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ACTIVE POTASSIUM ABSORPTION IN RAT DISTAL COLON

BY JACOB H. SWEIRY* AND HENRY J. BINDER†
From the Department of Internal Medicine, Yale University, New Haven, CT 06510, USA

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SUMMARY

1. Active potassium (K^+) absorption in rat distal colon was investigated by measuring mucosal-to-serosal ($J_{K,ms}$) and serosal-to-mucosal ($J_{K,sm}$) $^{42}K^+$ fluxes (μequiv h^{-1} cm^{-2}) across isolated stripped mucosa under short-circuit conditions in normal and dietary Na-depleted animals. As previously demonstrated, removal of Na^+ from both mucosal and serosal solutions bathing the normal colon slightly increased net K^+ absorption as a result of inhibition of $J_{K,sm}$ without affecting $J_{K,ms}$, while in the Na-depleted group net K^+ secretion ($-0{\cdot}54 \pm 0{\cdot}11$) was converted to a marked net K^+ absorption ($1{\cdot}68 \pm 0{\cdot}30$, $P < 0{\cdot}001$).

2. In both groups of animals in Na^+-free Ringer solution, $J_{K,ms}$ exhibited saturable and linear components, while $J_{K,sm}$ was a linear function of $[K^+]$. Estimated affinity constants (mM) for saturable net K^+ absorption were similar in normal ($0{\cdot}52 \pm 0{\cdot}12$) and Na-depleted ($0{\cdot}67 \pm 0{\cdot}11$) animals; however, there was a greater than 3-fold increase in the saturable flux (J_{max}) from $0{\cdot}54 \pm 0{\cdot}04$ in the normal colon to $1{\cdot}78 \pm 0{\cdot}08$ μequiv h^{-1} cm^{-2} in Na-depleted animals.

3. Mucosal orthovanadate (100 μM) inhibited $J_{K,ms}$ in both normal (control, $0{\cdot}66 \pm 0{\cdot}05$ *vs.* orthovanadate, $0{\cdot}36 \pm 0{\cdot}03$ μequiv h^{-1} cm^{-2}, $P < 0{\cdot}001$) and Na-depleted animals (control $1{\cdot}20 \pm 0{\cdot}13$ *vs.* orthovanadate $0{\cdot}77 \pm 0{\cdot}07$ μequiv h^{-1} cm^{-2}, $P < 0{\cdot}01$) without affecting $J_{K,sm}$ or the short-circuit current. In the Na-depleted group mucosal omeprazole or SCH28080 (100 μM), inhibitors of gastric K^+–H^+-ATPase, insignificantly or slightly reduced (by 10%) $J_{K,ms}$ respectively; in contrast, mucosal ouabain (1 mM) markedly inhibited $J_{K,ms}$ (control, $1{\cdot}61 \pm 0{\cdot}16$ *vs.* ouabain, $0{\cdot}83 \pm 0{\cdot}98$ μequiv h^{-1} cm^{-2}, $P < 0{\cdot}001$).

4. Mucosal Na^+ appeared to be a competitor of K^+ uptake across the apical membrane.

5. These results indicate that dietary Na-depletion increases electroneutral K^+ absorption by increasing its transport capacity and suggest that the mechanism of this active K^+ absorption process may involve an apical K^+-ATPase with properties that are unlike the gastric K^+–H^+-ATPase but similar, in part, to Na^+–K^+-ATPase.

* Present address: Biomedical Sciences Division, King's College London, Campden Hill Road, London W8 7AH.
† To whom reprint requests should be addressed.

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INTRODUCTION

Studies performed in rat distal colon under short-circuit conditions have provided evidence for active absorption and secretion of K^+ depending on the physiological state of the animal and the prevailing *in vitro* conditions (Foster, Zimmerman, Hayslett & Binder, 1983; Foster, Hayslett & Binder, 1984). Thus, in the normal colon there is net K^+ absorption which is electroneutral, Na^+ independent and, in part, Cl^- independent. In contrast, dietary K^+ loading (Foster, Sandle, Hayslett & Binder, 1986) or dietary Na-depletion (Foster *et al.* 1984) induces a substantial net K^+ secretory process which is electrogenic, Na^+ and Cl^- dependent, but amiloride insensitive. We have recently further characterized the K^+ secretory process in these animals with enhanced plasma levels of aldosterone and have established that K^+ secretion involves both K^+ uptake across the basolateral membrane via Na^+–K^+–$2Cl^-$ co-transport and the Na^+–K^+ pump, and tetraethylammonium (TEA)-inhibitable apical K^+ channels for K^+ exit (Sweiry & Binder, 1988, 1989). In Na^+-free Ringer solution, however, aldosterone-induced K^+ secretion is abolished and there is a substantial rate of K^+ absorption (Foster *et al.* 1984). Therefore elevated levels of aldosterone not only stimulate K^+ secretion but in Na^+-free Ringer solution enhance net K^+ absorption, which is the result of a marked increase in mucosal-to-serosal K^+ movement. It is unknown, however, whether this increase in K^+ absorption is related to an alteration in the kinetic properties of the K^+ transport process.

Since the active K^+ absorptive process is electroneutral, Na^+ independent and, in part, Cl^- independent, a K^+–H^+ exchange process has been suggested (Foster *et al.* 1984) and a reasonable candidate appears to be the K^+-activated ATPase isolated from brush-border membrane of rabbit colon by Gustin & Goodman (1981). This ATPase is inhibited by orthovanadate but not by ouabain, findings that are similar to the K^+-dependent ATPase activity which is inhibited by omeprazole (Wallmark, Jaresten, Larsson, Ryberg, Brandstrom & Fellenius, 1983; Scott & Sundell, 1985; Lorentzon, Jackson, Wallmark & Sachs, 1987) and SCH28080 (Scott & Sundell, 1985; Beil, Harkbarth & Sewing, 1986; Scott, Sundell & Castrovilly, 1987; Wallmark, Briving, Fryklund, Munson, Jackson, Mendlein, Rabon & Sachs, 1987) in gastric parietal cells. However, there are other candidates, for example, a K^+-sensitive, SCH28080-inhibitable proton pump has been described in rabbit distal colonic membranes (Kaunitz & Sachs, 1986) and a K^+–H^+ process mechanism has been identified in rat ileum (Binder & Murer, 1986). A recent study in the guinea-pig distal colon has demonstrated that this tissue secretes protons by a K^+-dependent, ouabain-inhibitable mechanism (Suzuki & Kaneko, 1987). Whether or not K^+ absorption in rat distal colon resembles any of these processes is not known.

