Consequences of Potassium Recycling in the Renal Medulla

EFFECTS ON ION TRANSPORT BY THE MEDULLARY THICK ASCENDING LIMB OF HENLE'S LOOP

JOHN B. STOKES, with the technical assistance of IVAN LEE and ALLEN WILLIAMS,
Laboratory of Epithelial Transport and Kidney Physiology, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52242

ABSTRACT The consequences of K recycling and accumulation in the renal medulla were examined by measuring the effect of elevated K concentration on ion transport by the medullary thick ascending limb of Henle's loop. Perfused and bathed in vitro, thick limbs from both mouse and rabbit displayed a graded, reversible reduction of transepithelial voltage after increasing K concentration from 5 to 10, 15, or 25 mM. The effect was reproducible whether osmolality was 328 or 445 mosmol/kg H_2O, and whether K replaced Na or choline. Net chloride absorption and transepithelial voltage were reduced by almost 90% when ambient K concentration was 25 mM. When either lumen or bath K was increased to 25 mM, net Na absorption was reduced. There was spontaneous net K absorption when perfusate and bath K concentration was 5 mM. Analysis of transepithelial K transfer after imposition of chemical gradients demonstrated rectification in the absorptive direction. Absorption of K by this segment provides a means to maintain high medullary interstitial concentration. Accumulation of K in the outer medulla, by reducing NaCl absorption, would increase volume flow through the loop of Henle and increase Na and water delivery to the distal nephron. K recycling thus might provide optimum conditions for K secretion by the distal nephron.

INTRODUCTION

Recent micropuncture experiments have demonstrated that K is recycled to the renal medulla (1–3). The recycling process is dramatically illustrated when, under conditions of acute or chronic K loading, K delivery to the bend of the loop of Henle exceeds 100% of the filtered load (1, 2, 4). The currently accepted pathways for the recycling process involve K secretion by the distal nephron (1, 5), passive absorption across the outer medullary collecting tubule (OMCT)[1] (6) and secretion into the pars recta or descending limb (1, 4). K concentration in the medullary interstitium can thus reach 35–50 mM (1). The consequences of the accumulation of K in the renal medulla have not been addressed. The present experiments were designed to examine the effect of increased K concentration on NaCl absorption across the medullary thick ascending limb of Henle's loop (MTALH), a structure likely to be surrounded by K concentrations considerably in excess of 5 mM. In addition, they examine K transport by the MTALH, a subject that has received little attention to date. The results indicate that elevated K concentrations in the perfusing and bathing fluids reversibly reduce net NaCl absorption by the MTALH. In addition, K is absorbed by a process independent of transepithelial voltage (V_T). The absorption of K by MTALH provides a mechanism whereby K can recycle within the medulla. The resulting accumulation of K can reduce NaCl absorption by MTALH and thereby reduce NaCl content of the medulla. The consequences of K recycling are appropriate for facilitating excretion of K loads.

A portion of this work was presented at the 1981 Annual Meeting of the American Society of Nephrology, Washington, DC.

Received for publication 8 December 1981 and in revised form 9 March 1982.

[1] *Abbreviations used in this paper*: J_v, volume absorption; MTALH, medullary thick ascending limb of Henle's loop; OMCT, outer medullary collecting tubule; P_a^o, apparent permeability coefficient (when V_T is 0); U_{max}, maximum urine osmolality; V_T, transepithelial voltage.

METHODS

Isolated segments of MTALH were dissected either from Swiss-Webster mice (18–24 g) or female New Zealand White rabbits (1.2–3.0 kg). The general procedure has been previously described (7–9). The MTALH from the rabbit was dissected from the inner stripe of the outer medulla. The distinction between inner stripe and outer stripe is not as clear in the mouse but attempts were made to dissect segments from the inner portion of the outer medulla. Dissection from this portion of the medulla increases the likelihood of obtaining a homogeneous epithelium since the cortical portion of the thick ascending limb of Henle's loop is different from the medullary portion in both the mouse (10) and the rabbit (9).

The tubules were dissected in a solution identical to that in which they were subsequently bathed. One of two solutions was used. Solution 1 contained (in millimolars) NaCl, 120; $NaHCO_3$, 25; Na acetate, 10; KCl, 5; $MgSO_4$, 1; $CaCl_2$, 1.8; Na_2HPO_4, 2.3; glucose, 8.3; and L-alanine, 5. This solution had an osmolality of 328±4 mosmol/kg water. Solution 2 contained in addition 25 mM urea, 20 mM choline chloride, and 30 mM NaCl, and had an osmolality of 445±4 mosmol/kg water. All solutions were gassed with 95% oxygen and 5% carbon dioxide so that the pH was 7.4. Except where specifically indicated, all dissection and bathing solutions contained 5% vol/vol bovine calf serum. The tubules were perfused with the same solution as the bath, but without serum. The perfusate also contained 0.2–0.3 mg/ml FD&C green dye 3 (Keystone Aniline and Chemical Co., Chicago, IL) to improve visualization and, when flux studies were performed, 75–100 μCi/ml [*methoxy*-^{3}H]inulin exhaustively dialyzed to remove substances of a molecular weight < 3,000 (11). Using this volume marker, net volume absorption (J_v) was calculated by the standard expression (8) and tubules showing a $J_v > \pm 0.1$ nl·mm^{-1}·min^{-1} were discarded. The 1.1 ml bath was continuously changed at 0.5 ml/min.

After dissection, the tubules were transferred to a plastic chamber where they were perfused using concentric glass pipettes. The tubule was connected to the pipettes and the temperature was raised to 37°C over 2 min where it was maintained (±0.5°C) for the duration of the experiment. V_T, referenced to the bath, was measured continuously using circuitry as previously described (8). When asymmetric solutions were imposed across the tubule, the circuit voltage was corrected for the calculated liquid junction potential (0.6 mV). All voltages reported are thus transepithelial voltages.

The tubules were perfused for 45 min or until V_T was stable, whichever was longer. Rabbit MTALH generally displayed a gradual decay in V_T after reaching a peak immediately after warming (9). In all experiments, V_T was stable 90 min after warming. Mouse MTALH displayed this temporal decrement in V_T less frequently. The reason(s) for the difference is not known. During flux studies, perfusion rates were maintained by hydrostatic pressure at 4.0–5.3 nl/min. When only V_T was measured, perfusion rate was maintained at >10 nl/min to avoid flow-dependent effects (12).

Because net volume flux was near zero, net solute fluxes (J_i, peq·mm^{-1}·min^{-1}) were calculated according to the expression

$$J_i = \dot{V}_L([i]_b - [i]_L)/L,$$

where $\dot{V}_L$ is the collection rate (nanoliters per minute), measured directly by a constant volume pipette, and is equal to the perfusion rate; L is the length of the tubule (millimeters); and $[i]_b$ and $[i]_L$ are the concentrations of the solute in the perfused and collected fluid, respectively (picoequivalents per nanoliter).

