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Cyclic AMP-Induced Changes in Membrane Conductance of *Necturus* Gallbladder Epithelial Cells

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Summary. Enhanced cellular cAMP levels have been shown to increase apical membrane Cl^- and HCO_3^- conductances in epithelia. We found that the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX) increases cAMP levels in *Necturus* gallbladder. We used conventional open-tip and double-barreled Cl^--selective microelectrodes to study the effects of IBMX on membrane conductances and intracellular Cl^- activities in gallbladders mounted in a divided chamber and bathed with Ringer's solutions at 23°C and pH 7.4. In HCO_3^--free media, 0.1 mM IBMX added to the mucosal medium depolarized the apical membrane potential V_a, decreased the fractional resistance F_R, and significantly reduced intracellular Cl^- activity (a^i_{Cl}). Under control conditions, a^i_{Cl} was above the value corresponding to passive distribution across the apical cell membrane. In media containing 25 mM HCO_3^-, IBMX caused a small transient hyperpolarization of V_a followed by a depolarization not significantly different from that observed in HCO_3^--free Ringer's. Removal of mucosal Cl^-, Na^+ or Ca^{2+} did not affect the IBMX-induced depolarization in V_a. The basolateral membrane of *Necturus* gallbladder is highly K^+ permeable. Increasing serosal K^+ from 2.5 to 80 mM, depolarized V_a. Mucosal IBMX significantly reduced this depolarization. Addition of 10 mM Ba^{2+}, a K^+ channel blocker, to the serosal medium depolarized V_a and, essentially, blocked the depolarization induced by IBMX. These results indicate that mucosal IBMX increases apical HCO_3^- conductance and decreases basolateral K^+ conductance in gallbladder epithelial cells via a cAMP-dependent mechanism. The latter effect, not previously reported in epithelial tissues, appears to be the major determinant of the IBMX-induced depolarization of V_a.

Key Words *Necturus* gallbladder · apical membrane potentials · intracellular chloride activities · apical membrane conductances · basolateral membrane K^+ conductance · double-barreled Cl^--selective microelectrodes · IBMX

Introduction

Cyclic adenosine-3′,5′-monophosphate (cAMP) inhibits the absorption of fluid and electrolytes by isolated gallbladders from a number of animal species (Heintze, Petersen & Heidenreich, 1974; Frizzell, Dugas & Schultz, 1975; Petersen & Reuss, 1983). The inhibitory effects observed are essentially the same whether cAMP itself, its derivatives (e.g. 8-Br-cAMP, dibutyryl cAMP) or agents that increase intracellular cAMP levels (e.g. theophylline) are added to the medium bathing the tissue. This strongly indicates that it is the role of cAMP as an intracellular messenger that determines its effect on fluid and electrolyte absorption.

Microelectrode studies with isolated gallbladders from *Necturus* (Duffey et al., 1981; Petersen & Reuss, 1983) showed that when 8-Br-cAMP was added to the solution bathing the mucosal surface of the tissue, the apical membrane potential of the epithelial cells (V_a) depolarized by about 12 to 15 mV. At the same time the apparent resistance ratio of the apical and basolateral cell membranes (measured as the ratio of the changes in the apical and basolateral cell membrane potentials, $\Delta V_a/\Delta V_{bl}$, induced by a transepithelial current pulse) decreased dramatically. Petersen and Reuss (1983) observed essentially identical electrophysiological changes when cAMP or theophylline was added to the medium bathing the serosal surface of *Necturus* gallbladder. These changes suggested that the basic electrophysiological event underlying the depolarization of V_a might be an increase in P_{Na} and/or P_{Cl} (the conductive permeabilities of the apical cell membrane to Na^+ and Cl^-). An increase in P_{Cl} could account, at least in part, for the apparent reduction by cAMP of Na^+ and Cl^- entry across the apical membrane of the epithelial cells. This was noted in a number of studies and was attributed to cAMP-induced inhibition of a coupled NaCl transport process that is considered to be a prime factor in NaCl absorption by the gallbladder (Frizzell et al., 1975; Diez de los Rios, Derose & Armstrong, 1981; Petersen,

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Osswald & Heintze, 1982). To explore this hypothesis, Petersen and Reuss (1983) examined the effect of theophylline on ionic conductances in the apical membrane of *Necturus* gallbladder epithelial cells. They reported that their experiments showed no significant effect of theophylline on P_{Na}. However, following addition of this compound to the serosal bathing solution, there appeared to be a large increase in P_{Cl}. Petersen and Reuss (1983) concluded that a cAMP-induced increase in P_{Cl} is probably responsible, in large measure, for the inhibitory effects of this compound on fluid and electrolyte absorption by the gallbladder and other epithelia. However, a number of observations indicate that the effects of cAMP on membrane conductances in epithelia are more complex than the experiments of Petersen and Reuss (1983) would appear to suggest. In guinea pig gallbladder, cAMP elicits a secretion of K^+, Na^+ and HCO_3^- (Heintze et al., 1979). This response cannot be explained by an increase in P_{Cl} alone but would appear to require a cAMP-induced increase in P_{HCO_3}, the apical membrane HCO_3^- conductance (Stewart, Goetz & Heintze, 1982). In another leaky epithelium, the choroid plexus of the frog, Saito and Wright (1983*a*) found that the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine (IBMX), which presumably exerts its effects via an increase in intracellular cAMP, induces a large increase in P_{HCO_3}.

In terms of its overall electrophysiological effects (a depolarization of the apical cell membrane and a marked decrease in the apparent apical/basolateral membrane resistance ratio) the action of IBMX on the choroid plexus (Saito & Wright, 1983*b*) is strikingly reminiscent of the effect of theophylline on *Necturus* gallbladder. Because of this we examined the effect of IBMX (added to the mucosal medium) on membrane conductances in *Necturus* gallbladder. Since cAMP and theophylline have been reported to decrease the intracellular Cl^- activity (a^i_{Cl}) of gallbladder epithelial cells (Diez de los Rios et al., 1981; Petersen & Reuss, 1983) and since this decrease has been interpreted as a direct consequence of an increase in P_{Cl} (Petersen & Reuss, 1983), we also investigated the effects of IBMX on this parameter. Finally, the response of tissue cAMP levels in *Necturus* gallbladder to IBMX was measured directly. The results of these experiments are presented herein. A preliminary account of some of them was presented elsewhere (Armstrong, Zeldin & Corcia, 1984).

Materials and Methods

Necturus maculosus were obtained from Graska Biological Supplies (Oshkosh, Wis.). They were kept in a large aquarium at 4°C. Animals were killed by a blow on the head and a double transection of the spinal cord was performed. The abdominal cavity was opened by median incision. The gallbladder was removed, cleaned of adhering tissue, emptied, cut longitudinally and rinsed in the control Ringer's solution. This contained, in mM; 100 NaCl, 2.5 KCl, 1.0 $CaCl_2$ and was buffered at pH 7.4 with 10 mM HEPES. During all experiments this solution was bubbled continuously with 100% O_2.

To determine the effect of IBMX on tissue cAMP content, individual gallbladders were incubated at room temperature (23 ± 1°C) for 30 min in 2 ml of either the control medium or one of the same composition that contained 0.1 mM IBMX (Sigma Chemical Company, St. Louis, Mo.). After 30 min, 0.2 ml of 60% perchloric acid was added to each incubation vessel. The tissue was then homogenized *in situ* and the cAMP content of the homogenate plus its bathing medium was assayed by the protein binding assay of Gilman (1970) as modified by Shahed et al. (1979).

For electrophysiological experiments, the gallbladder was mounted as a flat sheet (mucosal surface upwards) in a divided Lucite® chamber. The mucosal and serosal surfaces of the tissue were separately and continuously superfused by a simple gravity perfusion device. The effective exposed area of gallbladder was 0.38 cm^2. Stopcock silicone grease (Dow Corning) was used to prevent edge damage to the tissue and leakage of fluid between the mucosal and serosal compartments. The serosal surface of the tissue was supported by a stainless steel grid. A negative hydrostatic pressure of approximately 20 cm H_2O was applied to the serosal compartment. This served to attach the bladder firmly to its supporting grid and to assist the flow of bathing fluid through the serosal compartment. During an experiment, the mucosal solution, the serosal solution, or both, could be changed by means of manual stopcocks located close to the appropriate chamber inlets.

