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Research Article

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The Nature of Transtubular Na and K Transport in Isolated Rabbit Renal Collecting Tubules

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ABSTRACT In order to investigate the mechanism of Na and K transport, rabbit cortical collecting tubules were perfused in vitro and the concentrations of Na and K in lumen and bathing fluid and the transtubular electrical potential difference (PD) were measured.

When the perfusate and external bath contained 150 Na–5 K (mEq liter^{-1}), the sodium concentration decreased and the potassium concentration increased by an approximately equal amount in collected tubular fluid. The transtubular electrical potential was equal at both ends of the tubule in the steady state and ranged between 21 and 67 mv, lumen negative. In all tubules perfused at rates less than 0.5 nl min^{-1}, the K concentration of the collected fluid was higher and the Na concentration lower than that predicted for electrochemical equilibrium between lumen fluid and external bath, evidence for active transtubular transport of both cations. These results differ from those observed in rat distal tubule in which potassium secretion is passive.

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INTRODUCTION

In the mammalian kidney, potassium filtered at the glomerulus is reabsorbed in the proximal tubule and loop of Henle. The potassium which appears in the final urine is derived principally from secretion in the distal nephron (1). On the basis of clearance and stop-flow studies it has been concluded that potassium secretion in the distal nephron is dependent on the availability of sodium in the luminal fluid (2–4). This has been interpreted as indicating that Na^+ entering the distal nephron is reabsorbed, at least in part, by an active carrier mediated process involving exchange for K and H ions (5). The distal nephron consists of three anatomically distinct segments: the distal convoluted tubule, the cortical collecting tubule, and the papillary collecting duct (6). In two of these, the distal convoluted tubule and papillary collecting duct, net K secretion and Na absorption have been observed directly by micropuncture (7, 8). In contrast to earlier inferences, however, no evidence of active K transport was obtained (7, 9, 10) and it was implied that net secretion of K^+ observed in clearance studies in the mammal was not a consequence of active transport anywhere in the nephron. Since the cortical collecting tubule does not approach the surface of the kidney, its electrolyte transport characteristics cannot be studied by micropuncture. However, it can be removed from the kidney by microdissection and perfused successfully in vitro (11).

In the present studies the tubule perfusion technique was used to examine the nature of Na and K transport in isolated fragments of rabbit cortical collecting tubule. Evidence for both active K^+ secretion and Na^+ reabsorption was obtained.

METHODS

Fragments of collecting tubules 0.5–3 mm long were dissected from slices of rabbit kidney cortex in oxygenated medium as described previously (11). Before the experiment the animals were maintained on standard laboratory chow and allowed free access to water.

Experiments were performed at room temperature (23–24°C). Unless specified otherwise the external bathing medium contained 115 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 10 mM Na acetate, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 5% v/v calf serum (Microbiological Sciences Inc., Yonkers, N. Y.), and 5.5 mM dextrose. The

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TABLE I

Perfusion Solutions

Designation	NaCl	K_2HPO_4	KCl	$CaCl_2$	$MgSO_4$	Raffinose
			mmoles/liter^{-1}			
150 Na-5 K	150	2.5	0	1.0	1.2	0
140 Na-15 K	140	2.5	10	1.0	1.2	0
115 Na-40 K	115	2.5	35	1.0	1.2	0
40 Na-115 K	40	2.5	110	1.0	1.2	0
40 Na-5 K	40	2.5	0	1.0	1.2	180
8 Na-5 K	8	2.5	0	1.0	1.2	238
0 Na-5 K	0	2.5	0	1.0	1.2	246

All solutions isosmotic with the external bath.

final osmolality was 290 mOsm kg^{-1}. The pH was maintained at 7.4 by gassing with 5% CO_2-95% O_2. The composition of the perfusion solutions is given in Table I; all were isosmotic with the external bathing fluid.

Method of tubular perfusion. The method for the simultaneous tubular perfusion and recording of the transtubule electrical potential difference (PD) at the perfusion end reported previously (12) was used. As shown diagrammatically in Fig. 1, the inner perfusion pipet (on the left) was advanced into the lumen (250–500μ) until a stable potential was observed. Care was taken to assure that no perfusion fluid leaked retrograde between the inner wall of the tubule and the glass pipet. The electrical potential was measured by connecting a Ag-AgCl wire in the rear of the perfusion pipet to the input of a Cary vibrating reed electrometer (Cary Instruments, Monrovia, Calif.). The bath was connected by a bridge to a second (reference) Ag-AgCl electrode which in turn was connected in series through a Heath (Heath Company, Benton Harbor, Mich.) VR-1 voltage reference source to ground. The perfusion end and reference Ag-AgCl electrodes were immersed in identical solutions (labeled *A*). The inner diameter of the perfusion pipet tip was made as large as possible (12–15 μ) in order to eliminate dead space between the tubular wall and inner pipet and to reduce the electrical tip resistance. In all studies, before attachment of the tubule to the pipets, the perfusion pipet was immersed in a bath containing the perfusion solution (*A* in Fig. 1) and the voltage reference source was adjusted so that no electrode potential asymmetry was registered by the electrometer. In none of the experiments, some of which lasted 12 hr, did this zero base line drift more than 3 mv.

In the initial studies fluid was infused into the tubule lumen at a constant rate using a piston pump. Owing to the effect of small variations in room temperature, this closed perfusion system could not be used conveniently to deliver fluid at rates less than 1 nl min^{-1}. It was found that rates as low as 0.01 nl min^{-1} could be obtained using a constant pressure system. To achieve constant pressure perfusion the back end of the perfusion pipet was attached to a fluid-filled polyethylene tube open to the atmosphere. The height of the fluid column above the level of fluid in the external bath was varied from 0 to 30 cm of H_2O. The pressure head needed to deliver the desired rate of flow was different in each tubule. The pressure in the tubule lumen was not measured. The perfusion pressures listed in the results refer to the pressure in the perfusion pipet rather than to the presumably smaller pressure in the tubule lumen.

