Functional Profile of the Isolated Uremic Nephron

POTASSIUM ADAPTATION IN THE RABBIT CORTICAL COLLECTING TUBULE

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ABSTRACT As a renal function declines in patients and experimental animals with chronic renal disease, potassium homeostasis is maintained by a progressive increase in potassium secretion by the surviving nephrons, a phenomenon known as potassium adaptation. To determine the nephron site and the underlying mechanisms responsible for this phenomenon, studies were performed on normal and 75% nephrectomized rabbits maintained on normal or high-potassium diets. Cortical collecting tubules (CCT) were dissected from the normal and remnant kidneys and perfused in vitro in an artificial bath solution. In normal CCT mean (±SE) net K secretion, J_K, (peq/cm per s) was 1.26±0.43 (normal diet) and 3.27±0.66 (high-K diet). In uremic CCT, J_K was 3.55±0.60 (normal diet) and 6.83±0.58 (high-K diet). By reducing the dietary intake of potassium in proportion to the reduction of renal mass in these uremic animals, the adaptation in K secretion was prevented (J_K: 1.22±0.40). Transepithelial potential difference was similar in CCT from normal and uremic animals on a normal diet despite the fact that J_K was significantly greater in the latter group. However, in both normal and uremic CCT, the increase in J_K caused by potassium loading was associated with an increase in luminal negativity. Uremic CCT underwent significant compensatory hypertrophy regardless of the dietary intake or potassium secretory rates. Plasma aldosterone levels were elevated only in the uremic-high potassium rabbits suggesting that a mineralocorticoid effect on the CCT may be exaggerated when potassium loading is superimposed upon decreased excretory capacity. The activity of Na-K ATPase was comparable in normal and uremic CCT from rabbits on either normal or high-K diets indicating that potassium adaptation may occur independently of changes in the activity of this enzyme. Intracellular potassium content measured chemically and by ^{42}K exchange, was not significantly altered in either normal or uremic CCT when dietary potassium intake was increased, despite the fact the J_K was increased under these circumstances.

These data indicate that the CCT is an important site of potassium adaptation in the surviving nephrons of animals with reduced renal mass. This adaptation is an intrinsic property of the CCT and is expressed in the absence of a uremic milieu. Potassium adaptation by the uremic CCT is not fixed according to the degree of compensatory hypertrophy but varies according to the excretory requirements of the animal. Transepithelial potential difference and circulating aldosterone levels contribute to the adaptation but neither factor can entirely account for the phenomenon. Potassium adaptation by the CCT occurs in the absence of changes in Na-K ATPase activity and intracellular potassium content.

INTRODUCTION

The course of chronic progressive renal disease in man and animals is typically characterized by an adaptation in renal potassium excretion that maintains serum potassium concentration within normal limits despite a significant reduction in glomerular filtration rate (1). Although additional nonrenal mechanisms participate in this adaptation (2, 3), the progressive increase in potassium excretion per surviving nephron appears to be the principal mechanism whereby potassium homeostasis is maintained.

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The exact site in the nephron and the mechanisms underlying this enhanced renal potassium secretory ability have not been adequately defined. Micropuncture studies by Bank and Aynedjian (4) in subtotally nephrectomized rats have shown that the augmented addition of potassium to the final urine occurs at a site beyond the accessible distal convoluted tubule. It was not possible from these observations to determine whether this resulted from an increase in potassium secretion by the collecting tubule or from an increased contribution of deeper nephrons to the potassium content of the final urine.

The present studies were designed to evaluate the role of the cortical collecting tubule (CCT)[1] in the potassium adaptation of uremia. Net potassium transport was measured in isolated perfused CCT from normal and uremic rabbits maintained on diets of different potassium content. Our results indicate that this nephron segment plays an important role in the potassium adaptation of uremia. To define the cellular mechanisms underlying these functional changes, a number of potentially important factors were investigated. These included the relationship of intrinsic adaptations in potassium transport by the CCT to excretory requirements, the role of humoral factors, including aldosterone, and the contributions of compensatory hypertrophy, transtubular potential difference, Na-K ATPase, and intracellular potassium content to the patterns of potassium secretion by the CCT.

METHODS

Experimental animals

Four groups of female, white New Zealand rabbits, 2–3 kg in weight, were used for each of the different studies outlined below:

Normal–normal potassium (NK) group. Normal rabbits maintained on an ad lib diet of Purina rabbit chow containing 1.19% K and 0.22% Na (Ralston Purina Co., St. Louis, Mo.) and distilled water.

Normal–high-K group. Normal rabbits maintained on the above diet supplemented with 100 mM/liter KCl in the drinking water for 10–14 d before study.

Uremic–NK group. Uremic rabbits with solitary remnant kidneys maintained on the standard diet described above.

Uremic–high-K. Uremic rabbits with solitary remnant kidneys on a standard diet supplemented with KCl in the drinking water for 10–14 d.

An additional group of uremic rabbits, in which dietary potassium intake was reduced in proportion to the reduction in renal mass, was included in certain of the studies, i.e., proportionally reduced potassium (uremic–PRK). These animals were placed on a zero potassium diet (ICN Nutritional Biochemicals, Cleveland, Ohio). The drinking water contained KCl 20 meq/liter and NaCl 40 meq/liter. The latter was necessary to maintain sodium intake in a range comparable to that observed in the other groups.

