J. Membrane Biol. 25, 115–139 (1975)

Electrical Properties of the Cellular Transepithelial Pathway in *Necturus* Gallbladder

I. Circuit Analysis and Steady-State Effects of Mucosal Solution Ionic Substitutions

Luis Reuss and Arthur L. Finn

Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Received 8 May 1975; revised 30 August 1975

Summary. Microelectrode techniques were employed to measure the electrical resistance of the cell membranes and the shunt pathway, and the equivalent electromotive forces (EMF's) at both cell borders in *Necturus* gallbladder epithelium. The cell is, on the average, 57 mV negative to the mucosal solution and 59 mV negative to the serosal solution. The transepithelial potential (V_{ms}) ranges from 0.5 to 5 mV, serosal solution positive. Assuming that the shunt EMF (V_s) is zero with standard Ringer's bathing both sides of the tissue, both cell membrane EMF's are oriented with the negative pole toward the cell interior and are 39.9 ± 3.6 mV (apical, V_a), and 69.4 ± 1.8 mV (basal-lateral, V_b). The values of the resistances of the cell membranes and the shunt are similar to those previously reported by others: apical (R_a), $3350 \pm 390\ \Omega\ cm^2$, basal-lateral ($R_b$) $2750 \pm 320\ \Omega\ cm^2$, shunt ($R_s$), $480 \pm 50\ \Omega\ cm^2$. Ionic substitutions on the mucosal side produce changes in both EMF and resistance of the apical membrane and the shunt pathway. Increasing K concentration to 112 mM reverses V_a and greatly reduces R_a. Complete Na replacement with an inert nonpermeant cation slightly increases V_a and R_a. These results indicate that across the apical membrane $P_K > P_{Na}$. Analogous measurements of V_s indicate cation permselectivity, with $P_K > P_{Na} > P_{choline} \sim P_{TEA} \sim P_{methylglucamine}$. In general, changes in V_s are very similar to the changes in V_{ms}, indicating that the latter measurements yield adequate information on the properties of the shunt. The fact that $P_{Na} > P_{Cl}$ across the shunt rules out the possibility that V_{ms} is generated by a NaCl concentration gradient across the limiting junction.

Low resistance ("leaky") sodium transporting epithelia are characterized by the existence of a shunt pathway of high ionic conductance [3, 10, 16, 43] in parallel with a cellular pathway. In *Necturus* gallbladder, it has been shown that the shunt pathway is intercellular [17, 18]. Leaky epithelia such as kidney proximal tubule [4, 5, 19], gallbladder [7, 22, 28, 41] or upper intestine [2, 35], characteristically exhibit a low transepithelial potential difference. This has been interpreted as an indication that the transepithelial transport process is "neutral" or "electrically

silent" [8, 9]. However, the measurement of shunt conductances 1 to 2 orders of magnitude higher than the cellular conductance suggests the alternative explanation of dissipation, through the shunt, of any electrical gradient generated by transepithelial transport. In fact, the transepithelial potential across gallbladders of several species increases when the conductance of the shunt pathway is selectively reduced by triaminopyrimidinium [29]. However, the possibility remains that this correlation between shunt resistance and transepithelial potential is unrelated to the transport process.

The potential profile across this kind of epithelium is characterized by a cell negative to both bathing solutions [17, 20, 35, 43]. The mechanisms of generation of the potentials at each cell border are unclear. Although the high K permeability of the basal-lateral membrane could account for most of its potential, in some tissues a parallel electrogenic or rheogenic mechanism of sodium extrusion has been suggested [37, 42]. Less information is available on the apical membrane, where Na entry in some preparations increases after the addition of glucose or amino acids [26, 35], and where the Na and K permeabilities are unknown. Even the orientation of the electromotive force generated at this border is controversial. The measurement of a negative potential across the apical cell membrane does not prove that the electromotive force (EMF) generated at this border has the same polarity: if the orientation of the apical EMF were such as to make the cell positive to the mucosal solution, it is still possible to have a negative apical membrane potential if the EMF generated at the basal-lateral membrane is high enough, and the resistance of the shunt low enough [36]. An apical EMF oriented with the positive pole toward the cell interior would suggest a high Na permeability and a mainly passive mechanism of sodium entry. On the other hand, if the EMF is oriented with the negative pole toward the cell interior, the apical Na permeability has to be low relative to that of other ions, suggesting a different Na transport mechanism at this level.

Electrical analyses of leaky epithelia are complicated by the presence of the low resistance shunt, and by the fact that the shunt exhibits ionic permselectivity. Thus, unilateral changes in bathing solution composition can alter the ipsilateral membrane potential in a complex fashion, in which the membrane current generated is partially shunted, and the new potential produced in the shunt itself influences the cell potential. In order to obtain the transference numbers for individual ions across the cell membrane it is necessary to evaluate the change in equivalent EMF

at that membrane (and not only the change in measured potential) as a function of the external ionic concentrations. This requires the measurement of the resistances of the cell membranes and the shunt pathway, in addition to the potentials across both cell membranes. From the changes in cell membrane and shunt resistances after changes in external solution it is also possible to calculate their relative ionic permeabilities, independently of the changes in electrical potentials.

In order to make such measurements, the epithelium must be structurally simple, in order to apply a precise mathematical treatment to its cable properties, and to have relatively big cells, in order to tolerate prolonged microelectrode impalements without significant leakiness or deterioration of the cell potentials. The gallbladder of *Necturus* fulfills these requirements [17] and was in consequence chosen for these studies. In summary, these microelectrode experiments are intended to provide a complete circuit analysis of a typical leaky epithelium, to determine the influence of cation substitutions on the electromotive forces generated at the cell borders and across the shunt pathway, and thus to obtain new insight into the mechanisms of production of the cellular and transepithelial electrical potentials, and their relationship to transepithelial electrolyte transport.

The calculation of equivalent EMF's and permeability coefficients depends on several difficult-to-test assumptions, such as constancy of intracellular ion activities and contralateral cell membrane EMF (after mucosal solution changes), and the value of the shunt EMF under control conditions. In this and the following paper [33] three different kinds of experiments were performed: measurements of steady-state potentials and resistances, measurements of rapid changes in potentials with microelectrode impalements of several cells, and continuous recording from single cells during changes in the mucosal solution. It will be shown that the results of these different experimental procedures and assumptions are internally consistent and validate each other.

Materials and Methods

Necturi (*Necturus maculosus*) were obtained from Mogul-Ed Co., Oshkosh, Wisconsin, and kept in tap water, at 4° C, in the dark. Animals were either decapitated or anesthetized by immersion in 1.5% urethane. The gallbladder was removed, opened longitudinally, washed in standard Ringer's solution, pinned to a cork ring (with enough stretch to avoid macroscopic foldings), and mounted on a lucite ring with an exposed area of 0.63 cm^2. The preparation was then set up horizontally in a lucite chamber (mucosal side upwards),

and was supported on an agar-Ringer's cylinder or a nylon mesh. The serosal solution was continuously changed (from an oxygenated reservoir) by perfusion under negative pressure. The mucosal solution was either continuously replaced by gravity superfusion or changed at 10 to 20 min intervals. All experiments were performed at room temperature ($22 \pm 1°$ C).

Solutions

Standard Ringer's solution had the following composition (mM): NaCl 109.2; KCl 2.5; $CaCl_2$ 0.89; $NaHCO_3$ 2.38; pH about 7.7, gassed with room air. All replacements were isosmolal. Na and/or K were replaced with choline, tetraethylammonium (TEA), or N-methyl-D-glucamine. Calcium and bicarbonate concentrations were kept constant.

