Cyclic AMP-induced Chloride Permeability in the Apical Membrane of *Necturus* Gallbladder Epithelium

KARL-UWE PETERSEN and LUIS REUSS

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

ABSTRACT The effects of theophylline, 8-Br-cAMP, and cAMP on *Necturus* gallbladder epithelium were investigated using microelectrode techniques. Each of these substances depolarized the cell membranes by ~15 mV and decreased the apparent ratio of apical to basolateral membrane resistances to a value not significantly different from zero. Examination of the ionic selectivity of the apical membrane by ion substitutions in the mucosal bathing medium revealed a large increase in Cl permeability with no apparent changes in K and Na permeabilities. Intracellular Cl activity ($a\mathrm{Cl_i}$) was measured using Cl-sensitive liquid ion-exchanger microelectrodes. Under control conditions, $a\mathrm{Cl_i}$ was ~20 mM, 2.5 times higher than the value expected for equilibrium distribution ($a\mathrm{Cl_i^{eq}}$). After addition of 8-Br-cAMP, $a\mathrm{Cl_i}$ decreased within <60 s to ~13 mM, a value not significantly different from $a\mathrm{Cl_i^{eq}}$. Virtually identical results were obtained with theophylline. Under control conditions, luminal Cl removal caused $a\mathrm{Cl_i}$ to fall at an initial rate of 1.8 mM/min, whereas in tissues exposed to 8-Br-cAMP or theophylline a rate of 11.6 mM/min was observed. The apical membrane Cl transference number was estimated from the change of $a\mathrm{Cl_i}$ upon exposure to 8-Br-cAMP as well as from the changes in apical membrane potential during variation of the luminal Cl concentration. The results, 0.91 and 0.88, respectively, are indicative of a high Cl permeability of the apical membrane during cAMP. This effect may explain, at least in part, the complete inhibition of fluid absorption produced by theophylline in this tissue. Moreover, enhancement of apical membrane Cl permeability may account for a variety of cAMP effects in epithelial tissues.

INTRODUCTION

One of the most intriguing features of NaCl absorption by mammalian and amphibian gallbladders is the mutual dependence of Na and Cl entry across the apical membrane (Cremaschi and Hénin, 1975; Frizzell et al., 1975; Reuss

Address reprint requests to Dr. Luis Reuss, Dept. of Physiology and Biophysics, Washington University School of Medicine, 660 South Euclid Ave., St. Louis, MO 63110. Dr. Petersen's current address is Abteilung Pharmakologie der RWTH Aachen, Schneebergweg, D-5100 Aachen, Federal Republic of Germany.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/83/05/0705/25 $1.00 705
Volume 81 May 1983 705–729

and Grady, 1979; Garcia-Diaz and Armstrong, 1980; Rose and Nahrwold, 1980; Ericson and Spring, 1982). This entry step is believed to be the target of cyclic adenosine-3′,5′-monophosphate (cAMP) in its inhibition of NaCl absorption (Frizzell et al., 1975; Diez de los Rios et al., 1981; Petersen et al., 1982). Whereas NaCl absorption is reduced by ~50% in rabbit gallbladder by cAMP (Frizzell et al., 1975), in guinea pig gallbladder, secretion of Na, K, and HCO_3 is observed (Heintze et al., 1979). To explain HCO_3 secretion in this epithelium, a cAMP-induced increase in apical membrane HCO_3 conductance has been proposed (Stewart et al., 1982). So far, both interpretations of the cAMP effects, i.e., inhibition of co-transport and increase of apical membrane conductance, have remained hypothetical. The present study investigates the mechanism of the cAMP effects using conventional and ion-sensitive microelectrode techniques in *Necturus* gallbladder. In this epithelium, cAMP treatment has been reported to reduce intracellular Na and Cl activities (Diez de los Rios et al., 1981) and to induce a sizeable short-circuit current (Duffey et al., 1981). These observations are consistent with inhibition of NaCl entry and anion secretion. Our results suggest that the dual action of cAMP, i.e., transport inhibition and induction of secretion, may be at least in part secondary to the induction of a dominant Cl conductance in the apical cell membrane.

MATERIALS AND METHODS

Preparations and Solutions

Mudpuppies (*Necturus maculosus*) of both sexes were purchased from Nasco Biological Supply (Fort Atkinson, WI). Animals were anesthetized with 0.1% tricaine-methanesulfonate (Sigma Chemical Co., St. Louis, MO). Gallbladders were quickly excised and horizontally mounted mucosal side up, as described previously (Reuss and Finn, 1975*a*). The exposed area was 0.44 cm^2. The solutions used for continuous perfusion of the mucosal and serosal compartments contained (mM): 109.2 Na, 3.5 K, 1.0 Ca, 113.7 Cl, and 1.0 HEPES buffer. In Na-free solution, Na was substituted with tetramethylammonium (TMA). In Cl-free media, KCl was replaced with K_2SO_4 (osmotically balanced with sucrose), NaCl was substituted for by either Na-cyclamate or Na-isethionate, and $CaCl_2$ was replaced with Ca-cyclamate or Ca-gluconate. All these solutions were bubbled with air and titrated to a pH of 7.4.

In some experiments gallbladders were cannulated and used as sac preparations for gravimetric determination of fluid absorption as described by Diamond (1962). All experiments were performed at room temperature (~23°C).

Electrodes and Electrical Measurements

The techniques for measurement of transepithelial potential difference (V_{ms}) and resistance (R_t) using current pulses of ~50 $\mu A/cm^2$ were the same as previously described (Reuss and Finn, 1975*a*). V_{ms} is referred to the serosal side; apical (V_{mc}) and basolateral (V_{cs}) membrane potentials are referred to the adjacent solutions. Conventional microelectrodes were pulled from inner-fiber glass capillaries (Hilgenberg, Malsfeld, Federal Republic of Germany), filled with 3 M KCl, and used as detailed elsewhere (Reuss and Grady, 1979). Effects of experimental maneuvers were monitored by keeping a microelectrode in the same cell until a new steady state of membrane potentials was achieved. Only such single-cell experiments were included in the data.

All values of V_{ms} and V_{mc} recorded during mucosal solution changes were corrected for the corresponding liquid junction potentials. To do this, the potential of a Ringer-agar bridge was compared with that of a saturated KCl flowing junction, assuming a constant potential of the latter.

The preparation of Cl-selective microelectrodes has recently been described (Reuss et al., 1983). Briefly, micropipettes were pulled from the same inner-fiber glass capillaries and had the same tip size as the conventional microelectrodes. They were dried at 200°C for ~2 h and exposed to hexamethyldisilazane (Sigma Chemical Co.) for at least 30 min. The tips were then filled by injection with ion-exchange resin (Corning 477913; Corning Medical, Medfield, MA). A fine inner electrode tip filled with the reference solution was inserted into the outer (resin-filled) microelectrode and positioned within ~100 μm of its tip. Connection to the electrometer (FD 223; W-P Instruments, Inc., New Haven, CT) was made by means of a chlorided silver wire immersed and sealed into the reference solution in the shank of the outer electrode. The use of an inner electrode, first described by Orme (1969), allowed us to reduce the length of the resin column and thus electrode resistance and noise. Electrodes were calibrated in single-salt solutions of NaCl and, during the experiment, in Ringer's solution diluted in isomolar sucrose solution. Slopes ranged from 52 to 58 mV for a 10-fold change in Cl activity. The selectivity sequence for various Cl substitutes was cyclamate > Cl > isethionate > sulfate. Generally, several cells in a given tissue were impaled with both conventional and Cl-sensitive microelectrodes to confirm the recorded values. Then, with both microelectrodes in cells, solutions were changed or drugs were added to obtain continuous traces of both electrode potentials. This procedure, used in most experiments, allowed us to monitor the old and new steady states as well as the transition between them. Criteria for successful impalements were the same as recently described (Weinman and Reuss, 1982*a*). In particular, paired impalements with KCl and resin microelectrodes were accepted only if they yielded equal steady-state voltage deflections across the basolateral membrane upon passage of a transepithelial current pulse. Traces were digitized by a signal averager (model 1074; Nicolet Instrument Corp., Madison, WI), analyzed with a microcomputer, and plotted with an *X-Y* plotter. In some experiments, a macroelectrode was used to monitor external Cl activity close to the tissue. For this purpose, a Teflon-coated silver wire was chlorided at its tip. The slope was ~58 mV for a 10-fold change in Cl activity.

Drugs and Chemicals

Cyclic AMP, 8-Br-cAMP, theophylline, cyclamic acid and its Na and Ca salts, HEPES, and Na-isethionate were obtained from Sigma Chemical Co. All other chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA).

Statistics

Statistical analysis was performed using the *t* test for paired or unpaired data as appropriate.

