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# Mechanisms of Cation Permeation across Apical Cell Membrane of *Necturus* Gallbladder: Effects of Luminal pH and Divalent Cations on $K^+$ and $Na^+$ Permeability

Luis Reuss, Laurence Y. Cheung, and Timothy P. Grady

Department of Physiology and Biophysics and Department of Surgery, Washington University School of Medicine, St. Louis, Missouri 63110

**Summary.** Conventional microelectrode techniques were combined with unilateral mucosal ionic substitutions to determine the effects of luminal pH and luminal alkali-earth cation concentrations on apical membrane cation permeability in *Necturus* gallbladder epithelium. Acidification of the mucosal solution caused reversible depolarization of both cell membranes and increase of transepithelial resistance. Low pH media also caused: (*a*) reduction of the apical membrane depolarization induced by high K, and (*b*) increase of the apical membrane hyperpolarization produced by Na replacement with Li or N-Methyl-D-glucamine. These results, in conjunction with estimates of cell membrane conductances, indicate that acidification of the luminal solution produces a reduction of apical membrane K permeability ($P_K$). Addition of alkali earth cations ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, or $Ba^{2+}$) produced cell membrane depolarization, increase of relative resistance of the luminal membrane and reduction of the apical membrane potential change produced by a high-K mucosal medium. These results, as those produced by low pH, can be explained by a reduction of apical membrane $P_K$. The effects of $Ba^{2+}$ on membrane potential and relative apical membrane $P_K$ were larger than those of all other four cations at all concentrations tested (1–10 mM). The effect of $Sr^{2+}$ was significantly larger than those of $Mg^{2+}$ and $Ca^{2+}$ at 10 mM, but not different at 5 mM. The reduction of $P_K$ produced by mucosal acidification appears to be mediated by: (*a*) nonspecific titration of membrane fixed negative charges, and (*b*) an effect of luminal proton activity on the apical K channel. Divalent cations reduce apical membrane $P_K$ probably by screening negative surface charges. The larger magnitude of the effects of $Ba^{2+}$ and $Sr^{2+}$ can be explained by binding to membrane sites, in the surface or in the K channel, in addition to their screening effect. We suggest that the action of luminal pH on K secretion in some segments of the renal tubule could be mediated in part by this pH-dependent K permeability of the luminal membrane.

The apical membrane of gallbladder epithelial cells has been shown to be mostly K-permeable in both rabbit and *Necturus* (Hénin & Cremaschi, 1975; Reuss & Finn, 1975*a, b*; Van Os & Slegers, 1975). In frog, rabbit and *Necturus* gallbladder, noise analysis experiments indicate that the K transport sites in the luminal membrane can be characterized as channels (Van Driessche & Gögelein, 1978; Gögelein, 1980).

Electrophysiologic studies in *Necturus* gallbladder suggest a smaller, but sizeable Na permeability of this membrane (Reuss & Finn, 1975*b*; Van Os & Slegers, 1975; Graf & Giebisch, 1979). These results suggest that transepithelial transport requires electroneutral Na entry from the mucosal solution into the cells. Tracer influx measurements in rabbit gallbladder (Cremaschi & Henin, 1975; Frizzell, Dugas & Schultz, 1975) and intracellular ion-selective microelectrode measurements in rabbit (Duffey, Turnheim, Frizzell & Schultz, 1978) and *Necturus* (Graf & Giebisch, 1979; Reuss & Grady, 1979*b*; Reuss & Weinman, 1979; Garcia-Diaz & Armstrong, 1980) indicate that Na and Cl entry depend on the presence of the other ion in the mucosal medium. It has been proposed that electroneutral NaCl cotransport can account for these observations. A more complicated process, involving perhaps $Na^+ - H^+$ and $Cl^- - HCO_3^-$ exchange cannot be ruled out from the presently available data.

Intracellular K activity is higher than predicted for passive distribution in both *Necturus* (Reuss & Weinman, 1979; Reuss, Weinman & Grady, 1980) and rabbit gallbladder (Gunter-Smith, Duffey & Schultz, 1980). These observations indicate that under

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normal transport conditions a K diffusional net flux should be observed from cells to mucosal medium. The net transepithelial K flux can be small, however, because of K back-diffusion through the intercellular pathway, which has been shown to have a high K conductance (Reuss & Finn, 1975*a, b*; Van Os & Slegers, 1975; Reuss & Weinman, 1979). The additional possibility of uphill K uptake at the luminal membrane has also been considered (Reuss & Weinman, 1979).

The experiments described in this paper are part of a detailed study of the mechanisms of cation transport across the luminal membrane of *Necturus* gallbladder epithelial cells. We have examined the effect of pH and divalent cations in the mucosal bathing medium on diffusional cation permeabilities, by means of conventional intracellular microelectrode techniques combined with ionic substitutions in the mucosal bathing medium. Our results indicate that acidification of the luminal solution produces a decrease of K permeability and an increase in the relative Na permeability ($P_{Na}/P_K$). We suggest that these effects are caused by: (*a*) titration of fixed negative surface charges in the membrane, and (*b*) a direct effect of the high proton activity on the K transport sites. Divalent cations also reduced relative apical membrane $P_K$, with the effectiveness sequence $Ba^{2+} > Sr^{2+} > Ca^{2+} \sim Mg^{2+}$. The relative magnitudes of the effects suggest a mixed mode of action of these agents: screening of negative surface charges in the apical membrane (common to all of them) and binding to these charges or to the K transport sites (probably $Ba^{2+}$ and $Sr^{2+}$ only).

## Materials and Methods

*Necturus* gallbladders were mounted horizontally in a modified Ussing chamber which permits positioning of microelectrodes (ME) in the mucosal bathing medium. Impalements were performed with motorized remote control micromanipulators (Stoelting, Chicago, Ill.) under microscopic observation. Transepithelial potential ($V_{ms}$) and cell membrane potentials (apical: $V_{mc}$, basolateral: $V_{cs}$) were measured with extracellular and intracellular electrodes as described before (Reuss & Finn, 1975*a, b*; Reuss, 1978; Reuss & Weinman, 1979). Cell membrane potentials were referred to the respective bathing medium; $V_{ms}$ was referred to the serosal solution. Intracellular ME were filled with 3 M KCl and had tip resistances of 25 to 50 MΩ. NaCl-Ringer's had the following composition (mM): NaCl, 109; KCl, 2.5; $NaHCO_3$, 2.4; $CaCl_2$, 1.0. This solution bathed the serosal side in all experiments. The mucosal side was exposed to bathing media of pH values ranging from 8 to 4, buffered with sodium phosphate (total concentration 3 mM). At each pH, mucosal solution ionic substitutions included replacement of all NaCl with KCl, to estimate the apical membrane relative K permeability, and replacement of all NaCl with LiCl or N-methyl-D-glucamine chloride, to estimate the apical membrane relative Na permeability. Divalent cations ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, and $Ba^{2+}$) were added to the mucosal bathing medium as the respective chloride salts, to final concentrations of 1, 5 or 10 mM. Control experiments with sucrose added to similar final osmolalities did not cause significant changes of the properties of the tissue. Since prolonged removal of $Ca^{2+}$ from the mucosal bathing medium frequently results in irreversible changes of the properties of the tissue (decrease of transepithelial potential and loss of transepithelial selectivity), the control medium always contained 1 mM $CaCl_2$.

All ionic substitutions were made while the ME was maintained in a cell, and were followed by return to the control bathing medium. Resistances were measured by techniques previously described in detail (Reuss & Finn, 1975*a*; Reuss & Grady, 1979*a*). The transepithelial resistance ($R_t$) was measured from the voltage deflection produced by a transepithelial square pulse of 25 to 50 μA. The ratio of membrane resistances ($R_a/R_b$) was measured from the ratio of the voltage deflections produced across the apical and basolateral membrane by the same pulse. Finally, to estimate absolute membrane resistances, intraepithelial cable analysis was performed (Frömter, 1972; Reuss & Finn, 1975*a*; Reuss & Grady, 1979*a*). After stable cell potentials had been obtained with two ME, current pulses of $2\text{–}5 \times 10^{-9}$ A were applied through one of them. The resulting voltage deflection ($\Delta V_x$) was recorded with the second ME, as a function of the distance ($x$). To circumvent time-dependent changes when comparing cable analysis results at different pH values, the mucosal solution was changed (from pH 8 to pH 6) while the electrodes were kept in their intracellular positions. $\Delta V_x$ was thus measured in the same cell at pH 8, at pH 6 (when the cell potential had reached a steady state) and at pH 8 again. At least six such measurements were performed in each of four tissues. To reduce the current density required for these measurements, up to 16 pulses were applied, and the records of $\Delta V_x$ were averaged (1074 signal averager, Nicolet Instrument Corp., Madison, Wis.).

Effects of divalent cations on $V_{ms}$, $V_{mc}$, $V_{cs}$, $R_t$ and $R_a/R_b$ were measured under control conditions (NaCl bathing medium on both sides), during addition of divalent cations to the mucosal medium only, and after removal of these agents. When the effect of one divalent cation was compared with control (1 mM $CaCl_2$), all measurements were made in the same cell. Comparisons of different divalent cations were obtained from studies in the same tissues. To assess the selective K permeability of the apical membrane, KCl was substituted isomolarly for NaCl in the mucosal bathing medium while keeping a microelectrode in the cell, as described for the pH experiments.

In most experiments, apical membrane, basolateral membrane and transepithelial potential ($V_{mc}$, $V_{cs}$, and $V_{ms}$, respectively) were digitized and stored in the signal averager, read digitally to 0.1 mV and plotted (7010 B X-Y recorder, Hewlett Packard, San Diego, Calif.).

Results are expressed as means ± SE. Statistical comparisons were done by conventional paired data analysis.

