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

II. Ionic Permeability of the Apical Cell Membrane

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Summary. Microelectrode techniques were employed to study the ionic permeability of the apical cell membrane of *Necturus* gallbladder epithelium. Results obtained from continuous records in single cells, and from several cellular impalements shortly after a change in solution, were similar and indicate that both the apical membrane equivalent electromotive force (V_a) and electrical resistance (R_a) strongly depend on external [K]. Cl substitutions produced smaller effects, while the effects of Na substitutions with N-methyl-D-glucamine on both V_a and R_a were minimal. These results indicate that the permeability sequence of the apical membrane is $P_K > P_{Cl} > P_{Na}$. From the calculated absolute value of P_{Na} it is possible to estimate the diffusional Na flux from the mucosal solution into the cells (from the cell potential and an assumed intracellular Na concentration). The calculated flux is roughly three orders of magnitude smaller than the measured net transepithelial flux in this tissue and in gallbladders of other species. Thus, only a minimal portion of Na entry can be attributed to independent diffusion. From estimations of the electrochemical potential gradient across the apical membrane, Cl transport at that site must be active. At the serosal cell membrane, Na transport takes place against both chemical and electrical potentials, while a significant portion of the Cl flux can be passive, if this membrane has a significant Cl conductance. The changes in shunt electromotive force and in transepithelial potential after mucosal substitutions were very similar, indicating that transepithelial bi-ionic potentials yield appropriate results on the properties of the shunt pathway.

The ionic permeability of cell membranes can be estimated from the changes in electrical potential measured after changes in external and/or internal concentration of one or more ions [13, 14, 19], if their permeation is determined by electrochemical potential gradients alone. This information is particularly important in transporting epithelia, since it can provide direct clues as to the mechanism of transmural ion transport. However, several difficulties—in addition to those present in isolated cells—are encountered in these tissues. These, partly discussed in the pre-

ceding paper [17], can be summarized as follows: in all epithelia thus far studied it has been shown that the transmural route of ion permeation consists of a cellular pathway, constituted in monolayered epithelia by the two cell membranes in series, in parallel with a paracellular shunt pathway whose conductance varies according to the tissue and whose anatomical location almost certainly corresponds to the limiting junctions in series with the lateral intercellular spaces [1, 7, 8, 18, 19]. Consequently, a minimum equivalent circuit for the tissue has to include six elements: a resistor and an electromotive force (EMF) at each cell border and at the shunt pathway. It is immediately obvious that a unilateral change of ionic concentrations in the bathing solution can alter the electrical potential across the ipsilateral cell membrane in a complex way, which includes changes in its own EMF (whose current can be partially shunted through the paracellular pathway), in the shunt EMF, or even in the EMF of the opposite membrane (e.g., if the cell composition changes, or some kind of interdependence exists between the potentials of the two membranes). It seems reasonable to assume that measurements performed a short time after a unilateral change in solution composition will not allow cellular ion concentrations to change significantly. However, the present information indicates that the EMF of the ipsilateral membrane and the EMF of the shunt are likely to change, and thus the changes in membrane potential are not necessarily equal to the changes in its EMF. It is then necessary to calculate the changes in EMF at each cell membrane and at the shunt—as a function of changes in ion concentration—in order to estimate their ionic permeabilities.

This calculation requires one to know the electrical resistances of the three elements of the circuit. Although their measurement is technically possible in several epithelia [7, 16, 18, 21], the length of time required excludes the likelihood of constant cell composition after changes in ionic concentrations of the bathing solutions. An alternative approach consists of the measurement of all elements under control conditions and the continuous recording of the changes in potential and some resistances during the ionic substitution. Given reasonable assumptions to be discussed below, it is possible to obtain the EMF's from these data. This procedure was employed, in the experiments reported here, to determine the ionic permeability of the apical (luminal) membrane of *Necturus* gallbladder. The results obtained from these short-term measurements compare favorably with the steady-state determinations reported in the accompanying paper [17].

Materials and Methods

The experimental techniques were as described in the preceding paper [17]. All changes in composition of the solutions were isomolar, and calcium and bicarbonate ion concentrations were kept constant. Fast changes in the composition of the mucosal solution were achieved by gravity superfusion through a glass pipette placed close to the recording microelectrodes. The protocol usually included, first, a complete measurement of potentials and resistances under control conditions (standard Ringer's on both sides). This included the measurement of the total transepithelial resistance (R_t), the voltage divider ratio ($a = R_a/R_b$), the voltage spread into the epithelium (all as described in the preceding paper), and the transepithelial (V_{ms}), apical (V_{mc}), and basal-lateral (V_{cs}) potentials. Subsequently, changes in ionic composition of the mucosal solution were performed, and electrical measurements carried out in one of two ways: (a) five to eight cells were impaled shortly before and immediately after the change in mucosal solution, and V_{ms}, V_{mc}, V_{cs}, R_t, and R_a/R_b were recorded in the two conditions; (b) a microelectrode was kept in a cell during the superfusion, and all cell parameters were obtained from the single record. Cations employed to replace Na were K or N-methyl-D-glucamine (NMDG). Cl was replaced with methylsulfate or gluconate.

Theory

The resistances of the cell membranes (R_a, R_b) and the shunt pathway (R_s), and the equivalent EMF's at both cell borders (V_a, V_b) were calculated – with Ringer's solution on both sides – according to Eqs. (4), (5), (6), (10) and (11) of the preceding paper [17]. In addition, several values of V_s (assuming several possible NaCl concentration gradients across the limiting junction, according to Machen and Diamond [15]), were substituted in Eqs. (12)–(14) to examine the influence of the assumption $V_s = 0$ on the control values of V_a and V_b, and on the changes of V_a.