RESULTS

Effects of cAMP, 8-Br-cAMP, and Theophylline on Cell Membrane Potentials and Resistances and Fluid Absorption

As illustrated in Fig. 1, addition of 10^{-3} M 8-Br-cAMP to the mucosal bath caused a rapid depolarization of both cell membranes and a dramatic decrease

of the apparent ratio of membrane resistances ($a \equiv \Delta V_{mc}/\Delta V_{cs}$). These effects reversed slowly upon removal of this agent. The steady-state effects of cAMP (6×10^{-3} M, serosal side), 8-Br-cAMP (10^{-3} M, mucosal side), and theophylline (3.3×10^{-3} M, serosal side) on cell potentials and resistances were virtually identical (Table I). V_{mc} and V_{cs} were reduced by ~15 mV and V_{ms} rose by ~0.4 mV (lumen more negative). No significant changes in transepithelial resistance (R_t) were observed at the time at which membrane potentials reached steady-state values (Table I). However, after a prolonged exposure to

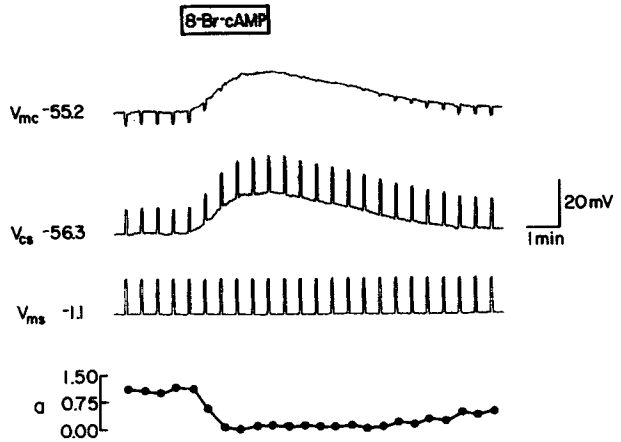

FIGURE 1. Effects of 8-Br-cAMP (10^{-3} M, mucosal side) on membrane potentials in *Necturus* gallbladder. V_{ms}, V_{mc}, and V_{cs} are the potential differences across the whole epithelium and the apical and basolateral membranes, respectively. The vertical deflections resulting from transepithelial current pulses (~50 $\mu A/cm^2$) allow one to calculate the transepithelial resistance (V_{ms} trace) and the apparent ratio of membrane resistances (a) (V_{mc} and V_{cs} traces). This ratio has been calculated for each current pulse, after correcting for voltage drops in the solution, and is plotted at the bottom. The initial potential values are given at the beginning of each trace. Upward drifts denote a change to more positive values. The tissue was exposed to 8-Br-cAMP during the period indicated by the rectangle at the top. Note the fall in a after exposure to the nucleotide.

theophylline there was a 50% increase in R_t (data not shown). The apparent ratio of apical over basolateral membrane resistances (R_a/R_b) dropped to a value not distinguishable from zero regardless of its value before addition of the agents (Table I).

The effects of 8-Br-cAMP became apparent almost immediately after its application (Fig. 1), whereas those of theophylline and cAMP started with delays of ~2 and 6 min, respectively. With cAMP, it took considerably more time to reach maximum depolarization than with either of the other substances. In some cases, a new steady state was achieved only after ~40 min. Apart from the change of R_t, which reversed only slowly, all effects were readily reversible after removal of the agents as illustrated in Fig. 1 for 8-Br-cAMP.

To ascertain whether elevation of intracellular cAMP inhibits fluid transport in *Necturus* gallbladder as in other epithelia, fluid absorption was measured gravimetrically under control conditions (60 min), during serosal addition of 3.3×10^{-3} M theophylline (45 min, starting 15 min after exposure to the drug), and after removal of theophylline (60 min). The results in eight gallbladders were 3.6 ± 0.8, 0.4 ± 0.6, and 2.5 ± 0.3 $\mu l/cm^2 \cdot h$. The absorption rate during theophylline was significantly different from the values obtained before and after exposure to the drug ($P < 0.05$ and $P < 0.025$, respectively).

Effects of Theophylline on Apical Membrane Ionic Conductances

The effect of cAMP, i.e., apical membrane depolarization in conjunction with a decrease of R_a/R_b, could result from increases in apical Na permeability

TABLE I

EFFECTS OF cAMP, 8-Br-cAMP, AND THEOPHYLLINE ON ELECTRICAL PROPERTIES OF *Necturus* GALLBLADDER EPITHELIUM

	V_{ms}	V_{mc}	V_{cs}	R_t	R_a/R_b
	mV	*mV*	*mV*	$\Omega\ cm^2$	
Control	−1.2±0.2	−75.2±2.6	−76.5±2.6	130±30	2.0±0.5
Theophylline ($n = 6$)	−1.8±0.2	−59.5±1.9	−61.2±3.2	150±30	0*
Control	−0.7±0.2	−70.0±3.5	−70.7±3.5	110±10	2.7±0.6
8-Br-cAMP ($n = 11$)	−1.0±0.2	−55.6±3.0	−56.7±3.0	120±10	0.1±0.1
Control	−0.9±0.5	−64.6±1.0	−65.6±0.6	90±10	2.7±1.5
cAMP ($n = 4$)	−1.1±1.5	−50.2±1.0	−51.3±1.8	80±10	0*

Means ± SEM are shown. Drug addition was as follows: theophylline, 3.3×10^{-3} M, serosal side; 8-Br-cAMP, 10^{-3} M, mucosal side; cAMP, 6×10^{-3} M, serosal side. Data during drug exposure were collected as soon as membrane potentials attained a new steady state. With the exception of V_{ms} during cAMP and R_t throughout, all parameters during drug treatment were significantly different from their control values ($P < 0.001$).

* In these experiments, R_a/R_b was so close to zero that its absolute value could not be determined with certainty.

(P^a_{Na}) and/or Cl permeability (P^a_{Cl}). To test these possibilities, single-ion substitutions were performed in the mucosal bathing medium. The largest changes were seen upon removal of Cl from the mucosal solution, as shown in Fig. 2. Under control conditions, mucosal cyclamate-for-Cl substitution caused hyperpolarization of the apical membrane by 3.6 ± 1.8 mV and rendered the lumen more negative by 1.4 ± 0.9 mV ($n = 5$). The relative magnitudes of these changes indicate a higher paracellular permeability for Cl than for cyclamate. Replacement of luminal Cl with isethionate yielded virtually identical changes (data not shown). The effects on membrane potentials under control conditions are difficult to interpret. They are not consistent with the observations of Reuss and Finn (1975*b*), which were interpreted to indicate a sizeable Cl permeability of the luminal membrane under control conditions. It seems that the effect of Cl removal from the mucosal solution is not due to Cl electrodiffusion, since its sign is opposite to the one expected from the

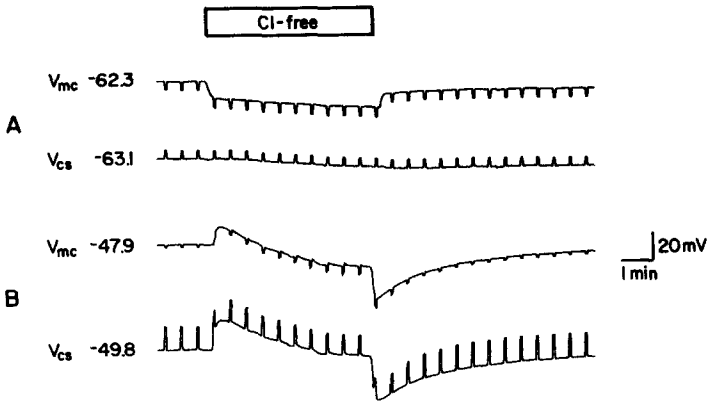

FIGURE 2. Effects on membrane potentials of mucosal Cl replacement with cyclamate under control conditions (A, upper two traces) and during serosal exposure to 3.3×10^{-3} M theophylline (B, lower two traces). Abbreviations as in Fig. 1. The apical membrane hyperpolarization produced by Cl^- removal under control conditions is exaggerated by the liquid junction potential, so that only the V_{cs} trace directly indicates the change in membrane potential. Note the drift of V_{cs} to more negative values when Cl is removed under control conditions and the changes in the current-induced deflections in V_{mc} and V_{cs} after removal and re-admission of Cl in the presence of theophylline.

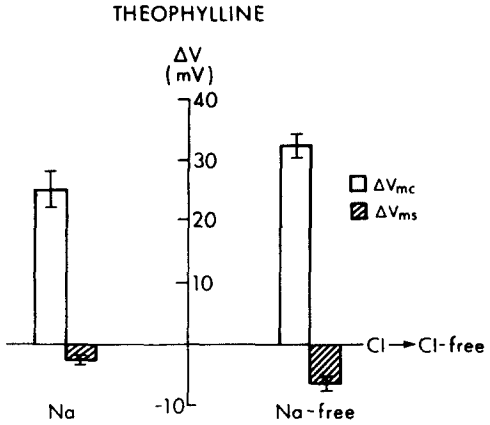

FIGURE 3. Effects of mucosal Cl replacement with cyclamate on apical membrane (V_{mc}) and transepithelial potential (V_{ms}) in the presence of 3.3×10^{-3} M theophylline (serosal side). Left: experiments in the presence of Na ($n = 5$); right: experiments during mucosal Na replacement with tetramethylammonium (TMA) ($n = 4$). Bars represent changes in potentials on removal of the luminal Cl in the two conditions and are means ± SEM of the depolarization peaks. They were corrected for junction potentials. Since the extracellular Cl activity has not reached its final value at the time of the peak depolarization (see Fig. 12), the correction probably leads to overestimates. The uncorrected ΔV_{mc} values of +16.1 ± 3.1 mV (Na present) and +22.7 ± 2.0 mV (Na-free) are the lower limits of the induced changes. All changes induced by Cl removal were significant ($P < 0.025$), whereas the response of the apical membrane to Cl removal was not significantly changed by the absence of mucosal Na.

change in apical membrane Cl diffusion potential. Further analysis of this issue is provided in the Discussion.