## Results

### *Effects of Luminal pH Changes on Cell Membrane Potentials*

Exposure of the mucosal surface of the gallbladder to low-pH media caused rapid and reversible depolarization of both cell membranes, with no significant changes of transepithelial potential. Fig. 1 shows a typical record of membrane potentials during a transient exposure to pH 6 of a tissue bathed in pH 8 Ringer's solution at the beginning and end of the trace. Lower pH of the mucosal medium sometimes resulted in spontaneous oscillations of cell membrane

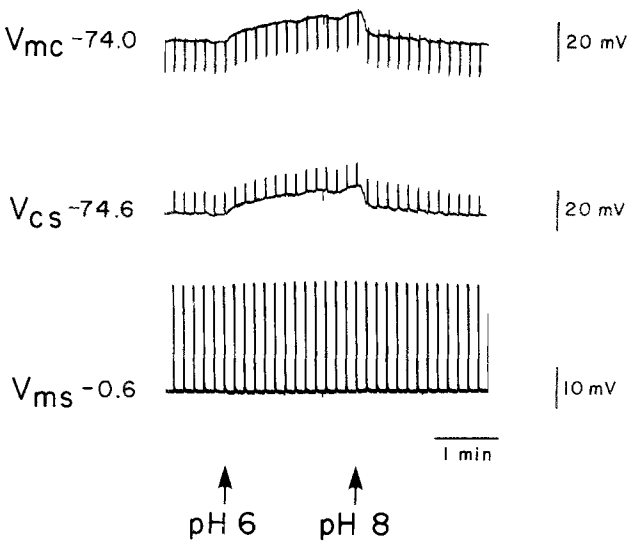


**Fig. 1.** Effects of low luminal medium pH on membrane potentials. Records start with a microelectrode in the cell, at pH 8. $V_{mc}$ = apical membrane potential (cell-lumen); $V_{cs}$ = basolateral membrane potential (cell-serosa); $V_{ms}$ = transepithelial potential (mucosa-serosa). Values indicated at the beginning of the traces are in mV. Voltage deflections were produced by transepithelial pulses of 50 μA; exposed area of tissue was 0.5 $cm^2$. Superfusion of the luminal surface with a pH 6 NaCl-Ringer's solution (first arrow) resulted in depolarization of both cell membranes, and slight increase of $R_t$ (shown by the deflections in the $V_{ms}$ trace). These changes were completely reversed by superfusion with a pH 8 medium (second arrow)

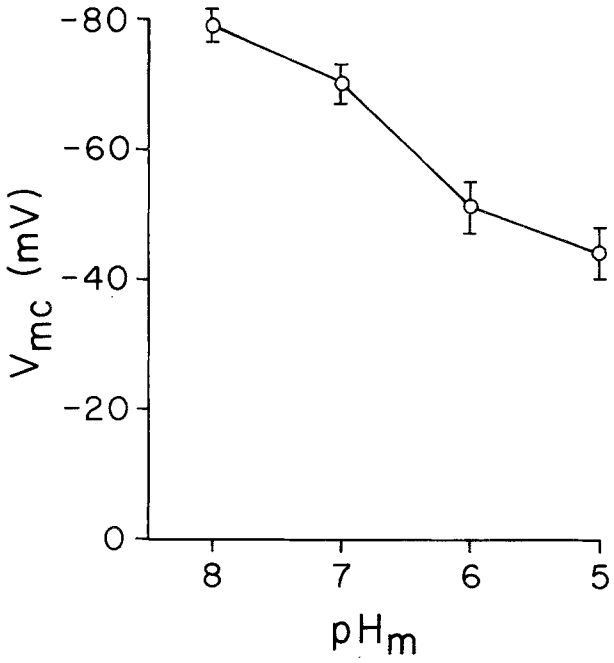


**Fig. 2.** Effect of luminal pH ($pH_m$) on apical membrane potential ($V_{mc}$). Means ± SEM of 12 experiments. The differences between pH 8 and pH 7, pH 7 and pH 6, and pH 6 and pH 5 were statistically significant ($p < 0.025$, $< 0.001$ and $< 0.025$, respectively)

potentials. This phenomenon was observed in 25% of the experiments at pH 5 and in 64% of the experiments at pH 4. Similar membrane potential oscillations have also been observed rarely by us in tissues incubated at pH 8 (*unpublished observations*). Frömter (1972) also described cyclic changes in cell membrane potential in two of 53 *Necturus* gallbladders studied at pH 7.4. The values communicated in this paper exclude the cases in which oscillations were observed.

Steady-state values of $V_{mc}$ as a function of mucosal medium pH are shown in Fig. 2. $V_{mc}$ falls when the mucosal solution is acidified, in the pH range of 8 to 5. In four tissues in which no membrane potential oscillation was seen, $V_{mc}$ values were $-40.1 \pm 6.9$ mV and $-54.8 \pm 8.0$ mV at pH 4 and 5, respectively. The data shown in Fig. 2 suggest a larger change in $V_{mc}$ in the range of pH values from 7 to 6. However, there was considerable variation of the relative magnitudes of the changes in the three intervals explored. $V_{mc}$ changes were $9.2 \pm 3.1$ mV in the range of pH 8 to 7, $18.3 \pm 3.6$ in the range of 7 to 6, and $6.9 \pm 2.5$ in the range of 6 to 5. Paired analysis revealed no significant difference between the changes of the first and second interval ($0.10 < p < 0.20$); the second and third interval differed significantly ($p < 0.025$). Although the conclusion of a highest sensitivity of $V_{mc}$ to mucosal pH in the pH range 7–6 is not certain from these data, similar results in K and Na-substitution experiments lend support to this notion (*see below*).

### *Effects of Divalent Cations on Membrane Potentials*

Addition of alkali-earth cations to the mucosal bathing medium resulted in depolarization of both cell membranes, with no consistent change of transepithelial potential. These effects were rapid and reversible. As shown in Fig. 3, the changes produced by $Mg^{2+}$, $Ca^{2+}$ and $Sr^{2+}$ (5 mM) did not differ from each other. The effect of $Ba^{2+}$ at this concentration was significantly larger than those of $Mg^{2+}$, $Ca^{2+}$ and $Sr^{2+}$. The magnitude of the depolarization produced by $Mg^{2+}$ and $Ca^{2+}$ at 10 mM was larger than the respective effects at 5 mM. Again there was no statistically significant difference between the effects of $Mg^{2+}$ and $Ca^{2+}$. However, the depolarization produced by $Sr^{2+}$ at 10 mM was significantly larger than that of $Mg^{2+}$ or $Ca^{2+}$, but smaller than that of $Ba^{2+}$.

$Ba^{2+}$ had a larger effect than the other alkali-earth cations at 1,5 and 10 mM. At 1 mM, $Ba^{2+}$ decreased $V_{mc}$ by $6.7 \pm 2.6$ mV ($n = 8$, $p < 0.05$), whereas $Mg^{2+}$, $Ca^{2+}$ and $Sr^{2+}$ had no significant effects. For the 1 mM $Ca^{2+}$ experiments, the control mucosal bathing

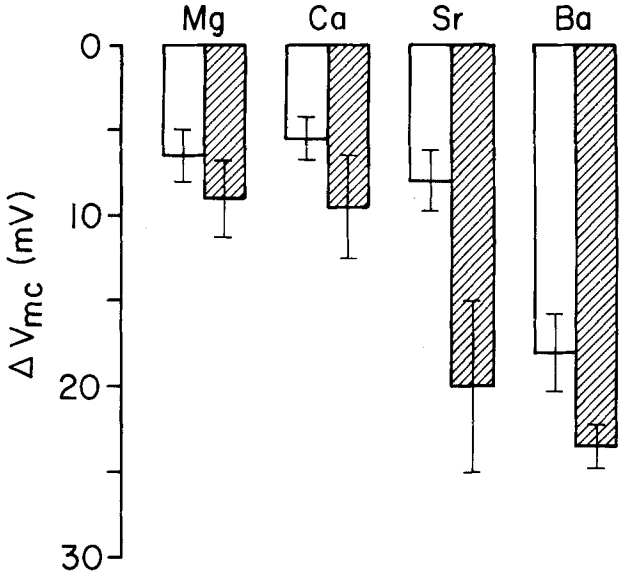


**Fig. 3.** Comparison of the magnitude of the apical membrane depolarization ($\Delta V_{mc}$) produced by alkali-earth cations in the mucosal solution at concentrations of 5 mM (open bars) and 10 mM (hatched bars). Number of experiments: at 5 mM, 8 (Mg), 9 (Ca), 8 (Sr), and 10 (Ba); at 10 mM, $n=4$ for each cation

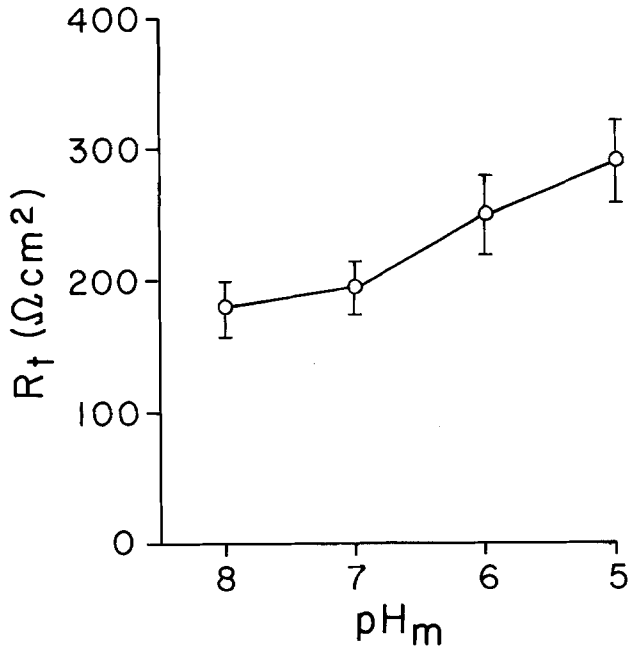


**Fig. 4.** Effect of luminal pH ($pH_m$) on transepithelial resistance ($R_t$). Means ± SEM of 12 experiments

solution was nominally Ca-free. The tissue was exposed to this medium briefly. $V_{mc}$ values were $-75.4 \pm 4.6$ and $-77.9 \pm 4.7$ mV in Ca-free and 1 mM $Ca^{2+}$, respectively.