The present study was therefore designed to provide additional understanding of the mechanism responsible for active K^+ absorption across the apical membrane. Thus, the kinetics of K^+ absorption in normal and Na-depleted rats were compared to determine whether the augmented K^+ absorption observed in dietary Na-depleted (hyperaldosterone) animals in Na^+-free Ringer solution represents alteration in the affinity of the apical membrane K^+ carrier or induction of new carrier proteins. The induction of carrier proteins is not an unlikely possibility since elevated levels of aldosterone induce a significant increase in Na^+–K^+-ATPase activity in rat

distal colon (Kashgarian, Taylor, Binder & Hayslett, 1980). In addition we also used a series of inhibitors to determine whether an apical transport ATPase is involved in this K^+ absorptive process. Part of this work has been presented in abstract form (Binder & Sweiry, 1988; Sweiry & Binder, 1988).

METHODS

Animals. Two groups of non-fasting male Sprague–Dawley rats weighing 220–280 g were used in this study. Group 1 consisted of animals fed a standard Agway Prolab 3000 diet containing 0·44 g Na^+/110 g food. Group 2 consisted of rats fed a paste diet (prepared in our laboratory) to which Na^+ was not added. The Na^+-deficient diet was given for a period of 7–9 days prior to the experiments to induce secondary elevation of aldosterone; previous studies have demonstrated that such animals have plasma aldosterone levels that are approximately 100-fold greater than normal animals (Halevy, Budinger, Hayslett & Binder, 1986). This group of rats will be referred to as the Na-depleted group.

Tissue preparation. The procedure has been described in detail previously (Binder & Rawlins, 1973; Foster *et al.* 1983). Briefly, the whole colon was removed under ether anaesthesia and washed with Ringer solution. The distal colon between a large lymph node close to the colorectal junction and some smaller nodes at the proximal end was stripped of the serosa and part of the underlying muscular layers, and divided into three equal segments of which the two more proximal pieces were mounted in Lucite chambers and bathed on both sides by equal volumes (8 ml) of an appropriate Ringer solution.

Electrical and flux measurements. The transepithelial potential difference, short-circuit current (I_{sc}) and conductance were either measured or calculated as described previously (Foster *et al.* 1983, 1984; Sweiry & Binder, 1989). The tissues were then paired on the basis of a conductance difference of $< 10\%$. Transmural fluxes of K^+ from mucosa to serosa or serosa to mucosa were measured under short-circuit conditions using $^{42}K^+$ as described previously (Foster *et al.* 1983, 1984; Sweiry & Binder, 1989). Briefly, $^{42}K^+$ fluxes were performed beginning 50 min after the addition of $^{42}K^+$. Preliminary studies have demonstrated that constant $^{42}K^+$ fluxes were obtained by 50 min and persisted for at least 100 min. In those experiments that examined the effect of putative transport inhibitors two additional 15 min flux periods were determined beginning 12 min after the addition of the inhibitor. In all experiments the two 15 min flux periods were identical and were therefore combined to obtain a single experimental value. In limited studies mucosal-to-serosal flux of Na^+ ($J_{Na,ms}$) was determined using $^{22}Na^+$. Four 15 min flux periods were performed: periods I and II were controls, and periods III and IV followed the addition of a transport inhibitor which was given a 12 min equilibration period.

Under short-circuit conditions, net K^+ transport ($J_{K,net}$) is active and defined as the difference between mucosal-to-serosal flux ($J_{K,ms}$) and serosal-to-mucosal flux ($J_{K,sm}$). Positive and negative values of net flux represent active absorption and active secretion, respectively. In some experiments in the Na-depleted group only $J_{K,ms}$ was measured, since in Na^+-free Ringer solution this flux of K^+ is markedly enhanced and K^+ movement is in the opposite direction; $J_{K,sm}$ is relatively small and represents paracellular K^+ movement (see Fig. 2*A*).

Solutions. The Ringer solution contained (mM): NaCl, 115; $NaHCO_3$, 25; K_2HPO_4, 2·4; KH_2PO_4, 0·4; $CaCl_2$, 1·2; $MgCl_2.6H_2O$, 1·2; glucose, 10. In Na^+-free experiments, NaCl and $NaHCO_3$ were replaced by equimolar choline chloride and choline bicarbonate, respectively. In experiments in which the effect of Ba^{2+} was tested, K_2HPO_4 and KH_2PO_4 were replaced by equimolar (5·2 mM) KCl, in order to prevent precipitation of barium phosphates. In the kinetic experiments K_2HPO_4 and KH_2PO_4 were removed and replaced by KCl which was varied as required, with appropriate adjustment of choline chloride to keep the osmolarity constant. The concentration of K^+ used in these studies was between 0·1 and 15·0 mM. All solutions were maintained at 37 °C and were continuously gassed with 95% O_2–5% CO_2 to a pH of 7·4.

Preparation of drugs and other agents. Drugs or other agents were added from concentrated stock solutions to give the desired concentration. Omeprazole was dissolved in 0·1 N-H_2SO_4 and SCH28080 in 10% dimethylsulphoxide (DMSO) prior to their addition to the chambers.

Materials. ^{42}K (as KCl) and ^{22}Na (as NaCl) were purchased from New England Nuclear (Boston, MA) and Amersham International PLC, respectively. Bumetanide was a gift from Hoffman La

Roche. Omeprazole and SCH28080 were gifts from AB Hassle (Sweden) and the Schering–Plough Corporation (New Jersey), respectively. All other chemicals were purchased from the Sigma Chemical Co., St Louis, MO.