Electrical Measurements

a) Potentials. The transepithelial potential (V_{ms}) was measured with a 600-A Electrometer (Keithley Instruments, Aurora, Ohio) by means of Ag-AgCl electrodes connected to both bathing solutions through agar-Ringer's bridges. Apical and basal-lateral membrane potentials (V_{mc} and V_{cs}, respectively) were measured with glass microelectrodes, filled with 4 M K acetate as previously described [31], amplified by M-4 differential amplifiers (WP Instruments, Hamden, Connecticut) recorded on a storage oscilloscope (Tektronix, Inc., Beaverton, Oregon), and photographed. All potentials measured with nonidentical composition of the solutions were corrected for the liquid junction potentials measured in the same system when the tissue was substituted by a short agar-Ringer's bridge.

Cellular impalements were performed with mechanical micromanipulators, under visual control with an inverted phase-contrast microscope (Leitz Wetzlar, Germany), at 200 or 320×. Impalements were accepted when the change in potential was abrupt, and the recorded intracellular value stable within 2 mV. In some experiments, these criteria were validated by adding the measurement of the "input resistance" of the tissue (by passing short 10^{-9} A d.c. pulses through the microelectrode, before and after the impalement, and measuring the increase in microelectrode-to-ground resistance after the impalement), and the voltage divider ratio across the cellular pathway, measured by passing transepithelial d.c. pulses (*see below*, and Fig. 1).

b) Resistances. The transepithelial resistance (R_t) was calculated from the voltage deflection (ΔV_{ms}) measured in response to a transepithelial current pulse of density i (60 μA/cm^2 or less):

$$R_t = \Delta V_{ms}/i. \tag{1}$$

ΔV_{ms} was measured as the instantaneous voltage deflection (50 msec after the start of the current pulse). At this time, the potential has reached a pseudosteady state and the current voltage plot is linear well beyond the current densities employed. Longer pulses produce deviations from linearity that will be discussed elsewhere.

The voltage divider ratio across the cellular pathway ($a = R_a/R_b$) was calculated as the ratio of instantaneous voltage deflections produced across the apical and basal-lateral membranes (ΔV_{mc} and ΔV_{cs}, respectively) by a transepithelial d.c. pulse:

$$a = R_a/R_b = \Delta V_{mc}/\Delta V_{cs} \tag{2}$$

where R_a = apical membrane resistance and R_b = basal-lateral membrane resistance. The mean value of a for a given preparation was obtained from at least six measurements in different cells.

The specific resistance for current flow from the cells to the bathing solutions (R_z) was calculated from cable analysis of the intraepithelial spread of current applied into a

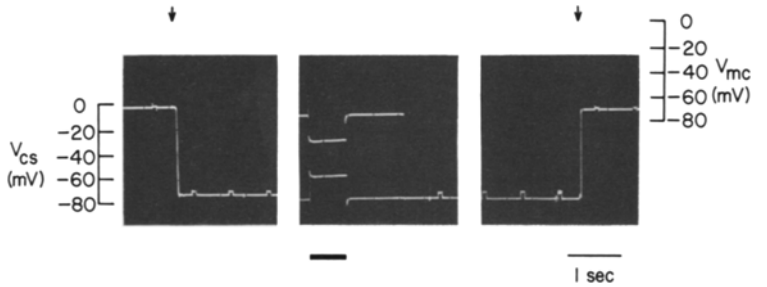

Fig. 1. Cellular impalement and measurement of the voltage divider ratio. The record starts on the left with the microelectrode in the mucosal solution and the reference electrode in the serosal solution. Between the upper arrows, the electrode is in the cell, and the tracing corresponds to the basal-lateral membrane potential (V_{cs}). In the middle section of the record, the potential across the apical membrane (V_{mc}) was also recorded, and a 100 μA transepithelial pulse (bar) passed to measure the ratio $\Delta V_{mc}/\Delta V_{cs}$. To measure the input resistance, 10^{-9} A pulses were passed through the microelectrode before, during, and after the impalement. The electrode was kept slightly unbalanced in the extracellular position. Note the progressive increase in the deflection after the impalement. There is a 15–30 sec delay between panels

cell. Current pulses of 10^{-8} to 2×10^{-8} A were intracellularly applied with a microelectrode and the steady-state voltage deflections produced in other cells were recorded, with a second microelectrode, as a function of the interelectrode distance (measured with a calibrated eyepiece). At least eight cells were explored in each preparation.

The voltage deflections (V_x) were plotted as a function of the distance (x) and fitted to the Bessel function K_o. The best fit yields the space constant of the preparation (λ) and the constant A. From these values, and the intracellularly applied current (i_o), R_z was calculated according to

$$R_z=\frac{2\pi A\lambda^2}{i_o} \tag{3}$$

(for further details, *see* [14, 17, 31, 38]).

Calculation of the Resistances of the Cell Membranes and the Shunt

According to the equivalent circuit shown in Fig. 2 (right), the values of R_a, R_b, and R_s can be calculated from:

$$R_t=(R_a+R_b)\,R_s/(R_a+R_b+R_s) \tag{4}$$

$$a=R_a/R_b \tag{5}$$

$$R_z=R_a\cdot R_b/(R_a+R_b) \tag{6}$$

where R_t, a, and R_z are experimentally determined.

Calculation of the Equivalent Electromotive Forces Generated at the Cell Membranes and the Shunt

As shown in Fig. 2, the cell membranes and the shunt pathway can be represented each by a battery in series with a resistor. Each equivalent electromotive force (V_a, V_b,

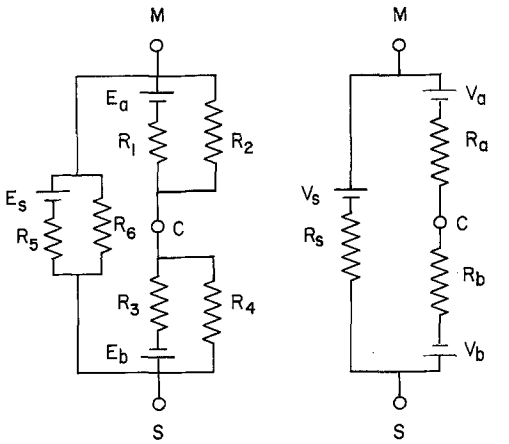

Fig. 2. "Steady state" equivalent circuit for *Necturus* gallbladder epithelium. M, C, and S represent mucosal solution, cell, and serosal solution, respectively. On the left, the electromotive forces generated at the cell membranes (E_a, E_b) and at the shunt pathway (E_s) are shown with the internal (R_1, R_3, R_5) and the external (R_2, R_4, R_6) resistances at each site. The simplified circuit on the right corresponds to the replacement at each cell border and the shunt of the two resistors by their equivalents (R_a, R_b, R_s) and of the electromotive forces by their equivalents (V_a, V_b, V_s), e.g., $R_a = R_1 \cdot R_2/(R_1 + R_2)$, and $V_a = E_a \cdot R_2/(R_1 + R_2)$

V_s) corresponds to the potential that would be measured across a given membrane or the shunt if it were the only element of the circuit, i.e., if no other elements were contributing to or shunting its potential. Thus each EMF may represent a combination of diffusion potentials and rheogenic pumps. The lumped resistances (R_a, R_b, R_s) correspond to all parallel ionic pathways through each membrane and the shunt.

Under control conditions (Ringer's solution bathing both sides of the tissue), V_s was assumed to be zero. It certainly must be close to zero, given the permselectivity of the shunt and a reasonable estimation of the salt concentration gradient across the limiting junction (*see below*).