From the changes in potentials, R_t, and a after a change in solution, the new resistances (R'_a, R'_s) and EMF's (V'_a, V'_s) were calculated, assuming that R_b and V_b remain constant:

$$R'_a = a' \cdot R_b \tag{1}$$

$$R'_s = \frac{(R'_a + R_b)\, R'_t}{R'_a + R_b - R'_t} \tag{2}$$

$$V'_a = \frac{V'_{mc}(R'_a + R_b + R'_s) - R'_a(V_b - V'_s)}{R_b + R'_s} \tag{3}$$

$$V'_s = \frac{V'_{cs}(R_b + R'_s) - (V'_{mc} R_b + V_b R'_s)}{R_b}. \tag{4}$$

When a single ion is partially substituted by a nonpermeant species, an ion-dependent partial potential ratio (T_i) can be defined, from the changes in apical membrane EMF, according to Strickholm and Wallin [19]:

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

where $\Delta V_a = V_a - V'_a$, C_1 and C_2 are the ion concentrations before and after the change in solution, and R, T, z, and F have their usual meanings. The denominator represents the change in potential that would be measured if the membrane were perfectly permselective for the species i. In addition, absolute permeabilities can be calculated if the sum of partial potential ratios for all ions equals 1, and if the mean ion concentrations can be estimated [9]:

$$P_i = \frac{RT\, T_i\, g_t}{z^2 \bar{c} F^2} \tag{6}$$

where P_i=permeability coefficient (cm sec^{-1}), g_t=total membrane conductance, and $\bar{c}$=mean ion concentration in the membrane.

Eq. (6) is only applicable to a membrane with no rheogenic (electrogenic) pumps. If such pumps exist across the apical membrane, the derived permeability coefficients may be significantly in error. However, since there is no unequivocal evidence either for or against the existence of such a mechanism, we have chosen to use these calculations as a means of testing whether or not Na entry can occur by diffusion alone.

As an additional test, a series of experiments was performed to compare results of single ion substitutions with substitutions at constant product of K and Cl concentrations in the mucosal solution. These experiments (if K and Cl are passively distributed) should yield results independent of changes in intracellular ionic activities (*see below*).

Results

The time course, magnitude and reversibility of the changes in membrane potential and resistance during a K-for-Na substitution on the mucosal side are illustrated in Fig. 1. As shown by the records, the cell potentials reach a pseudo-steady state value within 1–2 sec after the change in solution. Coincidently with the membrane potential changes, the total transepithelial resistance (R_t) and the voltage divider ratio across the cel-

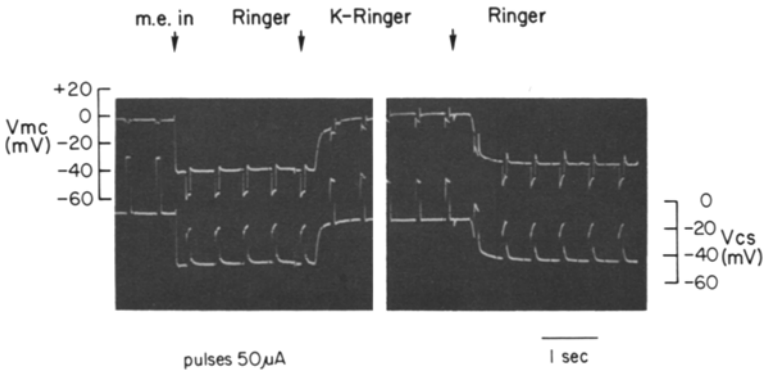

Fig. 1. Typical record showing changes in apical membrane potential (V_{mc}, upper tracing), basal lateral membrane potential (V_{cs}, lower tracing), and voltage divider ratio (R_a/R_b, ratio of the voltage deflections ΔV_{mc} and ΔV_{cs}), in a Na-K-Na substitution on the mucosal side. The record starts on the left with both microelectrodes in the mucosal solution. At the first arrow, a cell was impaled and the potential across each membrane recorded as indicated. At the second arrow, the mucosal solution was changed from standard Ringer's (2.5 mM K) to 111 mM K. At the third arrow, the mucosal solution was changed back to 2.5 mM K. The abrupt voltage changes in both tracings (ΔV_{mc} and ΔV_{cs}) are produced by transepithelial d.c. pulses. Their ratio equals R_a/R_b; from the sum of the deflections, the transepithelial resistance can be calculated. Note that both membranes depolarize with the increase in K concentration, while $\Delta V_{mc}/\Delta V_{cs}$ decreases and $(\Delta V_{mc}+\Delta V_{cs})$ also decreases. All changes are reversible. The delay between the two panels was about 10 sec

lular pathway (R_a/R_b) decrease. The changes in potentials and resistances are completely reversible.

In some tissues, such as the one in which this record was obtained, the deflections ΔV_{mc} and ΔV_{cs}, produced by the transepithelial pulses, did not approximate square waves (such as in Fig. 2), but showed time dependence. The direction of the transients was always the same as in the Figure (i.e., with Ringer's on both sides, ΔV_{mc} decreases and ΔV_{cs} increases, as functions of time, during the current pulse). This pattern appeared to be more frequent in gallbladders with spontaneously lower values of cell membrane potentials. The voltage divider ratio, in these cases, was calculated as the ratio of instantaneous voltage deflections, since the value taken at this time is constant over a wide range of current density. It can be shown that later measurements yield a sublinear relationship between $(\Delta V_{mc}/\Delta V_{cs})$ and the transepithelial current density. This, and other nonlinear properties of the tissue (*see* ref. [17]), will be described in detail elsewhere.

The effects of several K concentrations in the mucosal solution are shown in Fig. 2. It is notable that the changes in both membrane poten-

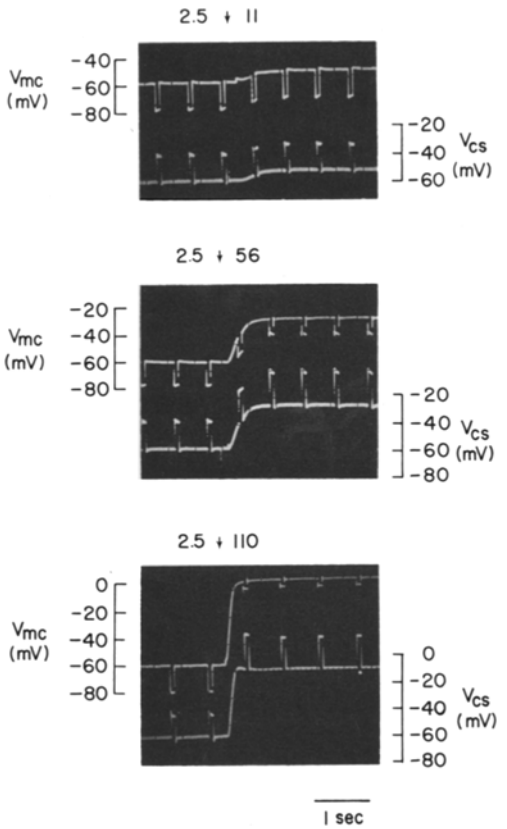

Fig. 2. Effect of changes in K concentration in the mucosal solution on cell potentials, voltage divider ratio and transepithelial resistance. The records start with the microelectrode in the cell. At the arrow, K concentration was changed as indicated (mM/liter). The deflections in both recordings (ΔV_{mc} and ΔV_{cs}) were produced by transepithelial d.c. pulses of 80 $\mu A\,cm^{-2}$. Note that progressively larger increases in mucosal K concentration produce larger depolarization of both cell membranes and more marked reduction of $\Delta V_{mc}/\Delta V_{cs}$

tials and resistances are proportional to the final K concentration on the outside.