As illustrated in Fig. 2 (bottom) and summarized in Fig. 3, in the presence of theophylline, removal of Cl from the apical surface (cyclamate replacement) resulted in a rapid depolarization of both cell membranes, followed by repolarization and a concomitant increase of R_a/R_b. Re-exposure to Cl medium gave rise to a fast hyperpolarization of both cell membranes, followed by a slow depolarization towards the values before Cl removal and a decline of R_a/R_b to virtually zero. In three further experiments, essentially the same changes were seen in preparations treated with mucosal 8-Br-cAMP (10^{-3} M), when Cl in the mucosal perfusate was replaced with cyclamate (data not

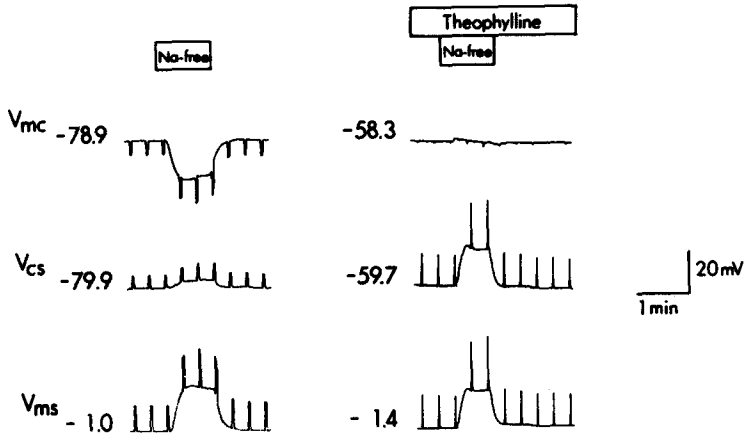

FIGURE 4. Effects of isomolar mucosal Na replacement with TMA on membrane potentials in the absence and in the presence of 3.3×10^{-3} M theophylline (serosal side). Abbreviations are as in Fig. 1. Note that in the presence of theophylline the *IR* drop produced by the Na/TMA bi-ionic shunt potential occurs almost exclusively at the basolateral membrane.

shown). These findings indicate that theophylline, cAMP, and 8-Br-cAMP effect a substantial increase of P^a_{Cl}.

The effects of complete luminal Na replacement with tetramethylammonium (TMA) are illustrated in Fig. 4 and summarized in Fig. 5. In theophylline-treated tissues, the increase in V_{mc} produced by this maneuver was significantly smaller than under control conditions. This effect is not consistent with a rise of P^a_{Na}, which would result in a larger apical membrane hyperpolarization in response to Na removal. The effect on V_{ms} was essentially the same under the two conditions, i.e., a lumen-positive change of ~24 mV. The changes in membrane potentials elicited by luminal Na substitution with a large cation result mainly from the *IR* drops generated across the cell membranes by the lumen-positive bi-ionic paracellular potential ($P_{Na} > P_{TMA}$). This diffusion potential tends to hyperpolarize the apical membrane and to depolarize the basolateral membrane in proportion to the ratio of membrane

resistances (Reuss and Finn, 1975*b*). After theophylline, R_a/R_b approaches zero and therefore most of the voltage drop must occur across the basolateral membrane (Fig. 4).

The large rise of P^a_{Cl} in the presence of theophylline must result in a reduction of the partial K conductance of the apical membrane, if P^a_K remains constant. Under control conditions, partial luminal K-for-Na substitution (final K concentration 58 mM) depolarized the apical membrane by ~38 mV (Figs. 6 and 7). This value as well as the concomitant change in V_{ms} (Fig. 7) are in agreement with previous studies (Reuss and Finn, 1975*b*) and indicate a high K permeability of both the apical membrane and the paracellular

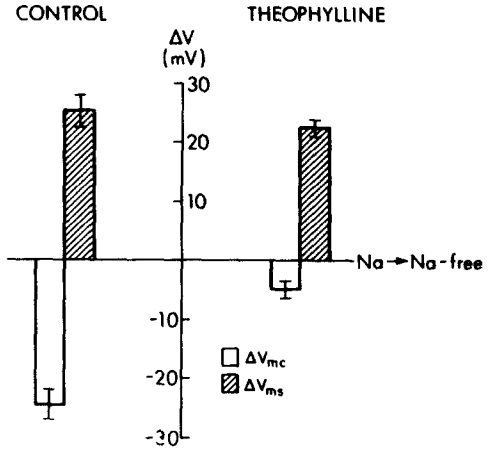

FIGURE 5. Effects of isomolar mucosal Na replacement with TMA on apical membrane potential (V_{mc}) and transepithelial potential (V_{ms}) under control conditions and in the presence of 3.3×10^{-3} M theophylline (serosal side). Values represent means ± SEM of the induced changes ($n = 5$) and were corrected for junction potentials. Theophylline significantly reduced the change of V_{mc} produced by Na removal ($P < 0.001$).

pathway. In the presence of theophylline, as shown in Fig. 6, the same increase in luminal K concentration caused a much smaller depolarization. In three out of six experiments, two distinct rates of depolarization were observed: a rapid change followed by a slow further depolarization. In the other three experiments, no such transition was evident. As considered in the Discussion, the slow sustained change of V_{mc} induced by high luminal K suggests net Cl entry across the apical membrane. To quantify the apical membrane potential changes attributable to the change of extracellular K per se, measurements were carried out shortly after the substitution, i.e., at the moment that a steady state was reached under control conditions and at the same time during exposure to theophylline. The V_{mc} changes thus determined are summarized in Fig. 7.

The diminished effect of high mucosal K on membrane potentials in

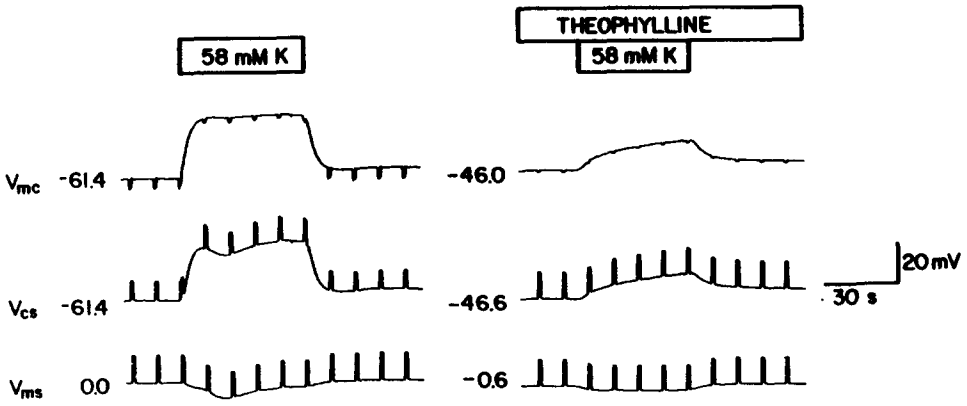

FIGURE 6. Effects of high mucosal K (58 mM, isomolar substitution of Na) on membrane potentials in the absence and in the presence of 3.3×10^{-3} M theophylline (serosal side). Abbreviations are as in Fig. 1. Note that in the presence of theophylline, the initial fast depolarization is followed by a slower but sustained decrease of both cell membrane potentials.

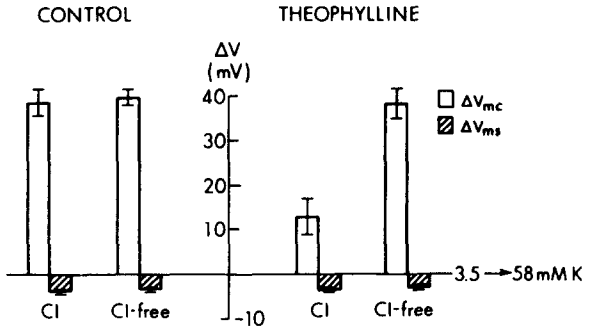

FIGURE 7. Effects of high mucosal K (58 mM, isomolar substitution of Na) on apical membrane (V_{mc}) and transepithelial potential (V_{ms}) under control conditions and in the presence of 3.3×10^{-3} M theophylline (serosal side), before ($n = 6$) and after ($n = 4$) luminal Cl removal (cyclamate substitution). The values shown are means ± SEM of the K-induced changes and were corrected for junction potentials. See text for mode of data collection during K elevation in theophylline-treated tissues (Cl present). All K-induced changes were significant ($P < 0.025$ or better). Note that the response of V_{mc} to K elevation was significantly reduced by theophylline ($P < 0.025$) when Cl was present, but remained unchanged in the absence of Cl.

theophylline-treated tissues could be due to a true reduction of apical membrane K permeability in parallel with the increase in P^a_{Cl} or could simply reflect a lowering of the partial K conductance. To distinguish between these possibilities, the luminal K-for-Na substitution was repeated after Cl removal from the mucosal solution. As evident from Fig. 7, Cl removal restored the V_{mc} and V_{ms} responses to high K in the presence of theophylline. Note that

the concomitant changes in V_{ms} are small and of similar magnitude in the four sets of experiments. Hence, changes in paracellular equivalent electromotive force cannot account for the reduced effect of high K in the presence of theophylline (Cl present).

Effects of 8-Br-cAMP and Theophylline on Intracellular Cl Activity

Intracellular Cl activity in *Necturus* gallbladder epithelium has been shown to exceed the value predicted for equilibrium distribution across the apical membrane (Reuss and Weinman, 1979; Garcia-Diaz and Armstrong, 1980).