*Effects of Luminal pH Changes on Resistances*

Acidification of the mucosal solution caused a progressive increase of the transepithelial resistance in

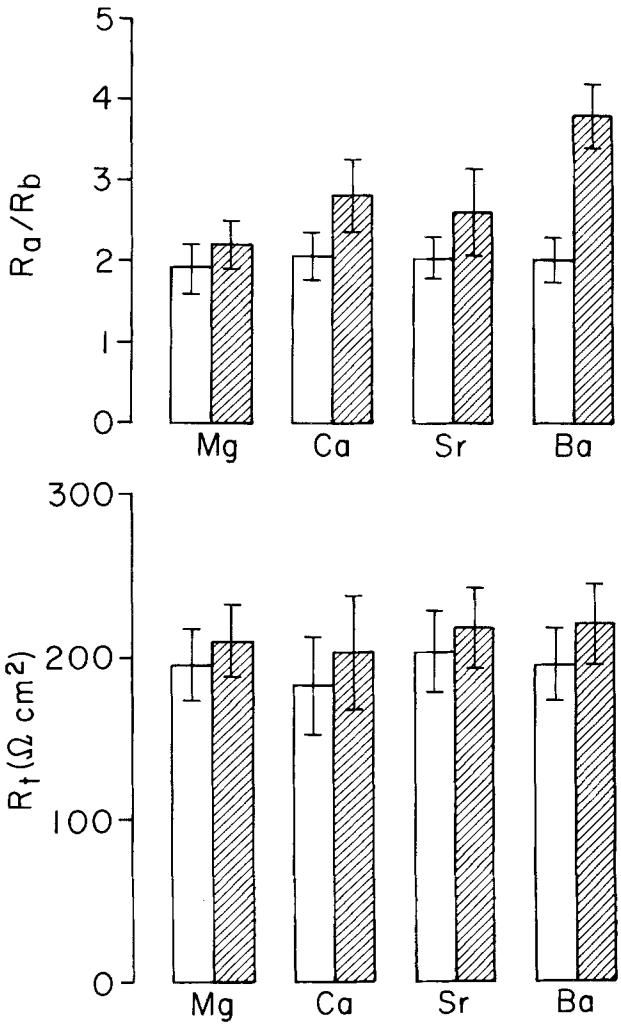


**Fig. 5.** Effects of alkali-earth cations on ratio of cell membrane resistances ($R_a/R_b$, upper graph) and transepithelial resistance ($R_t$, lower graph). Open bars indicate control values, and hatched bars test values during exposure of the apical surface of the tissue to a 5 mM concentration of the respective divalent cation. $R_a/R_b$ values were significantly different from control in $Ca^{2+}$ ($p<0.05$) and $Ba^{2+}$ ($p<0.005$). $R_t$ increased significantly in all cases ($p<0.025$ or better). Number of experiments as in Fig. 3 (5 mM series)

the range of pH from 8 to 5, as illustrated in Fig. 4. In the four experiments in which no membrane potential oscillations occurred at pH 4, $R_t$ was $370 \pm 65$ Ω cm$^2$. In the same tissues, $R_t$ was $290 \pm 30$ Ω cm$^2$ at pH 5. Since $R_t$ depends essentially on the properties of the limiting junctions (Frömter & Diamond, 1972; Frömter, 1972; Reuss & Finn, 1975a), these results suggest that low pH reduces limiting junction ionic permeability, and are consistent with the demonstration of a reduction of paracellular Na conductance at low pH in gallbladder of several species (Wright & Diamond, 1968; Moreno & Diamond, 1974).

The ratio of cell membrane resistances ($R_a/R_b$) did not change significantly in the pH range of 8 to 5. In individual tissues, increases or decreases of

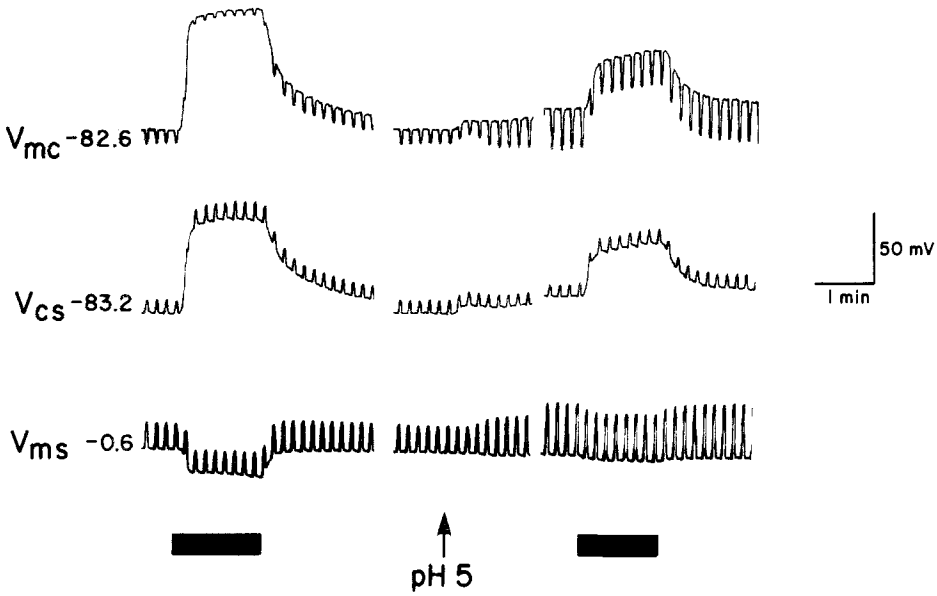


**Fig. 6.** Effect of K-for-Na mucosal substitution on potentials and resistances at pH 8 and 5. Symbols and format as in Fig. 1. Exposure to KCl-Ringer's indicated by the lower bars. In the middle panel, the start of superfusion with NaCl-Ringer's (pH 5) is indicated by the arrow. Note the depolarization of the cell membranes, the increase of transepithelial resistance and, in this case, ratio of membrane resistances ($R_a/R_b$) upon mucosal solution acidification. Exposure to K-Ringer's at pH 5 causes smaller changes of $V_{mc}$, $V_{cs}$, $V_{ms}$ and $R_a/R_b$

$R_a/R_b$ could be observed upon acidification of the mucosal medium in a reproducible fashion, but the direction of the change was unpredictable.

Cable analysis experiments were performed, as described above, in four tissues at pH values of 8 and 6. In every case, $\Delta V_x$ was larger at pH 6. The space constant for current spread increased from $263 \pm 80$ to $337 \pm 94$ μm. $R_z$, the resistance equivalent to $R_a$ and $R_b$ in parallel (Frömter, 1972; Reuss & Finn, 1975*a*) was $1460 \pm 240\ \Omega\,cm^2$ at pH 8 and $2690 \pm 480\ \Omega\,cm^2$ at pH 6. These results indicate that the pathway for current flow out of the cells increases its resistance at the lower pH value. Inasmuch as $R_a/R_b$ does not change, both $R_a$ and $R_b$ increase. The increase of $R_a$ can be readily attributed to an effect of extracellular $H^+$ activity on the membrane ionic conductance. The increase of $R_b$ may represent an increase in basolateral membrane resistance, or a reduction in width of the lateral intercellular spaces. $R_b$, in our equivalent circuit, is the equivalent resistance of the basal and lateral portions of the cell membrane; the lateral membranes are in series with the interspaces. A detailed analysis of this problem has been published by Boulpaep and Sackin (1980). At the present time, we cannot distinguish between these two possibilities, i.e., changes of basolateral membrane or lateral intercellular spaces resistance. However, it seems certain that the conductance of the luminal membrane decreases when the mucosal bathing medium is acidified.

*Effects of Divalent Cations on Resistances*

The effects of the four alkali-earth cations (5 mM) on transepithelial resistance ($R_t$) and ratio of cell membrane resistances ($R_a/R_b$), are shown in Fig. 5. $Ca^{2+}$ and $Ba^{2+}$ significantly increased the ratio of cell membrane resistances by 38 and 90%, respectively. No significant changes were observed with $Mg^{2+}$ and $Sr^{2+}$.

The transepithelial resistance changes produced by all four alkali earth cations were small (7 to 12% of the control values), but statistically significant. No significant differences were noted when one cation was compared to another.