The effects of Cl-gluconate or Na-NMDG substitutions on electrical potentials and resistances are also fast and reversible.

Effects of Single Ion Substitutions in the Mucosal Solution on Cell Membrane Potentials and Equivalent EMF's

The changes in measured potentials across each cell membrane and across the whole epithelium, as functions of the K concentration in the

Table 1. Effects of K concentration in the mucosal solution on transepithelial and cell membrane electrical potentials

$[K]_m$ (mM/liter)	V_{mc} (mV)	V_{cs} (mV)	V_{ms} (mV)
2.5	58.7±3.8	60.1±3.8	1.4±0.4
11	45.0±2.1	47.0±2.1	2.0±0.3
56	20.0±2.0	27.5±1.8	7.5±1.7
111	6.3±1.4	20.7±2.5	14.4±2.5

Cell potentials measured 1 to 6 min after the change in mucosal [K]. Five to eight impalements were performed in each preparation at each concentration. $n=5$ gallbladders. Means and SEM determined from the means in each preparation.

mucosal solution, are shown in Table 1. Both V_{mc} and V_{cs} decrease pari-passu with the increase in external K concentration. However, the changes in V_{mc} are greater than the changes in V_{cs} at each step. The change in V_{ms} ($V_{ms}=V_{cs}-V_{mc}$) can be attributed to the change in V_a, that generates current flow through the shunt, tending to make the mucosal solution more negative, and to a bi-ionic potential produced in the shunt itself by its different permeabilities to Na and K ($P_K > P_{Na}$, *see below* and [17]), that also tends to make the mucosal side negative. The measurements of V_{ms} and the cell potentials were taken 1 to 6 min after the change in mucosal solution, when the value of V_{ms} is stable. Occasionally, there is a hyperpolarizing transient of 2 to 3 mV that lasts for a few seconds. It will be shown below that most of the change in V_{ms} is secondary to the shunt bi-ionic potential and not to the change in apical membrane EMF. The changes in V_{cs} are essentially simultaneous with the changes in V_{mc}. They can be entirely attributed, given the low resistance of the shunt, to the current generated by the reduction of V_a and the shunt bi-ionic potential (V_s).

From the measurements of resistances and potentials, assuming that V_b does not change, the values of V_a and V_s after each substitution were calculated. The values of V_a at several K concentrations, shown in Fig. 3, indicate a strong dependence of V_a on external K concentration. If the relationship shown in Fig. 3 is assumed to be a straight line, the least-squares fit of all experimental points yields a slope ($\Delta V_a/\log [K]_m$) of 42 mV, when V_s is assumed to be zero under control conditions. The lower three curves show the effect on V_a of assuming different control values of V_s (from $P_{Na}/P_{Cl}=3.18$ across the shunt, and from NaCl concentration gradients of 5, 10, and 20 mM across the limiting junction).

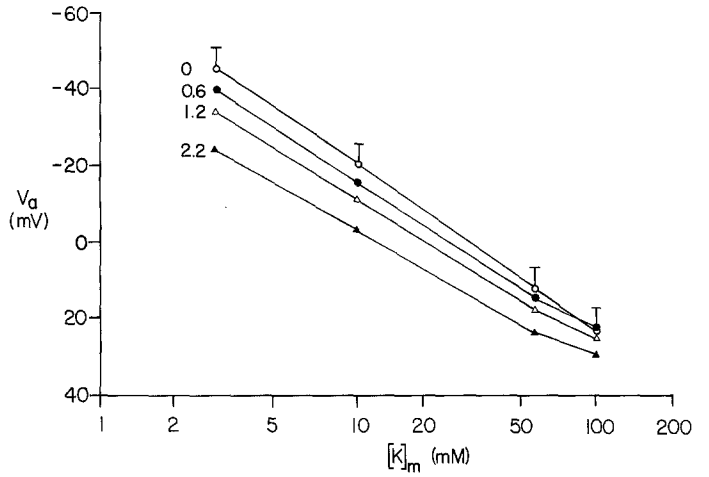

Fig. 3. Apical membrane EMF (V_a) as a function of K concentration in the mucosal solution ($[K]_m$). Values calculated from the experiments shown in Table 1, according to Eq. (3). Numbers on the left of each curve indicate the assumed value of V_s under control conditions (2.5 mM K); 0.6, 1.2, and 2.2 mV correspond to 5, 10, and 20 mM NaCl concentration gradients across the limiting junction. V_s calculated from $t_{Na}/t_{Cl}=3.18$ (*unpublished observations*). $n=5$ preparations. Isomolar Na-K replacements

For instance, if the NaCl concentration gradient were 10 mM, as calculated for the rabbit gallbladder [15], V_s would be 1.2 mV, and the slope (calculated in the same way) would be 37 mV. As discussed below, the existence of such a shunt EMF would not appreciably alter our conclusions on the permselectivity of the apical membrane. The calculated values of V_s in the same experiments are close to those of V_{ms}, indicating, as stated above, that the transepithelial potential alteration produced by the change in V_a is small when compared to the alteration in V_{ms} produced by the change in V_s, i.e., the bi-ionic potential generated in the shunt itself.

Table 2 summarizes the effects of changes in Cl concentration in the mucosal solution on V_{mc}, V_{cs}, and V_{ms}. As external Cl is reduced, both V_{mc} and V_{cs} decrease. In contrast to the observations in Na-K substitutions, the change in V_{cs} is now greater than the change in V_{mc} at each step. This observation, reflected by the changes in V_{ms}, indicates a significant contribution of V_s to the potentials across the cell membranes. The polarity of both V_{ms} and the calculated value of V_s after the substitution show that methylsulfate and gluconate are slightly more permeant than Cl across the paracellular pathway. Thus, the developed bi-ionic potential tends to increase the value of V_{mc} and to decrease the value of V_{cs}.