Remnant kidneys, approximately one-fifth of total renal mass, were created by selective ligation of branches of the left renal artery and contralateral nephrectomy as described (5, 6). Uremic animals were studied 3–4 wk after this surgical procedure.

Balance studies

Balance studies were performed on seven normal–NK, six normal–high-K, seven uremic–NK, five uremic–high-K, and six uremic–PRK rabbits to obtain a representative evaluation of the amount of Na and K ingested and to determine the contribution of urinary excretion to external potassium balance. Animals were placed on their respective diets for 10–14 d before the performance of balance studies. They were then housed in metabolic cages for 4 d. During this period, the total sodium and potassium ingested and excreted in the urine per 24 h was measured. Feces were not collected for analysis. The results are expressed as the mean of four 24-h measurements.

Potassium transport by isolated CCT

CCT were obtained from 12 normal–NK, 9 normal–high-K, 11 uremic–NK, 9 uremic–high-K, and 6 uremic–PRK rabbits. Rabbits were killed by cervical dislocation and the normal or the remnant kidneys rapidly removed. A 1-mm thick cross section was transferred to a dish of chilled bath solution (see below) and collecting tubules were dissected from the cortex as described (6). Blood was sampled from the femoral artery at the time of sacrifice for determination of blood urea nitrogen and potassium concentrations.

Tubule segments were transferred to a bath solution containing (in millimoles per liter) NaCl 115, KCl 5, $NaHCO_3$ 25, Na acetate 10, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.0, dextrose 5.5, and 5% vol/vol normal rabbit serum (Microbiological Associates, Walkersville, Md.). The bath was maintained at 37°C and bubbled continuously with 95% O_2–5% CO_2. Osmolality was maintained constant by adding deionized water to the bath throughout the experiment (7).

Tubule segments were perfused through concentric pipettes at a rate of ≅5 nl/min. Both ends of the tubule were insulated with Sylgard 184 (Dow Corning Corp., Midland, Mich.). Transepithelial potential difference (PD) was monitored throughout the experiment using the perfusion pipette as the luminal electrode (7). The tip of this pipette was advanced ≅150 μm beyond the Sylgard seal.

The perfusate used in all experiments contained (in millimoles per liter), NaCl 150, K_2HPO_4 2.5, $CaCl_2$ 1.0 and $MgSO_4$ 1.2, pH 7.4. The osmolality of the bath and the perfusate was identical. Tubules were perfused for 90 min before the initiation of the experiments. During this period, the PD attained stability and both normal and uremic CCT became impermeable to water (6, 7).[2] Perfusion pressure was ±5 cm H_2O in all experiments.

Five timed collections of fluid emerging from the collecting end of the tubule were made under mineral oil into a calibrated constriction pipette. Samples of collected fluid as well as two samples of perfusate were analyzed for potassium concentration using a helium-glow photometer (American Instrument Co., Travenol Laboratories Inc., Silver Springs, Md.). Assuming zero net water flux (6), net flux of potassium (J_K) is: $J_K = V_L (C_L - C_O)$, where V_L is the collection rate, and C_L and C_O the concentrations of potassium in collected and

[1] *Abbreviations used in this paper*: CCT, cortical collecting tubule(s); J_K, net potassium flux; NK, normal potassium; PD, potential difference; PRK, proportionally reduced potassium.

[2] This was confirmed in two tubules in each of the high-K groups and in the uremic–PRK group.

perfused fluids, respectively. Positive values indicate net flux from bath to lumen (secretion). Values were factored for both tubule length and luminal surface area. Measurements of length and internal diameter were made with a calibrated reticle in the ocular of the microscope. The result for each tubule is expressed as the mean of five collections.

Na–K ATPase activity in CCT

Na–K ATPase activity was measured in CCT from 12 normal–NK, 10 normal–high-K, 9 uremic–NK, and 9 uremic–high-K rabbits by a modification of the method described by Cortas and Walser (8) for assaying isolated toad bladder cells. CCT were dissected in a modified protein-free bath medium (see above) in which NaCl replaced all the NaH_2PO_4. A total length of ≅5–8 mm was transferred to individual wells of a Microtest Terasaki tissue culture plate (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). An additional 10–15 mm was assayed for protein content as described (6); recovery for this assay in the range of 1.5–3 μg is 103±6% (SD) (n = 25). Excess medium was carefully aspirated and 2 μl of hypotonic preincubation medium added. This contained 5 mM EDTA, 40 mM Tris, 30 mM histidine HCl, 120 mM sucrose, 0.1% deoxycholic acid, adjusted with HCl to pH 7.4. Tubule segments were preincubated in this medium for 2 h at 0°C.[3] It was found that maximal activity of the enzyme in both normal and uremic CCT was obtained after 2 h of preincubation. Preincubation for a 3rd h neither increased nor decreased activity further. 10 μl of reaction mixture were then added to each well. For the assay of total Na–K ATPase activity, this mixture contained 100 mM NaCl, 10 mM KCl, 10 mM NaN_3, 2 mM $MgCl_2$, and 40 mM Tris HCl, p 7.0. For the assay of ouabain insensitive-ATPase (Mg-ATPase) the KCl was omitted and ouabain was added to achieve a final concentration of 1 mM. The tissue was incubated for 10 min at 37°C in these media and the reaction was then started by the addition of 2 μl of 10.5 mM Tris–ATP. The plates were incubated for 10 min at 37°C, after which the reaction was stopped with 4 μl of 10% TCA.