*Effects of Mucosal K-for-Na Substitutions*

Fig. 6 illustrates the effect of acidification of the mucosal medium on the dependence of the apical membrane potential on external K concentration: At lower pH, the membrane potential changes produced by transient exposure to high-K medium were significantly reduced. This effect was reversible. Fig. 7 summarizes results from experiments in which the effects of high K could be compared at 1 pH unit intervals between 8 and 5. As shown for the baseline apical membrane potential, the slope of the plot of $\Delta V_{mc}$ (the change produced by brief exposure to high K) and pH appeared to be maximal in the range from pH 7 to pH 6. The changes observed at pH 6 were

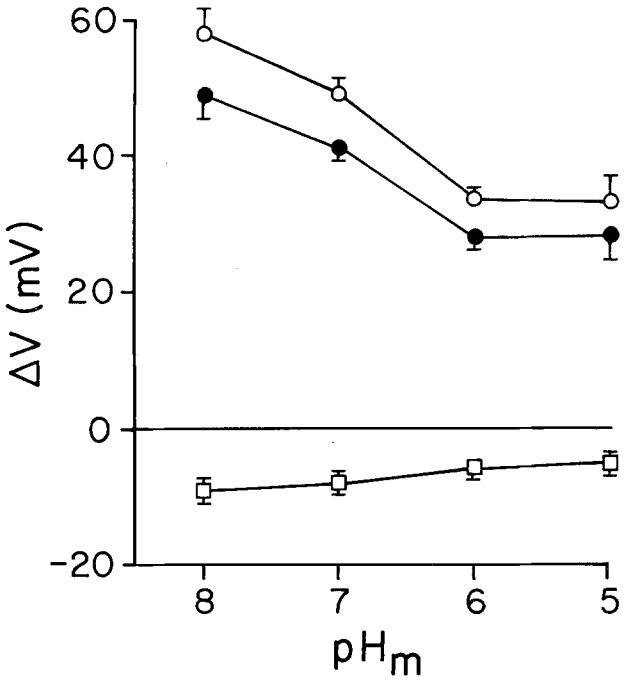


**Fig. 7.** Effects of mucosal solution pH ($pH_m$) on electrical potential changes produced by exposure to K-Ringer's on the mucosal side. Open circles: apical membrane potential changes ($\Delta V_{mc}$); closed circles: basolateral membrane potential changes ($\Delta V_{cs}$); squares: transepithelial potential changes ($\Delta V_{ms}$). Note that $\Delta V_{mc}$, $\Delta V_{cs}$, and $\Delta V_{ms}$ decrease as luminal pH is reduced. The changes appear to be larger in the interval from pH 7 to pH 6

not significantly different from those observed at pH 5. The changes of transepithelial potential produced by mucosal exposure to K-Ringer's were reduces slightly by lower pH. At least in part, this reduction is a consequence of the decrease of apical membrane relative K permeability (*see* Discussion). The basolateral membrane depolarization in these experiments is a consequence of intraepithelial current flow (because of the fall of apical membrane equivalent electromotive force) and follows rather closely the changes of $V_{mc}$.

Similarly to the effect of mucosal acidification, the change of $V_{mc}$ produced by exposure to high-K medium was significantly smaller in the presence of divalent cations, as illustrated in Fig. 8. Again, the effect of $Ba^{2+}$ was larger than those of $Mg^{2+}$, $Ca^{2+}$ and $Sr^{2+}$ (Fig. 9).

To evaluate further the effect of divalent cations on apical membrane $P_K$, $R_a/R_b$ was measured during exposure to K Ringer's in the presence an in the absence of divalent cations. Even though $R_a/R_b$ was on the mean greater in the presence of the divalent cation than in its absence, statistical significance was achieved only in the $Ba^{2+}$ and $Ca^{2+}$ series. This result is evidenced in the $V_{mc}$ and $V_{cs}$ traces of Fig. 8 by comparing the deflections caused by transepithelial current pulses.

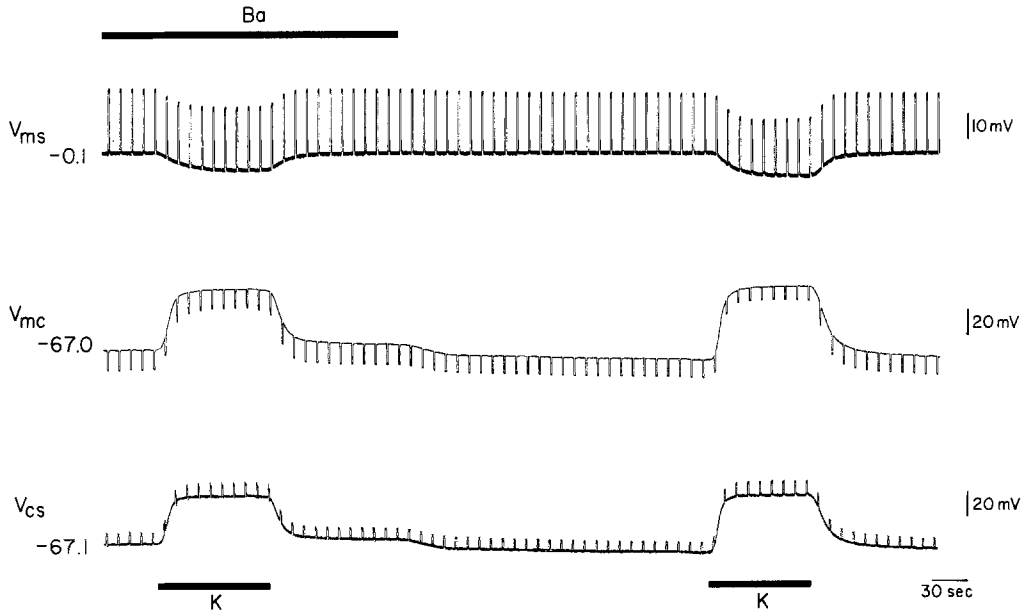


**Fig. 8.** Effect of $Ba^{2+}$ (5 mM) on membrane potentials and resistances. A microelectrode was in the cell throughout the traces. Top trace ($V_{ms}$): transepithelial potential; middle trace ($V_{mc}$): apical membrane potential; lower trace ($V_{cs}$): basolateral membrane potential. Values of potentials at the beginning of the records are shown on the left. Voltage deflections in all three traces were produced by transepithelial current pulses. The tissue was exposed to $BaCl_2$ from the beginning of the traces, as indicated by the top bar. During the periods indicated by the lower bars (labeled K) NaCl was substituted with KCl on the mucosal side. The following effects are illustrated: *a*) removal of Ba causes hyperpolarization of both cell membranes, with little change of $V_{ms}$; *b*) the changes of $V_{ms}$, $V_{mc}$ and $V_{cs}$ produced by high-K medium are smaller in the presence of $Ba^{2+}$ than in its absence; *c*) the voltage deflections in the $V_{mc}$ trace are larger in the presence than in the absence of Ba, during exposure to Na or K medium

*Effects of Na Substitutions with Less Permeant Cations*

Fig. 10 shows the effects on cell membrane and transepithelial potentials of complete substitutions of NaCl with N-methyl-D-glucamine chloride (NMDG-Cl) in the luminal side, at mucosal pH values of 8 and 5, respectively. At pH 8, the changes of cell membrane potentials are in opposite directions (the apical membrane hyperpolarizes, whereas the basolateral membrane depolarizes). This result indicates that the main mechanism of these membrane potential changes in a change of paracellular equivalent emf. Since at the junctions Na is more permeant than NMDG, the mucosal solution substitution causes a mucosa-positive Na/NMDG biionic potential (Reuss & Finn, 1975*a*, *b*). This conclusion is supported by the increase of transepithelial resistance produced by the substitution. A qualitative change of this pattern was observed at pH 5: the transepithelial potential change produced by NMDG was reduced, and both $V_{mc}$ and $V_{cs}$ increased (i.e., the cell became more negative to both bathing media). Results of similar experiments, at pH values ranging from 8 to 5, are shown in Fig. 11. The main result in this series is the progressive increase of the $V_{mc}$ change produced by exposure to NMDG, accompanied by a decrease, and then reversal, of the $V_{cs}$ change. The results at low pH cannot be explained on the basis of a paracellular diffusion potential alone: they indicate that at high mucosal proton activity the change of equivalent emf of the apical membrane produced by Na-NMDG substitutions was increased. We conclude, therefore, that the relative Na permeability of the membrane is increased when the mucosal pH is lowered.

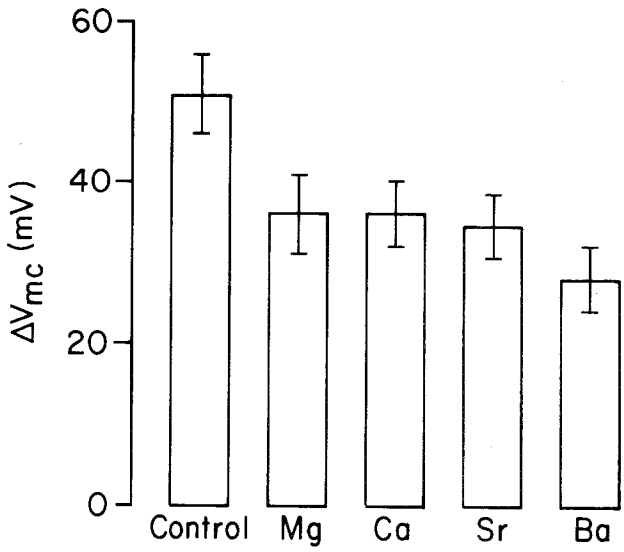


**Fig. 9.** Effects of alkali earth cations (5 mM, mucosal side) on the change of apical membrane potential produced by high-K mucosal medium ($\Delta V_{mc}$). All values were obtained in the same 5 tissues. $\Delta V_{mc}$ was significantly different from control ($p < 0.025$ or better) in all four series. Changes produced by $Mg^{2+}$, $Ca^{2+}$ and $Sr^{2+}$ were not significantly different from each other. $Ba^{2+}$ caused a significantly greater reduction of $\Delta V_{mc}$ than those produced by $Mg^{2+}$, $Ca^{2+}$ or $Sr^{2+}$ ($p < 0.05$ or better)

N-methyl-D-glucamine is a base (pK *ca.* 9.7). It is possible, therefore, that its effect on membrane potentials is not entirely caused by Na substitution, but also by penetration of the uncharged species into