Collection of tubular fluid. In the initial studies (using pump perfusion) the free end of the tubule was sucked into a glass micropipet in order to collect tubule fluid as described in an earlier paper (11). It was subsequently noted that though contact between the outer wall of the tubule and the glass holding pipet was adequate to prevent leakage of fluid between the collecting pipet and the external bath it did not provide adequate electrical insulation. In later studies (using constant pressure perfusion) insulation was achieved by cannulating the collecting end of the tubule in the same manner as the perfusion end. This method of cannulating both ends of the tubule is shown diagrammatically in Fig. 1. The perfusion assembly was attached first and flow induced in order to distend the tubule lumen. After attachment of the outer pipet at the collecting end (on the right), an inner pipet was advanced into the lumen for 300–500 μ. The latter was open at its distal end to allow introduction of other glass capillaries for removal of fluid samples and measurement of PD. Oil was layered over the collected fluid to prevent evaporation. The electrical PD was measured by inserting into the collected tubular fluid a glass capillary bridge filled with the appropriate tubule perfusion fluid and sealed at the tip with hardened agar (4 g/100 ml). A Ag-AgCl electrode in the capillary bridge was connected to the input of a model 600A Keithley electrometer (Keithley Instruments Inc., Cleveland, Ohio). The ground of this electrometer was connected directly to the Ag-AgCl reference electrode. Electrode symmetry was checked throughout the experiment and any imbalance subtracted from the transtubular PD readings. The correction was less than 3 mv in all studies except those in which raffinose replaced all of the sodium chloride in the perfusion

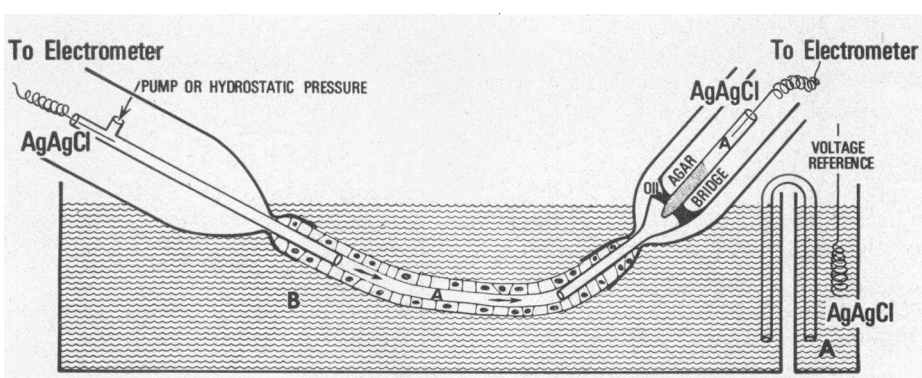

FIGURE 1 Arrangement for measuring electrical potential difference across isolated collecting tubules. See Methods for details.

fluid (0 Na-5 K solution). In these the asymmetry was 3–4 mv positive because of the presence of agar in the tip of the glass capillary bridge.

Interpretation of the measurements of PD is complicated by the presence of liquid junction potentials. The recorded PD is the sum of the transtubular PD and the liquid junction potentials in series with it. Liquid junction potentials could occur in the following two places in the present experiments: (*a*) between the bath solution (*B* in Fig. 1) and the solution (*A* in Fig. 1) containing the reference Ag/AgCl electrode; and (*b*) between the collected perfusion fluid and the agar capillary bridge inserted into it. Liquid junction potentials between the different fluids at these junctions were estimated in the conventional manner using bridges of 3 M KCl agar to minimize all other liquid junction potentials. The liquid junction potentials were less than ±2 mv with all fluids except those in which raffinose replaced NaCl in the perfusion fluid in which case they were 5.5 mv (usual bathing medium positive to 0 Na-5 K) or less. The transtubular PD's noted in the text were not corrected for the estimated liquid junction potentials since the magnitude of the correction was so small as not to alter the interpretation of the results.

To collect tubular fluid the potential measuring bridge was removed. The collected tubular fluid was then removed by suction and discarded and the glass surface of the inner pipet which had been exposed to the tubular fluid was rinsed several times with distilled water. The next collection of tubular fluid was then discarded. Subsequently all of the tubular fluid was drawn into a double constriction volumetric pipet as it appeared. This pipet contained a small sample and a larger diluting chamber arranged in series. The tubule fluid was sucked into the first or sample chamber (volume 1 nl). The rate of flow at the collecting end of the tubule, V_L, was determined by dividing the volume of the sample compartment in the calibrated pipet (1 nl) by the time required for appearance of sufficient tubule fluid to fill it completely. In order to reduce the number of manipulations, the potential recording electrode was reintroduced into the collecting end only after two or more samples had been collected.

Measurement of sodium and potassium in collected fluid. The sodium and potassium concentration of the tubule fluid was measured using a Vurek-Bowman helium glow photometer (13). The 1 nl sample of collected fluid was diluted with solution containing 0.03 M $CsNO_3$ and 0.005 M $NH_4H_2PO_4$ by drawing the latter into the double chamber constriction pipet containing the collected sample until the second compartment containing approximately 30 nl was filled. The small drop of diluted sample was stored under mineral oil until it was read in triplicate using 5- to 7-nl aliquots. Sodium and potassium concentrations were derived by interpolation from a standard curve which was constructed using solutions containing 150 Na-5 K, 110 Na-45 K, or 40 Na-110 K mmoles/liter^{-1} and a blank. The standards were diluted in the special micropipet and processed along with the collected samples. The coefficient of variation of the standard solutions diluted and read in this manner was 3.7% for sodium and 5.7% for potassium. The sodium and potassium concentrations of the bath and perfusate were measured with an Instrument Associates (Port Washington, N. Y.) flame photometer and osmolality with a Bowman-Aminco osmometer (Silver Springs, Md.).