Inorganic phosphorus (P_i) was measured spectrophotometrically by a modification of the method of Polley (9). 13 μl of the assay sample was added to 130 μl of molybdate reagent and 10 μl of freshly prepared diluted stannous chloride solution (9). The absorption at 660 nm was read after 10 min with a Zeiss spectrophotometer (Carl Zeiss, Inc., New York). Readings were corrected for blank values that were obtained by measuring the absorption of the reaction mixtures alone.

The following assay conditions were established: (*a*) Enzyme activity proceeded linearly for at least 30 min. (*b*) At a medium concentration of 7 mM potassium, enzyme activity increased with increasing sodium concentrations to achieve maximal values at 70 mM sodium. Activity declined to ≅75% of maximal activity as medium sodium concentration was further increased to 120 mM. (*c*) Using medium concentrations of 70 mM sodium and 7 mM potassium, maximal enzyme activity was obtained at an ATP concentration of 1.5 mM. At a concentration of 6 mM ATP, activity declined to 73% of maximal activity. (*d*) P_i generation was linearly related to tubule length over a range of 1–12 mM (see Results). (*e*) The difference between two assays performed on the same sample was <10% of either value.

The assays reported here were conducted at medium concentrations of 70 mM Na, 7 mM K, and 1.5 mM ATP. Each assay was performed in duplicate. Na–K ATPase activity is expressed as the difference between total and ouabain-insensitive ATPase activities.

Intracellular potassium content of CCT

Intracellular potassium content of normal and uremic CCT obtained from rabbits on standard and high-K diets was determined by two independent methods.

(a) Chemical determination of intracellular potassium. Cellular potassium content was measured in six animals from each group according to the technique described by Grantham et al. (10). CCT were dissected in the bath medium described above and an approximate total length of 5–10 mm transferred to each of two wells of a Microtest Terasaki tissue culture plates. The plates were incubated at 37°C for 30 min in a humidified atmosphere of 95% O_2–5% CO_2. The tubule segments were then photographed for the determination of total length and removed from the wells with a fine acid-washed glass needle to which they readily adhered.

To remove the bulk of extracellular sodium, the segments were carefully agitated for 2 min in a chilled isotonic solution containing (in millimoles per liter) choline Cl 150, K_2HPO_4 2.5, Mg SO_4 1.2, $CaCl_2$ 1.2, pH 7.4. This maneuver removes extracellular electrolytes without significantly affecting intracellular electrolyte content (9).[4] The tubules were then lifted from the medium and allowed to dry in the air.

The tip of the glass needle with the adherent tubules was cut off with a pair of fine forceps and the tissue positioned under mineral oil in a 0.5-μl droplet of diluent used for the helium-glow photometer (0.03 M $CsNO_3$ and 0.005 M $NH_4H_2PO_4$ in distilled water). They were allowed to extract for 24 h at room temperature and the eluate was analyzed for K concentration with the helium-glow photometer. Although Na concentration was always significantly lower than K concentrations in any given sample, the wide variability and poor reproducibility of the sodium values in our hands, precludes their usefulness and are not reported here. Estimation of K concentrations on the other hand gave reproducible results, duplicate samples yielding values that did not differ by more than 10–15%. Recovery for potassium in the concentration range 0.5–2.5 meq/liter was 104±5% (SD) (n = 20). No correction for extracellular K was made.

(b) Isotopic determination of intracellular potassium. ^{42}K was used to determine the content of "exchanged" potassium in CCT under steady-state conditions according to the method described by Burg and Abramow (11). These authors reported a single compartment for ^{42}K in the normal rabbit collecting tubule.

Tubules were dissected from the kidneys of six animals in each group and transferred to incubation wells as described above. Duplicate samples of tubules were incubated for 30, 60, and 90 min in bath medium that contained ^{42}K (300–400 μCi/ml) and [^{14}C]inulin (50 μCi/ml; New England Nuclear, Boston, Mass.). The chemical potassium concentration of this medium was 5 meq/liter.

At the end of each incubation period, the wells were

[3] The use of a hypotonic medium and the detergent deoxycholate are required to disrupt cell membranes and to remove extraneous proteins and lipids from the membranes to unmask catalytic activity of the preparation. We have previously used a combination of hypotonic preincubation plus repeated freezing and thawing to disrupt the cell membranes of collecting tubules (6). The latter maneuver was not employed in the present study because Cortas and Walser (8) reported that freezing results in loss of enzyme activity of 70–100%.

