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Dependence of Cell Membrane Conductances on Bathing Solution HCO_3^-/CO_2 in *Necturus* Gallbladder

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Summary. The effects of bathing solution HCO_3^-/CO_2 concentrations on baseline cell membrane voltages and resistances were measured in *Necturus* gallbladder epithelium with conventional intracellular microelectrode techniques. Gallbladders were bathed in either low HCO_3^-/CO_2 Ringer's solutions (2.4 mM HCO_3^-/air or 1 mM HEPES/air) or a high HCO_3^-/CO_2 Ringer's (10 mM HCO_3^-/1% CO_2). The principal finding of these studies was that the apical membrane fractional resistance (fR_a) was higher in tissues bathed in the 10 mM HCO_3^-/CO_2 Ringer's, averaging 0.87 ± 0.06, whereas fR_a averaged 0.63 ± 0.07 and 0.48 ± 0.08 in 2.4 mM HCO_3^- and 1 mM HEPES, respectively. Intraepithelial cable analysis was employed to obtain estimates of the individual apical (R_a) and basolateral membrane (R_b) resistances in tissues bathed in 10 mM HCO_3^-/1% CO_2 Ringer's. Compared to previous resistance measurements obtained in tissues bathed in a low HCO_3^-/CO_2 Ringer's, the higher value of fR_a was found to be due to both an increase in R_a and a decrease in R_b. The higher values of fR_a and lower values of R_b confirm the recent observations of others. To ascertain the pathways responsible for these effects, cell membrane voltages were measured during serosal solution K^+ and Cl^- substitutions. The results of these studies suggest that an electrodiffusive Cl^- transport mechanism exists at the basolateral membrane of tissues bathed in a 10 mM HCO_3^-/1% CO_2 Ringer's, which can explain in part the fall in R_b. The above observations are discussed in terms of a stimulatory effect of solution $[HCO_3^-]/PCO_2$ on transepithelial fluid transport, which results in adaptive changes in the conductive properties of the apical and basolateral membranes.

Key Words chloride transport · equivalent circuit · cable analysis · fluid transport · buffers

Introduction

To understand the mechanisms responsible for transcellular ion transport in epithelia an assessment of the conductive properties of the apical and basolateral cell membranes is required. Typically, this problem has been addressed by representing the epithelium with equivalent electrical circuit formalisms, and estimating the cell membrane resistances and equivalent electromotive forces utilizing electrophysiologic techniques. Early microelectrode studies in *Necturus* gallbladder epithelium employed flat-sheet intracellular cable analysis in an effort to obtain estimates of the electrical resistances of the apical and basolateral membranes (Frömter, 1972; Reuss & Finn, 1975*a*, 1977). In these studies, the apical membrane resistance averaged 3 to 5 kΩ · cm², whereas the basolateral membrane resistance was somewhat lower, averaging 2 to 4 kΩ · cm². Thus, the cell membrane resistance ratio (apical/basolateral = R_a/R_b) was found to range between about 1 and 2, and in fact, there was general agreement in the literature as to this estimate (van Os & Slegers, 1975; Suzuki & Frömter, 1977; Graf & Giebisch, 1979; Reuss, Bello-Reuss & Grady, 1979).

However, in more recent studies utilizing either cable analysis or impedance analysis (Suzuki et al., 1982; Kottra & Frömter, 1984*a*,*b*), R_a/R_b was reported to be higher than previous estimates from the same laboratory (Frömter, 1972). The DC cell membrane resistance ratio averaged about 6.5 under control conditions in these studies. In addition, the increase in R_a/R_b was found to be primarily attributable to a markedly lower basolateral membrane resistance. Suzuki et al. (1982) first suggested that the apparent discrepancy in the early versus later estimates of the cell membrane resistance ratio could be due to hypoxic experimental conditions in the earlier studies (Frömter, 1972). The oxygen deficiency of the tissue would cause a decrease in transepithelial fluid transport and collapse of the lateral intercellular spaces leading to an underestimation of the cell membrane resistance ratio. In later studies designed to test this idea directly, confirmatory evidence could not be obtained (Kottra & Frömter, 1984*b*). It was suggested instead by Kottra and Frömter (1984*b*) and later by Petersen and Reuss (1985) that the differences in ionic composition of the bathing solutions, and specifically in HCO_3^- con-

centration, could account for the results. In this regard, it has been a recent observation in this laboratory (Poler & Reuss, 1987, Reuss, 1987; Reuss, Costantin & Bazile, 1987), that when tissues are bathed in a HCO_3^-/CO_2-rich Ringer's solution (10 mM HCO_3^-/1% CO_2) the cell membrane resistance ratio is higher than measured previously, when either a low HCO_3^- (2.4 mM) air-equilibrated or a HEPES-buffered Ringer's solution was used (Reuss, 1979; Reuss et al., 1979; Weinman & Reuss, 1982; Petersen & Reuss, 1983, 1985).

In view of these differences, the present studies were undertaken to establish the direction and magnitude of the effects of solution buffers on the steady-state electrical properties of the epithelial cells. The apical fractional resistance ($fR_a = R_a/(R_a + R_b)$) was measured in studies in which the epithelium was bathed in one of the above-mentioned Ringer's solutions. The results of these studies then led us to reevaluate the equivalent electrical circuit parameters of apical and basolateral membranes in tissues bathed in 10 mM HCO_3^-/1% CO_2 Ringer's. We found that the fR_a is indeed higher and the basolateral membrane resistance lower than previously measured, in agreement with the observations of others (Suzuki et al., 1982; Kottra & Frömter, 1984*b*). It was also found that part of the increase in fR_a was because of an increase in apical membrane resistance. Lastly, we provide evidence for a conductive Cl^- transport mechanism at the basolateral membrane that can account, at least in part, for the observation of a lower R_b in tissues bathed in the 10 mM HCO_3^-/1% CO_2 solution.

Materials and Methods

Mudpuppies (*Necturus maculosus*) were obtained from Nasco Biologicals (Ft. Atkinson, Wis.) or Kon's Scientific (Germantown, Wis.) and kept in a large refrigerated aquarium (5 to 10°C). The animals were anesthetized with tricaine methanesulfonate and the gallbladders excised and mounted horizontally in a modified Ussing chamber (exposed area = 0.5 cm^2) as previously described (Reuss & Finn, 1975*a*,*b*; Weinman & Reuss, 1984). Upper (~0.5 ml) and lower (~1.0 ml) chamber volumes were continuously exchanged at flow rates of 10 to 40 ml/min and 5 to 10 ml/min, respectively. All studies were performed at room temperature.

Serosal solution ion substitutions were conducted with the epithelium mounted serosal-side-up. The chamber assembly and mounting procedure were identical to those used as if the tissue was mounted mucosal-side-up. Following mounting of the tissue in the ring assembly that eventually fit into the chamber, dissection of the subepithelial connective tissue was done under microscopic observation using fine forceps and a dissection instrument fashioned from a stainless steel pin bent at the end into a small hook. A patch of about 30 to 60% of the total exposed surface area was largely cleared of connective tissue. The remaining serosal connective tissue layer in the central region of the patch was thin enough to allow for penetration by the microelectrode and for rapid solution exchange at the basolateral surface of the cells.

