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# Potassium/Proton Exchange in Brush-Border Membrane of Rat Ileum

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**Summary.** These experiments were designed to determine whether proton-driven $^{86}$Rb uptake was present in apical membrane vesicles prepared from rat ileum. The uptake of $^{86}$Rb was approximately 300 to 350% greater in the presence of a 100-fold $H^+$ gradient than in its absence and was greater at 1, 2 and 5 minutes ("overshoot") than that at 90 minutes. Proton-driven $^{86}$Rb uptake was decreased by 20% in TMA-nitrate compared to that in TMA-gluconate. 0.3 mM amiloride did not significantly inhibit proton-driven $^{86}$Rb uptake; in contrast, proton-driven $^{22}$Na uptake was significantly inhibited by 0.3 mM amiloride by 34%. Similarly, 25 mM KCl inhibited proton-driven $^{86}$Rb uptake more than that of $^{22}$Na, while the inhibition of proton-driven $^{22}$Na uptake by 25 mM NaCl was greater than that of $^{86}$Rb. In additional studies intravesicular acidification measured by acridine orange fluorescence was demonstrated in the presence of an outwardly directed K gradient. These studies demonstrate that a proton gradient stimulates $^{86}$Rb uptake and a K gradient induces intravesicular acidification; and that these fluxes are mediated by a K/H exchange distinct from Na/H exchange which is also present in this membrane. We conclude that a specific exchange process for K/H is located in ileal apical membrane vesicles.

**Key Words** ileum · colon · vesicle transport · Na/H exchange · K/H exchange · acridine orange · amiloride

## Introduction

Active potassium absorption has recently been identified in the distal colon of both rat and rabbit [5, 11, 22]. These studies indicate that active potassium absorption is electroneutral and is both sodium and chloride independent; observations consistent with K/H exchange [5]. In addition to the recent evidence suggesting that a K/H exchange is present in the distal colon there are a few other experimental observations that suggest its existence in intestinal epithelial cells. In the guinea pig colon acid secretion has been measured which is potassium-dependent [18]. White has proposed that the mechanism of $HCO_3$ absorption in the *Amphiuma* jejunum is a result of a K/H exchange and has recently observed that omeprazole, which inhibits both gastric K-ATPase and acid secretion, also inhibits $HCO_3$ absorption [20, 21].

The present studies were initiated to determine whether a K/H exchange could be identified in intestinal apical brush-border membrane vesicles. Ileal apical membrane vesicles were studied in view of the relatively high protein yield from rat ileum. However, we were unable to study apical membranes from rat colon because of a much lower yield.[1] The substantial protein required for the experiments to detect intravesicular acidification with acridine orange precluded the use of colonic vesicles.

These studies demonstrate that a proton gradient stimulates $^{86}$Rb uptake and that a potassium gradient induces intravesicular acidification. It is likely that a K/H exchange is present in apical brush-border membranes of the rat ileum.

## Materials and Methods

Nonfasting male Wistar rats weighing between 200 and 300 g were used in all experiments. Following sacrifice the distal 40% of the small intestine was removed, washed and everted. The intestine was sliced into 1-cm segments and incubated in a solution containing (in mM): NaCl, 115; $NaHCO_3$, 25; KCl, 5; and $CaCl_2$, 1 for 30 min with continuous oxygenation with $O_2/CO_2$ (95%/5%). Epithelial cells were removed by vibration for 3 min (Chemap E, Chamap AG Männedorf ZH, Switzerland) and the suspension was filtered across a Buchner funnel. The filtrate was homogenized (Sorvall Omnimix, full speed) for 3 min in a solution containing (in mM): mannitol, 60; EGTA, 1; Tris-HCl, 2.4; pH 7.1. Tissue from four rats was homogenized in 150 ml of buffer.

Apical brush-border vesicles were prepared by a $Mg^{2+}$-EGTA precipitation method [1]. Following homogenization (*see above*) $MgCl_2$ (final concentration: 10 mM) was added and the

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[1] H.J. Binder, G. Stange, H. Murer, B. Stieger and H.P. Hauri. Sodium-proton exchange in brush border membranes of rat colon. *Am. J. Physiol.* (*submitted for publication*).