[4] Identical values were obtained on tubules from the same animal if agitation proceeded for 1, 2, 3, or 4 min.

photographed for determination of tubule length, transferred to a vial containing 50 μl of distilled water, and were counted in a Packard gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Appropriate corrections were made for isotope decay. Upon completion of counting, the vial containing the tubule segments was transferred to counting vials containing 10 ml of Aquasol liquid scintillation fluid (New England Nuclear). Samples were counted for ^{14}C after 7 d, by which time decay of the ^{14}K was complete.[5] The volume of extracellular fluid contaminating the tissue sample was calculated for the [^{14}C]inulin counts. The ^{42}K counts contained in this volume of medium were subtracted from the tissue counts to give total corrected counts.

Exchanged potassium was calculated as $^{42}K_t/L$ (SA_K), where $^{42}K_t$ is the total corrected radioactivity of the tissue (counts/min), L, the total length of the tubules, and SA_K the specific activity of ^{42}K in the medium (cpm/meq). Because no significant differences were observed in any of the groups for values obtained at 30, 60 and 90 min of incubation, the results are expressed as the mean of the three values. Achievement of steady-state values by 30 min were also observed by Burg and Abramow (11).

Values for intracellular potassium content using both the chemical and the isotopic methods described above were expressed per millimeter length.

Indices of hypertrophy

The degree of hypertrophy that occurred in the CCT of the remnant kidney was assessed by two methods. The total cell volume per unit length of CCT was measured during perfusion by measuring the internal and external diameters of the tubule and assuming that the CCT has a cylindrical configuration. Since all groups of CCT were perfused at similar rates and pressures, the degree of distention of the lumen is assumed to be similar in all. Protein content per unit length was measured in the nonperfused tubules.

Plasma aldosterone concentrations

To evaluate the role of endogenous aldosterone levels in potassium secretion by the CCT, plasma aldosterone concentration was determined by radioimmunoassay using the method described by Mayes et al. (12) on femoral arterial samples obtained at the time of sacrifice of the animals used for the studies of net potassium transport (Tables I and II).

Statistics

Results from different groups of animals were compared by means of an unpaired *t* test. In the balance studies, the differences between intake and excretion were compared for each animal using paired *t* analysis. Results are expressed as mean±SE.

RESULTS

Balance studies. The mean (±SE) of Na and K ingested and excreted in the urine per 24 h are depicted in Fig. 1. On a standard laboratory diet the normal rabbits ingested 39.0±4.97 meq K/d; uremic rabbits maintained on the same diet ingested 28.1±3.61 meq/d. When 100 mM KCl was added to the drinking water, normal rabbits ingested 59.4±13.9 and uremic rabbits 57.0±7.8 meq/d. In the uremic–PRK animals potassium intake was reduced to 7.3±1.2 meq/d. In each group, except the uremic–high-K animals, urinary excretion of Na and K was not significantly different from ingested Na and K. In the uremic–high-K animals, urinary K excretion represented 87% of the ingested K ($P < 0.05$). The difference in this group is presumably caused by augmented fecal K excretion (2). Na intake was approximately the same in all groups of animals. In the normal rabbits on NK and high-K diets serum potassium concentration was 6.0±0.3 and 5.9±0.4 meq/liter, respectively. In the uremic rabbits these values were 6.1±0.3 and 6.4±0.4 meq/liter.

J_K in isolated perfused CCT. J_K and transepithelial PD were simultaneously measured after an equilibration period of 90 min. Results for each experiment are listed in Tables I and II and the mean±SE results depicted in Fig. 2. In normal CCT, J_K was significantly higher in tubules obtained from animals maintained on high-K diets than from animals on normal diets ($P < 0.02$), whether this was expressed per unit length or per unit luminal surface area. Similarly, the mean±SE PD, which was −13±6 mV (lumen negative) in normal–NK tubules was −29±6 mV in normal–high-K tubules but the difference was not significant because of the wide variation of the normal values.

J_K in uremic–NK tubules was approximately twice as great as that observed in CCT from normal rabbits on the same diet ($P < 0.02$) despite the fact the mean PD was approximately the same in the two groups of tubules. In CCT obtained from uremic rabbits on high-K-diets, J_K was increased twofold over the value obtained in uremic–NK tubules (<0.01). This was associated with a marked increase in PD ($P < 0.001$). In the uremic–PRK tubules, J_K and PD were not significantly different from values obtained in normal CCT

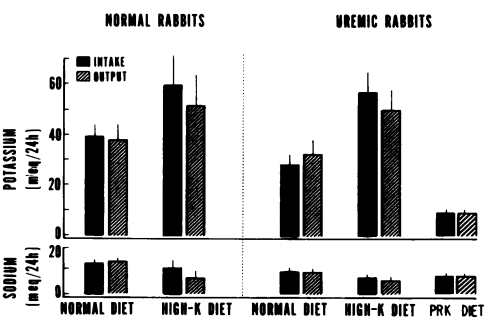

FIGURE 1 Balance studies on normal and uremic rabbits maintained ad libitum diets of normal, high-K and PRK content and normal sodium content.

[5] Unidentified decay products of ^{42}K produce scintillation that complicates the determination of [^{14}C]inulin. The percentage of total counts due to ^{14}C was determined by counting aliquots of ^{14}C-containing medium prepared with and without ^{42}K and correcting the total counts by this factor. Counts from [^{14}C]inulin exceeded 75% of the total counts in all cases.