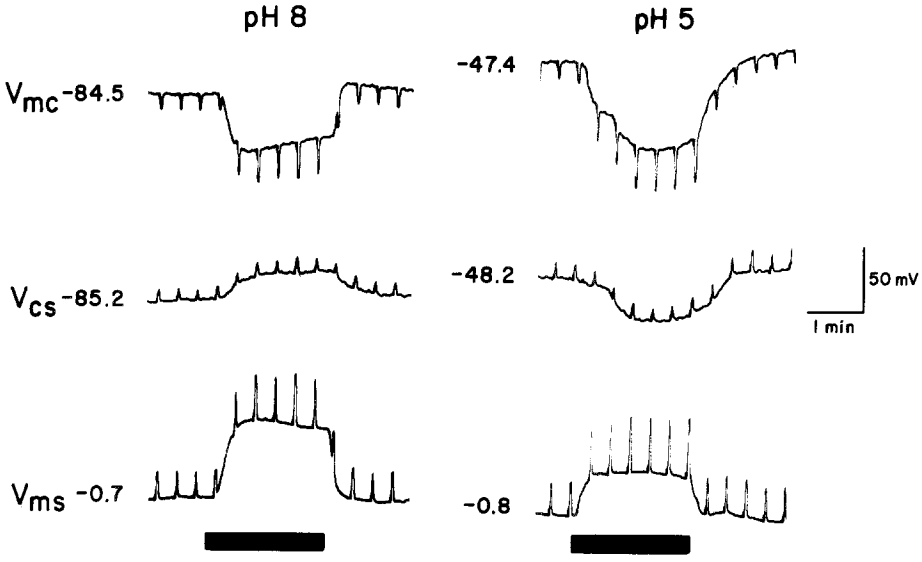


**Fig. 10.** Effect of NMDG-for-Na mucosal substitution on potentials and resistances at pH 8 and 5. Symbols and format as in Fig. 1. Records were obtained in the same cell. Note that at pH 5 the change of $V_{mc}$ during exposure to NMDG (lower bar) was larger and that the change of $V_{cs}$ was reversed in polarity, as compared to the effects of the same substitution at pH 8. In both conditions, during NMDG $V_{ms}$ changed in the mucosa-positive direction, $R_t$ increased and $R_a/R_b$ also increased

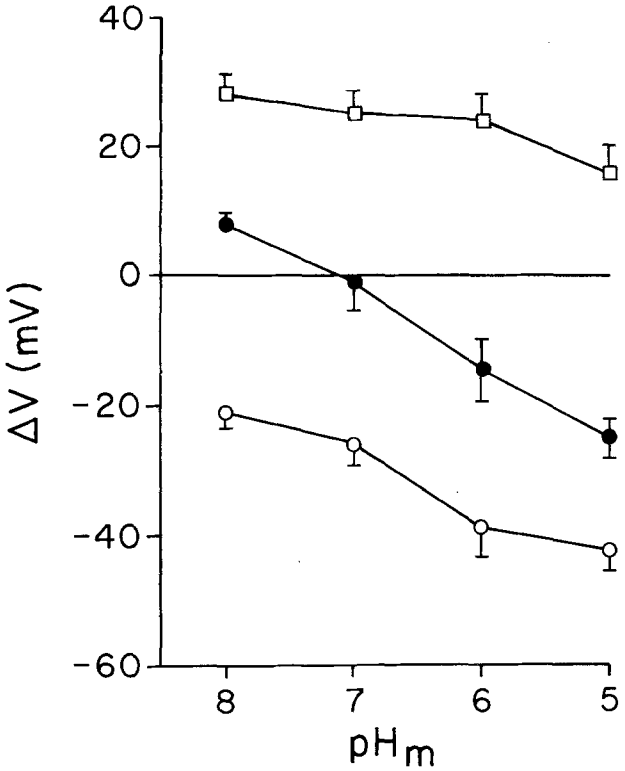


Fig. 11. Effect of mucosal solution pH ($pH_m$) on membrane potential changes ($\Delta V$) produced by mucosal substitution of Na with NMDG. $\Delta V_{mc}$: open circles; $\Delta V_{cs}$: closed circles; $\Delta V_{ms}$: squares

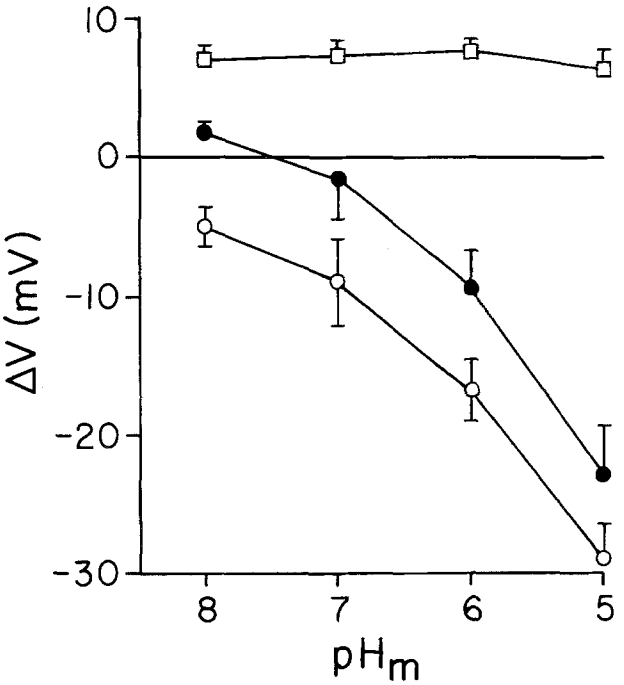


Fig. 12. Effect of mucosal solution pH ($pH_m$) on membrane potential changes. ($\Delta V$) produced by mucosal substitution of Na with Li. Symbols as in Fig. 11. Note difference in scale with Fig. 11

the cells and consequent change of intracellular pH. To examine this possibility, similar experiments were performed with LiCl replacing isomolarly NaCl. The results, shown in Fig. 12, were smaller, but qualitatively similar to those obtained with NMDG in that the $V_{mc}$ change increased progressively with the pH reduction, while the $V_{cs}$ change decreased and then reversed. The slopes in the different pH intervals, however, differed: in NMDG substitutions the maximum slope was seen in the interval of pH 7 to pH 6, whereas in Li substitutions the slope increased continually with decreasing pH.

### *Effect of Ionic Strength on K-Dependence of Apical Membrane Potential*

The pH effects on the apical membrane effects of $H^+$ and divalent cations described above could be the result of titration ($H^+$) or screening (divalent cations) of membrane fixed sites. The end result of both processes would be a surface potential change in the positive direction, resulting in changes in the cation and anion concentration profiles near the membrane surface: titration of the membrane makes the surface potential less negative (or more positive) at lower pH, cation concentrations decrease and anion concentrations increase. The concentrations at the interface itself are directly related to the permeability. Therefore, this process is expected to reduce cation permeability and increase anion permeability. The observed effect of luminal pH on cation permeabilities cannot be entirely explained by such a mechanism, however, since all permeability coefficients (for species of the same valence) should increase proportionally and the experimental observation is a decrease of relative $P_K$ and an increase of relative $P_{Na}$.

The possibility of a surface potential effect was studied further by measuring the changes of cell membrane potentials produced by increasing mucosal solution K activity from 2 to 8 mM under two experimental conditions (Szabo, Eisenman, McLaughlin & Krasne, 1972): low ionic strength medium (all NaCl replaced isoosmotically with sucrose) and high ionic strength medium (all NaCl replaced isomolarly with NMDG-sulfate, plus sucrose to make the solution isoosmotic). All four bathing media were buffered to pH 8.0, and K activities were measured with a K-selective electrode. The result of this experiment is shown in Fig. 13. At low ionic strength, the $V_{mc}$ change produced by elevation of extracellular K was increased, when compared with that observed at high ionic strength. This result is consistent with the hy-

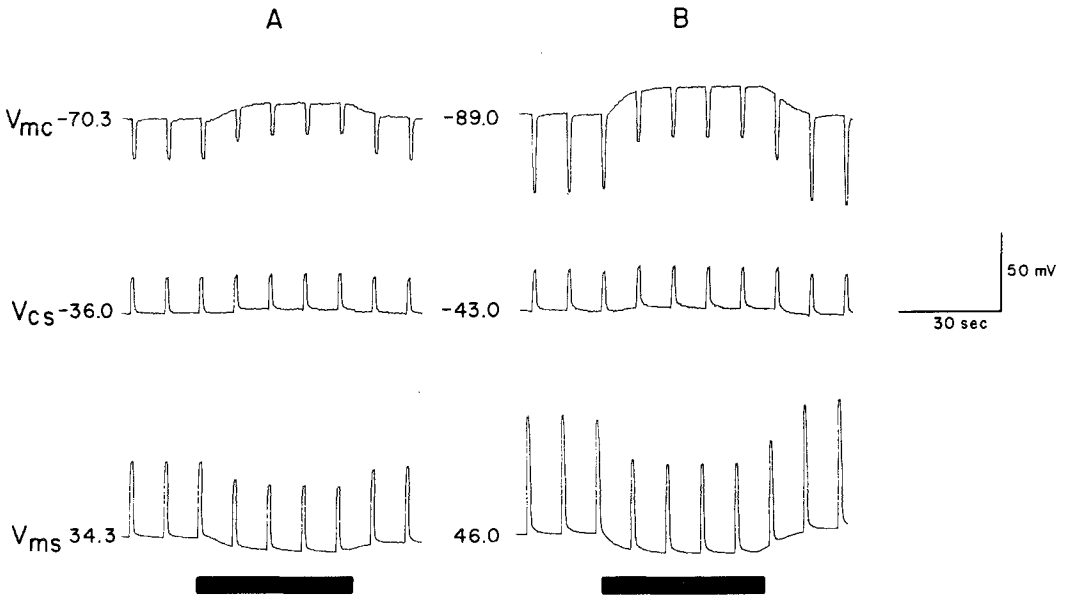


**Fig. 13.** Effect of an increase of luminal K activity from 2 to 8 mM (lower bars) during incubation at high ionic strength (*A*) and at low ionic strength (*B*). Both sets of records were obtained in the same cell

pothesis of a surface potential and suggests that the net surface charge is negative, since screening resulted in a decrease of apparent K permeability.