The concentration of solute was determined by two different techniques. In experiments where only net chloride absorption was measured, chloride concentration was determined by microtitration (model F-25, WP Instruments, Inc., New Haven, CT). Three samples each of perfusate and collected fluid for each period were stored overnight in water-equilibrated oil. Samples stored in this way maintain stable concentrations for up to a week. For each sample, chloride concentration was determined in quadruplicate. Thus, for each period reported, the net chloride flux represents the difference between 12 determinations each of perfused and collected fluid. The coefficient of variation for a single sample was generally <2%.

In the experiments where net fluxes of Na, K, and Cl were determined, electron probe microanalysis was used. The procedure was similar to that previously reported (13) with minor modifications. The instrument used was a newer model (Applied Research Laboratories, Div. Bausch & Lomb, Sunland, CA, EMX-SM) interfaced with a Tracor Northern (Middleton, WI) computer. Simultaneous determination of Na, K, and Cl were conducted using wavelength dispersive spectrometers. Minimum detection limits and the coefficients of variation for each ion were similar to the values previously reported (13). Net fluxes for each period were determined from three samples each of perfused and collected fluid with each sample being determined in sextuplicate.

The experimental procedure consisted of two or three periods. In the control period potassium concentration of the perfusate and bath was 5 mM. In the experimental period potassium concentration was raised to 10, 15, or 25 mM. In experiments where only V_T was measured, a recovery period was obtained. When solution 1 was used, potassium replaced sodium. When solution 2 was used, potassium replaced choline. In all experiments where the perfusate was changed, a sham change was executed to be certain that changing the perfusate by itself did not result in a change in V_T. Any tubule having a change in $V_T > \pm 0.5$ mV after this sham change was discarded. All V_T and flux measurements were obtained after a minimum stable period of at least 10 min.

Statistical analysis was conducted by either paired or unpaired Student's *t* test as appropriate. Data are expressed as mean±SEM. Unless otherwise stated, significant differences have a *P* value of <0.05.

RESULTS

Effects of K concentration on V_T of mouse MTALH. Fig. 1 displays the results of increasing potassium concentration in both perfusate and bath solutions of the mouse MTALH from 5 mM to 10, 15, or 25 mM. In these experiments, where control and recovery periods used solution 1 (328 mosmol/kg water) K replaced Na. To evaluate whether the decrement in V_T was due to the increase in ambient K concentration or due to the decrease in Na concentration, a series of experiments was conducted on mouse MTALH where K replaced choline rather than Na. The results of these experiments are shown in Fig. 2. In this latter set of experiments the more hypertonic solution (445 mosmol/kg water) was designed to simulate more closely the com-

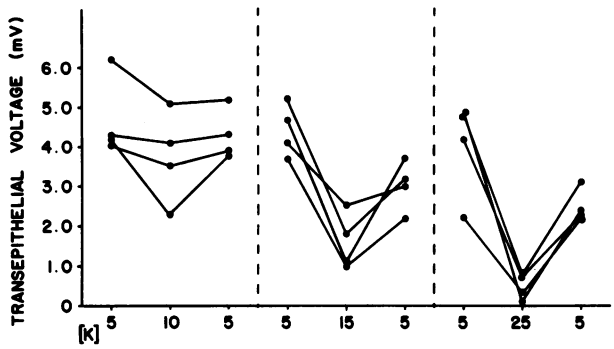

FIGURE 1 Effect of increasing K concentration ([K], in millimolars) in bath and lumen on transepithelial voltage across MTALH of the mouse. Onset of the reduction was within a minute and stabilization was achieved within 10 min. Recovery was similar in time course. In these experiments solution 1 was used (328 mosmol/kg H_2O) and K replaced Na.

position of the outer medullary interstitium (14). The mean values for each group are shown in Table I. It is clear that increasing ambient K concentration reduced the magnitude of V_T in a reversible fashion. The reduction in V_T often began as quickly as the exchanges could be made and continued for another 5–10 min at which time the V_T stabilized. On occasion, the reduction in V_T did not stabilize for 20 min. Recovery of V_T was equally rapid, requiring 5–10 min for stabilization.

When the results are considered together, the reduction in V_T must have been due to the increase in K concentration and not to changes in ambient Na or choline concentrations. The change in voltage cannot be attributed to the generation of diffusion potentials or liquid junction potentials since the substitutions were made symmetrically. It is likewise evident that the effect is reproducible under conditions of hypertonicity or near isotonicity (Figs. 1 and 2). Although

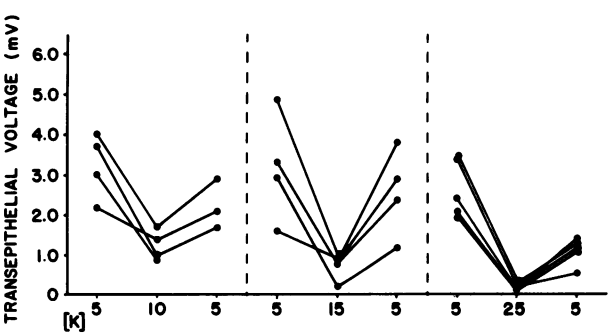

FIGURE 2 Effect of increasing K concentration (in millimolars) in bath and lumen on transepithelial voltage across MTALH of the mouse. Time course was similar to experiments shown in Fig. 1. In these experiments solution 2 was used (445 mosmol/kg H_2O) and K replaced choline.

both groups responded to K in a generally similar fashion, there are some indications that the groups might be different. The mean V_T of tubules perfused originally in solution 1 was 4.4±0.4 mV while the mean value for the tubules perfused in solution 2 (hypertonic) was 3.1±0.2 mV. The difference is significant ($P < 0.01$ by unpaired analysis) and is consistent with the findings of Hebert et al. (15) that an acute increase in osmolality of solutions bathing the mouse MTALH reduces the vasopressin-stimulated increment in V_T. A second observation that suggests that the two groups may not be strictly comparable is that although 25 mM K produced a reduction in V_T of ~90% in both groups, the response to 10 mM K appears different. It is possible that a hypertonic environment renders the tubule more sensitive to small increments in K concentration. Despite these suggestions that the osmolality of the environment might influence the performance of the MTALH, it is clear that acute changes in ambient K concentration can produce reversible reductions in V_T across the mouse MTALH.

Effect of K concentration on V_T and J_{Cl} absorption across rabbit MTALH. To determine if the effect of elevated K concentration was operative in the rabbit MTALH as well as the mouse, K concentration in both lumen and bath was raised to either 10 or 25 mM in eight experiments. The results are depicted in Fig. 3. Solution 1 was used in these experiments and the results are comparable to the results in the mouse (Fig. 1). There was an insignificant effect of 10 mM K, while the effect of 25 mM K was unambiguous and at least partially reversible. The time course was similar to that of the mouse.