In addition to the control HCO_3^--free medium already described, the following perfusion media were employed: a solution in which 25 mM $NaHCO_3$ replaced an equivalent amount of NaCl, a solution which contained 85 mM NaCl and 10 mM $BaCl_2$ (together with KCl and $CaCl_2$), a solution from which $CaCl_2$ was omitted and which contained 10 mM EGTA (Ca^{2+}-free medium), a Na^+-free medium in which NaCl was replaced by Tris-Cl, and a Cl^--free medium in which Cl^- was completely replaced by gluconate. All media had a pH of 7.4. HCO_3^--free solutions were bubbled with 100% O_2. Solutions containing HCO_3^- were bubbled with a 95% O_2/5% CO_2 mixture.

Single-barreled open-tip microelectrodes were pulled from "Kwik-fil" borosilicate capillary glass tubing (o.d. 1.2 mm, i.d. 0.8 mm, W.P.I., New Haven, Conn.) in a vertical puller (Model 700C, David Kopf Instruments, Tujunga, Calif.) and were filled with 3 M KCl. Their tip diameters were about 1 μm and their tip resistances ranged from 10 to 20 MΩ when their tips were immersed in Ringer's solution.

Double-barreled microelectrodes (in which one barrel was an open-tip microelectrode, its companion being a Corning 477913 liquid ion-exchanger Cl^--selective microelectrode) were used to monitor intracellular Cl^- activity (a^i_{Cl}). These microelectrodes were fabricated as described by Corcia and Armstrong (1983) and had overall tip diameters of approximately 1 μm.

The Cl^--selective barrels of these microelectrodes were calibrated in solutions containing 10, 20, 50 and 100 mM KCl. Their slopes (S) ranged from 50.0 to 61.3 mV/decade change in Cl^- activity (mean value 56.0). a^i_{Cl} was calculated from the equation

$$a^i_{Cl} = a^o_{Cl} 10\ (V_a - V_{Cl})/S$$

where V_a is the apical membrane potential measured by the open-tip barrel and V_{Cl} is the change in potential recorded by the Cl^--selective barrel following cell impalement. a^o_{Cl} is the calculated Cl^- activity in the external medium, assuming an activity coefficient of 0.76.

The electrical circuitry used in these experiments has been described in detail elsewhere (Garcia-Diaz, Corcia & Armstrong, 1983; Garcia-Diaz, Stump & Armstrong, 1984). The transepithelial potential V_T was continuously monitored by two calomel half-cells, one connected to the mucosal and one connected to the serosal bathing medium *via* Ringer's-agar bridges. The tissue was kept in the open-circuit mode except for brief periods when transepithelial current pulses (I_T) were delivered *via* two AgCl-coated silver rings. These were integral components of the tissue chamber. Microelectrodes were connected through Ag/AgCl wires and a guarded coaxial cable to a high impedance ($>10^{15}\Omega$) FET-input electrometer (Analog Devices 515L) with capacitance neutralization. An electronic current-clamp device (Garcia-Diaz et al., 1984) was used to control the I_T pulses and calculate transepithelial resistance and the fractional apical voltage ratio F_R, i.e. the ratio between the deflections in V_a and V_T ($\Delta V_a/\Delta V_T$) produced by the I_T pulses. Each pulse had a duration of either 1.1 or 4.4 sec and the interval between successive pulses was 2.2 or 14 sec. Pulses were delivered in the serosal-to-mucosal direction and had an intensity of 15 μA (currently density 39.5 μA cm^{-2}). The electronic current-clamp device provided compensation of R_T and F_R for the resistance of the external solutions. All potentials were measured with reference to the mucosal bathing solution. The five parameters measured (R_T, V_T, F_R, V_a and V_{Cl}) were recorded in digital panel meters. In addition, the last four were recorded continuously on a four-channel strip-chart recorder (Gould Brush, Model 240).

Microelectrodes were advanced perpendicular to the tissue with a Narishige MO-10 hydraulic micro-manipulator and cells were impaled through their mucosal membranes. The criteria used to determine the acceptability of individual impalements are discussed in detail elsewhere (Armstrong & Garcia-Diaz, 1981; Garcia-Diaz et al., 1983).

Results

Tissue cAMP Levels-Effect of IBMX

In five gallbladders incubated for 30 min at 23°C in the control HCO_3^--free medium, the mean tissue cAMP content was 996 ± 93 (SEM) pmol/g tissue wet weight. The average cAMP content of five gallbladders incubated in an otherwise identical medium containing 0.1 mM IBMX was 2532 ± 348 pmol/g tissue wet weight. The average control value for tissue cAMP found in these experiments is similar to the normal values reported for several mammalian (rat) tissues in which cAMP was assayed by the same technique. These ranged from 1060 pmol/g tissue wet weight in the lung to 1687 pmol/g wet weight in the kidney (Allmann & Kleiner, 1980). The increase in tissue cAMP following exposure of the gallbladders to 0.1 mM IBMX was highly significant ($P < 0.001$). Therefore, although the present experiments give no indication concerning the distribution, between the epithelial cells and the subepithelial tissues, of this increase, it seems reasonable to suppose that, in the former, intracellular cAMP was substantially larger following exposure to IBMX.

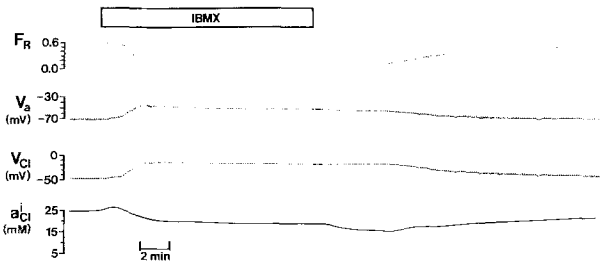

Fig. 1. Top three tracings. Recordings showing the effect of IBMX (0.1 mM, present in the mucosal medium during the period indicated by the horizontal bar) on F_R, V_a and V_{Cl} in *Necturus* gallbladder maintained (at 23°C) between identical HCO_3^--free Ringer's solutions. Bottom tracing: Calculated values of a^i_{Cl}. For explanation of symbols, *see* text

IBMX-Induced Changes in Apical Membrane Potential and Intracellular Chloride Activity

Figure 1 shows an impalement, with a Cl^- double-barreled microelectrode, of an epithelial cell in a gallbladder superfused on both sides with the control HCO_3^--free Ringer's solution used in these experiments. It is apparent from this figure that, following the addition of 0.1 mM IBMX to the mucosal medium, V_a (second tracing from top), measured by the open-tip barrel, rapidly depolarized. In the example shown in Fig. 1, V_a declined from its initial value of −72 to −51 mV. At the same time, F_R (top tracing) rapidly fell from its initial value of 0.59 to a final value of 0.11. It is likely that the onset of these changes is more rapid than Fig. 1 indicates. The delay (about 1 min) between the time that the mucosal medium was changed to one that contained IBMX and the appearance of perceptible changes in V_a and F_R includes the interval required for complete replacement of the fluid in the mucosal half-chamber. When the mucosal fluid was again replaced by the control medium, V_a and F_R returned, slowly at first and then more rapidly, to values (−69 mV and 0.56, respectively) that were close to those initially observed, thus demonstrating the reversibility of the effects of IBMX on V_a and F_R.