The potentials are then given by

$$V_{mc} = \frac{V_a(R_b + R_s) + V_b R_a}{R_a + R_b + R_s} \tag{7}$$

$$V_{cs} = \frac{V_b(R_a + R_s) + V_a R_b}{R_a + R_b + R_s} \tag{8}$$

$$V_{ms} = \frac{(V_b - V_a) R_s}{R_a + R_b + R_s} \tag{9}$$

where the polarities are defined as follows: V_{mc}, V_a = mucosal solution − cell; V_{cs}, V_b = serosal solution − cell; V_{ms} = serosal solution − mucosal solution. According to Eqs. (7)–(9), it is possible to calculate V_a and V_b if the resistances and the potentials across the cell membranes and the entire epithelium are known:

$$V_a = V_{mc} - V_{ms} \frac{R_a}{R_s} \tag{10}$$

$$V_b = V_{cs} + V_{ms}\frac{R_b}{R_s}. \tag{11}$$

If the bathing solutions are changed so that they are not identical, $V_s \neq 0$, and it can be shown that

$$V'_{mc} = \frac{V''_a(R'_b + R'_s) + R'_a(V'_b - V'_s)}{R'_a + R'_b + R'_s} \tag{12}$$

$$V'_{cs} = \frac{V'_b(R'_a + R'_s) + R'_b(V'_a + V'_s)}{R'_a + R'_b + R'_s} \tag{13}$$

$$V'_{ms} = \frac{R'_s(V'_b - V'_a) + V'_s(R'_a + R'_b)}{R'_a + R'_b + R'_s} \tag{14}$$

where $'$ indicates values after a change in bathing solution.

After a change in the mucosal solution, it can be assumed that initially the EMF at the serosal border (V_b) remains constant, provided that changes in intracellular composition are slow. Within this assumption, V'_s and V'_a can be calculated, according to Eqs. (12)–(14), as follows:

$$V'_s = \frac{V'_{cs}(R'_b + R'_s) - (V'_{mc}R'_b + V_bR'_s)}{R'_b} \tag{15}$$

$$V'_a = \frac{V'_{mc}(R'_a + R'_b + R'_s) - R'_a(V_b - V'_s)}{R'_b + R'_s}. \tag{16}$$

All statistical comparisons were made by paired analysis of tissues studied under control and experimental conditions. Means $\pm$ SEM are given throughout the paper.

Results

Cell Membrane Potentials

Our experience in this tissue has been that most frequently the cell potential remains stable after the impalement, or rarely increases slightly for a few seconds. Decreases in potential were taken as an indication of inadequate impalement. The stability of the intracellular record is illustrated in Fig. 1. The mean value for V_{cs} was 59.4 ± 1.9 mV (44 to 72 mV), for V_{mc} 57.2 ± 2.3 mV (39 to 71 mV), and for V_{ms}, 2.2 ± 0.4 mV (0.5 to 5.0 mV), with Ringer's solution on both sides. These values are similar to those previously reported by Frömter [17]. A highly significant correlation was demonstrated between the value of the transepithelial potential and the shunt resistance. In 14 experiments in which both measurements were performed, the least-squares fit yielded the equation $V_{ms} = (-1.5 \pm 0.4) + (7.8 \pm 0.7)\ R_s$, $r = 0.955$, $p < 0.001$ (V_{ms} in mV, R_s in kΩ cm^2). This would be predicted from Eq. (9). Concomitant increases in transepithelial potential and transepithelial resistance have been observed by Moreno [29] in the frog gallbladder under the action of TAP,

Table 1. Steady-state changes in cell and transepithelial potentials after ionic substitutions in the mucosal solution

Mucosal solution	V_{mc} (mV)	V_{cs} (mV)	V_{ms} (mV)
Ringer's	58.9±1.0	60.5±0.9	1.6±0.2
K	4.3±1.2	25.0±3.1	20.9±3.5
Difference	54.6±2.2	35.5±3.4	−19.3±3.5
p	<0.001	<0.005	<0.01
Ringer's	59.4±5.8	61.1±5.0	1.7±0.9
TEA	73.1±5.3	47.6±3.7	−25.6±3.2
Difference	−13.7±4.2	13.5±2.3	27.3±3.0
p	<0.05	<0.005	<0.001
Ringer's	57.7±3.2	59.2±2.8	1.5±0.5
N-methyl-D-glucamine	72.2±4.7	55.2±3.0	−16.5±2.7
Difference	−14.5±1.7	3.8±0.9	18.0±2.3
p	<0.005	<0.025	<0.005

Mucosal bathing solution identified by the main cation. Ringer's solution on the serosal side. Concentrations of Ca^{2+} and HCO_3^- constant on both sides. Values corrected for liquid junction potentials. Ringer's-K, $n=4$; Ringer's-TEA, $n=5$; Ringer's-N-methyl-D-glucamine, $n=4$. Polarities defined in Materials and Methods.

an agent that presumably acts specifically at the shunt pathway, blocking its Na conductance.

Changes in the ionic composition of the mucosal solution produced changes in V_{mc}, V_{cs}, and V_{ms}. These changes usually consisted of two distinct phases: an initial, fast change, with a time constant of less than one second, and a slow component with a time constant of several minutes. From observations in other tissues, it can be presumed that the latter is at least partly due to changes in intracellular composition [25, 39]. Ideally, the quasi-steady state reached after the fast component, in response to small alterations in the external concentrations, should be used to estimate ionic permeabilities. However, the determination of the EMF's requires procedures that take at least 30 min in each experimental condition. The alternative is to calculate the resistances immediately after the change in solution, making additional assumptions. Both approaches were employed. In this paper we will report the results obtained from steady-state measurements. The results calculated from the quasi-steady state just after the fast changes in potential will be discussed in the accompanying paper [33].

Steady-state transepithelial and cell membrane potentials with several mucosal solution compositions are given in Table 1. Increases in K con-

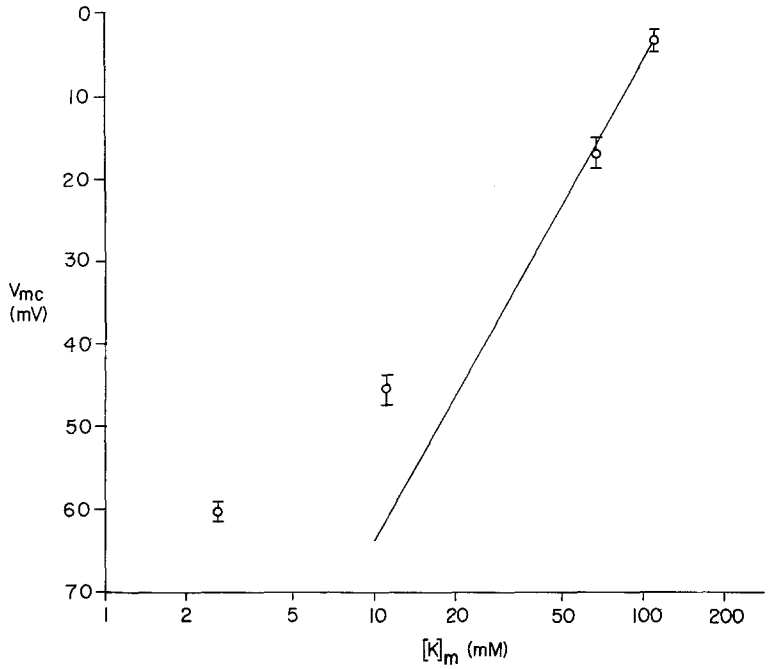

Fig. 3. Changes in V_{mc} as a function of K concentration in the mucosal solution. Each value corresponds to the mean ± SEM of six impalements in the same preparation, 1 to 10 min after exposure to each mucosal solution. Solid line gives the slope for a potassium electrode

centration depolarize both the apical and basal-lateral membrane. Fig. 3 shows the results of a typical experiment in which cell potentials were measured 1 to 10 min after exposure to different K concentrations on the mucosal side. Note that at the higher K concentrations the slope of V_{mc} as a function of external K is close to the value predicted for a K electrode, indicating that the apical membrane has a high K permeability. In addition, as shown in Table 1, the direction of the change in transepithelial potential indicates that $P_K > P_{Na}$ across the shunt pathway. It will be shown that the changes in V_{mc} and V_{ms} are very similar to the changes in the corresponding equivalent electromotive forces. When mucosal Na is replaced with choline or TEA (*see* Table 1), V_{mc} increases significantly, V_{cs} decreases (both changes being smaller than in the Na-for-K substitution), and V_{ms} reverses its polarity, changing an average of 27.3 mV. As a first approximation, this indicates that the effect of mucosal Na concentration on the apical membrane potential is far less than that of K. In addition, the change in transepithelial potential indicates that $P_{Na} > P_{TEA}$ (across the shunt). A similar result

(V_{ms} changes from 2.8 ± 0.7 to -17.6 ± 2.6) is obtained when Na is replaced with choline.