Although the precise mechanisms involved in the generation of the lumen-positive V_T in the thick ascending limb are not completely understood, there is considerable evidence that changes in the magnitude of the V_T reflect changes in the magnitude of NaCl absorption (9, 10, 15–19). To determine directly whether such was the case in the present experiments, net chloride absorption (lumen-to-bath flux) was measured in MTALH dissected from normal rabbits before and after increasing both perfusate and bath K concentration from 5 to 25 mM. The results of 10 such experiments together with six control experiments (where the perfusate was sham changed) are displayed in Table II. Solution 1 was used and K replaced Na. Both V_T and J_{Cl} were reduced by 86%, a value comparable to the decrements in V_T produced by 25 mM K in the mouse MTALH (Figs. 1 and 2). Thus, a reduction of V_T reflects a reduction of NaCl absorption.

Evaluation of simultaneous net Na, K, and Cl fluxes. The simultaneous determination of net Na, K, and Cl fluxes has not been reported for the MTALH of the rabbit. Table III displays the mean V_T, the mean

TABLE I

Effect of Increasing Ambient K Concentration on Mean Voltage (mV) across MTALH of the Mouse

	Control	10K	Recovery	Control	15K	Recovery	Control	25K	Recovery
		(n = 4)			(n = 4)			(n = 4)	
Solution 1, 328 mosmol/kg	4.7 ±0.5	3.8 ±0.6	4.3 ±0.3	4.4 ±0.3	1.6* ±0.3	3.0 ±0.3	4.0 ±0.6	0.5* ±0.2	2.6 ±0.2
		(n = 4)			(n = 4)			(n = 5)	
Solution 2, 445 mosmol/kg	3.2 ±0.4	1.2* ±0.2	2.2 ±0.4	3.2 ±0.7	0.7* ±0.2	2.6 ±0.5	2.6 ±0.3	0.2* ±0.1	1.3 ±0.3
All results	4.0 ±0.4	2.5* ±0.6	3.4 ±0.5	3.8 ±0.4	1.2* ±0.2	2.8 ±0.3	3.3 ±0.4	0.3* ±0.1	1.9 ±0.3

Values are mean±SEM. In solution 1, K replaced Na while in solution 2, K replaced choline.

* Significantly different from both control and recovery values by paired analysis ($P < 0.05$).

difference in concentration between perfused and collected fluid, and the net (absorptive) fluxes of Na, K, and Cl, determined by electron probe microanalysis for 14 tubules perfused and bathed with solution 1. There was a small but significant net absorption of K. In addition, there was no difference between the net absorption of the sum of Na and K (101±10) and the net absorption of Cl (109±12). These results provide no evidence for a significant flux of another anion (such as HCO_3^-) although the technique is not sufficiently sensitive to detect a small net flux of an unmeasured species. These data support the notion that the measurement of the change in net Cl transport (under symmetrical conditions) reflect changes in NaCl transport (10, 12, 20).

To evaluate the effect of an asymmetric increase in K concentration the 14 tubules, after measurement of their steady-state base-line transport, had either their bath or perfusate K raised to 25 mM with a concomitant reduction in Na. The purposes of this maneuver were to examine the "sidedness" of an effect and to examine net K fluxes in the presence of a chemical concentration gradient.

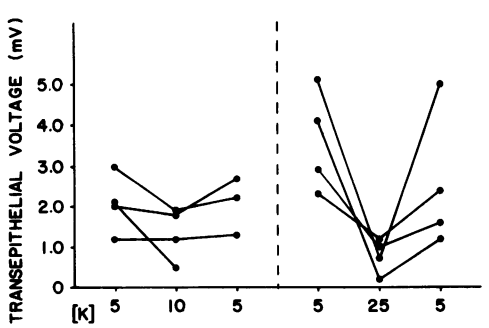

FIGURE 3 Effect of increasing K concentration (in millimolars) in bath and lumen on transepithelial voltage across MTALH of the rabbit. Time course was similar to the experiments conducted on mouse MTALH (Figs. 1 and 2). Solution 1 was used (328 mosmol/kg H_2O) and K replaced Na.

The results of the experiments where lumen K was raised to 25 mM are displayed in Table IV. There was an increase in net K efflux, a striking reduction in net Na efflux, and no significant change in Cl efflux. Since the direction of the change in Na and K transport was appropriate for their respective concentration gradients, one cannot be certain whether the increase in luminal K reduced active transport of NaCl. The reduction in V_T from 3.1±1.0 to 0.8±0.2 mV suggests that it did, but one cannot be completely certain (vide infra). In any event, it is obvious that this maneuver reduced the net NaCl absorption and enhanced net KCl absorption.

The results of the experiments where bath K was raised to 25 mM are depicted in Table V. There was a net K influx, consistent with the direction of the K concentration gradient, a reduction of Cl efflux to near zero, and a significant reduction of net Na efflux. The reduction in Na efflux occurred despite the favorable chemical gradient. This result suggests that the active transport of NaCl was reduced by increasing bath K.

The mechanism of the reduction of Na efflux by increasing either bath or lumen K to 25 mM cannot be deduced with certainty from these experiments. The reduction in Na absorption when bath K was raised to 25 mM (Table V) is consistent with the notion that transcellular NaCl efflux was reduced, since net Na efflux fell (despite a favorable chemical gradient) and Cl efflux was nearly abolished. The reduction of V_T from 3.3±0.5 to 1.7±0.6 mV supports this interpretation. The interpretation of the effects of raising

TABLE II

Effect of Increasing Both Perfusate and Bath K Concentration from 5 to 25 mM on V_T and J_{Cl} across MTALH of the Rabbit

Experiment	L	$\dot{V}$	V_T Control	V_T 25K	J_{Cl} Control	J_{Cl} 25K
	mm	*nl/min*	*mV*		*peq·mm⁻¹·min⁻¹*	
Effect of 25K						
1	0.65	4.5	4.8	0.2	67.4	9.2
2	1.1	4.3	2.0	0.1	152	2.0
3	1.1	4.7	2.0	0.2	90.1	−2.5
4	1.25	4.5	1.1	1.9	68.6	16.8
5	0.7	4.8	4.6	0.6	77.3	2.4
6	0.8	4.6	1.1	0.1	38.6	25.9
7	0.4	4.1	2.5	0.0	47.9	21.2
8	0.75	4.4	4.1	0.2	97.4	6.6
9	0.65	4.3	2.9	0.4	73.5	6.1
10	0.9	4.6	2.8	1.0	67.1	21.3
Mean	0.8	4.5	2.8	0.4	78.0	10.9
±SEM	0.1	0.1	0.4	0.2	9.9	3.1
P			<0.002		<0.001	
Effect of sham change						
1	1.0	4.8	1.6	2.4	50.1	98.1
2	1.2	4.4	6.3	6.5	170	143
3	0.4	4.8	3.5	4.0	118	124
4	0.95	4.4	2.5	1.8	41.4	38.7
5	0.5	4.5	2.2	1.7	86.3	54.4
6	0.45	4.1	2.8	2.2	28.2	43.9
Mean	0.8	4.5	3.2	3.1	82.2	83.6
±SEM	0.1	0.1	0.7	0.8	22.1	18.1
P			NS		NS	

