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Electrical Properties of the Cellular Transepithelial Pathway in *Necturus* Gallbladder: III. Ionic Permeability of the Basolateral Cell Membrane

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Summary. The ionic permeability of the basolateral membrane of *Necturus* gallbladder epithelium was studied with intracellular microelectrode techniques. After removal of most of the subepithelial tissue (to reduce unstirred layer thickness), impalements were performed from the serosal side, and ionic substitutions were made in the serosal solution while a microelectrode was kept in a cell. Thus, it was possible to obtain continuous (and reversible) records of transepithelial and cell membrane potentials and to measure intermittently the transepithelial resistance and the ratio of cell membrane resistances. From these data and the mean value of the equivalent resistance of the cell membranes in parallel (obtained from cable analysis in a different group of tissues), absolute cell membrane and shunt resistances and equivalent electromotive forces (emf's) were calculated. From the changes of basolateral membrane emf (E_b) produced by the substitutions, the conductance (G) and permeability (P) of the membrane for K, Cl and Na were estimated. Potassium-for-sodium substitutions produced large reductions of both cell membrane potentials, of E_b, and of the resistance of the basolateral membrane (R_b), indicating high G_K and P_K. Chloride substitution with isethionate or sulfate resulted in smaller changes of cell membrane potentials and E_b and in no significant change of R_b, indicating small but measurable values of G_{Cl} and P_{Cl}. Sodium substitutions with N-methyl-D-glucamine (NMDG) resulted in cell potential changes entirely attributable to the biionic potential produced in the shunt pathway ($P_{Na} > P_{NMDG}$), and in no significant changes of R_b or E_b, indicating that G_{Na} and P_{Na} are undetectable. The question of the mechanism of Cl transport across the basolateral membrane was addressed by comparing the mean rate of transepithelial Cl transport (J_{Cl}^{net}) and the predicted passive Cl flux across the basolateral membrane (from the membrane Cl conductance, potential, and Cl equilibrium potential). The conclusion is that only a very small fraction of the Cl flux across the basolateral membrane can be electrodiffusional. Since the paracellular Cl conductance is also too low to account for J_{Cl}^{net}, these results suggest the presence of a neutral mechanism of Cl extrusion from the cells. This could be a NaCl pump, a downhill KCl transport mechanism, or a Cl-HCO_3 exchange mechanism.

Key words: Gallbladder, epithelium, salt transport, chloride transport, membrane permeability.

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To understand the mechanisms of ion transport by epithelia, it is necessary to determine the properties of the cell membranes, in particular their ionic permeabilities. A method suitable for this purpose is to measure changes of membrane potentials and resistances produced by alterations in the ionic composition of the media bathing the tissue *in vitro*. In leaky epithelia (Frömter & Diamond, 1972) this analysis is far more complicated than in isolated cells for the following reasons: (i) The potential measured across a cell membrane depends not only on its active and passive properties, but also on the equivalent electromotive forces (emf's) and resistances of both the opposite cell membrane and the intercellular transepithelial pathway. This is due to the high conductance of the latter, which results in circular current flow within the epithelium (Boulpaep, 1971; Schultz, 1972). (ii) When the bathing medium is substituted only on side of the tissue, one may expect changes of the emf and resistance not only at the ipsilateral cell membrane, but also at the intercellular (shunt) pathway. (iii) The presence of sizable anatomic unstirred layers on the basolateral side of most of the epithelial tissues employed for studies *in vitro* (frog skin, intestine, urinary bladder, gallbladder) results in very slow changes of cell membrane potentials when the serosal (or inner) bathing medium is changed. The likelihood of alterations in cellular composition under these conditions makes the analysis difficult.

The experiments reported in this paper were undertaken to characterize the ionic permeability of the basolateral membrane of the gallbladder of *Necturus maculosus*. This epithelium is typically leaky (Frömter, 1972; Reuss & Finn, 1975*a*), and transports NaCl isosmotically from mucosa to serosa (Hill & Hill, 1978*b*) at a mean rate of about $12\,\mu l \cdot cm^{-2} \cdot h^{-1}$ (Reuss, Bello-Reuss & Grady, 1979). The luminal cell membrane has high K permeability. Chloride and Na are less permeant (Reuss & Finn, 1975*b*; Van Os & Slegers, 1975).

The approach employed in this study was similar to the one used previously to characterize the luminal membrane (Reuss & Finn, 1975*b*), except that the cells were impaled from the serosal side after removal of most of the subepithelial layers. This procedure resulted in considerable shortening of the time required to reach steady-state changes in cell membrane potentials when the serosal solution was substituted.

The results indicate that the basolateral membrane has a high K permeability and a low Cl permeability. Sodium permeability is negligible. Even though the electrochemical gradient is favorable for passive Cl transport from cell to serosal solution, the low Cl permeability of the

membrane indicates that only a small fraction of the transepithelial Cl flux could result from simple diffusion across this membrane. These results are consistent with previous observations in *Necturus* and rabbit gallbladder (Hénin & Cremaschi, 1975; Reuss & Finn, 1975*a*; Van Os & Slegers, 1975). In those experiments, no attempt was made to reduce the anatomic serosal unstirred layer or to consider electrical parameters other than the cell membrane and transepithelial potentials.

Materials and Methods

Necturi were obtained from Mogul-Ed Co., Oshkosh, Wisc., and kept at 4 °C. The gallbladders were removed, opened, washed, and pinned to a cork ring, mucosa down, on top of a piece of dialysis membrane. The subepithelial tissue was removed under microscopic observation. The tissues were then mounted on a modified Ussing chamber (serosa up), and incubated in Ringer's solution (Na-Ringer) of the following composition (in mmol per liter): NaCl, 109.2; KCl, 2.5; $CaCl_2$, 1.0; $NaHCO_3$, 2.4. The pH was about 8.0 after equilibration with room air. All experiments were performed at 24 ± 1 °C. NaCl in the serosal medium was replaced isosmotically with KCl (K-Ringer), N-methyl-D glucamine (NMDG-Ringer), Na isethionate (Ise-Ringer) or Na_2SO_4 (SO_4-Ringer). Sucrose was added to SO_4-Ringer to keep a constant osmolarity. All solutions were titrated to a final pH equal to that of Na-Ringer. Since the K activity coefficient in Na_2SO_4 solutions is lower than in NaCl solutions (Dietschy & Moore, 1964; Lewis, Wills & Eaton, 1978), the K activity in SO_4-Ringer was measured with a 93-19 K-selective electrode (Orion Research Inc., Cambridge, Mass.), and adjusted to the value in NaCl-Ringer.