Table 1 summarizes the maximal effects of IBMX on V_T, V_a, F_R and R_T observed in these experiments. All the impalements included in this ta-

Table 1. Effect of IBMX on electrophysiological parameters of *Necturus* gallbladder[a]

	V_T (mV)	V_a (mV)	R_T (Ωcm^2)	F_R
Control	−0.22 ± 0.1	−59 ± 3	174 ± 21	0.52 ± 0.02
IBMX	0.52 ± 0.21	−46 ± 2	202 ± 28	0.15 ± 0.01
Δ	0.74 ± 0.19	13 ± 2	28 ± 13	−0.37 ± 0.03
P	<0.01	<0.005	n.s.	<0.001

[a] IBMX (0.1 mM) added to the HCO_3^--free mucosal bathing medium. Average values ± SEM are shown for 17 impalements in seven gallbladders.

Table 2. Effect of IBMX on a^i_{Cl} in *Necturus* gallbladder epithelial cells[a]

	V_a (mV)	a^i_{Cl} (mM)	a^{eq}_{Cl}
Control	−58 ± 4	21.7 ± 1.8	8.6 ± 1.1
IBMX	−48 ± 2	18.3 ± 1.2	13.5 ± 1.2
Δ	10 ± 3	−3.4 ± 0.7	—
P	<0.05	<0.02	—

[a] Average values ± SEM are shown for eight impalements in five gallbladders from the experiments summarized in Table 1. a^{eq}_{Cl} is the intracellular Cl^- activity (calculated from the Nernst equation) that corresponds to an equilibrium distribution of Cl^- across the apical cell membrane.

ble were made with double-barreled microelectrodes. The major findings illustrated in the table are as follows: After exposure of the mucosal surface of the tissue to IBMX, V_T increased significantly from a small serosal negative value (−0.22 mV) to a small serosal positive value (0.52 mV), V_a depolarized by a highly significant amount (13 ± 2 mV), F_R decreased significantly from 0.52 to 0.15 and R_T did not change significantly.

The lower two panels in Fig. 1 illustrate the changes in a^i_{Cl} that followed exposure of the mucosal surface of the tissue to IBMX. The upper of these two panels is a tracing of V_{Cl}, the intracellular potential registered by the Cl^--selective barrel of the double-barreled microelectrode. It is important to note that, in the experiment illustrated by Fig. 1 and similar experiments in the present series, V_a and V_{Cl} were recorded simultaneously but independently with respect to the same external reference electrode. Therefore, V_{Cl} at any point in a tracing, such as that shown in Fig. 1, includes the corresponding value of V_a. For this reason the bottom panel of Fig. 1 is included. The curve shown in this panel reflects the time course of a^i_{Cl}, calculated from the observed data and provides a direct representation of the changes in this parameter that were found during the experiment.

As expected on the basis of earlier observations with cAMP (Diez de los Rios et al., 1981), 8-Br-cAMP, and theophylline (Petersen & Reuss, 1983), IBMX added to the mucosal medium caused a marked decline in a^i_{Cl} (25.7 to 19.6 mM). It is, however, noteworthy that this decline was much slower than the concomitant depolarization of V_a. Furthermore, following withdrawal of IBMX from the mucosal solution, recovery of a^i_{Cl} close to its initial level was also much slower than the corresponding recovery in V_a. Indeed, in the experiment illustrated in Fig. 1 (and in other experiments not shown), a^i_{Cl} continued to decline for several minutes after the control medium, without IBMX, was readmitted to the mucosal half of the chamber, and remained at a relatively low level at a time when V_a had already repolarized to a significant extent. In the experiment shown in Fig. 1, the minimum value of a^i_{Cl} (16.1 mM) was recorded when V_a had repolarized from −51 to −56 mV.

In the experiments summarized in Table 1, eight impalements in 5 different gallbladders were maintained for a sufficient time (at least 10 min) to record a significant decrease in a^i_{Cl}. The results of these impalements are summarized in Table 2. This table also includes the intracellular Cl^- activities (a^{eq}_{Cl}) that would be expected if the Cl^- ion was in electrochemical equilibrium across the apical membrane of the gallbladder epithelial cell. These were calculated from the appropriate form of the Nernst equation.

The average a^i_{Cl} value for *Necturus* gallbladder under control conditions (21.7 mM) is well within the range of values (about 12 to 31 mM) reported for this tissue by other investigators (Reuss & Grady, 1979; Garcia-Diaz & Armstrong, 1980; Diez de los Rios et al., 1981; Fisher, Persson & Spring, 1981; Zeuthen, 1982; Petersen & Reuss, 1983). Also, as found in these earlier investigations, a^i_{Cl} under control conditions was well above a^{eq}_{Cl}. Following exposure of the tissue to IBMX, a^i_{Cl} declined significantly ($P < 0.02$) and reached a value much closer to the corresponding a^{eq}_{Cl} level. It seems reasonable to suppose that, had the tissues been exposed to IBMX for longer times than those corresponding to the data of Table 2, a^i_{Cl} would have reached a level that did not differ from the corresponding a^{eq}_{Cl} values. Thus, the results shown in Table 2 are in essential agreement with those reported for the action of cAMP, 8-Br-cAMP and theophylline on a^i_{Cl} in *Necturus* gallbladder (Diez de los Rios et al., 1981; Petersen & Reuss, 1983).

Table 3. Effect of IBMX (0.1 mM) on V_a and F_R in Cl^-- and Na^+-free Ringer's[a]

Conditions	V_a (mV)			F_R			Impalements/ gallbladders
	Control	IBMX	Δ	Control	IBMX	Δ	
Cl^--free	−65 ± 3	−52 ± 3	13 ± 1	0.32 ± 0.01	0.20 ± 0.01	−0.12 ± 0.01	7/3
Na^+-free	−62 ± 3	−48 ± 3	13 ± 4	0.46 ± 0.5	0.16 ± 0.03	−0.30 ± 0.09	6/3

[a] Average values ± SEM are shown.

Ionic Basis of IBMX Effects on V_a

The simplest explanation for the depolarization of V_a, and the concomitant decrease in F_R induced by mucosal IBMX in the present studies, would be an increase in the conductive permeability of the apical cell membrane to one or more ions, so distributed under control conditions that this increase would generate a depolarizing current across the membrane. Since a^i_{Cl} is above equilibrium under these conditions (Table 2), an increase in P_{Cl}, such as that reported by Petersen and Reuss (1983) could be responsible for the effects of IBMX on V_a and F_R shown in Fig. 1 together with the IBMX-induced decrease in a^i_{Cl} (Table 2). If this were so, one would predict that when a^i_{Cl} is reduced to zero or near zero levels during immersion of the tissue in a Cl^--free medium, the IBMX-induced depolarization of V_a should disappear or at least be greatly attenuated. To test this prediction, gallbladders were superfused for at least 60 min on both sides with Cl^--free solutions (*see* Materials and Methods). Cells were impaled with single-barreled open-tip microelectrodes and V_a was permitted to stabilize. The mucosal medium was then replaced by a similar Cl^--free medium that contained IBMX and the resulting change in V_a was monitored.

The results of these experiments are summarized in Table 3. It is apparent from this table that the average depolarization induced by IBMX (13 ± 1 mV) under Cl^--free conditions was identical with that observed in control media (Table 1). It is of interest that, in Cl^--free conditions, the initial value of F_R and the IBMX-induced change in this parameter (Table 3) were both considerably smaller than they were in normal Ringer's (Table 1). Although this may be due in part to the relatively small number of experiments included in Table 3, it could reflect the fact that, in *Necturus* gallbladder, removal of Cl^- from the mucosal bathing medium enhances apical membrane K^+ conductance, P_{Ka} (Garcia-Diaz et al., 1983). In these experiments, R_T did not change significantly (322 ± 20 to 327 ± 16 Ωcm^2) following exposure of the tissue to IBMX. V_T increased (in a serosal positive direction) from −0.89 ± 0.10 to −0.59 ± 0.09 mV.