It will be shown that although the changes in V_{ms} are close to the calculated changes in V_s, the changes in V_{mc} are opposite in direction to the changes in V_a, because of peculiar effects of these replacement cations on the permeability properties of the membrane. This fact made it necessary to use other cations to replace Na. N-methyl-D-glucamine (NMDG) was shown to yield results that are consistent with pure Na replacement. As shown in Table 1, the effects of this substitution on the potentials are in the same direction as observed in TEA medium, but the magnitude of the change in V_{cs} is significantly smaller.

Resistances Under Control Conditions

The values of cell membrane and shunt resistances (with Ringer's solution bathing both sides of the tissue) are similar to those reported by Frömter [17, 18]. The mean space constant for the intraepithelial spread of current in a series of 14 preparations was 363 μm (range 210 to 520 μm), and the voltage divider ratio (R_a/R_b) 1.26, with a range from 0.77 to 1.99. The calculated resistances in these 14 preparations were (in $\Omega\,\text{cm}^2$): $R_t = 450 \pm 40$, $R_a = 3350 \pm 390$, $R_b = 2750 \pm 320$, and $R_s = 480 \pm 50$. The ratio of the mean resistances of the cellular pathway ($R_a + R_b$) to the shunt (R_s) is approximately 12.

Circuit Analysis Under Control Conditions

In the same series of 14 preparations, the equivalent electromotive forces at the membranes were calculated from the measured potentials and resistances, according to Eqs. (10) and (11). These results, summarized in Table 2, indicate that both EMF's are oriented with the negative pole facing the cell interior, as might have been presumed from the high K dependence of V_{mc}. In addition, the value of V_b is significantly higher than the value of V_a. Because of this difference in EMF's, current flows from the basal-lateral to the apical membrane, through the low resistance shunt pathway. This current hyperpolarizes the apical membrane with respect to the value of V_a, i.e., $V_{mc} > V_a$, while V_b is partially shunted, i.e., $V_b > V_{cs}$. At the limit, as R_s approaches zero, V_{mc} and V_{cs} would become equal, regardless of the absolute values of V_a and V_b,

Table 2. Equivalent electromotive forces at both cell borders

Cell membrane	Measured potential (mV)	Equivalent EMF (mV)	Difference (mV)	p
Apical	54.8±2.3	39.9±3.6	15.0±2.2	<0.001
Basal-lateral	57.3±1.9	69.4±1.8	−12.1±1.4	<0.001

Ringer's on both sides. $n=14$ preparations. Polarities defined in Materials and Methods.

while as R_s approaches infinity, V_{mc} would become equal to V_a and V_{cs} equal to V_b.

Resistances After Mucosal Ionic Substitutions

The increase in mucosal K concentration reduces significantly the spread of intracellularly applied current into the epithelium. A typical experiment at 2.5 and 110 mM in the same preparation is shown in Fig. 4*A*. The direction of his change is what would be predicted from the high K conductance of the apical membrane [i.e., reduction in R_a and R_z; *see* Eq. (6)]. However, both at 110 and 11 mM mucosal K, the value of R_z, calculated from the fit to the Bessel function, was lower than the minimum that would be predicted if $R_t=R_a+R_b$, i.e., if $R_s=\infty$. Thus, the use of Eqs. (4)–(6) yielded in every case negative values of R_s, and impossibly low values for R_a and R_b for the observed R_t. The most likely explanation for this observation is that the cells uncouple electrically when exposed to high external K concentration, i.e., the internal resistance of the epithelial sheet increases. This is consistent with the demonstration of uncoupling induced by cell depolarization (and its reversal by repolarization) in other systems [30, 34]. In addition, the "input resistance" of the cells either remained constant or increased after the change of mucosal solution. This observation is consistent with our explanation for the reduction in the voltage spread, and indicates that such reduction cannot be entirely secondary to the drop in R_a (if that were the case, we would expect the "input resistance" to decrease). In this situation, the use of a thin sheet model (with low internal resistance) is inappropriate. However, it is still possible to calculate all resistances, from the measurements in Ringer's solution and the values of R_t and R_a/R_b in high K, if one assumes that R_b remains constant. The potential error introduced by this assumption is calculated below. The results

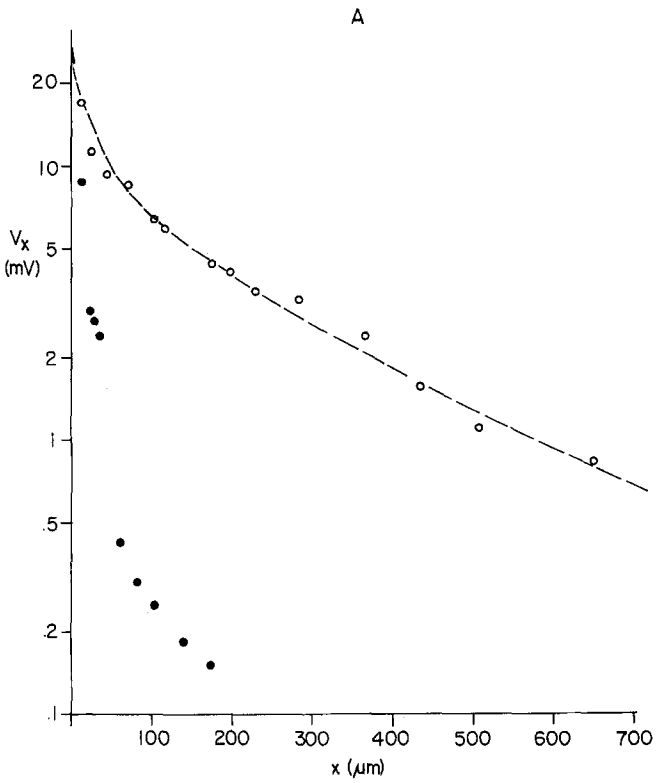

Fig. 4. Spread of intracellularly applied current under control conditions and after exposure to K or choline mucosal solutions. (*A*) Open circles, Ringer's; filled circles, 110 mM K. The curve depicts the Bessel function K_o; $\lambda = 400$ μm. (*B*) Open circles, Ringer's; filled circles, choline. Best fits yield λ of 210 μm (control) and 400 μm (choline)

obtained with 110 mM K are compared to the control values in Table 3. R_a is decreased to one-third of control ($p < 0.05$). No significant changes in R_t or R_s were observed in the steady state (15–45 min after the change in solution), although immediately after the substitution R_t is diminished and R_s is also shown to be reduced if a continuous record from a single cell is used for the calculation. This is consistent with the microscopic observation that the lateral intercellular spaces appear to close after the change in solution.