Electrical Measurements

Potentials and resistances were measured as previously described (Reuss & Finn, 1975*a*, *b*; 1977). Glass microelectrodes with inner fiber were filled with 3 M KCl or 4 M potassium acetate and sometimes beveled by the technique of Odgen, Citron and Pierantoni (1978). Only electrodes with tip resistances greater than ca. 30 MΩ (usually 40 to 50 MΩ) proved adequate for impalements from the serosal side. In most cases, a few layers of connective tissue remained after the dissection. Lower resistance microelectrodes broke, became plugged, or simply did not penetrate these layers. Extracellular potential measuring electrodes were Na-Ringer-agar or 3 M KCl-agar bridges connected to calomel half cells or Ag-AgCl pellets. Extracellular current-passing electrodes were Ag-AgCl pellets connected to the bathing media with Na-Ringer-agar bridges. All potential measurements during substitutions were corrected for the respective liquid junction potentials as described before (Reuss, 1978).

Cell impalement were performed with motorized, remote control micromanipulators (Stoelting, Chicago, Ill.), under direct observation, at 200–400×, with a MS inverted microscope (Nikon Inc., Garden City, N.Y.) or a Biovert inverted microscope (Reichert, Austria). Intracellular (apical membrane: V_{mc}, basolateral membrane: V_{cs}) and transepithelial (V_{ms}) potentials (*see* Fig. 1) were measured with high impedance ($>10^{12}\,\Omega$) electrometers, provided with digital readouts to 0.1 mV, and displayed on a storage

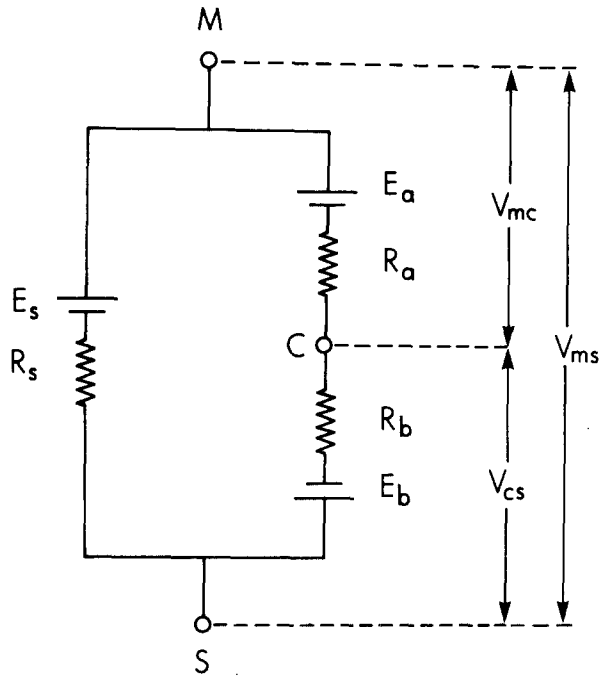

Fig. 1. Equivalent electrical circuit for *Necturus* gallbladder epithelium. Each element of the circuit is represented as a Thévenin equivalent, i.e., an equivalent electromotive force (E) in series with an equivalent resistance (R). The subscripts refer to the apical membrane (a), the basolateral membrane (b), and the paracellular or shunt pathway (s). The emf values can result, in principle, from diffusional mechanism and/or electrogenic pumps. E_s is expected to have a very low value when the tissue is bathed with identical solutions on both sides *(see Discussion)*. The right hand side of the figure defines the measured potentials: transepithelial (V_{ms}), apical membrane (V_{mc}), and basolateral membrane (V_{cs})

oscilloscope (Tektronik, Beaverton, Ore.) and on a three-channel pen recorder (Brush 2400, Gould Inc., St. Louis, Mo.). The reference was the serosal solution.

The transepithelial resistance (R_t) and the ratio of apical to basolateral membrane resistance (R_a/R_b) were measured from the deflections produced in V_{ms}, V_{mc}, and V_{cs} by calibrated transepithelial current pulses, usually $50\,\mu A \cdot cm^{-2}$, after appropriate corrections for the voltage drops in the solutions.

Experimental Procedure

Experiments were started at least 30 min after mounting the tissue. Both bathing solutions were exchanged continuously. After an acceptable impalement was obtained, the serosal solution was changed from Na-Ringer to K-, Ise-, SO_4- or NMDG-Ringer. The microelectrode was kept continuously in the cell, and V_{ms}, V_{mc}, V_{cs}, R_t and R_a/R_b were recorded. After stable new values of cell potentials were obtained, the serosal solution was changed back to Na-Ringer. Only fully reversible records were accepted.

Statistics

Direct data (potentials, R_t and R_a/R_b) are reported as means ± SE. Calculated values of emf's and resistances were obtained from means in each group of experiments, as described in *Results*. Unless otherwise indicated, statistical comparisons were made by conventional paired data analysis.