Measurement of net fluid absorption. In experiments 37 and 38, albumin-^{131}I (150 μCi ml^{-1}) was included in the perfusate as a volume marker (11). The rate of perfusion, V_o, was calculated as $V_o = S_L/[S_o]t$, where S_L is the total amount of isotope collected, $[S_o]$ the concentration of isotope in the perfusion solution, and t the time. The rate of net fluid absorption per unit of luminal surface area (A) is $(V_o - V_L)/A$ where V_L is the rate of appearance of fluid in the collecting pipet. No leakage of albumin-^{131}I into the external bath was detected. Samples of sufficient activity

TABLE II

Transtubular Electrical Potential Difference and Electrolyte Concentrations in Collected Tubular Fluid

Exp.	Mean contact time	PD_o	PD_L	Na_L	K_L
	sec	*mv*		*mEq liter^{-1}*	
A					
34*	3	−60	−66	149	6.1
1	11	−11		154	8.5
2	12	−19		133	7.7
3	15	−19		152	5.1
4	18	−35		132	12.9
5	18	−29		132	10.3
6	19	−22		141	9.3
7	19	−13		142	9.9
8	21	−21			9.9
9	21	−39		136	17.5
10	25	−32			8.7
11	28	−31		134	20.5
12	30	−40		131	24.8
13	30	−11			9.5
14	31	−23		153	7.8
15	33	−22		142	21.5
16	34	−8		132	15.6
17	36	−25		136	14.1
18	38	−24		137	16.9
19	38	−40		146	14.7
20	38	−47		129	10.1
21	38	−15		144	12.8
22	44	−45		125	28.4
23	44	−28		129	25.4
24	44	−26		143	15.4
25	47	−33		130	22.7
37*	130	−57	−57	122	58.0
26	145	−31		127	20.2
37*	198	−67	−67	69	100
34*	302	−57	−61	109	57.0
38*	609	−34	−30	66	106
32*	622	−21	−24	66	108
37*	747	−43	−43	46	143
34*	777	−53	−55	67	118
33*	1360	−33	−42	78	87.0
B					
36*	1048	−51	−47	14	139
43*	545	−45	−42	13	133

A, perfusion solution Na 150-K 5.

B, perfusion solution No. 36 Na 115-K 40 and No. 43 Na 40-K 110.

* Double cannula.

were counted in a Packard gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.); lower activity in a low-background Geiger system (Nuclear-Chicago, Des Plaines, Ill.).

Mean contact time (T) was calculated as $T = \pi r^2 L/V_L$ where r is tubular luminal radius. Tubule radius and length were measured with a calibrated reticle in the ocular of the microscope. Since V_o was not measured in all experiments, V_L was used to estimate T. This introduces an error which is greatest at slow perfusion rates owing to the fact that some fluid is absorbed. A maximal error of 40% in the estimation of T at times longer than 300 sec could be accounted for on this basis. This does not influence the interpretation of the results.

RESULTS

The concentration of Na and K in the collected tubular fluid and the transtubular PD reached a steady state after 180–200 min of perfusion at a constant rate. When the perfusate and bath both contained 150 Na-5K, the lumen was negative with respect to the external bath (Table II). In the steady state the PD ranged between −8 and −67 mv in individual tubules and was generally higher in tubules perfused at slow rates (Table II A). In the seven tubules in which both ends were cannulated (as in Fig. 1), 11 simultaneous measurements of steady-state potential were made at the two ends of the tubule at different perfusion rates (Table II A and B). The mean difference between the PD at the two ends was only 1.1 mv.

The concentrations of Na and K in the collected tubular fluid during perfusion with 150 Na-5 K solution are indicated in Table II A. The results are listed in order of increasing contact time. The relationship between contact time and the concentrations of sodium and potassium in collected fluid is also shown in Fig. 2. In most tubules the concentration of sodium in the collected fluid was less and that of potassium greater than in the perfusion solution, and the magnitude of the change in the Na and K concentrations was directly related to the mean contact time. Since contact times longer than 1500 sec could not be reliably achieved, it was not possible to determine the maximal change in sodium and potassium concentration when the perfusate was 150 Na-5 K. In two studies designed to determine whether greater concentration differences could be developed, an isotonic perfusate was used in which part of the sodium had been replaced quantitatively with potassium. As shown in Table II B, under these circumstances the concentration of sodium fell to 13 and that of potassium rose to 133 mEq liter^{-1}.

The increase in K concentration was not a consequence of absorption of K-free fluid. When the contact time was less than 100 sec, net fluid absorption could not be detected. At slower perfusion speeds with 150 Na-5 K as the perfusion fluid (Experiments 37, 38) although absorption was measurable (34 and 25×10^{-6} cm^3 cm^{-2} min^{-1}), it could only have accounted for a two-fold increase in K concentration (from 5 to 10 mEq/liter). The observed changes in Na and K concentration in the collected fluid must therefore reflect net transtubular transport of these ions.

It is notable that in all of the double cannula studies the PD's measured at both ends of the tubule were virtually identical despite marked differences in the Na^+

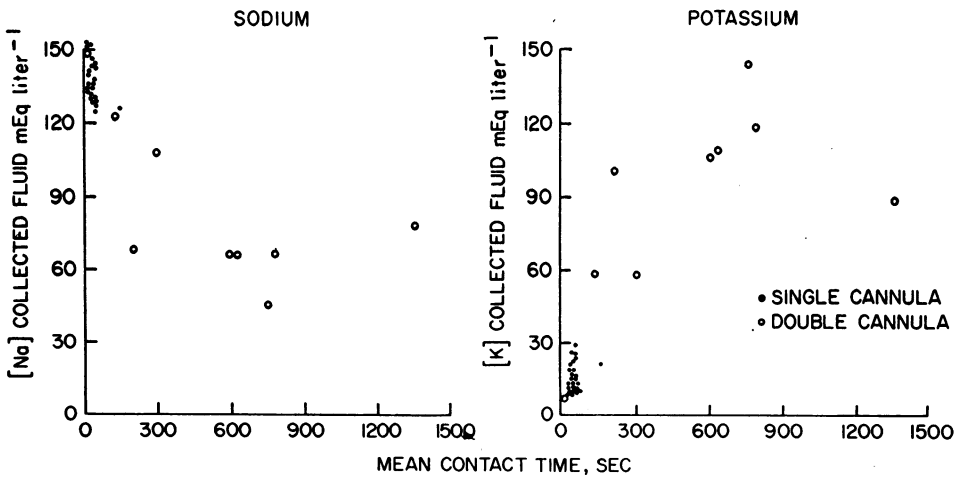

FIGURE 2 Effect of contact time on the electrolyte concentration of collected tubular fluid. Single cannula with pump perfusion ●. Each point represents a different tubule. Double cannula technique with perfusion at various pressures ○. Each point represents a single perfusion rate in one of the five tubules studied.