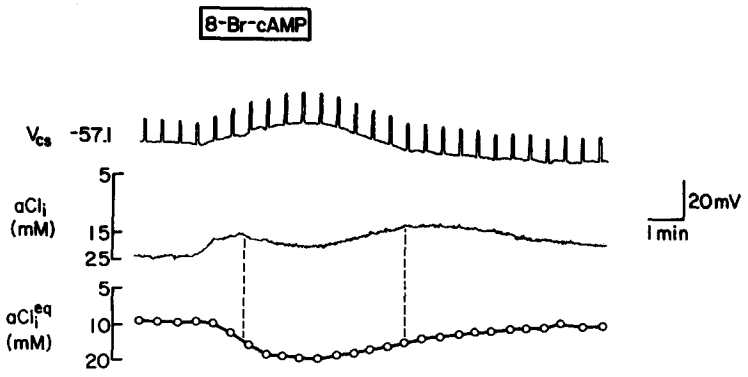

FIGURE 8. Effects of 10^{-3} M 8-Br-cAMP on basolateral membrane potential (V_{cs}) and intracellular Cl activity (aCl_i). 8-Br-cAMP was present in the mucosal solution for the time indicated. The bottom curve depicts the intracellular Cl equilibrium activity (aCl_i^{eq}) calculated for the apical membrane. Addition of 8-Br-cAMP caused a rapid fall of intracellular Cl activity towards the value predicted for equilibrium across the apical membrane. Starting with the first and ending with the second vertical broken line, the traces of aCl_i and aCl_i^{eq} were virtually congruent. Before addition of 8-Br-cAMP, aCl_i exceeded aCl_i^{eq}; after the first broken line, they run parallel; after the second one, aCl_i again rose over aCl_i^{eq}. See text for further explanation.

This is due to uphill, Na-dependent uptake at this membrane. Because cAMP induces a large Cl permeability in the apical membrane, one would expect that if Na-dependent Cl entry persisted in the presence of cAMP, it would result in Cl backflux to the luminal solution by simple electrodiffusion. Therefore, the intracellular Cl activity (aCl_i) should approach equilibrium (aCl_i^{eq}) during exposure to cAMP, regardless of whether the Na-dependent Cl influx is affected or not. This possibility was tested using Cl-selective liquid ion-exchanger microelectrodes. Fig. 8 illustrates an experiment in which 10^{-3} M 8-Br-cAMP was added to the mucosal perfusate. Within <60 s, the ratio of aCl_i over aCl_i^{eq} declined from 2.3 to ~1.0, i.e., intracellular Cl activity reached equilibrium across the apical membrane. This point is marked with a vertical broken line in Fig. 8. From here on, intracellular Cl activity remained at equilibrium and further apical membrane depolarization was accompanied

by a rise in aCl_i. The continuing fall of cell membrane potential may be due to electrodiffusive K efflux from the cell (see Discussion). Removal of 8-Br-cAMP caused a prompt return of cell potential to pre-cAMP values, most likely initiated by a decrease of P^a_{Cl}. aCl_i declined in response to the more negative value of V_{mc}. When the cell membrane potential reached its control (pre-cAMP) value, aCl_i slowly began to increase above aCl_i^{eq}. The second vertical broken line in Fig. 8 marks this point. By this time, P^a_{Cl} had presumably dropped to a value too low to permit Cl equilibration across the apical membrane. In other words, for the NaCl entry process to raise aCl_i to values above equilibrium, a low P^a_{Cl} is required. The time course of the changes in aCl_i described here and depicted in Fig. 8 was similar in all experiments of this kind.

TABLE II

EFFECTS OF THEOPHYLLINE AND 8-Br-cAMP ON INTRACELLULAR Cl ACTIVITIES IN *Necturus* GALLBLADDER EPITHELIUM

				*a*Cl		
	V_{ms}	V_{cs}	V_{Cl}	a_i	a^{eq}_{ap}	a^{eq}_{bl}
	mV	*mV*	*mV*	*mM*	*mM*	*mM*
Control	−0.7±0.2	−62.6±2.0	−23.4±1.4	17.3±1.2*	7.6±0.6	7.5±0.6
Theophylline (n = 7)	−1.1±0.2	−51.6±2.2	−3.8±0.8	12.4±1.1	12.0±1.2	11.6±1.1
Control	−0.8±0.3	−60.8±1.7	−24.3±1.3	20.3±1.6*	8.1±0.6	8.0±0.6
8-Br-cAMP (n = 8)	−1.0±0.3	−51.5±2.2	−4.2±2.1	13.3±1.2	12.0±1.1	11.6±1.0

Mean values ± SEM are shown. V_{Cl} is the signal of the Cl-sensitive microelectrode, a_i is the measured intracellular Cl activity; a^{eq}_{ap} and a^{eq}_{bl} are the calculated intracellular Cl activities for equilibrium across the apical (ap) and basolateral (bl) membranes, respectively. In the theophylline group, values are the means of 21 and 19 validated simultaneous impalements before and during theophylline, respectively. In the 8-Br-cAMP experiments, all values before and during 8-Br-cAMP were recorded in single cell impalements. In all measurements, the conventional and the Cl-sensitive microelectrodes were kept in the cells simultaneously. V_{ms}, V_{cs}, V_{Cl}, and a_i during either theophylline or 8-Br-cAMP were significantly different from their control values ($P < 0.025$ or better).

* Significantly different from a^{eq}_{ap} at $P < 0.001$.

Table II summarizes the effects of 8-Br-cAMP and theophylline on intracellular Cl activity. Under control conditions, aCl_i exceeded aCl_i^{eq} across the apical membrane (aCl^{eq}_{ap}) by a factor of 2.4 ± 0.1 (n = 15). Either 8-Br-cAMP (n = 8) or theophylline (n = 7) reduced this ratio to 1.1 ± 0.1, i.e., Cl was passively distributed across the apical membrane under these conditions.

For a further demonstration of the large increase in apical Cl permeability, tissues were exposed to Cl-free solution (cyclamate substitution) on the mucosal side, as illustrated in Fig. 9. In control experiments, Cl removal resulted in an initial fall of aCl_i at a rate of 1.8 ± 0.5 mM/min (n = 4). In tissues exposed to either 10^{-3} M 8-Br-cAMP or 3.3×10^{-3} M theophylline (pooled data, n = 8), the decline of aCl_i was significantly accelerated (11.6 ± 0.8 mM/min, $P < 0.001$). The apparent steady-state aCl_i, measured in four tissues, fell over a 4-min period from 13.1 ± 1.5 to 3.6 ± 0.8 mM. After adding back Cl

to the luminal solution, $a\mathrm{Cl}_i$ recovered at a rate similar to that observed during Cl removal.

In theophylline-treated tissues, the changes in intracellular Cl activity following rapid changes of luminal Cl concentration can explain the transient character of the resulting depolarization or hyperpolarization of the apical membrane (Fig. 2). Such alterations of intracellular Cl activity during changes in luminal Cl should be reduced if at the same time the product of extracellular K and Cl concentrations is kept constant. Hence, if the above interpretation is correct, reduction of luminal Cl at constant KCl product should yield a

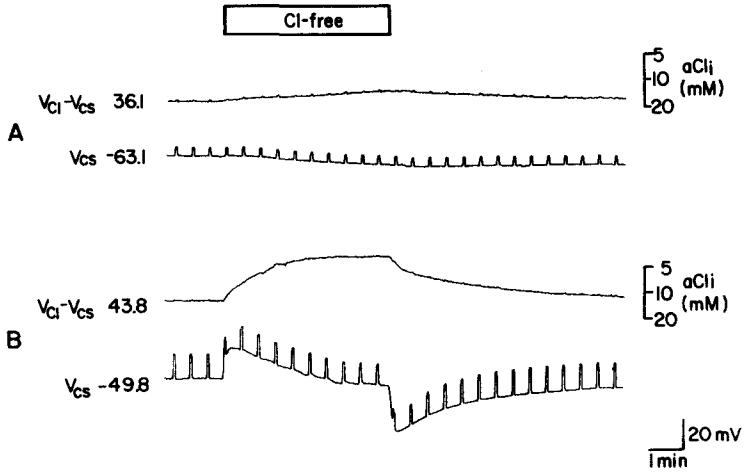

FIGURE 9. Changes of intracellular Cl activity produced by Cl replacement with cyclamate on the mucosal side, under control conditions (A, upper two traces) and during serosal exposure to 3.3×10^{-3} M theophylline (B, lower two traces). Experiments are the same as in Fig. 2. The difference between the signal of the Cl-sensitive microelectrode and the basolateral membrane potential ($V_{Cl} - V_{cs}$) and the basolateral membrane potential (V_{cs}) are shown. Note the lower value of $a\mathrm{Cl}_i$ in theophylline as compared with control conditions and the virtual absence of current-induced deflections in the differential traces.

sustained depolarization of both membranes. This was tested in the experiment illustrated in Fig. 10. A 10-fold reduction of Cl in the mucosal bath at constant K elicited biphasic changes in V_{mc} and V_{cs} comparable to those illustrated in Fig. 2. In contrast, a 10-fold increase in luminal K concentration concomitant with the Cl reduction resulted in a sustained depolarization of both cell membranes after a small initial overshoot.

Properties of the cAMP-induced Apical Cl Permeability

All of the above experiments indicate that cAMP induces an electrodiffusive pathway for Cl in the apical membrane. The possibility was considered that electrodiffusional transport of Cl might be accomplished by a carrier combining, for example, one Na^+ with two Cl^- to form a negatively charged complex.

In this case the changes of V_{mc} induced by mucosal Cl removal (cf. Fig. 2) should depend on the presence of luminal Na. However, as illustrated in Fig. 11, removal of Na from the mucosal bath failed to affect the changes in V_{mc} induced by addition and withdrawal of luminal Cl. Fig. 3 compares the effects of mucosal Cl removal on V_{mc} in the presence and in the absence of luminal Na. There was no significant difference in the depolarization elicited by omission of Cl from the mucosal bath.