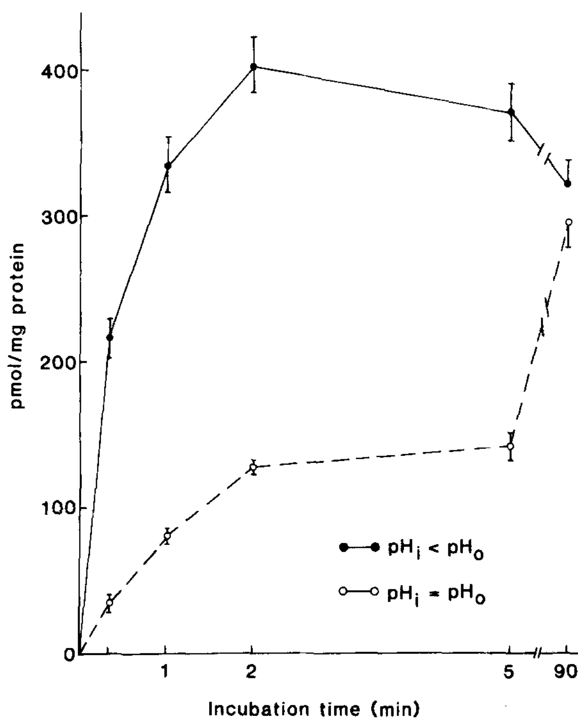


**Fig. 1.** Time course of $^{86}$Rb uptake in the presence and absence of a proton gradient. Membranes loaded with 300 mM mannitol and 50 mM MES adjusted to pH 5.5 with Tris were incubated in either 300 mM mannitol, 50 mM MES adjusted to pH 5.5 with Tris, and 0.1 mM $^{86}$RbCl (○) or 300 mM mannitol, 50 mM HEPES adjusted to pH 7.5 with Tris, and 0.1 mM $^{86}$RbCl (●) for 20 sec, 1, 2, 5 and 90 min. This is a representative experiment and presents mean ± SD of four to five replicate samples. The experiment was repeated four times on different membrane preparations

homogenate was allowed to sit on ice for 15 min. The homogenate was centrifuged at 5,000 rpm (Sorvall, SS-34) for 15 min, the supernatant was then centrifuged at 15,000 rpm for 30 min. The resulting pellet was resuspended in a solution containing (in mM): mannitol, 60; Tris-HCl, 5; pH 7.1 and homogenized in a glass-Teflon®-Potter. $MgCl_2$ was again added (final concentration: 10 mM) to the suspension which remained on ice for an additional 15 min. After a centrifugation at 5,000 rpm for 15 min the supernatant was centrifuged at 15,000 rpm for 30 min. The pellet was again resuspended in the buffer that would be used in the transport studies. A final centrifugation step was made for 30 to 40 min at 15,000 rpm. Membranes were frozen in liquid nitrogen until used. Preliminary experiments indicated that transport in frozen membrane preparations was equivalent to that in freshly prepared samples.

Aminopeptidase M (EC 3.4.11.2) was assayed as a specific brush-border enzyme marker [13] and its mean enrichment in the isolated membranes compared to that in the initial homogenate was 9.1 ± 0.9.

$^{86}$Rb and $^{22}$Na uptake was determined by rapid filtration using Sartorius filters of 0.65 μm pore diameter that had been soaked in distilled water. When uptake for periods shorter than or equal to 10 sec was measured, a short-time uptake apparatus (reference 9, Innovativ Labor, Adliswil, Switzerland) was used. Incubations were carried out at 20°C. The buffer compositions are provided in the legends to the figures. The ice-cold "stop solution" contained mannitol, 150 mM; K-gluconate, 150 mM; Tris-Cl, 5 mM, pH 5.5. Protein was measured by the Bio Rad method. Aminopeptidase M was measured as described by Haase et al. [8].

In other studies intravesicular acidification was determined with acridine orange [16]. The fluorescence of acridine orange was measured by a Shimadzu RF 510 spectrofluorometer. The experiments were performed at 37°C according to an earlier description [3]. The buffer compositions will be given in the legends to the figures. The sensitivity (gain) of the fluorometer recordings was kept constant throughout an experiment. However, between the different experiments sensitivity of recording was different; e.g. in the experiments where we used ionophores the sensitivity was only one third (Fig. 7) of that where we observed mainly the intrinsic properties (Figs. 8 and 9).

All experiments were performed at least three times with qualitatively identical results. $^{22}$NaCl and $^{86}$RbCl were obtained from New England Nuclear Co. (Boston, Mass.). All chemicals were purchased at analytical grade purity.

## Results

Figure 1 demonstrates $^{86}$Rb uptake as a function of time in the presence ($pH_i < pH_o$) and absence ($pH_i = pH_o$) of a proton gradient. In the absence of a proton gradient $^{86}$Rb uptake was very slow, reaching at 5 min approximately 40% of the uptake at 90 min. In contrast, the imposition of a 100-fold outwardly directed proton gradient resulted in a marked enhancement of $^{86}$Rb uptake. After 20 sec $^{86}$Rb uptake in the presence of a proton gradient was 328 ± 27% greater than that in its absence. Between 1 and 5 min uptake was greater than that observed at equilibrium (overshoot).