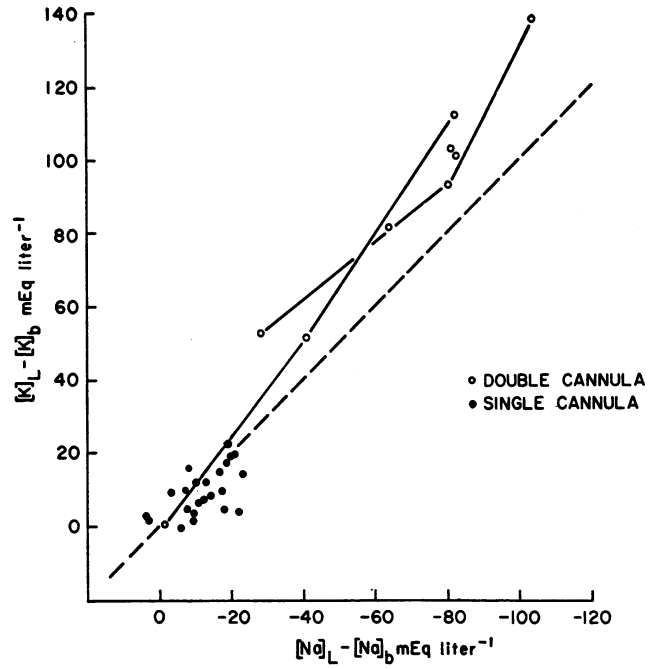

FIGURE 3 Relation between the change in sodium and potassium concentrations of collected tubular fluid. Lines connect data points from individual tubules at different perfusion rates. Identity between sodium loss and potassium gain is depicted by the dashed line. Subscripts L and b refer to lumen and bath. Perfusate, 150 Na-5 K.

and K^+ concentrations at the two ends.[1] Apparently the luminal membrane does not act either as a simple K^+ or Na^+ electrode over the range of concentrations studied and in this regard differs from other epithelial cells such as amphibian skin (14) and bladder (15) in which the outer membrane has the characteristics of a sodium electrode. On the other hand alterations in [K] on the serosal border do change the PD as does virtual elimination of Na^+ from the perfusion solution (v.i.).

The relation between the change in sodium and potassium concentrations in individual tubules infused with 150 Na-5 K solution is shown in Fig. 3. In general, the concentration of sodium decreased as the concentration of potassium increased in the luminal fluid. When the contact time was prolonged to permit maximal changes in concentration, the gain of potassium was slightly greater than the loss of sodium.

[1] Any difference in PD between the two ends of the tubule should have been apparent despite the fact that the ends are in electrical contact through the luminal fluid. The length constant for the collecting tubule is approximately 800 μl (Helman, Grantham, Burg, and Orloff. Unpublished observations) which is one-half of the length of the average tubule studied. Therefore approximately 80% of any difference in PD between the ends of the tubule should have been detected.

It has been shown in an earlier study that ouabain depolarizes isolated collecting tubules (12). By analogy with the effects of the glycoside in other tissue, this was interpreted as indicative of inhibition of active Na and K transport in this tissue as well. Direct evidence for this conclusion is contained in the representative study in Fig. 4. The perfusate and external bath both contained 150 Na-5 K. The perfusion rate was constant through the entire study. After addition of ouabain, the PD fell to zero or became slightly positive and both net K secretion and Na absorption were completely inhibited.

Isosmotic perfusates containing raffinose and different concentrations of sodium were infused in order to determine (*a*) the steady-state concentration of Na in the lumen and (*b*) the effect of a low concentration of Na in the lumen on K transport. As shown in Table III the initial concentration of Na^+ in the perfusate was higher, lower, or equal to the final steady-state value in the collected fluid. The mean steady-state Na concentration at the contact times given in Table III was 6.5 mEq liter^{-1} and was independent of the initial Na concentration. The mean K concentration in the fluid collected from these tubules (21.5 mEq liter^{-1}) was considerably less than in experiments in which the 150

Na-5 K perfusate was used. (With 150 Na-5 K perfusate [Table II A] and comparable contact times [greater than 100 sec], the mean K concentration in the collected fluid was 88.5 mEq liter^{-1}.)

The electrical PD was generally oriented so as to favor the movement of cations into the lumen. In order to determine whether the PD was sufficiently great to account for the potassium accumulation in the tubular fluid, it was necessary to relate the transtubular distribution of potassium to the existing electrical potential difference. The relation between the electrical PD across a membrane and the passive distribution of permeant ions at electrochemical equilibrium is given by the Nernst equation. When applied specifically to transtubular potassium,

$$E_m = E_k = -59 \log \frac{[K]_l}{[K]_b}$$

where E_m is the transtubular PD in mv, E_K is the K equilibrium potential, and [K] is the steady-state potassium concentration in the lumen (1) or external bath (b). If potassium distribution across the collecting tubule were entirely passive, then in the steady state with zero net K flux the potassium equilibrium potential, E_K, calculated from the Nernst equation would equal the measured transtubular electrical PD (E_m). A condition of zero net K flux was approximated when a raffinose-containing solution was infused at a rate slow enough to allow the K in the collected fluid to reach a stable value. As shown in Table III, the calculated E_K was more negative than the electrical potential measured at either end of the tubule indicative or uphill or active transport of potassium from bath to lumen. Similarly, in the steady state the mean value of E_{Na} also differed from the measured electrical PD (Table III) and is consistent with active transport of Na from lumen to bath.