Results

Removal of the subepithelial tissue resulted in a considerable reduction of unstirred layer delays upon substitutions of the serosal

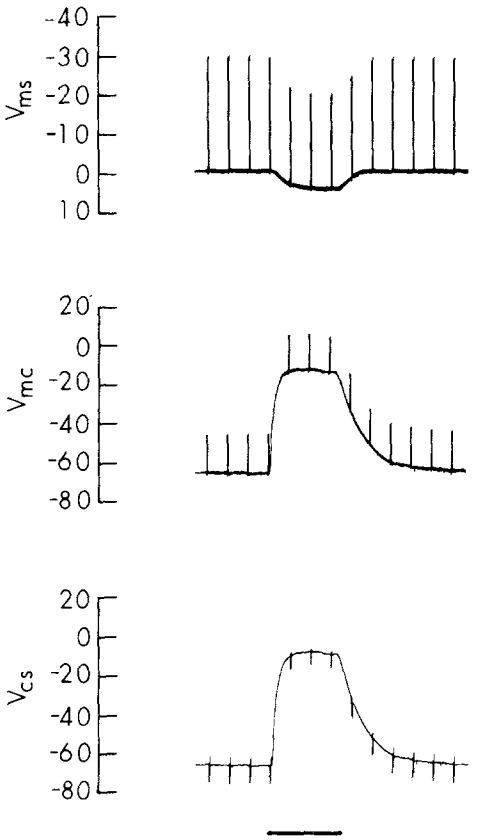

Fig. 2. Effect of increasing serosal K concentration from 2.5 to 111.7 mM (complete substitution of KCl for NaCl) on potentials and resistances. The records start with a microelectrode in a cell. V_{ms}, V_{mc} and V_{cs} are the transepithelial, apical membrane, and basolateral membrane potential, respectively. Short vertical deflections are the voltage changes produced by transepithelial current pulses of 25 $\mu A \cdot cm^{-2}$, at 15-sec intervals. During the period indicated by the bar, serosal Na-Ringer was replaced by K-Ringer. V_{mc} and V_{cs} decreased by about 54 and 59 mV, respectively. V_{ms} reversed from −0.6 to +4.2 mV (reference, serosal solution). Note also the decrease of R_t during exposure to K-Ringer, as shown by smaller deflections of V_{ms}. Finally R_a/R_b decreased. After the seventh pulse, the serosal solution was changed back to Na-Ringer. All changes were reversible

solution. The time required for steady-state depolarization after increases of K activity in the serosal solution ($[K]_s$) was shortened from about 5 min to about 30 sec (*see* Fig. 2).

Effects of $[K]_s$ *on Membrane Potentials and Resistances*

A typical record of cell membrane and transepithelial potentials before, during, and after an increase of $[K]_s$ is shown in Fig. 2. Note that both cell membranes depolarized and that V_{ms} reversed (becoming mucosa positive). The transepithelial resistance fell and R_a/R_b rose during exposure to K-Ringer. All of these changes were completely reversible, even at this high $[K]_s$. The magnitude of the depolarization suggests a high basolateral membrane K permeability.

The results of twelve experiments in which K-Ringer was substituted for Na-Ringer are summarized in Table 1. As already shown in Fig. 2, the main observations are: large depolarization of both cell membranes, mucosa-positive change of V_{ms}, reduction of R_t and increase of R_a/R_b

In five experiments, impalements were performed from the mucosal side in tissues in which no subepithelial dissection was performed. The steady-state V_{cs} change upon exposure to K-Ringer was not different from the value shown in Table 1 (59.5 ± 1.9 mV, $P > 0.2$, nonpaired). In four of these experiments two distinct phases of depolarization could be observed: the first, fast, took about 30 sec and amounted to ca. two thirds of the final change; the second, slow, lasted for 5 min or more. The fact that the values at $t \sim 5$ min are similar to the steady-state values obtained at a shorter time from serosal impalements indicates that the final value, and not the initial change, should be used to estimate the K conductance and permeability of the membrane.

Table 1. Effects of serosal K-Ringer on transepithelial and cell membrane potentials, ratio of cell membrane resistances, and transepithelial resistance

Serosal medium	V_{ms} (mV)	V_{mc} (mV)	V_{cs} (mV)	R_a/R_b	R_t ($\Omega \cdot cm^2$)
Na-Ringer	-0.8 ± 0.2	-72.3 ± 1.1	-73.1 ± 1.2	2.13 ± 0.24	160 ± 8
K-Ringer	3.2 ± 0.5	-14.1 ± 1.6	-10.9 ± 1.8	2.91 ± 0.38	122 ± 8
Δ	-4.0 ± 0.5	-58.2 ± 1.5	-62.2 ± 1.6	-0.78 ± 0.16	38 ± 5
P	<0.001	<0.001	<0.001	<0.001	<0.001

$N = 12$ experiments. Measurements performed as described in Fig. 2. Mucosal bathing medium was Na-Ringer. V_{ms} referred to serosal solution. V_{mc} and V_{cs} referred to the corresponding bathing solution.

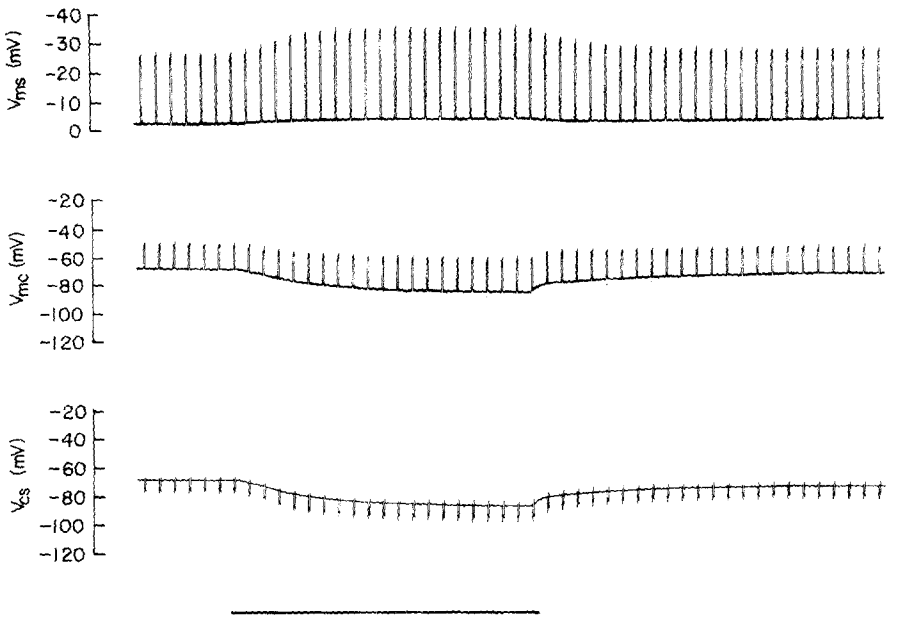

Fig. 3. Effect of exposure to K-free serosal solution on potentials and resistances. Notation as in Fig. 2. Exposure to K-free solution (indicated by lower line) results in hyperpolarization of both cell membranes and a slight reduction of transepithelial potential. R_t and R_a/R_b increase. Interval between pulses: 10 sec. All changes are reversible

Finally, in three experiments in nonstripped tissues the serosal solution was substituted by Na-Ringer containing no K. In all three cases an increase of V_{cs} of ca. 20 mV was observed. An example is shown in Fig. 3. Note that both V_{cs} and V_{mc} increase, V_{ms} also increases, R_t rises, and R_a/R_b falls moderately. All of these results are in agreement with those from the high-K experiments.