RESULTS

Net K^+ absorption ($0{\cdot}24 \pm 0{\cdot}05$ μequiv h^{-1} cm^{-2}) occurred in normal animals when both sides of the distal colon were bathed by Na^+-containing Ringer solution, while

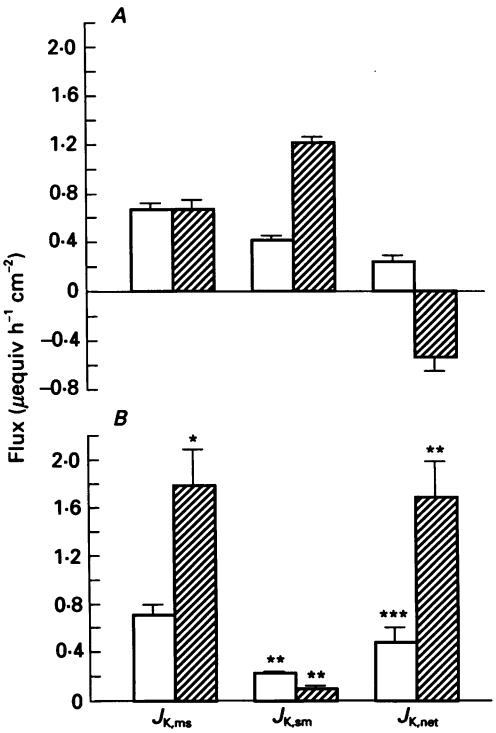

Fig. 1. The effect of aldosterone on K^+ transport in Ringer (*A*) and Na^+-free Ringer (*B*) solutions. In experiments in Na^+-free Ringer solution, Na^+ was replaced by choline. Results are means ± S.E.M. and represent in normal animals (□) eleven and nine tissue pairs in Ringer and Na^+-free Ringer solutions, respectively, and five tissue pairs in each experimental condition in Na-depleted animals (▨). *P* represents an unpaired *t* test comparing results in Ringer and Na-free Ringer solutions. Sodium depletion induced active K^+ secretion in Ringer solution. Removal of Na^+ in normal animals resulted in a small increase in $J_{K,net}$ as a result of a decrease in $J_{K,sm}$. In the absence of Na^+, Na depletion substantially enhanced active K^+ absorption. *, $P < 0{\cdot}005$; **, $P < 0{\cdot}001$; ***, $P < 0{\cdot}05$.

in the Na-depleted group net K^+ secretion ($-0{\cdot}54 \pm 0{\cdot}11$ μequiv h^{-1} cm^{-2}) was observed (Fig. 1*A*). The absence of Na^+ from both bathing solutions significantly reduced $J_{K,sm}$ but only slightly increased net K^+ absorption in the normal colon since $J_{K,ms}$ was not altered. In contrast, in the Na-depleted group net K^+ secretion was converted to net K^+ absorption ($1{\cdot}68 \pm 0{\cdot}30$ μequiv h^{-1} cm^{-2}), due to both inhibition of $J_{K,sm}$ and (unlike the normal colon) a marked increase in $J_{K,ms}$ (control, $0{\cdot}70 \pm 0{\cdot}09$

vs. Na^+-free, $1{\cdot}78 \pm 0{\cdot}30$ μequiv h^{-1} cm^{-2}, $P < 0{\cdot}005$) (Fig. 1*B*). The short-circuit current (I_{sc}) in the normal group was $0{\cdot}9 \pm 0{\cdot}1$ and 0 μequiv h^{-1} cm^{-2} ($P < 0{\cdot}001$) in Ringer and Na^+-free Ringer solution, respectively, while in the Na-depleted group the I_{sc} was $3{\cdot}5 \pm 0{\cdot}6$ μequiv h^{-1} cm^{-2} in Ringer solution and was also reduced to 0 ($P < 0{\cdot}001$) in Na^+-free Ringer solution. Similarly, the absence of Na^+ in the Ringer solution reduced the tissue conductance in both the normal (control, $6{\cdot}2 \pm 0{\cdot}3$ *vs.* Na^+-free, $4{\cdot}8 \pm 0{\cdot}4$ mS cm^{-2}, $P < 0{\cdot}02$) and Na^+-depleted (control, $10{\cdot}9 \pm 1{\cdot}6$ *vs.* Na^+-free, $4{\cdot}9 \pm 0{\cdot}8$ mS cm^{-2}, $P < 0{\cdot}005$) groups. These results are in agreement with previous studies from our laboratory (Foster *et al.* 1984).

Concentration dependence of potassium transport

To understand the mechanism of the marked increase in $J_{K,ms}$ observed in Na-depleted animals in Na^+-free Ringer solution the kinetic characteristics of unidirectional K^+ fluxes in mucosal and serosal Na^+-free Ringer solution were investigated in both normal and Na-depleted groups. Figure 2*A* illustrates the concentration dependence of $J_{K,ms}$ and $J_{K,sm}$ in normal and Na-depleted groups. In both groups $J_{K,ms}$ exhibited a saturable and a linear component; however, K^+ movement in the Na^+-depleted group was markedly greater than that in the normal colon throughout the concentration range employed. Unlike $J_{K,ms}$, $J_{K,sm}$ was a linear function of $[K^+]$ in both groups (see legend to Fig. 2*A* for linear regression results) and this movement of K^+ most probably represents passive paracellular transfer, which has been described in detail in the rabbit distal colon (McCabe, Smith & Sullivan, 1984). Moreover, in normal animals $J_{Na,sm}$ is a linear function of $[Na^+]$ over the range 4–140 mM-Na^+ (J. H. Sweiry & H. J. Binder, unpublished observations) and analysis of these data gave an apparent permeability to Na^+ (P_{Na}) of $8{\cdot}47 \times 10^{-8}$ cm s^{-1}. The ratio P_{Na}/P_K is 0·71, in good agreement with the free diffusion coefficient ratio (D_{Na}/D_K) of 0·77 at 25 °C (*Handbook of Chemistry and Physics*, 1974–1975). Assuming that passive K^+ movement in the mucosal-to-serosal and serosal-to-mucosal direction are equal, subtraction of $J_{K,sm}$ from $J_{K,ms}$ yields active absorption of K^+ which is a saturable function of $[K^+]$, as shown in Fig. 2*B*. A direct fit of these data (by non-linear regression) to the Michaelis–Menten kinetics equation ($J_K = J_{max}[K^+]/(K_m + [K^+])$, where J_K is the flux at a given value of [K], J_{max} is the saturable flux and K_m is the value of $[K^+]$ that gives half-maximal saturable K^+ transport) yielded similar affinity constants (K_m) in normal ($0{\cdot}52 \pm 0{\cdot}12$ mM) and Na-depleted ($0{\cdot}67 \pm 0{\cdot}11$ mM) animals; however, there was a substantial difference in the J_{max} values: $0{\cdot}54 \pm 0{\cdot}04$ *vs.* $1{\cdot}78 \pm 0{\cdot}08$ μequiv h^{-1} cm^{-2}, in normal and Na-depleted animals, respectively.

