chronic 3/4 nephrectomy rats. The values along the distal tubule were closely comparable with those in the normal control rats, and were significantly lower than was found in the 3/4-nephrectomy rats studied 24 h after renal ablation. The EF_{Na}, shown to the right of the figure, averaged 0.72%, a value higher than in the normal controls (0.15%) but close to that in the 24 h 3/4 nephrectomy rats (0.69%).

Fig. 6 shows the data for fractional K^+ excretion in the same six chronic 3/4-nephrectomy animals. The regression lines for the normal controls and 24 h 3/4-nephrectomy animals have been included for comparison. As in the previous groups of rats, progressive reabsorption of K^+ was observed along the length of the distal tubule. Approximately 7% of the filtered K^+ remained in the lumen at the end of the distal tubule. A sharp rise in potassium content occurred beyond the end of the distal tubule, the average urinary EF_K being 30.6%.

The results of the experiments in which potassium was either completely eliminated from the diet for 24 h, or was infused i.v. to acutely elevate serum K^+ are shown in Fig. 7. These animals were studied 4–8 days after 3/4 nephrectomy. GFR for the remnant kidney averaged 3.21±0.22 ml/min per kg and C_{PAH} 10.29±0.98 ml/min per kg in these six rats. In the three animals deprived of potassium for 24 h before micropuncture

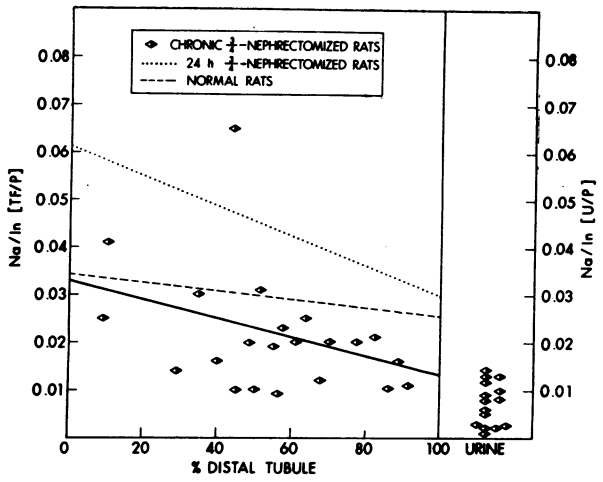

FIGURE 5 Fraction of filtered sodium remaining in distal tubule and final urine of chronic 3/4-nephrectomized rats. The solid line is the linear regression line for this group.

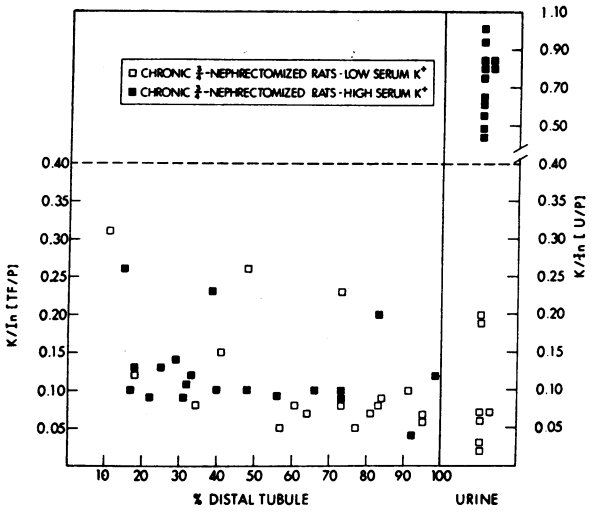

FIGURE 7 Fraction of filtered potassium remaining in distal tubule and final urine of chronic 3/4-nephrectomized rats. "Low K^+" rats were deprived of potassium for 24 h before study. "High K^+" rats were infused with 0.15 M KCl during study.

study, serum K^+ averaged 3.31 meq/liter (range 2.4–3.8). Blood pH ranged from 7.36 to 7.47 and plasma HCO_3^- from 18.4 to 20.0 meq/liter. In the three animals infused i.v. with 0.15 M KCl throughout the micropuncture study, serum K^+ averaged 5.94 meq/liter (range 4.5–7.5). Blood pH ranged from 7.29 to 7.48 and serum HCO_3^- from 14.3 to 19.2 meq/liter. As shown in Fig. 7, progressive reabsorption of potassium was observed along the distal tubule in both the low potassium and high potassium rats, without any discernible difference between the two groups. In the rats deprived of potassium, there was no addition of potassium to the urine beyond the distal tubule. In sharp contrast, a large increase in the excreted fraction of potassium occurred between the end of the distal tubule and the final urine in the rats infused with KCl.