Solution 1 was used in all experiments. The increase in K concentration was accompanied by an equimolar reduction in Na concentration. Abbreviations used in this table: L, length; $\dot{V}$, collection rate (equal to perfusion rate); J_{Cl}, net chloride absorption; control and 25K refer to the periods where K concentration was 5 and 25 mM, respectively.

lumen K to 25 mM are more complicated (Table IV). The reduction in net Na efflux might have been due to either the reduction in transcellular NaCl efflux or an increase in Na backflux through the paracellular pathway or a combination of these effects. The reduction of V_T from 3.1±1.0 to 0.8±0.2 mV supports the notion that transcellular NaCl absorption was reduced. This reasoning is based on the relative cation permeability, determined by Greger (21), of the cortical thick limb of the rabbit. If the voltage response to these maneuvers were due only to the biionic diffusion potential, raising bath K should have increased (rather than reduced) V_T by 0.9 mV. Likewise, raising perfused K should have reduced V_T by only 0.9 mV. Thus, although the effect of elevated K from either the lumen or bath is consistent with the notion that transcellular NaCl efflux was reduced, this conclusion must be considered tentative until there is more information regarding alterations in intracellular events.

Analysis of K flux. As demonstrated in Table III, under base-line conditions there was a consistent net reabsorption of K. This result is in contrast to the results in the cortical thick ascending limb of Henle's loop of the rabbit (22) where, under similar circumstances, K secretion was generally observed. The evaluation of whether this transport process is passive can be approached by using the Goldman equation (23), an integrated form of the Nernst-Plank equation, which assumes a constant electric field (i.e., the electrical potential is a linear function of distance). The expression is written

$$-J_i = \frac{PzFV_T}{RT}\left[\frac{C_b - \bar{C}_l e^{-zFV_T/RT}}{e^{zFV_T/RT} - 1}\right],$$

where J_i is the net flux of ion i (picoequivalents per millimeter per minute) where a positive value indicates absorption, P the permeability coefficient[2] (nanoliters per millimeter per minute), C_b the concentration of i in the bath, $\bar{C}_l$ the arithmatic mean concentration of i in the lumen, z the valence of i, F the Faraday, R the gas constant, and T the absolute temperature. This expression allows the prediction of net flux for any epithelium (membrane) where V_T, the permeability coefficient and the concentration gradients are known if the flux proceeds entirely by simple diffusion.[3]

Under control conditions there was net absorption of K (Table III). Since absorption of a cation in the presence of a lumen-positive V_T might be owing to diffusion and not active transport, the apparent permeability coefficient was calculated using the above expression and the measured values for each tubule. The mean apparent permeability coefficient was 6.13 $\times 10^{-4}$ cm/s. This value is an order of magnitude larger

[2] The permeability coefficient can be converted to the more traditional units of centimeters per second by multiplying by 2.66×10^{-5}, which assumes an internal diameter of the tubule of 20 μm.

[3] The designation of permeability coefficient means that transport proceeds entirely by simple diffusion. A value for P can be calculated despite no a priori knowledge of the mechanism of transport. For purposes of comparing lumen-to-bath and bath-to-lumen fluxes in the presence of a measurable V_T and a concentration gradient, P can be calculated and called an apparent permeability coefficient at $V_T = 0$. In the present experiments the calculated apparent permeability coefficients are not the true permeability coefficient since they do not conform to the patterns predicted by simple ionic diffusion.

TABLE III

Base-line Transport Characteristics of Rabbit MTALH (n = 14)

V_T	$[Na]_b - [Na]_L$	J_{Na}	$[K]_b - [K]_L$	J_K	$[Cl]_b - [Cl]_L$	J_{Cl}
mV	*meq/liter*	*peq·mm^{-1}·min^{-1}*	*meq/liter*	*peq·mm^{-1}·min^{-1}*	*meq/liter*	*peq·mm^{-1}·min^{-1}*
3.2	17.1	97.1	0.7	3.8	19.3	109
±0.3	±1.6	±9.8	±0.2	±0.3	±2.0	±12

Values are mean±SEM.

J_{Na}, J_K, and J_{Cl} are the net transport rates of Na, K, and Cl, respectively.

$[Na]_b - [Na]_L$, $[K]_b - [K]_L$, and $[Cl]_b - [Cl]_L$ are concentration differences between perfused and collected fluid.

All values are significantly >0 ($P < 0.01$).

than the permeability coefficient for Na of 6.27×10^{-5} cm/s determined by Rocha and Kokko (12) using bath-to-lumen tracer fluxes. The discrepancy suggests that potassium transport might not be entirely explained by paracellular diffusion, for a 10-fold selectivity of K over Na has not been previously described. However, the magnitude of the V_T and J_K measurements[4] is such that small errors create large errors in the calculated apparent permeability. The standard error is sufficiently large so that no firm conclusion can be reached.

The measurement of net K fluxes after an imposed chemical gradient allows a considerably more accurate determination of the apparent permeability coefficient. Table VI displays the K flux data in tubules where such a concentration gradient was imposed. It is clear that the net fluxes of K under the two conditions are significantly different. Although the individual experiments are not shown, there was no overlap in these values. Statistical analysis of V_T showed no difference between the two groups. However, since a small V_T might produce a significant difference in the flux ratio if the true permeability coefficient were large, the apparent permeability coefficient (P_a^o) was calculated for each experiment. These values were likewise significantly different from each other (Table VI). If potassium were transported entirely by simple diffusion, the ratio of these numbers should be unity. The ratio is 2.17, a value closer to the ratio of the unidirectional fluxes for Na (1.71) measured isotopically (12).

The analysis of the discrepancy between the potassium fluxes can be conducted in another way. If one assumes that all K transport occurs by simple diffusion, i.e., that the apparent permeability coefficient measured from lumen-to-bath and bath-to-lumen are in fact not different, then one can calculate the mean V_T necessary to effect the measured net K fluxes. This voltage would have to be ~10 mV. Given an upper limit of 2 mV at the perfused end, the voltage at the distal end would have to be ~22 mV, an extremely unlikely occurrence since the (bath-to-lumen) NaCl concentration gradient required to produce this voltage is >4 (12) and the maximal concentration gradient at the distal end of these tubules was only 1.3. Thus, the absorption of K that occurred under control conditions was most likely not due entirely to the lumen positive V_T. Rather, these data provide presumptive evidence for a transcellular absorptive process for K.

Mechanism of the effect of K on NaCl absorption.

TABLE IV

Effect of Increasing Perfused K Concentration to 25 mM on Net Ion Transport across Rabbit MTALH (n = 7)

	L	V_T	J_{Na}	J_K	J_{Cl}
	mm	*mV*		*peq·mm^{-1}·min^{-1}*	
Control		3.1	102	2.9	104
		±1.0	±16	±1.2	±17
	0.9				
	±0.1				
25K		0.8	12.7	59.5	80.7
		±0.2	±5.9	±3.8	±7.8
P*		<0.002	<0.002	<0.001	NS

* P values represent comparison of 25K period vs. control.