Solutions

In one group of studies (*see* Table 1) gallbladders were bathed in one of three salt solutions specifically chosen to allow for comparison with steady-state recent (Reuss, 1979; Reuss et al., 1979; Weinman & Reuss, 1982; Petersen & Reuss, 1983, 1985) and earlier results (Reuss & Finn, 1975*a*,*b*; Reuss & Finn, 1977). All experiments in this series were done in the same group of mudpuppies and over a three-week period of time (May–June) to avoid batch-to-batch differences and/or seasonal variability. The 10 mM HCO_3^-/1% CO_2 solution contained (in mM): 90 NaCl, 10 $NaHCO_3$, 2.5 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, and 0.5 NaH_2PO_4. This solution was equilibrated with a gas mixture of 1% CO_2/99% air and had a pH of ~7.65. The 2.4 mM HCO_3^- solution contained: 109.2 NaCl, 2.4 $NaHCO_3$, 2.5 KCl, and 1.0 $CaCl_2$. After equilibration with air, the pH was ~8.4. The 1 mM HEPES solution contained: 109.2 NaCl, 2.5 KCl, 1.0 $CaCl_2$ and 1.0 HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). After titration with NaOH and equilibration with air its pH was 7.7. With the exception of the studies summarized in Table 1, the control Ringer's was the 10 mM HCO_3^-/1% CO_2 solution. Ion substitutions were done by partial isomolar replacements of a salt of the control 10 mM HCO_3^-/1% CO_2 Ringer's. 25 mM K-Ringer's (K-Ringer's) was made by isomolar replacement of NaCl with KCl. In the low chloride (8.1 mM Cl^-) solutions 90 mM NaCl was replaced with Na^+ gluconate (gluconate Ringer's), Na^+ isethionate (isethionate Ringer's), Na^+ cyclamate (cyclamate Ringer's) or 45 mM Na^+ sulfate (sulfate Ringer's). Sucrose (45 mM) was included in the sulfate Ringer's to keep osmolarity constant. Both gluconate and sulfate Ringer's solutions contained an additional 6 mM $CaSO_4$.

Electrical Measurements

Transepithelial voltage (V_{ms}), and apical (V_{mc}) and basolateral (V_{cs}) membrane voltages were measured as described previously (Reuss & Finn, 1975*a*,*b*; Reuss et al., 1987). With the tissue mounted mucosal-side-up, V_{ms} and V_{cs} were measured with respect to serosal ground and V_{mc} was calculated as the electrical difference ($V_{cs} - V_{ms}$). With the tissue mounted serosal-side-up, electrical ground was the mucosal solution and transepithelial voltage is referred to as V_{sm}. Accordingly, V_{cs} was calculated as $V_{mc} - V_{sm}$. The reference electrode in both groups of studies (mucosal- or serosal-side-up) was a Ag-AgCl pellet separated from the solution by a short Ringer's-agar bridge. To minimize liquid junction potentials arising from solution changes, the ipsilateral electrode was a calomel half-cell connected to the bathing solution by a flowing saturated-KCl bridge consisting of a ~2 mm length of Ultrawick glass capillary (WPI, New Haven, Conn.) pulled to a 0.5 to 1 mm OD tip. Intracellular conventional microelectrodes were prepared from inner-fiber borosilicate glass and filled with either 1 M KCl or 3 M KCl. Their tip resistances ranged from 20 to 100 MΩ.

The transepithelial resistance, R_t ($\Delta V_{ms}/I_t$), and the apparent apical membrane fractional resistance, fR_a ($\Delta V_{mc}/\Delta V_{ms} = R_a/(R_a + R_b)$), were calculated from the changes in transepithelial and cell membrane voltages produced by a calibrated transepithelial DC pulse, I_t (50 $\mu A/cm^2$), which was provided by a

constant current source and applied through Ag-AgCl electrodes. The pulse duration was 1 sec and the change in voltage was measured at 600 msec following the onset of the pulse. Appropriate corrections were made for series resistances. Electrical resistances and equivalent emf's of the "lumped" equivalent circuit model of *Necturus* gallbladder epithelium illustrated in Fig. 1 were determined by intracellular current injection and cable analysis as described in detail elsewhere (Frömter, 1972; Reuss & Finn, 1975*a*, 1977). In brief, intracellular current pulses (I_o) of different intensities were applied via an intracellular microelectrode (*see below*) and the steady-state voltage deflections (ΔV_x) at several radial distances (x) were measured in adjacent cells (7 to 14 impalements) with a second microelectrode. The best fit of plots of ΔV_x *vs.* x to the appropriate Bessel function allowed determinations of the tissue space constant (λ) and the constant A (*see* Frömter, 1972). From these values, the parallel arrangement of apical and basolateral membrane resistances, $R_z = R_a \cdot R_b/(R_a + R_b)$ was calculated according to (Frömter, 1972):

$$R_z = 2\Pi A\lambda^2/I_o. \quad (1)$$

The electrical parameters defined in Fig. 1 were then calculated using equations found in Reuss and Finn (1975*a*).

The I_o *vs.* ΔV_x relationship was essentially linear over an I_o range of ±20 nA, provided that the interelectrode distance (x) was greater than ~50 μm (Fig. 2*B*). As shown in Fig. 2(*A*), at $x <$ 50 μm (equivalent to 2 to 5 cells between electrodes), and when I_o was both greater than about 10 nA and passed in a direction to depolarize the cell membranes, a deviation from linearity was observed. With hyperpolarizing current, the I_o-ΔV_x relationship was linear even at small distances x. A similar nonlinear relationship has been reported by Frömter (1972). This observation can be explained by a voltage-dependent conductance at the apical membrane (García-Díaz, Nagel & Essig, 1983) which is activated when V_{mc} is depolarized by intracellular current injection. In theory, this voltage-dependent attenuation of ΔV_x should be apparent at all interelectrode distances. However, in practice, the attenuation becomes immeasurably small beyond about 50 μm for two reasons: 1. Inasmuch as $g_b \gg g_a$, the fraction of I_o leaving the cells across the apical membrane is small relative to that across the basolateral membrane. Therefore, a voltage-dependent increase in g_a will have a minimal effect on the intraepithelial spread of current. 2. In the absence of voltage-dependent changes in R_a, ΔV_x falls off very sharply in the first few cells from the point of current injection (i.e., the ΔV_x *vs.* x relationship is described by a zero-order Bessel function, *see above*). Thus, the influence of voltage-dependent changes in R_a on ΔV_x will decrease with larger interelectrode distances and hence will be localized to a region of one or a few cells. In all cable experiments (Table 2) I_o ranged from ±5 to ±16 nA and ΔV_x was normalized to an I_o of 10 nA. To minimize nonlinear behavior, a lower I_o was most often used at small interelectrode distances.

Statistical Analysis

Summary values are expressed as the mean ± the standard error of the mean (SEM). Significant differences between groups were determined by conventional t-tests for paired or unpaired data as appropriate. Linear regression (and correlation coefficient, r) were employed to assess significant correlations between parameters. In all cases, a value of $P < 0.05$ was considered significant.