Table 2. Effects of Cl concentration in the mucosal solution on transepithelial and cell membrane electrical potentials

$[Cl]_m$ (mM/liter)	V_{mc} (mV)	V_{cs} (mV)	V_{ms} (mV)
2.5	55.3±1.1	49.6±3.7	−5.7±3.6
11	58.5±1.1	53.3±3.2	−5.3±2.9
56	63.4±2.1	62.2±2.0	−1.2±0.7
111	65.1±2.6	66.5±2.5	1.4±0.6

Cell potentials measured 1 to 6 min after the change in mucosal [Cl] (isomolar replacement with methylsulfate or gluconate). Five to eight impalements were performed in each preparation at each concentration. $n=4$ gallbladders. Means and SEM determined from the means in each preparation.

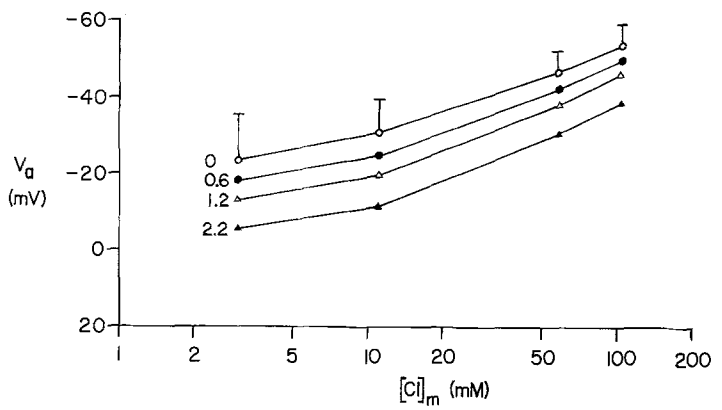

Fig. 4. Apical membrane EMF (V_a) as a function of Cl concentration in the mucosal solution ($[Cl]_m$). *See* legend to Fig. 3. $n=4$ preparations. Isomolar replacements of Cl with gluconate or methylsulfate

The values of V_a, at four external Cl concentrations, are shown in Fig. 4. The slope of the line, calculated in the same way as in K-Na substitutions, is 19 mV when V_s (under control conditions) is assumed to be zero. Again, the other curves have approximately the same slope if different values are assumed for V_s. Although V_a depends on external Cl, such dependence is not as prominent as that on mucosal K concentration.

The effect of external Na concentrations on the potentials was studied by partial substitutions of the cation with N-methyl-D-glucamine. The results are shown in Table 3. When Na concentration is decreased, V_{mc} augments while V_{cs} diminishes slightly. The transepithelial potential

Table 3. Effects of Na concentration in the mucosal solution on transepithelial and cell membrane electrical potentials

$[Na]_m$ (mM/liter)	V_{mc} (mV)	V_{cs} (mV)	V_{ms} (mV)
2.5	72.2±4.7	55.7±2.9	−16.5±2.7
11	68.5±4.7	55.9±3.1	−12.6±2.0
56	62.0±3.6	57.7±2.6	−4.3±1.8
111	57.7±3.2	59.2±2.8	1.5±0.5

Cell potentials measured 1 to 6 min after the change in mucosal [Na] (isomolar replacement with N-methyl-D-glucamine). Five to eight impalements were performed in each preparation at each concentration. $n=4$ gallbladders. Means and SEM determined from the means in each preparation.

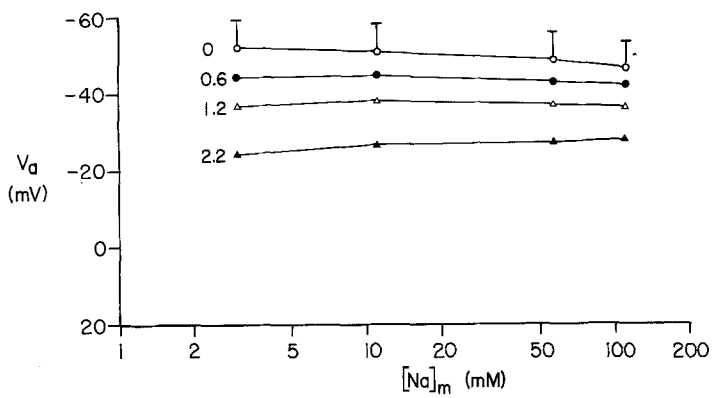

Fig. 5. Apical membrane EMF (V_a) as a function of Na concentration in the mucosal solution ($[Na]_m$). *See* legend to Fig. 3. $n=4$ preparations. Isomolar replacements of Na with N-methyl-D-glucamine

(V_{ms}) reverses, the serosal potential becoming progressively negative as mucosal Na is replaced by the nonpermeant cation. The bi-ionic potential across the shunt has a mucosa-positive polarity, and the resulting IR drops tend to hyperpolarize the apical membrane and depolarize the basal-lateral membrane. The observation that V_{mc} and V_{cs} change in opposite direction can only mean that the cell membrane IR drops produced by the bi-ionic shunt potential (V_s) are greater than those generated by the change in V_a. V_a values at different Na concentrations are shown in Fig. 5. The changes are small, indicating that the Na permeability of the apical membrane is very low when compared with P_K or P_{Cl}. As in the two previous series of experiments, the changes in V_s and V_{ms}

Table 4. Changes in cell and transepithelial potentials, apical membrane EMF and apical membrane resistance, obtained from continuous records in single cells

$[K]_m$ (mM/liter)	V_{mc} (mV)	V_{cs} (mV)	V_{ms} (mV)	V_a (mV)	V_s (mV)	R_a (Ω cm^2)
2.5	57.5±3.3	59.3±2.8	1.9±0.6	40.1±6.2	0	4,290±510
111	3.0±1.2	21.1±2.9	18.1±2.7	−14.4±2.6	13.6±2.7	1,230±270

$n=7$ gallbladders (one cell explored in each preparation). R_a is calculated from the voltage divider ratio in the same cell before and after the change in solution. Potentials and resistances measured 3 sec after the change in solution (compare Fig. 2, lower panel).

after each substitution were very similar. This is easily understood if one considers that R_s is small if compared to R_a or R_b, and that ΔV_s was greater than ΔV_a in these substitutions, as indicated above.