As indicated above when the concentration of sodium in the perfusate was equal to that in the bath (150 Na-5 K), both the transtubular electrical potential difference and the maximum luminal concentration of potassium were higher than when the raffinose perfusate was used. The relation between E_m and calculated E_K using the 150 Na-5 K perfusate was also determined. As noted in Fig. 5 the discrepancy between E_m and E_K became greater as the contact time was prolonged. Thus, as luminal K approached its maximal steady-state concentration, the concentration of the ion was consistently greater than that predicted for electrochemical equilibrium.

In the above experiments active K secretion was apparent only at slow perfusion rates at a time when the transtubular hydrostatic pressure difference was presumably low. To determine if K is also secreted actively at higher transtubular hydrostatic pressure differences and flow rates, tubules were infused at relatively rapid rates with a solution containing K at a concentration 15 mEq liter^{-1} or greater (Table IV).

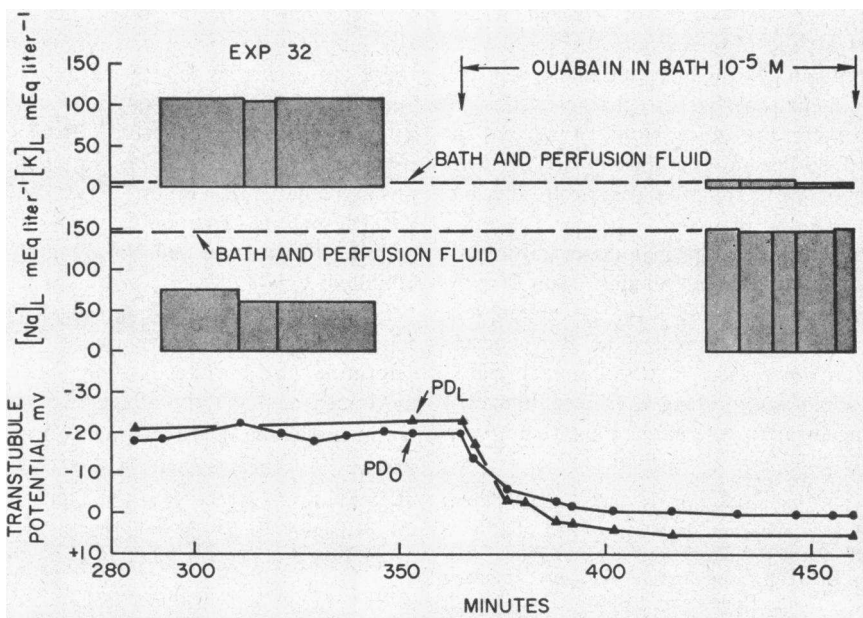

FIGURE 4 Effect of ouabain on transtubular electrical potential difference and sodium and potassium concentrations of collected tubular fluid. PD was recorded at both the perfusion end (PD_o) and collecting end (PD_L).

TABLE III

The Transtubular PD and Sodium and Potassium Concentrations of Collected Tubular Fluid in the Balanced State

Exp.	Perfusate	Contact time	$[Na]_L$	$[K]_L$	PD_0	PD_L	E_K	E_{Na}
		sec	*mEq liter*$^{-1}$		*mv*		*mv*	
42	0 Na-5 K	403	5.3	9.6	+11	−2	−17	+85
40	0 Na-5 K	598	6.6	40.5	+3	−2	−54	+80
41	8 Na-5 K	113	8.4	11.2	−7	−8	−21	+74
39	40 Na-5 K	780	5.7	24.9	−5	−5	−41	+84
Mean			6.5	21.5	0	−4	−33	+81

The K concentration of the collected tubular fluid was higher than that infused and the potassium equilibrium potential exceeded the measured transtubular PD indicative of active K secretion under these circumstances as well.[3]

From comparison of Tables II and III it is apparent that net K secretion and transtubule PD were both reduced when the concentration of Na in the collected fluid was less than 10 mEq liter^{-1}. Additional studies were performed to examine this apparent dependence of both P D and net K^+ accumulation on the intraluminal concentration of Na^+. In order to allow maximal changes of luminal electrolyte concentration to occur, the mean contact time was progressively increased in individual tubules by reducing the perfusion pressure. Eventually the pressure was lowered to a point at which flow of tubular fluid stopped. Two representative experiments are shown in Fig. 6. The solid circles refer to a study in which raffinose was substituted for all but 43 mEq/liter of Na in the perfusion solution; the open circles to a study in which K was substituted for all but 40 mEq/liter of Na. In both the PD exceeded −50 mv when the perfusion rate was rapid and the concentration of Na in the collected fluid was virtually equal to that infused. As the contact time was prolonged by reduction in perfusion pressure, the Na^+ concentration in the collected fluid fell below 20 mEq/liter and the PD diminished. The maximal net increase in K_L under these circumstances was only 20 and 23 mEq liter^{-1}. Additional studies in which the effect of stopped flow on PD was examined are listed in Table V. The interruption of flow caused the PD to become more positive even when the perfusate was 150 Na-5 K.

Changes in the concentration of potassium at the blood border of most epithelial cells has a pronounced effect on net electrolyte transport and PD. In both frog skin (14) and toad bladder (15), elevation of the K concentration in the medium bathing the blood surface reduces the transepithelial PD. This has been interpreted as indicating that the transepithelial PD is in part a K diffusion potential arising from the K concentration difference between cell interior and inner bathing solution. Since the outer surface of frog skin has the characteristics of a Na^+ electrode, Koefoed-Johnsen and Ussing (14) postulated that a neutral Na-K exchange pump at the blood border of the cell maintains the intracellular Na and K concentration gradients but is only indirectly responsible for the transepithelial PD. The latter is assumed to be the sum of the Na diffusion PD across the outer cell membrane and the K diffusion PD across the inner cell membrane. The PD will only be affected by inhibition of pump activity insofar as this

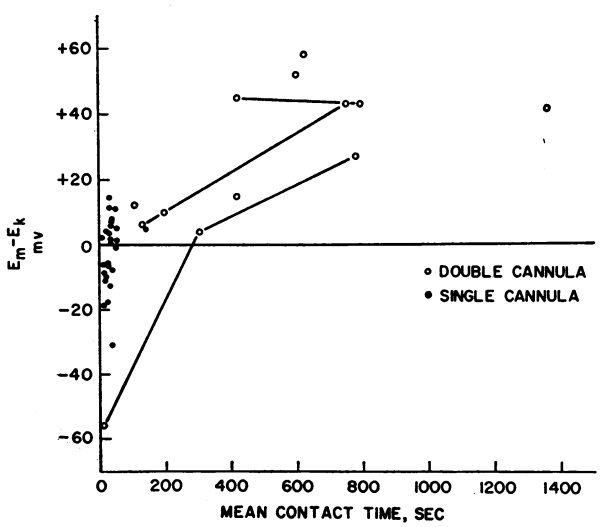

FIGURE 5 The effect of contact time on the difference between the transtubular PD (E_m) and potassium equilibrium potential (E_K) in collecting tubules. Lines connect data points in individual tubules perfused at different rates. Positive values of E_m-E_K indicate that K transport occurred against an electrochemical gradient.