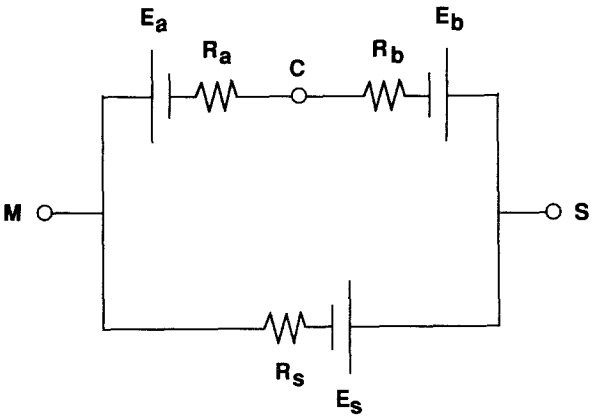

Fig. 1. Equivalent electrical circuit of *Necturus* gallbladder epithelium. *M*, *C*, and *S* represent mucosal, cellular and serosal compartments, respectively. This simple "lumped" model depicts two parallel pathways for transepithelial current flow: a cellular pathway consisting of the series arrangement of apical and basolateral membranes and a paracellular pathway, denoted by subscripts *a*, *b*, and *s*, respectively. Each electrical analog is represented by a Thévenin equivalent, composed of an equivalent electromotive force (E) in series with an equivalent resistance (R). As noted in the text, E_s is assumed to be equal to zero when the tissue is bathed with symmetrical solutions. It is also assumed that all the shunt resistance is localized at the level of the tight junctions of the cells. It should be stressed that to the extent that the resistance of the lateral intercellular space is not negligible in comparison to R_s and $R_a + R_b$, then a "distributed" electrical model of the epithelium is more appropriate (Clausen et al., 1979; Boulpaep & Sackin, 1980; Essig, 1982; Kottra & Frömter, 1984*b*). The measured fR_a in our experiments is therefore an "apparent" fR_a

Results

Effect of Solution Buffer Composition on fR_a

The aim of these studies was to determine if the apparent fR_a is higher in tissues bathed in 10 mM HCO_3^-/1% CO_2 Ringer's than in tissues incubated in either a 2.4 mM HCO_3^- air-equilibrated Ringer's solution or a 1 mM HEPES air-equilibrated Ringer's solution. The control values of membrane voltages and resistances of tissues bathed continuously in one of these three solutions are summarized in Table 1. The major finding was a significantly higher fR_a in gallbladders bathed with 10 mM HCO_3^-/1% CO_2, compared with either of the other two bathing media. The fR_a averaged 0.87 ± 0.06 in 10 mM HCO_3^-/1% CO_2 which agrees well with recent measurements from this laboratory (Poler & Reuss, 1987, Reuss, 1987; Reuss et al., 1987) and others (Suzuki et al., 1982; Kottra & Frömter, 1984*b*). Kottra and Frömter (1984*b*) and Petersen and Reuss (1985) have suggested that high values of fR_a may

Table 1. Membrane voltages and resistances of gallbladders bathed in solutions buffered with 1 mM HEPES, 2.4 mM HCO_3^- or 10 mM HCO_3^-/1% CO_2[a]

Solution	V_{ms} (mV)	V_{mc} (mV)	V_{cs} (mV)	fR_a	R_t ($\Omega \cdot cm^2$)
1 mM HEPES	0.0 ± 0.1	−67 ± 4	−67 ± 4	0.48 ± 0.08	210 ± 20
(n = 7)	(−0.4, 0.5)	(−53, −82)	(−53, −82)	(0.17, 0.84)	(130, 300)
2.4 mM HCO_3^-	−0.2 ± 0.2	−75 ± 2	−75 ± 2	0.63 ± 0.07	180 ± 10
(n = 7)	(−0.7, 0.3)	(−62, −82)	(−63, −83)	(0.24, 0.77)	(140, 220)
10 mM HCO_3^-/1% CO_2	0.3 ± 0.2[b]	−68 ± 1[b]	−68 ± 1[b]	0.87 ± 0.06[c]	160 ± 10
(n = 7)	(−0.1, 0.9)	(−65, −73)	(−64, −72)	(0.51, 0.99)	(110, 200)

[a] Values are mean ± SEM (range).
[b] Significantly different from 2.4 mM HCO_3^- ($P < 0.05$, unpaired t-test).
[c] Significantly different from both 1 mM HEPES and 2.4 mM HCO_3^- Ringer's ($P < 0.05$, unpaired t-test).

There were no significant differences between the 1 mM HEPES and 2.4 mM HCO_3^- series.

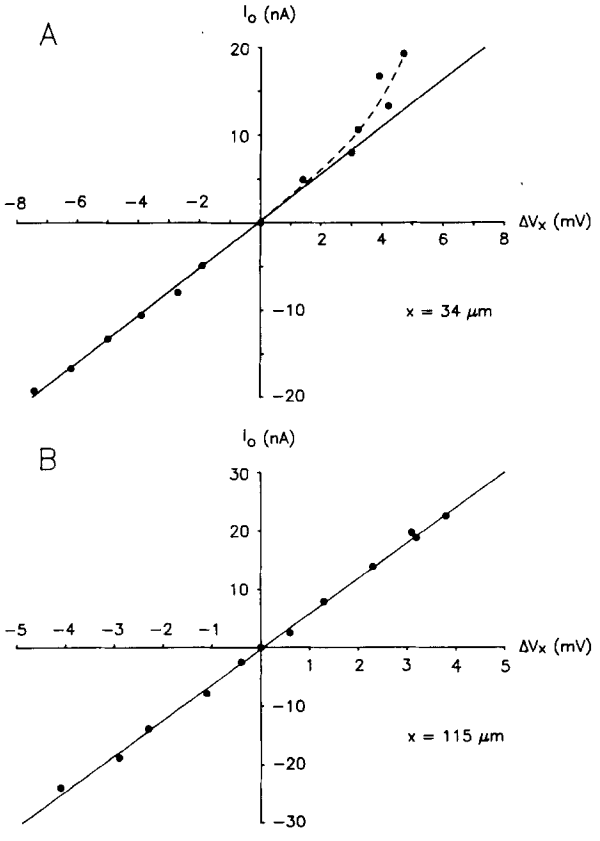

Fig. 2. Relationship between intracellular current injection (I_o) and the cell membrane voltage deflection (ΔV_x) measured at interelectrode distances of 34 μm in one tissue (Panel *A*) and 115 μm in another tissue (Panel *B*). Positive values of I_o result in depolarization of apical and basolateral membranes. Conversely, when I_o is negative, ΔV_x is negative in polarity. Solid lines: linear least-squares fit of lower 9 points (panel *A*) or all points (panel *B*); dashed line denotes nonlinear behavior with large depolarizing currents

be related to solution HCO_3^- concentration, and indeed fR_a increases progressively as solution HCO_3^- concentration is increased (r = 0.68, n = 21, $P <$ 0.01).