In an attempt to estimate the apical membrane Cl permeability, luminal Cl concentration was elevated stepwise (10 → 25, 25 → 50, and 50 → 100 mM) in tissues exposed to theophylline. A fast mucosal perfusion rate was

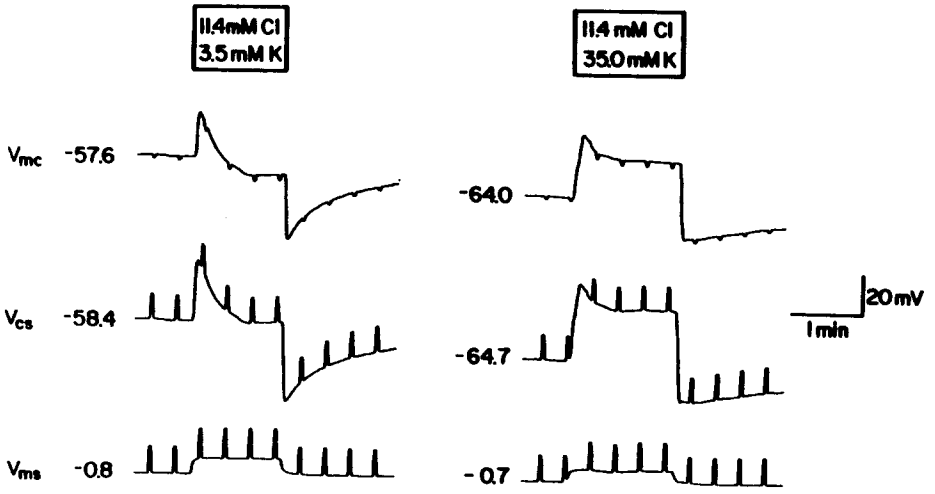

FIGURE 10. Effects of partial mucosal Cl replacement with cyclamate on membrane potentials in the presence of 3.3×10^{-3} M theophylline (serosal side). Abbreviations are as in Fig. 1. The traces on the left show the effects of a 10-fold Cl reduction at constant K in the mucosal bath; the traces on the right illustrate an experiment in which Cl was reduced concomitantly with a 10-fold increase in luminal K concentration (constant product). This experiment is representative of four tissues in which essentially the same results were obtained.

used to minimize unstirred layer effects on the build-up of the new Cl activity next to the tissue. An Ag-AgCl electrode was positioned close to the tissue to measure directly the actual Cl activity in the fluid layer adjacent to the membrane. Fig. 12 compares the apical hyperpolarization induced by the stepwise increase of Cl activity with the changes observed in the Cl electrode potential (V_{Cl}).

One approach to evaluate the apical membrane Cl permeability would be based on the peak change in V_{mc} after re-exposure to Cl. The presence of a substantial K permeability, however, allows Cl to enter the cell across the apical membrane (Fig. 9). The onset of this Cl influx a few seconds after Cl addition to the lumen is responsible for the hyperpolarization slowing down

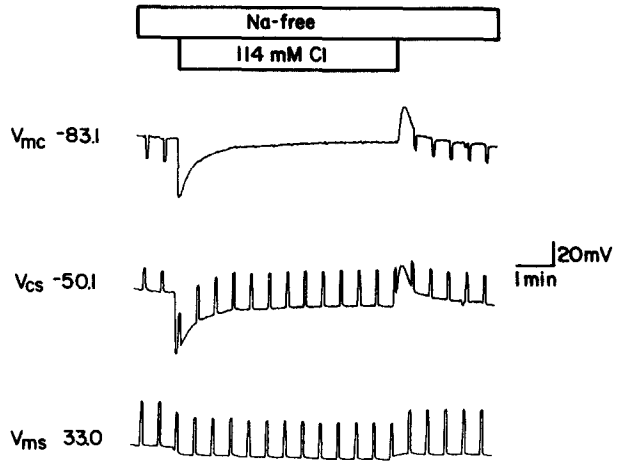

FIGURE 11. Effects of mucosal Cl on potentials of a theophylline-treated tissue exposed to Na-free solution on the mucosal side. Abbreviations are as in Fig. 1. At the beginning and end of the records, the luminal perfusate contained TMA and cyclamate instead of Na and Cl, respectively. In the middle portion, cyclamate was replaced with Cl for the time indicated. As in the presence of Na (Fig. 2B), Cl addition and removal hyperpolarized and depolarized, respectively, both cell membranes.

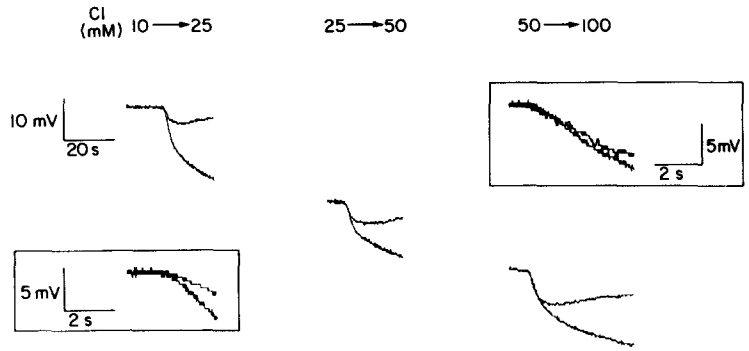

FIGURE 12. Apical membrane hyperpolarization induced by stepwise increases of luminal Cl concentration in theophylline-treated tissues. All records show the potential across the apical membrane and that of a miniature Ag-AgCl electrode positioned in the mucosal solution close (~100 μm) to the impaled cell. These two traces were superimposed at the time of Cl addition. V_{mc} is the upper and V_{Cl} is the lower trace in each pair. The three slower traces show consecutive elevations of luminal Cl concentration during a single-cell impalement, between the concentrations indicated at the top. The boxed inserts illustrate the same experiments at a faster sampling rate in the same tissue. Note the difference in the scales.

and finally reversing. Hence, the use of the maximum change of V_{mc} for assessment of apical Cl permeability must lead to an underestimate. In contrast, the initial slope of V_{mc} gives a more accurate measure of apical Cl permeability.

The main diagram in Fig. 12 shows superimposed records of V_{mc} and V_{Cl} during a single cell impalement. Each stepwise addition of Cl to the luminal perfusate resulted in initial V_{mc} and V_{Cl} changes that were very similar and appeared almost congruent at the higher Cl concentrations. This similarity continued until the point where V_{mc} broke away from V_{Cl} because of the changes in intracellular ionic composition. With a faster sampling rate, the differences between the initial slopes of V_{mc} and V_{Cl} became measurable. Examples are given in the inserts of Fig. 12. From these data we calculated the ratios of the initial slopes of V_{mc} and V_{Cl}. As explained in the Discussion,

TABLE III

APICAL MEMBRANE Cl TRANSFERENCE NUMBERS (T_{Cl}) IN THE PRESENCE OF THEOPHYLLINE

		Cl		
		10 → 25	25 → 50	50 → 100
		mM	*mM*	*mM*
T_{Cl}	A	0.59±0.08	0.74±0.04	0.88±0.04*
	B	0.52±0.08	0.62±0.06	0.69±0.06*

Mean values ± SEM are shown ($n = 6$). Tissues were exposed to 3.3×10^{-3} M theophylline in the serosal bath. After complete Cl removal from the mucosal side (cyclamate substitution), Cl was added stepwise as indicated. Line A gives T_{Cl} calculated as $\Delta V_{mc}/\Delta V_{Cl}$ using the initial slopes of V_{mc} and V_{Cl}. Line B gives the values obtained when the maximum change of V_{mc} and the change of V_{Cl} at the same time were employed for calculation of $\Delta V_{mc}/\Delta V_{Cl}$. At each step, the two sets of values differed significantly ($P < 0.025$).

* Significantly different from (10 → 25) and (25 → 50) ($P < 0.05$).

because of the low apical membrane resistance in the presence of cAMP, the change in V_{mc} is essentially equivalent to the change in the apical membrane equivalent electromotive force (E_a). Hence, the ratio of the slopes is an approximation of the Cl transference number (T_{Cl}) under such conditions. A value of 1.0 would indicate a perfect Cl electrode. Table III (line A) gives the T_{Cl} values of the apical membrane calculated from the three steps of Cl addition to the lumen. The T_{Cl} of 0.88 for the step 50 → 100 mM Cl indicates a large predominance of the Cl permeability. It significantly exceeds T_{Cl} calculated from the other two steps. This difference is presumably due to the presence of a second permeant ion, K. As the luminal Cl concentration is reduced, the partial K conductance increases until, after complete removal of luminal Cl, it represents the major part of apical membrane conductance (Fig. 7). Line B of Table III lists T_{Cl} obtained when the peak changes in V_{mc} were used to calculate $\Delta V_{mc}/\Delta V_{Cl}$. In agreement with the above discussion, these values are significantly lower than those derived from the initial slopes.

The preceding analysis is valid only if during intracellular cAMP elevation the apical membrane is impermeable to the Cl substitute used. This is probably the case for the following reasons. First, although our liquid Cl exchanger microelectrodes exhibited the selectivity sequence cyclamate > Cl > isethionate > sulfate, isomolar Cl replacement with any of these anions resulted in similar apparent changes of $a\mathrm{Cl}_i$ (data not shown), which suggests no sizable entry of these anions (in particular cyclamate, see Fig. 9) during the substitution of luminal Cl. Second, the possibility of anion permeation correlated inversely with this selectivity sequence can be ruled out because identical changes of R_a/R_b were observed when Cl was replaced with any of the three anions in tissues treated with theophylline or cAMP.

DISCUSSION

The principal finding of the present study is the dramatic rise of electrodiffusional Cl permeability of the apical membrane (P^a_{Cl}) after addition of cAMP, 8-Br-cAMP, or theophylline. This effect is responsible for the decrease of apical membrane partial K conductance, the reduction to essentially zero of the apparent ratio of membrane resistances (R_a/R_b), and the depolarization of both cell membranes. This increase of P^a_{Cl} may explain, at least in part, the inhibition of NaCl absorption. Although our data suggest the possibility that the intracellular cAMP level might regulate fluid transport in vivo, this issue is not addressed in this paper.