This stimulation of $^{86}$Rb uptake by an outwardly directed proton gradient could represent either a K/H exchange or uptake of $^{86}$Rb driven by a vesicle inside-negative potential created by the outward movement of $H^+$ through a proton-conductive pathway. To reduce or eliminate electrodiffusional coupling between the two ion fluxes $^{86}$Rb uptake was determined in the presence of TMA nitrate (inside and outside), an anion with a permeability considerably higher than that of gluconate (*see* Discussion, ref. 6). $^{86}$Rb uptake in the presence of nitrate was compared to its uptake in the absence of nitrate, i.e. in TMA gluconate. The uptake of $^{86}$Rb in the presence of TMA gluconate (Fig. 2) was almost identical to that observed in Fig. 1, i.e. uptake in the absence of either gluconate or nitrate. In the presence of TMA nitrate there was an approximate 20% decrease in $^{86}$Rb uptake compared to that in the presence of TMA gluconate. $^{86}$Rb uptake in the absence of a proton gradient was identical in the presence of either TMA gluconate or TMA nitrate. This observation together with the observation of a low K

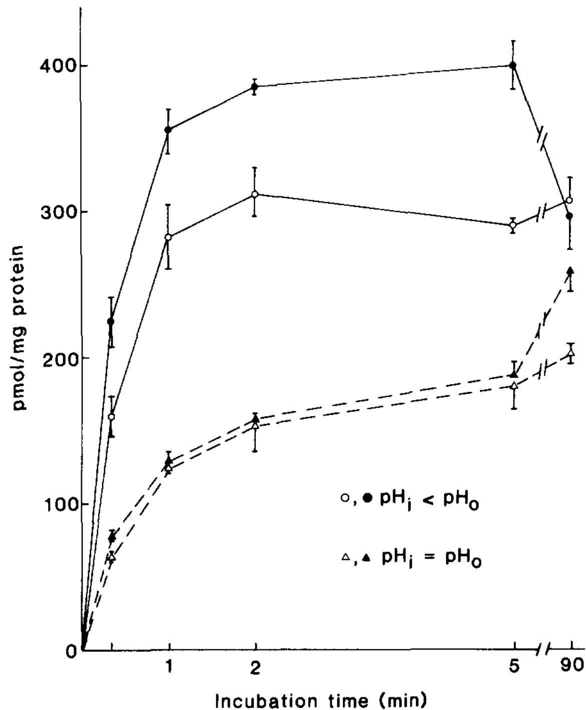


**Fig. 2.** Effect of TMA-nitrate (open symbols) and TMA-gluconate (solid symbols) on $^{86}$RbCl uptake in the presence and absence of a proton gradient. Membranes loaded with 300 mM mannitol, 50 mM MES adjusted with Tris to pH 5.5 and 20 mM TMA-gluconate were incubated in either 300 mM mannitol, 50 mM MES adjusted with Tris to pH 5.5, 20 mM TMA-gluconate and 0.1 mM $^{86}$RbCl (▲) or 300 mM mannitol, 50 mM HEPES adjusted with Tris to pH 7.5, 20 mM TMA-gluconate and 0.1 mM $^{86}$RbCl (●). In parallel experiments membranes loaded with 300 mM mannitol, 50 mM MES adjusted to pH 5.5 with Tris, 20 mM TMA-nitrate were incubated in 300 mM mannitol, 50 mM MES adjusted to pH 5.5 with Tris 20 mM TMA-nitrate and 0.1 mM $^{86}$RbCl (△) or 300 mM mannitol, 50 mM HEPES adjusted to pH 7.5 with Tris, 20 mM TMA-nitrate and 0.1 mM $^{86}$RbCl (○). Incubations were for 20 sec, 1, 2, 5 and 90 min. This is a representative experiment and presents mean ± SD of four to five replicate samples. The experiment was repeated three times on separate membrane preparations