Cell membrane voltages were lower and V_{ms} was more mucosa-positive in the 10 mM HCO_3^-/1% CO_2 studies in comparison with the 2.4 mM HCO_3^- studies. These data, taken together with the higher fR_a, can be explained by a lower value of basolateral membrane equivalent emf (E_b) and a reduction in intraepithelial current flow (*see below* and Discussion). Although the average cell membrane voltage and fR_a values were somewhat higher in tissues bathed in 2.4 mM HCO_3^- in comparison with tissues bathed in 1 mM HEPES, they did not differ statistically. Importantly, the membrane voltages and resistances in both conditions were quite similar to previous estimates obtained in this and other laboratories in which the bathing solution composition was either identical or very similar to those used in the present experiments (for references, *see* Materials and Methods).

On rare occasions a rather low estimate of fR_a was obtained in gallbladders bathed in 10 mM HCO_3^-/1% CO_2 (Table 1). This could be related to a high endogenous cAMP level in these gallbladders resulting in an increase in apical membrane chloride conductance (g_{Cl}) (Petersen & Reuss, 1983). The values of V_{ms} in all three groups of studies were not statistically different from zero, a result which suggests that, assuming that $E_s = 0$, $E_a = E_b$. In individual tissues, however, V_{ms} frequently differs from zero, which denotes the existence of a finite intraepithelial current loop attributable to $E_s \neq 0$ and/or $E_a \neq E_b$. Lastly, we note that no significant cor-

Table 2. Equivalent circuit parameters of gallbladders bathed in 10 mM HCO_3^-/1% CO_2[a]

Resistances	R_z ($\Omega \cdot cm^2$)	fR_a	R_t ($\Omega \cdot cm^2$)	R_a ($\Omega \cdot cm^2$)	R_b ($\Omega \cdot cm^2$)	R_s ($\Omega \cdot cm^2$)
	900 ± 100 (610, 1170)	0.90 ± 0.02 (0.81, 0.97)	190 ± 20 (130, 280)	11240 ± 2640 (4810, 20610)	1010 ± 120 (690, 1370)	200 ± 20 (130, 280)

Voltages and emf's	V_{ms} (mV)	V_{mc} (mV)	V_{cs} (mV)	E_a (mV)	E_b (mV)
	0.0 ± 0.2 (0.7, −0.3)	−66 ± 1 (−63, −71)	−66 ± 1 (−63, −71)	−68 ± 10 (−37, −109)	−66 ± 1 (−61, −70)

[a] Values are mean ± SEM (range) of $n = 6$ experiments. Calculations of R_z involved estimates of A and λ which averaged 2.1 ± 0.2 mV and 266 ± 21 μm, respectively (*see* Materials and Methods for details).

relation was found between R_t and bathing solution HCO_3^- concentration ($r = -0.42$, $n = 21$).

Intracellular Cable and Equivalent Circuit Analysis

A reexamination of the cable properties of the epithelium was done to quantify the absolute changes in apical and basolateral membrane resistances responsible for the higher fR_a measured in tissues bathed in 10 mM HCO_3^-/1% CO_2 Ringer's. The results of six circuit analysis experiments performed under these conditions are summarized in Table 2. The apical membrane resistance was found to be two- to fourfold higher and the basolateral membrane resistance was found to be one-half to two-thirds of the values obtained in previous studies done in low HCO_3^--low CO_2 Ringer's solutions (Frömter, 1972; Reuss & Finn, 1975*a*, 1977; Reuss, 1979). The lower value of R_b confirms the recent findings of Frömter and colleagues (Suzuki et al., 1982; Kottra & Frömter, 1984*b*).

The differences in apical and basolateral cell membrane resistances were associated with alterations in apical and basolateral membrane emf's. Compared to earlier estimates (Reuss & Finn, 1975*a*, 1977; Reuss, 1979, *see* Discussion), E_a was hyperpolarized and E_b was depolarized, the net result being $E_a \simeq E_b$.

The results described above, namely greater values of R_a and E_a and smaller values of R_b and E_b, can, in principle, be explained by a reduction of the relative conductance of a low emf pathway (more positive than E_K) at the apical membrane and an increase in the relative conductance of a low emf pathway at the basolateral membrane. To test the latter possibility, we performed basolateral solution ionic substitutions.

Serosal Solution Potassium Substitutions

Investigations of the ionic permeability of the basolateral membrane of *Necturus* gallbladder have demonstrated that this membrane is primarily K^+ conductive with at most a very small Cl^- conductance (Reuss, 1979; Fisher, 1984). To test if the same is true in tissues bathed in 10 mM HCO_3^-/1% CO_2 Ringer's, we performed serosal solution K^+ and Cl^- substitutions. A typical record of the changes in cell membrane and transepithelial voltages upon elevation of serosal solution K^+ concentration is shown in Fig. 3. In this experiment, control values of V_{cs} and V_{sm} were −74 and 0.2 mV, respectively. A 10-fold elevation of serosal solution K^+ concentration resulted in a rapid and reversible depolarization of V_{cs} by 39 mV and a serosa-negative change in V_{sm} by 1.5 mV. As summarized in Table 3, V_{cs} and V_{mc} depolarized by 40 ± 2 mV and 39 ± 2 mV, respectively, within about 10 sec, which demonstrates that the basolateral membrane remains potassium selective under the conditions used in this study. The serosa-negative change in V_{sm} and the fall in R_t indicate that $P_K > P_{Na}$ at the paracellular pathway (van Os & Slegers, 1975; Reuss & Finn, 1975*b*). After returning to control Ringer's, both cell membranes hyperpolarized beyond control values for about 5 min. Although the mechanism of this response remains unknown, possible explanations include an increase in cell membrane P_K in response to an elevation in intercellular Ca^{2+} activity (García-Díaz et al., 1983) or to cell swelling induced by KCl entry across the basolateral membrane (Reuss, 1983; Larson & Spring, 1984).

During elevation of serosal solution $[K^+]$ there was no significant reduction in fR_a, although it fell in every case (Table 3). This is an unexpected find-