It seems clear, therefore, that the effects of IBMX on V_a and F_R observed in our experiments cannot be accounted for by an increase in apical P_{Cl} alone. One should be aware, however, that the experiments in Cl^--free media reported above do not rule out an IBMX-induced increase in apical membrane P_{Cl} such as that reported by Petersen and Reuss (1983) following exposure of *Necturus* gallbladder to theophylline. They do, however, show that under the conditions of our experiments, any changes in V_a arising from an increase in P_{Cl}, if this occurs, are completely overshadowed by the influence of some other event on this parameter. It is of interest to note that in the experiments in which Petersen and Reuss (1983) demonstrated a hyperpolarizing response of V_{Cl} to removal of Cl^- from the mucosal medium, Cl^- removal was performed after the tissue had been exposed to theophylline, i.e. at a time when other theophylline-induced effects would already have occurred.

Experiments were also performed in which, following impalement, the mucosal solution was replaced by one that was Na^+-free (*see* Materials and Methods). When the electrical parameters (V_a, V_T, R_T) had stabilized, the mucosal medium was again replaced, this time by an identical solution that contained 0.1 mM IBMX. It is evident (Table 3) that this maneuver had no effect on the depolarization of V_a or the decrease of F_R by IBMX. Hence, it may be concluded that a change in apical membrane P_{Na} is not a major determinant of these changes.

Under Na^+-free conditions V_T (−26.5 ± 3.2 mV) and R_T (633 ± 75 Ωcm^2) were quite high. This is to be expected since Na^+, for which the paracellular shunt pathway has a relatively high permeability, was unilaterally replaced by Tris, for which the shunt pathway has a much lower permeability (Reuss, 1979*b*). Following exposure of the tissue to IBMX, V_T increased significantly by 5.1 ± 1.0 mV in the direction of serosal positivity and R_T declined

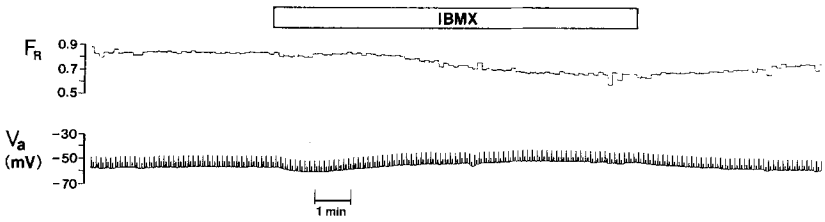

Fig. 2. Effect of IBMX (0.1 mM added to the mucosal medium at the time indicated by the horizontal bar) on F_R and V_a in *Necturus* gallbladder superfused with identical Ringer's solutions containing 25 mM HCO_3^-

Table 4. Effect of IBMX (0.1 mM) on V_a in Ringer's containing 25 mM HCO_3^-[a]

	V_a (mV)	P
Control	−67 ± 2	
IBMX (maximum)	−70 ± 2	
ΔV_a	−3 ± 1	<0.05
IBMX (final)	−59 ± 2	
ΔV_a (maximum-final)	11 ± 2	<0.005

[a] Average values ± SEM are shown for 11 impalements in five gallbladders.

significantly by 57 ± 14 Ωcm². These changes are consistent with an IBMX-induced decrease in shunt resistance under these conditions. Note that in these and subsequent experiments where the tissue was exposed at a given time to mucosal and serosal bathing media of widely different composition, the appropriate electrical parameters were corrected for liquid junction potentials (Garcia Diaz et al., 1983).

The experiments described so far were performed in HCO_3^--free media. The possibility remained that an increase in apical membrane P_{HCO_3} could be the cause of the observed depolarization by IBMX. This could occur if, under normal conditions, endogenously produced HCO_3^- accumulated within the cell to a level exceeding that required for its equilibrium distribution across the apical membrane. In this situation, the opening by IBMX of a HCO_3^- channel in the apical membrane (Saito & Wright, 1983*b*) could result in depolarization of V_a.

To test this possibility, gallbladders were first equilibrated with normal HCO_3^--free Ringer's. Following this, the superfusate was changed to a solution that contained 25 mM HCO_3^-. If the IBMX-induced depolarization is due to an increase in apical membrane P_{HCO_3}, one might predict that addition of a relatively large concentration of HCO_3^- to the external medium would, in all likelihood, alter the direction of the transmembrane electrochemical gradient for this ion and lead to a hyperpolarization rather than a depolarization of V_a following exposure of the tissue to IBMX. Figure 2 shows the result of an experiment designed to test this prediction. It will be seen that, following impalement in a medium containing 25 mM HCO_3^-, the steady-state V_a was −57 mV. Addition of 0.1 mM IBMX caused a transient hyperpolarization of V_a (maximum value −61 mV). Following this, V_a depolarized from −61 to −51 mV. In this experiment, F_R declined from its initial value of 0.82 to 0.60.

Table 4 summarizes the changes in V_a observed during 11 impalements with five gallbladders. This table shows that in HCO_3^- media, mucosal IBMX caused a significant hyperpolarization of V_a (−3 ± 1 mV). This was followed by a larger depolarization. The final steady-state value of V_a in these experiments was 8 mV below its initial value. The difference between the maximum value of V_a following exposure of the cells to IBMX and the final steady-state value of V_a in the presence of this agent (11 ± 2 mV) did not differ significantly from the corresponding changes observed under other experimental conditions (Tables 1 and 3). The effects of IBMX on the other electrophysiological parameters monitored during these experiments can be summarized as follows: F_R decreased from 0.78 ± 0.08 to 0.61 ± 0.06 ($P < 0.005$), V_T (referenced to the mucosal solution) increased from −0.78 ± 0.28 to 0.94 ± 0.18 mV ($P < 0.005$) and R_T increased from 167 ± 12 to 190 ± 16 Ωcm² ($P < 0.025$).

The initial hyperpolarization of V_a by IBMX, observed in these experiments, indicates that this agent does increase apical membrane P_{HCO_3}. However, the fact that this hyperpolarization was followed by a depolarization identical to that observed under other conditions clearly shows that an increase in apical membrane P_{HCO_3} cannot alone account for the IBMX-induced effects observed in this study. It is interesting that the only significant increases in R_T observed during the present experiments were found in media containing HCO_3^-. This agrees with the observations of Duffey et al. (1981) and of Petersen and Reuss (1983). The former authors observed that 8-Br-cAMP induced a significant increase in R_T when gallbladders were immersed in a medium that contained HCO_3^- and suggested that this might reflect an effect of 8-Br-cAMP on the paracellular shunt resistance. Petersen and Reuss (1983), working with HCO_3^--free me-

dia, failed to observe any changes in R_T. Our results could be interpreted as suggesting a role for HCO_3^- in the paracellular effects reported by Duffey et al. (1981). However, further speculation concerning this possibility seems unwarranted until additional data are on hand.

In a final attempt to probe the role of apical membrane conductance changes in the IBMX-induced depolarization of V_a, the effect of Ca^{2+} removal in solutions containing 25 mM HCO_3^- was explored. In these experiments, freshly excised gallbladders were initially mounted between identical control Ringer's solutions. When the transepithelial electrical characteristics had stabilized, a cell was impaled. As soon as V_a reached a steady state, the mucosal superfusate was changed to one from which Ca^{2+} had been omitted and to which in some cases 10 mM EGTA had been added. When V_a again reached a steady state[1], this solution was changed to a similar solution containing 0.1 mM IBMX and the resultant change in V_a was measured. Finally, the mucosal chamber was again perfused with the control medium and V_a was allowed to recover to its initial value. The results obtained in these experiments were essentially similar to those shown in Fig. 2 and Table 4, i.e., a hyperpolarization of V_a (3 ± 1 mV) followed by a more pronounced depolarization (11 ± 2 mV). In eight impalements with three gallbladders, the difference between the maximum value of V_a and its steady-state value in the presence of IBMX was 13 ± 2 mV. F_R declined from a control value of 0.52 ± 0.03 to 0.29 ± 0.07.