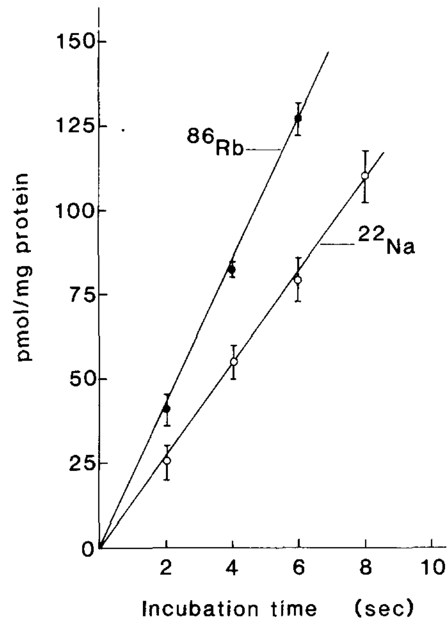


**Fig. 3.** Uptake of $^{86}$Rb and $^{22}$Na in the presence of a proton gradient as a function of time. Membranes loaded with 300 mM mannitol, 50 mM MES adjusted with Tris to pH 5.5 and 20 mM TMA-nitrate were incubated in 300 mM mannitol, 50 mM HEPES adjusted with Tris to pH 7.5, 20 mM TMA-nitrate and 0.1 mM $^{86}$RbCl (●) or 300 mM mannitol, 50 mM HEPES adjusted with Tris to pH 5.5, 20 mM TMA-nitrate and 0.1 mM $^{22}$NaCl (○) for 2, 4, 6, 8 and 10 sec. This is a representative experiment and presents mean ± SD of five replicate samples. The experiment was repeated three times on separate membrane preparations

conductance (*see below,* Fig. 7) suggests that it is unlikely that proton-driven $^{86}$Rb uptake represents indirect coupling via proton gradient-dependent alterations in the transmembrane electrical potential difference. All further tracer experiments were performed in the presence of nitrate in order to minimize electrodiffusional coupling between proton and rubidium fluxes.

To characterize $^{86}$Rb uptake further studies were performed to determine the period in which the initial uptake velocity was linear. Since proton-driven $^{86}$Rb uptake could also represent uptake by the Na/H exchange process, studies of proton-driven $^{86}$Rb and $^{22}$Na uptake were performed in parallel to compare their characteristics. Figure 3 presents the results of $^{86}$Rb and $^{22}$Na uptake at incubation periods of 2 to 8 sec. $^{86}$Rb uptake was linear for at least 6 sec and $^{22}$Na uptake for at least 8 sec. Therefore, in studies to determine the kinetics of $^{86}$Rb uptake and the effect of potential inhibitors $^{86}$Rb and $^{22}$Na uptake was measured in incubations that lasted 6 sec. The linearity of the uptake also indicates constant driving forces during this uptake period, i.e. despite the intrinsic proton conductance (Fig. 7 and ref. 3) the present ΔpH was sufficiently maintained during this time.

The uptake of $^{86}$Rb was determined at several concentrations between 0.75 and 50 mM. The results shown in Fig. 4 suggest the presence of a saturable component of $^{86}$Rb uptake but at higher Rb concentrations it is evident that $^{86}$Rb uptake still increases, i.e. saturation is apparently not reached. Since it is likely that the data in Fig. 4 represents both saturable and diffusional components, $^{86}$Rb uptake was measured at several concentrations in the absence of a proton gradient to determine whether the diffusional component might represent proton

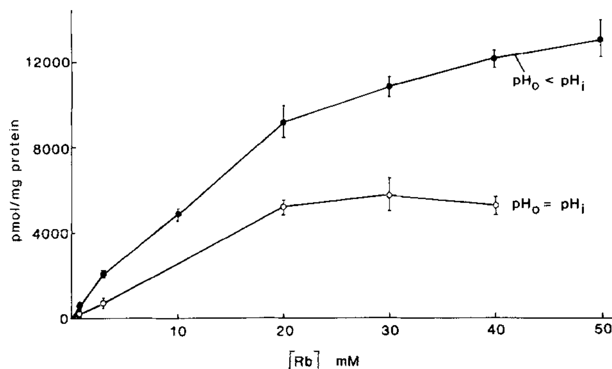


**Fig. 4.** Uptake of $^{86}$Rb in the presence (●) or absence (○) of a proton gradient. Membranes loaded with 300 mM mannitol, 50 mM MES adjusted with Tris to pH 5.5 and 20 mM TMA-nitrate were incubated for 6 sec in 300 mM mannitol, 50 mM HEPES adjusted to pH 7.5, 20 mM TMA-nitrate and either 0.75, 3.0, 10.0, 20.0, 30.0, 40.0 or 50.0 mM $^{86}$RbCl (●) or in 300 mM mannitol, 50 mM MES adjusted to pH 5.5, 20 mM TMA-nitrate and either 0.75, 3.0, 10.0, 20.0, 30.0, 40.0 or 50.0 mM $^{86}$RbCl (○). This is a representative experiment and presents mean ± SD of five replicate samples. The experiment was repeated five times on separate membrane preparations