In Fig. 8 are presented fractional potassium excretion data obtained in three normal rats fed a regular rat pellet diet and infused with 0.15 M KCl during micropuncture study. Serum K^+ concentration ranged from 4.7 to 7.7 meq/liter during the period of distal tubular fluid and urine collections. As in the other groups of rats, progressive reabsorption of K^+ was observed along the length of the distal convoluted tubule. Approximately

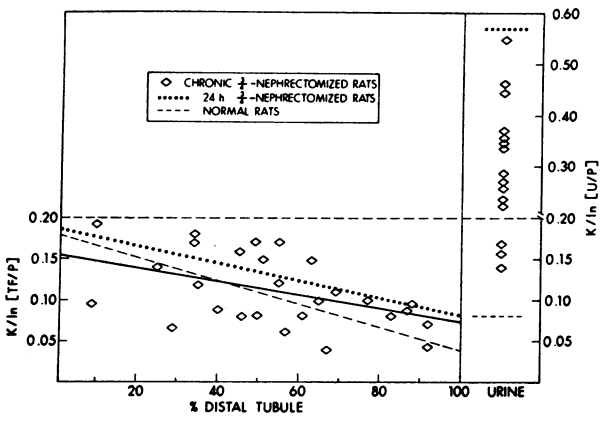

FIGURE 6 Fraction of filtered potassium remaining in distal tubule and final urine of chronic 3/4-nephrectomized rats. The solid line is the linear regression line for this group. The regression lines for the other groups are shown for comparison, as well as the mean K/In (U/P) values.

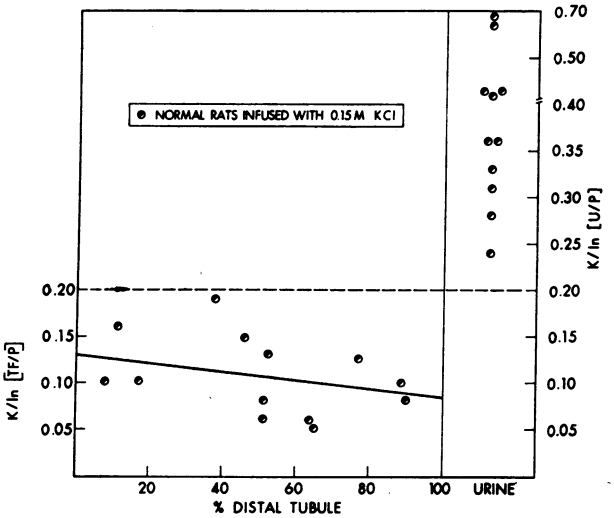

FIGURE 8 Fraction of filtered potassium remaining in distal tubule and final urine of normal rats infused with 0.15 M KCl.

8% of the filtered K^+ remained in the lumen at the end of the distal tubule. Urinary K^+ ranged from 24.0 to 66.1% of the filtered K^+, indicating that a large increase in urinary K^+ occurred beyond the distal tubules.

DISCUSSION

The results of the present study suggest that the collecting ducts play a major role in the adaptation in potassium excretion which occurs in response to a reduction in nephron mass. In 3/4-nephrectomized animals studied 24 h or 10–14 days after renal ablation, the distal convoluted tubules were found to reabsorb potassium progressively along their length, with the result that only about 8% of the potassium in the glomerular filtrate remained at the end of the distal tubule (Figs. 3 and 6). Between the end of the distal tubule and the final urine, a large addition of potassium occurred in the 3/4-nephrectomized rats, but not in the normal controls maintained on the same intake of potassium. We assume that the K^+ added beyond the distal tubule was secreted by the collecting ducts. The alternative explanation is that deeper nephrons were contributing large amounts of potassium to the urine, perhaps by addition of K^+ to their distal tubular fluid. Although we have no evidence against this interpretation, there is on the other hand no reason to postulate that juxtamedullary nephrons function differently from superficial nephrons with respect to potassium excretion.