Effect of ATPase inhibitors on potassium absorption

A possible role for an ATPase in K^+ absorption was investigated by first examining the effect of orthovanadate, a transport ATPase inhibitor with an IC_{50} (concentration giving 50% maximal inhibition) of 5–20 μM for a variety of ATPases (see review by Schuurmans-Stekhoven & Bonting, 1981) including the K^+-ATPase in cortical collecting tubules (Doucet & Marsy, 1987; Garg & Narang, 1988). Figure 3*A* illustrates the effect of 100 μM-orthovanadate, added to the mucosal Ringer solution bathing the normal distal colon. In these studies net K^+ absorption was abolished

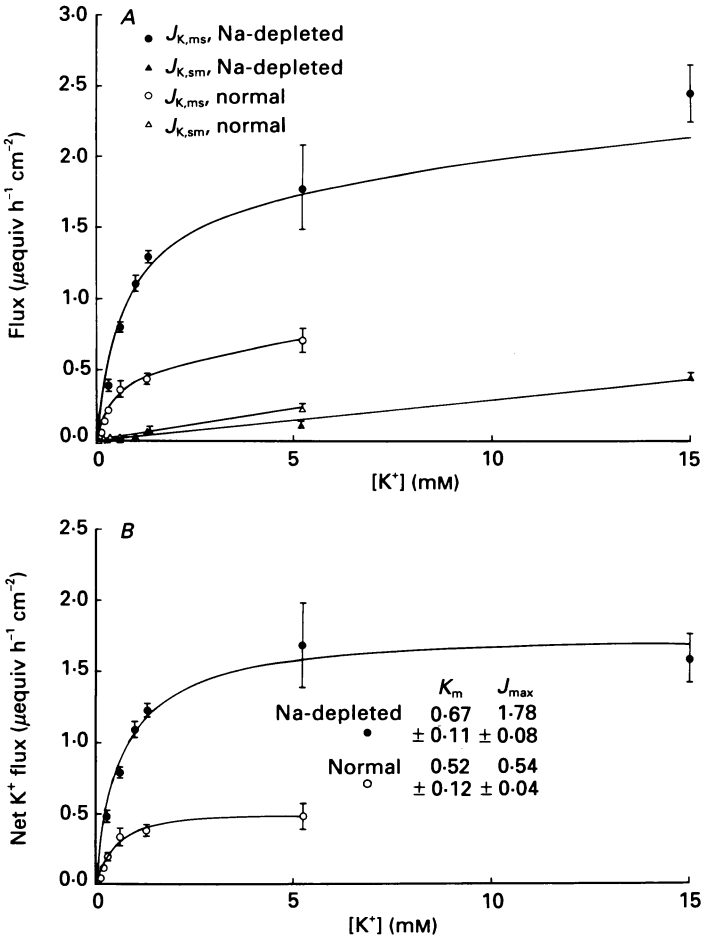

Fig. 2. Kinetics of K^+ transport in normal and Na-depleted animals in Na^+-free Ringer solution. *A*, dependence of unidirectional K^+ fluxes on $[K^+]$ when both serosal and mucosal $[K^+]$ were changed. Linear regression analysis of the serosal-to-mucosal ($J_{K,sm}$) K^+ movement gave the following equations: $J_{K,sm} = (0{\cdot}043 \pm 0{\cdot}001)[K^+] - (0{\cdot}003 \pm 0{\cdot}001)$ in normal animals, and $J_{K,ms} = (0{\cdot}029 \pm 0{\cdot}002)[K^+] - (0{\cdot}01 \pm 0{\cdot}01)$ in Na-depleted animals. The curves are drawn based on the estimated kinetics constants obtained by a direct fit of the data to the Michaelis–Menten equation (see text) plus the linear term obtained from the serosal-to-mucosal data. *B*, saturation kinetics of net K^+ absorption. The data were fitted to the Michaelis–Menten equation by non-linear regression (see text) and the kinetic parameters so obtained are shown. The curves were drawn based on the kinetics parameters estimated from the regression analysis. K_m is expressed in mM and J_{max} as μequiv h^{-1} cm^{-2}. All results are means ± S.E.M. from six tissue pairs in each group.

solely as a result of significant reduction in $J_{K,ms}$ without affecting $J_{K,sm}$ or I_{sc}. In order to investigate the effect of orthovanadate on K^+ absorption in the Na-depleted animal in normal Ringer solution, active net K^+ secretion was abolished by the addition of 30 mM-TEA to the mucosal side (Binder & Sweiry, 1988; Sweiry & Binder, 1989). Under these conditions mucosal orthovanadate also markedly inhibited net K^+ absorption, again solely as a result of inhibiting $J_{K,ms}$ without

affecting $J_{K,sm}$ (Fig. 3*B*). The I_{sc}, which in this tissue represents electrogenic Na^+ absorption (Foster *et al.* 1983), was not altered by orthovanadate (Fig. 3). The effect of ouabain, an inhibitor of Na^+–K^+-ATPase, was examined in the normal colon in Ringer solution, and the results in Fig. 4 reveal that there was a marked reduction in $J_{K,ms}$ such that net K^+ absorption was abolished; $J_{K,sm}$ and I_{sc} were unaffected by the mucosal addition of ouabain.

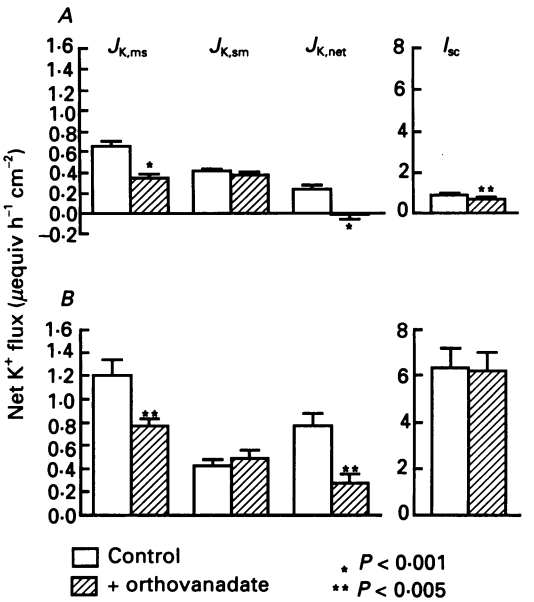

Fig. 3. Effect of orthovanadate on K^+ transport and the I_{sc} in normal (*A*) and Na-depleted (*B*) animals. Orthovanadate (100 μM final concentration) was added to the mucosal Ringer solution. In the Na-depleted group TEA (30 mM) was added to the mucosal solution at the beginning of the experiment to abolish K^+ secretion. Results are means ± S.E.M. from eleven and five tissue pairs in normal and Na-depleted animals, respectively. *P* represents paired *t* test comparing post-orthovanadate data with pre-orthovanadate (or control) data.