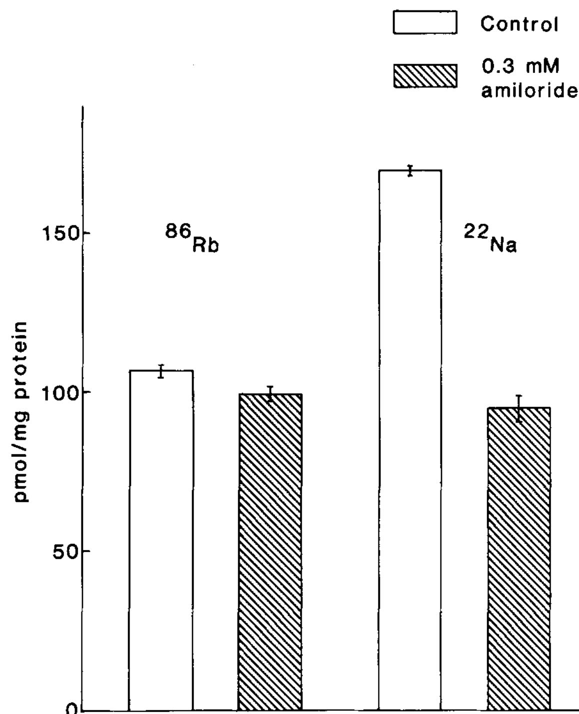


**Fig. 5.** Effect of 0.3 mM amiloride on the uptake of $^{86}$Rb and $^{22}$Na. Membranes loaded with 300 mM mannitol, 50 mM MES adjusted with Tris to pH 5.5 and 20 mM TMA-nitrate were incubated for 6 sec in 300 mM mannitol, 50 mM HEPES adjusted with Tris to pH 7.5, 20 mM TMA-nitrate, 0.1 mM $^{86}$RbCl with and without 0.3 mM amiloride (left panel) or in 300 mM mannitol, 50 mM HEPES adjusted with Tris to pH 7.5, 20 mM Tris-nitrate, 0.1 mM $^{22}$Na with and without 0.3 mM amiloride (right panel). This is a representative experiment presenting mean ± SD of four to five replicate samples. The experiment was repeated three times on separate membrane preparations. In each experiment proton-driven $^{86}$Rb and $^{22}$Na uptake was demonstrated to be at least 4 times greater than uptake in the absence of a proton gradient

gradient-independent $^{86}$Rb uptake. $^{86}$Rb uptake under these conditions also demonstrated a saturable component (Fig. 4). Thus, it is not possible to correct for diffusional uptake by subtracting the uptake in the absence of a proton gradient from that in the presence of a proton gradient. It is therefore difficult to estimate the kinetic parameter for K/H exchange (e.g. apparent $K_m$; from Hanes-Wolff-plots we obtained in five experiments values between 9 and 22 nM based on uncorrected uptake values).

Amiloride is known to inhibit Na/H exchange in several membrane systems [10]. In order to distinguish ΔpH-dependent rubidium flux from flux via Na/H exchange studies were performed to compare the effect of 0.3 mM amiloride on $^{86}$Rb and $^{22}$Na uptake. As shown in Fig. 5, 0.3 mM amiloride significantly inhibited $^{22}$Na uptake by 34.3 ± 9.0% while only minimally affecting $^{86}$Rb uptake. The inhibition of $^{22}$Na uptake by amiloride was much greater than that of $^{86}$Rb. These studies suggest that $^{86}$Rb uptake is not mediated by the Na/H exchange but rather by a separate and distinct K/H exchange.

To obtain additional evidence to support the concept that there are separate K/H and Na/H exchange processes, experiments were designed to determine the effect of KCl and NaCl on both $^{86}$Rb and $^{22}$Na uptake at concentrations greater than their apparent $K_m$. Figure 6 presents the results of this experiment. 25 mM KCl inhibited $^{86}$Rb uptake more than it inhibited $^{22}$Na uptake. In contrast, the inhibition of $^{22}$Na uptake by 25 mM NaCl was greater than that of $^{86}$Rb. These results are also consistent with K/H exchange that is distinct from Na/H exchange.

The initial experiments with acridine orange demonstrated that the ileal brush-border membranes contain a high proton conductance but apparently only minimal potassium conductance (Fig. 7). In vesicles preloaded with K-gluconate the addition of valinomycin (trace 3), i.e. efflux of potassium and a concomitant vesicle inside-negative membrane potential produced a large quenching of acridine orange fluorescence due to proton influx. On the other hand, the addition of a protonophore, FCCP, in the absence of valinomycin (trace 2) was unable to provoke an alteration in acridine orange fluorescence quenching which indicates that the intrinsic potassium conductance is low (for details *see* reference [3]). The absence of a significant potassium diffusion potential in potassium-preloaded ileal brush-border vesicles suspended in potassium-free solu-