The finding of progressive reabsorption of potassium along the distal convoluted tubule in normal rats is in agreement with the observations of Marsh, Ullrich, and Rumrich (6), but conflicts with several studies by Malnic, Klose, Giebisch, and de Mello Aires (7–9). These latter authors have reported that under a variety of different experimental conditions, tubular fluid potassium concentration rises at the end of the distal tubule to levels severalfold higher than plasma concentration, and that there is net addition of potassium to the tubular fluid by the distal convoluted tubule. Only under conditions of a low potassium diet (7), or severe metabolic or respiratory acidosis (9) did net reabsorption of potassium occur in the distal tubule. The reason for the difference between our observations and those of Malnic and co-workers is not clear, but several possibilities can be considered. First, it seems unlikely that dietary factors can account for the differences, since we observed distal potassium reabsorption in normal animals ingesting 5 meq K^+/day and an amount of Na^+ approximately equal to that in a regular rat pellet diet (see Methods). Distal potassium reabsorption was also observed in rats eating a regular pellet diet and infused with 0.15 M KCl (Fig. 8). In these latter experiments, serum K^+ concentration was elevated to as much as 7.5 meq/liter. Wright, Strieder, Fowler, and Giebisch (10) reported net secretion of K^+ by the distal tubules of rats chronically fed a high potassium diet or infused with a much larger K^+ load than used in the present study, but because of these differences in protocol, the results of the present study cannot be compared with theirs. A second possibility which should be considered is acid-base changes, since acidosis has been found to inhibit secretion of K^+ by the distal tubule (9). However, this cannot account for our findings, since only the 24 h 3/4-nephrectomy rats manifested metabolic acidosis. The control rats had no significant acid-base disturbance, and the chronic 3/4 nephrectomy rats manifested a mild respiratory alkalosis. A third consideration is the level of circulating aldosterone. This also cannot account for the absence of distal K^+ secretion in our animals, since *d*-aldosterone was infused continuously throughout each experiment, starting 60–90 min before micropuncture collections were obtained. Although we cannot provide a definitive explanation for the difference between our findings and those of Malnic et al. (7–9), a possible answer lies in the recent finding by Tisher [2] that the histologic characteristics of tubules identified as "distal convolutions" on the surface of the kidney during micropuncture vary from strain to strain. In Sprague-Dawley rats, the strain used in the present study, approximately 75–85% of the so-called "distal tubules" on the surface of the kidney are lined by epithelium that is characteristic of the distal convoluted tubule. In certain strains of Wistar rats, however, about half of the surface "distal tubules" are lined by epithelium characteristic of the distal convoluted tubule, while the remaining tubules are lined by epithelium resembling that of the cortical collecting duct. It is of interest that in the study by Marsh, Ullrich, and Rumrich (6), in which distal tubular K^+ reabsorption was found, as in the present study, Sprague-Dawley rats were used. The possibility therefore exists that what has been thought to be secretion of K^+ by the distal convoluted tubules is actually a collecting duct phenomenon. If this explanation is correct, our findings indicate that the distal convoluted epithelium has primarily a reabsorptive function with regard to potassium, even in the presence of acute K^+ loading and large amounts of *d*-aldosterone, and that the major site of alterations in urinary K^+ excretion is the collecting ducts. Hierholzer (11) came to the conclusion that collecting ducts of hamsters secrete K^+ under appropriate experimental conditions, on the basis of retrograde microcatheterization studies of single collecting ducts.

The mechanism of K^+ addition to the urine by the collecting ducts of rats is not certain. Grantham, Burg, and Orloff (12) demonstrated in the isolated perfused-tubule preparation that the collecting ducts of rabbits actively secrete K^+ against an electrochemical gradient.

[2] Tisher, C. C. Personal communication.

Although an active secretory process has not been demonstrated for the rat-collecting duct, the present observations are compatible with the view that a substrate-dependent transport system may be present in this species. In the normal control rats on a potassium intake of 5 meq/day, no definite reabsorption or secretion of K^+ by the collecting ducts was evident (Fig. 3). When normal rats were infused with 0.15 M KCl, however, a clear increase in urinary K^+ occurred beyond the distal tubule (Fig. 8). Thus, the collecting ducts of normal rats appear to be capable of secreting K^+ when serum K^+ is acutely elevated. In the 3/4-nephrectomized rats, the secretory process also appeared to be sensitive to changes in serum K^+ concentrations. When serum K^+ was either normal or elevated (Figs. 3, 6 and 7), collecting duct secretion was observed, but when potassium was eliminated from the diet and serum K^+ concentration fell to an average value of 3.3 meq/liter, no secretion by the collecting ducts was evident (Fig. 7). Thus, in both the normal and 3/4-nephrectomized rats, a collecting duct secretory mechanism seemed to be demonstrable which was responsive to changes in potassium intake and/or serum K^+ concentration. Without measurements of the electrical profile of the collecting ducts, it is not possible to state whether this mechanism is an active or passive one. However, based on the study by Grantham, Burg, and Orloff (12), the likelihood seems to be that it is an active process. In any case, collecting duct K^+ secretion appears to be of major importance for homeostasis of body potassium.