Control solutions contained 5 mM K in both perfusate and bath. Increase in K concentration accompanied by equivalent decrease in Na concentration.

Abbreviations as in Tables I and II.

[4] At perfusion rates that are sufficiently slow to allow a NaCl concentration gradient to develop at the collection end of the tubule, the V_T at the distal end will be more positive than at the proximal end. The greatest bath-to-lumen concentration gradient measured in these experiments was 1.3. If the Na/Cl permeability ratio is between 2.2 (10, 21) and 6.1 (12), the V_T at the distal end of the tubule will be as much as 3.5 to 5.5 mV greater than the proximal end and the mean V_T will be greater than reported. Because of biologic variability and uncertainty of the V_T at the distal end, and the inherent errors in measuring ion concentrations, mean V_T can not be precisely assessed.

TABLE V
Effect of Increasing Bath K Concentration to 25 mM on Net Ion Transport across Rabbit MTALH (n = 7)

	L	V_T	J_{Na}	J_K	J_{Cl}
	mm	*mV*		*peq·mm⁻¹·min⁻¹*	
Control		3.3	92.2	4.7	114
		±0.5	±12.5	±1.0	±19
	0.8				
	±0.1				
25K		1.7	57.3	−33.5*	4.9
		±0.6	±5.3	±3.0	±10.2
P		<0.05	<0.05	<0.001	<0.001

Abbreviations as in Tables I and II.
* Negative flux represents secretion.

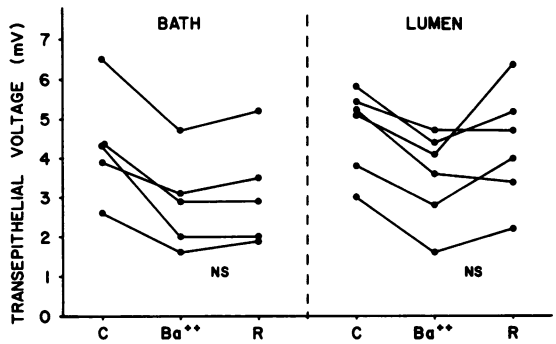

FIGURE 4 Effect of 4 mM Ba^{++} on transepithelial voltage across the mouse MTALH. Solution 1 was used (328 mosmol/kg H_2O). C and R indicate stable control and recovery values, respectively. (Ba^{++} effect from bath, $P < 0.001$; from lumen $P < 0.002$. Recovery NS).

The most likely explanation for the K effect on NaCl transport involves membrane depolarization. If such is the case, membrane depolarization by another mechanism should produce a similar effect. Previous reports have shown that Ba^{++} administered in millimolar concentrations can reduce membrane K conductance of several cell types (24–27) and by so doing depolarize the membrane (25, 28). In addition, Greger and Schlatter (29) have shown that Ba^{++} produces such an effect in the cortical thick limb of the rabbit. Thus, Ba^{++} should mimic the effects of elevated K concentration.

It became apparent that the effect of Ba^{++} would not be as straightforward as the effect of high K. First, there was no effect of Ba^{++} when the bath contained 5% bovine serum. For this reason, the serum was omitted from the bath, a maneuver that on several occasions caused a reduction in V_T in both rabbit and mouse MTALH. Thus, the Ba^{++} experiments were conducted on mouse MTALH that had been dissected in 5% serum solution but was perfused and bathed in solution 1 (osmol = 328) that contained no serum. Three tubules treated in this fashion developed a V_T that gradually fell from >5 mV initially to <2 mV at 90 min, despite having an epithelium that appeared normal. These tubules were discarded. The results of the effect of 4 mM Ba^{++} on V_T of the acceptable experiments are displayed in Fig. 4. Ba^{++}, when added to the bath as $BaCl_2$, produced a reduction in V_T from 4.3±0.6 to 2.9±0.5 mV ($P < 0.01$). The change usually began within a minute and was complete within 10 min. When Ba^{++} was added to the perfusate, V_T fell from 4.7±0.4 to 3.5±0.5 mV ($P < 0.002$) with a similar time course. Recovery was inconsistent. Despite the differences between the effects of high K and Ba^{++}, the similarities between the direction of the change in V_T and the time course of the change are consistent with the notion that both high K and Ba^{++} might be producing their effects by depolarizing the membrane.[5]

TABLE VI
Net K Flux across Rabbit MTALH in Presence of a Concentration Gradient

Perfused [K] (mM) Bath [K] (mM)	25 5	5 25	*P*
	(*n* = 7)	(*n* = 7)	
V_T (mV)	0.8 ±0.2	1.7 ±0.6	NS
$[K]_0 - [K]_L$ (mM)	10.5 ±0.8	−6.2 ±0.4	<0.001
J_K (peq·mm^{-1}·min^{-1})	59.5 ±3.8	−33.5 ±3.0	<0.001
P_a^0 (cm/s × 10^{-5})	12.8 ±0.5	5.9 ±0.5	<0.001

Values are mean±SEM.
P values compare absolute values of column 1 to column 2 using unpaired analyses. P_a^0 is the calculated apparent permeability coefficient (when $V_T = 0$) for K. Other abbreviations as in Tables I, II, and III.

[5] In the cortical thick limb of the rabbit, Ba^{++} depolarizes the cell membrane by reducing K conductance, and inhibits the calculated short circuit current (an estimate of the magnitude of NaCl absorption) (29). However, the influence of these events on V_T is complicated by uncertainties regarding the magnitude of the change in transepithelial resistance. Van Driessche and Zeiske (30) have demonstrated a possible reduction in shunt resistance in the frog skin. Greger and Schlatter (29) have demonstrated a large increase in transepithelial resistance of the cortical thick limb, most probably owing to reduction of the transcellular K conductance. This latter effect would explain the lesser change in V_T observed in the present Ba^{++} experiments (compared with the K experiments) if the cellular K conductance participated in the shunting of V_T generated by active NaCl transport.

DISCUSSION

The results of the present experiments demonstrate that an acute increase in ambient K concentration to ranges found in vivo (1) can produce a substantial reduction in V_T and NaCl absorption across the MTALH of both the mouse and the rabbit. The effect is reversible and occurs under hypertonic and near isotonic conditions (Figs. 1 and 2).

The rate of net NaCl absorption across the MTALH has been shown to be stimulated by vasopressin (10, 15–17) and β-adrenergic agonists (31). It can be reduced by prostaglandin E_2 (9) and hypertonicity (15). The present results add another link to our understanding of the control of NaCl absorption by the loop of Henle and, as a consequence, the control of medullary tonicity. Perhaps most importantly, they provide the first data addressing the functional significance of K recycling to the renal medulla.