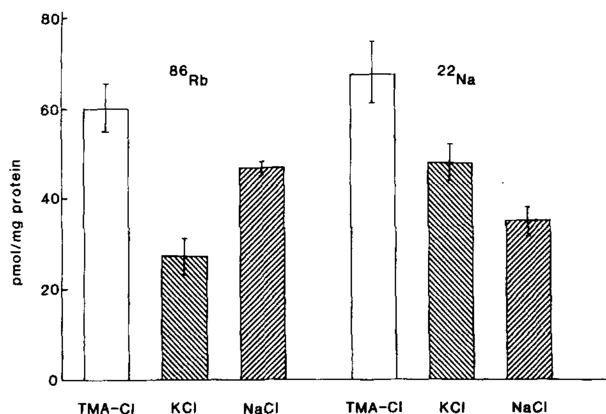


**Fig. 6.** Effect of 25 mM KCl and 25 mM NaCl on the uptake of $^{86}$Rb (left panel) and $^{22}$Na (right panel). Membranes loaded with 300 mM mannitol, 50 mM MES adjusted with Tris to pH 5.5, and 20 mM TMA-$NO_3$ were incubated for 6 sec with 300 mM mannitol, 50 mM HEPES adjusted with Tris to pH 7.5, 20 mM TMA-nitrate and 0.1 mM $^{86}$RbCl or 0.1 mM $^{22}$NaCl plus either 25 mM TMA-Cl, 25 mM KCl or 25 mM NaCl. Preliminary experiments revealed that 25 mM TMA-Cl did not alter either $^{86}$Rb or $^{22}$Na uptake. This is a representative experiment representing mean ± SD of four replicates. The experiment was repeated three times on separate membrane preparations. In each experiment proton-driven $^{86}$Rb and $^{22}$Na uptake was demonstrated to be at least 4 times greater than uptake in the absence of a proton gradient

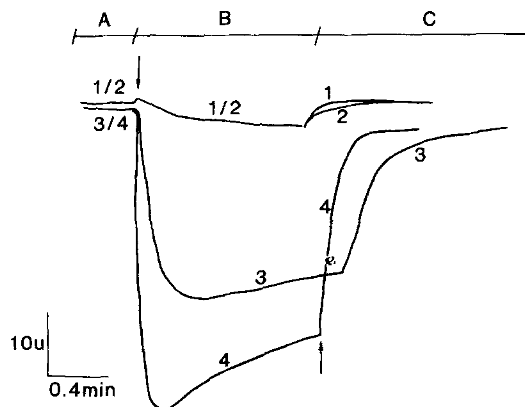


**Fig. 7.** K-gradient-dependent formation of a ΔpH in apical membrane vesicles by protonophore (FCCP) and/or K ionophore (valinomycin). Vesicles loaded with 100 mM K-gluconate, 50 mM TMA-gluconate and 10 mM HEPES adjusted with Tris to pH 7.5 were injected (↓) into solution containing 150 mM TMA-gluconate, 10 mM HEPES adjusted with Tris to pH 7.5, 6 μM acridine orange and 1% ethanol plus in trace 1, no ionophore; in trace 2, 2.5 μg/ml FCCP; in trace 3, 2.5 μg/ml valinomycin; and in trace 4, 2.5 μg/ml FCCP and 2.5 μg/ml valinomycin. Period A represents baseline fluorescence; Period B, development of intravesicular acidification; Period C, release of fluorescence (i.e. recovery of intravesicular acidification) following addition at (↑) of 50 mM K-gluconate

tions is important as it indicates that indirect electrodiffusional coupling between potassium (and most probably also rubidium) and proton fluxes are minimal and that the observations made on the effect of proton gradients on rubidium flux (Figs. 1–6) or of potassium gradients on proton fluxes (Fig. 8) are the manifestations of directly coupled fluxes.

Figure 8 demonstrates that the imposition of an outward-directed K gradient by the addition of K-containing vesicles to a K-free solution resulted in a decrease in acridine orange fluorescence (i.e. intravesicular acidification). Further, the elimination of the K gradient by addition of K to the incubation solution resulted in an increase in acridine orange fluorescence (trace 1). It is of interest that also the addition of Na (trace 2) is able to reverse the fluorescence quenching similar to the addition of K. The addition of TMA (trace 3) instead of either K or Na has no influence. Furthermore, we observed no difference in the fluorescence quenching whether we used gluconate or nitrate (*data not shown*) indicating the absence of significant electrodiffusional coupling between potassium and proton fluxes which was probably related to the low K-conductance (*see above*). The release of fluorescence quenching after the injection of Na or K was slow and incomplete. Experiments with prolonged incubation periods (*data not shown*) or by addition of nigericin (*see* Fig. 9) showed that recovery is only at about 60 to 70%. The reason for this incomplete recovery is not clear but could be related to dye bindings to the membranes injected.