[3] In a preliminary report the "single cannula" experiments in Fig. 5 were incorrectly interpreted to indicate that K was passively distributed (16). Clearly the contact time in those studies was of insufficient length to permit maximal accumulation of K in the lumen.

TABLE IV

The Transtubular PD and Sodium and Potassium Concentrations of Collected Tubular Fluid during Relatively Rapid Infusion of "High-K" Perfusates

Exp.	Perfusate	Contact time	$[Na]_L$	$[K]_L$	PD_o	PD_L	E_K
		sec	*mEq liter*$^{-1}$		*mv*	*mv*	*mv*
28	140 Na-15 K	31	135	24.5	−13		−41
30	140 Na-15 K	28	133	18.6	−19		−34
43	40 Na-110 K	6	37	114	−56	−73	−80

results in dissipation of the concentration gradients across the pertinent borders. This view of the origin of the PD has been challenged by Bricker, Biber, and Ussing(17) and Essig and Leaf (18) who consider it a manifestation of an electrogenic pump. As noted above the luminal surface of the collecting tubule is not a simple Na^+ or K^+ electrode. Although a marked decrease in the Na^+ concentration in the perfusion solution will effect a fall in the transepithelial PD (Fig. 6), this is undoubtedly associated with a fall in Na^+ transport and thus the result does not permit distinction between the two models for the origin of the PD.

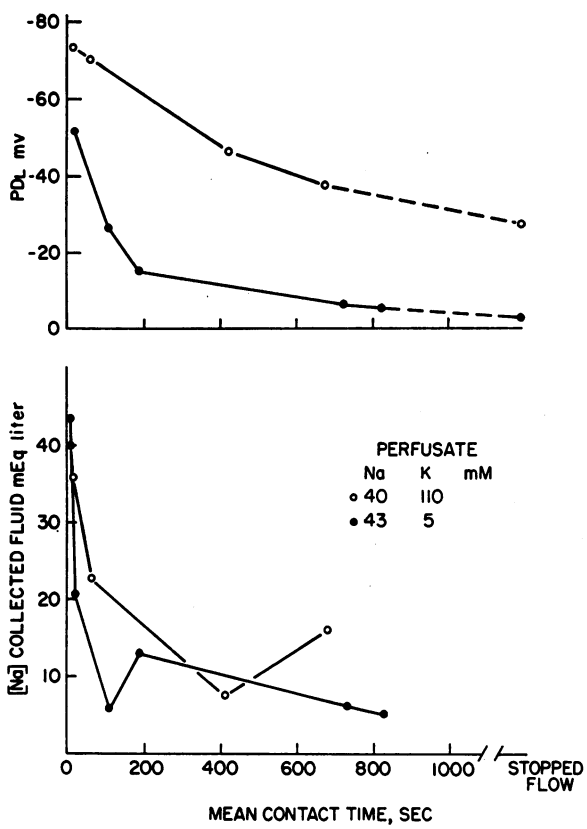

FIGURE 6 Relation between electrical PD or the sodium concentration of collected tubular fluid and the contact time in two tubules. PD_L is the transtubular PD measured at the collecting end of the tubule.

The effect on the transtubular PD of changing the K concentration in the external bath was tested in collecting tubules by quantitatively substituting potassium for sodium or sodium for potassium in the external medium. The single cannula method was used in order to maintain constant high perfusion rates and minimize changes in electrolyte concentration of the luminal fluid. Increasing the potassium concentration from 3.2 to 9.5 mEq liter^{-1} had little effect on the transtubular PD (Fig. 7) whereas increasing the concentration above 10 mEq liter^{-1} of potassium caused the PD to fall. The depolarization in high K solutions was reversible. In four studies in which the [K] concentration was greater than 10 mEq liter^{-1} (Fig. 7), the average slope of the linear portion of the individual lines relating PD to log external [K] was −41 mv per decade change in [K] concentration. Although these results, similar to those in amphibian skin and bladder, may indicate that the transepithelial PD is in part due to the K^+ concentration difference across the blood surface of the epithelial cell, they do not exclude participation of an electrogenic Na^+ pump in the genesis of the overall PD.

Indirect evidence favoring existence of an electrogenic pump to account for a portion of the PD was obtained by examining the time course of the change in PD induced by elimination of K from the serosal bathing medium. A representative study is illustrated in Fig. 8. It can be seen that removal of virtually all of the K^+ from the bath (final concentration 0.2 mEq/liter) re-

TABLE V

Effect of Stopped Flow on Electrical Potential

Exp.	Perfusate	PD_L with flow*	PD_L no flow	Δ
		mv	*mv*	
37	150 Na-5 K	−60	−40	+20
38	150 Na-5 K	−40	−16	+24
39	40 Na-5 K	−51	−5	+46
43	40 Na-110 K	−73	−27	+46