Since K recycling was first described by Battilana et al. (1), its nature has received increasing attention. The unambiguous evidence of K secretion by the thin descending limb of Henle's loop or the pars recta (1, 4, 32) has prompted a series of experiments investigating the pathways for this recycling. It is now generally accepted that the K secreted by the distal convoluted tubule and cortical collecting tubule is in part reabsorbed across the outer medullary and/or papillary collecting tubule (1, 2, 6) and accumulates in the medullary interstitium. This accumulation allows a concentration gradient to develop that causes net K secretion into the thin descending limb of Henle's loop (33) or pars recta (34, 35). K recycling is enhanced in chronic K administration (1) as well as acute K administration (2) and is reduced by K deprivation (3).

The concentrations of K used in these experiments are probably within the range of concentrations found in vivo. Dobyan et al. (3) have reported a mean K concentration at the bend of the loop of 25 mM in young Munich-Wistar rats fed a normal diet, and a concentration of 9 mM in rats deprived of K for 3 d. Battilana et al. (1) have reported similar values for normal rats and a mean concentration of 37 mM for rats fed a high K diet. Concentrations in vasa recta blood were ~10 mM greater than loop fluid (1, 36). Although there is no clear way to extrapolate K concentrations found at the bend of the loop to the concentrations in the outer medulla, the range used in the present study is a reasonable estimate of the range of concentrations likely surrounding the medullary thick ascending limb of Henle's loop. Individual values reported by Jamison et al. (37) for the Sprague-Dawley rat and deRouffignac and Morel (32) for the psammomys support the notion that this range of concentrations may be found under normal physiologic conditions.

The reduction of NaCl absorption across the MTALH by elevated K concentration is consistent with the effects of acute infusion of K on Na excretion. Vander (38) described a "direct" effect of K on Na excretion by the kidney. Brandis et al. (39), using micropuncture techniques, found that fluid absorption across the proximal convoluted tubule was reduced by increased peritubular K concentration. Reduction in fluid absorption was not observed by increasing peritubular K concentration similarly in the isolated, perfused rabbit proximal tubule (40). Kahn and Bohrer (41), using clearance techniques, postulated a major effect on the "distal convoluted tubule" (diluting segment). Wright et al. (42) examined distal tubule Na absorption and found that most of the natriuretic effect occurred proximal to the superficial distal convoluted tubule. They showed that Na delivery to the early distal tubule was increased by acute K infusion as well as chronic administration of a high K diet.

The diuretic effect of a high K diet is also widely recognized, although the explanation for this phenomenon is not completely understood. The osmotic diuresis (NaCl and KCl) may play a role under some circumstances. However, Battilana et al. (1) demonstrated a reduced urine osmolality in chronically K-loaded rats without an increase in solute excretion. These findings suggest that chronic K-loading might impair the generation of a maximum urine osmolality (U_{max}). An impairment in U_{max} is consistent with the present results regarding reduced NaCl absorption across the MTALH, an effect that would lead to a diminished medullary interstitial solute content.

K transfer across MTALH. In addition to providing information regarding the effect of K on NaCl absorption, the present experiments provide evidence that K transfer across the MTALH cannot be accounted for by an entirely passive process. Under baseline conditions, when ambient K concentration was 5 mM, K was absorbed (Table IV). This result is different from that reported by Burg and Bourdeau (22) for the cortical thick ascending limb of the rabbit. Under similar conditions they found a small net secretion.[6]

Since the net absorption of K in the presence of a lumen-positive V_T might be owing completely to diffusion, the data were analyzed according to the Goldman flux equation (23). These results (Table VI) clearly indicate that there is rectification of K movement

[6] The present results also contrast with preliminary results reported by Work and Schafer (43). Using tracer measurements of Rb flux they reported a larger secretory component than absorptive component. The reasons for these apparent discrepancies are not clear.

across the MTALH in the absorptive direction. The mechanism of such a rectification process cannot be discerned from the present experiments. Nevertheless, its presence is consistent with the magnitude of the net absorption of K under control conditions when K concentration was 5 mM (Table IV).

The significance of K absorption by the MTALH is most readily appreciated if it is viewed within the context of the presumed pathways of K recycling as depicted schematically in Fig. 5. There appear to be four discrete steps. (*a*) K is secreted by the distal convoluted tubule and the cortical collecting tubule; (*b*) a portion of this secreted K is passively reabsorbed across the OMCT (6) and raises the interstitial K concentration; (*c*) K enters the pars recta or thin descending limb of Henle's loop; (*d*) the recycling process is amplified by absorption across the MTALH. This last step may be important in regulating the magnitude of K accumulation in the medulla. Although it is clear that K can diffuse across the outer medullary collecting duct into the interstitium, the permeability of this epithelium to K is low (6) so there is some doubt as to whether the magnitude of K reabsorption across OMCT could explain interstitial K concentration of up to 50 mM (1). A large K reabsorption across OMCT would also be counterproductive under conditions when the K excretory mechanisms should be operating maximally, i.e., under conditions of K loading. The absorption of K across the MTALH would effectively minimize the requirements for K absorption across OMCT and would amplify K accumulation in the medullary interstitium.

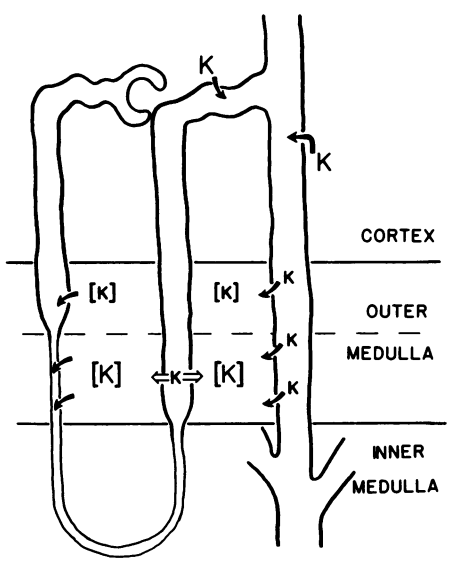

FIGURE 5 Schematic representation of K recycling to the renal medulla. (*a*) K is secreted by the distal nephron. (*b*) Small amount is passively reabsorbed by the outer medullary collecting tubule. (*c*) Secretion into the pars recta or descending limb of Henle's loop. (*d*) Absorption by MTALH.

K effect on NaCl absorption. The mechanism whereby increasing K concentration reduced V_T and NaCl absorption is most likely related to depolarization of the cell membrane. Most mammalian cells have as a major component of their membrane voltage a K diffusion potential. Because barium depolarizes cell membranes by reducing K conductance (24–27, 29), the results of the effects of barium (Fig. IV) are consistent with the notion that cell depolarization may be the mechanism whereby an elevation in the ambient K concentration might reduce NaCl absorption. Depolarization of the cell might reduce NaCl absorption by reducing the electrochemical gradient for Cl exit across the basolateral membrane. According to our current perceptions of the mechanism of NaCl absorption by the thick ascending limb of Henle's loop, NaCl enters the cell by an electrically neutral mechanism across the apical membrane and exits across the basolateral membrane (15, 21, 29, 44–46). A conductive Cl exit step would be voltage-sensitive and would provide a convenient explanation for the present results.