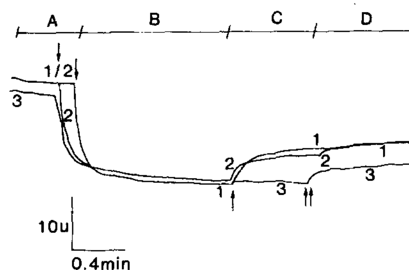


**Fig. 8.** K-gradient-dependent formation of a ΔpH in apical membrane vesicles and its elimination by K-, Na-, or TMA-gluconate. Vesicles loaded with 100 mM K-gluconate, 50 mM TMA-gluconate and 10 mM HEPES adjusted with Tris to pH 7.5 were injected into solution containing 150 mM TMA-gluconate, 10 mM HEPES adjusted with Tris to pH 7.5, 6 mM acridine orange, and 1% ethanol. Period A represents baseline fluorescence; Period B, development of intravesicular acidification; Period C, release of fluorescence (i.e. recovery of intravesicular acidification) following the addition at (↑) of 50 mM K-gluconate in trace 1, 50 mM Na-gluconate in trace 2, or 50 mM TMA-gluconate in trace 3. Period D represents an additional period of release of fluorescence with the injection at (⇈) of 50 mM K-gluconate in trace 2; 50 mM Na-gluconate in trace 3; in trace 1 no additional injection was made

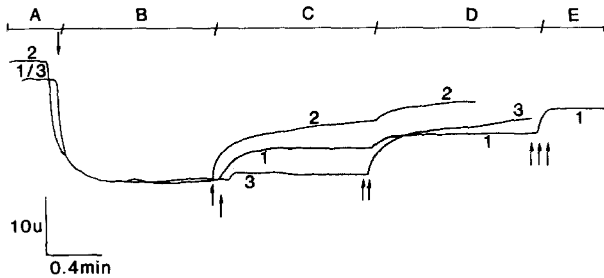


**Fig. 9.** Na-gradient dependent formation of a ΔpH in apical membrane vesicles and its elimination by K-, Na-, or TMA-gluconate or by nigericin. Vesicles loaded with 100 mM Na-gluconate, 50 mM TMA-gluconate and 10 mM HEPES adjusted with Tris to pH 7.5 were injected at (↓) into solution containing 150 mM TMA-gluconate and 10 mM HEPES adjusted with Tris to pH 7.5, 6 $\mu$M acridine orange and 1% ethanol. Period A represents baseline fluorescence; Period B, development of intravesicular acidification; Period C, D and E, release of fluorescence (i.e. recovery of intravesicular acidification). In trace 1, 50 mM Na-gluconate was added at (↑); 50 mM K-gluconate at (↑↑); and 2.5 $\mu$g/ml nigericin (↑↑↑). In trace 2, 50 mM K-gluconate was added at (↑); and 50 mM Na-gluconate at (↑↑). In trace 3, 50 mM TMA-gluconate was added at (↑) and 50 mM K-gluconate at (↑↑)

In parallel experiments we have measured in an analogous manner Na/H exchange. Figure 9 demonstrates that the imposition of an outward-directed Na gradient resulted in a decrease in acridine orange fluorescence (i.e. intravesicular acidification). The Na gradient-induced fluorescence quenching is released by injection of either Na (trace 1 in period C) or K (trace 2 in period C). This figure also contains a few additional controls as indicated in the figure legend. For example the addition of TMA-gluconate (trace 3, period C) is unable to release the fluorescence quenching and the addition of nigericin via K/H exchange immediately releases the fluorescence quenching (trace 1 in period E).

The observation that the fluorescence quenching induced either by an outwardly directed K gradient (Fig. 8) or by a Na gradient (Fig. 9) can be released by an addition of either K or Na to the extravesicular compartment suggests that Na/H exchange and K/H exchange operate in the same vesicles and not in two separate subpopulations.

## Discussion

These studies provide evidence that a K/H exchange is present in brush-border membrane vesicles of the rat ileum. An outward-directed proton gradient stimulated $^{86}$Rb uptake (Fig. 1 and 2) and an outward-directed K gradient induced intravesicular acidification (Fig. 8). This coupling of Rb and proton movement does not appear to be related to an electrodiffusional coupling of the two fluxes. K/H exchange is distinct from Na/H exchange process which is also present in this epithelium and these two exchange processes are most probably located in the same membrane (*see* Figs. 8 and 9).

Since Na/H exchange is present in rat small intestine [12], it is possible that the uptake of Rb in these studies could be mediated by the Na/H exchange mechanism. However, our present studies do not support the presence of a single proton-cation exchange mechanism but rather two separate processes. First, 0.3 mM amiloride significantly inhibited proton-driven $^{22}$Na uptake while only minimally inhibiting $^{86}$Rb uptake (Fig. 5). Second, 25 mM Na inhibited $^{22}$Na uptake more than $^{86}$Rb uptake but 25 mM K inhibited $^{86}$Rb uptake more than $^{22}$Na uptake (Fig. 6). These observations are more consistent with separate and distinct transport processes rather than a single common mechanism.