Several factors which might influence K^+ secretion by distal segments of the nephron need to be considered in evaluating the difference between the normal control and the 3/4-nephrectomy rats, since on the same K^+ diet and Ringer infusion, only the 3/4-nephrectomy rats demonstrated K^+ secretion. It seems unlikely that differences in circulating levels of aldosterone or acid-base disturbances can account for the observations. Urine pH and HCO_3^- concentrations were closely comparable in all three groups of animals. We cannot entirely rule out the possibility that differences in Na^+ delivery to the collecting duct had some effect on K^+ secretion. In the 24 h 3/4-nephrectomy rats, a larger than normal fraction of filtered sodium reached the beginning of the distal tubule, and by the end of the distal tubule, the fraction was still somewhat higher than in the controls. If collecting duct K^+ secretion is influenced by the rate of sodium delivery to that segment of the nephron (13), the higher rate of K^+ secretion in the 24-h rats might be attributable in part to this mechanism. Similarly, in the chronic 3/4-nephrectomy rats, the absolute rate of Na^+ delivery to the collecting ducts was in all likelihood elevated, due to the rise in GFR and the essentially normal fractional reabsorption of Na^+ in the distal tubule. Thus, in these animals as well, sodium delivery rate may have played some role in determining K^+ secretion by the collecting ducts. It is clear, however, that sodium delivery is not the sole factor, since in the chronic 3/4-nephrectomy rats given a normal sodium but K^+-free diet, the collecting ducts secreted little or no K^+ (Fig. 7).

Although both the 24 h and chronic 3/4-nephrectomy rats demonstrated collecting duct K^+ secretion, several differences between these two groups of animals are noteworthy. First, the 24-h rats had a significant degree of azotemia, hyperkalemia, and metabolic acidosis. In addition, as mentioned above, fractional reabsorption of sodium and water by segments of the nephron before the distal tubule was considerably lower than in animals studied 10–14 days after 3/4 nephrectomy. The reduced fractional reabsorption suggests that the 24-h rats may have been undergoing an osmotic diuresis of an even greater extent than one would expect from the reduction in nephron mass. Presumably, both the 24 h and the chronic 3/4-nephrectomy rats were excreting more urea per nephron than before renal ablation. Yet, fractional water reabsorption was lower in the 24-h animals than in the chronic animals. One explanation for the difference between the two groups is that the 24-h rats may have been in a catabolic condition after surgery. Thus, the elevated BUN in these animals may have been due not only to the reduction in renal mass but also to increased protein breakdown. An excessively elevated BUN might, in turn, have resulted in a moderate urea diuresis in the 24-h rats. The hyperkalemia and acidosis may have also been contributed to in part by post-operative catabolism. Whatever the correct explanation, it seems that the chronic animals are more representative of the steady-state situation expected in patients with chronic renal disease. It is important to note, however, that K^+ secretion by the collecting ducts was evident as early as 24 h after renal ablation. In the study by Schultze and co-workers (2), enhanced K^+ excretion by the remnant kidney of the dog was found as early as 18 h after contralateral nephrectomy, perhaps due to a similar mechanism of collecting duct secretion.

Silva, Hayslett, and Epstein (14) have reported that augmented potassium excretion in normal rats fed a high potassium diet is associated with an increase in Na-K-ATPase in the renal cortex and outer medulla. More recently, these investigators have found a similar increase in Na-K-ATPase in the remnant kidney of 3/4-nephrectomized rats (15). If this enzyme is involved with active K^+ transport by the collecting ducts, their findings suggest that changes in Na-K-ATPase may account for the adaptation in K^+ excretion which the normal kidney undergoes when potassium intake is

chronically increased, and the adaptation of the remaining nephrons of the remnant kidney. A reasonable hypothesis is that increased K^+ uptake by the collecting duct cells across their peritubular membrane leads to induction of Na-K-ATPase synthesis. This might result in an enhanced capacity for K^+ transport into the collecting duct lumen, thereby increasing potassium excretion and maintaining body potassium within a narrow range. Further studies are needed to determine the validity of this concept.

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