Duffey et al. (1981) found membrane depolarization, a drop of R_a/R_b, and an increase of R_t after addition of 8-Br-cAMP. Our results confirm and explain their observations on membrane potentials and R_a/R_b. However, R_t did not change significantly in our experiments (Table I). This difference seems to be due to the slow change of R_t under these conditions (see Fig. 1 in Duffey et al., 1981). These authors measured R_t when it reached a maximum, whereas our data were collected as soon as membrane potentials stabilized, ~5 min after addition of the drugs. After prolonged exposure to theophylline, R_t increased by ~50% in our experiments, which agrees with the conclusion that the junctional ionic permeability is decreased (Duffey et al., 1981). Using solutions containing 10 mM HCO_3, the same authors recorded a sizeable short-circuit current (I_{sc}) when they exposed *Necturus* gallbladder an I_{sc} to 8-Br-cAMP. No significant I_{sc} was induced by 8-Br-cAMP in the present experiments where nominally HCO_3-free solutions were used. However, when the baths contained 25 mM HCO_3, an I_{sc} was measured (Petersen and Reuss, unpublished observations) that was comparable to that reported by Duffey et al. (1981). Using 6×10^{-3} M cAMP in the same epithelium, Diez de los Rios et al. (1981) failed to observe changes in membrane potentials. The reason for this discrepancy with our results and those of Duffey et al. (1981) is unclear.

Ion Permeabilities of the Apical Membrane

Under control conditions, the apical membrane equivalent electromotive force (E_a) in *Necturus* gallbladder epithelium is mainly the result of a K diffusion potential. Other ion permeabilities, however, are large enough to prevent this

cell border from being a pure K electrode, in which case E_a would equal the K equilibrium potential of ~98 mV. Both Van Os and Slegers (1975) and Reuss and Finn (1975*b*) presented evidence for a Na permeability in the apical membrane. In addition, Reuss and Finn (1975*b*) obtained a depolarization of the apical membrane after mucosal Cl removal. This suggested a considerable Cl permeability. For unknown reasons, the present study is at variance with these results. Replacement of luminal Cl with cyclamate or isethionate (data not shown) caused a slow hyperpolarization of both cell membranes, which agrees with a recent report in which gluconate or methylsulfate was used as a substitute (Corcia et al., 1982). Given the outward Cl gradient under these conditions, a fast depolarization would be expected after Cl removal if Cl transport across the luminal membrane occurred by both coupled NaCl influx and electrodiffusional Cl permeation. In contrast, a small, slow hyperpolarization was observed. A possible explanation for this result is an effect of mucosal solution Cl concentration on P_K. Our results in *Necturus* gallbladder (Petersen and Reuss, unpublished observations), similar to observations in guinea pig gallbladder (Heintze et al., 1979; Petersen et al., 1981), suggest the presence of a Cl-HCO_3 exchange in the apical membrane. Therefore, luminal exposure to a Cl-free solution can result in elevation of intracellular HCO_3 concentration, and thus intracellular pH, because of a net HCO_3 influx. Apical membrane P_K is sensitive to extracellular (Reuss et al., 1981) and probably intracellular pH (Weinman and Reuss, 1982*a*). Hence, Cl removal from the apical side could increase P^a_K and thus V_{mc} by elevating intracellular pH. A similar interpretation was recently proposed by Guggino et al. (1982) to account for the hyperpolarization of both cell membranes after bilateral gluconate-for-Cl substitution in *Necturus* proximal tubule. Thus, Cl permeability, although presumably present in the apical membrane of *Necturus* gallbladder (Corcia et al., 1982), cannot be determined directly by means of Cl substitutions because of the possibility of concomitant changes in other ion conductances. The possibility that the Cl substitutes have a higher apical permeability than Cl is not supported by the effects of Cl removal on intracellular Cl activity under control conditions. Cyclamate, isethionate, and sulfate yielded the same changes in apparent $a\mathrm{Cl}_i$ (data not shown); although the Cl ion-exchanger is highly selective for cyclamate (see Materials and Methods).

It should be noted that for similar reasons apical Na permeability is likely to be underestimated when assessed from the results of luminal Na replacement. Recent studies (Weinman and Reuss, 1982*a*, *b*) have shown that removal of Na from the mucosal bath causes intracellular acidification. This in turn will reduce K permeability and membrane potentials.

Mechanism of the Cell Membrane Potential Changes Produced by cAMP

The initial depolarization of the apical membrane produced by cAMP can be easily explained by the increase in P^a_{Cl}, since under control conditions $E_K > E_{Cl}$. Therefore, the rise in P^a_{Cl} displaces apical membrane equivalent electromotive force and potential, E_a and V_{mc}, respectively, towards E_{Cl}, i.e., to less

negative values. The steady-state depolarization of the apical membrane when $a\mathrm{Cl}_i = a\mathrm{Cl}_i^{eq}$ is probably the result of two phenomena: first, a reduction of the hyperpolarizing *IR* drop across the apical membrane produced by the disparity in apical (E_a) and basolateral (E_b) equivalent electromotive forces ($E_b > E_a$); and second, the fall of $a\mathrm{K}_i$ (Duffey et al., 1981; Petersen and Reuss, unpublished observations), which is secondary to the cAMP-induced depolarization. Note that the fall of $a\mathrm{K}_i$, which is presumably secondary only to the depolarization, will decrease the values of both E_a and E_b. The same events, i.e., the decreases of R_a and $a\mathrm{K}_i$, account for the parallel depolarization of the basolateral membrane.

During elevation of intracellular cAMP, removal of Cl from the mucosal perfusate gave rise to a rapid cell-positive change of V_{mc} as a consequence of both the large electrochemical gradient favoring Cl exit and the high P_{Cl}^a. As Cl removal also causes a rapid Cl efflux from the cells, intracellular Cl activity falls, E_{Cl} decreases, and with it the contribution of Cl to the membrane potentials. Therefore, V_{mc} approaches the value before cAMP elevation. Adding Cl back to the mucosal bathing medium rapidly hyperpolarized the apical membrane, which is again a reflection of the high apical Cl permeability. The slow fall of V_{mc} toward the value recorded before Cl removal results from Cl entry and the return of the intracellular Cl activity to equilibrium values.

The effects of Cl replacement and addition in the presence of cAMP are what would be expected for a membrane with high electrodiffusive Cl and K permeability, as described by Hodgkin and Horowicz (1959) for single skeletal muscle fibers. Their analysis is applicable also to the apical membrane of cAMP-treated gallbladder cells, although $a\mathrm{K}_i$ remains above electrochemical equilibrium. In a system featuring a cell membrane that is permeable to two ions, i and j, changes in the extracellular activity of only one of them (i) will give rise to biphasic changes of the membrane potential. The first phase reflects the instantaneous change in E_i that leads to a shift of the membrane potential toward the new value of E_i but away from E_j. This initiates the second phase where both ions, i and j, cross the membrane until a new steady-state value of the membrane potential is attained. This was observed during elevation of luminal K at constant Cl (Fig. 6) and during removal of luminal Cl at constant K (Fig. 2). After substitution of luminal Cl with an impermeant anion, mucosal K elevation does not cause a net K flux across the apical membrane and hence only a monophasic change in membrane potential is observed. Also in agreement with the preceding analysis, Cl reduction on the mucosal side at constant product of K and Cl concentrations greatly reduced the secondary changes in apical membrane potential (Fig. 10). Substitutions at constant KCl product prevent secondary membrane potential changes in frog muscle, a finding interpreted to indicate constancy of intracellular ionic composition (Hodgkin and Horowicz, 1959).

There was no measurable effect of theophylline on either P_{Na}^a or P_K^a, as indicated by the ion-replacement experiments. The response to elevation of K in the mucosal perfusate was significantly reduced in the presence of theo-

phylline. This could be taken as evidence in favor of an additional theophylline effect on P^a_K. However, after omission of luminal Cl the effects of mucosal exposure to high K were fully restored (Fig. 7). Hence, disregarding the possibility of a voltage-dependent K conductance in the luminal membrane, the diminished response to high K in the presence of Cl can be ascribed to a reduction of partial K conductance secondary to the increase in P^a_{Cl}, with P^a_K being unchanged. Furthermore, the effects of elevating external K in Cl-free medium support the conclusion that theophylline did not affect P^a_{Na}. With P^a_{Na} increased, the depolarizing effect of high K in Cl-free solution would have been decreased because of the concomitant reduction of Na concentration and the lower value of T_K. The only alternative explanation is an exactly proportional rise of both P^a_{Na} and P^a_K. Such permeability changes would have to be much smaller than those of P^a_{Cl}, since re-exposure to luminal Cl after foregoing Cl removal was sufficient to reduce the apparent ratio of membrane resistances (R_a/R_b) to zero, regardless of whether Na was present (see Figs. 2 and 11).