Effects of $[Na]_s$ *on Membrane Potentials and Resistances*

Replacements of serosal Na with the large cation NMDG resulted in a larger change of transepithelial potential than of cell membrane potentials (Table 2). As seen also in Table 2, R_a/R_b was unchanged by exposure to NMDG-Ringer, whereas, in contrast, R_t (and therefore R_s) increased significantly. It will be shown that all of these effects can be explained by the larger shunt permeability for Na as compared to NMDG, and that the basolateral membrane permeability for Na is, with this method, undetectable.

Table 2. Effects of serosal NMDG-Ringer on transepithelial and cell membrane potentials, ratio of cell membrane resistances, and transepithelial resistance

Serosal medium	V_{ms} (mV)	V_{mc} (mV)	V_{cs} (mV)	R_a/R_b	R_t ($\Omega \cdot cm^2$)
Na-Ringer	-0.6 ± 0.1	-68.2 ± 2.4	-68.7 ± 2.4	1.85 ± 0.20	171 ± 5
NMDG-Ringer	-14.8 ± 0.8	-58.1 ± 2.3	-72.9 ± 1.8	1.91 ± 0.18	245 ± 1
Δ	$+14.2 \pm 0.7$	-10.1 ± 0.7	$+4.2 \pm 1.0$	-0.06 ± 0.05	-74 ± 1
P	<0.001	<0.001	<0.005	NS	<0.001

$N = 11$ experiments. Mucosal bathing medium was Na-Ringer. Polarities are as defined in Table 1.

Table 3. Effects of serosal Cl substitutions on transepithelial and cell membrane potentials, ratio of cell membrane resistances, and transepithelial resistance

Serosal medium	V_{ms} (mV)	V_{mc} (mV)	V_{cs} (mV)	R_a/R_b	R_t ($\Omega \cdot cm^2$)
Na-Ringer	-0.5 ± 0.1	-67.8 ± 3.1	-68.3 ± 3.0	2.15 ± 0.31	161 ± 7
Ise-Ringer	$+2.7 \pm 0.6$	-67.3 ± 2.7	-64.6 ± 3.0	2.09 ± 0.30	182 ± 5
Δ	-3.2 ± 0.6	-0.5 ± 1.1	-3.7 ± 0.9	0.06 ± 0.14	-21 ± 3
P	<0.005	NS	<0.025	NS	<0.001
Na-Ringer	-0.4 ± 0.2	-64.6 ± 2.1	-65.1 ± 2.2	1.66 ± 0.29	180 ± 26
SO_4-Ringer	$+2.6 \pm 1.0$	-64.7 ± 1.7	-62.1 ± 1.5	1.62 ± 0.31	188 ± 28
Δ	-3.0 ± 0.4	0.1 ± 1.2	3.0 ± 1.0	0.04 ± 0.06	-8 ± 2
P	<0.005	NS	<0.05	NS	NS

$N = 7$ experiments (Ise-Ringer), 5 experiments (SO_4-Ringer). Mucosal bathing medium was Na-Ringer. Polarities are as defined in Table 1.

Effects of $[Cl]_s$ *on Membrane Potentials and Resistances*

The results of changes of serosal Cl activity ($[Cl]_s$) are shown in Table 3. V_{cs} decreased by 3.7 mV when all NaCl was replaced with sodium isethionate, and by 3.0 mV when sodium sulfate was employed to replace NaCl.

These results suggest a small, but finite, basolateral membrane Cl permeability. Note, however, that the transepithelial potential change was of similar magnitude. This indicates a higher shunt permeability for Cl than for isethionate or sulfate.

Effects of $[K]_s$, $[Na]_s$ *and* $[Cl]_s$ *on Basolateral Membrane Equivalent* emf

Absolute values of cell membrane and shunt resistances are necessary to calculate the changes of basolateral membrane emf (E_b) produced by

external ionic substitutions. With serosal impalements, cable analysis is not possible. Therefore, to estimate the changes of E_b, the following procedure was employed: cable analysis was performed, with impalements from the mucosal side, in eight tissues, during the same period of the year as the experiments reported here. The mean value of equivalent resistance of the cell membranes in parallel (R_z) was employed, together with the mean values of R_t and R_a/R_b obtained in Na-Ringer, in the tissues impaled from the serosal side, to calculate control values of R_a, R_b and R_s (shunt resistance), as previously described (Reuss & Finn, 1975*a*, Eqs. (4)–(6); *see also* Fig. 1).

From R_a, R_b and R_s, and the measured potentials, the cell membrane emf's (apical, E_a; basolateral E_b) were calculated (Reuss & Finn, 1975*a*, Eqs. (10) and (11)). These equations are correct if E_s (shunt emf) is zero when the tissue is bathed with Na-Ringer in both sides. For a justification of this assumption, *see Discussion* (and Reuss & Finn, 1975*a*).

Upon going to a new serosal bathing medium, changes of both the emf and resistance of both the basolateral membrane and the shunt can take place. The luminal membrane, however, will not be immediately affected. Assuming that R_a remains constant, the values of R_b and R_s can be calculated as follows:

$$a' = R_a/R_b' \tag{1}$$

$$R_s'^{-1} = R_t'^{-1} - (R_a + R_b')^{-1} \tag{2}$$

where $'$ indicates values in the substituted serosal solution. The new values of E_b and E_s are given by

$$E_b' = \frac{V_{cs}'(R_a + R_b') - R_b'(E_a + V_{ms}')}{R_a} \tag{3}$$

$$E_s' = V_{ms}' + (E_a - V_{mc}')\frac{R_s'}{R_a}. \tag{4}$$

The derivation of Eqs. (3) and (4) is outlined in the *Appendix*.