The effect of transport ATPase inhibitors was examined in the Na-depleted group under conditions in which K^+ absorption was maximal and in the absence of active K^+ secretion. These conditions are present when the mucosal and serosal solutions are Na^+ free. Under these conditions $J_{K,ms}$ is greatly enhanced and $J_{K,sm}$ is reduced to paracellular movement (see Fig. 2*A*). Figure 5 shows that orthovanadate (100 μM) again reduced $J_{K,ms}$ to a value comparable to that seen in normal Ringer solution (Fig. 3). Omeprazole or SCH28080 (100 μM), both of which are potent gastric K^+–H^+-ATPase inhibitors (Wallmark *et al.* 1983; Lorentzon, Ekiundh, Brandstrom & Wall mark, 1985; Beil *et al.* 1986), insignificantly or very slightly reduced $J_{K,ms}$, respectively. In contrast, ouabain (1 mM) markedly reduced $J_{K,ms}$ by approximately 50%. Thus, ouabain inhibited mucosal-to-serosal K^+ transport regardless of whether Na^+ was (Fig. 4) or was not (Fig. 5) present in the mucosal bathing solution. Because a residual K^+ flux was always present following mucosal addition of orthovanadate or ouabain in the Na-depleted animals (Figs 3 and 5), the effect of the simultaneous

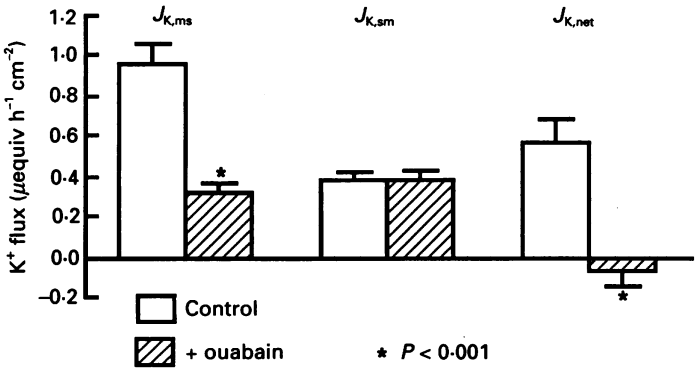

Fig. 4. Effect of mucosal ouabain on K^+ transport in normal rat distal colon. Ouabain was added to Ringer solution to give a final bath concentration of 1 mM. The short-circuit current was unaffected by ouabain: control, 0·63±0·08 *vs.* ouabain, 0·70±0·03 μequiv h^{-1} cm^{-2}. Results are means±S.E.M. from seven tissue pairs. *P* represents paired *t* test comparing post-ouabain to control period results.

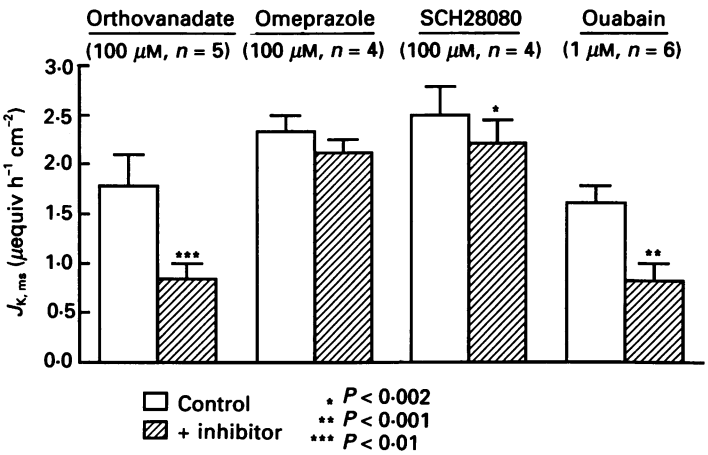

Fig. 5. Effect of several transport ATPase inhibitors on mucosal-to-serosal K^+ flux in Na-depleted animals in Na^+-free Ringer solution. Each inhibitor was separately added to the mucosal bath to give the final concentrations shown. Results are means±S.E.M. *P* represents paired *t* test comparison of the post-inhibitor to control period results.

presence of both of these inhibitors was further examined. Under these conditions $J_{K,ms}$ was reduced to a value (0·82±0·06 μequiv h^{-1} cm^{-2}, $n = 8$) not significantly different from that obtained in the presence of either orthovanadate or ouabain alone (Fig. 5).

Effect of sodium on potassium absorption

Enhancement of K^+ absorption when Na^+ was absent from the Ringer solution in the Na-depleted group suggests that there may be a direct competition between the two cations for the K^+ transport process at the apical membrane. Thus, the effect of Na^+ on K^+ absorption was established by determining K^+ fluxes at 0·6 mM-K^+ in

either 0 or 140 mM-mucosal Na^+. To determine maximal rates of K^+ absorption in Na-depleted animals experiments were performed in which K^+ secretion had been abolished by serosal ouabain (in normal colon serosal ouabain did not alter $J_{K,ms}$) (Sweiry & Binder, 1989). Figure 6 shows that 140 mM-Na^+ in the mucosal Ringer solution produced substantial reduction in $J_{K,ms}$ when K^+ was present at 0·6 mM but that the effect of Na^+ at 5·2 mM-K^+ was either negligible (in the normal colon) or relatively small (in Na-depleted animals).

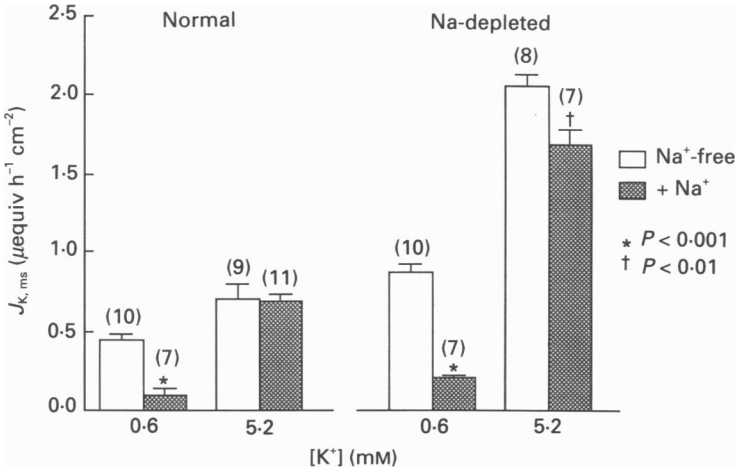

Fig. 6. Effect of Na^+ on K^+ absorption in normal and Na-depleted animals. Open (control) histograms represent results obtained in mucosal and serosal Na^+-free (choline replacement) Ringer solution. In normal animals the effect of Na^+ was studied with Na^+ present in both mucosal and serosal Ringer solutions. In the Na-depleted group the effect of Na^+ was studied under conditions in which K^+ secretion was abolished and K^+ absorption was maximal. Thus, in the 0·6 mM-K^+ experiments, Na^+ was present only in the mucosal bathing solution and ouabain (1 mM) was added to the serosal side; for the 5·2 mM-K^+ group the conditions were similar but Na^+ was present in both Ringer solutions. Results are means ± S.E.M., number in parentheses being the number of tissues studied. P represents paired t test comparing the control Na^+-free results with 140 mM-Na^+.