## Discussion

In *Necturus* gallbladder, acidification of the mucosal bathing solution causes depolarization of both cell membranes. These membrane potential changes are proportional to the degree of acidification in the pH range of 8 to 5, and are entirely reversible. The effects of low pH on potentials are accompanied by a decrease in transepithelial conductance and a decrease in the equivalent conductance of the pathways for current flow out of the cell. These results indicate that increased proton concentration in the mucosal bathing medium causes a decrease in overall ionic permeability of both the apical membrane and the limiting junctions. The concomitant cell membrane depolarization suggests that the main mechanism of the $H^+$ effect is a reduction of apical K permeability. Comparison of the apical membrane potential changes in the different pH intervals, and of the magnitudes of $V_{mc}$ changes produced by K-Na and NMDG-Na substitutions suggest maximum effects of pH in the range of 7 to 6. These data, however, cannot be directly employed to estimate the pK of the titratable sites in the membrane, because (*a*) the pH at the membrane surface itself is unknown, and (*b*) more than one chemical entity in the membrane could be responsible for the effects observed.

Alkali-earth cations ($Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, and $Ba^{2+}$) also cause cell membrane depolarization, which is accompanied by a reduction of apical membrane total conductance.

### *Effects of Luminal pH on K Permeability*

From the values of potentials and resistances at pH 8 and pH 6 (levels at which cable analysis experiments were performed) the equivalent electromotive forces of the two cell membranes (apical: $E_a$, basolateral: $E_b$) were calculated, assuming that the equivalent emf of the paracellular pathway ($E_s$) is zero, and independent of mucosal pH, when the tissue is exposed to NaCl-Ringer's on both sides. The equations necessary for this calculation have been published before (Reuss & Finn, 1975*a*). The results were: at pH 8, $E_a = -6.33$ mV, $E_b = -85.0$ mV; at pH 6, $E_a = -6.0$ mV, $E_b = -74.2$ mV. These values, although tentative because of the assumptions involved, are consistent with the notion that mucosal acidification reduces the relative K permeability of the apical membrane. Under control conditions the cell membrane potentials are closer to $E_K$ than to $E_{Cl}$ or $E_{Na}$ (Reuss & Weinman, 1979); whereas $E_K$ is *ca.* $-96$ mV, $E_{Cl}$ and $E_{Na}$ are about $-24$ and $+33$ mV, respectively. Therefore, a reduction of the K transference number at either membrane shifts the membrane potentials to values closer to $E_{Cl}$ and $E_{Na}$, i.e., causes cell depolarization. It is uncertain whether $E_b$, the equivalent emf of the basolateral membrane, is changed by acidification of the mucosal bathing medium. The data given above

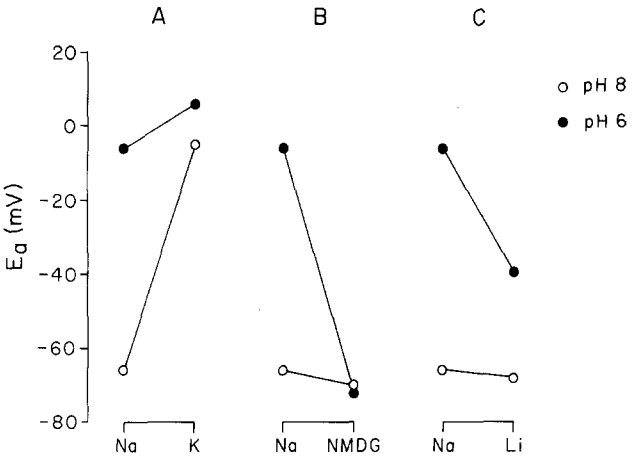


**Fig. 14.** Calculated values of apical membrane equivalent electromotive force ($E_a$) during exposure to Na-Ringer's and during Na replacements on the mucosal side at pH 8 and pH 6. Note that the change of $E_a$ produced by K-Ringer's is decreased at the lower pH, whereas the changes of $E_a$ produced by NMDG or Li are increased at the lower pH

show a decrease of 10.8 mV, but the uncertainties involved in the calculation do not allow us to ascribe significance to this result. In conclusion, our results indicate that acidification of the mucosal solution causes a decrease of apical membrane $t_K$. Since the total membrane conductance decreases as well, the inescapable conclusion is a reduction of $P_K$.

Confirmation of this interpretation was obtained in ionic substitution experiments. As shown before (Reuss & Finn, 1975*a*, *b*; Van Os & Slegers, 1975) exposure of the apical surface of the tissue to a high-K bathing medium caused large depolarization of both membranes and a mucosa-negative change of transepithelial potential. These results have been interpreted in detail with an equivalent electrical circuit consistent of three Thévenin equivalents, one for each cell membrane and one for the paracellular pathway (Reuss & Finn, 1975*a*, *b*). We have also shown that, when mucosal exposure to K-Ringer's is brief, intracellular K activity does not change appreciably (Reuss *et al.*, 1980). Thus, the relative permeabilities of the apical membrane and the paracellular pathway can be assessed in these experiments from potentials and resistances, assuming unchanged properties of the basolateral membrane. Within this assumption, $E_a$ was calculated at pH 8 and pH 6, during exposure to Na-Ringer's and K-Ringer's, as described before (Reuss & Finn, 1975*b*). The results are shown in Fig. 14 (*A*). The change of $E_a$ produced by transient exposure to K-Ringer's was 61.2 mV at pH 8 and only 11.8 mV at pH 6. At the latter pH, therefore, $t_K$ is largely reduced. Again, although the results of these calculations are uncertain because of the assumptions involved, they are in qualitative agreement with those obtained directly from the analysis of the $V_{mc}$ changes. Therefore, resistance changes not considered in the direct analysis of the membrane potentials do not alter qualitatively the conclusion advanced above. The calculation outlined yields also the change of $E_s$ produced by exposure to K-Ringer's. This was 4.8 mV at pH 8 and 4.5 mV at pH 6 (mucosa-negative in both cases), indicating that $P_K/P_{Na}$ (paracellular) is greater than 1 in both conditions and remains essentially unchanged. Thus, the pH dependence of the $V_{ms}$ changes produced by exposure to K-Ringer's (Fig. 7) depends mostly on the loss of apical membrane selectivity, and not on the paracellular effect of low pH. Moreno and Diamond (1974) observed a shift of the alkali metal transepithelial permeability ratios toward free solution values at low pH in gallbladders of several species. This result, obtained from transepithelial measurements alone, could be explained, at least for K-Na substitutions, by effects of proton activity on the apical membrane.

*Effects of Luminal pH on Na Permeability*

The membrane potential changes produced by mucosal Na substitutions with NMDG or Li show an increased relative Na permeability in response to acidification of the mucosal bathing medium, since this experimental perturbation resulted in increased hyperpolarization of the luminal membrane when the tissues were exposed to NMDG- or Li-Ringer's (Figs. 10, 11 and 12). These results are difficult to interpret quantitatively because of both membrane resistance changes and the low baseline $P_{Na}$ of the apical membrane (Reuss & Finn, 1975*b*; Van Os & Slegers, 1975). Most of the membrane potential changes during exposure to NMDG or Li on the mucosal side result from the paracellular biionic potential and not from $E_a$ changes. Low pH enhanced the $V_{mc}$ change produced by exposure to NMDG or Li concomitantly with a reversal of the change of $V_{cs}$. These observations are excellent indications of the progressively greater contribution of $E_a$ to the membrane potential changes as the pH of the luminal medium is lowered. As done above for K-Na substitutions, cell membrane emf values were calculated at pH 8 and 6 during exposure to Na-Ringer's, NMDG-Ringer's and Li-Ringer's, within the same assumptions (*see above*, and Reuss & Finn, 1975*b*). The results, shown in Fig. 14 (*B* and *C*), indicate that, at pH 8, $E_a$ increased by 3.6 mV during exposure to NMDG and by 1.4 mV during exposure to Li. At pH 6, the analogous changes were 66.1 mV (NMDG) and 33.4 mV (Li). Both sets of results indicate that at pH 6 the relative Na permeability of the apical

membrane is increased when compared with that at pH 8. In addition, these data suggest a small, but sizeable, apical membrane Li permeability, since NMDG-Na substitutions caused larger membrane potential and equivalent emf changes than Li-Na substitutions.

The increased relative Na permeability of the membrane could be explained in part by the reduction of $P_K$. If at low pH $P_{Na}$ remains constant or decreases less than $P_K$, then $P_{Na}/P_K$, $t_{Na}$, and the dependence of $E_a$ and $V_{mc}$ on mucosal Na concentration should increase, as observed. Interestingly, as the ratio $P_K/P_{Na}$ decreases (at low pH values), the ratio $P_{Na}/P_{Li}$ increases, but at all pH values explored the apparent permeabilities were $P_K > P_{Na} > P_{Li}$. The meaning of this sequence is uncertain at the present time, since we do not know whether all three ions permeate the membrane through the same pathway. It has been established that K transport at the apical membrane of frog, rabbit, and *Necturus* gallbladder is through a channel (Van Driessche & Gögelein, 1978; Gögelein, 1980). However, the specific mechanisms of conductive Na and Li translocation have not yet been characterized.

### *Effects of Alkali-Earth Cations on K Permeability*

The effects of $Ba^{2+}$ on K permeability have been studied in a number of excitable tissues (for references, *see* Eaton & Brodwick, 1980), and some epithelia (Pacífico, Schwartz, MacKrell, Spangler, Sanders & Rehm, 1969; Ramsay, Gallagher, Shoemaker & Sachs, 1976; Nagel, 1979). The studies of Nagel (1979) in isolated frog skin have demonstrated that $Ba^{2+}$ reduces basolateral membrane K permeability, similarly to the effects reported in excitable tissues (Eaton & Brodwick, 1980). Nagel calculated that the total conductance of the basolateral membrane was about 30% of control during exposure to 0.5 mM $Ba^{2+}$, an effect significantly larger than the one reported here.