The change of E_b produced by the ionic substitution can be employed to estimate the ion-dependent partial potential ratio (T_i):

$$T_i = \frac{\Delta E_b}{\frac{RT}{zF}\ln\frac{C_1}{C_2}} \tag{5}$$

where ΔE_b is the change of E_b produced by the substitution and C_1 and C_2 are the external activities of the ith ion before and after the change in solution (*see* Reuss & Finn, 1975*b*).

From T_i, the partial ionic conductance (G_i) and the ionic permeability coefficient (P_i) can be calculated:

$$P_i = \frac{RTG_i}{z^2 \bar{C}_i F^2} \tag{6}$$

where G_i is the partial conductance of the ith ion ($= T_i \cdot G_b$; $G_b = R_b^{-1}$), $\bar{C}_i$ is the mean activity of the ith ion, and R, T, z and F have their usual meanings. Equation (6) is used under the assumptions of constant field and no contribution of electrogenic pumps to the potentials (*see Discussion*).

The method described above is strictly valid only for small changes around the baseline potentials and for small concentration changes. The former condition was fulfilled when Cl was replaced or when NMDG was substituted for Na, but not when K-Ringer was used to replace Na-Ringer. Therefore, a series of experiments was done in which the tissues were exposed to four different serosal K activities, keeping K + Na constant and Cl constant. Since P_{Na} across the basolateral membrane is zero (*see* above), an independent estimate of P_{Cl}/P_K was obtained, assuming constant field, no electrogenic pumps, and $T_K + T_{Cl} = 1$:

$$\Delta E_b = \frac{RT}{zF} \ln \frac{K_o^i + P' Cl_i}{K_o^f + P' Cl_i} \tag{7a}$$

where K_o^i and K_o^f are the initial and final K activities in the serosal solution, P' is P_{Cl}/P_{Na}, Cl_i is the intracellular Cl activity and R, T, z, and F have their usual meanings. The mean value of Cl_i in a different group of tissues (Reuss, 1979) was employed. Cl_i was assumed to remain constant during the time required for the measurement.

Because of the small changes of potentials observed during Cl and Na substitutions (Tables 2 and 3) it was not possible to study the effect of small changes in the activity of these ions. This experimental limitation is unimportant in Na-NMDG substitutions, because E_b did not change (*see* below). Its significance in Cl substitutions will be discussed later.

Dependence of E_b on $[K]_s$

The results of K for Na substitutions were employed to calculate the values shown in Table 4. During exposure to K-Ringer, E_b decreased by

Table 4. Calculated resistances and electromotive forces during exposure of the serosal side to Na-Ringer or K-Ringer (*A*), or to Na-Ringer or NMDG-Ringer (*B*)

	Serosal medium	R_a ($\Omega \cdot cm^2$)	R_b ($\Omega \cdot cm^2$)	R_s ($\Omega \cdot cm^2$)	E_a (mV)	E_b (mV)	E_s (mV)
A)[a]	Na-Ringer	5480	2570	163	−45.4	−85.7	0
	K-Ringer	5480	1880	124	−45.4	−0.2	2.5
B)[b]	Na-Ringer	4990	2700	175	−54.0	−76.4	0
	NMDG-Ringer	4990	2600	253	−54.0	−75.1	−14.6

[a] Data calculated from means in Table 1 as described in *Methods*. $N=12$ experiments. From the E_b change, $T_K=0.94$. E_s referred to serosal solution. E_a and E_b referred to the corresponding bathing solution.

[b] Values calculated from means in Table 2. $T_{Na}=-0.01$ (not significantly different from zero if calculated for individual tissues).

85.5 mV (yielding a mean basolateral T_K of 0.94) and E_s changed by 2.5 mV (mucosa positive), indicating that across the shunt $P_K > P_{Na}$, as observed previously (Reuss & Finn, 1975*a*, Van Os and Slegers, 1975). The computation of T_K from ΔE_b during Na- substitutions assumes implicitly that Na does not contribute to the potential (i.e., that $T_{Na}=0$). Support for this assumption was provided by Na-NMDG substitution

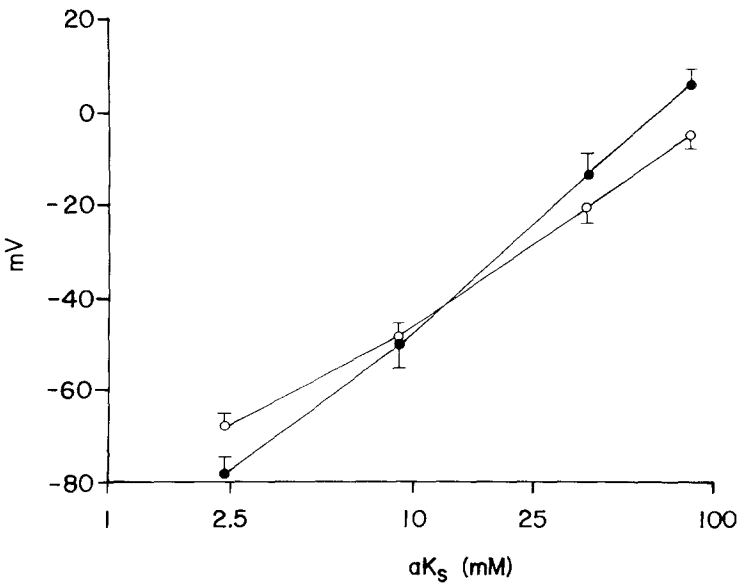

Fig. 4. Basolateral membrane potential (V_{cs}, open circles) and basolateral membrane equivalent emf (E_b, filled circles) as functions of serosal K activity. Values for each K activity obtained in the same cell. $N=4$ tissues. Points were joined by straight lines

experiments (*see* below). The changes of R_b and R_s produced by exposure to K-Ringer (Table 4) are confirmatory of a high P_K (as compared to P_{Na}) at both the basolateral membrane and the shunt.