In summary, the experiments presented up to this point provided evidence for an IBMX-induced increase in apical membrane P_{HCO_3}. However, they failed to uncover any specific conductance change at the apical cell membrane that could account for the marked depolarization of V_a and the concomitant decrease in F_R observed in the present study. It is unlikely that these observations reflect an effect of IBMX or cAMP on P_{Ka}, the K^+ permeability of the apical membrane. It has been found (Reuss, Cheung & Grady, 1981; Garcia-Diaz et al., 1983) that, in *Necturus* gallbladder, an increase in P_{Ka} reduces F_R as one would predict. However, under these conditions, V_a hyperpolarizes. Conversely, a decrease in P_{Ka} depolarizes V_a and increases F_R. These considerations strongly suggest that the principal event underlying the IBMX-induced decreases in V_a and F_R occurs, not at the apical membrane, but at some other location in the gallbladder epithelium. This raises two possibilities. The first is that the effects of IBMX are the result of a cAMP-mediated change in the properties of the paracellular shunt pathway. The second possibility is that they arise from a reduction, by intracellular cAMP, in the ionic conductance of the basolateral cell membrane. The latter would most probably reflect a decrease in basolateral K^+ permeability, P_{Kbl}, since this accounts for more than 90% of the total basolateral membrane conductance (Reuss, 1979*a*).

In a general sense, the IBMX-induced reductions in V_a and F_R found in this study can readily be interpreted in terms of a decrease in P_{Kbl}. Such a decrease would reduce F_R which, at least approximately, reflects the ratio $Ra/(Ra + Rbl)$ where Ra and Rbl are the resistances of the apical and basolateral cell membranes, respectively (*see* Fig. 5*a*). At the same time, a decrease in P_{Kbl} would lower the basolateral cell membrane potential (V_b) and, because the paracellular shunt pathway in *Necturus* gallbladder is highly conducting, this reduction in V_b should induce a concomitant decrease in V_a. It seemed appropriate therefore to explore this interpretation in more detail. For this purpose, two experimental approaches were chosen. The first was to investigate the effect of IBMX on the depolarization of the basolateral cell membrane by K^+ ions. In terms of the well-known constant field equation of Goldman (1943), one would predict that a specific decrease in P_{Kbl} following exposure of the tissue to mucosal IBMX would attenuate the depolarization of V_b and the concomitant depolarization of V_a induced by an increase in the K^+ concentration of the serosal medium. The second approach involved the use of Ba^{2+} to decrease P_{Kbl}. Ba^{2+} is known to block K^+-conducting channels in a number of epithelial cell membranes (Nagel, 1979). It was reasoned that, following the reduction of P_{Kbl} by serosal Ba^{2+}, the effect of mucosal IBMX on V_a and F_R should be significantly attenuated.

To determine the effect of mucosal IBMX on the depolarization of V_a by serosal K^+, gallbladders were mounted between identical control Ringer's solutions containing 2.5 mM KCl and cells were impaled through their mucosal surfaces with open-tip microelectrodes. During a single impalement, the serosal medium was changed to one that contained 80 meq K^+/liter (this was prepared by replacing 77.5 mM NaCl in the control medium with KCl). Following the establishment of a new steady-state V_a value, this high K^+ medium was replaced by the

[1] In these experiments a small depolarization of V_a (average value 5 mV) was observed when Ca^{2+} was removed from the mucosal superfusate. This was not studied in detail. There was no appreciable change in the other electrophysiological parameters recorded. No appreciable differences were noted between solutions with and those without EGTA. The results given here are the pooled averages of experiments performed with and without this agent.

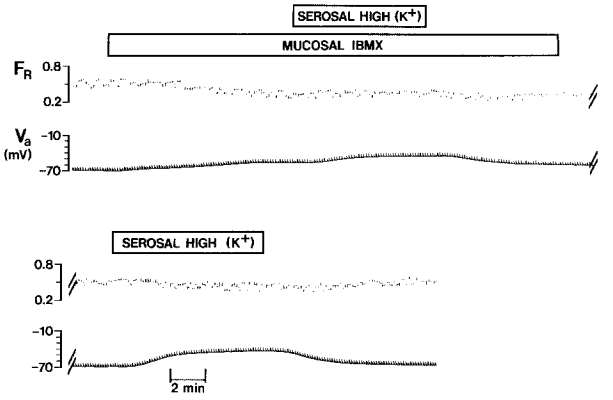

Fig. 3. Effect of 0.1 mM IBMX on the depolarization of V_a induced by increasing the KCl concentration of the serosal medium from 2.5 to 80 mM

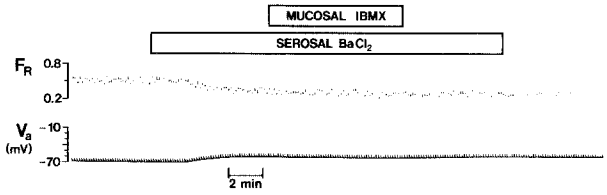

Fig. 4. Inhibition by Ba^{2+} (10 mM $BaCl_2$, present in the serosal bathing medium during the period indicated) of the effects of 0.1 mM IBMX on F_R and V_a

control Ringer's and V_a was allowed to recover to its initial value. At this time the mucosal medium was replaced by an otherwise identical solution containing 0.1 mM IBMX. When V_a and F_R had declined to a new steady state, the serosal solution was again replaced by the high K^+ (80 mM KCl) medium and the depolarizing effect of this solution on V_a was measured in the presence of mucosal IBMX. Normal Ringer's solutions were then admitted to both the mucosal and the serosal compartments of the tissue chamber and V_a was again allowed to recover. Finally, another depolarization of V_a was elicited with the high K^+ (serosal) medium and V_a was allowed to recover.

Figure 3 shows segments from a tracing of one of these experiments. The upper panel of the figure illustrates the changes in V_a and F_R that followed exposure of the mucosal surface of the tissue to IBMX and subsequent exposure of its serosal surface to a medium containing 80 mM KCl. The lower panel shows the change in V_a and F_R that resulted from an increase in serosal K^+ concentration alone. It is evident from Fig. 3 that mucosal IBMX markedly attenuates the decrease in V_a that results from the reversible depolarization of V_b by K^+ ions.

In all, five experiments of this kind were performed with three gallbladders. The results are summarized in Table 5. The salient findings in these experiments were as follows: As expected (Reuss, 1979*a*), when serosal [K^+] was increased from 2.5 to 80 mM, V_a was strongly and reversibly (Fig. 3) depolarized. At the same time F_R increased by about 20%, R_T decreased and V_T became more negative with respect to the mucosal bathing solution. All these changes were significant ($P < 0.05$). Again, with one exception, the effects of mucosal IBMX alone, i.e. a depolarization of V_a, a decrease in F_R and no change in R_T, were as expected on the basis of Table 1. The exception was that no significant change in V_T was observed in the experiments shown in Table 5. In the presence of mucosal IBMX, the depolarization of V_a induced by high serosal [K^+] was significantly smaller than that observed under control conditions. This is consistent with a cAMP-induced decrease in P_{Kbl} following exposure of the tissue to IBMX.

The experiments with Ba^{2+} were performed as follows: Following impalement (with an open-tip microelectrode) of a cell in a bladder exposed on both sides to normal Ringer's solutions, the serosal bathing medium was replaced by one that contained 10 mM $BaCl_2$ (*see* Materials and Methods). Subsequently, the mucosal medium was replaced by a similar solution that contained 0.1 mM IBMX. Figure 4 illustrates the results obtained in one such experiment. It is clear from this figure that, when Ba^{2+} was added to the serosal compartment of the tissue chamber, V_a and F_R decreased. These decreases are consistent with a decrease, in the presence of Ba^{2+} of P_{Kbl}. It is also clear from Fig. 4 that subsequent addition of IBMX to the mucosal bathing solution had no apparent effect on V_a or F_R. The results, with respect to V_a and F_R, of four experiments of this kind in four different gallbladders are shown in Table 6. They confirm the conclusions suggested by Fig. 4, i.e. that, in the presence of serosal Ba^+, mucosal IBMX had no significant effect on V_a or F_R. In these experiments, neither IBMX nor Ba^{2+} had any effect on R_T. Ba^{2+} did increase V_T significantly (from 0.7 to 1.9 mV). We interpret this as further evidence that the effects of IBMX on the electrical parameters of *Necturus* gallbladder (Table 1) are primarily the result of a cAMP-mediated decrease in P_{Kbl}.