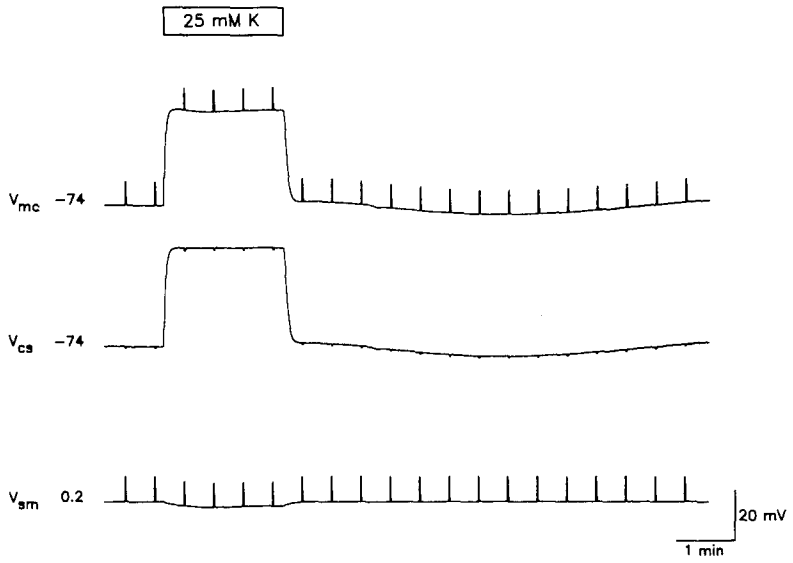

Fig. 3. Effect of serosal K Ringer's (25 mM) on transepithelial (V_{sm}) and cell membrane voltages (V_{mc} and V_{cs}) in tissues incubated in 10 mM HCO_3^-/1% CO_2 Ringer's. Initial voltages are given to the left of each trace. Vertical deflections are the result of 1-sec transepithelial current pulses (50 $\mu A/cm^2$) applied at 30-sec intervals to obtain resistance estimates. Note the long time course of hyperpolarization of cell membrane voltages following return to control after the exposure to K Ringer's

Table 3. Effects of elevation of serosal $[K^+]$ on membrane voltages and resistances[a]

Condition	V_{sm} (mV)	V_{mc} (mV)	V_{cs} (mV)	fR_a	R_t ($\Omega \cdot cm^2$)
Control	0.4 ± 0.2	−72 ± 1	−72 ± 1	0.90 ± 0.02	170 ± 20
K-Ringer's	−1.2 ± 0.2	−33 ± 2	−32 ± 3	0.85 ± 0.04	160 ± 20
Difference	−1.5 ± 0.1[b]	39 ± 2[b]	40 ± 2[b]	−0.05 ± 0.03	−10 ± 2[b]

[a] Values are mean ± SEM of $n = 5$ experiments. Difference was calculated as experimental − control.
[b] $P < 0.05$, paired t-test. Changes in V_{sm}, V_{mc} and V_{cs} represent the initial changes in voltage and were measured at about 10 sec following exposure of the tissue to K Ringer's. Resistance measurements were obtained near the end of the 2-min exposure to K Ringer's.

ing, inasmuch as raising serosal $[K^+]$ is expected to increase fR_a by decreasing basolateral R_b. The lack of increase in fR_a can be explained by a voltage-dependent decrease in R_a (García-Díaz et al., 1983). Although this interpretation was not tested in detail, in one experiment we clamped the tissue for 1 min with a transepithelial constant-current pulse (200 $\mu A/cm^2$) during exposure to high-serosal $[K^+]$. The fR_a was determined by superimposing 1-sec pulses on the current clamp. When current was passed in the mucosa-to-serosa direction, causing a hyperpolarization of V_{mc} and thus reversing the serosal K^+-induced depolarization, fR_a increased. Current application in the serosa-to-mucosa direction (depolarization of V_{mc}) had the opposite effect, that is, a further fall in fR_a. These results are consistent with a voltage-dependent K^+ conductance at the apical membrane which is activated by elevating serosal solution $[K^+]$. This effect reduces fR_a and obscures the increase in this parameter expected from the K^+-induced decrease in R_b.

Serosal Solution Chloride Substitutions

To test for a possible basolateral Cl^- conductance, serosal $[Cl^-]$ was reduced from 98.1 to 8.1 mM while measuring membrane voltages and resistances. The responses of V_{mc}, V_{cs} and V_{sm} to partial replacement of Cl^- with either gluconate or sulfate are illustrated in Fig. 4. Although smaller than the changes elicited by K^+ substitutions, reducing $[Cl^-]$ caused a significant depolarization of V_{cs}. As summarized in Table 4, gluconate and sulfate Ringer's had similar effects on membrane voltages. The time course of the V_{cs} change was variable. Following an initial rapid depolarization, which occurred in about 15 sec and was somewhat slower than the V_{cs} response to K-Ringer's (~10 sec), V_{cs} either secondarily depolarized further (Fig. 4, gluconate Ringer's), hyperpolarized slightly (*not shown*), or remained essentially unchanged (Fig. 4, sulfate Ringer's). The secondary changes were small and most often in the depolarizing direction. For example, in the gluconate Ring-

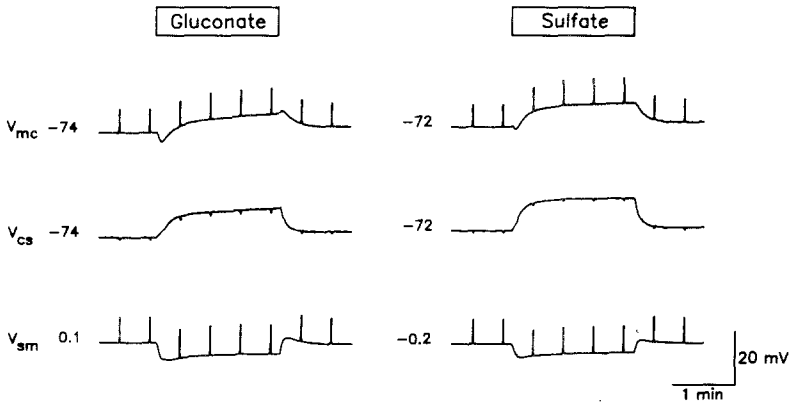

Fig. 4. Effects of serosal solution Cl^- reduction (98.1 to 8.1 mM) on V_{mc}, V_{cs} and V_{sm} using gluconate and sulfate as Cl^- substitutes. The voltage records were obtained from the same cell impalement as in Fig. 3. Format as in Fig. 3

Table 4. Effects of reduction of serosal [Cl^-] on membrane voltages and resistances[a]

Condition	V_{sm} (mV)	V_{mc} (mV)	V_{cs} (mV)	fR_a	R_t ($\Omega \cdot cm^2$)
Control	0.2 ± 0.1	−71 ± 1	−72 ± 1	0.88 ± 0.04	160 ± 20
Gluconate Ringer's	−7.1 ± 0.4	−72 ± 1	−65 ± 1	0.85 ± 0.04	200 ± 30
Difference	−7.3 ± 0.4[b]	−1 ± 1	6 ± 1[b]	−0.03 ± 0.01[b]	40 ± 10[b]
Control	0.2 ± 0.1	−70 ± 2	−71 ± 2	0.88 ± 0.04	160 ± 20
Sulfate Ringer's	−5.4 ± 0.3	−68 ± 2	−63 ± 1	0.87 ± 0.03	180 ± 30
Difference	−5.6 ± 0.4[b]	2 ± 1	8 ± 1[b]	−0.01 ± 0.01	30 ± 10[b]
Control	0.2 ± 0.1	−71 ± 2	−71 ± 2	0.87 ± 0.04	150 ± 20
Isethionate Ringer's	−4.9 ± 0.3	−72 ± 2	−67 ± 2	0.86 ± 0.04	170 ± 20
Difference	−5.1 ± 0.4[b]	−1 ± 1	4 ± 1[b]	−0.01 ± 0.00	20 ± 4[b]
Control	0.2 ± 0.1	−72 ± 1	−72 ± 1	0.92 ± 0.01	160 ± 20
Cyclamate Ringer's	−7.0 ± 0.4	−77 ± 1	−70 ± 1	0.92 ± 0.01	190 ± 30
Difference	−7.2 ± 0.4[b]	−5 ± 1[b]	2 ± 1	0.00 ± 0.01	30 ± 10[b]

[a] Values are mean ± SEM of $n = 6$ experiments. Difference was calculated as experimental − control.
[b] $P < 0.05$, paired t-test with control data. Changes in V_{sm}, V_{mc} and V_{cs} represent the initial changes in voltage and were measured at about 15 sec following exposure of the tissue to low-Cl Ringer's. Resistance measurements were obtained near the end of the 2-min exposure to low-Cl Ringer's. Note that with cyclamate Ringer's there was no significant V_{cs} change.

er's studies V_{cs} fell by an additional 2 ± 1 mV over 2 min after an initial depolarization of 6 ± 1 mV (Table 4).