To validate the procedure employed in the previous experiments, i.e., the use of several impalements shortly after the change in mucosal solution, continuous intracellular records during the substitution were obtained in several preparations, such as those shown in Figs. 1 and 2. The effects of increasing K concentration from 2.5 to 111 mM are shown in Table 4. A comparison of these results with those in Table 1 and Fig. 1 indicates no significant differences. This observation proves that the results detailed above, obtained a few minutes after the change in solution, do not differ from those calculated from measurements performed a few seconds after the substitution. As the latter results make it necessary to calculate the resistance from the voltage divider ratio measured in only one cell, they were not used for the estimation of permeability coefficients detailed below.

Finally, given the fact that the external substitutions of a single permeant ion may alter intracellular concentrations rapidly (according to the absolute permeability), in some experiments K and Cl mucosal concentrations were altered keeping their product constant. Under these conditions, if internal K and Cl activities are close to equilibrium (and thus the ions are passively distributed) no changes in intracellular activities should take place when external substitutions are performed. The results, summarized in Table 5, are again very similar to the previous ones, and suggest (if the assumption is correct) that no important shifts in intracellular ionic activities took place during the lapse in which the previous measurements were performed.

Table 5. Effect of mucosal K and Cl substitutions at constant product on transepithelial and cell membrane electrical potentials

$[K]_m$ (mM/liter)	$[Cl]_m$ (mM/liter)	V_{mc} (mV)	V_{cs} (mV)	V_{ms} (mV)
2.5	114	55.6±1.8	56.6±1.8	1.0
11	26	44.9±1.5	49.9±1.5	5.0
56	5.1	25.1±1.0	32.1±1.0	7.0
111	2.6	4.5±0.6	23.5±0.6	19.0

Cell potentials measured 1 to 6 min after the change in mucosal solution. Means±SEM from 12 impalements at each concentration. $n=2$ gallbladders.

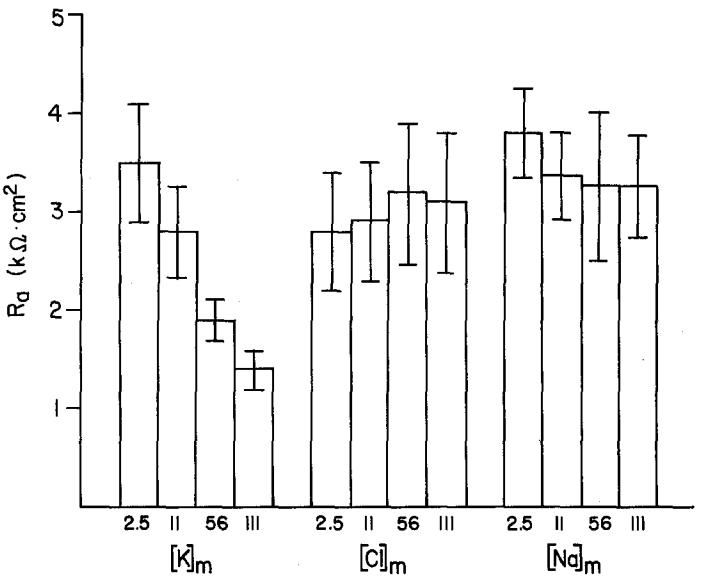

Fig. 6. Electrical resistance of the apical membrane (R_a) as a function of external K, Cl, and Na concentrations. Number of experiments as indicated in Figs. 3–5. R_a calculated from the values of R_t and R_a/R_b after the change in mucosal solution, assuming that R_b remains unchanged. *See text*

Effects of Single Ion Substitutions in the Mucosal Solution on the Electrical Resistance of the Apical Membrane

The resistances of the cell membranes and the shunt were calculated from the transepithelial resistance, the voltage divider ratio across the cellular pathway, and cable analysis, in preparations bathed by standard Ringer's solution on both sides. After ionic substitutions, R_a was calculated from the new values of R_t and R_a/R_b, assuming that R_b remains constant.

The results are summarized in Fig. 6. It can be seen that R_a is strongly dependent on external K concentration, being reduced to a

mean value of 40% of control when [K] is increased from 2.5 to 111 mM. In the Cl-gluconate substitutions no significant changes were observed. In the Na-NMDG replacements, the differences were significant only between the two extreme concentrations. Even if this is a real change, it is considerably smaller than the one observed with Na-K substitutions.

These results are consistent with the effects of the ions on V_a, and indicate that the K conductance of the membrane is higher than g_{Cl} and g_{Na}, at any of the concentration ranges explored.

Discussion

The results described above indicate that the apical (mucosal) cell membrane of *Necturus* gallbladder is mainly potassium permselective. This conclusion can be obtained from both the measurement of the electrical resistance and the calculation of the equivalent EMF at that membrane, as functions of K concentration in the mucosal solution.

Dependence of Apical Membrane Potential and Equivalent EMF on Mucosal Solution Ionic Concentrations

The results shown in Figs. 3–5 indicate that the apical membrane equivalent EMF depends on the mucosal solution ionic concentrations following the sequence K > Cl > Na. If one assumes that the generation of such an EMF is entirely diffusional (i.e., no rheogenic pumps are present in the system), the permeabilities of the membrane for the three ions also follow the same sequence.

Our measurements are based on several assumptions, some of which were discussed in the preceding paper [17]. Figs. 3–5 illustrate the error that might be generated by assuming that $V_s=0$ when the tissue is bathed by Ringer's solution on both sides. As seen by the slight changes in slope, this assumption, if in error, would introduce a small change in our estimation of the relative permeabilities, but would not significantly alter the sequence $P_K > P_{Cl} > P_{Na}$ or the ratio P_K/P_{Na}, since in both cases the slope of V_a as a function of the external cation concentration decreases when V_s is assumed to be different from zero. A second important assumption is that V_b does not change shortly after a change in composition of the mucosal solution. This cannot be fully validated at

Table 6. Ion-dependent partial potential ratios (T's) calculated from single-ion substitutions

T	Concentration range (mM)		
	2.5–11	11–56	56–112
T_K	0.67 ± 0.11	0.79 ± 0.06	0.66 ± 0.05
T_{Cl}	0.23 ± 0.06	0.38 ± 0.09	0.42 ± 0.08
T_{Na}	0.01 ± 0.01	0.06 ± 0.01	0.10 ± 0.01

T's calculated according to Eq. (5). Substitutions were: K-Na ($n=5$), Cl-gluconate or methylsulfate ($n=4$), Na-NMDG ($n=4$).

present, but two lines of evidence appear to indicate that it is in general correct: first, the resulting values of V_a are relatively time-independent, and if a change in V_b does take place as a result of changes in intracellular ion concentrations, one would expect it to produce a time-dependent change in the calculated V_a; second, in theory V_b might change, if it is generated by rheogenic Na transport, as a function of intracellular Na activity; if such were the case, bilateral reductions in Na concentration would depolarize both borders, but the effect of NMDG-for-Na substitution was a slight increase in both cell membrane potentials. Our final assumption is that R_b does not change after a mucosal solution substitution. This assumption was discussed in the preceding paper [17].