* Mean contact time <800 sec

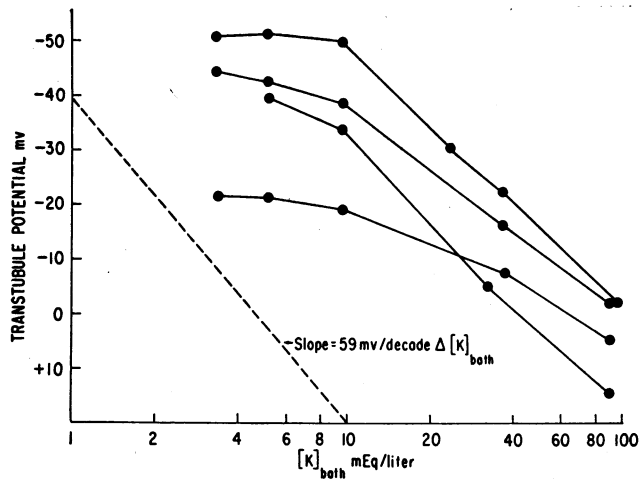

FIGURE 7 Relation between transtubule PD and potassium concentration of the external bath.

sulted in a rapid decrease in PD with ultimate reversal of the sign, the lumen becoming positive with respect to the bath. In six experiments using the single cannula method, the initial base line PD was −19 mv with 5 mEq liter^{-1} of K in the bath. When the K was removed from the bath, the PD decreased and eventually reached a mean steady-state value of +6 mv. The time course of the fall in PD in the representative study illustrated in Fig. 8 *A* is plotted on a logarithmic scale in Fig. 8 *B*. The rate of decrease of potential was rapid initially, after which it declined more slowly. Within 0.5–1.5 min after the removal of external potassium, the change to a new steady-state PD was on the average 51% complete in the six experiments. The change of the PD after 2 min was logarithmic as shown in Fig. 8 B having an average half-time of 6.1 ±0.9 min (SEM) in the six experiments. In four of the experiments 5mM potassium was reintroduced in the external bath. As illustrated in Fig. 8 A the transtubule PD transiently became more negative (average peak change −15 mv) and then re-

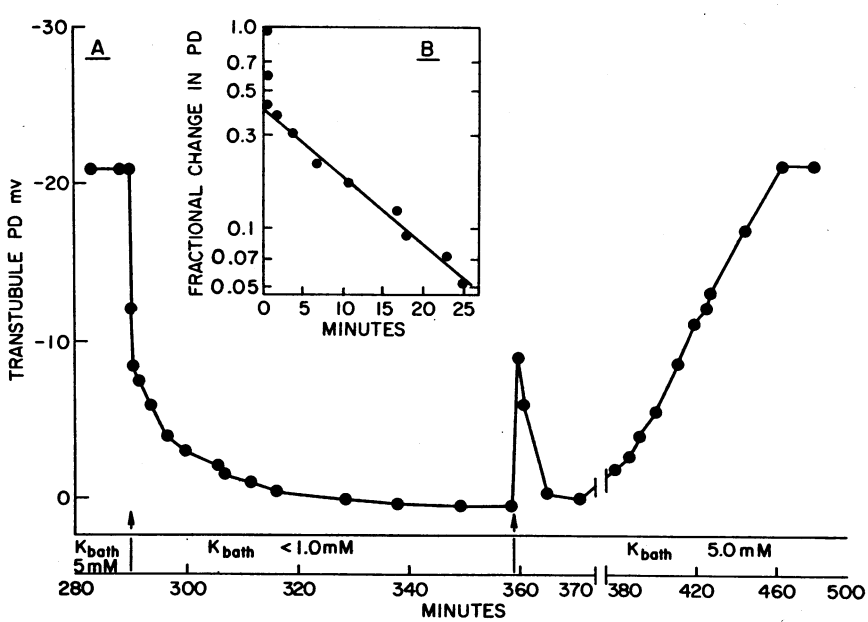

FIGURE 8 (*A*) Time course of electrical potential difference after removal of potassium from the external bath. (*B*) Semilogarithmic plot of the early points in *A*. Zero time in *B* is 287 min in *A*.

turned within a few minutes to the previous low value. After this the potential gradually returned to its original negative value (provided the tubule was incubated in the regular medium for sufficient time [Fig. 8 A]). Removal of K from the bathing solution also resulted in a decrease in net Na transport from lumen to bath. In three experiments the net transport of sodium decreased 81% ±10 (SEM) from its base line value in 5 mEq liter^{-1} medium to the new steady state in zero K medium.

The above results are suggestive of the participation of an electrogenic pump in the generation of the transepithelial potential. Admittedly removal of K^+ would be expected to lower the PD whether it were a diffusion potential generated by a linked Na-K exchanger as Koefoed-Johnsen and Ussing (14) originally postulated or a PD generated by an electrogenic Na^+ pump dependent on K^+. The time course of the depolarization in the collecting tubule however, in our view, is of sufficient rapidity to preclude dissipation of a diffusion gradient as the responsible phenomenon. The PD fell 50% in approximately 1 min whereas the $t_{\frac{1}{2}}$ for cellular K^+ exchange in cortical collecting tubules is of the order of 12 min (10). It is acknowledged that this interpretation of the data is not unique and in the absence of knowledge of the concentration and potential profile in the pertinent electrolyte transport pools cannot be cited as incontrovertible evidence for existence of an electrogenic pump in this tissue.

DISCUSSION

It has been assumed for some time that virtually all of the filtered K^+ is reabsorbed in the proximal nephron in the mammal (5). Subsequent secretion in the distal nephron by an active process involving obligatory carrier-mediated exchange with Na^+ as the counterion was posited to account for the appearance of K^+ in the final urine. This view of the mechanism of K^+ secretion was deduced from the results of both clearance (2) and stop-flow studies (3, 4) in the mammal and renal-portal perfusion experiments in the chicken (20). In the latter the secretory phenomenon was shown to be saturable, a result indicative of a carrier-mediated process.