Our conclusion that $Ba^{2+}$ blocks K permeability at the luminal membrane is based on the observations of *a*) membrane depolarization, *b*) increase in apical membrane resistance (relative to that of the basolateral membrane), and *c*) decrease of the apical membrane potential and resistance changes induced by luminal K for Na substitution.

The magnitude of the membrane potential changes produced by $Ba^{2+}$ and other divalent cations in *Necturus* gallbladder epithelium was rather small when compared with other tissues, such as frog skin (*see below*). However, it should be noted that the apical membrane potential in leaky epithelia such as the gallbladder will tend to be maintained, even if the relative K permeability of the membrane falls, by intraepithelial current flow. If the equivalent electromotive force of the paracellular pathway is assumed to be zero, $V_{mc}$ can be described by the following equation:

$$V_{mc} = \frac{E_a(R_b + R_s) + E_b R_a}{R_a + R_b + R_s} \tag{1}$$

where $E$'s are equivalent electromotive forces, $R$'s are resistances, and the subscripts denote apical membrane (*a*), basolateral membrane (*b*), and paracellular pathway (*s*) (*see* Reuss & Finn, 1975*a*). If $Ba^{2+}$ causes a decrease in $E_a$ and an increase in $R_a$ proportionally larger than the increase of the denominator, the voltage drop produced by $E_b$ across $R_a$ would be in fact increased. This is shown by the comparison of the measured changes of $V_{mc}$ and the calculated changes of $E_a$ at different $Ba^{2+}$ concentrations. The calculations of $E_a$ were carried out under the following assumptions: *a*) $R_b = 2{,}000\ \Omega\ cm^2$ (Frömter, 1972; Reuss & Finn, 1975*a*; Reuss, Bello-Reuss & Grady, 1979); *b*) $E_s = 0$ (*see above*, and Reuss & Finn, 1975a); *c*) $R_b$ and $E_b$ are not changed by the addition of $Ba^{2+}$ to the mucosal medium. $R_a$, $R_s$, $E_a$ and $E_b$ were calculated as previously described (Reuss & Finn, 1975*a*, *b*), from the values of potentials, $R_t$ and $R_a/R_b$. The results ($E_a$, mV) were $-64.9$ (control), $-56.0$ (1 mM $Ba^{2+}$), $-37.8$ (5 mM $Ba^{2+}$), and $-23.9$ (10 mM $Ba^{2+}$). At all concentrations of $Ba^{2+}$ tested, the decrease of $E_a$ was larger than the decrease of $V_{mc}$. A similar result (*not shown*) was obtained by comparing the $V_{mc}$ and $E_a$ changes produced by high mucosal K: in the presence of $Ba^{2+}$, the K-dependent reduction of $E_a$ was larger than the reduction of $V_{mc}$. Quantitation of blocking effects of ion permeabilities such as the one produced by $Ba^{2+}$ require calculation of $E_a$. This determination is technically difficult, because measurements of resistances require intraepithelial cable analysis (Frömter, 1972; Reuss & Finn, 1975*a*). Furthermore, the value of $E_s$ is uncertain when the tissue is exposed to asymmetric bathing media (Reuss & Grady, 1979*a*). For these reasons, a qualitative approach has been preferred in this discussion. It is clear, however, that changes of $V_{mc}$, although smaller, reflect the effects of $Ba^{2+}$, and the other divalent cations tested, on $E_a$.

The effects of $Ba^{2+}$, observable at 1 mM, can be contrasted with those in tight epithelia, in which the apical membrane is mainly Na-permeable. In toad urinary bladder (Ramsay *et al.*, 1976), and frog skin (Nagel, 1979) $Ba^{2+}$ had no effect on the electrical properties of the tissue from the apical border at concentrations up to 1 mM.

The effects of $Ba^{2+}$ on membrane potentials and relative apical $P_K$ are dose-dependent in the range

of 1 to 10 mM. These concentrations are much higher than effective doses in excitable tissues or basolateral membrane of frog skin (Nagel, 1979). Comparison or our results with those of Nagel (1979) suggests different properties of the respective K transport sites. This conclusion is supported by experiments in which $Ba^{2+}$ was added to the serosal side of *Necturus* gallbladder. In these studies (Reuss and Grady, *unpublished*) $Ba^{2+}$ was effective at concentrations of 0.1 mM.

### *Mechanism of the Effect of Luminal pH on Apical Membrane Cation Permeability*

The effects described above could result from several possible mechanisms of action of the increased $H^+$ activity in the luminal solution. First, changes in extracellular pH ($pH_e$) could cause intracellular pH ($pH_i$) changes and these in turn could be responsible for the effects observed. This possibility can be ruled out, because in experiments in which the apical surface is exposed, at constant $pH_e$, to permeant acids (e.g., propionic acid) the effects on membrane potential and $t_K$ are opposite to the ones described above. These results will be described in detail separately. We can conclude, therefore, that the effects observed are genuinely produced by $pH_e$ changes.

A second possibility to consider is that the reduction of K permeability at low pH is caused by titration of fixed negative charges in the membrane, and therefore by a surface potential change. This question is really twofold: (*a*) is the membrane negatively charged? and (*b*) can the pH effect on cation permeabilities be explained by titration of such charges? A tentative answer to the first question was obtained from the experiment shown in Fig. 13. A reduction of ionic strength of the bathing medium produced an increase in the depolarization caused by a step change of mucosal K concentration. To circumvent the possibility of "shunting" effects by other ions, N-methyl-D-glucamine sulfate was the main solute of the high ionic strength solution. (The main solute of the low ionic strength solution was sucrose.) Definitive interpretation of this experiment will require additional studies, since we have not ruled out the possibility of effects of NMDG or sulfate mediated by other mechanisms than the change of ionic strength. Within these limitations, the result supports the notion of a negatively charged membrane. The high ionic strength solution is expected to "screen" the charges and thus to reduce the surface potential, and apparent monovalent cation permeability (Szabo *et al.*, 1972). The pH effects observed cannot be entirely attributed to a nonspecific effect on membrane charge. Such an effect would not be expected to change the apparent permeability ratios of ions of the same valence. Therefore, our results suggest that $H^+$-dependent K permeability changes are mediated, at least in part, by an effect of $H^+$ activity on the transport site (channel) itself.

Gögelein (1980) has observed that mucosal acidification reduces apical membrane K current fluctuations in *Necturus* gallbladder, even though the effect was evident only at pH 5 or lower. These experiments are not entirely comparable to ours, because the pH was reduced in both bathing media.

### *Mechanisms of Effects of Divalent Cations on Apical Membrane $P_K$*

The observation that at a 5 mM concentration, $Mg^{2+}$, $Ca^{2+}$, and $Sr^{2+}$ depolarized the apical membrane and reduced its relative $P_K$ similarly suggests a common mechanism of action of divalent cations on $P_K$, independent of ionic size. In principle, a reduction of $P_K$ in these experiments could result from: *a*) a change of surface potential of the membrane by a "screening" mechanism; *b*) a change of surface potential by binding of the divalent cations to fixed "nonspecific" negative sites in the membrane, and *c*) an interaction with the K channel. Only the first of these mechanisms is independent of ionic size.

The similarity of the effects of $Mg^{2+}$, and $Ca^{2+}$ at 5 and 10 mM suggests a nonspecific effect, i.e., a mechanism of action consistent with screening of surface negative charges. As stated above, the effects of mucosal solution pH changes and the effects of high and low ionic strength solutions on apparent K permeability support the notion that the apical membrane of *Necturus* gallbladder has a net negative surface charge. One would therefore expect a screening effect of divalent cations at the concentrations employed in this study (D'Arrigo, 1978). The behavior of $Ba^{2+}$ and $Sr^{2+}$ was different from those of $Mg^{2+}$, and $Ca^{2+}$. $Ba^{2+}$ had a greater effect on $P_K$ than all other ions at all concentrations tested. $Sr^{2+}$, although not exhibiting different effects at 5 mM, was more effective than $Mg^{2+}$, and $Ca^{2+}$, and less effective than $Ba^{2+}$, at 10 mM. These results suggest that $Ba^{2+}$ and $Sr^{2+}$ have effects other than screening of surface changes. Such effects could result from binding of these ions, to nonspecific sites in the membrane (in which case the surface potential would decrease by both screening and binding), or to the K channel itself. We cannot distinguish between these two possibilities with the available data. The ionic radius of $Ba^{2+}$ (1.34 Å) is practically the same as the one of $K^+$ (1.33 Å), and therefore $Ba^{2+}$ penetration in the channel would be expected. Our data support but do not prove this mechanism. The possibility of binding of $Ba^{2+}$ to nonspecific membrane sites cannot be excluded, and is in fact, consistent with the rather

low effectiveness of this cation. $Sr^{2+}$, at 10 mM, was more effective than either $Mg^{2+}$ or $Ca^{2+}$. This observation suggests that the effect of $Sr^{2+}$ at this concentration may include a binding mechanism in conjunction with screening of membrane surface charges.

### *Paracellular Effects of Divalent Cations and Luminal Medium pH*

The paracellular effects of the divalent cations tested did not differ significantly from one another. At 5 mM, the transepithelial resistance increased by 7 to 12%. At 10 mM, the effects were on the mean slightly larger, but not statistically different from those at 5 mM. Since the transepithelial conductance of this tissue is dominated by the paracellular conductance (Frömter, 1972; Reuss & Finn, 1975*a*) it is likely that most of the increase of $R_t$ is caused by an increase in paracellular and not transcellular resistance. A reduction of junctional complex cation permeability is the most likely explanation of this result. Our results indicate also that acidification of the mucosal bathing solution results in an increase of $R_t$. In addition, both low pH and high $Ca^{2+}$ decrease the transepithelial dilution potential in mammalian gallbladder (Wright & Diamond, 1968). The effect of low pH is caused by a decrease of Na conductance in gallbladders of several species (Moreno & Diamond, 1974).