The magnitude of the initial depolarization of V_{cs} followed the sequence sulfate > gluconate > isethionate > cyclamate. This result could be due to a finite permeability of the Cl^- electrodiffusive pathway to some of the anion substitutes tested, a partial inhibition of the transport mechanism, or perhaps systematic differences in pH or Ca^{2+} activity in the low-Cl^- solutions. Finite permeability of anion-selective channels to Cl^- substitutes has been found in the basolateral membrane of rabbit urinary bladder (Hanrahan, Alles & Lewis, 1985) and certain anion substitutes have been observed to inhibit Cl^- efflux in skeletal muscle (Bretag, 1987).

A negative correlation was found between the magnitude of the response of V_{cs} to low-Cl^- Ringer's and the magnitude of the K^+-induced depolarization, which was statistically significant ($P < 0.05$) for each of the Cl^- substitutes tested. However, there were no significant correlations between the baseline membrane voltage and the magnitude of the depolarization of V_{cs} induced either by the low-Cl^- or by the high-K^+ Ringer's solutions. The changes in apical fractional resistance with low-Cl^- Ringer's were small and only in the case of gluconate was a significant fall in fR_a observed (Table 4). This result indicates that the contribution of the chloride-conductive pathway to R_b is small.

The response of V_{sm} to the reduction in serosal chloride was remarkably similar regardless of which anion substitute was used. As shown in Fig. 4, V_{sm} changed rapidly in the serosa negative direction over the first several seconds, which is consistent with a solution bi-ionic potential. Subsequently, V_{sm}

Table 5. Equivalent electrical circuit parameters in *Necturus* gallbladder epithelium[a]

Solution $[HCO_3^-]/PCO_2$ or Air	Technique	fR_a	R_t ($\Omega \cdot cm^2$)	R_a ($\Omega \cdot cm^2$)	R_b ($\Omega \cdot cm^2$)	E_a (mV)	E_b (mV)	Reference
2.4/-	DC Cable	0.64[b]	307	4470	2880			Frömter (1972)
2.38/Air	DC Cable	0.56[b]	450 ± 40	3350 ± 390	2750 ± 320	39.9 ± 3.6	69.4 ± 1.8	Reuss & Finn (1975*a*)
2.38/Air	DC Cable	0.45[b]	400[b]	3510 ± 420	4260 ± 460	38.7 ± 3.6	90.5 ± 7.6	Reuss & Finn (1977)
2.4/Air	DC Cable	0.68[c]	170[c]	5160[c]	2458[c]	50.6[c]	77.7[c]	Reuss (1979)
10/2.5%	Square-Wave Pulse	0.86 ± 0.025	82 ± 6.7	1220 ± 406	201 ± 79			Suzuki et al. (1982)
10/2.5%	DC Cable	0.86 ± 0.019	83 ± 8.5	960 ± 221	127 ± 16			Suzuki et al. (1982)
10/1%	AC Impedance	0.87[b]	153	3470	225			Kottra & Frömter (1984*b*)
10/1%	DC Cable	0.90 ± 0.02	190 ± 20	11240 ± 2640	1010 ± 120	68 ± 10	66 ± 1	Present Study

[a] Values are either mean or mean ± SEM. The Ringer's solutions used in these studies contained the following principal ions in mM: 101–115.4 Na^+; 2.5–3 K^+; 93.5–118.7 Cl^-; 0.89–2.7 Ca^{2+}. All studies were done at room temperature.
[b] Calculated from mean values in the references quoted, from the relations: $a/(a + 1) = fR_a$, where $a = R_a/R_b$, or $R_t = (R_a + R_b)R_s/(R_a + R_b + R_s)$.
[c] Data summarized from Tables 1–5 (Reuss, 1979).

returned slowly towards control over the next 2 min, but always remained serosa-negative to control. This secondary change in V_{sm} can be explained by dissipation of the junction potential as the fluid within the lateral intercellular spaces exchanges with the low-Cl^- Ringer's. The changes in V_{sm} are mostly due to a change in E_s, which in principle must also cause IR drops across both cell membranes. Consistent with this view, the initial response of V_{mc} was in the hyperpolarizing direction (Fig. 4). The contribution of ΔE_s to ΔV_{cs} ($\Delta V_{cs'}$) is given by:

$$\Delta V_{cs'} = \Delta E_s[R_b/(R_a + R_b + R_s)]. \quad (2)$$

This contribution is small because of the low value of R_b relative to $(R_a + R_b + R_s)$. Therefore, the change in V_{cs} must arise essentially from a change in E_b. Accordingly, we tentatively conclude that in 10 mM HCO_3^-/1% CO_2 Ringer's significant electrodiffusive Cl^- permeability exists at the basolateral membrane of these cells.

Discussion

The major conclusions drawn from these studies are that the steady-state conductances of the apical and basolateral membranes of *Necturus* gallbladder epithelial cells depend on the nature and concentration of the buffers present in the bathing media, in particular the HCO_3^-/CO_2 concentrations. In addition, we can tentatively conclude that in 10 mM HCO_3^-/1% CO_2 Ringer's the basolateral membrane has a sizable electrodiffusive Cl^- permeability. Finally, as will be discussed below, we believe that intracellular cable analysis and the DC transcellular voltage divided ratio can be useful in the electrophysiologic analysis of this epithelium. Our results suggest that the voltage dependence of the apical membrane conductance poses a greater problem than the width of the lateral intercellular spaces in electrical modeling of the system. In this section, we will discuss first the use and interpretation of fR_a determinations, then we will address the apparent differences in the literature pertaining to equivalent circuit parameters, and finally we will justify our conclusions concerning adaptive changes of basolateral membrane-conductive pathways and their possible physiological significance.