Significance. The direct implications of the present experiments are that K recycling would produce natriuresis and diuresis. Although these effects are often observed after acute K infusion into the intact animal, the complete (teleological) significance may relate to the diuretic and natriuretic effects only indirectly. The major importance of K recycling may have more to do with facilitating K excretion than with producing a Na and water diuresis. As we currently understand K secretion by the distal nephron, there are two major determinants. In the distal convoluted tubule K secretion is positively correlated with axial volume flow (47). In the cortical collecting tubule, K secretion is, in large part, regulated by the absorption of Na (48), a process that is enhanced by mineralocorticoid hormone (48–50) and is dependent on an adequate concentration of Na in the tubular fluid (48, 51). By reducing NaCl absorption across the MTALH, K accumulation in the medulla would increase volume flow through the loop of Henle (by virtue of reducing water absorption across the thin descending limb) and thus increase fluid delivery to the distal convoluted tubule. Simultaneously, Na delivery (and presumably Na concentration in the tubular field) to the cortical collecting tubule would also be increased. This shift in the location of Na absorption together with an appropriate increase in aldosterone secretion (52) would maximize K secretion by the cortical collecting tubule. Thus, an increase in K secretion by the distal nephron would, by virtue of K recycling to the medulla, create

conditions in the distal nephron that would permit maximal K excretion.

In summary, the accumulation of K in the renal medulla can inhibit the absorption of NaCl by the MTALH. This action would have as an immediate effect a reduction in medullary solute content and consequently an enhancement of Na and H_2O delivery to the distal nephron. The ultimate importance of K recycling might be the provision of adequate Na delivery and volume flow to the distal nephron so that K secretion can proceed maximally. The mechanism whereby K produces this reduction in NaCl absorption is probably via depolarization of the cell. The presence of absorptive rectification of K raises new possibilities for the involvement of K in the process of NaCl absorption by the MTALH.

ACKNOWLEDGMENTS

I appreciate the secretarial assistance of Ms. Sydney Harned and the thoughtful critique of the manuscript by Dr. Gerald DiBona and Dr. Michael Welsh.

This work was supported in part by grants from the National Institutes of Health AM-25231, HL-14388, and RR-05372; a grant from the Iowa Heart Association; and a grant from the National Kidney Foundation of Iowa.

REFERENCES

1. Battilana, C. A., D. C. Dobyan, F. B. Lacy, J. Bhattacharya, P. A. Johnston, and R. L. Jamison. 1978. Effect of chronic potassium loading on potassium secretion by the pars recta or descending limb of the juxtamedullary nephron in the rat. *J. Clin. Invest.* **62:** 1093–1103.
2. Arrascue, J. B., D. C. Dobyan, and R. L. Jamison. 1981. Potassium recycling in the renal medulla: effects of acute potassium chloride administration to rats fed a potassium-free diet. *Kidney Int.* **20:** 348–352.
3. Dobyan, D. C., F. B. Lacy, and R. L. Jamison. 1979. Suppression of potassium recycling in the renal medulla by short-term potassium deprivation. *Kidney Int.* **16:** 704–709.
4. Jamison, R. L., F. B. Lacy, J. P. Pennell, and V. M. Sanjana. 1976. Potassium secretion by the descending limb or pars recta of the juxtamedullary nephron in vivo. *Kidney Int.* **9:** 323–332.
5. Wright, F. S. 1977. Sites and mechanisms of potassium transport along the renal tubule. *Kidney Int.* **11:** 415–432.
6. Stokes, J. B. 1982. Na and K transport across the cortical and outer medullary collecting tubule of the rabbit: evidence for diffusion across the outer medullary portion. *Am. J. Physiol.* **242**(Renal Fluid Electrolyte Physiol. 11): F514–F520.
7. Burg, M., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* **210:** 1293–1298.
8. Stokes, J. B., and J. P. Kokko. 1977. Inhibition of sodium transport by prostaglandin E_2 across the isolated, perfused rabbit collecting tubule. *J. Clin. Invest.* **59:** 1099–1104.
9. Stokes, J. B. 1979. Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle. Selective inhibition of the medullary portion. *J. Clin. Invest.* **64:** 495–502.
10. Hebert, S. C., R. M. Culpepper, and T. E. Andreoli. 1981. NaCl transport in mouse medullary thick ascending limbs. I. Functional nephron heterogeneity and ADH-stimulated NaCl cotransport. *Am. J. Physiol.* **241**(Renal Fluid Electrolyte Physiol. 10): F412–F431.
11. Schafer, J. A., S. L. Troutman, and T. E. Andreoli. 1974. Volume reabsorption, transepithelial potential differences, and ionic permeability properties in mammalian superficial proximal straight tubules. *J. Gen. Physiol.* **64:** 582–607.
12. Rocha, A. S., and J. P. Kokko. 1973. Sodium chloride and water transport in the medullary thick ascending limb of Henle. Evidence for active chloride transport. *J. Clin. Invest.* **52:** 612–623.
13. Stokes, J. B., M. J. Ingram, A. D. Williams, and D. Ingram. 1981. Heterogeneity of the rabbit collecting tubule: localization of mineralocorticoid hormone action to the cortical portion. *Kidney Int.* **20:** 340–347.
14. Ullrich, K. J., K. Kramer, and J. W. Boylan. 1961. Present knowledge of the counter-current system in the mammalian kidney. *Prog. Cardiovasc. Dis.* **3:** 395–431.
15. Hebert, S. C., R. M. Culpepper, and T. E. Andreoli. 1981. NaCl transport in mouse medullary thick ascending limbs. III. Modulation of the ADH effect by peritubular osmolality. *Am. J. Physiol.* **241**(Renal Fluid Electrolyte Physiol. 10): F443–F451.
16. Hall, D. A., and D. M. Varney. 1980. Effect of vasopressin on electrical potential difference and chloride transport in mouse medullary thick ascending limb of Henle's loop. *J. Clin. Invest.* **66:** 792–802.
17. Sasaki, S., and M. Imai. 1980. Effects of vasopressin on water and NaCl transport across the in vitro perfused medullary thick ascending limb of Henle's loop of mouse, rat, and rabbit kidneys. *Pfluegers Arch. Eur. J. Physiol.* **383:** 215–221.
18. Hebert, S. C., R. M. Culpepper, and T. E. Andreoli. 1981. NaCl transport in mouse medullary thick ascending limbs. II. ADH enhancement of transcellular NaCl cotransport; origin of transepithelial voltage. *Am. J. Physiol.* **241**(Renal Fluid Electrolyte Physiol. 10): F432–F442.
19. Kokko, J. P. 1974. Membrane characteristics governing salt and water transport in the loop of Henle. *Fed. Proc.* **33:** 25–30.
20. Seldin, D. W., J. M. Rosen, and F. C. Rector, Jr. 1975. Evidence against bicarbonate reabsorption in the ascending limb, particularly as disclosed by free-water clearance studies. *Yale J. Biol. Med.* **48:** 337–347.
21. Greger, R. 1981. Cation selectivity of the isolated perfused cortical thick ascending limb of Henle's loop of rabbit kidney. *Pfluegers Arch. Eur. J. Physiol.* **390:** 30–37.
22. Burg, M. B., and J. E. Bourdeau. 1978. Function of the thick ascending limb of Henle's loop. *In* New Aspects of Renal Function. H. G. Vogel and K. J. Ullrich, editors. Exerpta Medica, Amsterdam. pp. 91–102.
23. Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27:** 37–60.
24. Armstrong, C. M., and S. R. Taylor. 1980. Interaction of barium ions with potassium channels in squid giant axons. *Biophys. J.* **30:** 473–488.
25. Hermsmeyer, K. 1976. Ba^{++} and K^+ alteration of K^+ conductance in spontaneously active vascular muscle. *Am. J. Physiol.* **230:** 1031–1036.
26. Nagel, W. 1979. Inhibition of potassium conductance