TABLE I

J_K and Transepithelial PD in CCT Obtained from Normal Rabbits Maintained on Normal and High-K Diets

	BUN	Perfusion rate	Luminal diameter	J_K	J_K	PD
	mg/dl	*nl/min*	*μm*	*peq/s/cm length*	*peq/s/cm² luminal surface area*	*mV*
Normal diet	15.9	4.28	23.0	1.62	224.2	−65
	25.4	10.51	24.6	5.40	716.2	−21
	10.6	3.24	23.4	2.89	418.1	−24
	9.5	5.89	19.9	0.84	133.6	−6
	11.4	2.76	26.5	0.93	113.9	−25
	13.8	9.00	22.0	0.61	88.3	−20
	13.8	6.09	18.3	0.35	63.6	+2
	13.9	3.99	17.8	0.64	116.0	+7
	14.5	7.71	15.6	0.73	150.3	−2
	13.2	7.30	22.2	0.28	40.2	+3
	14.7	8.98	17.8	0.19	35.2	−6
	22.1	8.16	26.6	0.63	74.9	−1
Mean±SE	14.9±1.3	6.49±0.73	21.5±1.0	1.26±0.43	181.2±57.2	−13±6
High-K diet	19.1	10.78	20.0	6.28	1001.3	−67
	13.9	5.98	15.6	3.55	724.1	−34
	13.5	4.91	22.2	2.84	406.4	−23
	13.5	6.53	17.6	1.23	221.6	−20
	10.2	7.98	13.3	2.77	661.7	−30
	16.2	6.86	15.5	1.02	207.2	−10
	18.3	9.45	15.6	3.39	694.4	−14
	14.7	6.58	17.8	6.57	1177.0	−39
	11.7	5.79	20.8	1.84	293.5	−25
Mean±SE	14.5±1.0	7.21±0.63	17.5±0.9	3.27±0.66	487.4±119.8	−29±6

BUN, blood urea nitrogen.

(normal diet). J_K was significantly lower than in the other two uremic groups ($P < 0.05$; $P < 0.001$).

Na–K ATPase activity of CCT. Fig. 3 depicts the linear relationship obtained between P_i generation and tubule length in 14 assays on normal CCT in the absence and presence of ouabain.

In Fig. 4, Na–K ATPase activity is shown for the four groups of animals studied. The mean value of ≅8 nmol $P_i/\mu g$ protein per h in normal CCT is very close to that reported by Schmidt and Habicht (13), who assayed Na–K ATPase on segments of the distal nephron of the rabbit using a different method (which also included deoxycholate pretreatment) and obtained values of 4–9 mol/kg dry wt per h. In the present study, no differences in Na–K ATPase activity could be found between any of the groups. Similarly, ouabain-insensitive ATPase activity was comparable in all groups. (Normal–NK, 4.3±0.5; normal–high-K, 5.6±0.6; uremic–NK, 6.4±0.9; uremic–high-K 5.8±1.0).

Intracellular potassium content of isolated CCT. Results of measurements of intracellular K content are listed in Table III. The mean values obtained by chemical and isotopic methods were closely comparable in all groups. CCT from normal and uremic rabbits on high-K diets did not have intracellular potassium contents that were significantly different from those on normal diets. Potassium content expressed per unit tubule length was greater in the uremic than in the normal CCT because of the increase in tubule size. When, however, values are expressed per unit protein content, (Table III) it is evident that they are closely comparable in the two groups.

Indices of compensatory hypertrophy. Two separate indices of hypertrophy were measured (Table IV). Cell volume/unit length was calculated from the measured luminal and external diameters of the tubules during perfusion. Measurements of protein content per unit length were measured in nonperfused tubules. A comparison between normal and uremic CCT revealed that there was a significant increase in both of the parameters measured in the latter. Dietary intake of potassium did not affect the degree of hypertrophy.

Plasma aldosterone concentrations. Radioimmunoassay of plasma aldosterone concentrations revealed a wide range of values in each group. In normal rabbits

TABLE II

J_K and Transepithelial PD in CCT Obtained from Uremic Rabbits Maintained on Normal, High-K, and PRK Diets

	BUN	Perfusion rate	Luminal diameter	J_K	J_K	PD
	mg/dl	*nl/min*	*μm*	*peq/s/cm length*	*peq/s/cm² luminal surface area*	*mV*
Normal diet	76.9	3.94	24.4	3.45	450.5	+1
	70.4	11.90	44.4	6.21	444.8	−8
	87.6	6.53	35.5	1.23	110.0	−1
	34.4	3.00	35.5	1.07	95.9	−7
	41.7	8.96	33.3	1.19	113.4	−7
	102.8	7.91	36.7	3.58	310.8	−6
	23.4	4.49	19.9	3.89	1106.5	−53
	45.2	7.69	19.6	7.72	1235.6	−25
	54.0	5.73	21.1	4.11	620.4	−3
	53.4	4.69	23.0	3.36	465.2	−1
	80.2	9.14	18.2	3.27	573.8	−19
Mean±SE	60.9±7.4	6.75±0.90	28.3±2.7	3.55±0.62	502.4±114.3	−12±5
High-K diet	57.1	5.95	22.2	7.62	1089.6	−60
	66.8	4.91	15.5	8.01	1640.1	−21
	35.8	6.18	23.0	8.86	1226.5	−42
	31.8	7.32	23.6	9.04	2114.3	−24
	121.9	6.88	22.2	5.52	791.5	−32
	56.1	6.49	26.6	5.36	640.3	−68
	23.2	9.82	26.6	7.08	845.2	−50
	48.1	6.62	20.1	6.11	962.2	−50
	66.7	7.61	20.0	3.92	624.7	−35
Mean±SE	56.4±9.7	6.86±0.45	22.2±1.15	6.83±0.58	1104.7±164.5	−42±5
PRK diet	150	7.04	33.3	2.58	247.1	−30
	80	5.34	22.3	0.44	63.3	−16
	68	7.80	22.0	2.29	331.8	−42
	94	9.91	17.7	0.45	180.5	−3
	55	5.50	35.5	0.52	46.8	−6
	36	5.59	33.4	1.09	104.3	−17
Mean±SE	80.5±16.1	6.86±0.73	27.3±3.1	1.22±0.40	162.3±45.7	−19±6