Intracellular Cl Activities

Intracellular Cl activity measured in the present study was ~20 mM, a value exceeding the Cl activity expected for passive distribution ($a\mathrm{Cl}_i^{eq}$) by a factor of ~2.5. These values are within the wide range of previous determinations of $a\mathrm{Cl}_i$ (14–31 mM) and its ratio over $a\mathrm{Cl}_i^{eq}$ (1.6–4.4) (Reuss and Grady, 1979; Garcia-Diaz and Armstrong, 1980; Fisher et al., 1981; Zeuthen, 1982). Both 8-Br-cAMP and theophylline caused a rapid fall of $a\mathrm{Cl}_i$ to values that were not significantly different from $a\mathrm{Cl}_i^{eq}$. A reduction of $a\mathrm{Cl}_i$ to $a\mathrm{Cl}_i^{eq}$ by serosal cAMP has recently been reported by Diez de los Rios et al. (1981). Since these authors observed no changes in V_{mc}, they interpreted their finding in terms of an inhibition of electroneutral NaCl entry across the apical membrane. However, given the enormous increase of P^a_{Cl} in the presence of cAMP, there is no need to invoke a direct cAMP effect on carrier-mediated Cl entry across the apical membrane to explain the fall of $a\mathrm{Cl}_i$.

Quantification of Apical Membrane Cl Permeability in the Presence of cAMP

The fast drop of $a\mathrm{Cl}_i$ upon addition of 8-Br-cAMP emphasizes the high P^a_{Cl} induced by the nucleotide. The rate of the initial change in $a\mathrm{Cl}_i$ was 11.3 ± 1.8 mM/min ($n = 8$). It is possible to estimate G^a_{Cl} roughly from the change in $a\mathrm{Cl}_i$ caused by 8-Br-cAMP.

For this calculation it was assumed (*a*) that all of the initial change in intracellular Cl^- activity produced by exposure to cAMP is by electrodiffusion through the apical membrane, (*b*) that the cell height is 34.6 μm (Spring and Hope, 1979), and (*c*) that cell volume does not change during this initial phase. Within these assumptions, a Cl^- flux and an equivalent Cl^- current (I_{Cl}) through the apical membrane can be determined, and the Cl^- conductance of the apical membrane (G^a_{Cl}) can be calculated from

$$G^a_{Cl} = I_{Cl}/(V_{mc} - E_{Cl}),$$

where E_{Cl} is the Cl equilibrium potential across the apical membrane. In this

group of eight tissues, I_{Cl} was 62.9 ± 10.1 $\mu A/cm^2$, V_{mc} was −62.0 ± 1.7 mV, and E_{Cl} was −37.1 ± 1.8 mV. The calculated value of G^a_{Cl} was 2.52 ± 0.28 mS/cm^2. Since the apical membrane conductance under control conditions is ~0.25 mS/cm^2 (Frömter and Diamond, 1972; Reuss and Finn, 1975*a*), the increase produced by cAMP is ~10-fold. Therefore, if cAMP only increases P^a_{Cl}, the Cl^- transference number (T_{Cl}) must increase to at least 2.52/2.77 = 0.91. Although the validity of these estimates hinges on several untested assumptions, strong support for this analysis is provided by the observed apparent ratio of cell membrane resistances in tissues exposed to cAMP (Table I) and by the similar results obtained from calculations based on the effects of Cl^- substitutions in the mucosal bathing medium on cell membrane potentials, as described below.

In an attempt to obtain a second, independent estimate of T_{Cl}, rapid changes of luminal Cl concentration were performed in tissues treated with theophylline. The induced apical membrane potential changes were compared with those of a Cl electrode close to the tissue in the mucosal bath. For reasons already discussed, the initial slopes of the induced changes in the potentials rather than peak changes were used for this assessment. The calculated T_{Cl} values, summarized in Table III, were 0.59, 0.74, and 0.88 for the Cl concentration steps 10 → 25, 25 → 50, and 50 → 100 mM, respectively. This means that at higher Cl concentrations the apical membrane functions almost like a Cl electrode, whereas at the lower concentrations the contribution of P^a_K becomes more significant. In this experimental approach there are several possible sources of error that need evaluation. The extracellular electrode and the impaled cell could be exposed to different Cl concentrations caused by imperfect mixing in the mucosal bath. However, both electrodes were positioned within the same field of view at a distance of no more than ~100 μm, which should minimize concentration differences between the two sites. In agreement with this notion, there was no significant delay between the onset of the Cl-induced changes of V_{mc} and V_{Cl}, even at the higher time resolution of the inserts in Fig. 12. A second uncertainty is the possibility of changes in aCl_i, which might already begin during the initial hyperpolarization, when slopes were measured. This possibility cannot be entirely excluded, but since elevation of aCl_i would blunt the Cl-induced hyperpolarization of the apical membrane, such an error would lead to an underestimate of T_{Cl}. A final point of concern is the paracellular current loop during hyperpolarization of the apical membrane. In the presence of theophylline, however, R_a is much smaller than R_b. Therefore, the contributions of basolateral and paracellular electromotive forces to the apical membrane potential are very small and it can be shown that the magnitude of the changes of V_{mc} and E_a upon changing the external Cl concentration are very similar. This holds true as long as R_b substantially exceeds the resistance of the paracellular pathway (R_s). If R_b and R_s were of similar magnitude, the change in E_a would be even higher than that in V_{mc}, resulting in an underestimate of T_{Cl}. To summarize, most of the possible errors in this determination of T_{Cl} would lead to an underestimate.

A third, less direct estimate of T_{Cl} in theophylline-treated mucosa is

available from an evaluation of T_K. In the presence of Cl, elevation of the luminal K concentration from 3.5 to 58 mM depolarized the apical membrane by 13.1 mV (Fig. 7). Since $\Delta V_{mc} \approx \Delta E_a$, T_K is directly given by the ratio of ΔV_{mc} over the change in potential expected for an ideal K electrode (58.7 log 58/3.5). Assuming that G^a_K and G^a_{Cl} are the only significant conductive pathways, T_{Cl} would be 0.82. This indirect assessment of T_{Cl} is less accurate because of the uncertainties involved in the exact determination of the K-induced depolarization of the apical membrane (see Results). However, the close agreement in the values of T_{Cl} obtained using two other independent methods (0.88 and 0.91) strongly supports the conclusion that, in the presence of elevated intracellular cAMP levels, Cl is the main permeant ion at the apical membrane.

The absolute value of P^a_{Cl} was calculated, according to constant field theory (Goldman, 1943), from

$$P^a_{Cl} = -\left[\frac{R^2T^2\,G^a_{Cl}}{z^3F^3\,V_{mc}}\right]\left[\frac{(1-\xi)\,\ln(Cl_o/Cl_i\xi)}{Cl_o - Cl_i\xi}\right],$$

where G^a_{Cl} is the chloride conductance of the apical membrane, $\xi = \exp(zF\,V_{mc}/RT)$, Cl_o and Cl_i are the extra- and intracellular Cl concentrations, respectively, V_{mc} has been already defined, and the other symbols have their usual meanings. For a derivation of this equation, see Schultz (1980). In Ringer's solution (Cl_o = 113.7 mM), the calculated value of P^a_{Cl} was 18.2 × 10^{-6} cm/s. This value was used to calculate T_{Cl} at lower luminal Cl concentrations, assuming that P^a_{Cl} is constant (independent of the Cl concentration), that G^a_K is constant and equal to 0.25 mS/cm^2 (the apical membrane conductance under control conditions), and that under steady-state conditions, i.e., before each change in solution, $aCl_i = aCl_i^{eq}$. The T_{Cl} values thus calculated were 0.82, 0.70, and 0.48 for the luminal Cl concentrations of 50, 25, and 10 mM, respectively. These values are in good agreement with the experimentally determined ones summarized in Table III. This analysis supports the notion that at low Cl concentrations the contribution of K to the membrane conductance is significant and accounts for the reduction of T_{Cl}.

Significance of the Increase in P^a_{Cl} for Transport in Gallbladder and Other Epithelia

The present study shows that cAMP inhibits fluid and electrolyte absorption in *Necturus* gallbladder as it does in mammalian gallbladder (Heintze et al., 1974; Frizzell et al., 1975) and small intestine (Field, 1971). This inhibitory effect has been ascribed to an action on the coupled NaCl influx across the apical membrane (Frizzell et al., 1979*a*). Direct measurements of NaCl uptake in the presence of cAMP have not been reported in gallbladder epithelium. Measurements of Cl uptake under the action of cAMP have been attempted in rabbit ileum (Nellans et al., 1973) and flounder intestine (Frizzell et al., 1979*b*). An inhibition was demonstrated only in the first species. The interpretation of these results is difficult because of the possibility of a cAMP-induced increase of P^a_{Cl}, which would drastically change the kinetics of Cl uptake. In rabbit ileum, the measurements were carried out over a period of

30–45 s, a time at which our observations show large changes of $a\mathrm{Cl}_i$, when the external Cl concentration is changed in the presence of theophylline (Fig. 9). Influx measurements at this time would yield a net, not a unidirectional, tracer flux across the apical membrane because the tracer efflux is no longer negligible. In addition, the intracellular Cl pool would be reduced during the preincubation in theophylline, and therefore the steady-state tracer uptake would be lower than the control value. This analysis does not exclude the additional possibility of a direct effect of cAMP on the carrier, but at least in the gallbladder the effect of cAMP on Cl transport is secondary, at least in part, to the increase of P^a_{Cl}.

In the presence of cAMP, the Cl that enters the cell across the apical membrane by carrier-mediated transport leaves it at the same border through the cAMP-induced Cl conductive pathway. In other words, inhibition of net Cl influx across the apical membrane by cAMP may result from elevation of Cl backflux (J_{cm}) rather than from reduction of influx (J_{mc}). Regardless of the mechanism of basolateral Cl exit from the cell, the fall of $a\mathrm{Cl}_i$ will result by itself in a reduction of Cl efflux from the cells to the serosal bath. This may account, at least in part, for the reduction of fluid absorption by cAMP. If the hypothesis of a neutral KCl exit across the basolateral membrane (Reuss, 1979; Reuss et al., 1980) is correct, the fall of $a\mathrm{K}_i$ and the concomitant reduction of the K chemical gradient across this membrane will contribute to transport inhibition.