The effect of the replacement of mucosal Na and K with choline, TEA, or NMDG is an increase in R_a. Fig. 4*B* illustrates the increase in voltage spread observed after a mucosal choline-for-Na substitution. The results obtained with either choline or TEA are very similar. In addition to the increase in R_z, R_a/R_b also increased significantly. In these experiments, R_b was calculated from the resistance measurements [Eqs. (4)–(6)], and not assumed to remain constant. As shown in Table 3, R_b in-

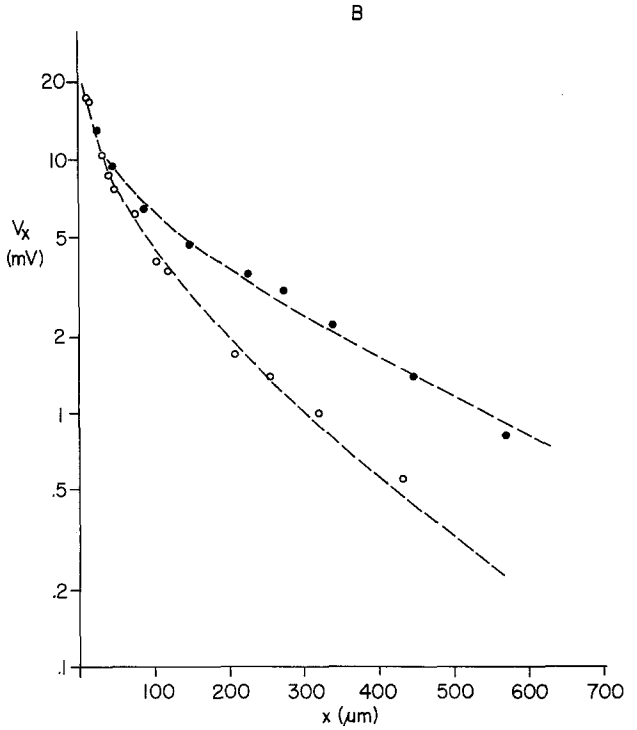

Fig. 4 B

Table 3. Effects of mucosal solution substitutions on the resistances of the cell membranes and the shunt

Mucosal solution	R_t	R_a	R_b	R_c	R_s
Ringer's	350±40	4,400±830	3,010±570	7,410±1,270	370±50
K	310±60	1,300±290	3,010±570*	4,310±840	350±70
Difference	50±70	3,100±580	—	3,100±580	20±80
p	NS	<0.02	—	<0.02	NS
Ringer's	390±40	3,490±760	2,720±600	6,210±1,330	420±60
TEA	700±80	11,500±1,960	3,390±520	14,890±2,310	740±90
Difference	320±50	8,010±1,320	670±100	8,680±1,280	320±50
p	<0.005	<0.005	<0.005	<0.005	<0.005
Ringer's	410±90	2,920±590	2,420±270	5,140±730	420±90
N-methyl-D-glucamine	640±150	4,070±680	2,420±270*	6,290±820	710±170
Difference	230±60	1,140±90	—	1,140±90	290±90
p	<0.05	<0.005	—	<0.005	<0.05

Resistances in Ω cm^2 of tissue. $R_c = R_a + R_b$; remaining abbreviations as in text. *R_b assumed to remain constant after the change in solution. Ringer's-K, $n=4$; Ringer's-TEA, $n=5$; Ringer's-N-methyl-D-glucamine, $n=4$.

creased (in TEA medium) by 25%, while the increases in R_a and R_s were 230% and 76%, respectively. When choline was used, R_b did not change significantly, while R_a and R_s increased similarly to the TEA results. NMDG substitutions, also shown in Table 3, produced augmentation of both R_a and R_s. The latter calculations were performed under the assumption that R_b remains constant after the change in mucosal solution. In a single experiment, the cable properties were studied in Ringer's and NMDG medium, and R_b—calculated independently in the two conditions—was shown to increase by 15%. As discussed below, the magnitude of these changes is too small to influence our conclusions. Thus, the assumption that R_b remains constant is, for practical purposes, correct. It is notable that the relative increase in R_a in the TEA and NMDG experiments differ almost by a factor of 6. This suggests that TEA alters the permeability of the apical membrane to other ions, and a similar conclusion can be obtained from the choline experiments. Further experimental evidence on this point is given below.

After both TEA and NMDG replacements, the increase in total transepithelial resistance is mainly the consequence of a higher shunt resistance, and this can be attributed to a low conductance for TEA or NMDG (as compared to Na conductance) across the shunt. This conclusion is consistent with the measured changes in V_{ms} in the same experiments (Table 1). In addition, closing of the lateral intercellular spaces might contribute to the increase in R_s.

These resistance measurements indicate that across the apical membrane $P_K > P_{Na}$, and that the Na permeability is higher than that of TEA, choline and NMDG. In addition, both TEA and choline appear to effect alterations of the properties of the apical membrane.

Equivalent Electromotive Forces After Mucosal Ionic Substitutions

V_a and V_b were calculated [according to Eqs. (10) and (11)] from measurements of electrical potentials and resistances in tissues incubated with Ringer's solution on both sides. Afterwards, the mucosal solution was changed, the measurements repeated, and the new values of the equivalent EMF's were calculated according to Eqs. (15) and (16). Results obtained in K, TEA, and NMDG mucosal media are compared to the control values in Table 4. When mucosal K was increased from 2.5 to 110 mM, the value of V_a changed by 54.3 mV, from 34.0 mV (cell negative) to 20.3 mV (cell positive). This mean change is almost identical to the mean change in measured potential across the apical membrane,

Table 4. Steady-state changes in equivalent electromotive forces after ionic substitutions in the mucosal solution

Mucosal solution	V_a (mV)	V_b (mV)	V_s (mV)
Ringer's	34.0±7.5	77.2±3.5	0[b]
K	−20.3±2.9	77.2±3.5[a]	15.3±3.6
Difference	54.3±7.2	–	−15.3±3.6
p	<0.005	–	<0.02
Ringer's	50.2±7.6	68.3±4.2	0[b]
TEA	17.2±3.5	68.3±4.2[a]	−29.1±3.8
Difference	33.0±10.1	–	29.1±3.8
p	<0.05	–	<0.005
Ringer's	46.7±5.1	68.2±3.4	0[b]
N-methyl-D-glucamine	50.3±5.5	68.2±3.4[a]	−20.2±2.2
Difference	−3.7±1.1	–	20.2±2.2
p	<0.05	–	<0.005

[a] V_b assumed to remain constant.
[b] V_s assumed to be 0 with standard Ringer's solution on both sides. *See* legend to Table 1.

of 54.6 mV, in the same experiments (Table 1). In addition, V_s was 15.3 mV after the increase in mucosal K concentration. This value is very similar to the mean change of V_{ms} (19.3 mV), as shown in Table 1.

When all mucosal Na was replaced with TEA, at constant K concentration, V_a decreased from 50.2 to 17.2 mV. This change is in the opposite direction to the change in V_{mc}, which increased from 59.4 to 73.1 mV (Table 1). This result, similar to the one obtained with choline instead of TEA, might indicate that the assumption concerning the constant value of V_b is wrong, or that choline and TEA, in addition to acting as Na substitutes, alter the properties of the apical membrane (the possibility of choline and TEA being more permeant than Na can be ruled out by the observed increase in R_a). In order to distinguish between these two possibilities, cell potentials were measured in several preparations before and after substitution of Na with TEA or choline in both mucosal and serosal media. After the substitution, V_s can be safely assumed to be 0 (there is no transepithelial transport and no asymmetries in composition of the bathing solutions), and V_a and V_b have to change in the same direction as V_{mc} and V_{cs}. In fact, one can predict that V_{mc} and V_{cs} will increase if TEA or choline are simple nonpermeant replacement cations (because the Na concentration gradient across both cell membranes is reversed). In TEA substitutions, V_{mc} changed from 63.5±2.2 mV to 37.7±0.8 mV, and V_{cs} changed from 64.5±2.2 mV to

37.7 ± 0.8 mV. In choline substitutions, the changes were from 64.7 ± 1.7 to 46.3 ± 2.4 mV, and from 66.2 ± 1.7 to 46.8 ± 2.4 mV, respectively. Thus, both TEA and choline bilateral substitutions decreased V_{mc}, V_{cs}, and V_{ms}. If $V_s = 0$ under control conditions, the decrease in V_{mc} and V_{cs} is necessarily secondary to the fall of both V_a and V_b. Furthermore, if $V_s \neq 0$ when Ringer's solution bathes both sides of the preparation, the changes in cell membrane potentials cannot be ascribed to changes in V_s alone, because V_{mc} and V_{cs} would change in opposite directions, and because it can be shown that the magnitude of such changes would be about 50% of the change in V_s. Thus, a change in V_s from 2 to 0 mV would not change the potentials across the cell membranes by more than 1 mV. Thus, this experiment demonstrates action of TEA and choline on the permeability of both membranes to other ionic species.