BUN, blood urea nitrogen.

on normal and high-K diets the values were 39±15 and 23±6 ng/100 ml, respectively. In uremic rabbits these values were 36±11 and 149±50 ng/100 ml. The latter value was significantly greater than those in the other three groups. ($P < 0.02$).

DISCUSSION

A considerable body of evidence has recently accumulated from micropuncture studies indicating that the collecting tubule is a major site of potassium secretion by the normal mammalian nephron (4, 14–22). In vitro studies on the isolated perfused CCT of the rabbit have demonstrated the existence of active potassium secretion in this segment (23–27). The ability of patients and experimental animals with renal disease to maintain potassium homeostasis despite significant reductions on functioning renal mass (1, 4, 14–22, 28–32) suggests that nephron sites which normally secrete potassium are able to augment this secretion to maintain potassium balance in chronic uremia. The site of this "potassium adaptation" has recently been the subject of a number of studies. Evidence suggestive of the fact that the collecting tubule may be the nephron site in which the major adaptation takes place has been presented by Bank and Aynedjian who studied rats with 75% nephrectomy (4). Micropuncture observations in these animals revealed that a large increase in tubular fluid potassium content occurred between the end of the superficial distal tubule and the final urine in the 75% nephrectomized animals but not in normal controls. Definitive evidence for collecting tubule potassium secretion was, however, not provided since the conventional micropuncture approach

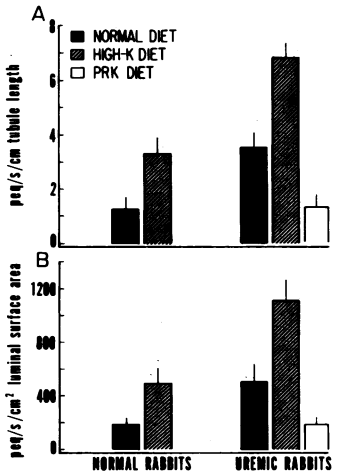

FIGURE 2 J_K (K secretion) of normal and uremic CCT from rabbits maintained on normal, high-K, and PRK diets. (A) J_K is expressed per unit tubule length and (B) it is expressed per unit luminal surface area.

used could not distinguish between collecting tubule secretion and an augmented contribution of deep nephrons to the final urinary composition. Similar results were obtained by Bengele et al. (14) who studied uninephrectomized rats and compared fractional delivery to the late superficial distal convoluted tubule with fractional excretion of potassium in the urine (14). In both the above studies there was no addition of potassium along the distal tubule. On the other hand, Kunau and Whinery (22) found that in 75%

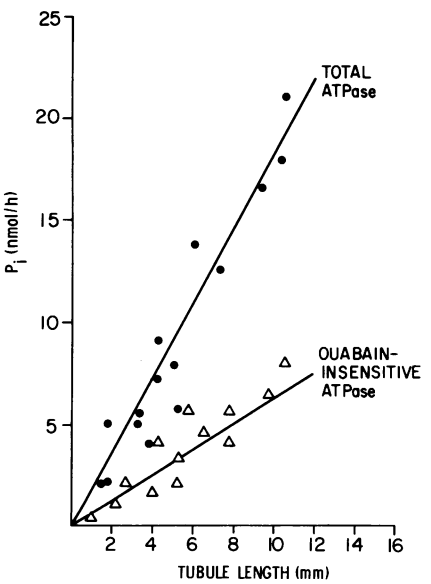

FIGURE 3 Assays for total ATPase and ouabain-insensitive ATPase in normal rabbit collecting tubules showing the linear relationship between tubule length and the generation of P_i from ATP.

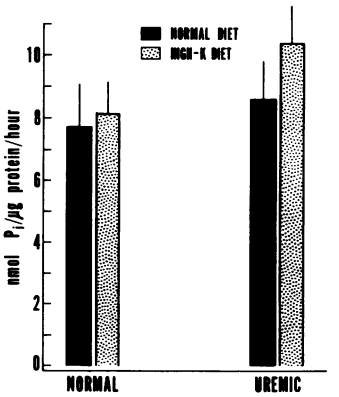

FIGURE 4 Na–K ATPase activity in normal and uremic CCT from rabbits maintained on normal and high-K diets.

nephrectomized rats there was a significant increase in potassium secretion in the superficial distal tubule as compared with normal controls and that net addition beyond the late distal tubule occurred only irregularly.