From the values of V_a as functions of external ionic concentrations, ion-dependent partial potential ratios were calculated, according to Eq. (5) [19]. The results of these calculations are shown in Table 6. T_K values are not statistically different in the three concentration ranges explored. T_{Cl} seems to be higher at higher external [Cl]. This effect might indicate that the Cl conductance is dependent on external Cl concentration, or that it is dependent on the membrane potential. Similarly, T_{Na} appears to increase with high mucosal Na concentrations. From the partial potential ratios when V_s is assumed to be zero, the relative values are $T_K/T_{Cl}/T_{Na} = 1.00:0.63:0.15$; if V_s is assumed to be 1.2 mV (under control conditions), $T_K/T_{Cl}/_{Na} = 1.00:0.75:0.10$.

In summary, we conclude that the luminal membrane of this epithelium has a high K permeability and a low Na permeability. Absolute values of the permeability coefficients will be estimated below.

Our calculations of V_a after ionic substitutions are performed under the assumption that V_b is not changed after mucosal substitutions. However, as shown in Tables 1–3, V_{cs}, the measured potential across the

basal-lateral membrane, changed in every instance. This is also illustrated in Figs. 1 and 2. Given the relative resistances of the shunt and the cell membranes [7, 8, 17], and the permselectivity of the shunt in this tissue and in gallbladders of other species [3, 10, 15], the changes in V_{cs} are predictable, because of the shunt current generated by changes in V_a, and because of the bi-ionic potential produced in the paracellular pathway when the mucosal solution is changed. These mechanisms have been fully described in *Necturus* proximal tubule [1]. In addition, a comparison of the changes in V_a and in V_{mc} in the three series of substitution shows that they differ (in Na-NMDG substitutions, by several-fold). Again, this indicates that single-sided replacements produce changes in the ipsilateral cell membrane potential in a complex way, in which at least two EMF's and two resistances can be altered. Thus, it is impossible to draw conclusions about the properties of the membrane without solving for the whole circuit. This is illustrated by our results of Na-NMDG replacements. As shown in Table 3, the changes in V_{ms} are greater than the changes in V_{mc}, at every concentration range. Given the ratio of R_s to R_a (*see* [17]), the fact that $\Delta V_{ms} > \Delta V_{mc}$ indicates that $\Delta V_s \gg \Delta V_a$; in other words, the changes in V_{mc} in these experiments were brought about mainly by changes in the shunt EMF, and not in the EMF of the cell membrane itself. Lack of understanding of this fact would induce a two- to three fold overestimation of T_{Na}. In tight epithelia, the magnitude of the error would be smaller. In fact, the higher the value of $R_s/(R_a+R_b)$, the less the deviation between ΔV_{mc} and ΔV_a. Unfortunately, most of the information available on ionic permeabilities of cell membranes in epithelia has been derived from measurements of membrane potentials and not equivalent EMF's [9, 11, 20]. The previous considerations clearly demonstrate the necessity of re-examining some of these issues. The direction of the deviation between ΔV_{mc} and ΔV_{cs} is not constant. If the only effect of the paracellular pathway were to "shunt" the alteration in electrical potential generated by the change in luminal solution, ΔV_{mc} would be always smaller than ΔV_a. The second mechanism by which the presence of a paracellular pathway produces misestimations of ΔV_a is the generation of a bi-ionic potential in the shunt itself, because of its permselectivity. When the polarity of this bi-ionic potential is the same as that of the change in V_a, ΔV_{mc} tends to be greater than ΔV_a (e.g., Na-NMDG substitutions); when the polarities of ΔV_a and ΔV_s are opposite, ΔV_{mc} tends to be smaller than ΔV_a (e.g., Cl-gluconate substitutions). The only situation, in a leaky epithelium, in which $\Delta V_a \sim \Delta V_{mc}$ is when $\Delta V_a \gg \Delta V_s$ for a given substitution. The con-

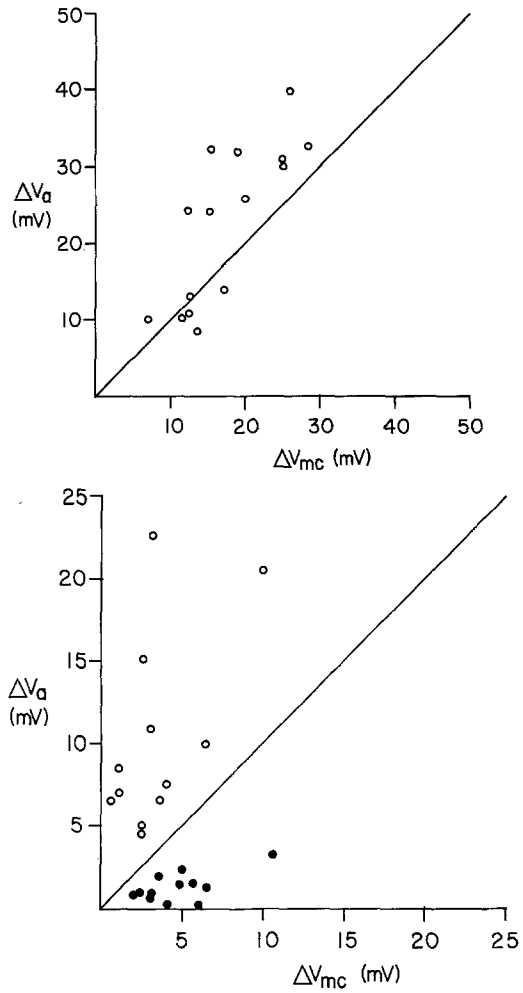

Fig. 7. Comparison between changes in apical membrane potential (ΔV_{mc}) and changes in apical membrane equivalent EMF (ΔV_a) after single ion substitutions on the mucosal side. Upper graph, Na-K substitutions. Lower graph, Cl-gluconate or methylsulfate substitutions (open circles) and Na-NMDG substitutions (filled circles). Each symbol corresponds to the effects of a change in concentration, in one of the three ranges explored, in a single preparation. Straight lines=identity lines. Note the wide deviations from identity, especially in Cl and Na substitutions with nonpermeant ions