In four experiments, the tissue was exposed to four different solutions in which NaCl was replaced with KCl, keeping both (Na+K) and Cl activities constant. The results are shown in Fig. 4. V_{cs} and E_b are plotted as functions of external K activity. As expected, the membrane potential changes are smaller than the membrane emf changes. The reason is current flow, through the intercellular pathway, from apical to basolateral membrane, which results in an IR drop in the latter ($=E_a \cdot R_b/(Ra + R_b + R_s)$). From ΔE_b in each K activity range P_{Cl}/P_K was calculated from Eq. (7), inserting $Cl_i = 36$ mM (Reuss, 1979). The values were 0.02, 0, and 0.02 in the low, middle, and high K-activity ranges, respectively. These values are in good agreement with the results shown in Tables 4 and 5 (*see* below).

Dependence of E_b *on* $[Na]_s$

The calculated changes of emf's during exposure to NMDG-Ringer indicate that the basolateral membrane is essentially impermeable to Na (mean $T_{Na} = -0.01$, not different from zero if calculated separately for each tissue). The shunt emf, in contrast, changed by 14.6 mV (mucosa negative) indicating that across the shunt $P_{Na} > P_{NMDG}$. The observation of a basolateral membrane Na permeability as low as that of the large cation NMDG lends support to the assumption of $T_{Na} = 0$ when calculating T_K and P_{Cl}/P_K from K-Na substitutions (*see* above).

Dependence of E_b *on* $[Cl]_s$

Resistances and emf values calculated from the data in Table 3 are summarized in Table 5. T_{Cl}, computed from these data, was 0.06 in the isethionate series and 0.04 in the sulfate series, i.e., more than one order of magnitude lower than T_K. The lack of significant changes of R_b in SO_4- or Ise-Ringer agrees with the low value of T_{Cl} calculated from the changes of E_b. Finally, the increase of R_s with both replacements indicates a higher shunt permeability for Cl than for sulfate or isethionate.

Table 5. Calculated resistances and electromotive forces before and during Cl substitutions on the serosal side

Serosal medium	R_a ($\Omega \cdot cm^2$)	R_b ($\Omega \cdot cm^2$)	R_s ($\Omega \cdot cm^2$)	E_a (mV)	E_b (mV)	E_s (mV)
Na-Ringer	5510	2560	164	−51.0	−76.1	0
Ise-Ringer	5510	2640	186	−51.0	−72.4	3.3
Na-Ringer	4660	2000	184	−52.0	−72.7	0
SO_4-Ringer	4660	2870	193	−52.0	−69.9	3.1

Values calculated from means in Table 3. $T_{Cl}=0.06$ (isethionate substitutions), $T_{Cl}=0.04$ (sulfate substitutions). Polarities are as defined in Table 4.

Discussion

The results described above indicate that the basolateral cell membrane of *Necturus* gallbladder epithelium is highly permeable to K, moderately permeable to Cl, and impermeable to Na. Quantitative analysis of the data and calculations of transference numbers and permeability coefficients required several assumptions that should be discussed.

To solve the circuit equations under control conditions it was necessary to assume that $E_s=0$ when the tissue is bathed with identical solutions on both sides. This assumption would appear to contradict the standing osmotic gradient hypothesis, which postulates a higher NaCl concentration in the intercellular spaces than in the mucosal bathing medium (resulting from active salt transport into the spaces). Since the limiting junctions are cation selective ($P_{Na}>P_{Cl}$, Reuss & Finn, 1975*a–b*) the concentration gradient would result in a mucosa-positive diffusion potential and E_s would not be zero. Early calculations by Machen and Diamond (1969) for the rabbit gallbladder predicted a 10 mM NaCl concentration gradient. However, recent evidence, which indicates that the osmotic water permeability of the gallbladder is one to two orders of magnitude greater than estimated from steady-state osmotic water fluxes, suggests strongly that the gradient must be considerably smaller (Wright, Smulders & Tormey, 1972). In addition, if $E_s\neq 0$ under control conditions, the absolute values of E_a and E_b are different from those calculated, but their changes upon unilateral ionic substitutions are essentially the same (Reuss & Finn, 1975*b*).

A second assumption is that serosal substitutions do not alter the properties of the luminal membrane, within the time frame of these

observations. Circumstantial evidence in favor of this assumption includes: (i) the lack of sizable changes in cell potential after the initial change (*see* Fig. 2), and (ii) the observation of only slow changes in intracellular ionic activities (in minutes) upon large alterations of external ionic concentrations (L. Reuss and S. Weinman, *unpublished* results).

Finally, for the calculation of ionic permeability coefficients it was assumed that the membrane potentials are entirely diffusional, i.e., that no electrogenic (rheogenic) pumps contribute to them. There is no published suggestion of any electrogenic pump located at the luminal membrane of gallbladder epithelium, but the possibility of a basolateral electrogenic Na pump has been advanced (Moreno & Diamond, 1976; Rose & Nahrwold, 1976; Rose, 1978). We have recently shown that in *Necturus* gallbladder ouabain insignificantly depolarizes the basolateral membrane at a time at which net fluid transport is completely blocked (Reuss *et al.*, 1979). Furthermore, when depolarization does occur, it can be accounted for by a fall in intracellular K activity and a reduction of basolateral membrane K permeability. The latter observation is consistent with findings in frog skin (Helman & Nagel, 1977). In sum, the possibility of a large electrogenic component of E_b seems unlikely. A "small" electrogenic pump would not alter qualitatively the conclusions concerning the mechanism of Cl transport across the basolateral membrane (*see* below).

Basolateral Membrane Ionic Permeability Coefficients

From the mean values of transference numbers and basolateral membrane conductances, ionic permeability coefficients (P_i's) were calculated according to Eq. (6). For the calculation of the mean ionic activities, the following intracellular values were employed (mM): Na, 19; Cl, 36; K, 90. These are means obtained with liquid ion exchanger microelectrodes (Reuss, 1979). The Na results agree well with the observations of J. Graf and G. Giebisch (*personal communication*), K.R. Spring (*personal communication*), and Zeuthen (1978) when the microelectrode was positioned near the basolateral membrane. The Cl result is comparable to the mean value of 30 mM found in rabbit gallbladder by Duffey *et al.* (1978).