by barium in frog skin epithelium. *Biochim. Biophys. Acta.* **552**: 346–357.
27. Sperelakis, N., M. F. Schneider, and E. J. Harris. 1967. Decreased K^+ conductance produced by Ba^{++} in frog sartorius fibers. *J. Gen. Physiol.* **50**: 1565–1583.
28. Ramsay, A. G., D. L. Gallagher, R. L. Shoemaker, and G. Sachs. 1976. Barium inhibition of sodium ion transport in toad bladder. *Biochim. Biophys. Acta.* **436**: 617–627.
29. Greger, R., and E. Schlatter. 1981. Presence of luminal K^+, a prerequisite for active NaCl transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pfluegers Arch. Eur. J. Physiol.* **392**: 92–94.
30. VanDriessche, W., and W. Zeiske. 1980. Ba^{++}-induced conductance fluctuations of spontaneously fluctuating K^+ channels in the apical membrane of the frog skin (*Rana temporaria*). *J. Membr. Biol.* **56**: 31–42.
31. Polhemus, R. E., and D. A. Hall. 1981. Effect of catecholamines on the potential difference and chloride efflux in the mouse thick ascending limb of Henle's loop. *Kidney Int.* **19**: 253.
32. deRouffignac, C., and F. Morel. 1969. Micropuncture study of water, electrolytes, and urea movements along the loops of Henle in psammomys. *J. Clin. Invest.* **48**: 474–486.
33. Rocha, A. S., and J. P. Kokko. 1973. Membrane characteristics regulating potassium transport out of the isolated perfused descending limb of Henle. *Kidney Int.* **4**: 326–330.
34. Wasserstein, A. G., and Z. S. Agus. 1981. Net K^+ secretion in proximal straight tubules (PST). *Kidney Int.* **19**: 261.
35. Work, J., S. L. Troutman, and J. A. Schafer. 1982. Transport of potassium in the rabbit pars recta. *Am. J. Physiol.* **242**(Renal Fluid Electrolyte Physiol. 3): F226–F237.
36. Johnston, P. A., C. A. Battilana, F. B. Lacy, and R. L. Jamison. 1977. Evidence for a concentration gradient favoring outward movement of sodium from the thin loop of Henle. *J. Clin. Invest.* **59**: 234–240.
37. Jamison, R. L., C. M. Bennett, and R. W. Berliner. 1967. Countercurrent multiplication by the thin loops of Henle. *Am. J. Physiol.* **212**: 357–366.
38. Vander, A. J. 1970. Direct effects of potassium on renin secretion and renal function. *Am. J. Physiol.* **219**: 455–459.
39. Brandis, M., J. Keyes, and E. E. Windhager. 1972. Potassium-induced inhibition of proximal tubular fluid reabsorption in rats. *Am. J. Physiol.* **222**: 421–427.
40. Cardinal, J., and D. Duchesneau. 1978. Effect of potassium on proximal tubular function. *Am. J. Physiol.* **234**(Renal Fluid Electrolyte Physiol. 3): F381–F385.
41. Kahn, M., and N. K. Bohrer. 1967. Effect of potassium-induced diuresis on renal concentration and dilution. *Am. J. Physiol.* **212**: 1365–1375.
42. Wright, F. S., N. Strieder, N. B. Fowler, and G. Giebisch. 1971. Potassium secretion by distal tubule after potassium adaptation. *Am. J. Physiol.* **221**: 437–448.
43. Work, J., S. L. Troutman, and J. A. Schafer. 1982. Rubidium transport in medullary thick ascending limb. *Kidney Int.* **21**: 292 (Abstr.).
44. Greger, R. 1981. Coupled transport of Na^+ and Cl^- in the thick ascending limb of Henle's loop of rabbit nephron. *Scand. Audiol. Suppl.* **14**: 1–15.
45. Greger, R. 1981. Chloride reabsorption in the rabbit cortical thick ascending limb of the loop of Henle: a sodium dependent process. *Pfluegers Arch. Eur. J. Physiol.* **390**: 38–43.
46. Frizzell, R. A., M. Field, and S. G. Schultz. 1979. Sodium-coupled chloride transport by epithelial tissues. *Am. J. Physiol.* **236**(Renal Fluid and Electrolyte Physiol. 5): F1–F8.
47. Good, D. W., and F. S. Wright. 1979. Luminal influences on potassium secretion: sodium concentration and fluid flow rate. *Am. J. Physiol.* **236**(Renal Fluid Electrolyte Physiol. 5): F192–F205.
48. Stokes, J. B. 1981. Potassium secretion by the cortical collecting tubule: relation to sodium absorption, luminal sodium concentration, and transepithelial voltage. *Am. J. Physiol.* **241**(Renal Fluid Electrolyte Physiol. 10): F395–F402.
49. O'Neil, R. G., and S. I. Helman. 1977. Transport characteristics of renal collecting tubules: influences of DOCA and diet. *Am. J. Physiol.* **233**(Renal Fluid Electrolyte Physiol. 2): F544–F558.
50. Schwartz, G. J., and M. B. Burg. 1978. Mineralocorticoid effects on cation transport by cortical collecting tubules in vitro. *Am. J. Physiol.* **235**(Renal Fluid Electrolyte Physiol. 4): F576–F585.
51. Grantham, J. J., M. B. Burg, and J. Orloff. 1970. The nature of transtubular Na and K transport in isolated rabbit renal collecting tubules. *J. Clin. Invest.* **49**: 1815–1825.
52. Cannon, P. J., R. P. Ames, and J. H. Laragh. 1966. Relation between potassium balance and aldosterone secretion in normal subjects and in patients with hypertensive or renal tubular disease. *J. Clin. Invest.* **45**: 865–879.