dition, in these experiments, closest to this description is the Na-K substitution, where $(P_K/P_{Na})_{apical} \gg (P_K/P_{Na})_{shunt}$. Even in this situation, however, some deviations do occur, and a plot of ΔV_a *vs.* ΔV_{mc} does not yield identity (Fig. 7, upper graph). In Cl-gluconate and Na-NMDG substitutions, the deviations are far more pronounced (Fig. 7, lower

graph). In fact, the use of ΔV_{mc} values to calculate relative permeabilities would yield $P_{Na} > P_{Cl}$, and, as shown, the opposite is true. In a recent study in rabbit gallbladder epithelium [12], although the results are consistent with high K conductance at both cell borders, the conclusions concerning permeabilities for other ions are unjustified, since the contribution of the shunt EMF to the membrane potential was neglected.

Dependence of Apical Membrane Resistance on Mucosal Solution Ionic Concentrations

The changes in R_a following mucosal solution substitutions strongly support our interpretation of the changes in EMF's. Only changes in K concentration in the mucosal solution produced significant changes at any concentration range. As shown in Figs. 1 and 2, the changes in the voltage divider ratio (R_a/R_b) following an increase or decrease in mucosal K concentration were complete within 1 or 2 sec. This observation makes unlikely a participation of changes in cell volume, shown to alter membrane resistances in other tissues [5], in the drop of R_a. In consequence, we conclude that the increase in apical membrane conductance observed after increases in K concentration in the mucosal solution is due to high K permeability of that membrane.

Cl and Na substitutions produce smaller changes in R_a. In fact, they were not significant (in the three concentration ranges) in Cl-gluconate substitutions, and only between 2.5 and 111 mM in Na-NMDG replacements. These results indicate (the same as the EMF changes) that apical membrane Cl and Na permeabilities are far lower than K permeability. Given the experimental error inherent in the measurement of R_a, it is difficult to ascribe significance to the observation that R_a changes were very small in both Cl and Na substitutions. From the changes in V_a one would expect R_a changes to be greater in Cl-gluconate than in Na-NMDG substitutions. This apparent discrepancy might simply indicate experimental error, but other possibilities cannot be ruled out. For instance, external Cl concentration might exert an effect on g_K, g_{Na}, or both. In this view, the effect of Cl concentration on the membrane EMF and conductance would be mediated by changes in the cation conductance of the membrane, i.e., at high Cl concentration g_K/g_{Na} would increase, and thus V_a would increase, and at low [Cl] g_K/g_{Na} would decrease, with a consequent decrease in V_a. Since the tendency of R_a if anything is to increase in high external Cl such

effects on cation conductances would be on g_{Na} rather than on g_K. Thus, in high Cl, g_{Na} decreases and R_a increases, while in low external Cl, g_{Na} increases and R_a decreases. This would be complicated to prove experimentally, but illustrates the difficulty in interpreting this kind of measurements: i.e., changes in V_a and R_a as functions of Cl concentration can be mainly secondary to an indirect effect through changes in g_{Na}. Thus, "apparent" transference numbers and permeabilities have to be taken as phenomenological coefficients only, and not as real indicators of a particular physico-chemical process taking place in the membrane.

Calculation of Ionic Permeability Coefficients Across the Apical Membrane

If the ion-dependent partial potential ratios (T's) are employed as transference numbers (t's), permeability coefficients can be calculated according to Eq. (6). It is necessary to assume a mean ion concentration in the membrane, and to assume as well that active transport across the apical membrane is neutral, i.e., no rheogenic pumps are present. As the profiles of individual ion concentrations are unknown, and no data are available on intracellular ion activities in *Necturus* gallbladder, we chose to assume linear profiles for every ion, starting with intracellular concentrations similar to those measured by Diamond in fish gallbladder [2] and Frizzell, Dugas and Schultz in rabbit gallbladder [6]. The influence of these assumptions on the calculated values is discussed below.

Table 7 summarizes the results of these calculations when the intracellular concentrations were assumed to be Na=60 mM/liter, K=85 mM/

Table 7. Ionic permeability coefficients of the apical membrane

P	Concentration range (mM)		
	2.5–11	11–56	56–112
P_K	12.15	14.87	12.53
P_{Cl}	4.19	5.54	4.63
P_{Na}	0.27	1.04	1.12

Permeability coefficients calculated from Eq. (6), assuming intracellular concentrations (Na=60, Cl=85, K=85 mM/liter), and linear ion concentration profile in the membrane. Membrane total conductances are the means of the values at the two external ion concentrations. P's are expressed as 10^{-7} cm sec^{-1}.

liter, and Cl = 85 mM/liter. Each P value was estimated at three concentration ranges, g_t being calculated from the arithmetic mean of the apical membrane resistances at the two external concentrations of the ion. P_K is at least one order of magnitude higher than P_{Na}. In addition, the absolute value of P_{Na} can be used to predict the Na flux from the mucosal solution into the cell, by assuming again a constant field in the membrane, and thus a constant Na concentration profile within the membrane and using the mean value of V_{mc} as the electrical term driving Na entry. The calculated flux represents then the maximum possible Na flux that can be expected on the basis of passive diffusion. The result of such a calculation is 2.1×10^{-12} M cm^{-2} sec^{-1} for $T_{Na} = 0.1$ (56–112 mM range), when Na_{cell} is assumed to be 60 mM. Measured *net* gallbladder mucosal to serosal Na fluxes are 2.3×10^{-9} in fish [2], 3.8×10^{-9} in rabbit [6], and 1.4 to 5.6×10^{-9} in *Necturus* (*unpublished*), all in M cm^{-2} sec^{-1}. These net M to S fluxes are the minimum influx across the apical membrane, because $J_{m \to cell} = J^{net}_{m \to s} + J_{cell \to m}$. In brief, the maximum predicted Na influx is three orders of magnitude smaller than the minimum Na influx obtained from transepithelial measurements. Our calculation of the value of J_{Na} depends strongly on the mean ion concentration in the membrane, and in consequence, on the intracellular concentration, if a linear profile in the membrane is assumed. However, even the assumption of an intracellular Na concentration of 1 mM instead of 60 mM yields a flux of only 2.0×10^{-11} M cm^{-2} sec^{-1}. This reasoning is based on the calculated value of P_{Na}, and in turn this calculation requires one to assume electroneutral active ion transport across the apical membrane. If one or more rheogenic pumps were present at this level, our P values could be significantly wrong. In any event, even if one ascribes the total conductance of the membrane to Na (under control conditions) and calculates the passive flux from the mucosal solution into the cell, the result (2.7×10^{-10} M cm^{-2} sec^{-1}, if Na_{cell} cell = 60 mM) is still one order of magnitude smaller than the measured net M to S Na flux. These results prove that Na entry into the epithelial cells in the gallbladder cannot be explained by independent diffusion.