The present studies were designed to evaluate, by direct measurement, whether net potassium secretion increases in cortical collecting tubules of animals with reduced nephron populations and whether such adaptations parallel the changes in potassium excretion which are induced by varying the dietary intake of potassium. Recent preliminary observations suggest the cortical, rather than the medullary collecting tubule, is probably responsible for the major adaptations in potassium secretion (33, 34). The present studies were confined to the cortical part of collecting tubule.

Our results indicate that, when compared with normal CCT, net potassium secretion is increased in CCT obtained from remnant kidneys of uremic rabbits on a normal diet whether net transport is expressed per unit length or per unit luminal surface area. If, however, dietary potassium intake is reduced in proportion to the reduction in renal mass, this adaptation is prevented and the potassium secretory rate remains within the normal range. In both the normal and the uremic CCT, J_K was approximately doubled when the potassium intake of the animals was increased twofold. The mean value for the net potassium secretory rate in CCT from uremic rabbits on a high potassium intake was more than five times greater than that observed in normal CCT from animals on a standard laboratory diet. Since the tubules were all studied more than 90 min after removal from the rabbits and were bathed and perfused with artificial solutions, it may be concluded that the observed adaptations are intrinsic to the tubular epithelium. It is possible that some element(s) of the uremic milieu may have induced changes in function that persisted in the in vitro situation, but it is apparent that no short-lived humoral influences are required for the expression of this adaptation.

TABLE III
Intracellular Potassium Content of Isolated CCT

	Normal rabbits		Uremic rabbits	
	Normal diet	High-K diet	Normal diet	High-K diet
Chemical determination				
Length, *pmol/mm*	73.2±10.3	81.9±8.6	113.9±13.4	90.5±7.7
Protein, *pmol/μg**	(385.2±54.2)	(431.0±45.2)	(392.9±46.2)	(291.9±74.8)
^{42}K exchange				
Length, *pmol/mm*	54.8±5.1	64.4±6.8	94.8±15.9	71.5±9.4
Protein, *pmol/μg**	(288.4±26.8)	(338.9±35.7)	(326.9±54.8)	(230.6±30.3)

Mean±SE values are shown.
* Values derived by factoring mean potassium content per unit length by the mean values for protein content per unit length listed in Table IV.

The significant compensatory hypertrophy that occurred in the uremic CCT does not appear to have contributed to this phenomenon because potassium secretion could be shown to be either normal or increased with comparable degrees of hypertrophy. A number of additional factors known to modulate potassium secretion (35) were examined. Tubule-fluid flow rate as a variable may be excluded from consideration because all groups were studied at comparable perfusion rates. The role of the transepithelial PD in altering net potassium excretion is more difficult to evaluate. In the present studies mean PD of the normal CCT was −13±6 mV; in uremic tubules from animals on the same diet, PD was −12±5 mV, and yet net potassium flux was nearly three times higher than in the normal CCT. Furthermore, comparable PD were obtained in the uremic–PRK tubules in which J_K was not significantly different from normal. On the other hand, PD was increased in both normal and uremic CCT to −29±6 and −42±5 mV, respectively, when the animals were placed on high potassium intakes. Although it is impossible to quantitate the exact contribution of the PD to net transport in the absence of unidirectional flux data, it is clear that the electrical gradient must influence passive movement of K across the luminal membrane to some extent. In the collecting tubule, where potassium permeability is relatively low, the effect of this electrochemical potential would influence potassium movement less than it would, for instance, in the distal tubule where potassium permeability is appreciably higher (36). The explanation for the differences in PD between the different groups of tubules, especially in the high-K uremic tubules, may lie in differences in the mineralocorticoid status of the animals (26, 37, 38).

Neither serum potassium concentration nor plasma aldosterone levels appear to be the principal modulators of the observed adaptations in potassium secretion. Serum potassium concentrations were similar in all groups of rabbits studied and plasma aldosterone levels were increased only in the uremic high-potassium animals. Identical observations have been made on normal and subtotally nephrectomized dogs maintained on normal and high-potassium diets in which plasma aldosterone levels are increased only in the uremic animals on high potassium intakes.[6] The ability of exogenous and endogenous mineralocorticoids to augment the in vitro secretion of potassium by normal CCT has recently been reported by O'Neill and Helman (26) and Schwartz and Burg (27). However, both normal–high-K CCT and uremic–NK CCT had higher potassium secretory rates than normal–

[6] M. Tuck and R. G. Schultze. Unpublished observations.

TABLE IV
Indices of Hypertrophy in CCT

	Normal		Uremic		
	Normal diet	High-K diet	Normal diet	High-K diet	PRK diet
Cell volume/mm, × 10^{-4} mm^3*	6.71±0.67	6.34±0.62	11.53±1.42	12.32±0.63	10.29±0.43
Protein content/mm, μg‡	0.19±0.2	0.19±0.01	0.29±0.05	0.31±0.05	0.25±0.02

* Measured in perfused CCT listed in Tables I and II.
‡ Measured in nonperfused CCT.