These studies with Na^+ suggest that at low K^+ concentration Na^+ may be transported via the vanadate-sensitive, ouabain-sensitive K^+ transport system. To test this possibility the effect of both vanadate and ouabain on Na^+ transport was determined at two K^+ concentrations: 0·6 and 5·2 mM. Figure 7*A* demonstrates that 100 μM-mucosal vanadate inhibited $J_{Na,ms}$ by 0·9 ± 0·2 μequiv h^{-1} cm^{-2}, when [K^+] was 0·6 mM. In contrast, when [K^+] was raised at 5·2 mM, $J_{Na,ms}$ was reduced by vanadate by 0·4 ± 0·1 μequiv h^{-1} cm^{-2}. The inhibition of $J_{Na,ms}$ by vanadate was significantly ($P < 0·05$) smaller at a [K^+] of 5·2 mM than at 0·6 mM. Almost identical results on $J_{Na,ms}$ were produced by mucosal ouabain (Fig. 7*B*). Ouabain (1 mM) inhibited $J_{Na,ms}$ by 27% at 0·6 mM-K^+ but did not alter $J_{Na,ms}$ at 5·2 mM-K^+.

Effect of putative potassium channel blockers on potassium absorption

Experiments were designed to examine the role, if any, of conductive pathways in regulating the movement of K^+ across the basolateral membrane. To evaluate the

effects of putative K^+ channel blockers on active K^+ absorption it is desirable to inhibit active K^+ secretion and increase $J_{K,ms}$ to a value close to its J_{max}. Therefore, experiments were performed in Na-depleted animals in the absence of Na^+ (see above). Thus, the effect of putative K^+ channel blockers, TEA, Ba^{2+} and Cs^+ on K^+

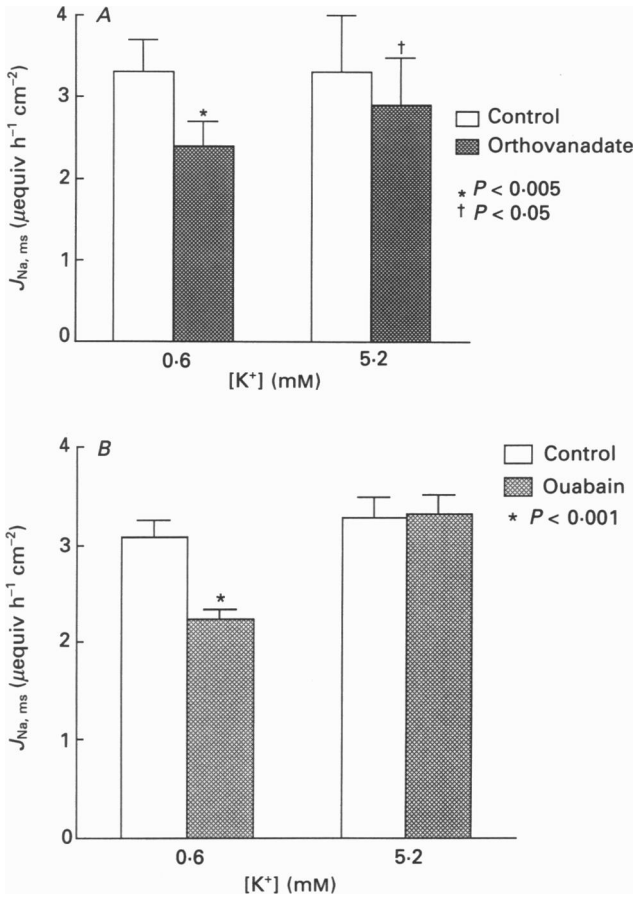

Fig. 7. Effect of orthovanadate and ouabain on mucosal-to-serosal Na^+ flux ($J_{Na,ms}$) in normal animals. 100 μM-orthovanadate (*A*) or 1 mM-ouabain (*B*) was added to the mucosal bath following the completion of two 15 min flux periods. After a 12 min equilibration period two additional 15 min flux periods were determined. In these experiments the Ringer solution contained 8 mM-Na^+ with variable concentrations of K^+ as shown. Results are mean ± S.E.M. from five tissues in each group in the orthovanadate studies and eight tissues in the ouabain experiments. *P* represents paired *t* test comparing the experimental period to the control period.

absorption was examined by adding these agents separately to the serosal bathing solution. Both TEA (30 mM) and Ba^{2+} (5 mM) only modestly reduced (but did not abolish) $J_{K,ms}$: control, 2·00 ± 0·23 *vs.* post-TEA, 1·75 ± 0·21 μequiv h^{-1} cm^{-2}, $P < 0·001$; control, 2·55 ± 0·12 *vs.* post-Ba^{2+}, 1·87 ± 0·17 μequiv h^{-1} cm^{-2}, $P < 0·001$). Caesium (15 mM) was ineffective ($J_{K,ms}$ was: control, 1·73 ± 0·19 *vs.* 1·70 ± 0·21 μequiv h^{-1} cm^{-2}).