The mean permeability coefficients calculated from the data in Tables 4 and 5 were: $P_K = 4.01$ and $P_{Cl} = 0.11 \times 10^{-6}$ cm·sec^{-1}. The mean value of P_{Cl}/P_K is 0.03, in good agreement with the range obtained from

partial substitutions of K for Na. In these experiments (Fig. 4) P_{Cl}/P_K ranged from 0 to 0.02.

These values are subject to uncertainties because of the possibility of cumulative errors in the measurements of cell membrane potentials and resistances and intracellular ionic activities. Some of these uncertainties have already been discussed. It should be clear, however, that regardless of the precision of the estimates of absolute permeability coefficients, the relative value of P_{Cl} (referred to P_K) was low in two independent series of experiments. To calculate P_{Cl}/P_K from the data shown in Fig. 4 it was assumed that $T_{Na}=0$, assumption supported by the results of NMDG for Na substitutions. It is possible, however, that a small, but finite T_{Na} was not detected in these experiments. If in fact $P_{Na}>0$, Eq. (7*a*) is incorrect, and P' (P_{Cl}/P_K) should be calculated from

$$\Delta E_0=\frac{RT}{zF}\ln\frac{K_o^i+P'Cl_i+P''Na_o^i}{K_o^f+P'Cl_i+P''Na_o^f} \tag{7b}$$

where $P''=P_{Na}/P_K$.

It can be shown that P' decreases if P'' is assumed to be larger than zero. Therefore, the main conclusion derived from these experiments, i.e., the basolateral membrane has a low Cl permeability, is independent of this assumption.

Mechanism of Salt Transport across the Basolateral Membrane

Sodium transport across the basolateral membrane is uphill, since it takes place against an electrochemical potential difference of about 2.47 kcal/mole ($V_{cs}=-68.7$ mV, $E_{Na}=37.8$ mV). Chloride transport, however, might be passive, since the intracellular Cl activity is well above equilibrium (measured activity, 36 mM; equilibrium activity, 8 mM). Cl efflux is downhill, with an electrochemical potential difference of about 1.05 kcal/mole ($V_{cs}=-68.3$ mV, $E_{Cl}=-22.8$ mV). The most important conclusion in this paper, in agreement with the calculations of Van Os and Slegers (1975) is that the basolateral membrane G_{Cl} is too low to explain Cl extrusion on the basis of simple diffusion. The mean rate of fluid transport (J_v) across the wall of *Necturus* gallbladder is $12.7\,\mu l\cdot cm^{-2}\cdot h^{-1}$ (Reuss *et al.*, 1979). Since transport is isosmotic (Hill & Hill, 1978*b*), the net transepithelial Cl flux (J_{Cl}^{net}) can be calculated to be $0.38\,neq\cdot cm^{-2}\cdot sec^{-1}$ or $38\,\mu A\cdot cm^{-2}$. Assuming that salt transport is

entirely transcellular, this is the *minimum* cell-to-serosa Cl flux, since it is the *net* flux across the membrane. We can compare this flux with the passive flux (J^P_{Cl}) predicted from V_{cs}, E_{Cl}, and the Cl conductance of the membrane:

$$J^P_{Cl} = (V_{cs} - E_{Cl})\, G_{Cl} \tag{8}$$

where E_{Cl} is the Cl equilibrium potential across the basolateral membrane and G_{Cl} is the Cl conductance of the membrane. Since $(V_{cs} - E_{Cl}) = -45.5\,\text{mV}$, and $G_{Cl}(= G_b \cdot T_{Cl})$ is $23.4\,\mu\text{mho} \cdot \text{cm}^{-2}$, $J^P_{Cl} = -1.1\,\mu\text{A} \cdot \text{cm}^{-2}$, where the negative sign indicates flux from cell to serosal solution. This calculated J_{Cl} value represents only 3% of the measured J^{net}_{Cl}. The potential sources of error in this calculation should be examined. Three parameters are employed: basolateral membrane resistance, Cl transference number, and Cl electrochemical gradient. The present value of basolateral membrane resistance ($2{,}560\,\Omega \cdot \text{cm}^2$, Table 3) compares well with previously published mean values of 2,880 (Frömter, 1972) and 2,750 (Reuss & Finn, 1975*a*). If G_b were larger by a factor of ten, still only 30% of J^{net}_{Cl} could be ascribed to simple diffusion. The same argument holds for T_{Cl}. The estimated value could be in error because it was determined over a large change of Cl activity (*see* above). However, even if $T_{Cl} = 1$ (i.e., if the membrane were a perfect Cl electrode) only 30% of J^{net}_{Cl} could be diffusional. From the observations of Van Os and Slegers (1975) in *Necturus* gallbladder, T_{Cl} would appear to be even smaller than calculated here. Finally, the quoted intracellular Cl activity value could be in error. However, if anything, the Cl activity in the cell is probably lower, because the measurements quoted were not corrected for the response of the Cl-sensitive microelectrode to other intracellular anions. As shown by Spring and Kimura (1978) in *Necturus* proximal tubule, an "intracellular Cl activity" of ca. 6 mM is measured after prolonged removal of external Cl, at a time when tissue Cl concentration is essentially zero. In addition, our intracellular Cl activity measurements are in excellent agreement with the values reported recently for rabbit gallbladder (Duffey *et al.*, 1978). Finally, even assuming that the only intracellular anion is Cl (and therefore that $a\,\text{Cl}_i \sim 100\,\text{mM}$), J_{Cl} would still be only $1.6\,\mu\text{A} \cdot \text{cm}^{-2}$, i.e., only 4% of J^{net}. In sum, the magnitude of the discrepancy between the measured transepithelial net Cl flux and the predicted diffusional Cl flux across the basolateral membrane is such that only large errors in all three parameters would prove the conclusion to be wrong. It appears safe, therefore, to conclude that Cl transport across the basolateral membrane is not by simple diffusion, but could result

from a neutral Cl transport mechanism, as first proposed by Diamond (1962). This could be a neutral NaCl pump, in parallel with a Na-K exchange pump, similar to the scheme proposed by Whittembury (1971) for renal proximal tubule, a "downhill" KCl cotransport mechanism, or a Cl-HCO_3 exchange system in parallel with a Na-K pump. The stimulation of fluid transport by bicarbonate in rabbit gallbladder (Diamond, 1964; Martin, 1974; Martin & Murphy, 1974) is consistent with this view. Such a mechanism would require HCO_3^- recycling through the membrane (Duffey *et al.*, 1978) since the gallbladder does not secrete HCO_3^- but absorbs it (Diamond, 1964; Wheeler, 1963; *see also* Cremaschi & Hénin, 1975).