For a cell Cl concentration of 85 mM/liter, Cl entry is against its electrochemical gradient, since V_{mc} is about 58 mV. Cl entry could be passive only at intracellular concentrations below about 11 mM (one tenth of the external Cl concentration). The possibility of a "neutral" NaCl influx (i.e., electrically silent), as suggested by Diamond [3] seems very likely, especially since complete replacement of mucosal Cl by a non-transported anion reduces Na M to S flux in gallbladder of *Necturus*

(*unpublished*) as well as in other species [2, 4]. Our conclusions are entirely consistent with recent Na influx determinations in rabbit gallbladder [6].

If similar calculations are done for the basal-lateral membrane, it can be easily shown that Na transport from the cell into the serosal solution has to be an active process, since it takes place against both chemical and electrical gradients. For Cl extrusion, the situation is not entirely clear, since the maximum predicted passive flux (from $V_{cs}=60$ mV, $T_{Cl}=1$, $Cl_{cell}=85$ mM/liter, and $R_b=2{,}750\ \Omega\ cm^2$) is 5.4×10^{-9} M cm^{-2} sec^{-1}. Evidently, then, a significant portion of the Cl flux across the basal-lateral membrane might be explained by simple diffusion, according to the estimated electrochemical potential gradient, if the membrane has a significant Cl conductance. A final conclusion regarding this mechanism will have to wait until reasonable estimations of Cl conductance are obtained.

The mechanism of generation of the potential across each cell membrane remains in doubt. K diffusion potentials operating at both borders would explain our results, given the fact that the basal-lateral membrane exhibits an even higher degree of K permselectivity than the luminal membrane. Thus, the transepithelial potential would be the consequence of $V_b > V_a$ when standard Ringer's solution bathes both sides of the cells [17]. If such were the case, Na-K exchange pumps operating at both borders would suffice to explain the maintenance of cation concentration gradients across the cell membranes. However, the alternative or additional possibility of a rheogenic Na pump at the basal-lateral border cannot be ruled out. Such a current source might contribute a significant fraction of the cell potential and generate the K concentration gradient from cell to medium, if the latter ion is passively distributed. It is interesting to note that, in rabbit gallbladder epithelial cells, chemical determinations of intracellular K concentration yield values close to those calculated from the measured electrical potential, if K is assumed to be at equilibrium [6]. However, the error involved in such a procedure may be considerable. The determination of intracellular ion activities with ion-selective microelectrodes should be helpful in answering this question and providing the data necessary to establish the site(s) of active ion transport.

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References

1. Boulpaep, E.L. 1971. Electrophysiological properties of the proximal tubule: Importance of cellular and intercellular transport pathways. *In:* Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 91. Schattauer-Verlag, Stuttgart
2. Diamond, J.M. 1962. The reabsorptive function of the gall-bladder. *J. Physiol.* **161**:442
3. Diamond, J.M. 1962. The mechanism of solute transport by the gallbladder. *J. Physiol.* **161**:474
4. Dietschy, J.M. 1964. Water and solute movement across the wall of the everted rabbit gallbladder. *Gastroenterology* **47**:395
5. Finn, A.L., Reuss, L. 1975. Effects of changes in the composition of the serosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Physiol.* **250**:541
6. Frizzell, R.A., Dugas, M.C., Schultz, S.G. 1975. Sodium chloride transport by rabbit gallbladder: Direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* **65**:769
7. Frömter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**:259
8. Frömter, E., Diamond, J.M. 1972. Route of passive ion permeation in epithelia. *Nature, New Biol.* **235**:9
9. Frömter, E., Müller, C.W., Wick, T. 1971. Permeability properties of the proximal tubular epithelium of the rat kidney studied with electrophysiological methods. *In*: Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 119. Schattauer-Verlag, Stuttgart
10. Gelarden, R.T., Rose, R.C. 1974. Electrical properties and diffusion potentials in the gallbladder of man, monkey, dog, goose and rabbit. *J. Membrane Biol.* **19**:37
11. Giebisch, G. 1961. Measurements of electrical potential differences on single nephrons of the perfused *Necturus* kidney. *J. Gen. Physiol.* **44**:659
12. Hénin, S., Cremaschi, D. 1975. Transcellular ion route in rabbit gallbladder. Electric properties of the epithelial cells. *Pflügers Arch.* **355**:125
13. Hodgkin, A.L., Horowicz, P. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**:127
14. Hodgkin, A.L., Katz, B. 1949. The effect of sodium on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**:37
15. Machen, T.E., Diamond, J.M. 1969. An estimate of the salt concentration in the lateral intercellular spaces of rabbit gall-bladder during maximal fluid transport. *J. Membrane Biol.* **1**:194
16. Reuss, L., Finn, A.L. 1974. Passive electrical properties of toad urinary bladder epithelium: Intercellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* **64**:1
17. Reuss, L., Finn, A.L. 1975. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. I. Circuit analysis and steady-state effects of mucosal solution ionic substitutions. *J. Membrane Biol.* **25**:115
18. Spenney, J.G., Shoemaker, R.L., Sachs, G. 1974. Microelectrode studies of fundic gastric mucosa: Cellular coupling and shunt conductance. *J. Membrane Biol.* **19**:105
19. Strickholm, A., Wallin, B.G. 1967. Relative ion permeabilities in the crayfish giant axon determined from rapid external ion changes. *J. Gen. Physiol.* **50**:1929
20. Whittembury, G., Sugino, N., Solomon, A.K. 1961. Ionic permeability and electrical potential differences in *Necturus* kidney cells. *J. Gen. Physiol.* **44**:689
21. Windhager, E.E., Boulpaep, E.L., Giebisch, G. 1967. Electrophysiological studies in single nephrons. *Proc. 3rd Int. Congr. Nephrol.*, Washington, 1966. p. 35. Karger, Basel-New York