In recent years direct evidence in support of this thesis was sought in micropuncture studies. It has been demonstrated that filtered K^+ is reabsorbed virtually completely in the proximal nephron and the loop of Henle and that K^+ in the final urine is derived largely from that secreted in the distal convoluted tubule of rats (7, 9), dogs (21), and monkeys (22). No evidence of active K^+ transport in the distal convolution was obtained however (9). Under all circumstances the concentration of K^+ in the tubule fluid was less than that required for electrochemical equilibrium between urine and peritubular fluid, consistent with passive entry of K^+ into the luminal fluid. The same deviation from electrochemical equilibrium, noted in the steady state during "stationary" microperfusion, is ascribed to opposing *active* reabsorption of the cation (10). Coupled exchange of Na^+ and K^+ thought to account for the interdependence of the two ions observed in clearance and stop-flow experiments was also excluded in the micropuncture studies. Under physiological conditions the delivery of Na^+ to the distal convolution could never be shown to be limiting for K^+ secretion. When the Na^+ content of the perfusate was artificially reduced by equimolar substitution of choline, the resultant fall in K^+ secretion was attributable to a concomitant reduction in the electrical driving force (the intraluminal PD) rather than to unavailability of Na^+ as a counter ion (10).

The present studies establish (*a*) that the cortical collecting tubule of the rabbit is the site of both active K^+ secretion and active Na^+ reabsorption, and (*b*) that these processes are interdependent. The results should not be interpreted as being at variance with those of the micropuncture studies alluded to above since they do not bear on the characteristics of Na^+ and K^+ transport in the distal convolution. It is our view that the dissimilarity in the results of the distal convolution micropuncture and isolated collecting tubule studies are not due to species or technical differences. Rather, they reflect specific differences in the function of the two segments. The histological dissimilarity of the two segments is in accord with this conclusion as are the divergent effects of ouabain in the distal convolution and collecting tubules. In the former ouabain inhibits net reabsorption and not net secretion (23), whereas in the latter net secretion is abolished by the glycoside. It may be that variable effects of ouabain on the balance between reabsorption in the distal convolution and secretion in the collecting tubule may account for the diverse effects of the glycoside on K^+ excretion in the intact animal (24, 25).

If it is permissible to extrapolate from observations in one species to another, then the present results are consistent with those of the clearance, stop-flow, and micropuncture studies. It is assumed that net NaCl absorption, passive K^+ secretion, and active reabsorption of K^+, transpire in the distal convolution whereas active exchange of Na^+ and K^+ as well as a minimal degree of NaCl absorption occur subsequently in the collecting tubule. Only in the latter segment is clearcut interdependence of Na^+ and K^+ transport demonstrable. In the present studies net active secretion of K^+ fell progressively as the concentration of $[Na^+]$ in the perfusate decreased below 30 mEq liter^{-1}. Even under these circumstances E_K exceeded E_m, evidence of persistent active secretion of the cation. Since the measured $[Na^+]$

concentration in the fluid entering the cortical collecting tubule in rat (9), dog (21), and monkey (22) and by analogy in rabbit is normally below this value (10–30 mEq liter^{-1}, this would account for the minimal degree of K^+ secretion into final urine observed in both micropuncture and clearance studies under normal conditions. In the absence of Na diuresis, the K/inulin concentration ratio at the end of the distal convolution was found to be equal to or higher than that in the final urine, indicative of negligible secretion of K^+ in the nephron beyond the distal convolution (7, 9, 21, 22). On the other hand, natriuresis induced by infusion of Na^+ salts or administration of diuretics undoubtedly increases the concentration of Na^+ in fluid delivered to the active transport site in the collecting tubule and thereby enhances K^+ secretion. Consonant with this is the observation that in contrast to results in nondiuretic animals K/inulin ratio during Na^+ diuresis in the rat, dog, and monkey is often higher in final urine than in samples obtained from the terminal portion of the distal convolution (7, 9, 21, 22).

The mechanism of Na^+ and K^+ transport in the collecting tubule is unknown. As stated above, these studies only establish the active nature of the two processes; they do not distinguish between any of the current models which have been postulated to account for transport in epithelial or other cells. K^+ secretion and Na^+ reabsorption are interdependent in this tissue as in others (18). Thus net secretion of K^+ decreases when the Na^+ concentration of luminal fluid is lowered; Na^+ reabsorption falls when K^+ is eliminated from the bathing medium. These results, though compatible with the presence of a linked carrier-mediated exchange process, cannot be cited as incontrovertible evidence for its existence. Similarly it is not possible on the basis of the available evidence to localize the active transport steps which result in the exchange of Na^+ for K^+ with certainty either to the luminal or peritubular membrane.

The origin of the transepithelial PD has been discussed above. It is our current view that either an independent Na^+ transport process or a nonneutral (non 1:1) Na-K exchanger may be the primary source of the PD. Since some net absorption of NaCl does occur in this segment in association with apparent reciprocal exchange of Na^+ for K^+, this is not an unreasonable hypothesis. We have previously reported that active Na^+ flux across the peritubular border of nonperfused collecting tubules (26) and mixed suspensions (27) of tubules greatly exceeds K^+ influx into these cells. The results, though incompatible with a simple-linked 1:1 exchanger pump at the peritubular border as originally concluded, may not be pertinent to the present studies which involve net transcellular exchanges from lumen to blood rather than exchange across a single-cell border. They certainly do not exclude the presence of a linked exchange at the luminal surface. When considered in concert with the results of removal of K^+ from the outer bathing solution which leads to rapid depolarization of tissue and inhibition of Na^+ transport (Fig. 8), they may be indicative of the existence of an electrogenic pump. It is assumed that K^+ is necessary for operation of the Na^+ pump, though not by virtue of its being a counterion in a 1:1 linked exchange process. Conclusive evidence for or against this view obviously requires further study.

Finally although net K^+ reabsorption in the collecting system was inferred from the results of the micropuncture studies (7, 9, 21, 22), no evidence of K^+ reabsorption in the cortical collecting tubule was obtained in the present studies or in the papillary collecting duct in an earlier report (8). It should be emphasized that our studies do not exclude this process since the experiments were not designed to investigate the possibility. On the other hand, it should be emphasized that the micropuncture evidence for K^+ reabsorption in the collecting tubule is not conclusive. The K/inulin ratio of surface distal convolutions only was compared to that of pelvic urine and was found to exceed the latter. If, as has been suggested, the function of surface tubules differs quantitatively to a significant degree from that of the deeper tubules (28), then one may reach erroneous conclusions from comparative analyses of pelvic urine derived from all nephrons, superficial and deep.

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