Our studies do not rule out the possibility of additional effects of cAMP on Na and/or Cl transport. Because of the discrepancies with the studies of others (Diez de los Rios et al., 1981), re-examination of the mechanisms of cation transport during exposure to cAMP will be necessary.

In a number of intestinal tissues, the response to cAMP features Cl secretion, e.g., in rabbit colonic mucosa (Frizzell and Heintze, 1979). As concluded from measurements of transepithelial parameters and the apparent ratio of membrane resistances, cAMP is believed to exert its effect by enhancing the apical Cl permeability, thereby permitting passive exit of Cl. More direct methods have been used to reach a similar conclusion in rabbit cornea, where stimulation of Cl secretion by adrenaline has been found to be associated with a sizeable rise of the apical (tear side) membrane Cl permeability (Klyce and Wong, 1977). The possibility of an effect of cAMP on cell membrane Cl permeability in epithelia that do not secrete Cl has also been suggested (Mandel, 1975; Field et al., 1980). Finally, increases of the conductance of smooth muscle cell membranes by the same agent have been interpreted in terms of a rise in Cl permeability (Tomita et al., 1974). The widespread occurrence of cAMP-induced increases in cell membrane Cl permeability suggests that this may be one of the main mechanisms by which cAMP exerts its physiological effects.

We thank S. A. Weinman for critical comments on the manuscript and Sue Eads for secretarial help.

This work was supported by grant AM-19580 from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. K.-U. P. was supported by a grant from the Deutsche Forschungsgemeinschaft (I3-Pe 280/1-1).

Received for publication 19 October 1982 and in revised form 18 December 1982.

REFERENCES

Corcia, A., J. F. Garcia-Diaz, and W. McD. Armstrong. 1982. Removal of luminal Cl alters K permeability in *Necturus* gallbladder. *Fed. Proc.* 41:1495. (Abstr).

Cremaschi, D., and S. Hénin. 1975. Na^+ and Cl^- transepithelial routes in rabbit gallbladder. Tracer analysis of the transports. *Pflügers Arch. Eur. J. Physiol.* 361:33–41.

Diamond, J. M. 1962. The reabsorptive function of the gallbladder. *J. Physiol. (Lond.).* 161:442–473.

Diez de los Rios, A., N. E. DeRose, and W. McD. Armstrong. 1981. Cyclic AMP and intracellular ionic activities in *Necturus* gallbladder. *J. Membr. Biol.* 63:25–30.

Duffey, M. E., B. Hainau, S. Ho, and C. J. Bentzel. 1981. Regulation of epithelial tight junction permeability by cyclic AMP. *Nature (Lond.).* 294:451–453.

Ericson, A.-C., and K. R. Spring. 1982. Coupled NaCl entry into *Necturus* gallbladder epithelial cells. *Am. J. Physiol.* 243:C140–C145.

Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3′,5′-AMP. *Am. J. Physiol.* 221:992–997.

Field, M., P. L. Smith, and J. E. Bolton. 1980. Ion transport across the isolated intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus*. II. Effects of cyclic AMP. *J. Membr. Biol.* 55:157–163.

Fisher, R. S., B.-E. Persson, and K. R. Spring. 1981. Epithelial cell volume regulation: bicarbonate dependence. *Science (Wash. DC).* 214:1357–1359.

Frizzell, R. A., and K. Heintze. 1979. Electrogenic chloride secretion by mammalian colon. *In* Mechanisms of Intestinal Secretion. H. J. Binder, editor. Alan R. Liss, Inc., New York. 101–110.

Frizzell, R. A., M. C. Dugas, and S. G. Schultz. 1975. Sodium chloride transport by rabbit gallbladder: direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* 65:769–795.

Frizzell, R. A., M. Field, and S. G. Schultz. 1979*a*. Sodium-coupled chloride transport by epithelial tissues. *Am. J. Physiol.* 236:F1–F8.

Frizzell, R. A., P. L. Smith, E. Vosburgh, and M. Field. 1979*b*. Coupled sodium-chloride influx across brush border of flounder intestine. *J. Membr. Biol.* 46:27–39.

Frömter, E., and J. M. Diamond. 1972. Route of passive ion movement in epithelia. *Nat. New Biol.* 235:9–13.

Garcia-Diaz, J. F., and W. McD. Armstrong. 1980. The steady-state relationship between sodium and chloride transmembrane electrochemical potential differences in *Necturus* gallbladder. *J. Membr. Biol.* 55:213–222.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27:37–60.

Guggino, W. B., E. L. Boulpaep, and G. Giebish. 1982. Electrical properties of chloride transport across the *Necturus* proximal tubule. *J. Membr. Biol.* 65:185–196.

Heintze, K., K.-U. Petersen, and O. Heidenreich. 1974. Inhibition of fluid transport in the isolated gallbladder of the guinea-pig by isoprenaline, theophylline and cyclic adenosine 3′,5′-monophosphate. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 285:151–163.

Heintze, K., K.-U. Petersen, S. H. Saverymuttu, and J. R. Wood. 1979. Effects of bicarbonate on fluid and electrolyte transport by the guinea-pig gallbladder: a bicarbonate-chloride exchange. *J. Membr. Biol.* 45:43–59.

Hodgkin, A. L., and P. Horowicz. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibers. *J. Physiol. (Lond.)*. 148:127–160.

Klyce, S. D., and R. K. S. Wong. 1977. Site and mode of adrenaline action on chloride transport across the rabbit corneal epithelium. *J. Physiol. (Lond.)*. 266:777–799.

Mandel, L. J. 1975. Actions of external hypertonic urea, ADH and theophylline on transcellular and extracellular solute permeabilities in frog skin. *J. Gen. Physiol.* 65:599–615.

Nellans, H. N., R. A. Frizzell, and S. G. Schultz. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* 225:467–475.

Orme, F. W. 1969. Liquid ion-exchanger microelectrodes. *In* Glass Microelectrodes. M. Lavallée, O. F. Schanne, and N. C. Hébert, editors. John Wiley & Sons, New York. 376–403.

Petersen, K.-U., H. Osswald, and K. Heintze. 1982. Asymmetric release of cyclic AMP from guinea-pig and rabbit gallbladder. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 318:358–362.

Petersen, K.-U., J. R. Wood, G. Schulze, and K. Heintze. 1981. Stimulation of gallbladder fluid and electrolyte absorption by butyrate. *J. Membr. Biol.* 62:183–193.

Reuss, L. 1979. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. III. Ionic permeability of the basolateral cell membrane. *J. Membr. Biol.* 47:239–259.

Reuss, L., and A. L. Finn. 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. Membr. Biol.* 25:115–139.

Reuss, L., and A. L. Finn. 1975*b*. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. II. Ionic permeability of the apical cell membrane. *J. Membr. Biol.* 25:141–161.

Reuss, L., and T. P. Grady. 1979. Effects of external sodium and cell membrane potential on intracellular chloride activity in gallbladder epithelium. *J. Membr. Biol.* 51:15–31.

Reuss, L., and S. A. Weinman. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membr. Biol.* 49:345–362.

Reuss, L., L. Y. Cheung, and T. P. Grady. 1981. Mechanisms of cation permeation across apical cell membrane of *Necturus* gallbladder: effects of luminal pH and divalent cations on K^+ and Na^+ permeability. *J. Membr. Biol.* 59:211–224.

Reuss, L., P. Reinach, S. A. Weinman, and T. P. Grady. 1983. Intracellular ion activities and Cl transport mechanism in bullfrog corneal epithelium. *Am. J. Physiol.* In press.

Reuss, L., S. A. Weinman, and T. P. Grady. 1980. Intracellular K activity and its relation to basolateral membrane ion transport in *Necturus* gallbladder epithelium. *J. Gen. Physiol.* 76:33–57.

Rose, R. C., and D. L. Nahrwold. 1980. Electrolyte transport in *Necturus* gallbladder: the role of rheogenic Na transport. *Am. J. Physiol.* 238:G358–G365.

Schultz, S. G. 1980. Basic Principles of Membrane Transport. Cambridge University Press, Cambridge. 119.

Spring, K. R., and A. Hope. 1979. Fluid transport and the dimensions of cells and interspaces of living *Necturus* gallbladder. *J. Gen. Physiol.* 73:287–305.

Stewart, C. P., R. Goetz, and K. Heintze. 1982. Electrogenic HCO_3 secretion by guinea-pig gallbladder. *In* Electrolyte and Water Transport across Gastrointestinal Epithelia. R. M. Case, A. Garner, L. A. Turnberg, and J. A. Young, editors. Raven Press, New York. 209–214.

Tomita, T., Y. Sakamoto, and M. Ohba. 1974. Conductance increase by adrenaline in guinea pig taenia coli studied with voltage clamp method. *Nature (Lond.)*. 250:432–433.

Van Os, C. H., and J. F. G. Slegers. 1975. The electrical potential profile of gallbladder epithelium. *J. Membr. Biol.* 24:341–363.

Weinman, S. A., and L. Reuss. 1982*a*. Na^+-H^+ exchange at the apical membrane of *Necturus* gallbladder: extracellular and intracellular pH studies. *J. Gen. Physiol.* 80:299–321.

Weinman, S. A., and L. Reuss. 1982*b*. Intracellular pH and sodium activity in *Necturus* gallbladder: relationship between Na-H exchange and apical transport. *Fed. Proc.* 41:1495. (Abstr.)

Zeuthen, T. 1982. Relations between intracellular ion activities and extracellular osmolarity in *Necturus* gallbladder epithelium. *J. Membr. Biol.* 66:109–121.