DISCUSSION

These present experiments were designed to explore the apparent phenomenon that Na^+ depletion not only induces active K^+ secretion but when the secretory process is inhibited (by removal of Na^+ from the bathing solution or other manoeuvres that impair K^+ uptake across the basolateral membrane), Na-depletion also significantly stimulates active K^+ absorption to levels above those observed in the normal rat. Although these present studies were performed in dietary Na-depleted animals with elevated plasma aldosterone levels, it is most likely that active K^+ absorption is stimulated by aldosterone since continuous subcutaneous infusion of aldosterone via Alzet minipumps for 7 days also stimulates active K^+ secretion and active K^+ absorption (in the presence of serosal ouabain) (Turnamian & Binder, 1989). This absorptive process is electroneutral, Na^+ independent and, in part, Cl^- independent, and as a result we have proposed that it may represent K^+–H^+ exchange (Foster *et al.* 1984). This ability of aldosterone to regulate absorption and secretion of K^+ occurs in the distal, but not proximal, colon of the rat, and the physiological importance of this phenomenon is not yet apparent, nor is it known whether these two oppositely directed transport processes occur in the same or different cells. In the present study we have characterized the transepithelial K^+ movement by determining the kinetics of this process and establishing a role for both an ATPase and Na^+ in mediating K^+ transport.

Kinetics of potassium absorption

This study demonstrates that K^+ absorption in the distal colon of both normal and Na-depleted rats in Na^+-free Ringer solution is carrier-mediated and conforms to Michaelis–Menten saturation kinetics. A non-saturable linear process which most probably represents paracellular diffusion is also present. The affinity constants (K_m) for the saturable component were similar in the normal (0·52 mM) and Na-depleted (0·67 mM) animals (Fig. 2*B*); however, J_{max} in the Na-depleted rat (1·78 μequiv h^{-1} cm^{-2}) was more than 3-fold higher than that in the normal colon (0·54 μequiv h^{-1} cm^{-2}). These results indicate that Na-depletion increases K^+ transport capacity, most probably by increasing apical membrane K^+ transport proteins, rather than by inducing a new or altering the existing transport system in the distal colon. Since these transepithelial K^+ fluxes describe K^+ movement across both apical and basolateral membranes, it is possible that the increase in J_{max} seen in the Na-depleted group represents an increase in exit of K^+ across the basolateral membrane and not solely an increase in apical membrane uptake of K^+. The only other available kinetic data on colonic K^+ transport are from the normal rabbit distal colon where, also under short-circuit conditions, addition of serosal ouabain inhibited K^+ secretion and unmasked an active net K^+ absorptive process (McCabe, Cooke & Sullivan, 1982; Wills & Biagi, 1982). This mucosal-to-serosal K^+ movement is Na^+ independent (Plass, Gridl & Turnheim, 1986) and saturable with a K_m of 2·3 mM and J_{max} of about 1 μequiv h^{-1} cm^{-2} (McCabe *et al.* 1984).

Role of an apical ATPase in potassium absorption

In recent years there have been descriptions of K^+-ATPases, K^+ absorption and K^+-dependent proton secretion in several epithelia including gastric parietal cells (Sachs, Chang, Rabon, Schackman, Lewin & Saccomani, 1976; Forte & Lee, 1977; Wallmark *et al.* 1983; Lorentzon *et al.* 1985, 1987; Beil *et al.* 1986), mammalian colon (Gustin & Goodman, 1981; Kaunitz & Sachs, 1986; Suzuki & Kaneko, 1987; Perrone & McBride, 1988), reptile bladder (Husted & Steinmetz, 1981) and colon (Halm & Dawson, 1984), amphibian jejunum (Imon & White, 1984) and renal distal tubule (Doucet & Marcy, 1987; Hayashi & Katz, 1987*a*, *b*). Studies of the cellular mechanism of gastric acid secretion initiated investigation of K^+–H^+-ATPase and its relationship to electroneutral K^+ transport. However, characterization of the properties of these several disparate systems reveal some similarities but, with rare exception, no two systems appear identical.

The role of K^+–H^+-ATPase in acid secretion by gastric parietal cells has been characterized (Sachs *et al.* 1976; Forte & Lee, 1977; Lorentzon *et al.* 1985, 1987). This K^+–H^+-ATPase and proton secretion are inhibited by orthovanadate, a transport ATPase inhibitor (see review: Schuurmans-Stekhoven & Bonting, 1981) but not by ouabain. Omeprazole, a substituted benzimidazole, appears to interact with the sulphydryl groups of the K^+–H^+-ATPase and has an IC_{50} of about 2 μM against rabbit gastric K^+–H^+-ATPase (Scott *et al.* 1987), while SCH28080 (IC_{50} = 1·3–3·0 μM) appears to be a competitive inhibitor of the high-affinity K^+ site of the gastric ATPase (Beil *et al.* 1986; Scott *et al.* 1987).

In the reptile bladder and mammalian distal tubule there have been a few descriptions of both active K^+ absorption and K^+-ATPase activity. Husted & Steinmetz (1981) reported in the turtle bladder a luminal-located K^+ absorptive process that was Na^+ dependent and inhibited by mucosal ouabain, suggesting that Na^+–K^+-ATPase was involved in this process. A role for apical Na^+–K^+-ATPase in mediating K^+ absorption was also implicated by the studies of Hayashi & Katz (1987*a*, *b*) in which K^+ depletion enhanced Na^+–K^+-ATPase and tubular reabsorption of K^+ in the rat kidney. In the rabbit distal collecting ducts, however, a ouabain-insensitive K^+-ATPase activity has been demonstrated (Doucet & Marsy, 1987; Garg & Narang, 1988). Of considerable interest was that this enzyme was inhibited by vanadate, omeprazole and SCH28080, a pattern of inhibition that is in marked contrast to these present results in the rat distal colon (Figs 4–6). Whether the ATPase in the rabbit kidney is associated with K^+ absorption in these experiments is not known.

Gustin & Goodman (1981) made the initial suggestion that a similar K^+–H^+-ATPase was present in the mammalian colon by demonstrating ouabain-insensitive, vanadate-sensitive K^+-ATPase activity in apical membranes of rabbit distal colon. An additional study of ATPase in rabbit colon was reported by Kaunitz & Sachs (1986) who described two proton ATPases: a vanadate-sensitive but *N*-ethylmaleimide (NEM-) insensitive and an NEM-sensitive, vanadate-insensitive enzyme. It was not established whether either or both of these enzymes were located on the brush-border membranes.