The acridine orange studies provide important information regarding the relative conductances of K and H in these membranes (Fig. 7). We concluded that under control conditions there is limited K conductance compared to the proton conductance since intravesicular acidification was not increased by FCCP, a proton ionophore, but was increased by valinomycin. Thus, it would be unlikely that $^{86}$Rb uptake is a result of its movement through a K conductance pathway. There is additional evidence that indicates that an exchange process is responsible for the stimulation of $^{86}$Rb uptake by the presence of a proton exchange rather than parallel H and K conductances that are linked by the development of an intravesicular potential. Nitrate is an anion whose permeability is considerably higher than that of gluconate [6] and was used to minimize the membrane potential. Figure 2 demonstrates that approximately 20% of $^{86}$Rb uptake was abolished by the presence of nitrate. Further, the presence of nitrate was unable to alter intravesicular acidification in the acridine orange experiments (*data not shown*). Therefore, the low K conductance and the use of nitrate as a permeant anion makes it unlikely that our observation on K(Rb)/H exchange are the product of electrodiffusional coupling between the two ion fluxes.

Interest in K/H exchanges has been stimulated by studies of gastric acid secretion. Forte and Lee and Sachs et al. have provided evidence that gastric acid secretion is mediated by a K/H exchange which represents the transport function of the ouabain-insensitive K-ATPase [4, 17, 23]. In this model K and Cl are secreted across the parietal cell apical membrane. K is recycled via a K/H exchange resulting in H-Cl secretion. Substituted benzimidazole derivatives (e.g. omeprazole) inhibit both K-

ATPase and gastric acid secretion [15]. Although K/H exchange processes have been identified in bacteria and *Amphiuma* erythrocyte mutants [2, 14], the present observations are the first direct demonstration of a K/H exchange in nongastric mammalian membranes. Several studies in intact intestinal tissue suggest the presence of such a mechanism. In the rat distal colon active K absorption is present in an $HCO_3$-Ringer's solution and is not inhibited by the removal of either Na or Cl [5]. K absorption in this epithelium is thus electroneutral and Na and Cl independent are consistent with K/H exchange. Similarly, in the rabbit distal colon ouabain inhibits active K secretion which is present under basal conditions and unmasks active K absorption [11, 22]. Since short-circuit current is zero in the presence of ouabain, K/H exchange is compatible with this observation. A ouabain-insensitive K-ATPase has also been identified in studies by Gustin and Goodman in apical membranes from rabbit distal colon [7]. There is additional experimental evidence suggestive of the presence of a K/H transport process in the intestine. First, in the guinea pig colon Suzuki has demonstrated acid secretion which is inhibited by the removal of K [18]. Second, in studies of electrolyte transport in the *Amphiuma* jejunum White and coworkers have proposed that $HCO_3$ absorption in this tissue is also a result of a K/H exchange [20, 21]. Recently, White has reported that omeprazole both inhibited $HCO_3$ absorption and decreased intracellular K activity [20]. However, to date active K transport in the colon has not been inhibited by omeprazole.

It is generally accepted at the present time that small intestinal K movement is not mediated by an active transport process but is affected by passive driving forces and bulk water flow [19]. Thus, despite the compelling evidence that a K/H exchange is present in the ileal brush-border membrane and operates in parallel to the Na/H exchange, a ready explanation for its physiological role is not apparent. Therefore, we would like to speculate regarding its function in the ileal epithelial cell: 1) Although, no evidence for mediated K transport in ileum is available, it is important to note that in the colon, removal of sodium either increases or unmasks K absorption [5, 11, 22]. Therefore, it is possible that, in the absence of sodium, K absorption might also be unmasked in the ileum. 2) In the absence of luminal K conductance, K/H exchange could represent a mechanism for K secretion across the apical membrane which would be further increased by a recycling of protons generated at the luminal surface by the Na/H exchange. On the other hand, protons produced intracellularly could serve as driving force for electroneutral K absorption. 3) If we accept the existence of a third ion exchange mechanism namely a $Cl/HCO_3$ exchange system, the K/H exchange could represent a regulatory device for uncoupling Na and Cl absorption by virtue of decreasing the driving force for the $Cl/HCO_3$ exchange. 4) Finally, it is possible that K/H exchange is not involved in transcellular K transport but rather in cell volume regulation. It is evident, that all these possibilities do not exclude that the K/H exchange is a regulatory entity and that our study on apical ileal vesicles represent an activated state of the K/H exchange while in the intact tissue it may be either active or quiescent and its activity regulated by unknown cellular factor(s). We hope that the present observation of a K/H exchange in rat apical membrane vesicles stimulates experimental work on intact tissue in order to define the physiological role of K/H exchange.

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