When NMDG was used as Na substitute in the mucosal solution, V_a increased from 46.7 to 50.3 mV (Table 4). Although the change is in the same direction as the change in V_{mc} (Table 1), the latter increased by 14.5 mV. The difference is given by the contribution of V_s (-20.2 mV, Table 4) to V_{mc} after the substitution. Unlike the TEA and choline experiments, NMDG-for-Na substitution yielded congruent changes in V_a and R_a. Furthermore, an experiment was performed in which V_{mc}, V_{cs}, and V_{ms} were measured in the same preparation before and after a bilateral NMDG-for-Na substitution. As expected for an inert nonpermeant cation, V_{mc} increased from 52.7 ± 1.0 to 53.9 ± 0.6 mV, V_{cs} increased from 54.2 ± 1.0 to 54.9 ± 0.6 mV, and V_{ms} fell from 1.5 to 1.0 mV.

The calculated values of V_s after mucosal substitutions of Na with TEA and NMDG were -29.1 and -20.2 mV, respectively (Table 4). In both cases, these mean values are close to the measured changes in V_{ms} in the same experiments: 27.3 mV after TEA substitutions and 18.0 mV in the NMDG experiments (Table 1). This observation indicates that in this tissue the ionic selectivity of the shunt pathway can be properly derived from measurements of transepithelial potentials.

Discussion

Equivalent Circuit

A complete equivalent circuit for this epithelium should include one EMF for every ion at each cell border and the shunt, internal and external (shunting) resistances at these three sites, and capacitances at both cell membranes and the shunt. Presently, no solution is obtainable with

Table 5. Effect of assumed NaCl concentration gradients across the limiting junctions on the calculated values of V_s, V_a, and V_b

NaCl concentration gradient (mM)	V_s (mV)	V_a (mV)	V_b (mV)
0	0	39.9	69.4
5	−0.6	33.2	75.0
10	−1.2	29.3	78.2
20	−2.2	21.9	84.3

V_s calculated from $t_{Na}/t_{Cl}=3.18$ (across the shunt). Eqs. (12)–(14) were solved for V_a and V_b, inserting the calculated value of V_s and mean values of resistances and potentials from 14 experiments under control conditions.

such detail. Thus, the following simplifications were employed: (1) All resistance measurements are taken in the quasi-steady state (e.g., 50 msec after the start of the current pulse in the measurements of R_t and R_a/R_b). Thus, capacitances are not considered. (2) Each cell membrane, and the shunt pathway, are represented by a battery and an ohmic resistor in series. Each EMF (V_a, V_b, V_s in Fig. 2) is equal to the voltage drop that would be measured across that structure if it were the only element of the circuit (e.g., for a cell membrane, if R_s were infinite), and is the sum of the contribution of diffusion potentials and rheogenic pumps at that site. Even with these simplifications, a complete solution of the circuit shown in Fig. 2 is impossible, unless some additional assumptions are made. These were as follows: (1) with Ringer's solution on both sides, V_s is assumed to be 0; (2) after a K-for-Na substitution on the mucosal side, R_b and V_b are assumed to remain constant; (3) after a choline, TEA, or NMDG-for-Na replacement, V_b is assumed to remain constant. The potential error involved in the use of the first assumption can be evaluated by a calculation of V_a and V_b at several values of V_s. These values were chosen from assumptions of gradients in NaCl concentration which might reasonably exist across the limiting junction, within the framework of the standing osmotic gradient hypothesis [11, 28], and from the Na and Cl transference numbers across the shunt. The results of this calculation at concentration gradients of 0, 5, 10, and 20 mM are shown in Table 5. As V_s is opposite in polarity to V_{ms}, because $P_{Na}>P_{Cl}$ across the shunt, the higher value of V_s the lower the calculated value of V_a and the higher the calculated value of V_b. Note that a 10 mM gradient across the limiting junction was calculated by Machen and Diamond [28] for the rabbit gallbladder, under maximal transport, from

dilution potentials. Even a 10 mV error in the calculation of V_a, if such a gradient exists, does not modify our conclusions concerning the ionic permeabilities of the apical membrane, as shown below. The assumption concerning R_b after a K-for-Na-mucosal substitution cannot be tested directly, but the possible error involved can be calculated, similarly to the validation of an analogous procedure in the toad urinary bladder [31]. If R_b decreased to half of the control value, R'_s would be 10% higher than calculated, and R'_a even lower than calculated. On the other hand, increases in R_b would produce overestimations of R'_s and underestimations of R'_a. In the event that the mechanism of a change in R_b is an increase in cell volume (produced by net K uptake from the mucosal solution), the direction of the change in R_b would probably be a decrease, as suggested by the effect of hyposmolal solutions on the resistances of the cell membranes in toad urinary bladder epithelium [15] and in this tissue (*unpublished observations*). When choline is used to replace Na in the mucosal solution, the value of R_b can be determined from voltage spread experiments, and was in fact not different from that found in Ringer's, while all other resistances increased significantly. In TEA-for-Na substitutions, R_b increased by about 25%. This change, although statistically significant, is small when compared with the more than twofold increases of R_a in the same experiments.

The assumption that V_b remains constant after a mucosal solution substitution is also difficult to test. At least two mechanisms might produce changes in V_b after ionic substitution at the opposite border of the cell: changes in ionic gradients across the membrane, or some kind of interdependence of the potentials at both cell borders, independent of the shunt current, as shown for the toad urinary bladder [32]. An argument against the first possibility is provided by the fact that measurements within a few seconds after a K-for-Na mucosal substitution yield values of membrane potentials and voltage divider ratio very similar to those found after a much longer time, whereas changes in V_b mediated by diffusion should be time dependent. The second possibility, i.e., a change in V_b secondary to the change in V_a, not due to alterations in ionic concentrations, and analogous to the one observed in toad urinary bladder, cannot be tested presently. In any event, the influence of assumed changes in V_b on the calculated values of V'_a and V'_s (after K-for-Na substitutions) is shown in Table 6. Even if V_b changes by as much as 20 mV in either direction, it is clear that our conclusions concerning the relative permeabilities of the apical membrane and the shunt would not be altered.

Table 6. Effect of assumed changes of V_b on the calculated values of V'_a and V'_s after mucosal K-for-Na substitutions

V'_b (mV)	V'_s (mV)	V'_a (mV)
53.5	17.4	−8.0
63.5	16.3	−12.3
73.5	15.1	−16.6
83.5	14.0	−20.9
93.5	12.8	−25.2

V_b calculated from mean resistances and potentials in four experiments (73.5 mV). V_s and V_a calculated according to Eqs. (15) and (16) from the mean resistances and potentials measured after exposure to 110 mM K, assuming a constant value of V_b, and allowing 10 or 20 mV changes in each direction.