Discussion

The effects of mucosal IBMX on the apical cell membrane in *Necturus* gallbladder reported herein closely resemble the corresponding effects observed with other agents that elevate intracellular

Table 5. Effects of high serosal $[K^+]$ in the presence and absence of mucosal IBMX (0.1 mM)[a]

Conditions	V_T (mV)	V_a (mV)	R_T (Ωcm^2)	F_R
Control	−0.01 ± 0.5	58 ± 3	272 ± 16	0.43 ± 0.02
High $[K^+]$	−4.5 ± 0.4[b]	40 ± 4	235 ± 17	0.52 ± 0.03
Δ_1	−4.5 ± 0.4[c]	18 ± 2[c]	−36 ± 11[c]	0.09 ± 0.03[c]
IBMX	0.1 ± 0.5	49 ± 3	279 ± 27	0.26 ± 0.02
IBMX + high $[K^+]$	−4.4 ± 0.3[b]	39 ± 4	237 ± 22	0.31 ± 0.01
Δ_2	−4.5 ± 0.8[c]	10 ± 3[c]	−42 ± 11[c]	0.05 ± 0.02

[a] Average values (± SEM) for five impalements in three gallbladders.
[b] Corrected for liquid junction potential (*see* Garcia-Diaz et al., 1983).
[c] $P < 0.05$.

Table 6. Effect of IBMX (0.1 mM) on V_a and F_R in the presence of serosal $BaCl_2$ (10 mM)[a]

Conditions	V_a (mV)	F_R
Control	61 ± 9	0.46 ± 0.04
IBMX	52 ± 7	0.22 ± 0.04
Δ	9 ± 2	−0.24 ± 0.06
Control	63 ± 9	0.43 ± 0.03
Ba^{2+}	56 ± 7	0.30 ± 0.03
Δ	7 ± 1	−0.13 ± 0.03
Ba^{2+} + IBMX	55 ± 8	0.21 ± 0.06
Δ	1 ± 1[b]	−0.10 ± 0.06[b]

[a] Average values ± SEM for four impalements in four gallbladders.
[b] No significant change ($P > 0.05$).

cAMP levels (Duffey et al., 1981; Petersen & Reuss, 1983). Moreover, IBMX, in our experiments, caused a significant increase in tissue cAMP. In other cell types, the effects of IBMX on such parameters as membrane conductances have been linked to the increase in intracellular cAMP it elicits (Klein & Kandel, 1978, 1980; Kaczmarek, Jenning & Strumwasser, 1978; Saito & Wright, 1983*a*). It seems reasonable, therefore, to propose that enhanced intracellular cAMP is the effector of the changes in membrane conductance and intracellular Cl^- activity observed in the present study.

The only effect of IBMX on apical membrane conductances that is apparent from our experiments is induction of a permeability to HCO_3^- or an increase in a small, pre-existing P_{HCO_3}. This agrees with the findings of Saito and Wright (1983*b*) in the choroid plexus. The absence of any observable effect on P_{Na} is in agreement with the results obtained by Petersen and Reuss (1983) in their experiments with theophylline. Although no apparent effect on apical P_{Cl} was found in the present study, Petersen and Reuss (1983) have presented convincing evidence that cAMP does open channels in the apical cell membrane that are permeable to Cl^-. Our failure to observe any increase in apical P_{Cl} (Table 3) is probably due to the fact that, under the conditions of the present experiments, the effects of such an increase on V_a and F_R were small compared to those arising from other changes in membrane permeability that occurred at or about the same time, in particular the decrease in P_{Kbl} (Figs. 3, 4 and Tables 5, 6). On the basis of our results, as well as those of Petersen and Reuss (1983) and of Saito and Wright (1983*a*) one might speculate that cAMP opens anion-selective channels in the apical cell membrane that are permeable to both HCO_3^- and Cl^-. If so, our results (Fig. 2 and Table 4) would suggest that these channels are more selective to HCO_3^- than to Cl^-. In this context, it is of interest to note that the experiments of Petersen and Reuss (1983) were performed in HCO_3^--free media. Further investigation is needed to establish whether the increase in P_{HCO_3} observed in this study and the increase in P_{Cl} reported by Petersen and Reuss (1983) are due to the opening by cAMP of an anion-selective channel permeable to both these ions or to the opening by this nucleotide of separate HCO_3^--permeable and Cl^--permeable channels. In either event it seems clear that, under the conditions of the present study, these changes in apical membrane permeability cannot account for more than a small fraction of the observed effects of IBMX on V_a and F_R. Furthermore, as already mentioned in the Results section, earlier studies in this laboratory and elsewhere (Garcia-Diaz et al., 1983; Reuss et al., 1981) have shown that the simultaneous decreases in V_a and F_R induced by IBMX (*see* e.g. Table 1) do not match the changes in these parameters that one would predict from an increase or a decrease in P_{Ka}. This would appear to rule out an IBMX-induced change in P_{Ka} as the major mechanism underlying the results reported herein. Thus, one may conclude

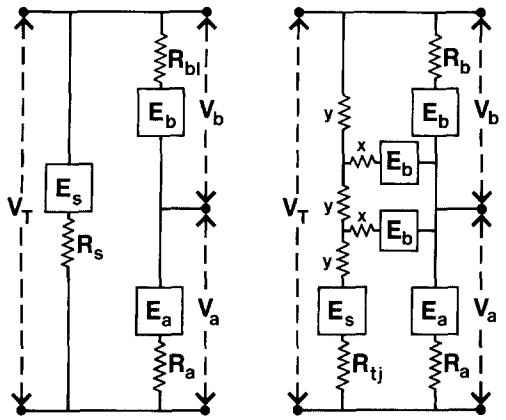

Fig. 5. Lumped (*a*) and distributed (*b*) electrical equivalent circuit models for gallbladder epithelium. For detailed explanation *see* text

that the principal site of the electrophysiological response of *Necturus* gallbladder epithelium to IBMX is not at the apical cell membrane but lies elsewhere in the epithelial cell layer, e.g. the paracellular shunt pathway or the basolateral cell membrane.

Analysis of equivalent electrical circuit models for gallbladder epithelium yields further insights into this question. Two such models are diagrammed in Fig. 5. A simple lumped equivalent circuit that has been widely used for electrophysiological analysis of the gallbladder and other leaky epithelia (*see* e.g. Reuss & Finn, 1975) is shown in Fig. 5*a*. In this model, E_a and R_a, E_b and R_{bl}, and E_s and R_s represent the emf's and resistances across the apical cell membrane, the basolateral cell membrane, and the shunt pathway, respectively, V_a and V_b are the measured potential differences between the cell interior and the mucosal and serosal bathing solutions. V_T is the measured potential differences across the whole epithelium.

Figure 5*b* is the distributed equivalent circuit model proposed by Boulpaep and Sackin (1980). This model is similar to Fig. 5*a* except that R_s and R_{bl} are respresented, not as single resistors, but as distributed resistive networks. R_s is the sum of two components, R_{tj} and R_{lis}, the resistances of the tight junctions and lateral intercellular spaces, respectively. R_{lis} is distributed along the length of the lateral intercellular space and is modelled in Fig. 5*b* as a set of resistive elements ($y, y \ldots$) in series. Similarly, R_b, the lateral component of R_{bl}, is modelled as a set of parallel resistors ($x, x \ldots$) through the area of the basolateral cell membrane that bounds the lateral intercellular space. Boulpaep and Sackin (1980) showed that the model depicted in Fig. 5*b* is appropriate when R_{lis} is a significant fraction of R_s and that, the further R_s diverges from the limiting condition $R_{tj} \rightarrow R_s$, the greater will be the deviation between the predictions of the lumped circuit model (Fig. 5*a*) and the actual behavior of the epithelium.