Paracellular Cl Transport

An objection to the preceding considerations is that a sizable fraction of J_{Cl}^{net} could be paracellular. Since the transepithelial potential is mucosa-negative, J_{Cl} through the shunt could be driven by $V_{ms}(J'_{Cl})$. In addition, if part of fluid transport is through the shunt, Cl could also be transported by entrainment of water and salt fluxes in the junctions, i.e., by solvent drag (J''_{Cl}).

The possibility of a sizable paracellular J_{Cl} driven by the transepithelial potential is ruled out by the following calculation:

$$J'_{Cl} = V_{ms} G^*_{Cl} \tag{9}$$

where G^*_{Cl} is the paracellular Cl conductance.

From T_{Cl} (shunt) = 0.2 (Reuss, 1978), $R_s = 160\,\Omega \cdot cm^2$ (Tables 2 and 4), and $V_{ms} = 0.8$ mV (maximum mean V_{ms} value, Table 1), $J'_{Cl} = 1\,\mu A \cdot cm^{-2}$. Suzuki and Frömter (1977) found lower values of transepithelial resistance than previously reported (mean $= 55\,\Omega \cdot cm^2$). If their value were correct, J'_{Cl} would be ca. $3\,\mu A \cdot cm^{-2}$, i.e., still only 8% of J_{Cl}^{net}. In rabbit gallbladder, the transepithelial potential is oriented in the wrong direction to account for net Cl absorption through the shunt (Cremaschi & Hénin, 1975; Frizzell, Dugas & Schultz, 1975).

Solvent drag is a possibility that cannot be analyzed with certainty at the present time, because of lack of information on both the relative magnitudes of transcellular and paracellular water fluxes and the Cl reflection coefficient at the junction. J''_{Cl} is described by the equation:

$$J''_{Cl} = \beta \times J_v (1 - \sigma_{Cl})\, C_{Cl} \tag{10}$$

where β is the fraction of J_v which flows through the junctions, σ_{Cl} is the Cl reflection coefficient and C_{Cl} is the mean Cl concentration.

For known values of J_v and C_{Cl}, J''_{Cl} depends on β and σ_{Cl}. In order to ascribe to this mechanism more than 90% of J^{net}_{Cl} (only in this condition could all of J_{Cl} at the basolateral membrane be passive), the product $\beta(1-\sigma_{Cl})$ would have to be at least 0.9. In other words, solvent drag could account for the fraction of J^{net}_{Cl} which is not explained by passive transport through the basolateral membrane only if the two following conditions were fulfilled: (i) at least 90% of the transepithelial fluid transport is paracellular, and (ii) Cl reflection coefficient of the junctions is at most 0.1. It is not possible to rule out at present these possibilities, but they appear highly unlikely. σ_{Cl} in proximal tubule of the kidney (a tissue with far greater paracellular permeabilities for Na and Cl than the gallbladder) has been estimated to be about 0.7 (Schafer, Patlak & Andreoli, 1975).

Hill and Hill (1978*a*) have suggested that most of transepithelial fluid transport is paracellular, based on measurements of the rates of fluid and sucrose absorption in gallbladders of *Necturus*. These authors estimated a $\sigma_{sucrose}$ of 0.05. However, their experiments were done in hyposmotic media (50 mosmol/kg) and the possibility of an abnormally large tissue permeability under these conditions was not ruled out.

Comparison with Mammalian Gallbladder

The results reported here agree in most respects with those obtained with electrophysiological and radioactive tracer techniques in rabbit gallbladder. Large effects of external K on basolateral membrane potential were demonstrated by Hénin and Cremaschi (1975) and Van Os and Slegers (1975). Cremaschi and Hénin (1975) reported that radioactive Cl applied to the serosal medium does not enter the cells, unless the membrane potential is reduced. Sodium did not enter the cells from the serosal side in their experiments. As mentioned before, Hénin and Cremaschi (1975) also found that Cl replacement in the external media did not alter the cell membrane potential significantly. The overall evidence, therefore, suggests that in rabbit gallbladder, as in *Necturus*, Cl transport across the basolateral membrane is not by simple diffusion.

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Appendix

Derivation of Equation for the Calculation of E_b and E_s during Ionic Substitutions in the Serosal Bathing Medium

For definition of symbols, *see* Fig. 1. Shortly after a substitution in the serosal solution only, the new values of transepithelial and cell membrane potentials are given by:

$$V'_{ms} = \frac{(E'_b - E_a) R'_s + E'_s (R_a + R'_b)}{R_a + R'_b + R'_s} \tag{A1}$$

$$V'_{mc} = \frac{E_a (R'_b + R'_s) + R_a (E'_b - E'_s)}{R_a + R'_b + R'_s} \tag{A2}$$

$$V'_{cs} = \frac{E'_b (R_a + R'_s) + R'_b (E_a + E'_s)}{R_a + R'_b + R'_s} \tag{A3}$$

where the polarities are defined as follows: V_{ms} and E_s; mucosal solution-serosal solution; V_{mc} and E_a, cell-mucosal solution; V_{cs} and E_b, cell-serosal solution. The primes stand for values measured during exposure to the new serosal solution. R_a and E_a are assumed to remain constant during the brief time required for the measurement (*see Discussion*).

Equation (A1) can be rewritten:

$$E'_s = \frac{V'_{ms}(R_a + R'_b + R'_s) - (E'_b - E_a) R'_s}{R_a + R'_b}. \tag{A4}$$

Combining Eqs. (A4) and (A3):

$$E'_b = \frac{V_{cs}(R_a + R'_b) - R'_b (E_a + V'_{ms})}{R_a} \tag{A5}$$

which is Eq. (3) (*see Results*).

Combining Eqs. (A5) and (A2):

$$E'_s = V'_{ms} + (E_a - V'_{mc}) \frac{R'_s}{R_a} \tag{A6}$$

which is Eq. (4) (*see Results*).

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