Interpretation of fR_a Measurements

To compare fR_a values under steady-state conditions in solutions of different compositions, we carried out studies in the same group of mudpuppies and at the same time of the year, in order to eliminate a number of potential sources of error, including differences in experimental techniques between laboratories and/or investigators. The results of the present studies with 10 mM HCO_3^-/1% CO_2 are compared with earlier observations in Table 5. Clearly, the values of fR_a are much higher in solutions buffered with 10 mM HCO_3^-/1% CO_2 or with 10 mM HCO_3^-/2.5% CO_2. One interpretation of these results is that the previous measurements of fR_a underestimated the true value. That is, when tissues are bathed in a low HCO_3^-/CO_2 Ringer's, transepithelial ion transport could be significantly reduced, leading to a reduced rate of transepithelial fluid transport and consequently to closure of the lateral intercellular spaces. Under these conditions, if fR_a is calculated from a lumped model its value is underestimated because the distributed nature of the basolateral membrane-lateral intercellular space is ignored (Clausen, Lewis & Diamond, 1979; Boulpaep & Sackin, 1980; Essig, 1982). However, sev-

eral experimental observations indicate that a large contribution of the lateral intercellular spaces to the estimate of fR_a is unlikely under these experimental conditions.

First, we found no correlation between solution HCO_3^-/CO_2 concentrations and R_t (Table 1), which suggests that the different buffering conditions did not result in major changes in the resistance of the lateral intercellular spaces. Suzuki et al. (1982) reported an average R_t of about 83 $\Omega \cdot cm^2$ in gallbladders bathed with a 10 mM HCO_3^-/2.5% CO_2 solution. In a later study from the same laboratory, in which a 10 mM HCO_3^-/1% CO_2 solution was used, R_t was about double, averaging 153 $\Omega \cdot cm^2$ (Kottra & Frömter, 1984*b*). Since no differences in fR_a (measured by transepithelial direct current pulses) were observed between these two series of experiments, the contribution of the lateral spaces to the apparent R_b must have been either small or independent of solution PCO_2. This suggests that if the different R_t values reported are caused by changes in width of the spaces, this is not reflected in the value of fR_a.

Second, Kottra and Frömter (1984*b*), utilizing AC impedance analysis, demonstrated that only under rather extreme experimental conditions, namely complete cessation of chamber perfusion or replacement of solution O_2 with N_2, did the resistance of the lateral intercellular spaces increase significantly. Similar experimental conditions were necessary to elicit a significant increase in R_t (Kottra & Frömter, 1984*b*).

Third, in paired studies the fluid transport rate in low-HCO_3^- media is reduced by only about 40% compared with 10 mM HCO_3^-/1% CO_2 Ringer's (Reuss, 1984). Thus, it is unlikely that partial inhibition of transepithelial fluid transport would account for a large decrease in apical membrane fractional resistance. In conclusion, to the extent that the equivalent resistance of the basolateral membrane and the lateral intercellular space is distributed (Kottra & Frömter, 1984*b*), changes in the width of the space will result in underestimation of fR_a. However, the available experimental evidence indicates that this error is small and the estimates of fR_a under most experimental conditions are reasonable. Therefore, we considered the possibility that the observed differences in fR_a values are caused by changes in the cell membrane resistances themselves.

Estimates of Cell Membrane Resistances

Inspection of Table 5 reveals that the larger values of fR_a measured in the present studies (10 mM HCO_3^-/1% CO_2) are attributable to both an increase in R_a and a decrease in R_b in comparison to values obtained in low HCO_3^-/CO_2 Ringer's. However, our estimates of R_a and R_b were significantly greater than in other recent studies in which higher HCO_3^-/CO_2 bathing conditions were used. Suzuki et al. (1982), using both square-wave pulse analysis and intracellular DC cable analysis in tissues bathed with 10 mM HCO_3^-/2.5% CO_2, obtained similar results with both techniques. The values of R_a and R_b averaged about 1000 and 130 to 200 $\Omega \cdot cm^2$, respectively. In tissues bathed with 10 mM HCO_3^-/1% CO_2 Ringer's, Kottra and Frömter (1984*b*) utilized alternating current spectroscopy to estimate R_a and R_b, which averaged about 3500 and 230 $\Omega \cdot cm^2$, respectively. The results of Kottra and Frömter (1984*b*) are somewhat higher than those of Suzuki et al. (1982), but still lower than our estimates. Regarding the studies of Suzuki et al. (1982), we suggest that the transport properties of the epithelium may be quite different from ours because of the higher CO_2 tension of the bathing solution. We have found that elevation of solution CO_2 from 1 to 5%, at constant $[HCO_3^-]_o$, has marked effects on intracellular pH (Stoddard & Reuss, 1985, 1986). In addition, R_t averaged 83 $\Omega \cdot cm^2$ in the studies of Suzuki et al. (1982). We have on occasion measured R_t values below 100 $\Omega \cdot cm^2$, but this has been a rare occurrence. Thus, because of these R_t values and the possible differences in pH_i, a direct comparison of resistance estimates between the studies cited is not warranted.

Although very similar bathing media were employed, a major difficulty in comparing our resistance estimates with those of Kottra and Frömter (1984*b*) is the model dependency and the different assumptions involved in the two methodologies, i.e., AC impedance versus DC cable analysis. In addition, the AC impedance analysis has a rather limited degree of certainty in the estimates of cell membrane resistances, whereas the DC circuit analysis requires an accurate determination of fR_a to ultimately arrive at absolute values of R_a and R_b. It is also possible that discrepancies between their results and ours could be related to differences in experimental procedure between laboratories that we are unaware of.

Suzuki et al. (1982) reported cell membrane voltages averaging about −80 mV. This value is higher than our mean of −66 mV in 10 mM HCO_3^-/1% CO_2, which may suggest that our estimates of R_a and R_b could be compromised by leaky impalements. However, using their mean values of R_a and R_b from cable analysis, we calculate that in their hands $R_z \simeq 110\ \Omega \cdot cm^2$, which is considerably smaller than our estimate of 900 $\Omega \cdot cm^2$. Although

the authors did not report values of A or λ, it is clear from Eq. (1) (*see* Materials and Methods) that A, λ, or both, must be greater in our experiments than in theirs. This result is inconsistent with the possibility of leaky impalements in our experiments.

Permselectivity of Apical and Basolateral Membranes

Previous determinations of apical and basolateral membrane zero-current voltages have shown that $E_a < E_b$ when tissues are bathed in a low-HCO_3^-/CO_2 Ringer's (Table 5). In the present studies in 10 mM HCO_3^-/1% CO_2 Ringer's, E_a was found to be increased and E_b decreased from previous estimates, resulting in $E_a \simeq E_b$ (Tables 2 and 5). In low HCO_3^-, when $E_a < E_b$, assuming E_s to be zero with symmetric bathing solutions, cellular current flow from apical to basolateral membranes results in a mucosa-negative value of V_{ms}. In 10 mM HCO_3^-/1% CO_2, reduction of this cellular current flow to near zero ($E_a \simeq E_b$) should then result in a mucosa-positive shift in V_{ms}, a prediction which was confirmed in our studies (Table 1).

Parallel increases in R_a and E_a cannot be attributed to a decrease in apical membrane K^+ conductance alone. Previous studies have demonstrated a small but significant apical membrane electrodiffusive P_{Na} (van Os & Slegers, 1975; Reuss & Finn, 1975*b*). Hence, a reduction in apical g_K by itself should result in a fall in E_a. Recent patch-clamp studies suggest that the small electrodiffusive Na^+ permeability of the apical membrane may be attributable to Na^+ permeation across K^+ channels (Segal & Reuss, 1987). A reduction of the conductance of this channel together with an increase in its potassium permselectivity would explain the present observations.