The assumption that the observed effects of IBMX on the electrical parameters of *Necturus* gallbladder (Table 1) reflect alterations, by this agent, in the properties of the shunt pathway only, may now be compared with the predictions of Figs. 5*a* and 5*b*, respectively. First one may note that, under normal conditions, Fig. 5*a* can be assumed to apply to *Necturus* gallbladder since it has been reported that, in this tissue, R_{tj} accounts for a large fraction (perhaps as much as 98%) of R_s (Spring & Hope, 1978, 1979; Curci & Frömter, 1979). Figure 5*a* yields the following expressions for V_a, V_b and V_T, measured with respect to a fixed reference, e.g. the mucosal bathing solution:

$$V_a = E_a + iR_a \tag{1}$$

$$V_b = E_b + iR_{bl} \tag{2}$$

$$V_T = E_s + iR_s \tag{3}$$

where i, the current flowing through the loop depicted in Fig. 5*a*, is obtained as $(E_b - E_a - E_s)/(R_a + R_{bl} + R_s)$. If, following exposure of the tissue to IBMX, the ratio R_{tj}/R_{lis} remains such that Fig. 5*a* is still an acceptable basis for analysis, any IBMX-induced effect on the shunt pathway (e.g. an alteration in its overall resistance or its ionic permselectivity) can be modelled as a change in R_s, a change in E_s, or changes in both these parameters. The interpretation, in these terms, of the data exemplified in Table 1 encounters two major difficulties. First, it is difficult to account in terms of this model, for the marked decrease in the ratio $\Delta V_a/\Delta V_T$ (i.e. F_R) induced by IBMX. Second, the effects of IBMX on V_a, R_T and V_T found in the present study are not quantitatively compatible with a simple change in the magnitude of R_s or E_s. This can be shown as follows: In *Necturus* gallbladder $R_s \ll R_t$, where $R_t = R_a + R_{bl} + R_s$. Therefore, even relatively large changes in R_s will leave R_t virtually unchanged. Moreover, when the tissue is bathed on both sides by identical solutions, E_s, under control conditions is zero or close thereto (Reuss & Finn, 1975). Under these conditions, Eq. (1) predicts that, for a change in R_s only

$$\Delta V_a = E_a \cdot \frac{\Delta R_s}{R_t}. \tag{4}$$

Similarly, for a change in E_s only, Eq. (1) predicts that

$$\Delta V_a = -\Delta E_s \cdot \frac{R_a}{R_t}. \tag{5}$$

Equations (4) and (5) can be solved numerically by combining the average value of ΔV_a found in the present study (13 mV, *see* Table 1) with published estimates of the relevant parameters of Fig. 5*a*. The values chosen for the latter are shown in Table 7. Two widely disparate sets of values for R_a, R_{bl}, and R_s are shown in this table. One set (Frömter, 1972) is representative of the majority of estimates so far published (*see* Corcia & Armstrong, 1983). The other set (Suzuki et al., 1982) is included because it differs widely from other estiamtes of these parameters and because, fortuitously, it shows that even large uncertainties in these estimates do not invalidate the arguments presented herein.

Insertion of the two values of R_t obtained from Table 7 (7520 and 1512 Ωcm^2), together with $E_a = -27.2$ mV, into Eq. (4) gives estimates of 3628 and 771 Ωcm^2, respectively, for the change in R_s required to depolarize V_a by 13 mV. The corresponding changes in R_T, which is equal to $R_s(R_a + R_{bl})/R_t$, are 3384 and 668 Ωcm^2. Since R_T did not change appreciably following exposure of the tissue to IBMX (Table 1) it is clear that a change in R_s does not offer a likely explanation for the IBMX-induced depolarization of V_a noted in this study.

If one inserts in turn the two values of R_a from Table 7 in Eq. (5), one finds that a ΔE_s of -22 or -16 mV would be required to depolarize V_a by 13 mV. Assuming that, under control conditions, $E_s \rightarrow 0$, Eq. (3) shows that, for a change ΔE_s in this parameter.

$$\Delta V_T = \Delta E_s(R_a + R_{bl})/R_t. \tag{6}$$

With the appropriate numerical values of R_a, R_{bl} and R_t (Table 7), Eq. (6) predicts ΔV_T equal to -21 or -15 mV for ΔE_s values of -22 and -16 mV. The average observed value of ΔV_T was 0.74 mV (Table 1). Therefore it appears that, as already shown above for R_s, a change in E_s alone cannot account satisfactorily for the observed effects of IBMX on the electrical characteristics of *Necturus* gallbladder. It can also be shown, although the proof is tedious, that any probable combination of changes in R_s and E_s does not, in terms of Fig. 5*a*, suffice to explain quantitatively the findigns summarized in Table 1.

In principle, Fig. 5*b* permits an IBMX-induced decrease in $\Delta V_a/\Delta V_T$ to be reconciled with a change in shunt resistance alone. This requires only that R_{lis} increase by a sufficient amount, relative R_{tj} (Boulpaep & Sackin, 1980). An increase in R_{lis}, resulting from exposure of the tissue to IBMX, is not an unreasonable expectation. Increased intracellular cAMP is known to inhibit fluid absorption in gallbladder (Reuss, 1979*b*). This could cause collapsing of the lateral intercellular spaces, thereby increasing R_{lis}. However, to apply this prediction to the results shown in Table 1, one is forced to assume also a concomitant and equal decrease in R_{tj}, since R_T remains constant. Moreover, attempts, based on Fig. 5*b*, to predict the combination of effects shown in Table 1 from alterations in the shunt pathway only involve additional arbitrary assumptions that are not readily accessible to experimental study.

Table 7. Representative values for emf's and resistances in Fig. 5*a*[a]

	R_a	R_{bl}	R_s	E_a	E_b
a)	4500	2700	320		
				-27.2[b]	-74.2[b]
b)	1220	201	91		

[a] Resistances in Ωcm^2; emf's in mV (referred to mucosal bathing solution). *a*) from Fromter (1972); *b*) from Suzuki et al. (1982).
[b] Average values from Reuss (1979*b*).

In summary, neither the lumped (Fig. 5*a*) nor the distributed (Fig. 5*b*) equivalent circuit model for *Necturus* gallbladder epithelium allows a simple interpretation, based on alterations in the properties of the shunt pathway, of the electrophysiological effects of IBMX on this tissue. By contrast, analysis, in terms of Fig. 5*a*, of the assumption that these effects result from a cAMP-induced decreased in P_{Kbl} (i.e. an increase in R_{bl}) alone leads in a straightforward fashion to predictions that, allowing for the necessary approximations involved, are in good agreement with the results summarized in Table 1. This can be shown as follows: If R_{bl} changes to R^1_{bl}, where $R^1_{bl} = R_{bl} + \Delta R_{bl}$, the following expression can be derived from Eq. (1),

$$\Delta Va \cdot Rt(Rt + \Delta Rbl) = \Delta Rbl \cdot Ra(Ea - Eb). \tag{7}$$

Similarly, from Eq. (2) one obtains

$$\Delta V_b = (E_a - E_b)\frac{Rbl}{Rt} - \frac{R^1_{bl}}{R^1_t} \tag{8}$$

where $R^1_t = R_t + \Delta R_{bl}$.

Taking ΔV_a as 13 mV (Table 1), together with the appropriate values of $(E_a - E_b)$, R_a and R_t from Table 7, one obtains, from Eq. (7), $\Delta R_{bl} = 6463$ or 789 Ωcm^2. These values of ΔR_{bl} can now be combined with the appropriate values of R_a, R_{bl} and R_s (Table 7) to calculate ΔF_R and ΔR_T, the corresponding changes in F_R and R_T, and, utilizing Eq. (8), to obtain ΔV_{bl} for these conditions. Finally, ΔV_s can be obtained from Eq. (3) or, more simply, as $(\Delta V_a - \Delta V_{bl})$. The results of these calculations, together with the observed values of ΔF_R, ΔR_T, ΔV_{bl}, and

Table 8. Calculated (*a*, *b*) and observed (*c*) effects of IBMS on electrical properties of *Necturus* gallbladder[a]

	F_R			R_T (Ωcm^2)			ΔV_b (mV)	ΔV_s (mV)
	Control	IBMX	Δ	Control	IBMX	Δ		
a)	0.63	0.33	−0.30	306	313	7	14	−0.9
b)	0.86	0.55	−0.31	86	87	1	14	−1.0
c)	0.52	0.15	−0.37	174	202	28[b]	12	0.7

[a] *a* and *b* from Fromter (1972) and Suzuki et al. (1982), respectively. *c*, average values from Table 1.
[b] Not significant ($P > 0.05$).