Resistances of the Cell Membranes and the Shunt Pathway

The resistances of the cell membranes and the shunt pathway with Ringer's solution bathing both sides of the tissue are similar to those reported by Frömter and Diamond [17, 18]. Although our mean value for the resistance of the cellular pathway is somewhat smaller, and our value for the resistance of the shunt is somewhat higher, given the variability of the results and the additive sources of error in the experimental measurements, no real discrepancies are apparent. As shown in Table 3, the resistance of the apical membrane decreases significantly when the K concentration in the mucosal solution is increased. This change is almost complete within a few seconds and is completely reversible. The diminution of R_a can be ascribed to a high K permeability of the apical membrane. On the other hand, the effect of the replacement of Na with TEA, choline, or NMDG (in the mucosal solution) is an increase in R_a, implying that the latter ions are less permeant than Na. The change in resistance is again fast and reversible. However, the increase in R_a in TEA or in choline media is several-fold greater than the one observed in NMDG, and also greater than expected from an estimation of P_{Na}/P_K from V_a changes after Na-K and Na-NMDG substitutions. This is not the case when K or NMDG is used, where the measured and predicted changes in R_a are similar. These results suggest that choline and TEA affect the permeability properties of the membrane in addition to simply replacing Na. Furthermore, there is a decrease in V_a after mucosal Na is replaced by one of these cations. In the absence of such an effect, V_a should increase, because the Na concentration gradient across the

apical membrane is reversed. In fact, V_a increases when NMDG is employed to replace Na. Possible errors involving the presence of a bi-ionic potential across the shunt were ruled out by bilateral substitutions, described above. The results of these experiments indicate effects of TEA and choline on both cell membranes. In contrast, NMDG does not appear to alter the ionic permeability of the cell membranes. It has been shown in nervous tissue [1, 24] that the presence of TEA in the external solution brings about effects that can be interpreted as closing of K channels in the membrane. Other quaternary amines produce qualitatively similar effects. Given the high K permeability of the apical membrane, evidenced by the changes in R_a and V_a as functions of external K concentration, it seems certain that the magnitude of the effects of TEA or choline on the same two parameters can only be attributed to a decrease in K conductance produced by these ions. NMDG has an entirely different structure and does not exhibit these effects.

In summary, our measurements of R_a at several compositions of the mucosal solution indicate that the apical membrane is predominantly K-permeable, in contrast with the common observations in tight epithelia [6, 21, 27]. In fact, the only Na-transporting epithelium in which data showing this cation permselectivity at the apical membrane has been communicated is the proximal tubule of *Necturus* kidney [23], but later studies in the same preparation showed that the apical membrane is Na permselective [3].

The resistance of the shunt pathway exhibits time dependent changes after K-Na replacements. In every instance, the immediate effect of the increase in mucosal K concentration is a decrease in R_s, presumably due to a higher permeability of the limiting junction for K than Na, as in gallbladders of other species [8, 12]. Shortly after the change in solution, R_t is observed to increase, sometimes to a value higher than control, while microscopic observation reveals apparent closing of the lateral intercellular spaces. This effect might be secondary both to cell swelling (high K permeability of the apical membrane) and reduction of transepithelial salt transport [13, 40].

The replacement of mucosal Na with TEA or choline produced in every instance an increase in R_s, attributable to a higher shunt permeability for Na than for the replacing cation. This observation is consistent with the change in transepithelial potential. From dilution and bi-ionic potentials measured across the tissue, the permeability ratios across the shunt were calculated. If these values are employed to predict a change in shunt resistance after mucosal Na substitution with choline, the meas-

ured increase in R_s is higher than the calculated value, indicating that in addition to the increase in resistance at the limiting junction produced by the lower permeability, a change in geometry at some segment of the shunt has taken place. Again, it is likely that the width of the lateral intercellular spaces has decreased as a consequence of reduced rate of transepithelial transport produced by the absence of Na in the mucosal solution. Thus, the measurements of R_s after cation substitutions in the mucosal solution indicate that the limiting junction has the permeability sequence $P_K > P_{Na} > P_{Ch} \sim P_{TEA}$.

Cell Membrane EMF's and Transepithelial Potential Under Control Conditions

Under control conditions, the measured potential across the apical membrane (V_{mc}) is higher than the equivalent EMF at that membrane (V_a), while the measured potential across the basal-lateral membrane (V_{cs}) is lower than the equivalent EMF at that border (V_b). Both EMF's are oriented with the negative pole toward the cell interior. As stated before, the low value of R_s explains why the difference between V_{mc} and V_{cs} is only 2 mV, while the difference between V_a and V_b is about 30 mV. Under open circuit conditions, current flow through the shunt is in the S to M direction ($V_b > V_a$), and thus the apical membrane is hyperpolarized while the basal-lateral membrane depolarizes.

The difference between the absolute values of V_b and V_a with identical bathing solutions requires additional discussion. Experiments not reported here show that the basal-lateral membrane of this epithelium is (as the apical membrane) predominantly K permeable. The fact that the potentials measured at both borders are different—with the same ion concentration gradients—might in principle indicate: (1) the presence of a third EMF (i.e., $V_s \neq 0$), (2) different permselectivities of the cell membranes, or (3) presence of one or more rheogenic or electrogenic pumps. The first possibility can be ruled out, because—given the relative Na and Cl permeabilities of the shunt—a diffusion potential produced by a NaCl concentration gradient across the limiting junction (higher salt concentration within the intercellular space) would increase the apical and reduce the basal-lateral potential. In addition, the polarity of this shunt EMF would be opposite to the measured transepithelial potential. In fact, if such a potential exists, the real magnitude of the asymmetry is even more than calculated (Table 5). The available infor-

mation does not allow us to distinguish between the second and third possibilities. The difference between V_b and V_a is consistent with a higher K permselectivity at the basal-lateral membrane: accordingly, the relative Na and Cl permeabilities (as compared to P_K) across this membrane should be lower than the analogous values at the apical membrane. This is in fact the case, as will be published separately, but these estimations start – by necessity – with the assumption that no rheogenic pumps are present. Alternatively, our results would be explained by the presence of a rheogenic pump at one or both membranes: for instance, an inwards cation rheogenic pump at the luminal border (reducing V_a, the apical membrane EMF), an outwards cation rheogenic pump at the serosal border (increasing V_b, the basal-lateral membrane EMF), or both. However, definitive proof of such a mechanism requires determination of intracellular ion activities.

The existence of a significant, although small, transepithelial potential reflects the difference between V_b and V_a. If V_s is different from zero with Ringer's solution on both sides, its polarity is opposite to the polarity of the transmural potential, because $P_{Na} > P_{Cl}$ across the shunt. Similar to what has been shown in gallbladders from other species [22], if a diffusion potential exists across the limiting junction, an independent mechanism of higher magnitude and opposite orientation has to be postulated to explain the polarity of V_{ms}. In fact, only the rabbit gallbladder has the serosa-negative transepithelial potential that might be explained by a higher NaCl concentration in the lateral intercellular spaces than in the mucosal solution [28]. Although the transepithelial potential in gallbladder and other leaky epithelia is abolished by ouabain, cold or metabolic inhibitors [22, 28] coincidentally with the reduction in transepithelial transport, a cause-effect relationship between potential and transmural electrolyte transport has not been proven. It is possible that the major portion of, or even all, net transepithelial NaCl flux is secondary to a mechanism by which Na and Cl fluxes are tightly coupled in a "neutral" or "electrically silent" way, as initially proposed by Diamond [8, 9]. Gallbladders of different species have transmural potentials ranging from about -2 to about 7.5 mV. A univocal interpretation of these potentials as indications of the primary step of salt transport would require one to postulate "electrogenic" sodium transport when V_{ms} is negative, "electrogenic" chloride transport when V_{ms} is positive, and neutral NaCl transport when V_{ms} is 0. However, the overall characteristics of the function of these tissues are very much the same, independently of the species: transport of an approximately isotonic NaCl solu-

tion, at high rate, in the M to S direction. This interpretation could be challenged by direct evidence of the existence of rheogenic pumps. However, the presence of an inwards rheogenic K pump at the mucosal membrane, unrelated to transmural transport, would explain the transepithelial potential profile even if NaCl transport is neutral. In this view, the difference in p.d.'s among species could reflect different ionic permselectivities of the cell membranes, and not qualitatively different pump mechanisms.

Changes in EMF's After Mucosal Solution Substitutions

K-for-Na substitutions produced changes in V_a very similar to the measured V_{mc} changes in the same preparations (Tables 1 and 4). This result indicates that appropriate ionic permeability ratios across the apical membrane can be obtained from the measurement of the changes in its potential after these substitutions in the mucosal solution. In addition, the changes in V_{ms} under the same experimental conditions were similar to the changes in V_s. This means that transepithelial measurements yield adequate information on the permselectivity of the shunt. The two sets of EMF measurements are consistent with the resistance measurements discussed above: at the apical membrane and the shunt, $P_K > P_{Na}$. When Na in the mucosal solution was replaced with choline or TEA, V_a decreased significantly. This change is opposite in direction to the change in V_{mc} (compare Tables 1 and 4). As stated, we interpret this result as an indication of reduction of the K permeability of the apical membrane, and arguments obtained from resistance measurements were detailed above. NMDG-for-Na substitutions produce a moderate increase in V_a, indicating that P_{Na} is low at this membrane.