There have been several studies of active K^+ absorption in the rat and rabbit distal

colon, but a comprehensive description of this absorptive process has not previously been reported. In the rabbit colon serosal ouabain was found to unmask active K^+ absorption which was inhibited by 2,4-dinitrophenol but was insensitive to mucosal ouabain (McCabe *et al.* 1982, 1984; Wills & Biagi, 1982). Plass *et al.* (1986) also described in the rabbit distal colon Na^+-independent active K^+ absorption which was inhibited by vanadate and mersalyl, a sulphydryl reagent, but was not altered by mucosal ouabain. In rat distal colon Foster *et al.* (1984, 1986) identified an electroneutral Na^+-independent K^+ absorption, which was also enhanced by dietary Na-depletion and dietary K-depletion, and suggested that a K^+–H^+ exchange might be present in this tissue. Our present studies were designed to evaluate the role of an apical ATPase in modulating active K^+ absorption. Figures 3–5 demonstrate that in both normal and Na-depleted animals active K^+ absorption is markedly inhibited by mucosal vanadate and mucosal ouabain. It should be noted that in the presence of Na^+, Na-depletion stimulates a vanadate-insensitive component of K^+ absorption (Fig. 3), whereas in Na^+-free Ringer solution both a vanadate-sensitive and vanadate-insensitive K^+ absorption was enhanced by Na-depletion (J. H. Sweiry & H. J. Binder, unpublished observations). Of considerable interest is the fact that mucosal ouabain was inhibitory in the presence or absence of serosal Na^+. However, inhibitors of gastric K^+–H^+-ATPase, omeprazole and SCH28080, manifested at best only a marginal inhibition of active K^+ absorption. Although omeprazole requires acid activation to inhibit K^+–H^+-ATPase (Lorentzon *et al.* 1985; Wallmark *et al.* 1987), SCH28080 inhibits acid secretion even following buffering of the acid space generated in gastric glands, though buffering decreases the potency of the drug 10-fold (Lorentzon *et al.* 1985). In our experiments, performed at pH 7·4, SCH28080 was present at 100 μM, a concentration far in excess of the measured IC_{50} (0·5–1·3 μM) in gastric glands, so that it is clear that in rat distal colon SCH28080 has only a mild inhibitory effect on K^+ absorption (Fig. 5). Similarly, an effect of omeprazole on K^+ absorption in rabbit distal colon could not be demonstrated (Halm & Frizzell, 1986).

The inhibition of K^+ absorption in the rat distal colon in these present experiments does not exactly resemble the effects of these inhibitors on either K^+ transport or K^+-ATPase activity in gastric or rabbit colonic tissue, except for proton secretion in guinea-pig distal colon. A recent study by Suzuki & Kaneko (1987) in the isolated, short-circuited guinea-pig distal colon has identified a mucosal acidification process that is inhibited by mucosal ouabain, by mucosal orthovanadate and by removal of mucosal K^+ but unaffected by absence of mucosal Na^+. These authors, therefore, proposed the presence of a K^+–H^+ exchange in the apical membrane of the guinea-pig distal colon. Thus, there are considerable similarities between the proton secretory process in the guinea-pig distal colon and our present results.

Effect of sodium on potassium absorption

Mucosal Na^+ suppressed K^+ absorption when K^+ was present at low (0·6 mM) concentration, but at high K^+ concentration (5·2 mM) the effect of Na^+ was either negligible (in normal animals) or small (in Na-depleted animals) (Fig. 6). In the rat distal colon the predominant mechanism of Na^+ absorption is via Na^+–H^+ exchange (Halevy *et al.* 1986). The present results demonstrate that Na^+ decreased $J_{K,ms}$ at a

K^+ concentration of 0·6 mM, but not at 5·2 mM, suggesting that at low K^+ concentration Na^+ may also be transported via the vanadate-sensitive, ouabain-sensitive K^+ transport system. The results presented in Fig. 7 reveal that vanadate inhibited $J_{Na,ms}$ by 28% at a K^+ concentration of 0·6 mM but by only 12% at 5·2 mM, while ouabain decreased $J_{Na,ms}$ by 27% at 0·6 mM-K^+ but not at 5·2 mM-K^+. Thus, it would appear that a small fraction of Na^+ is transported by an apical vanadate-sensitive, ouabain-sensitive ATPase that preferentially transports K^+. In contrast, in rabbit distal colon the removal of Na^+ from the mucosal bathing solution did not significantly affect $J_{K,ms}$ (Plass *et al.* 1986); it should be noted, however, that this study was performed at a K^+ concentration of 5·4 mM.

Potassium exit across the basolateral membrane

The mechanism(s) for K^+ exit across the basolateral membrane is not known. While in rabbit distal colon serosal Ba^{2+} decreased $J_{K,ms}$ and stimulated net K^+ secretion ($J_{K,sm}$), suggesting that K^+ exit through the basolateral membrane is conductive (Halm & Frizzell, 1986), serosal Ba^{2+} did not alter $J_{K,ms}$ in another study in the rabbit distal colon (McCabe *et al.* 1984). Based on the low (12–25% inhibition of K^+ absorption) sensitivity of $J_{K,ms}$ to serosal K^+ channel blockers, our results indicate that the major fraction of K^+ movement across the basolateral membrane may not occur via K^+ conductive pathways. However, it is possible that there is K^+ movement across the basolateral membrane through K^+ conductive channels that are not blocked by Ba^{2+}, Cs^+ or TEA. From their detailed analysis in rabbit gall-bladder epithelium Gunter-Smith & Schultz (1982) concluded that K^+ exit across the basolateral membrane is largely non-conductive and may be coupled to the movement of Cl^-. A similar conclusion was reached in studies in *Necturus* gall-bladder (Reuss, Weinman & Grady, 1980; Corcia & Armstrong, 1983) and indeed a basolateral membrane KCl co-transport process has been demonstrated in this epithelium (Reuss, 1983) and very recently in rabbit proximal tubule (Sasaki, Ishibashi, Yoshiyama & Shiigai, 1988). Removal of mucosal Cl^- (J. H. Sweiry & H. J. Binder, unpublished observations) or both mucosal and serosal Cl^- (Foster *et al.* 1984) reduced $J_{K,ms}$ by approximately 30–55% in the rat distal colon. Although these results in Cl^--free Ringer solution may be evidence of a Cl^--dependent process, we speculate that K^+ movement across the basolateral membrane may involve a Cl^--linked non-conductive process such as KCl co-transport.

In summary, the present study has demonstrated that dietary Na-depletion stimulates active electroneutral K^+ absorption by increasing the transepithelial transport capacity for K^+. Luminal Na^+ is a competitor of this active K^+ absorptive process which is markedly reduced by luminal orthovanadate and ouabain but not by omeprazole or SCH28080, suggesting that active K^+ absorption may involve an apical K^+-ATPase with properties that are unlike the gastric K^+–H^+-ATPase, but similar to the recently described K^+-dependent proton secretion in guinea-pig distal colon.

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