ΔV_s (from Table 1) are shown in Table 8. It is clear from this table that, considering the approximations involved in calculation, agreement between the observed results and those predicted from an IBMX-induced increase in R_{bl} are highly satisfactory.

Thus, equivalent circuit analysis strongly supports the conclusion that a decrease in P_{Kbl} plays a major role in the electrophysiological response of *Necturus* gallbladder to increased intracellular cAMP levels. This interpretation is further supported by the experiments with high serosal [K^+] and with Ba^{2+} (Tables 5 and 6). It will be seen from Table 5 that IBMX markedly attenuates the depolarization of V_a and the increase in F_R induced by high serosal [K^+]. These are the results that one would expect if IBMX reduced P_{Kbl} to a significant extent[2]. Similarly, it is apparent from Table 6 that Ba^{2+}, which is known to block K^+ channels in epithelial cell membranes, has effects on V_a and F_R that are very similar to those induced by IBMX. Furthermore, in the presence of serosal Ba^{2+}, the depolarizing effect of IBMX on V_a is virtually abolished and its effect on F_R is substantially reduced.

To our knowledge, a decrease in P_{Kbl} induced by increased intracellular cAMP levels has not previously been reported for the gallbladder or other epithelia. However, cAMP-dependent closure of K^+ channels (induced, *inter alia*, by external application of IBMX) has been reported in molluscan neural systems (Klein & Kandel, 1980; Adams & Levitan, 1982). The mechanism of this effect is not clear. There is strong evidence that, in molluscan neurons, phosphorylation of K^+-channel proteins by cAMP-activated protein kinases plays an important role in the regulation of membrane K^+ conductance. However, detailed interpretation of the regulatory role of protein phosphorylation is complicated by the fact that, depending on the animal species and the specific type of K^+ channel involved, K^+ conductance may be either decreased or enhanced by cAMP (Adams & Levitan, 1982; de Peyer et al., 1982). It is also unclear whether, or to what extent other intracellular responses to cAMP, e.g. changes in intracellular Ca^{2+} concentration and/or intracellular pH, are implicated in the modification of K^+ channel conductances by phosphorylation. To explore this question in the gallbladder, direct measurements with Ca^{2+}- and H^+-selective microelectrodes of intracellular pH and Ca^{2+} activity have been initiated.

Taken together, the permeability changes observed in the present experiments, i.e. an increase in apical membrane P_{HCO_3} (and P_{Cl}?) together with a decrease in basolateral P_K, offer an explanation for the cAMP-induced secretion of K^+ and HCO_3^- observed in guinea pig gallbladder (Heintze et al., 1979). Under appropriate conditions, i.e. where intracellular HCO_3^- concentration exceeds the level consistent with a purely passive distribution of this ion across the apical cell membrane, the opening of cAMP of HCO_3^--conducting channels in this mem-

[2] Although the results reported in Table 5 are in qualitative agreement with those reported earlier by Reuss (1979*a*) for the effect of high serosal [K^+] on *Necturus* gallbladder, there are significant quantitative discrepancies. In particular, since both sets of experiments were performed under virtually identical conditions, the depolarization of V_a (18 ± 2 mV) shown in Table 5 is substantially less than the value (about 35 to 40 mV) one would predict from Table 1 and Fig. 4 of Reuss' (1979*a*) paper. A complete accounting for this discrepancy is not possible on the basis of our results. Analysis of our Fig. 5*a* does however suggest a partial explanation at least. If one ignores the relatively small change in R_t induced by high serosal [K^+] and assumes $E_S \approx 0$, one obtains from Eq. (1) the expression $\Delta V_a = \Delta E_b \cdot R_a/R_t$ for a change in E_b only. Assuming that ΔE_b, in both Reuss' studies and ours, was the same for a given increase in serosal [K^+], the value of ΔV_a observed in each case would depend on R_a/R_t. In Table 1 of his paper, Reuss (1979*a*) gives 160 Ωcm^2 as the control value for R_s. The corresponding value from Table 5 is 272 Ωcm^2. More significantly, Reuss (1979*a*) found $R_a/R_{bl} = 2.13$ for these conditions. For the gallbladders used in the present study one obtains, from Table 5, $R_a/R_{bl} = 0.75$. Thus, R_a/R_t must have been considerably lower in our experiments than in those of Reuss (1979*a*). Conversely, the "attenuation" of ΔV_a with respect to E_b, must, in our experiments have been correspondingly higher.

brane would result in a net (secretory) HCO_3^- movement from the cell interior to the luminal fluid. Similarly, since both the apical and basolateral cell membranes are normally highly permeable to K^+, and since, under normal circumstances, net K^+ entry into the epithelial cells is mainly, if not exclusively, due to the operation of the basolateral Na^+/K^+ ATPase pump, a decrease in basolateral K^+ conductance could, by increasing the fraction of K^+ efflux that occurs *via* the apical membrane, give rise to a net secretion of K^+.

In common with other agents that elevate intracellular cAMP levels (Diez de los Rios et al., 1981; Petersen & Reuss, 1983) IBMX induces a decline towards its equilibrium value of a^i_{Cl} in *Necturus* gallbladder. Different interpretations of this effect have been proposed. Diez de los Rios et al. (1981), who did not observe the depolarizing effect of cAMP on V_a, considered that the decrease in a^i_{Cl} induced by this agent was due to an inhibition, by intracellular cAMP, of coupled electroneutral NaCl entry across the apical membranes of the epithelial cells. Petersen and Reuss (1983) proposed that Cl^- exit, *via* cAMP-induced Cl^- channels, across the apical membrane, could account for the observed decrease in a^i_{Cl}. They did not, however, entirely rule out the possibility that cAMP-induced inhibition of coupled NaCl entry contributed to this phenomenon. Our results (Table 4) would suggest that, under the conditions of the present experiments, Cl^- exit by electro-diffusion across the apical cell membrane may not alone be sufficient to account for the observed decline in a^i_{Cl} and that inhibition by cAMP of transapical NaCl entry and/or transapical Cl/HCO_3 exchange (Reuss & Constantin, 1984) may be important contributory factors in this process. However, further work is needed to clarify this point.

Finally, some comment on the failure, by Diez de los Rios et al. (1981), to observe the cAMP-induced depolarization of V_a reported by other workers (Duffey et al., 1981; Petersen & Reuss, 1983) and clearly demonstrated in the present study, seems appropriate. Although the experiments reported herein did not directly address this issue, they provide some evidence that points to a possible explanation of the discrepancy. It should be noted that, in the experiments of Diez de los Rios et al. (1981), gallbladders were initially mounted in divided chambers between identical Ringer's solutions. V_a and a^i_{Cl} were then measured under these conditions, with single-barreled open-tip and Cl^--selective microelectrodes. Following this, the serosal solution was replaced by one that contained cAMP and, *after at least 60 min*, V_a and a^i_{Cl} were again measured with single-barreled microelectrodes. In the present experiments we found that, during prolonged impalements in the presence of IBMX, there was, occasionally, a significant spontaneous repolarization of V_a. This was not studied in detail, but in one experiment where this was noted, the impalement was allowed to continue. The results obtained were as follows: Following exposure of the tissue to IBMX, V_a depolarized, over 5 min, from an initial value of -73 to -51 mV. However, 40 min after reaching its minimal value of -51 mV, V_a had increased again to -72 mV. A cAMP-induced depolarization of V_a, followed by a spontaneous repolarization to its initial value (or close thereto) could account for the apparent constancy of V_a, during exposure of the tissue to cAMP, reported by Diez de los Rios et al. (1981).

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