The lower values of R_b and E_b observed in 10 mM HCO_3^-/1% CO_2 Ringer's cannot be explained by a singular effect on basolateral g_K. Although basolateral g_K may indeed increase (Petersen & Reuss, 1985), the observed fall in E_b indicates an increase in the relative conductance of a parallel pathway in the basolateral membrane with a zero-current voltage more positive than E_K. Evidence for this conclusion was obtained in studies where the average depolarization of V_{cs} in response to a 10-fold elevation in serosal $[K^+]$ was found to be 40 ± 2 mV, i.e., significantly less than predicted if the basolateral membrane were only permeable to K^+. As discussed above (*see* Results), the less-than-expected basolateral depolarization may be explained, at least in part, by the voltage dependence of the apical membrane P_K (García-Díaz et al., 1983). Because E_a and R_a cannot be assumed to remain constant during the elevation of serosal $[K^+]$, estimates of basolateral membrane potassium partial ionic conductance, g_K, or ionic permeability, P_K, cannot be made with certainty. Nevertheless, the results of these studies suggest that although the basolateral membrane is primarily K^+ conductive, other ion(s) must contribute to the basolateral membrane potential and zero-current voltage.

The most direct evidence in support of a significant electrodiffusive Cl^- pathway at the basolateral membrane is the observation that reducing serosal $[Cl^-]$ causes a measurable fall in V_{cs}. The depolarization averaged ca. 7 mV when Cl^- was lowered from 98.1 to 8.1 mM and either gluconate or sulfate were used as Cl^- substitutes (Table 3). In similar studies utilizing 2.4 mM HCO_3^-, Reuss (1979) found a very small basolateral Cl^- conductance. Because E_b was found to be near E_K in that study, changes in V_{cs} as a consequence of alterations in serosal $[Cl^-]$ would be expected to be small, i.e., under these bathing conditions basolateral g_{Cl} is low (*see below*). Fisher (1984) carried out analogous experiments in 10 mM HCO_3^-/1% CO_2, and did not observe depolarizations comparable to those reported here. Fisher's experiments involved the use of two conventional microelectrodes: one to measure basolateral membrane voltage and the other passed through a neighboring cell to a position just below the basolateral membrane to detect the voltage changes occurring in the subepithelial tissue and hence to correct the intracellular voltage records for subepithelial junction potentials. The observed changes in V_{cs} in response to reductions in serosal solution $[Cl^-]$ were complex and interpreted as inconsistent with a simple electrodiffusive Cl^- transport mechanism at the basolateral membrane. Fisher's interpretation relies on the assumption that the subepithelial electrode yields an accurate correction for the junction potential change. This is questionable because the electrode is not a flowing one and hence the composition of its tip solution is unknown. We suggest that the expected basolateral depolarization upon lowering $[Cl^-]$ is masked by solution junction potential changes in the initial 30 sec following the ionic substitution [*see* Fig. 2 of Fisher (1984)].

Other results from our laboratory are also consistent with a basolateral electrodiffusive Cl^- transport mechanism in *Necturus* gallbladder epithelium. Experimental procedures that result in a decreased rate of Cl^- entry into the cells via Cl^-/HCO_3^- exchange, i.e., pharmacologic inhibition (Reuss et al., 1987) or reduction of mucosal $[Cl^-]$ (Reuss, 1984; Reuss & Costantin, 1984), cause cell membrane hyperpolarization and cellular alkalinization. The hy-

perpolarization can be accounted for by changes in basolateral membrane g_{Cl} and E_{Cl}, but not by an effect of intracellular pH on g_K (Stoddard & Reuss, 1985; Stoddard & Reuss, *unpublished observations*). We have also shown that acidification of the basolateral solution by reducing $[HCO_3^-]$ at constant pCO_2 results in a marked increase in the depolarization of V_{cs} in response to reductions in serosal $[Cl^-]$ (Stoddard & Reuss, 1986).

Finally, we will discuss the possible physiologic significance of the lower values of R_b and E_b in tissues bathed in 10 mM HCO_3^-/1% CO_2 Ringer's. Recent studies in *Necturus* gallbladder, have demonstrated that the transepithelial fluid transport rates, and by inference the rates of transepithelial sodium and chloride transport, are significantly increased by raising bathing solution HCO_3^-/CO_2 concentrations (Reuss, 1984; Petersen & Reuss, 1985). The steady-state intracellular Na^+ and Cl^- activities are unchanged under these various conditions (Weinman & Reuss, 1982; Reuss, 1984; Reuss & Costantin, 1984; Reuss & Petersen, 1985; Reuss et al., 1987), but pH_i is lower in 10 mM HCO_3^-/1% CO_2, i.e., about 7.35, compared with ca. 7.50 in low-HCO_3^- Ringer's (Weinman & Reuss, 1982; Reuss & Costantin, 1984; Reuss & Petersen, 1985; Reuss, 1987; Reuss et al., 1987). Assuming that intracellular $[HCO_3^-]$ is lower in the studies with low HCO_3^-, and since apical NaCl entry is by Na^+/H^+ and Cl^-/HCO_3^- exchange (Reuss & Stoddard, 1987), thermodynamic arguments indicate that apical Na^+ and Cl^- entry should be increased when solution HCO_3^-/CO_2 is increased. Consequently, the Na^+ and Cl^- transport rates at the basolateral membrane would be required to change accordingly. As discussed by Schultz (*see* in Reuss et al., 1984), stimulation of apical membrane ion transport in Na^+-absorbing or Cl^--secreting epithelia causes adaptive steady-state increases in both Na^+ pump rate and basolateral membrane K^+ conductance. Because of the electrical coupling between apical and basolateral membranes in such electrogenic systems, the increase in basolateral g_K tends to compensate for the depolarizing effect of the primary apical membrane conductance change. In addition, the requirement for enhanced K^+ recycling at the basolateral membrane is satisfied. In *Necturus* gallbladder epithelium, apical NaCl transport is primarily electroneutral (Reuss & Stoddard, 1987) and hence different adaptive changes may occur to accommodate increased rates of fluid transport while preserving intracellular homeostasis. Since the cell membranes are K^+ selective, increasing basolateral membrane P_K alone should hyperpolarize both cell membranes. The hyperpolarization, by itself, would tend to reduce the net basolateral conductive K^+ efflux. However, a concomitant increase in basolateral P_{Cl} would tend to maintain the cell membrane voltages constant, thereby increasing the basolateral electrodiffusive effluxes of both ions. Thus, both net Cl^- transport and K^+ recycling at the basolateral membrane will be increased *pari passu* with the increase in transepithelial fluid transport. The precise mechanisms of the changes in basolateral membrane-permselective properties remain unknown, but may be related to the changes in pH_i, HCO_{3i}^-, or perhaps secondary effects of Ca_i. Further studies are required to distinguish among these possibilities.

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