The changes in V_{ms} after mucosal Na is substituted with TEA, choline, or NMDG, are close to the calculated value of V_s after the substitution. This indicates (as in K for Na substitutions) that it is appropriate to calculate shunt permselectivities from transepithelial measurements, and that the shunt permeability for any of these cations is lower than its Na permeability.

Thus, the calculations of EMF's across the apical membrane and the shunt, after cation substitutions on the mucosal side, are consistent with resistance measurements, indicating that for both the apical membrane and the shunt, $P_K > P_{Na} > P_{NMDG}$.

This work was supported by Grant No. AM-17854 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

References

1. Armstrong, C.M. 1966. Time course of TEA^+-induced anomalous rectification in squid giant axons. *J. Gen. Physiol.* **50**:491
2. Barry, R.J.C., Eggenton, J. 1972. Membrane potentials of epithelial cells in rat small intestine. *J. Physiol.* **227**:201
3. Boulpaep, E.L. 1971. Electrophysiological properties of the proximal tubule: Importance of cellular and intercellular transport pathways. *In*: Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 91. Schattauer-Verlag, Stuttgart
4. Boulpaep, E.L., Seely, J.F. 1971. Electrophysiology of proximal and distal tubules in the autoperfused dog kidney. *Amer. J. Physiol.* **221**:1084
5. Burg, M.B., Orloff, J. 1970. Electrical potential difference across proximal convoluted tubules. *Amer. J. Physiol.* **219**:1714
6. Cereijido, M., Curran, P.F. 1965. Intracellular electrical potentials in frog skin. *J. Gen. Physiol.* **48**:543
7. Diamond, J.M. 1962. The reabsorptive function of the gallbladder. *J. Physiol.* **161**:442
8. Diamond, J.M. 1962. The mechanism of solute transport by the gallbladder. *J. Physiol.* **161**:474
9. Diamond, J.M. 1968. Transport mechanisms in the gallbladder. *In*: Handbook of Physiology: Alimentary Canal. Vol. 5, p. 2451. American Physiological Society, Washington, D.C.
10. Diamond, J.M., Barry, P.H., Wright, E.M. 1971. The route of transepithelial ion permeation in the gallbladder. *In*: Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 23. Schattauer-Verlag, Stuttgart
11. Diamond, J.M., Bossert, W.H. 1967. Standing gradient osmotic flow: A mechanism for coupling of water and solute transport in epithelia. *J. Gen. Physiol.* **50**:2061
12. Diamond, J.M., Harrison, S.C. 1966. The effect of fixed charges upon diffusion potentials and streaming potentials. *J. Physiol.* **183**:37
13. Dietschy, J.M. 1964. Water and solute movement across the wall of the everted rabbit gallbladder. *Gastroenterology* **47**:395
14. Eisenberg, R.S., Johnson, E.A. 1970. Three-dimensional electrical field problems in physiology. *Prog. Biophys. Mol. Biol.* **20**:1
15. Finn, A.L., Reuss, L. 1975. Effects of changes in the composition of the serosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Physiol.* **250**:541
16. Frizzell, R.A., Schultz, S.G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum. Influence of shunt on transmural sodium transport and electrical potential differences. *J. Gen. Physiol.* **59**:318
17. Frömter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**:259
18. Frömter, E., Diamond, J.M. 1972. Route of passive ion permeation in epithelia. *Nature, New Biol.* **235**:9
19. Frömter, E., Hegel, U. 1966. Transtubuläre Potentialdifferenzen an proximalen und distalen Tubuli der Rattenniere. *Pflügers Arch.* **291**:107
20. Frömter, E., Müller, C.W., Wick, T. 1971. Permeability properties of the proximal tubular epithelium of the rat kidney studied with electrophysiological methods. *In*: Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 119. Schattauer-Verlag, Stuttgart
21. Gatzy, J.T., Clarkson, T.W. 1965. The effect of mucosal and serosal solution cations on bioelectric properties of the isolated toad bladder. *J. Gen. Physiol.* **48**:647
22. Gelarden, R.T., Rose, R.C. 1974. Electrical properties and diffusion potentials in the gallbladder of man, monkey, dog, goose and rabbit. *J. Membrane Biol.* **19**:37

23. Giebisch, G. 1961. Measurements of electrical potential difference on single nephrons of the perfused *Necturus* kidney. *J. Gen. Physiol.* **44**:659
24. Hille, B. 1967. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. Gen. Physiol.* **50**:1287
25. Hodgkin, A.L., Horowicz, P. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**:127
26. Kokko, J.P. 1973. Proximal tubule potential difference. Dependence on glucose, HCO_3, and amino acids. *J. Clin. Invest.* **52**:1362
27. Leb, D.E., Hoshiko, T., Lindley, B.D. 1965. Effects of alkali metal cations on the potential across toad and bullfrog urinary bladder. *J. Gen. Physiol.* **48**:527
28. Machen, T.E., Diamond, J.M. 1969. An estimate of the salt concentration in the lateral intercellular spaces of rabbit gall-bladder during maximal fluid transport. *J. Membrane Biol.* **1**:194
29. Moreno, J.H. 1974. Blockage of cation permeability across the tight junctions of gallbladder and other leaky epithelia. *Nature* **251**:150
30. Politoff, A.L., Socolar, S.J. 1971. Uncoupling cell junctions in a glandular epithelium by depolarizing current. *Science* **172**:492
31. Reuss, L., Finn, A.L. 1974. Passive electrical properties of toad urinary bladder epithelium: Intercellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* **64**:1
32. Reuss, L., Finn, A.L. 1975. Dependence of serosal membrane potential on mucosal membrane potential in toad urinary bladder. *Biophys. J.* **15**:71
33. Reuss, L., Finn, A.L. 1975. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. II. Ionic permeability of the apical cell membrane. *J. Membrane Biol.* **25**:141
34. Rose, B. 1970. Junctional membrane permeability: Restoration by repolarizing current. *Science* **169**:607
35. Rose, R.C., Schultz, S.G. 1971. Studies on the electrical potential profile across rabbit ileum. Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. Gen. Physiol.* **57**:639
36. Schultz, S.G. 1972. Electrical potential differences and electromotive forces in epithelial tissues. *J. Gen. Physiol.* **59**:794
37. Schultz, S.G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum. II. The interaction between active sodium and active sugar transport. *J. Gen. Physiol.* **47**:1043
38. Shiba, H. 1971. Heavisides "Bessel Cable" as an electric model for flat simple epithelial cells with low resistive junctional membranes. *J. Theoret. Biol.* **30**:59
39. Strickholm, A., Wallin, B.G. 1967. Relative ion permeabilities in the crayfish giant axon determined from rapid external ion changes. *J. Gen. Physiol.* **50**:1929
40. Wheeler, H.O. 1963. Transport of electrolytes and water across wall of rabbit gallbladder. *Amer. J. Physiol.* **205**:427
41. Whitlock, R.T., Wheeler, H.O. 1964. Coupled transport of solute and water across rabbit gall-bladder epithelium. *J. Clin. Invest.* **43**:2249
42. Whittembury, G. 1971. Relationship between sodium extrusion and electrical potentials in kidney cells. *In:* Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 153. Schattauer-Verlag, Stuttgart
43. Windhager, E.E., Boulpaep, E.L., Giebisch, G. 1967. Electrophysiological studies in single nephrons. *Proc. 3rd Int. Congr. Nephrol.*, Washington 1966. p. 35. Karger, Basel-New York