NK CCT despite comparable plasma aldosterone levels in all three groups. These results are consistent with the previously documented ability of uremic animals to adapt to potassium independently of their mineralocorticoid status (1). Recent studies on uremic patients by Kahn et al. (31) have also failed to demonstrate any relationship between potassium excretion and plasma aldosterone levels under steady-state conditions.

The role of NA–K ATPase in the phenomenon of potassium adaptation has recently been reviewed by Silva et al. (39). These workers have reported an increase in the specific activity of this enzyme in response to potassium loading in normal rats (40) and comparable increases have been described by Schon et al. (41), and by Finkelstein and Hayslett (42) in rats with remnant kidneys. The increment in potassium excretion per surviving nephron and the rise in Na–K ATPase activity that occurred in these animals could be prevented by decreasing the dietary intake of potassium. In the present experiments, we have clearly demonstrated that both normal and uremic CCT can augment their intrinsic potassium secretory rates independently of any measurable changes in Na–K ATPase specific activity. In the hypertrophied uremic tubules, total enzyme activity per unit length increased in direct proportion to the increment in protein content so that specific activity remained constant. That the absolute increase in total enzyme activity per unit length caused by hypertrophy is not a key factor in determining the enhanced K secretory capacity of the uremic CCT, is demonstrated by the fact that an increase in the potassium intake of these uremic rabbits led to an approximate doubling of the rate of K secretion without any increase in the activity of the enzyme. It is possible, therefore, that the increases in both renal (41) and large intestinal (2) Na–K ATPase activity, which have been linked to potassium adaptation in uremia, are the result of the continued addition of potassium to a system with a reduced excretory capacity without there being a specific transcellular transport role for this adaptation. The demonstration by a number of workers, using different assay methods, that Na–K ATPase is found in highest activity in the thick ascending limb of the loop of Henle (43–46), a segment which is not regarded as a major potassium site, would lend support to this contention.

A number of important points are worthy of consideration when comparing the results of this study with previous studies. The segment of the nephron that we studied was the cortical segment of the collecting tubule. It is noteworthy that Silva et al. (39) had to raise the intake of potassium to ≅200 meq/kg body wt to achieve increments of only 25% in cortical enzyme activity in the rat. In the present study, the animals on a high-potassium intake were receiving ≅20–30 meq/kg body wt or one-fifth of the amount of potassium required to increase cortical enzyme activity in the rat. Thus the extent to which the system was stressed in the present experiments may have been inadequate to expose changes in enzyme activity. On the other hand, although Schon et al. (41) found a 30% increase in Na–K ATPase activity of the remnant kidneys of 75% nephrectomized rats on daily potassium intakes of only 20 meq/kg body wt, Finkelstein and Hayslett (42) failed to find any increase in cortical enzyme activity in the 65% nephrectomized rats that they studied. Thus, whether the change in Na–K ATPase activity in cortical nephron segments is significant in terms of potassium transport has been left unresolved by previous studies. Our inability to demonstrate a measurable increase in specific activity above control values while demonstrating marked differences in net potassium secretion, leads us to conclude that this enzyme does not play a key role in the renal potassium adaptation of uremia.

It is now generally accepted that establishment of a high intracellular potassium concentration by active transport across the peritubular membrane of the distal nephron is a major determinant of transepithelial potassium secretion (35). Studies of electrolyte content of other tissues in uremic man and animals including muscle (47), leukocytes (48), and erythrocytes (49) have all shown that intracellular potassium is low rather than high. It was of interest, therefore, to see whether the increased net transport rate of potassium by normal and uremic CCT from rabbits on high-potassium intakes was associated with an increased driving force across the luminal membrane produced by an elevation of intracellular potassium concentration. The shortcomings of measuring cellular potassium content in nonperfused renal tubules are (*a*) that "activity" rather than "concentration" would be the appropriate parameter to measure and (*b*) that steady-state intracellular potassium concentration in a nontransporting tubule may differ from that which obtains in a perfused tubule. Nevertheless, if the assumption is made that the relationship between concentration and activity is unchanged in tubules with differing transport rates, measurement of intracellular potassium content may provide meaningful information.

The present studies, using both chemical determination of potassium after leaching and isotopic determination of exchangeable potassium, revealed a wide degree of variability within each group. It is impossible to determine whether this was because of technical inadequacies or inherent variations in tubule segments. A similar degree of variation was observed by Grantham et al. (10) using the chemical method of determination on rabbit proximal straight tubules and by Burg and Abramow (11) using ^{42}K exchange in

rabbit collecting tubules. In our hands exchanged potassium constituted 75–80% of chemically determined potassium in each of the four groups studied. Intracellular potassium content per unit length was higher in the uremic than the normal CCT because of the increase in tubule size. When, however, the effect of dietary K intake was evaluated in each group, no differences were found by either method. If anything, the values tended to be lowest in the high-K uremic CCT.

The present studies demonstrate that the CCT is an important site of potassium adaptation in the surviving nephrons of rabbits with reduced renal function. This adaptation is intrinsic to the nephron, is expressed in the absence of a uremic environment and is not a fixed property of the hypertrophied nephron but varies according to the potassium excretory requirements of the animal.

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