### *Physiological Significance of the Results*

The gallbladder *in vivo* is not exposed to bulk luminal fluid of acid pH. However, the proton activity in the outer face of the apical membrane itself is unknown and could be significantly lower than in the bulk solution, because of the fixed negative charge of the membrane postulated above, and because of the possibilities of $H^+$ and/or $HCO_3^-$ transport through this membrane. Although not demonstrated to our knowledge in gallbladder, a $Na^+$-$H^+$ antiport seems to be a frequent feature in apical membranes of leaky epithelia. Furthermore, the gallbladder transports bicarbonate from mucosa to serosa. Both *in situ* and *in vitro* there are significant unstirred layers on both epithelial surfaces, and conceivably $HCO_3^-$ removal (by direct transport or $H^+$ secretion) could result in a local pH reduction, and in a decrease of apical membrane K permeability. This mechanism would tend to reduce the (downhill) K flux from cell to mucosal bathing medium.

It is tempting to speculate that a similar mechanism might be involved in the relationship between $H^+$ and $K^+$ secretion by the distal and collecting segments of the renal tubule. Although it is clear that these relations involve a host of phenomena (Giebisch, 1979), some observations are consistent with our results. Boudry, Stoner and Burg (1976) observed that acidification of the luminal fluid in rabbit cortical collecting tubules perfused *in vitro* caused a reduction of K secretion. In addition, Khuri (1979) has observed that acidification of the luminal fluid in *Necturus* proximal tubule causes cell depolarization. Other observations, reviewed in detail by Giebisch (1979) indicate that luminal pH and K secretion can be dissociated experimentally. Our results suggest that if luminal pH changes are one of the controlling factors of $K^+$ secretion, the mechanism of action of luminal pH can be an effect on apical membrane K permeability.

Our results indicate that divalent cations reduce $P_K$ at the apical membrane of *Necturus* gallbladder by two mechanisms: screening of surface negative charges (an effect similar to the one produced by acidification of the mucosal solution) and binding to nonspecific or specific (K channel) sites in the membrane. The latter mechanism seems appreciable only for $Ba^{2+}$ and $Sr^{2+}$, but we cannot rule out the possibility of binding of the other divalent cations. The concentration dependence of the effects of $Ba^{2+}$ on the apical membrane seems to reflect a lower affinity than the one observed at the basolateral membranes of this tissue (Reuss & Grady, unpublished) and tight epithelia (Pacifico *et al.*, 1969; Nagel, 1979). From this comparison we conclude that the molecular structures of apical and basolateral K channels in gallbladder are different. Finally, the moderate reduction of apical $P_K$ produced by increasing Ca concentration in the mucosal bathing medium can be contrasted with the effect of experimental perturbations presumed to raise intracellular $Ca^{2+}$ activity. Exposure to CN and treatment of the tissue with the Ca ionophore A23187 produce changes of cell membrane potential, cell membrane resistance and voltage responses to K for Na substitutions consistent with an increase of $P_K$ at both cell borders (Bello-Reuss, Grady & Reuss, 1980). Thus, high Ca, although in very different concentration ranges, increases $P_K$ from the inside of the membrane, but decreases $P_K$ from the outside. Preliminary experiments in which intracellular pH changes were induced at constant extracellular pH yielded similar results: low pH blocks $P_K$ from the outside and increases $P_K$ from the inside. No detailed explanation of these observations is available yet. Further investigation of the mechanisms involved in these effects will certainly enhance our understanding of the structure and mode of operation of the K channel in this and other epithelia.

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## References


Bello-Reuss, E., Grady, T.P., Reuss, L. 1980. Mechanism of the effect of cyanide on cell membrane potentials in *Necturus* gallbladder epithelium. *J. Physiol. (London) (in press)*

Boudry, J.F., Stoner, L.C., Burg, M.B. 1976. Effect of acid lumen pH on potassium transport in renal cortical collecting tubules. *Am. J. Physiol.* **230**:239

Boulpaep, E.L., Sackin, H. 1980. Electrical analysis of intraepithelial barriers. *In*: Current Topics in Membranes and Transport. Volume 13: Cellular mechanisms of renal tubular ion transport. F. Bronner, A. Kleinzeller and E.L. Boulpaep, editors. Chapter 12. Academic Press, New York

Cremaschi, D., Hénin, S. 1975. $Na^+$ and $Cl^-$ transepithelial routes in rabbit gallbladder. Tracer analysis of the transports. *Pfluegers Arch.* **361**:33

D'Arrigo, J. 1978. Screening of membrane surface charges by divalent cations: An atomic representation. *Am. J. Physiol.* **235**:C109

Duffey, M.E., Turnheim, K., Frizzell, R.A., Schultz, S.G. 1978. Intracellular chloride activities in rabbit gallbladder: Direct evidence for the role of the sodium-gradient in energizing "uphill" chloride transport. *J. Membrane Biol.* **42**:229

Eaton, D.C., Brodwick, M.S. 1980. Effects of barium on the potassium conductance of squid axon. *J. Gen. Physiol.* **75**:727

Frizzell, R.A., Dugas, M.C., Schultz, S.G. 1975. Sodium chloride transport by rabbit gallbladder. Direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* **65**:769

Frömter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**:259

Frömter, E., Diamond, J.M. 1972. Route of passive ion permeation in epithelia. *Nature, New Biol.* **235**:9

García-Díaz, J.F., Armstrong, W.McD. 1980. Intracellular Na activity and Na transport in *Necturus* gallbladder. *Fed. Proc.* **39**:4275a

Giebisch, G. 1979. Renal potassium transport. *In*: Membrane Transport in Biology. Volume IVA. Transport Organs. Chapter 5. G. Giebisch, D.C. Tosteson and H.H. Ussing, editors. Springer-Verlag, Berlin-Heidelberg-New York

Gögelein, H. 1980. Fluctuation analysis and impedance measurements in the gallbladder epithelium. M.D. Thesis, Catholic University of Leuven (Louvain), Louvain, Belgium

Graf, J., Giebisch, G. 1979. Intracellular sodium activity and sodium transport in *Necturus* gallbladder epithelium. *J. Membrane Biol.* **47**:327

Gunther-Smith, P.J., Duffey, M.E., Schultz, S.G. 1980. Intracellular potassium activities in rabbit gallbladder. *Fed. Proc.* **39**:4274a

Hénin, S., Cremaschi, D. 1975. Transcellular ion route in rabbit gallbladder. Electric properties of the epithelial cells. *Pfluegers Arch.* **355**:125

Khuri, R.N. 1979. Electrochemistry of the Nephron. *In*: Membrane Transport in Biology. Volume IVA. Transport Organs. Chapter 2. G. Giebisch, editor. Springer-Verlag, Berlin-Heidelberg-New York

Moreno, J.H., Diamond, J.M. 1974. Discrimination of monovalent inorganic cations by "tight" junctions of gallbladder epithelium. *J. Membrane Biol.* **15**:277

Nagel, W. 1979. Inhibition of potassium conductance by barium in frog skin epithelium. *Biochim. Biophys. Acta* **552**:346

Pacífico, A.D., Schwartz, M., MacKrell, T.N., Spangler, S.G., Sanders, S.S., Rehm, W.S. 1969. Reversal by potassium of an effect of barium on the frog gastric mucosa. *Am. J. Physiol.* **216**:536

Ramsay, A.G., Gallagher, D.L., Shoemaker, R.L., Sachs, G. 1976. Barium inhibition of sodium ion transport in toad bladder. *Biochim. Biophys. Acta* **436**:617

Reuss, L. 1978. Effects of amphotericin B on the electrical properties of *Necturus* gallbladder: Intracellular microelectrode studies. *J. Membrane Biol.* **41**:65

Reuss, L., Bello-Reuss, E., Grady, T.P. 1979. Effects of ouabain on fluid transport and electrical properties of *Necturus* gallbladder. Evidence in favor of a neutral basolateral sodium transport mechanism. *J. Gen. Physiol.* **73**:385

Reuss, L., Finn, A.L. 1975*a*. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. I. Circuit analysis and steady-state effects of mucosal solution ionic substitutions. *J. Membrane Biol.* **25**:115

Reuss, L., Finn, A.L. 1975*b*. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. II. Ionic permeability of the apical cell membrane. *J. Membrane Biol.* **25**:141

Reuss, L., Grady, T.P. 1979*a*. Triaminopyrimidinium ($TAP^+$) blocks luminal membrane K conductance in *Necturus* gallbladder epithelium. *J. Membrane Biol.* **48**:285

Reuss, L., Grady, T.P. 1979*b*. Effect of external sodium and cell membrane potential on intracellular chloride activity in gallbladder epithelium. *J. Membrane Biol.* **51**:15

Reuss, L., Weinman, S.A. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membrane Biol.* **49**:345

Reuss, L., Weinmann, S.A., Grady, T.P. 1980. Intracellular $K^+$ activity and its relation to basolateral membrane ion transport in *Necturus* gallbladder epithelium. *J. Gen. Physiol.* **76**:33

Szabo, G., Eisenman, G., McLaughlin, S.G.A., Krasne, S. 1972. Ionic probes of membrane structure. *Ann. N.Y. Acad. Sci.* **195**:273

Van Driessche, W., Gögelein, H. 1978. Potassium channels in the apical membrane of the toad gallbladder. *Nature (London)* **275**:665

Van Os, C.H., Slegers, J.F.G. 1975. The electrical potential profile of gallbladder epithelium. *J. Membrane Biol.* **24**:341

Wright, E.M., Diamond, J.M. 1968. Effects of pH and polyvalent cations on the selective permeability of gallbladder epithelium to monovalent ions. *Biochim. Biophys. Acta* **163**:57


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